The Development of a Novel Controlled Release Drug Delivery System

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by
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Abstract

The aim of this research was to formulate, characterise and assess the feasibility of a novel drug delivery system known as the *in situ* gelling matrix (ISGM) where a hydrophilic polymer is suspended in a non-aqueous solvent that converts into a gel when injected subcutaneously or intramuscularly thus giving a controlled release matrix for a drug. Although the concept has been patented with claims that this kind of drug delivery is achievable in theory for a wide variety of candidate substances, actual formulation studies for making a commercially viable product for this technology are completely lacking in practice. The research embodied in this thesis addresses this lack. Initial studies involved conducting a biocompatibility study using the HET-CAM (hens egg test – chorioallantoic membrane) test on a range of possible ingredients for the delivery system. The materials deemed biocompatible were then carried through to a screening process where the physical stability of the hydrophilic polymers in non-aqueous solvents was monitored. It was found that the hydrophilic polymers tested sedimented rapidly in the non-aqueous solvents indicating such a system was not physically stable. Consequently, density-inducing or viscosity-inducing agents were added to the non-aqueous solvents to retard the sedimentation rate. The addition of polycarbophil, a viscosity-inducing agent, clearly increased the viscosity of the system. However, undesirable formation of polycarbophil globules occurred during the manufacturing process, which caused batch-to-batch variations in the viscosity of the continuous phase. Various manufacturing methods were tested before arriving at the optimum procedure to prevent globule formation using a high speed dispersion tool. A final physical sedimentation analysis of candidate continuous phases and hydrophilic polymers was conducted for determining the ideal combination of ingredients to use in the system. These investigations finally led to the adoption of an optimum mix of components consisting of 10% (w/w) hydroxypropyl methylcellulose (HPMC) (the hydrophilic polymer) suspended in a continuous phase of propylene glycol (the non-aqueous solvent) containing 0.67% (w/w) polycarbophil (the viscosity inducing agent).

Using this mix of components, the *in situ* gelling matrix system was then subjected to various characterisation studies including infrared (IR), differential scanning calorimetry (DSC), ultraviolet-visible (UV-Vis) spectrophotometry and redispersion studies. The chemical stability of the hydrophilic polymer and the continuous phase (the non-aqueous solvent and polycarbophil) was monitored and were found to be chemically stable over a 9 month period.

The feasibility of the *in situ* gelling matrix technology as a controlled release device was assessed using the drug propranolol. *In vitro* drug release studies were conducted using a custom-built dissolution apparatus. The effect of various parameters such as the concentration of the hydrophilic gelling agent on the drug release rate was investigated. Increasing the concentration of the gelling agent in the formulation resulted in a slower rate of release. The drug release data were modelled using the Higuchi relationship and a power law relationship to compare the effects of the various parameters on the release rate

Stability studies on the drug in the *in situ* gelling matrix system were carried out by storing samples in accelerated ageing conditions of 40°C / 75% relative humidity for 4 weeks. During this time, the samples were analysed each week by high performance liquid chromatography (HPLC). These demonstrated that no apparent drug degradation had occurred over the 4-week period. This indicates that the drug propranolol in the *in situ* gelling matrix system is stable under ambient conditions for at least 4 weeks.

The results of this study demonstrated that the *in situ* gelling matrix technology is potentially viable as a drug delivery system and provide a practical methodology for the commercial development of such systems.

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List of Abbreviations

DSC	Differential Scanning Calorimetry
ESMS	Electrospray Mass Spectrometry
GRAS	Generally Recognised As Safe
HET-CAM	Hen’s Egg Test – Chorioallantoic Membrane
HPLC	High Performance Liquid Chromatography
HPMC	Hydroxypropyl Methylcellulose
IM	Intra-muscular
IR	Infra-Red
ISGM	*In Situ* Gelling Matrix
IV	Intravenous
LOD	Limit of Detection
LOQ	Limit of Quantitation
NMP	N-Methyl Pyrrolidone
PCP	Polycarbophil
PDA	Photodiode Array
PEG 400	Polyethylene Glycol 400
PEO	Polyethylene Oxide
PG	Propylene Glycol
PLA/PLGA	Poly-Lactide-Acid / Poly(Lactic-Co-Glycolic) Acid
PVA	Polyvinyl Alcohol
PVP	Polyvinyl Pyrrolidone
RI	Refractive Index

SC	Subcutaneous
SEC	Size Exclusion Chromatography
Sodium CMC	Sodium Carboxymethylcellulose
USP	United States Pharmacopoeia
UV-Vis	Ultraviolet-Visible

Glossary of Terms

Continuous Phase	The non-aqueous solvent and the viscosity inducing agent
Extra-vascular Coagulation	Precipitation of CAM proteins
Final Product / Formulation	The ISGM system and the drug
Haemorrhage	Bleeding of the CAM vessels
Hyperaemia	The swelling of small blood vessels resulting in increased blood flow
Intra-vascular Coagulation	The blood flow in the vessels will decrease and eventually stop as a result of thrombosis
ISGM system	The hydrophilic polymer suspended in the continuous phase
Lysis	The endothelial vessel structure is damaged

CHAPTER ONE

Introduction

When administering a drug, there is an optimum concentration range for which to gain the drug's maximum therapeutic benefit. Ideally the drug level in the body should remain between the maximum and minimum effective levels. Beyond the maximum level represents a toxic level and below the minimum the drug is no longer effective. With conventional methods (such as immediate release tablets), a single dose will result in the instant maximum release of the drug (perhaps above the maximum level) which then drops back below the minimum effective level (Freiburg and Zhu, 2004). Consequently multiple doses are required to maintain the average drug level within the optimum range (Figures 1.1a and 1.1b).

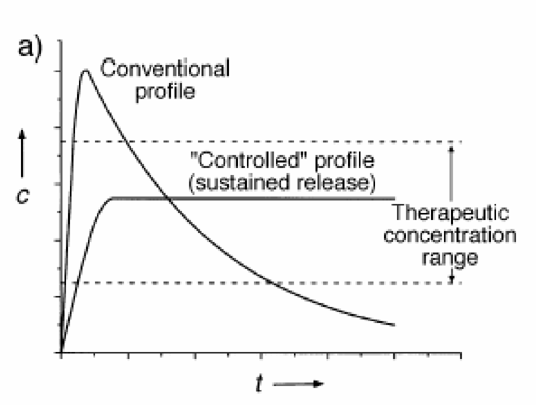


Figure 1.1a – Comparison of Conventional and Controlled Release Profiles
(Santini *et al.*, 2000)

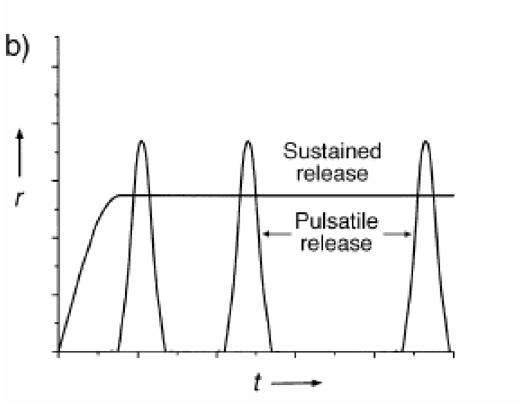


Figure 1.1b –Dosage Regime for Conventional and Controlled Release Systems
(Santini *et al.*, 2000)

To overcome the need for frequent dosing, controlled release drug delivery systems have been developed (Matschke *et al.*, 2002; Rothen-Weinhold *et al.*, 2000). Controlled release drug delivery systems first appeared in the 1960's and 1970's (Santini *et al.*, 2000; Banker, 1996). Since then there have been numerous systems developed and an entire field of scientific research is dedicated to the science and technology of controlled release drug delivery.

Controlled release drug delivery systems can be defined as chemical, mechanical or electromechanical devices which deliver drugs to the systemic circulation or to specific target sites in the body at pre-determined and controlled rates (Smolen, 1978; Smolen, 1981). The ideal controlled release drug delivery system would deliver the drug at a rate required by the body during the treatment period and would also deliver the drug specifically to the site of action. However, currently there is no such delivery system capable of fully achieving these aims.

Conventional methods of delivering drugs such as dissolution from a tablet are not able to predictably control the rate of release or control the site of action. Targeted drug delivery systems can control the site of action but are usually unable to dictate the release kinetics of the drugs in a predictable manner. Controlled release systems generally can control the rate of drug release but cannot control the fate of the drug once it has been released (Li *et al.*, 1987).

Controlled release drug delivery systems have been used extensively in human health. They also have wide application in the animal health industry. In 1998 controlled release dosage forms amounted to $1 billion out of a $7 billion market for veterinary pharmaceuticals. Of the $1 billion, 40% was estimated to be from parenteral (i.e., delivered outside of the gastrointestinal tract) sustained release products (Medlicott *et al.*, 2004). The major market for controlled release drug delivery in the veterinary field is disease prevention and control by delivery of antimicrobial and antiparasitic agents. Other areas applicable to the veterinary field are fertility control, oestrus synchronisation and supplementation of nutritional agents (Rothen-Weinhold *et al.*, 2000).

The advantages of controlled release drug delivery in the veterinary field are:

- The ease with which they are administered.
- Less stress to the animal due to reduced restraining.
- Less time and effort.
- Reduced handling and need for yarding of the animals.
- Reduced overall cost of treatment compared to the cost of multiple administration.
- Knowledge that the animal is being treated for the required length of time (i.e., doses are not missed which can occur if treatment involves a multiple dosage regime).
- The dose of drug administered is known unlike when the drug is added to food and water.
- Human exposure to veterinary compounds which may be unsafe to handle will be minimised.

Recent developments in dosage form design in the area of injectables for controlled release of drugs have focussed on the development of easily injectable systems that form solid implants following administration. Recently a new and novel *in situ* forming gel technology system has been described in the patent literature (Bunt *et al*., 2003). The patent describes a possible idea for a new *in situ* gelling matrix (ISGM) system, broadly described as a suspension of a hydrophilic polymer in a non-aqueous solvent.

The patent awarded to Bunt *et al*. (2003) describes a formulation for parenteral administration to a subject. The formulation described comprises at least one water miscible solvent, at least one gelling agent and at least one active agent. The gelling agent is in particulate form and suspended in the solvent. The patent describes the active agent in the formulation as being any of the following agents: antibacterial, antifungal, fungicidal, anti-inflammatory, antiparasitic, anti-neoplastic, analgesic, anaesthetic, antipsychotic, vaccine, central nervous system, growth factor, hormonal, antihistamine, osteoinductive, cardiovascular, antiulcer, bronchodilating, vasodilating, birth control, antihypertensive, anticoagulant, antispasmodic, fertility-enhancing and combinations thereof. The formulation is claimed to be administered via the parenteral route which is selected from the group consisting of subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intrastemal and intravenous and within a plant. In the case of a tree, a fungicide could be administered by injecting the formulation to fill a hole in the tree.

Although, the patent by Bunt *et al*. (2003) gives a broad description of what the ISGM system may incorporate, i.e., a suspension of any hydrophilic polymer in any non-aqueous solvent, no rational investigation has been conducted on the best mix of excipients to use in such an ISGM system. There are some obvious challenges and issues regarding the pharmaceutical formulation of this ISGM system. The ISGM system is described as a non-aqueous system, for which there is limited information in the literature and consequently extensive research is needed to find the ideal excipients to use so that the ISGM system could be formed as a physically stable suspension. In the case of aqueous suspensions, stabilisation might be achieved by adding hydrophilic polymers which thicken or gel in the presence of water. This could obviously not be done in the ISGM system under development in this study, as the system is based on a non-aqueous solvent. The difference lies in the fact that the hydrophilic polymer (which is added to the system) can only be *suspended* in the non-aqueous solvent and will only gel when the system comes into contact with aqueous fluids after injection into the body. The use of non-aqueous solvents thus becomes challenging in that the hydrophilic polymer must be also *maintained* in suspension without settling so that an acceptable time of dispersion stability is realised after shaking the ISGM system immediately prior to its injection into a living organism. To guarantee such a degree of dispersion stability, a further component modifying the non-aqueous solvent density or viscosity would thus be required. As a further complication, reactions may occur at the (subcutaneous or intramuscular) injection site due to unpredicted lack of biocompatibility between the non-aqueous solvent and living tissue. There is the possibility of allergic reactions and irritations.

Consequently, the initial thrust of the research described in this thesis was aimed at formulating the ISGM system to find the ideal combination of components to use for achieving a practical realisation of the claims set out in Bunt *et al.*'s (2003) patent. This required a cursory biocompatibility assessment of the excipients and a sedimentation analysis of the possible hydrophilic polymers to add to a range of candidate non-aqueous solvents. This led to an ideal combination of a hydrophilic polymer and a non-aqueous solvent which comprised a working system. The decision was made to assess the stability and drug delivery capability of the system using propranolol as a model drug.

The structure of this thesis is as follows:

Chapter 2 reviews the background and theory of some commonly used injection methods as well as the current state of technology in this area. Chapter 3 is the methodology section which outlines the experimental procedures followed in this study for achieving the aims set out in Chapter 1. The detailed discussion on the rationale of the studies undertaken is given in Chapter 4. Chapter 3 has been divided into sections each with a brief introduction. Section 3.1 mainly deals with biocompatibility of various candidate non-aqueous solvents and hydrophilic polymers. The excipient candidates which did not cause any significant immunological response were carried forward to a screening process, described in Section 3.2. The screening process involved investigating the physical stability of the hydrophilic polymers in non-aqueous solvents. Modifications to the solvents to improve physical stability by means of density- or viscosity-inducing agents were also investigated. Section 3.3 describes the characterisation of the ISGM system by carrying out experiments such as viscosity studies, ease of redispersion and water absorption studies. The chemical stability of the hydrophilic polymer and the continuous phase was also assessed. Section 3.4 describes characterisation of the final formulation by DSC, IR analysis and UV-Vis analysis. Once the formulation had been characterised, *in vitro* studies were conducted as described in Section 3.5. The *in vitro* studies involved addition of a drug to the ISGM system and measuring its release over time. The effects of various factors on the release rate of the drug were assessed. The stability of the formulation was assessed as described in Section 3.6. The purpose of the stability trial, carried out under accelerated ageing conditions, was to determine how the quantity of the drug in the system changed over time.

Chapter 4 contains the results and discussion of the experimental procedures described in Chapter 3. The final chapter of the thesis, Chapter 5, begins with a summary of all the findings along with recommendations for future research followed by a list of all the conclusions made as a result of this study.

CHAPTER TWO

Literature Review and Scope of this Project

There is currently considerable research on the development of new drug delivery systems. This wide interest is due to the following factors:

- The possibility of product lifecycle management for successful drugs by using controlled release drug delivery systems. The introduction of new drug entities to the market is increasingly expensive. Time is also a factor. The development of a new chemical entity takes approximately 10 - 12 years whereas a new drug delivery system only involves 3 - 4 years (Verma *et al*., 2002).

- New delivery systems are required to deliver genetically engineered pharmaceuticals such as peptides and proteins to the site of action without incurring significant immunogenicity or biological inactivation.

- Treatment of enzyme deficiencies and cancer therapies can be improved by more efficient targeting.

- The therapeutic efficacy and safety of drugs which are administered by conventional methods (such as immediate release tablets) can be improved by precise spatial and temporal placement in the body. This will reduce the size and number of doses (as discussed in Chapter 1).

Background

2.1 Routes of Drug Administration: The Parenteral Route

There are many possible routes of drug administration such as oral, rectal, transdermal and parenteral. The technology developed and investigated in this study is designed for administration via the parenteral route. Parenteral is derived from the Greek words *para* which means beside and *enteron* which means gut. Together these words mean something done outside of the intestine. Therefore parenteral products are those administered outside of the gut / gastrointestinal tract (Ansel and Popovich, 1990; Li *et al*., 1987).

The advantages of the parenteral route of drug administration are the rapid absorption of drugs (except for intravenous bolus and infusion administration as the drug is administered directly into the systemic circulation), avoidance of the first pass (hepatic) metabolic effect, the blood level of the drug is more predictable (as little is lost with intramuscular and subcutaneous injections and virtually none with intravenous) and consequently smaller doses can be administered. The disadvantage of parenteral delivery is that once the drug is injected, removal is practically impossible. In contrast, if a reaction or overdose occurs with other routes (such as the oral route), there is time between administration and drug absorption to remove the drug via induced vomiting for instance.

There are many routes of parenteral administration such as intramuscular (IM), subcutaneous (SC) and intravenous (IV) (Ansel and Popovich, 1990).

2.1a Intravenous Injections

With intravenous injections, normally an aqueous solution is injected into the venous system. The solution must not precipitate once injected into the circulatory system as this may produce an embolism (Aoki, 1994). An example of an intravenously injected controlled release delivery system is liposomes. Liposomes were first discovered in the 1960’s and have been extensively studied for the intravenous delivery of a variety of pharmaceutical compounds (Patil and

Burgess, 2005). There are currently more than 10 approved liposomal formulations on the market with several others in the advanced stage clinical trials (Crommelin and Storm, 2003).

Liposomes are complex emulsion like systems in which the drug is entrapped in the aqueous or lipid layer (which layer depends on the physicochemical properties of the drug). Liposomes are microparticles ranging in size from 0.03 to 10 µm containing a bilayer of phospholipids encapsulating an aqueous space. The polar head of the lipids face the aqueous phase and the hydrophobic hydrocarbon chains adhere together in the bilayer (Ranade and Hollinger, 1996) (Figure 2.1). In an aqueous environment the lipid molecules swell and self-assemble in a definitive and consistent orientation which is also known as a bi-layer structure.

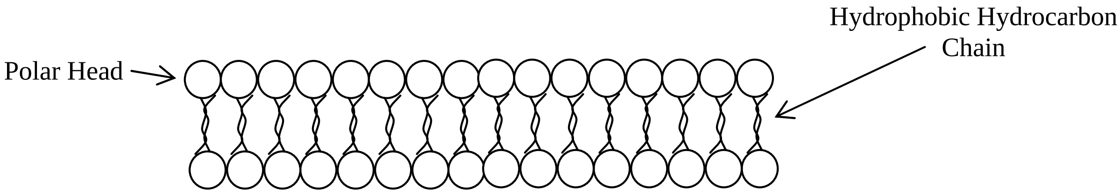


Figure 2.1 – The Lipid Bilayer

The disadvantages of liposomal products are they are expensive to manufacture and have limited stability (Feng *et al.*, 2004; Malmsten, 2006).

2.1b Intramuscular Injections

IM injections are made deep in the muscle (Figure 2.2). The site of injection is one where the chances of hitting a nerve or blood vessel are minimal. Drugs which are irritating to the SC site may be injected into muscle tissue (Ansel and Popovich, 1990).

2.1c Subcutaneous Injections

Subcutaneous injections are made into the subcutaneous layer below the skin (Figure 2.2). Upon injection the drug comes into contact with blood capillaries and diffuses or filters into them. In many animals, injections are made in areas of loose skin and where the SC site is more easily reached, such as the back of the neck. The injections are made into the space between the dermis and the underlying SC fat layer.

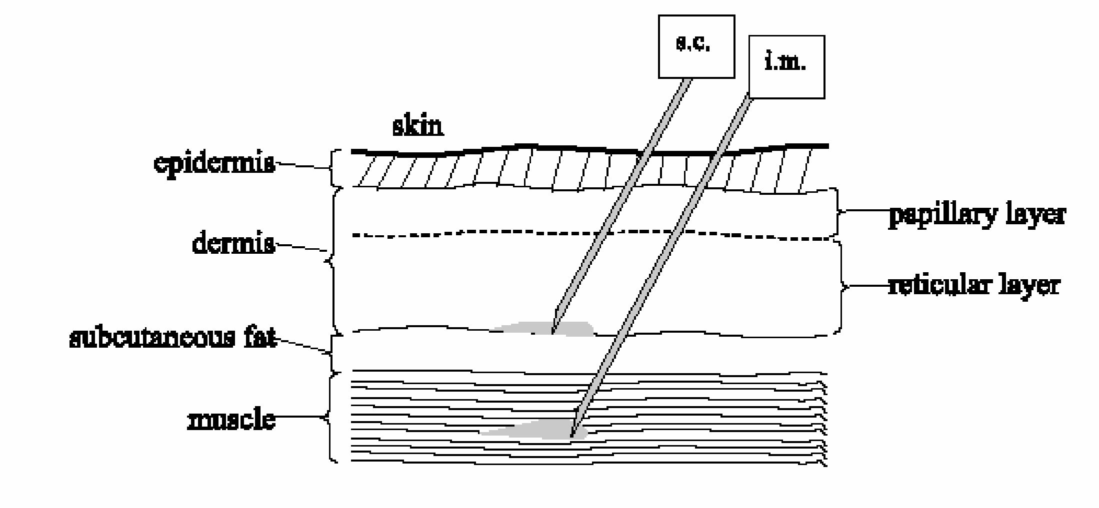


Figure 2.2 – Depiction of SC and IM Injections (Medlicott *et al*., 2004)

2.1d Preferred Injection Methods in Veterinary Drug Delivery

The IM and SC routes are the main routes of drug administration. The preferred route of administration depends on the animal and condition to be treated. The SC site is preferred in meat producing animals to minimise commercial losses due to injection site reactions and any drug residue that may be present in the muscle. If high doses of drug are administered then a withholding period is required when the tissue concentration exceeds the residue limit. Such problems can be avoided by injecting into the ear of an animal. This will reduce the drug residue in the muscle (for meat producing animals) (Brown, 2000).

The IM route may be more appropriate than the subcutaneous route for companion animals (for example cats and dogs) where the risk of slow release of drug into the muscle, tissue residue and some local tissue damage is not as important (compared to meat producing animals).

Different injection sites may give a difference in drug absorption rate. These differences will be due to factors such as tissue composition and flow rate and abundance of blood and lymphatic supplies.

The drug absorption rate tends to be faster at the IM site as the muscle is better perfused with blood than the SC layer.

2.2 Drug Release Mechanisms in Controlled Release Devices

With the exception of mechanical pumps controlled release devices use polymers in the rate control mechanism (Heller, 1987). Such devices can be classified into the following categories (Heller, 1987; Rathbone *et al.*, 2000; Ranade and Hollinger, 1996):

- Diffusion controlled devices
- Solvent controlled devices
- Chemically controlled devices

2.2a Diffusion Controlled Devices

There are two types of diffusion controlled systems – matrix devices and reservoir devices. With matrix devices, the drug is either dissolved or dispersed in the polymer. The drug is released from the matrix by diffusion. Reservoir systems consist of a drug core which can be in powdered or liquid form. The drug core is surrounded by a non-biodegradable polymeric material which the drug slowly diffuses through. Because the polymer is non-biodegradable, the device must be removed from the body after all the drug has been released. The other disadvantage of reservoir systems is that if the reservoir membrane was to rupture then a large amount of drug could be released into the bloodstream.

Polymeric membranes can be either dense or porous. The membrane properties (as well as the properties of the drug) control the rate of release from such a system. In systems incorporating dense membranes, the drug particles entrapped within the reservoir system dissolve from the solid state into the medium (which the drug is suspended in) and then diffuse through the medium to the polymer membrane. Once the drug molecules have partitioned into the polymer membrane, the drug then diffuses through to the outer surface of the membrane. The drug then partitions into the fluid surrounding the delivery device. The rate of drug release will depend on the solubility of the drug in the polymer and the thickness of the membrane (Rathbone *et al.*, 2000). In the case where the membrane is porous, the drug will diffuse through the fluid filled pores, rather than the polymer itself due to favouring the path of least resistance. In such a system the release rate can be manipulated by altering the thickness of the membrane or by altering the size and number of pores in the membrane.

2.2b Solvent Controlled Devices

Solvent controlled release devices are a result of solvent penetration. There are two types of solvent controlled systems – osmotic and swelling (Heller 1987; Ranade and Hollinger, 1996).

Osmotic controlled systems involve an external fluid containing a low concentration of drug moving across a semi-permeable membrane into a region within the device containing a high concentration of drug. The increased volume in the osmotic compartment forces the drug out through a small orifice.

In swelling controlled systems, a polymer which can hold a large volume of water is employed. When the device is placed in an aqueous environment water penetrates the matrix and the polymer consequently swells. As a result of the polymer swelling, chain relaxation takes place and the drug is able to diffuse through.

2.2c Chemically Controlled Devices

Chemically controlled devices can also be divided into two classes – “pendant-chain” systems or biodegradable (also known as bioerodible) systems.

In pendant-chain systems, a drug molecule is chemically linked to a polymer backbone. The drug is either linked directly or via a spacer-group to the polymer and drug release occurs by chemical hydrolysis or enzymatic cleavage in the presence of biological fluids and enzymes in the body.

Bioerodible systems are designed to have a drug which is dispersed throughout the polymer. As the polymer degrades, the drug diffuses out. The major advantages of such systems are that the device does not have to be removed after release and the drug does not have to be water soluble.

Current State of the Art in Injectable Technologies

2.3 Types of Controlled Release Drug Delivery Systems

The technology being researched in this thesis is intended for use in veterinary drug delivery. There are various controlled release drug delivery systems currently used in the veterinary industry. These include delivery systems such as injections, subcutaneous implants and microspheres (Rothen-Weinhold *et al.*, 2000; Okumu and Cleland, 2003; Medlicott *et al.*, 2004; Burgess and Hickey, 2005; Freiberg and Zhu, 2004).

2.3a Injections

Injections are the most common dosage form used in animals. Indeed, one of the highest selling animal products is an injectable suspension called Posilac®. Posilac® is administered once every 14 days to increase milk production in dairy cows (Medlicott *et al.*, 2004). The popularity of injections as a dosage form arises because they offer several advantages to the farmer or veterinarian. These advantages include:

- Cheap formulations.

- Ease of administration of a drug to a large number of animals (for IM and SC).
- Means of rapid administration of a drug to a large number of animals.
- Animals are not needle shy.
- No need for removal of the delivery system after administration.
- The blood level of the drug is more predictable (as little is lost with intramuscular and subcutaneous injections and virtually none with intravenous) and consequently smaller doses can be administered.

Despite the popularity of injectable use within the animal health industry these types of dosage forms do have inherent disadvantages. These disadvantages include:

- Once the drug is injected, removal is practically impossible (Okumu and Cleland, 2003). If a reaction or overdose occurred with other routes such as the oral route, there is time between administration and drug absorption to remove the drug where vomiting can be induced.
- Different injection sites may give a difference in drug absorption rate. These differences will be due to factors such as tissue composition and flow rate and abundance of blood and lymphatic supplies.
- Injections have inherent problems associated with them; the shape, size and nature of the depot depends on the formulation composition, volume injected, the system's rheological properties, relative dispersal rates of formulation components and the forces due to tissue movement and muscle contraction (Medlicott *et al.*, 2004).

However, the biggest problem associated with controlled release from an injectable drug delivery system is that the duration of action is short. Typically a controlled release injectable does not deliver a drug for longer than 7 days. Some may reach 14 days (e.g., Posilac®). However, many clinical conditions require much longer delivery periods than this. To date this problem has been overcome by the formulation of implants.

2.3b Subcutaneous Implants

The administration of implants involves the use of large gauge needles or surgical procedures. Consequently due to the invasive nature of administering an implant, a prolonged drug release of more than 3 months is preferred. The advantage of implants is they operate for long periods of time when implanted and are reported to provide a better control of release than injectable systems (Okumu and Cleland, 2003). Implants can also be removed unlike injections where once the drug has been injected, removal is practically impossible. Subcutaneous implants are typically placed into the subcutaneous tissue in the ear of animals since this is a non-edible tissue of the animal.

Examples of commercial implant delivery devices are given below.

SYNCRO-MATE-B®

Subcutaneous implants for the ear are used to deliver sufficient quantities of progestagens. The progestagens are incorporated in either hydron or silicone polymers (Rathbone *et al.*, 1998). The SYNCRO-MATE-B® is an example of a commercially available ear implant for cattle.

The DUROS® Implant

The DUROS® implant is a titanium alloy cylinder which is capped at one end by a semipermeable membrane and an exit port at the other end (Figure 2.3). The cylinder contains an osmotic engine and a piston and a drug formulation. The delivery of the drug is based on osmotic principles. The implant's membrane is permeable to water but not the osmotic solutes in the osmotic engine. Water diffuses into the cylinder due to the osmotic gradient which exists between the osmotic engine and the extra-cellular fluid. As a result of water entering the cylinder, the osmotic engine swells which displaces the piston reducing the volume of the compartment containing the drug formulation. Consequently the drug is delivered through the exit port at a controlled rate (as long as an osmotic gradient exists).

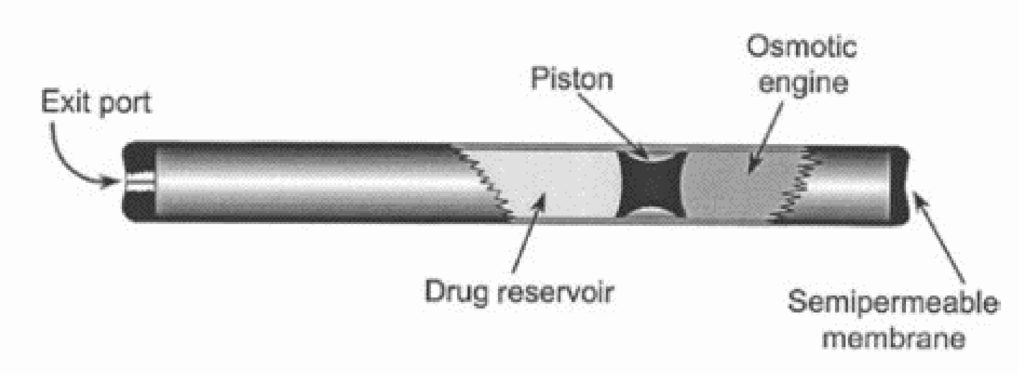


Figure 2.3 – The DUROS Implant (Wright *et al.*, 2003)

Ovuplant™

Ovuplant™ is used to stimulate ovulation within 48 hours in oestrous mares (Rathbone *et al.*, 2000). Ovuplant™ is a small biocompatible subcutaneous implant which works by augmenting the gonadotrophin releasing hormone which in turn increases the levels of two reproductive hormones (follicle stimulating hormone and leutinising hormone). The implant is inserted under the skin of the mare using a syringe-like applicator. The duration of action is short (2-3 days) and once the drug has been released, the implant is subsequently absorbed.

The systems described above are all examples of subcutaneous implants. However there are disadvantages with implants such as (Okumu and Cleland, 2003; Ranade and Hollinger, 1996):

- The implants have a fixed size and drug dose. Consequently application of this technology in the treatment of diseases where the drug dosing is based on weight or body surface area will be difficult.
- Administration involves large needles or surgical procedures.
- The implants are only useful for potent drugs.

As with injections, there are limitations with implants. Therefore it would be ideal to have a controlled release formulation that was easy to administer yet formed a depot from which drug can slowly diffuse that lasts for many weeks. Such a delivery system would offer the advantages of an injection (namely ease of administration), yet also the major advantage of an implant (prolonged delivery times).

In recent times in both the human and veterinary health industries, researchers have been exploring the possibility of developing delivery systems that meet this aim. Two major research directions have been followed. These are microspheres and *in situ* forming gels.

2.4 Microspheres

Research has been conducted in developing biodegradable polymer microspheres for drug delivery (Freiberg and Zhu, 2004). The advantage of microspheres is that they can be injected or ingested. Drug release from the microsphere is achieved by the drug leaching from the polymer or by degradation of the polymer matrix.

Microspheres are approximately 1 – 1000 µm in size. Drugs can be incorporated within the microsphere as an encapsulated core (microcapsule) or dispersed through out the microsphere (Burgess and Hickey, 2005).

Examples of products using microspheres as a delivery system are given below.

ProLease®

The ProLease® system was designed to deliver fragile biomolecules. The ProLease® system involves encapsulation technology. Therapeutic proteins are frequently administered by the parenteral route due to their low oral bioavailability and short *in vivo* half lives (Johnson and Herbert, 2003). Therefore such molecules are encapsulated in injectable biodegradable matrices which also provide a sustained release. The encapsulation process involves many steps such as production of lyophilised protein powder and extracting microspheres. It is essential to maintain the integrity and stability of the protein during the encapsulation process. Prior to delivery, the microsphere powder is dispersed in an aqueous viscous diluent. Upon injection, the encapsulated drug is released by a mechanism which depends on dissolution of the drug, diffusion of the drug out of the microsphere and hydrolysis and weight loss of the polymer which creates pores for continuous release of the drug from the microsphere matrix (Johnson and Herbert, 2003).

SMARTShot B12™

SMARTShot B12™ was the first commercially available micro-particle based veterinarian product. This product continuously releases vitamin B12 for over 200 days (Rathbone and Martinez, 2002; Medlicott *et al*., 2004; Matschke *et al*., 2002).

ProHeart®6

ProHeart®6 is a product used for protection against heartworm disease in dogs. The product is administered subcutaneously and provides release for 6 months (Rathbone and Martinez, 2002; Medlicott *et al*., 2004).

P+®

P+® (formerly called Lutamete Plus) is used in cyclic and late transitional phase non-cyclic mares (Blanchard *et al*., 1992; Jasko *et al*., 1993; Burns *et al*., 1990). P+® comprises of biodegradable microspheres containing estradiol and progesterone (Figure 2.4). The preparation is administered intramuscularly. The product was developed to release its load and then suddenly cease delivery resulting in a drop in progesterone levels.

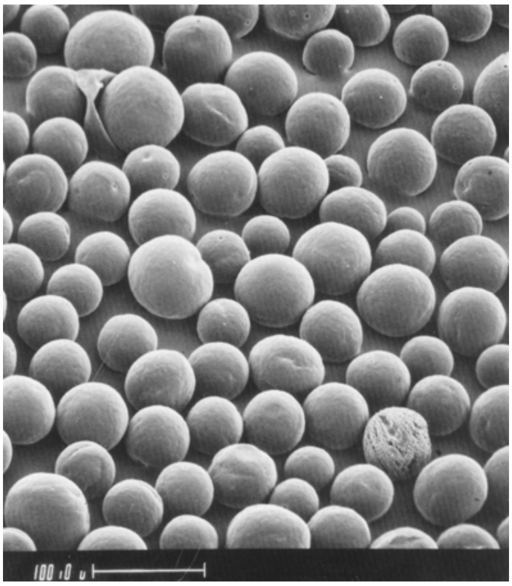


Figure 2.4 – The Product P+® Comprising of Microspheres
Containing Estradiol and Progesterone (Rathbone *et al*., 2000)

Ivermectin

A biodegradable microsphere preparation containing ivermectin has also been developed. The product provides prolonged release of ivermectin for control of livestock pests such as cattle ticks and horn flies (Miller, 2000; Medlicott *et al.*, 2004).

Despite the fact that microspheres are easy to administer via a syringe, have effective carrier characteristics and afford prolonged release of drug, they suffer from a drawback which is that the very large surface area that is created by the thousands of microscopic particles results in a very large burst effect. Also, they have a limited drug loading capacity (Matschke *et al.*, 2002).

The other disadvantage of microspheres is their short residence time at the absorption site. The residence time can be increased by coupling bioadhesion characteristics to microspheres. Such systems are referred to as bioadhesive microspheres. Bioadhesion is defined as the attachment of a synthetic or biological macromolecule to a biological tissue (Vasir *et al.*, 2003). Bioadhesive microspheres either consist entirely of a bioadhesive polymer or have an outer coating of the bioadhesive polymer (Mathiowitz *et al.*, 2001).

Because of the expense of manufacturing microspheres (due to emulsification, solvent handling and lyophilisation), *in situ* forming technologies have been developed as an alternative for the veterinary field (Dunn *et al.*, 1994; Matschke *et al.*, 2002).

2.5 *In Situ* Forming Gels

The *in situ* forming gel systems are designed such that they are fluid prior to injection. Once injected, the formulation responds to a change in the environment to give a high viscosity or solid depot at the injection site. The changes which can be affected to result in gelation or depot formation are temperature, loss of organic solvent by diffusion or hydration with water at the injection site. This type of injectable system is highly relevant to the present research.

Hydrogels are considered to be polymeric materials which are able to absorb more than 20% of its weight of water while maintaining a distinct 3-dimensional structure (Gupta *et al*., 2002). Hydrogels are also referred to as intelligent gels or smart hydrogels (Dagani, 1997). The smartness or intelligence is so given because the hydrogels can perceive the prevailing stimuli and respond by changing their physical or chemical behaviour which results in a controlled release of the entrapped drug (Gupta *et al*., 2002).

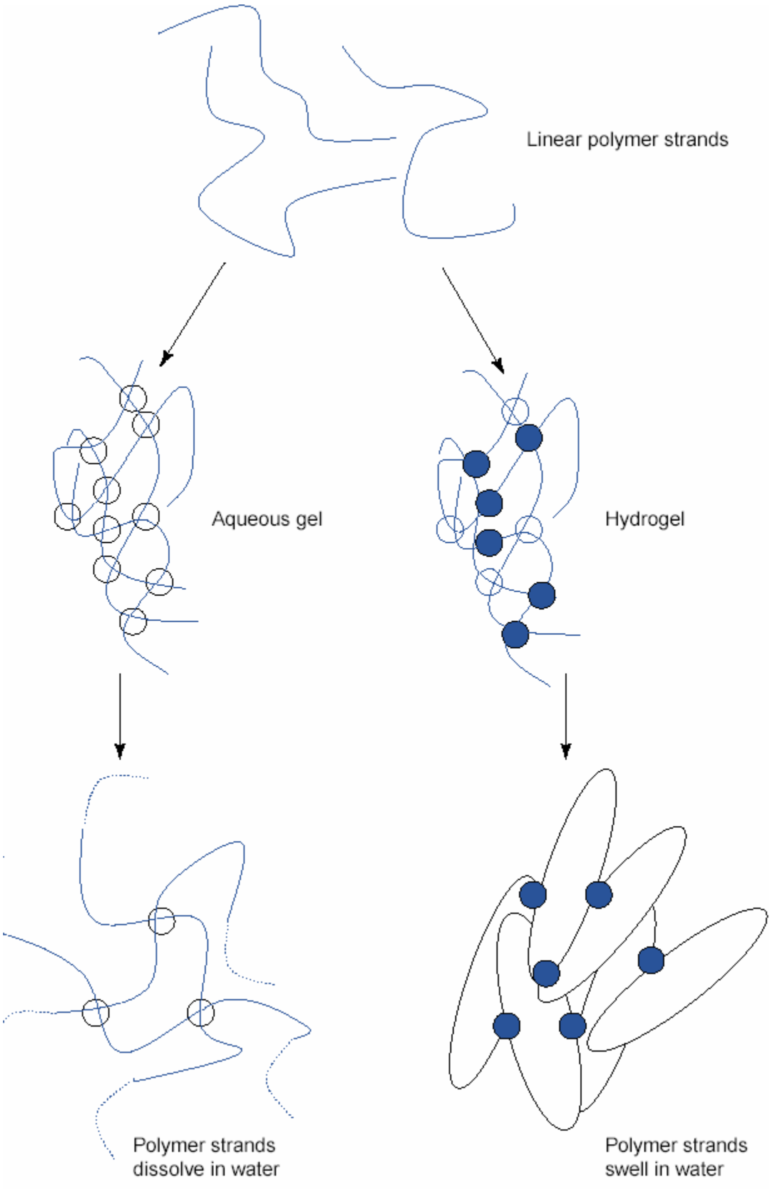


Figure 2.5 – Comparison of a Gel and a Hydrogel (Gupta *et al*., 2002)

Hydrogels and gels are similar chemically but are physically distinct (Figure 2.5). The difference between hydrogels and gels is that hydrogels can absorb large amounts of water and swell maintaining their 3-dimensional structure (Vasir *et al*., 2003; Jeong *et al*., 2002). In contrast gels are polymeric networks already swollen to equilibrium. The addition of further water results in dilution. Some gels are rigid enough to maintain their structure under a small amount of stress. However beyond this, gel fluidity is observed as well as a loss of polymer structure. The ability of the hydrogel to swell is due to the inherent cross-linking. Conventional gels may have small levels of cross-linking but this is reversible due to the weak physical forces.

A hydrogel can swell in response to many stimuli such as pH, ionic strength and temperature (Zhang *et al*., 2005). Hydrogels which respond to changes in pH are made up of polymeric backbones with ionic pendant groups. In an aqueous media at a certain pH and ionic strength the pendant groups (these groups are either acidic or basic) will ionise and develop fixed charges on the polymer network (Brannon-Peppas and Peppas, 1991; Tanaka, 1979; Peppas *et al*., 2000; Skouri *et al*., 1995). These fixed charges will generate electrostatic repulsion and the uptake of the solvent into the network is increased (Figure 2.6).

Hydrogels containing acidic pendant groups such as carboxylic groups ionise when the pH of the environment is higher than the pK_a of the ionisable group. As the system’s pH increases, the number of charges increases resulting in increased electrostatic repulsion between the negatively charged groups. Consequently the hydrophilicity of the network is increased giving greater swelling ratios. In contrast, hydrogels containing basic pendant groups such as amines will ionise when the pH of the environment is below the pK_b of the ionisable group. Therefore at low pH, ionisation increases giving increased electrostatic repulsion which increases the hydrophilicity of the network thus resulting in greater swelling ratios (Peppas *et al*., 2000).

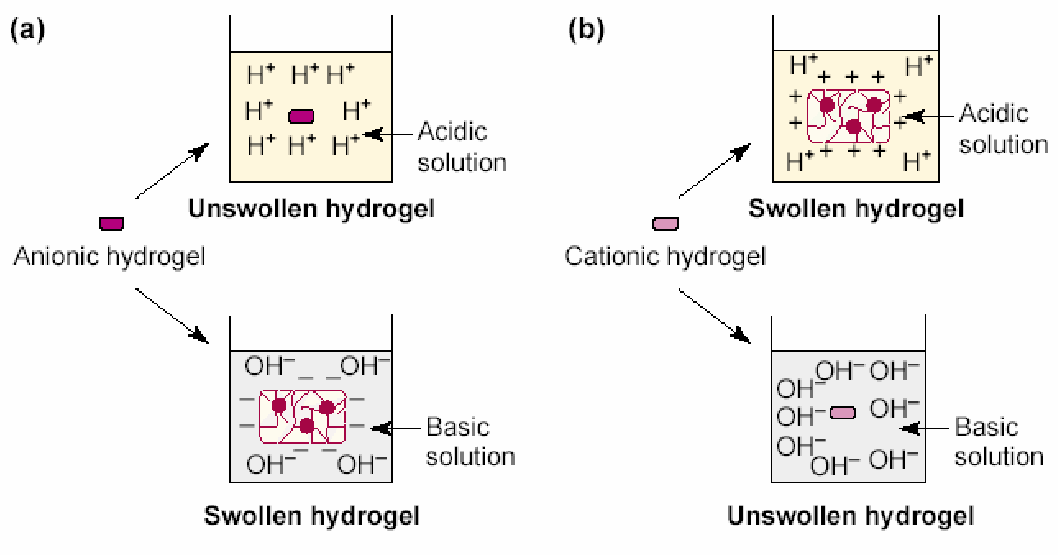


Figure 2.6 – Hydrogel Formation as a Result of a Change in pH (Gupta *et al*., 2002)

Hydrogel formation can also occur due to a change in ionic strength. When gellan gum is dispersed in an aqueous solution, it undergoes a liquid-gel transition at increased ionic strengths (Baeyens *et al*., 2000; Cohen *et al*., 1997). Divalent ions have been shown to be more effective in promoting sol-gel transitions than mono-valent ions (Baeyens *et al*., 2000).

Alginate with a high glucuronic acid content forms a 3-dimensional ionotropic hydrogel matrix. This hydrogel formation is generally by the preferential interaction with the calcium ions resulting in the formation of inhomogeneous gels. Properties such as mechanical strength and porosity of the hydrogel depend on factors such as the type of ionic cross-linker (bi- or poly-valent cations) and concentration and viscosity of the initial alginate solution (Cohen *et al*., 1997).

Gel formation as a result of a change in temperature is known as a thermosensitive hydrogel. There are two types of thermosensitive hydrogels – positive and negative. Negative thermosensitive gels have a lower critical solution temperature. Beyond the lower critical solution temperature, hydrogen bonding between the polymer and water becomes unfavourable (compared to polymer-polymer and water-water interactions). Consequently there is an abrupt transition as the solvated macromolecule dehydrates and changes into a structure which is more hydrophobic (Schild, 1992; Hoffman, 1997; Ruel-Gariepy and Leroux, 2004).

Positive thermosensitive gels have an upper critical solution temperature. Below this temperature, the hydrogel will contract.

Another means of gel formation is solvent-removal precipitation. This system consists of a water insoluble polymer dissolved in a water miscible solvent. When the formulation is injected into an aqueous environment, the solvent diffuses into the aqueous environment and water diffuses into the polymer matrix. The polymer, being water insoluble precipitates upon contact with water resulting in a solid polymeric implant (Hatefi and Amsden, 2002; Packhaeuser *et al*., 2004).

The release mechanisms from systems such as hydrogels are either diffusion controlled, solvent controlled or chemically controlled. These mechanisms were discussed earlier in Section 2.2. The most widely applicable mechanism to hydrogels is diffusion controlled. Swelling controlled release occurs when the drug diffusion is faster than polymer swelling. Chemically controlled release describes release which is determined by reactions that occur within the delivery matrix. Examples of such reactions include cleavage of polymer chains by hydrolytic or enzymatic degradation, reversible or irreversible reactions between the polymer network and drug and surface or bulk erosion (Lin and Metters, 2006).

The mechanism of gelling in the ISGM system investigated in this study involves hydration with water from the injection site. Polymers which swell by responding to the presence of water are not classed as “intelligent materials” unlike the cases discussed above where gel formation is a result of responding to stimuli such as pH and temperature (Colombo *et al*., 2000).

Upon injection of the formulation into the body, a steep water gradient is formed between the formulation and water interface. Consequently, as a result of this steep water gradient, water diffuses into the formulation. At the same time, the vehicle suspending the polymer diffuses out into the body.

The diffusion of water into the formulation, results in the hydrophilic polymer swelling. As a result of water absorption by the individual polymer chains (which were originally in an unperturbed state) the polymer chains end-to-end distance and radius of gyration expand to a new solvated state. This is due to the lowering of the transition temperature of the polymer (Colombo *et al*., 2000).

When the polymer matrix is exposed to water there are 3 distinct zones formed (Figure 2.7).

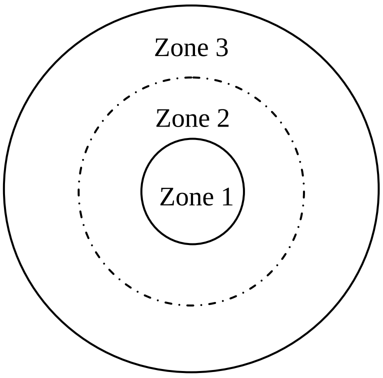


Figure 2.7 – The Three Zones Which Are Formed When a Polymer Matrix is Exposed to Water (Conti *et al.*, 2007)

Zone 1 represents the polymer which is still in a glassy state as water has not penetrated this region. The mobility for the macromolecules is low and leads to a low diffusion rate of water in this region. Zones 2 and 3 represent the polymer being in a rubbery state. The mobility of the polymer chains in these regions is higher than that of those in zone 1. Consequently as a result of increased mobility, the diffusion rate of water is higher (Gao and Fagerness, 1995). Drug dissolution will occur at the boundary between solid and dissolved drug where solid drug is found in zone 2 and dissolved drug in zone 3. The rate of movements of each boundary (for each zone) will depend on factors such as polymer hydration, swelling and dissolution behaviour (Ju *et al*., 1995).

On a molecular level, drug release is determined by water penetration, polymer swelling, drug dissolution, drug diffusion and matrix erosion. These factors depend on the interaction between water, polymer, matrix content and drug. Drug diffusion in the system occurs once water has penetrated into the matrix leading to polymer swelling and drug dissolution. The diffusion of water into the system decreases the polymer's glass transition temperature to the experimental temperature resulting in the glassy polymer transforming into a rubbery phase. The enhanced mobility of the polymer chains favours transport of water and therefore also the dissolved drug (Jamzad *et al.*, 2005).

Therefore swelling leads to changes in the polymer and drug concentration as well as increasing dimensions of the system. The viscous gel which is formed acts as a barrier to drug release thus slowing the release of the drug (Rathbone *et al.*, 2000). When the drug comes into contact with the water, the (hydrophilic) drug dissolves and diffuses out of the injected depot. The diffusion of the drug is a result of the concentration gradient of the drug.

As water continues to diffuse into the system the gel becomes progressively hydrated. Once sufficient water has accumulated into the depot, the polymer chains disentangle, detach from the gelled matrix and then dissolve (Lee and Peppas, 1987; Narasimhan and Peppas, 1997; Colombo *et al.*, 2000; Kavanagh and Corrigan, 2004) (Figure 2.8). The time for break down will depend on the chain length and degree of substitution of the polymer.

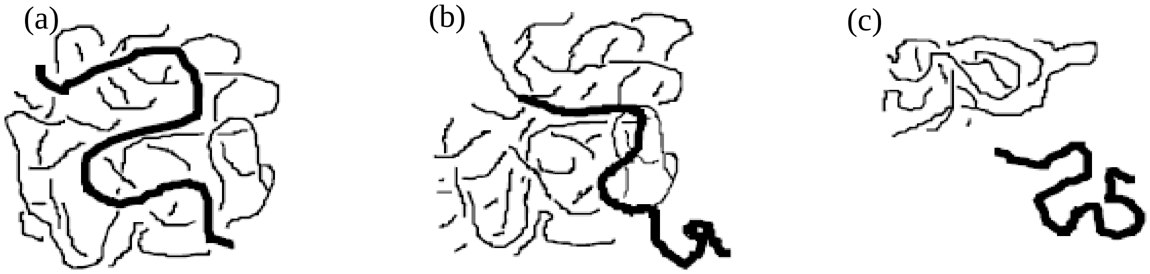


Figure 2.8 - Diagram Indicating the Successive Stages of Polymer Disentanglement. (a) System of Entangled Chains, (b) A Chain Disentangling from the System and (c) A Completely Disentangled Chain (Narasimhan and Peppas, 1997).

In the case where poorly water soluble drugs are used, drug release is by erosion of the matrix (Skoug *et al.*, 1993; Tahara *et al.*, 1995).

In recent years several systems have been investigated for both human and animal application and such examples are given below.

2.5a The Atrigel® System

The Atrigel® system consists of a biodegradable polymer dissolved in an appropriate solvent. Injection of the formulation results in the formation of a solid implant upon contact with water. The drug becomes entrapped in the solid polymer matrix. The drug is then released slowly over time as the polymer biodegrades (Dunn *et al*., 1990; Rathbone and Martinez, 2002).

Eligard®

Eligard® is a delivery system based on the Atrigel® technology and delivers leuprolide acetate for the treatment of prostate cancer. Eligard® consists of a biodegradable polymer dissolved in NMP. The formulation is then mixed with leuprolide and injected subcutaneously to form a solid depot at the site of injection (Dunn, 2003; Berges, 2005).

Deoxyrobe®

Deoxyrobe® is another example of an Atrigel® system (Dunn, 2003). Deoxyrobe® delivers doxycycline for the treatment of periodontal disease in companion animals.

2.5b The Alzamer® System

The Alzamer® depot technology consists of formulated drug particles, a solvent of low water miscibility and a biodegradable polymer. The polymer is dissolved in the solvent. Upon injection of the formulation, the depot comes into contact with the water in the physiological environment which causes the gel to undergo phase inversion resulting in a 2-phase gel implant being formed (Wright *et al*., 2003). The drug is released by diffusion. During the latter stages of release, the polymer biodegrades promoting drug release.

To date this technology has not been applied to veterinary application.

2.5c The SABER System

SAIB (Sucrose Acetate Isobutyrate) is a food additive (Reynolds and Chappel, 1988) used as an alternative to brominated vegetable oil in soft drinks. The high hydrophobicity and high viscosity properties of SAIB (Rathbone and Martinez, 2002) may be used to provide sustained drug delivery. The high viscosity SAIB is formulated as a low viscosity liquid which is achieved by mixing specific solvents (such as ethanol and propylene carbonate (Matschke *et al*., 2002)). The drug is then dispersed or dissolved in the SAIB/solvent mixture (Figure 2.9). If the solvent is water soluble the solvent will diffuse out when injected leaving a viscous depot containing the SAIB and drug. The use of a hydrophobic solvent results in a less viscous depot with slower diffusion of the solvent (Tipton, 2003). SAIB is less expensive to manufacture than other technologies such as implants and microspheres with PLGA (Section 2.5f). SAIB does not require expensive polymers, only small amounts of solvents (approximately between 15 and 35%) are needed and they have a short and easy method of manufacturing (Matschke *et al*., 2002).

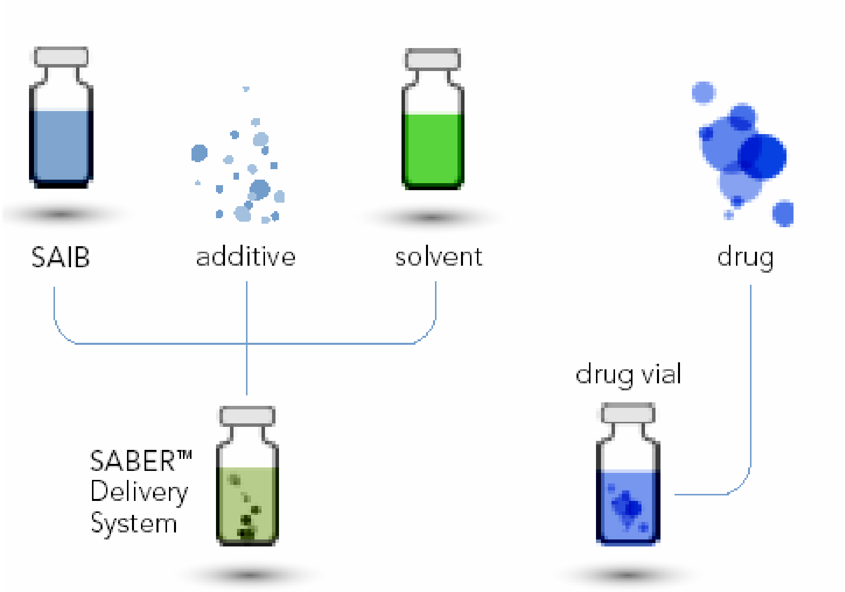


Figure 2.9 – The SABER Delivery System. The SAIB, additive and solvent are added together to give the SABER delivery system. The drug is then added to the SABER system and the formulation is then injected into the body (DURECT Corporation, 2006).

An example of a technology using SAIB, is a delivery system which provides prolonged release of deslorelin. Deslorelin is a potent gonadotropin-releasing hormone (GnRH) analogue used to induce ovulation at precise and predictable times in swine and mares prior to artificial insemination (Rothen-Weinhold *et al*., 2000).

2.5d Pluronic® 127

Pluronic® 127 (also known as Poloxamer 407) is a non-ionic triblock co-polymer consisting of polyoxyethylene and polyoxypropylene units (Moore *et al*., 2000). In an aqueous environment at room temperature, Pluronic® 127 exists as a liquid. However at 37°C it is able to transform into a reversible semi-solid gel (above a critical concentration of 20%) (Wang and Johnston, 1991; Mortensen and Pedersen, 1993).

A system similar to the Pluronic® 127 is the pH-sensitive polymeric hydrogel which is a polymer complex of polyacrylic acid, polymethacrylic acid and polyethylene glycol. The complex is barely soluble at pH's below 5.7. However addition of 50% ethanol as a co-solvent results in a fluid formulation which can be injected. Upon injection, the ethanol diffuses out of the formulation into the body and at the same time water diffuses into the formulation resulting in the sol-to-gel transition. However the use of such a large amount of ethanol is not desirable due to its local intolerability (Joshi *et al*., 1998).

Although this approach appears to hold promise as a delivery technology, to date no veterinary system has been developed.

2.5e Glycerol Monoleate

Glycerol monoleate is an unsaturated monoglyceride classed as a water insoluble amphiphilic lipid (Matschke *et al*., 2002). In the presence of water, glycerol monoleate forms liquid crystals. (Engstrom, 1990). The phase transformation of glycerol monoleate depends on the water content in the matrix. If the water content is only 0 – 5% (w/w), a reversible micellar phase is formed. In 5 – 20% w/w water content, a lamellar phase is observed. In the presence of excess water (35% w/w) cubic phase viscous gels are formed (Hyde *et al*., 1984). The cubic phase gel has a 3-dimensional network of curved lipid bilayers which are separated by a network of congruent water channels. The formation of cubic phase gels depends on the lipid concentration, water content and the temperature (Ganguly and Dash, 2004; Shah and Paradkar, 2005; Matschke *et al*., 2002)

2.5f PLA/PLGA Polymers

PLA/PLGA (poly-lactide-acid / poly(lactic-co-glycolic) acid) are synthetic polymers which are used in controlled release drug delivery systems. These polymers are made up of non-toxic monomers (glycolic and lactic acid) (Matschke *et al*., 2002). The benefits of using such polymers include their degradation products where hydrolytic degradation gives lactic and glycolic acid which are then subsequently metabolised to form water and carbon dioxide (Tice and Crossar, 1984; Lewis, 1990). Also the degradation time can vary from 1 month to several years. The time period is altered by changing parameters such as molecular weight and proportion of glycolic/lactic acid content ratio.

The PLA/PLGA polymers are dissolved in an organic solvent. The drug is either dispersed or dissolved in the solvent system. Upon injection, the solvent diffuses into the body leaving a semi-solid implant which is formed by precipitation of the polymer in an aqueous environment.

To date no veterinary system has been developed using this technology.

The patent awarded to Bunt *et al*. (2003) describes a formulation for parenteral administration to a subject. The formulation described comprises at least one water miscible solvent, at least one gelling agent and at least one active agent. The gelling agent is in particulate form and suspended in the solvent. Examples of water miscible solvents which may be used in the formulation include propylene glycol, polyethylene glycol 400 and ethanol. Examples of hydrophilic gelling agents which may be used in the formulation include hydroxypropyl methylcellulose, sodium carboxymethylcellulose, polyvinyl alcohol and polyvinyl pyrrolidone. Such materials are preferred due to their pharmaceutical and physiological acceptability and ability to form gels of desirable viscosity for use in such depot formation.

According to the patent, it is understood that administration of the formulation into the subject results in the solvent dispersing naturally into the subject fluids. The solvent being water miscible easily dissolves into such environments without leaving harmful residue levels. A water miscible solvent is a liquid which is capable of being dissolved in or mixing with water. This is essential as the water miscible solvent will not linger in the subject and therefore will not cause possible side effects. Water miscible solvents are often less aggressive than other non-aqueous solvents and therefore more physiologically acceptable. Upon dispersal of the solvent, the gelling agent particles coagulate to form a depot from which the active agent is released.

The patent also states that the suspension will remain largely stable (biologically, chemically and physically) when stored. Settling can occur but simple shaking of the formulation by means such as shaking by hand, will re-establish the desired particulate suspension.

This technology has clear advantages where a standard injection syringe can be used to administer the formulation. As a result of the natural depot forming characteristics of the formulation, the formulation can be administered to areas of a subject not easily reached using methods such as oral administration and insertion into a cavity or surgery.

However, though the patent describes an idea for a novel technology, there are deficiencies. The formulation uses water miscible solvents which the patents states are often less aggressive than other non-aqueous solvents and therefore more physiologically acceptable. However, there are various reports of uses of such solvents and contradictions in the literature regarding their biocompatibility. Therefore there is a need to assess the biocompatibility of such excipients. A possible major limitation of the technology may be the use of non-aqueous solvents which may present biocompatibility issues.

The patent describes the ISGM system as remaining largely stable and therefore the physical stability of the suspension needs to be assessed. Though the patent states that settling can occur, it is essential that if the hydrophilic polymer was to settle, the hydrophilic polymer does not form a hard compact mass which may be difficult or impossible to redisperse. The patent describes the formulation as one which can be easily redispersed by means such as shaking by hand. Therefore the ISGM system must be a physically stable suspension that can be easily redispersed if settling was to occur.

In addition though the concept of the technology described in the patent is sound, the feasibility of the technology has yet to be assessed where the ISGM system is incorporated with a drug to determine if the drug release rate is retarded upon formation of a gelled depot.

2.6 The Scope of the Current Study

The above literature review and description of commercial veterinary drug delivery products led to the identification of a gap in the existing research literature. Clearly, there is a need for an injectable drug delivery system that allows long-term predictable release of drugs. Such a system requires characteristics where the formulation is liquid prior to injection and gels upon contact with water to form a depot much like a semi-solid implant.

While Bunt *et al's* (2003) patent in principle describes such a technology, it lacks sufficient practical demonstration of a viable system. Furthermore, there has been no systematic study of the components that would make a suitable formulation, nor any methodology to develop such a system. Therefore the current study was devised to address these deficiencies.

There are many types of controlled release delivery systems currently available on the market as discussed above. However, there are many disadvantages associated with these delivery systems for the veterinary area. With subcutaneous implants, large gauge needles or surgical procedures are required. Microspheres are easy to administer, but they are very expensive to manufacture and are not economically viable for the veterinary industry. There is a need to have an easily deliverable controlled release system for the veterinary field.

Injectables are the ideal form of drug delivery as animals are not needle shy, the amount of drug administered is known and it is cheap and easy to administer. However, a problem with injections is that the duration of action is short. Implants have the advantage of long delivery times, but administration is disadvantageous as previously discussed in this section. Therefore a technology which has the combined advantages of being injectable (without serious side issues) and at the same time providing a drug-loaded slow release implant would be ideal. Such a technology would be easy to administer and at the same time form the semi-solid “depot” (the terminology denoting the “implant”) thus achieving longer release times. This type of technology is (as previously described) broadly classifiable as being in the area of *in situ* forming gels. The current technologies available in this area have the gelling agent *dissolved* in the actual solvent. Once administered, the solvent carrier disperses into the subject, leaving the gel implant material behind (after it has reacted with aqueous fluids and gelled). A major issue with this technology as it stands is that the solvents employed in these methods have needed to be aggressive in order to dissolve the gelling agent. Such solvents include dimethyl sulphoxide which is used due to its advantageous physical properties of high solubilising capability, low viscosity and water-miscibility. However the use of dimethyl sulphoxide is controversial due to its side effects such as local dose-related toxic effects on the vessel and pig brain tissue and systemic effects such as cardiovascular toxicity (Mottu *et al.*, 2000). Therefore the use of such solvents poses a problem in that they may be potentially toxic *in vivo* and cause further illness or ailments.

Consequently, there is a gap in this area of knowledge and a potential to develop a beneficial technology in the veterinary field with application to other areas if successful.

The ISGM technology developed in this study is different from conventional *in situ* gelling systems in that the gelling agent is *suspended* in a non-aqueous solvent as opposed to being *dissolved* as in current systems. Consequently, the use of aggressive solvents such as dimethyl sulphoxide are not required as the gelling agent is merely suspended in the non-aqueous solvent and will react with aqueous bodily fluids to gel, with the non-aqueous solvent diffusing away from the injection site without leaving harmful solvent residue levels.

A product which has a hydrophilic gelling agent suspended in a non-aqueous solvent has the potential to deliver a drug via the preferred route of injection and at the same time give longer release times (similar to that of an implant), without having to use aggressive solvents so would be highly beneficial. The use of non-aqueous solvents is not without its problems however. Aqueous vehicles are usually less irritating than non-aqueous vehicles. In the case of water or physiological saline, injection into the intramuscular tissue results in little or no tissue reaction (Baggot and Brown, 1998). A suitably innocuous non-aqueous solvent must be chosen as negative side effects may occur, including possible precipitation, pain, damage, inflammation and haemolysis upon injection (Gad *et al*., 2006, Rasmussen and Svendsen, 1976; Blom and Rasmussen, 1976). Irritation can give local inflammation, pain and tissue damage. Tissue damage is the reversible or irreversible change in the anatomy, biochemistry or physiology at the injection site as a result of introducing a formulation (Gad, 2000). Hence any non-aqueous solvents used in such an injectable technology must be non-irritating, non-toxic (in the amounts administered) and non-sensitising (Spiegel and Noseworthy, 1963; Ansel and Popovich, 1990).

There are many examples of non-aqueous solvents which can be used in a formulation such as fixed oils, ethanol, propylene glycol, glycerin and ethyl carbonate. Fixed oils (mainly mixtures of esters of unsaturated fatty acids which are fluid at 20°C) have been used as vehicles in parenteral formulations. Examples of fixed oils include peanut oil, sesame oil and corn oil. The toxicity of vegetable oil is relatively low. However, some patients have exhibited allergic reactions to vegetable oil. Fixed oils can also cause local tissue reactions such as cysts (Spiegel and Noseworthy, 1963) and rancidity may be an issue. Consequently the use of vegetable oil requires a label to indicate the specific oil included.

There is some controversy regarding the use of glycerin in parenteral formulations, as administration of glycerin to animals resulted in hemoglobinurea, hypotension, central nervous disturbances and weight loss (Spiegel and Noseworthy, 1963). Subcutaneous injection of ethanol is known to cause pain followed by anaesthesia.

As described in the examples above, there are issues regarding the use of non-aqueous solvents. Consequently there is a need to assess the biocompatibility of non-aqueous solvents. There are a number of vehicles available which meet the chemical and physical requirements of pharmaceutical formulation. However, there will be differences in species and routes of administration specific to the tolerances of these vehicles (Gad *et al*., 2006).

Assessment of Biocompatibility

It is important to have some means of assessing the irritation potential of solvents. This amounts to a need to assess the *biocompatibility* of the possible non-aqueous solvents to use in the formulation. There are many types of biocompatibility tests which can be conducted to determine the nature and extent of any immunological response such as the Draize eye test (Lambert *et al*., 1993; Balls *et al*., 1995; Cormier *et al*., 1996), the low volume eye test (Cormier *et al*., 1996; Lambert *et al*., 1993; Calvin, 1992; Bruner *et al*., 1992) and the HET-CAM test (Luepke and Kemper, 1986; Dannhardt *et al*., 1996; Balls *et al*., 1995; Dahl, 1999; Schlage *et al*., 1999). The HET-CAM test is described as a cross-over between *in vitro* and *in vivo* testing (Luepke and Kemper, 1986) and has the advantage that the CAM has the ability to simulate the pharmocokinetic profile of the substance tested which is not the case with an *in vitro* assay (Dannhardt *et al*., 1996). The HET-CAM test is suggested to be more sensitive than the Draize eye test. In addition, the HET-CAM is also advantageous in regard to the cost (it is less expensive) and does not involve the use of laboratory animals (Dahl, 1999). The HET-CAM test was thus chosen in this study to assess the biocompatibility of the possible excipients to use in the formulation (see Section 4.1b).

Consideration of a Model Drug for Use in the Studies

The testing of a new injectable *in situ* gelling technology is not complete without tests involving a model drug. The feasibility of the technology in acting as an *in situ* gelling system with controlled release characteristics is assessed herein using the model drug propranolol. Propranolol is a hydrophilic, non-selective, beta-adrenergic blocking agent that has been used in the treatment of hypertension, angina pectoris, tachycardia and many other cardiovascular disorders (Taylan *et al*., 1996; Thevis *et al*., 2001; Farooqi and Aboul-Enein, 1996). It is chemically known as 1-isopropylamino-3-(1-napthyloxy)-2-propanol (Figure 2.10) (Farooqi and Aboul-Enein, 1996) and its use in dog and cat veterinary medicine is well established and documented (Walker *et al*., 1997). The drug has a melting point of 162 – 165 °C and UV-Vis absorption maxima at 214 and 290 nm when dissolved in water.

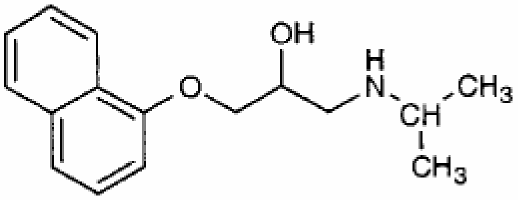


Figure 2.10 – Molecular Structure of Propranolol (Thevis *et al.*, 2001)

In a veterinary application, propranolol has been used in combination with phenobarbitone as a possible means of treating anxiety in dogs (Walker *et al*., 1997). Upon oral administration, propranolol undergoes extensive and highly variable hepatic first pass metabolism (Guyot and Fawaz, 2000). The propranolol is completely absorbed from the gastrointestinal tract where less than 5% is recovered in the faeces (Namedo and Jain, 2002). As a result of the metabolism, the oral bioavailability is low i.e. 30% (Riddel *et al*., 1987). The variability of propranolol plasma concentration is as much as 10 - 20 fold. There are alternative forms of administering propranolol. IV (intravenous) delivery is available for acute administration. Transdermal delivery is also possible and has the advantage that the propranolol is not subjected to the hepatic first pass metabolism. However the problem with transdermal delivery is that propranolol has a poor ability to permeate the skin in sufficient quantities to maintain the therapeutic levels. The skin has a horny outer layer known as the *stratum corneum*. The inability of propranolol to penetrate this skin barrier will be due to the low partitioning through the lipid matrix of the stratum corneum. Factors other than lipophilicty may also affect the permeability of propranolol through the skin such as skin protein binding and first pass metabolism in the skin.

Hence using propranolol in the current research for developing a novel injectable *in situ* gel forming technology is not only useful in its capacity as a model water soluble drug but could also provide an alternative route for delivering propranolol without considering a wholly transdermal route

Modelling

Modelling of the release data was carried out using the Higuchi and power law relationships. Though modelling in this study cannot be used to assess *in vivo* drug release behaviour (as there were restrictions imposed on the *in vitro* drug release system), modelling was used as comparative tool to assess the effect of various parameters. Complications such as swelling of the matrix, erosion of the polymer and irregular geometries of gel depots make mechanistic descriptions of the release difficult (as described in Section 4.5c). The Higuchi and power law relationships were used to model the release data as a means of comparing the effect of various parameters on the release rate.

Stability

The stability of the drug in the formulation was assessed by quantifying the amount of drug in the formulation over a period of 4 weeks under accelerated ageing conditions. In the case of the delivery of propranolol, the technology would likely be packaged as a two-component system where the dry powder is packaged separately along with the liquid for use at the time of reconstitution. Consequently a 4-week period was chosen to conduct the accelerated ageing stability study as this was considered to be a sufficient period of time to assess the stability of the formulation from a practical, commercial viewpoint.

CHAPTER THREE

Methodology

Throughout this research, reference will be made to the terms “the *in situ* gelling matrix (ISGM) system” and “the final formulation” (also referred to as the final product). The ISGM system comprises a dispersed phase and a continuous phase. The dispersed phase consists of a hydrophilic polymer that is suspended as *solid particles* in a continuous phase. The continuous phase consists mainly of a non-aqueous solvent but which may also include a density-inducing agent or viscosity-inducing agent dissolved in the solvent. Figure 3.1 is a schematic representation of an ISGM system.

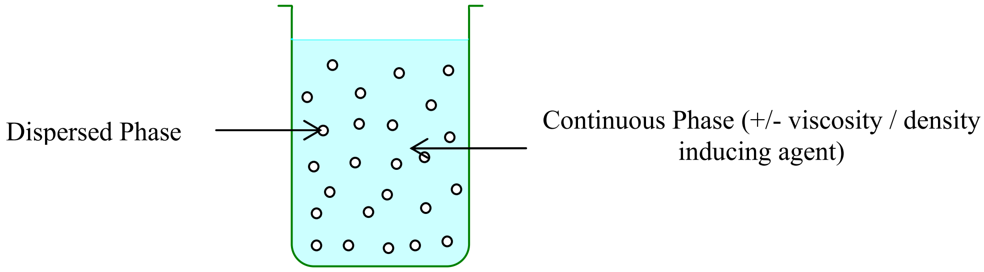


Figure 3.1 - Diagrammatic Representation of an ISGM System

“The final formulation” is defined as a system comprising the drug (in the case of this study, propranolol was chosen) present as a homogeneously dispersed saturated solution throughout the ISGM system (Figure 3.2).

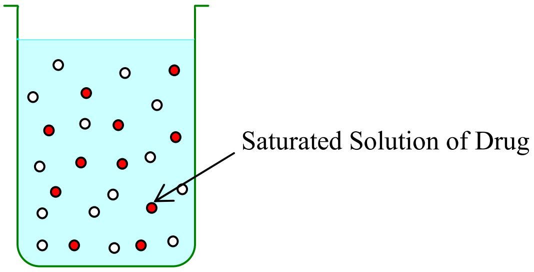


Figure 3.2 - Diagrammatic Representation of the Final Formulation

3.1 Structure, Properties and Biocompatibility of the Hydrophilic Polymers and Non-Aqueous Solvents Used in the Study

An investigation into the structure and properties of the hydrophilic polymers and the non-aqueous solvents was carried out. In order to determine which excipients to use for the ISGM system a biocompatibility study was conducted on the various hydrophilic polymers and non-aqueous solvents to ensure the excipients would not cause an adverse immunological response.

3.1a Structure and Properties

The structure and properties of the hydrophilic polymers and non-aqueous solvents used in the studies were compiled from literature sources and are listed in Tables 4.1 and 4.2 (Section 4.1a).

3.1b Biocompatibility

Biocompatibility studies were performed on all hydrophilic polymers (Table 3.1) and non-aqueous solvents (Table 3.2) investigated in this study using the HET-CAM (Hen's Egg Test – Chorioallantoic Membrane) test.

Table 3.1 - Polymers Investigated in this Study

Polymer
Xanthan Gum (Jungbunzlauer GmbH, Switzerland)
Sodium Carboxymethylcellulose (Sodium CMC) (BDH, UK)
Sodium Alginate (BDH, UK)
Polyvinyl Pyrrolidone (PVP) (BDH, England)
Hydroxypropylmethylcellulose (HPMC) (Shin-Etsu Chemical Co. Ltd, Japan)
Polyethylene Oxide (PEO) N12 (Dow Chemical Company, USA)
Polyethylene Oxide (PEO) N10 (Dow Chemical Company, USA)
Polyethylene Oxide (PEO) 303 (Dow Chemical Company , USA)
Polycarbophil (PCP) (Goodrich BF, England)
Polyvinylalcohol (PVA) (Sigma, USA)

Table 3.2 - Solvents Investigated in this Study

Solvent
Propylene Glycol (PG) (Sigma, USA)
Polyethylene Glycol 400 (PEG 400) (Sigma, USA)
Glycerin (Pronalys, Australia)
Alcohol (Ethanol) (Scharlau Chemie, Spain)
N-Methylpyrrolidone (NMP) (BDH, England)

Fertilised hen cobb eggs were obtained from Bromley Park Hatcheries Ltd., Tuakau, New Zealand. The eggs at the hatchery were stored in a cold room to ensure embryo development did not commence. Embryo development only starts at higher temperatures of approximately 37°C, and therefore the eggs were able to be transported and stored in the lab at room temperature (20°C). When fertilised hen eggs are produced, there is a maximum time period of 1 week upon within which embryo growth can proceed (Bromley Park Hatcheries Ltd., 2003). Beyond this time period, the success rate of chicken embryos developing in the fertilised eggs markedly decreases. It was thus critical to adhere to this time period in the HET-CAM studies.

The eggs were placed in an incubator (Contherm Stability Oven, Contherm, UK) (recorded as day 0) where the temperature was maintained at 36°C and the humidity at 60%. The humidity was controlled by placing a large tray of water at the bottom of the oven. The temperature and humidity of the oven were monitored using a digital thermometer/hygrometer (Tecpel, Taiwan). The eggs were rotated (mimicking the hen which turns eggs every hour whilst she lays on them) 180 degrees each day until day 3 to prevent the growing embryo from sticking to the side of the egg shell membrane. On day 3, the eggs were carefully cracked open into a growing chamber (i.e., a cylindrical plastic holder that was specifically designed for this application – Figure 3.3a). A polyethylene film, such as Gladwrap®, was placed on top and pushed down to ensure there was sufficient space for the egg contents to fall into and to be held in place using a circular sleeve (Figure 3.3b). The excess polyethylene film hanging below the circular sleeve was trimmed short to ensure minimum weeping out of the albumin (the egg white) from the plastic holder.

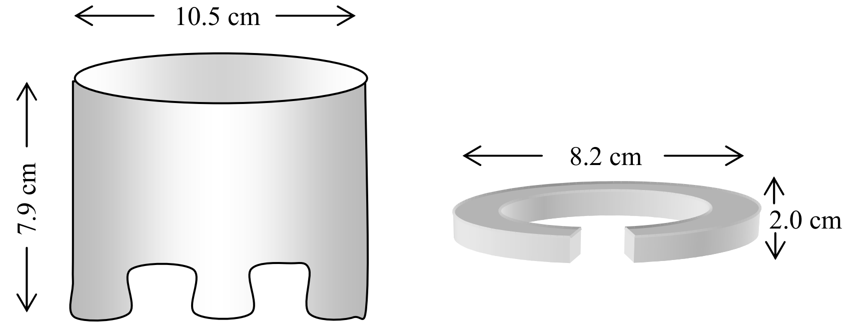


Figure 3.3a – The Chamber and Circular Sleeve Used to Incubate the Chicken Embryo

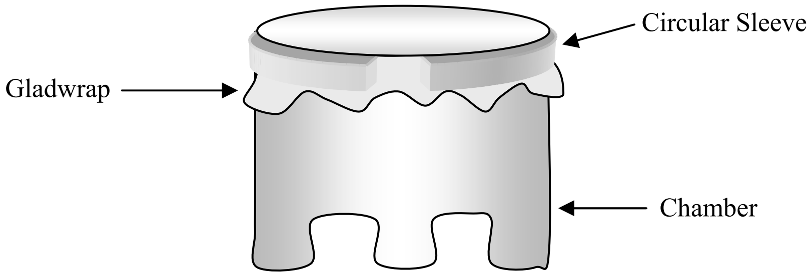


Figure 3.3b – The Final Appearance of the Assembled Chamber

The cracking process is important as the yolk and CAM must remain intact. Eggs with broken yolks or a damaged CAM had to be discarded as there was very little chance of the embryo surviving in such a case. Prior to cracking, the eggs were sprayed with 70% isopropanol as a sterilisation measure, as once the egg was cracked open, the embryo would no longer have the natural protection of the shell to prevent exposure to bacteria. The cracking technique used consisted of tapping the egg against the edge of the bench to produce a single crack. Immediately after, the shell was opened along the crack and the egg contents dropped into the chamber. Non-viable (eggs with a broken yolk) or non-fertilised eggs (where the heart and blood vessels were not observable) were discarded. The chambers containing the chicken embryos were then covered with a petri dish and placed in an incubator for 10 days. The actual HET-CAM test was performed on day 10 (the 10th day of incubation) by applying the materials to be tested onto the embryo and assessing their irritancy potential.

Different methodologies were used to apply the test materials depending on whether they were non-aqueous solvents or solid polymers. In the case of the non-aqueous solvents 300 μL of the test solvent was pipetted gently onto the CAM and the reaction of the blood vessels to the solvent was noted at pre-determined times (30 seconds, 2 minutes and 5 minutes) over a 5 minute period (this followed the method described by Schlage *et al.* 1999). In the case where the test material was a solid polymer, several different methods were adopted which were variations of the method described by Schlage *et al.* (1999). The first variation of the method was the so-called endpoint assessment method where sufficient (3-4 milligrams) solid polymer was sprinkled onto the CAM and carefully washed off with physiological saline after 30 seconds of exposure, and the time for any haemorrhage, hyperaemia and coagulation events to occur was observed over a 5 minute period. In another method, 3-4 milligrams of the solid polymer was sprinkled onto the CAM for a total exposure time of 5 minutes and then washed off (with physiological saline) to assess if any reaction had occurred. In yet another method, the solid polymer was dissolved in water and 300 μL was pipetted gently onto the CAM and left in contact with the CAM for 5 minutes. The reaction of the blood vessels was noted at fixed times (30 seconds, 2 minutes and 5 minutes) over a 5 minute period (this followed the method described by Schlage *et al.* 1999).

At the end of any exposure period, assessment of the irritancy potential of the material was carried out by observing the reaction at three fixed time points (0.5, 2.0 and 5.0 minutes). The time for the first appearance of any irritancy was recorded and allocated the score as shown in Table 3.3 (Schlage *et al.*, 1999).

Table 3.3 - Scoring System for Irritancy Potential

Irritancy	**Time (minutes)**		
	0.5	**2.0**	**5.0**
Hyperaemia	5	3	1
Haemorrhage	7	5	3
Coagulation	9	7	5

The scores allocated for each individual irritancy were totalled to give a cumulative score where 21 was the maximum possible total score. Generally, the score is high if irritancy is experienced in a small time-frame. The highest cumulative score of 21 is only possible if hyperaemia, haemorrhage and coagulation all occur within 30 seconds of applying the test substance which would indicate an extremely adverse reaction of the HET-CAM to the test substance, i.e. 5 (hyperaemia) + 7 (haemorrhage) + 9 (coagulation) = 21.

Table 3.4 shows how the cumulative score was used to define the severity of the reaction.

Table 3.4 – Severity of Reaction

Cumulative Score	**Severity of Reaction**
0.0 - 0.9	Practically None
1.0 - 4.9	Slight
5.0 - 8.9	Moderate
9.0 - 21.0	Strong

All tests were carried out in quadruplicate and three positive controls and one negative control were employed for each test. The positive controls were sodium dodecyl sulphate (Sigma, USA), ethanol and sodium hydroxide (Sigma, USA) which gave severely irritant reactions of lysis (disappearance of vessels), haemorrhage and coagulation, respectively. Although lysis was not included in the scoring system by Schlage *et al.* (1999), other authors have observed the phenomenon of lysis (Dahl, 1999; Wilson and Steck, 2000) and consequently lysis was included in this study as a control. The negative control employed in this study was water which produced zero change in the HET-CAM upon application. Results are summarised in Chapter 4 (Section 4.1b).

3.2 Formulation and Manufacture of the ISGM System

Those excipients not found by the HET-CAM tests to cause any severe immunological response were carried forward to the screening process. The screening process involved investigating the physical stability of the hydrophilic polymers in the non-aqueous solvents.

3.2a Sedimentation Behaviour of Hydrophilic Polymers in Non-Aqueous Solvents

The sedimentation behaviour of each hydrophilic polymer (see Table 3.5 for details) in pure non-aqueous solvent systems (Table 3.6) was determined in 6 mL glass vials (flat bottomed) (Samco Glass, Australia). An adhesive paper ruler was attached to the side of each vial and was used to measure the sediment height during the experiment. The paper ruler was constructed on computer. The printed paper ruler was checked against an actual plastic ruler to ensure the scale was accurate. The same ruler was used for each vial (numerous copies were printed onto adhesive paper) ensuring differences in readings did not occur between the different vials.

In preparing samples for sedimentation analysis, each test polymer was sieved to give particle sizes of less than 100 µm. 0.4 g of the test polymer was then added to the vial followed by 3.6 g of the pure non-aqueous solvent (10% w/w polymer concentration). Lids of the vials were tightly closed to prevent moisture entry as any exposure of the gelling agent to moisture will result in the system gelling which must be avoided, as a prematurely gelled ISGM system will be difficult or impossible to inject. The polymer was then homogeneously distributed throughout the solvent by means of a vortex mixer (Chiltern, Model MT17V, UK) for 2 minutes at the maximum speed setting of 8. The extent of homogeneity was observed by eye, ensuring no polymer remained “caked” on the bottom of the glass vial.

Table 3.5 - Polymers Investigated in these Studies

Polymer
Xanthan Gum[1,2,4]
Sodium Carboxymethylcellulose (Na CMC)[1,2,4]
Sodium Alginate[1,2]
Polyvinyl Pyrrolidone (PVP)[1]
Hydroxypropylmethylcellulose (HPMC)[1,2,4]
Polyethylene Oxide (PEO) N12[1,2]
Polyethylene Oxide (PEO) N10[1,2]
Polyethylene Oxide (PEO) 303[1,2,4]
Polycarbophil (PCP)[1,3]
Polyvinylalcohol (PVA)[1]

[P1] Polymers investigated in the pure solvent study
[2] Polymers investigated in glycerin modified continuous phase study
[3] Polymers investigated in polycarbophil modified continuous phase study
[4] Polymer used as viscosity inducing agent

Table 3.6 - Solvents Investigated in these Studies

Solvent
Propylene Glycol (PG)[1,3]
Polyethylene Glycol 400 (PEG 400)[1,3]
Glycerin[2]
Ethanol[1,3]

[1] Solvents investigated in pure solvent and glycerin modified continuous phase study.
[2] Solvent used to induce density/viscosity changes in the continuous phase.
[3] Solvents investigated in polycarbophil modified continuous phase study.

Upon uniform dispersion (in the vials) of the test polymer the vials were placed on shelves with a black background and the sedimentation process was observed and recorded by taking photographs (with a Canon MVX150i Digital Camera). The sedimentation process was monitored until the polymer had completely settled to the bottom of the vial. Sedimentation profiles (Section 4.2a) for each polymer/solvent system were subsequently collated from the photographs.

3.2b Effect of Glycerin on the Sedimentation Rate of Hydrophilic Polymers

The effect of viscosity and density of the continuous phase on the sedimentation rate of each of the dispersed hydrophilic polymers (Table 3.5) was determined by the addition of glycerin to each of the non-aqueous solvents listed in Table 3.6. Glycerin was used as a density modifier for the continuous phase as out of the vehicles which were deemed biocompatible (Section 4.1a), glycerin had the greatest density of 1.258 g mL^{-1} (Table 4.2). Table 3.7 shows the percentage composition of the resultant binary solvent systems investigated in the study.

Table 3.7 - Binary Solvent Systems Composition

Solvent:Glycerin Ratios (%)*						
0:100	10:90	30:70	50:50	70:30	90:10	100:0

*Each binary solvent system composition was prepared by weight by weight additions of the respective solvents.

Sedimentation behaviour was determined in 6 mL glass vials as described in Section 3.2a. 0.4 g of the test polymer was then added to the vial followed by 3.6 g of the binary solvent system (10% w/w polymer concentration). Lids of the vials were (as in the earlier experiments) tightly closed to prevent moisture entry and premature gelling of the hydrophilic gelling agent.

The state of homogenisation of the dispersion and sedimentation behaviour of the hydrophilic polymer was observed and then evaluated as described earlier in Section 3.2a.

The experimental errors for these procedures were calculated as the relative standard deviation in the percentage sedimentation height of the replicates, (where each experiment was carried out in duplicate).

3.2c Determination of the Potential Use of Polycarbophil as a Viscosity Inducing Agent in Non-Aqueous Solvents

Polycarbophil was one viscosity inducing agent of interest chosen for this study. Hence a preliminary qualitative evaluation of the use of polycarbophil as a viscosity inducing agent was conducted in which polycarbophil in various concentrations (0.50, 1.00 and 1.25% w/w) was added to propylene glycol by simple addition then shaken vigorously by hand for 1 minute. These preparations were then examined by eye by inverting the container and noting the flow characteristics (i.e. to check for evidence of viscosity increases in the solvent by the polycarbophil). It must be stated that the visual assessment of the viscosity of the continuous phase was merely for preliminary evaluation purposes. A viscosity analysis on the effect of polycarbophil concentration on the continuous phase viscosity was carried out as described in Section 3.2f.

3.2d Manufacturing Methods for Incorporating the Viscosity Inducing Agent into the Non-Aqueous Solvent

Various manufacturing methods were investigated to arrive at the optimum method for uniformly dispersing the viscosity inducing agent (polycarbophil) throughout the non-aqueous solvent. Propylene glycol was used as the non-aqueous solvent throughout this particular investigation. After identifying the best method for manufacturing the continuous phase, the other candidate non-aqueous solvents (ethanol and polyethylene glycol 400) were then investigated.

i. Magnetic Stirrer

The initial manufacturing process involved the use of a magnetic stirrer (Speedsafe, Hanna, UK). A known amount (50 g) of propylene glycol was placed into a 150 mL glass beaker at room temperature. The non-aqueous solvent was stirred rapidly using an 8 mm diameter x 30 mm length Teflon coated magnetic stirrer. 0.5% w/w polycarbophil was added slowly to the propylene glycol (over a period of 5 minutes) and stirring was continued until the experiment was terminated (after 30 minutes).

ii. Overhead Stirrer

In this method, 50 g of the non-aqueous solvent propylene glycol was placed into a 150 mL glass beaker at room temperature and stirred using an overhead stirrer (Heidolf, Model RZR1, Germany). The non-aqueous solvent was stirred from above using a three blade overhead stirrer at 2000 RPM. 0.5% w/w polycarbophil was added slowly (over a period of 5 minutes) to the propylene glycol and stirring was continued until the experiment was terminated (30 minutes).

iii. Overhead Stirrer Plus Heating

A known amount of propylene glycol (50 g) was placed into a 150 mL glass beaker. 0.5% w/w polycarbophil was added to the propylene glycol and mixed as described in Section 3.2d *ii*. After 30 minutes of stirring (at 2000 RPM), the mixture was placed on a heating mantle (IEC, Australia) and heated to temperatures of approximately 80°C while still being stirred by the overhead stirrer for a further 30 minutes.

iv. Overhead Stirrer Followed By Placing on a Roller at 40°C

A known amount of propylene glycol (50 g) was placed into a 150 mL glass beaker. 0.5% w/w polycarbophil was added to the propylene glycol and mixed as described in Section 3.2d *ii*. After 30 minutes of stirring (2000 RPM), the mixture was placed on a custom made roller in a 40°C heated room (the temperature was maintained by a thermostat). The room temperature was monitored by a digital thermometer (Tecpel, Taiwan). The mixture was rotated on the roller at 50 RPM for 14 days. No further manipulation of the mixture was conducted during this time.

v. Mortar and Pestle

A small amount of non-aqueous solvent (10 g) was placed in the pestle, followed by the addition of a known amount of polycarbophil (0.25 g – which would give an ultimate concentration of 0.5 % w/w). This was mixed using the mortar until a thick gel formed. The remaining solvent (40 g) was then added slowly in 10 even amounts over a period of 2 hours with continued mixing. During this period the solvent/polymer mixture was left to stand intermittently for 5 minutes to allow the gel to thicken. At intervals, a small amount (0.5 mL) of the dispersion was removed (using a plastic disposable pipette) and analysed for the presence of globules (refer to Section 4.5d *v* for a definition of what a globule observed in this study is). The presence of globules was assessed by tipping the glass vial onto its side. The thickness of the liquid layer becomes thin and globules of undissolved polymer can be observed if they exist.

vi. Mortar and Pestle Followed By Placing on a Roller at 40°C

The resultant mixture obtained when using the mortar and pestle technique (described in Section 3.2d *v*) was placed on a roller in a 40°C room. The mixture was left on the roller (rotating at 50 RPM) for 14 days. At intervals (every 24 hours), a small amount of the dispersion was removed and analysed for presence of globules (Section 3.2d *v*).

Control experiments investigated the amount of water uptake of the mixture during the mixing process by simple weighing (Sartorious 4-figure balance, Germany) at intervals of 20 minutes. The analysis was conducted in duplicate.

vii. Silverson® Homogeniser

A Silverson® L4RT (Silverson®, England) high speed homogeniser (Figure 3.4) was used to incorporate the polycarbophil into propylene glycol at speeds of 2000 RPM (low speed setting) 5000 RPM (medium speed setting) and 8000 RPM (high speed setting).

Figure 3.4 – The Silverson® Homogeniser

Figure 3.5a – The General Purpose Disintegrating Head

Figure 3.5b – The Square Hole High Shear Screen Head

Two types of heads (Figures 3.5a and 3.5b) were used in the mixing process - the general purpose disintegrating head and the square hole high shear screen head.

To mix the polycarbophil into the propylene glycol, the appropriate head of the Silverson® high speed homogeniser mixer was placed into the non-aqueous solvent (90 g in a 150 mL plastic vessel) and the mixer turned on to a speed of 8000 RPM. The high speed mixing capability allowed a greater amount of the continuous phase to be manufactured (i.e. 90 g compared to 50 g in the previous methods (Section 3.2d *i* – 3.2d *vi*) due to its greater stirring efficiencies which would allow a greater batch to be manufactured at one time. Though the volume was different the overall concentration of the polycarbophil was still the same at 0.5% w/w). The plastic vessel was submerged into an ice bath to prevent unwanted temperature increases of the propylene glycol. The polycarbophil (0.5% w/w) was added by continuously sprinkling small quantities from above over a period of 10 minutes. The polycarbophil was added in small amounts at a time to ensure the polymer was drawn into the workhead preventing clumping. The mixture was stirred for a period of up to 2 hours. At time points of 5, 10, 15, 20, 25, 60 and 120 minutes the mixing process was interrupted long enough to withdraw a small amount of the mixture which was analysed for the presence of globules (as outlined in Section 3.2d *v*).

viii. Ultra Turrax®

The Ultra Turrax® (T18 basic, IKA Works INC, USA) (Figures 3.6a and 3.6b) is similar to the Silverson homogeniser (in that it is a high speed homogeniser) but can operate at much higher speeds.

To mix the polycarbophil and the propylene glycol using the Ultra Turrax®, the probe was placed into the propylene glycol (90 g added into a 150 g plastic vessel) and the Ultra Turrax® turned on to a speed of 18000 RPM. The polycarbophil (0.5% w/w) was added by continuously sprinkling small quantities from above over a period of 2-3 minutes. The mixture was then stirred for a period of up to 1 hour and 45 minutes (which was found to be sufficient time to remove any globules which may have formed). At intervals of 20 minutes the mixing process was interrupted long enough to withdraw a small amount of the mixture which was analysed for the presence of globules.

Figure 3.6a – The Ultra Turrax® T18 Basic

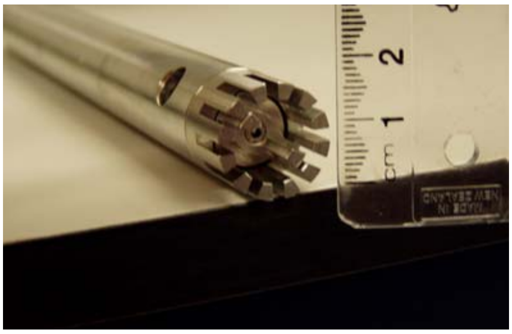


Figure 3.6b – The Ultra Turrax® Probe

3.2e Characterisation of Potentially Useful Continuous Phases

Following the outcomes of previous sections (discussed more in depth in Chapter 4 – Section 4.2e), continuous phases involving the particular non-aqueous solvents propylene glycol and polyethylene glycol 400 were prepared. These were manufactured according to the method outlined in Section 3.2d *viii.* where 0.5% (w/w) polycarbophil was added to 90 mL of each non-aqueous solvent.

i. Appearance of Continuous Phase

The appearance of each continuous phase manufactured in Section 3.2e was assessed by observing the colour, clarity and the presence of any globules every 24 hours over a period of 4 weeks. The experiment was carried out in duplicate.

ii. Physical Stability

The physical stability of each continuous phase was monitored for 4 weeks. Glass vials containing 7 mL of each of the 2 possible continuous phases were set aside against a black background at room temperature. The sedimentation process was observed and recorded by taking photographs (Canon MVX150i Digital Camera) every 48 hours over a period of 4 weeks. The experiment was carried out in duplicate.

3.2f Determination of the Effect of Polycarbophil Concentrations on Viscosity of the Continuous Phase (Polycarbophil Plus Propylene Glycol)

The effect of the viscosity inducing agent on the viscosity of the continuous phase was investigated by conducting viscosity measurements. Viscosity studies were conducted using a S10 vibro-viscometer (A&D, Japan). The viscometer has two sensor plates which vibrate like a tuning fork maintaining a constant frequency and amplitude. The viscometer measures the current which is varied to keep the constant vibration according to the viscosity change during the measurement. The sensor plates vibrate at 30 Hz at an amplitude of about 0.4 mm.

The viscometer was calibrated using glycerin standards (which were tightly sealed to prevent moisture entry and stored in a desiccator) having known viscosities of 104 and 500 mPa.s (Brookfield, USA). The sensitivity and robustness of the viscometer was determined by varying conditions such as temperature and volume of sample.

The effect of sample volume was assessed by measuring the viscosity of the 104 mPa.s glycerin standard using volumes of 9, 9.2, 9.4, 9.6, 9.8, 10.0, 10.2, 10.4, 10.6, 10.8 and 11 mL (where 10 mL was the required sample volume). A pipette was used to measure the desired volumes. The effect of filling the sample cup with solution using the 10 mL line (etched on the sample cup) compared to using a 10 mL pipette was also assessed. Experiments were conducted in triplicate.

The effect of temperature was determined by altering the temperature of the water bath from 24.6 to 25.6°C in increments of 0.1°C. Experiments were conducted in triplicate.

Upon developing and validating the method, viscosity readings were taken. The test solution was poured into the sample cup until the bottom meniscus was in line with the 10 mL mark. The sensor plates must be placed in the sample at the exact location for each reading. The correct position of the sensor plate for each sample was ensured by using a magnifying glass. The sample was left in the water jacket for 30 - 45 minutes or until a constant temperature reading was obtained on the viscometer. Once the sample temperature had equilibrated, a reading was taken every 5 seconds for 2 minutes.

3.2g Incorporation of the Hydrophilic Polymer into the Continuous Phase

To manufacture the ISGM system, the hydrophilic polymer was incorporated into the continuous phase. This was achieved by manufacturing the continuous phase by using the Ultra Turrax® method described in Section 3.2d *viii.* and then adding the hydrophilic polymer by addition of the required weight (10 % w/w) to a closed container (glass vial - Samco Glass, Australia) (at a rate of 0.2 g s^{-1}). The mixture was mixed by hand for 2 minutes which was sufficient time for the polymer to be homogeneously dispersed.

3.2h Sedimentation Behaviour of the Hydrophilic Polymers in the Continuous Phase

A physical sedimentation analysis was carried out on the ISGM systems described in Table 3.8 where the continuous phase was globule-free.

Table 3.8 – The ISGM Systems Investigated in the Physical Stability Study

Continuous Phase					**Hydrophilic Polymer**
Non-Aqueous Solvent	Polycarbophil Concentration				
PG	0.50	0.67	0.84	1.00	HPMC
PG	0.50	0.67	0.84	1.00	PEO 303

The sedimentation behaviour was determined in 6 mL glass vials (Samco Glass, Australia) as previously described in Section 3.2a. 0.4 g of the test polymer was then added to the vial followed by 3.6 g of the continuous phase (10% w/w polymer concentration). The vials were tightly closed to prevent moisture entry. Experiments were conducted in duplicate.

Homogeneous dispersion and observation of the sedimentation behaviour of the hydrophilic polymer was then evaluated as described in Section 3.2a over a period of 4 weeks.

The errors were calculated as the relative standard deviation in the percentage sedimentation height of the replicates, (where each experiment was carried out in duplicate).

The effect of the amount of polycarbophil present on the ease of redispersion of the ISGM system was determined by manually shaking the suspension. It must be stated that the visual assessment of the ease of redispersion of the continuous phase was merely for preliminary evaluation purposes. (An investigation into the ease of redispersion of the ISGM system was conducted in Section 3.3c).

The particle size of the hydrophilic polymers was determined using the Olympus BX60 (Olympus, Japan) optical microscope which was connected to a Nikon DS-

5Mc (Nikon, Japan) camera. The polymer was dusted over a coverslide and placed under a microscope and observed at 50 x magnification. A random area was chosen and photographs taken. 200 particles were selected from the magnified area and measured using the calibrated scale on the optical microscope.

3.3 Characterisation and Stability of the ISGM System

The ISGM system was characterised by carrying out experiments such as viscosity studies, ease of redispersion and water absorption studies. The chemical stability of the hydrophilic polymer and the continuous phase was also assessed.

3.3a Water Absorption Studies on the ISGM System

It is essential that water is not absorbed by the ISGM system as the water will cause the hydrophilic polymer to gel. Earlier studies (Section 3.2d *vi.* indicated when the continuous phase was open to the air, the hygroscopic non-aqueous solvent absorbed water leading to an increase in weight).

The extent of water absorption was monitored by placing 7 mL of the ISGM system contained in a glass vial (Samco, Australia) in a controlled environment which consisted of a humidity controlled oven, where the humidity was set to 60% and the temperature to 25°C. Over 7 weeks the vials were removed and weighed to determine the amount of water absorbed.

Variations of the ISGM system were also investigated to determine what effect the constituents had on the water intake. Each experiment involved altering one variable at a time. The HPMC concentration in the ISGM system was varied (Table 3.9) from 10% to 5 and 25% (keeping the polycarbophil concentration the same). The effect of the concentration of polycarbophil was also investigated by altering the concentration from 0.67% to 0.5, 0.84 and 1% (while keeping the HPMC concentration the same) (Table 3.9). All tests were conducted in triplicate.

Three sealed vials containing the ISGM system were also placed on the bench at room temperature in sealed vials to determine whether water absorption still occurred.

Table 3.9 – Tested Variations of the ISGM System

HPMC % (while keeping the PCP concentration the same)	**PCP % (while keeping the HPMC concentration the same)**
0	0.00
5	0.50
10	0.67
20	0.84
-	1.00

3.3b Chemical Stability

i. Hydrophilic Polymer (HPMC)

The stability of the hydrophilic polymer (HPMC) was monitored by SEC (size exclusion chromatography) based on previously reported methods (Crowley *et al.*, 2002; Vijayalakshmi *et al.*, 2005; Wittgren *et al.*, 2005; Liu *et al.*, 2004). An Amersham Bioscience HPLC instrument with a T-Load 16/60 Sephacryl S500 HR (Pharmacia LKB Biotechnology AB, Sweden) column was used. The column had a volume of 120 mL and a mean particle size of 47 µm. The column bed consisted of a cross-linked copolymer of allyl dextran and N,N-methylenebisacrylamide. PBS (phosphate buffered saline) (Sigma, USA) buffer (pH 6.74) was used as the mobile phase at a flow rate of 1 mL min^{-1}. The buffer was prepared by dissolving PBS tablets in Millipore ultra pure water (Millipore Company, 0.22 µm Millipore filter). The mobile phase was degassed using helium sparging (BOC) and filtered (0.2 µm pore size) (Nalgene Filtration Products, Nalge Nunc International, USA). A 0.1% (w/w) sample of HPMC was injected into the HPLC. The spectrum was monitored using a RI (refractive index) detector (Pharmacia LKB Biotechnology AB, Sweden).

SEC analysis was repeated on the Waters HPLC using the ultrahydrogel 1000 column (7.8 1D mm x 30 cm). A ultrahydrogel guard column (6 x 40 mm) was used to protect the column. 20 µL of 0.1% (w/w) HPMC was injected using a syringe (Hamilton, USA) into the system. 0.05% (w/w) sodium azide (Sigma, USA) (antibacterial agent) was used as the mobile phase at a flow rate of 0.9 mL min^{-1}.

To verify the detected peak was due to HPMC, the concentration of HPMC was halved to 0.05% and was injected into the HPLC system to determine if the detected peak would respond accordingly. The analysis was repeated 6 times to ensure results were reproducible.

To determine what effect a degraded sample of HPMC may have on the chromatogram, a sample of HPMC treated with 0.1 mol L^{-1} HCl (Scharlau, Spain) was also analysed. Treatment with HCl would result in acid hydrolysis of HPMC and any changes in the resulting chromatogram were noted.

Three samples of 10% (w/w) HPMC suspended in propylene glycol were prepared. An aliquot from each sample was taken, diluted with water, and injected into the HPLC. The prepared samples of HPMC dispersed in propylene glycol were then stored in a room maintained at 40°C / 75% relative humidity. Samples were then taken at time points of 3, 6 and 9 months to analyse the stability of HPMC. Analysis was carried out in triplicate.

To determine the molecular weight of HPMC, standards of dextran were run. The following molecular weight standards were used: 4 400, 50 000, 70 000, 110 000, 401 300 and 2 000 000.

ii. Continuous Phase

The stability of the continuous phase (the polycarbophil and the propylene glycol) could not be monitored by changes in molecular weight as polycarbophil has a very broad molecular weight range (Chen, 2000). Consequently the stability of the polymer was monitored by changes in viscosity (Chen, 2000). Viscosity readings were conducted using a SV 10 vibro-viscometer as outlined in Section 3.2f. The continuous phase was stored at room temperature (20°C) and the viscosity was measured over time at periods of 0, 3, 6 and 9 months. Measurements were conducted in triplicate.

3.3c Ease of Redispersion of the ISGM System

Twelve sealed glass vials containing 6 g of the ISGM system were stored in a cupboard in the dark at room temperature. 3 vials were removed each week for four weeks to assess the redispersibility of the ISGM system over time where the average of the 3 vials was taken. Redispersion was conducted by placing the vial in a rotary evaporator at 30 RPM. Cotton wool was placed alongside the vial to prevent the vial from breaking and to ensure the vial rotated end to end. The rotation was carried out for a total of five minutes and the extent of redispersion was observed (by measuring the height of the sediment which had not redispersed) every 30 seconds during the 5 minute period.

3.3d Appearance of the ISGM System after Administration into an Aqueous Environment

i. In Vitro Appearance of the ISGM System after Administration into an Aqueous Environment

A cellulose medium was prepared by addition of 3% (w/w) HPMC to a solution comprising of 50:50 water:ethanol. A cellulose medium was used as it formed a gel of suitable viscosity to allow injection of the formulation. The solution was stirred at 2000 RPM for 20 minutes using an overhead stirrer (Heidolf, Model RZR1, Germany). The ISGM system, with the inclusion of a blue dye, was injected (at a rate of 0.4 g s^{-1}) into the cellulose medium. A photograph of the gelled ISGM system was taken using a Canon MVX150i digital camera. This experiment was conducted at room temperature (20°C).

ii. In Situ Appearance of the ISGM after Administration into an Excised Calf Ear

The ISGM system was injected into the ear of a dead calf (20°C) using a 22 gauge needle to observe whether the injected suspension would gel in the aqueous environment of the tissues. The ear was used as the site of injection due to the ease of visualisation and excision. The purpose of this study was to determine whether the ISGM system would function as anticipated i.e. to determine whether the injected suspension would gel as a result of water from the surrounding tissue diffusing into the depot.

iii. In Vivo Appearance of the ISGM System after Administration into a Rat

The ISGM system was also injected into a rat. Ethics approval was gained from the Ruakura Animal Ethics Committee. The area posterior to the scapulae was shaved and swabbed with 70% ethyl alcohol. 0.5 mL of the ISGM system was injected using a 22 gauge needle. The rat was killed by CO_2 administration and then dissected to observe the appearance of the depot.

3.4 Formulation and Characterisation of the Final Product

The final formulation (which was the ideal mix of components containing the drug) for the ISGM system was characterised by carrying out experiments such as DSC (differential scanning calorimetry) studies, IR analysis and UV-Vis analysis. DSC studies were conducted to assess whether any potential incompatibilities existed between the drug and the excipients of the formulation and the DSC findings were then examined further by IR analysis.

3.4a Incorporation of the Drug into the ISGM System

To manufacture the final product, the drug (propranolol HCl (Sigma, USA)) was incorporated into the continuous phase. This was achieved by adding the required amount of drug to the continuous phase (manufactured using the Ultra Turrax® as described in Section 3.2d *viii*.) in a closed container and then mixing by shaking for 1 minute. The drug was weighed by difference to ensure the amount of drug transferred was known. Once the drug was incorporated, the hydrophilic polymer (10% w/w) was added and mixed by shaking for 2 minutes to ensure a homogeneously dispersed suspension. The properties of the drug are given in Section 4.4a.

3.4b Differential Scanning Calorimetry (DSC) Analysis

DSC (using a DSC 6, Perkin-Elmer, USA) studies were carried out to determine whether any potential interactions occur between the excipient and drug and hence whether any potential incompatibilities existed. The pure drug, the excipient alone and a 1:1 sample of excipient:drug were analysed. A 1:1 ratio was selected to maximise the likelihood of any interactions (Mura *et al*., 1998). The sample to be analysed was placed in an aluminium pan and spread out to ensure even coverage on the pan. An aluminium lid was placed on top and crimped using the DSC crimper. A crimped empty aluminium pan and lid was used as the reference. Handling of the pans was carried out using a pair of tweezers. The sample and reference were placed in the DSC instrument and a heating experiment conducted under the following conditions:

Temperature range: 25 – 250 °C

Heating Rate: 10 °C min^{-1}

3.4c Infra-Red (IR) Analysis

All FTIR spectra were acquired at 4 cm^{-1} resolution using a Digilab FTS-40 (Digilab FTS-40 Fourier Transform IR spectrometer, Massachusetts, USA) Fourier Transform infrared spectrometer which employed KBr optics and Win-IR version 4.14 Software (Galactic Industries Corporation, Massachusetts, USA). The KBr disc method was utilised to prepare solid samples for analysis. The drug, propranolol was added to dried KBr in a mortar and pestle in the approximate ratio of 1:5 and mixed to obtain a homogeneous mixture. The mixture was ground for 2 minutes. A sufficient amount (1-2 milligrams) of the mixture was placed in a holder between two metal discs and transferred to a hydraulic press to give a hard clear white disc. The resulting disc was then placed carefully (to avoid cracking or breaking of the disc) in a holder which was then positioned in the sample compartment of the infrared spectrometer. Absorbance spectra were acquired by ratioing sample single beam spectra against a spectrum of air (i.e. an empty sample compartment). Absorbance FTIR spectra were then baseline corrected to achieve a flat baseline. The following settings were used for all spectra:

- 4 cm^{-1} resolution
- 400 – 4000 cm^{-1} wavelength range
- Speed setting of 5 kHz for the DTGS (deuterated triglycine sulphate) detector
- Open aperture

3.4d Electrospray Mass Spectrometry (ESMS) Analysis

All ESMS analyses were conducted on an LCMS LCQ Advantage (Thermal Finnigan, USA) using the mass spectrometry function only. ESMS was used as a means of characterising the drug as well as determining whether drug was present in stability studies (Section 3.6). The spectra were collected under the following conditions:

- Vapour temperature 450ºC
- Sheath gas flow 80 mL min^{-1}
- Discharge current 5 µA
- Discharge potential of 4.38 kV.

An electrospray ionisation mode was used to collect the spectra. Mass spectra were acquired in the positive ion mode (Upthagrove *et al.*, 1999). Each spectrum was the average of 20 scans.

3.4e Ultra-Violet (UV) Analysis

All UV analyses were conducted on a Cary 1 UV spectrophotometer (Cary, USA). Samples of propylene glycol, polycarbophil, HPMC and propranolol were analysed using quartz 10 mm cells (Starna Ltd., England). Samples were scanned over the wavelength range of 200 – 400 nm at a speed of 600 nm min^{-1}.

3.5 Drug Release Studies on the Final Product

Once the formulation was characterised (Section 3.3 and 3.4), *in vitro* studies were conducted. The *in vitro* drug release studies were carried out to assess the feasibility of the formulation. The *in vitro* studies involved addition of a drug to the ISGM system and measuring its release over time. The effects of various factors such as the concentration of the hydrophilic gelling agent on the release rate of the drug were assessed. The release data was then modelled using the Higuchi equation and the power law relationships to compare the effects of parameters such as the concentration of the gelling agent on the drug release rate.

3.5a Drug Release Studies Conducted on the Hanson Dissolution Apparatus

Drug release studies were conducted on the final formulation (Section 4.2h) which consisted of a hydrophilic gelling agent HPMC and the chosen model drug propranolol in the continuous phase (comprising of the non-aqueous solvent propylene glycol and the viscosity inducing agent polycarbophil). 2 g of the formulation was drawn out with a 10 mL syringe and injected into the dissolution test medium. The formulation was injected at rate of 0.4 g s^{-1} each time as differing rates produced depots of differing surface area.

Dissolution studies were performed on a calibrated dissolution apparatus (Hanson Dissolution Apparatus, SR8Plus, USA). The dissolution test medium (water) was maintained at 37.0 ± 0.5°C. To each of the six vessels in the dissolution apparatus, 500 mL of dissolution test medium was added. The paddle blade was incorporated as the stirrer for each of the six vessels. A paddle was placed 25 mm from the bottom of each vessel as described in the protocol in the United States Pharmacopoeia (USP, 1994) method and set to a speed of 50 RPM. Upon injection into the vessel, the HPMC gelled giving a very viscous dissolution test medium posing problems of drawing adequate volumes using the narrow bore auto sampler. As a result samples were taken manually with a 2.5 mL pipette. The samples were collected and analysed by UV (Unicam, Helios Gamma, England) absorbance at 290 nm to determine the drug level.

i. Mode of Formulation Introduction to the Hanson Dissolution Apparatus

Initial release assessment studies were conducted using the USP Apparatus 2 (Paddle). The formulation was introduced into the vessel in one of two ways. The first method involved injecting the formulation from above the vessel in order to observe the effect of a dispersed injection. The second method involved injecting the formulation at the bottom of the vessel (after the addition of the dissolution test medium) thus forming an intact “bulb” (the second method of introducing the formulation was executed in a style that was anticipated to be the case *in vivo*). The run was conducted using 6 vessels.

ii. Effect of the Hydrophilic Gelling Agent

The effect of omitting the hydrophilic gelling agent HPMC from the formulation was also investigated. The trivial observation expected from this was obviously instant release of propranolol given there was no polymer to entrap the drug and retard its release rate. All formulations were introduced to the dissolution test medium by injection via a syringe onto the bottom of each of the 6 vessels.

iii. Basket Method (USP Apparatus I)

This experiment involved using a rotating basket instead of a paddle. The formulation was injected into the basket while it was held stationary in the dissolution test medium. After injection, the basket was rotated at 50 RPM for each of the 6 vessels.

iv. Reproducibility of Release Trials Conducted on the Hanson Dissolution Apparatus

The experiment was run in 6 vessels simultaneously and on 3 different days to assess the intra and inter-day reproducibility of the method.

3.5b In Vitro Drug Release Studies Conducted on the Modified Dissolution Apparatus

A different means of carrying out drug release was required where the injected formulation (i.e the final product) was not exposed to the stirrer and the surface area of the depot remained fixed. A novel system was developed to overcome these issues. A coagulating flocculating simulator (Boltac Ltd., New Zealand), capable of holding 6 vessels, was utilised as a mixing station. Each vessel (Perspex) was modified to allow space for a lid (Perspex) and a glass tube to be placed on top (Figure 3.7a). The use of the glass tube ensured the surface area of the depot exposed to the dissolution test medium remained fixed (as the injected formulation covered the whole area) and that it was no longer subjected to the stirrer. The glass tube was covered with a dialysis membrane (membra-cel, MW cut-off 14 000) held in place with an o-ring (see Figure 3.7b). The dialysis membrane was wide enough to cover the whole glass tube and the molecular weight cut-off value ensured the gelling agent could not diffuse through into the dissolution test medium. The dialysis membrane was hydrated prior to use and wrapped over the tube carefully to prevent breakage of the membrane and to ensure creases or folds did not form which would alter the surface area.

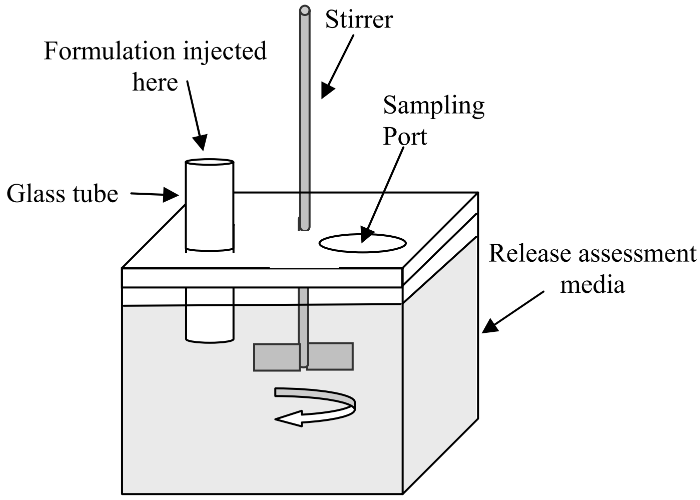


Figure 3.7a – Illustration of the Vessel in the Modified Dissolution Apparatus used to Conduct Drug Release Studies

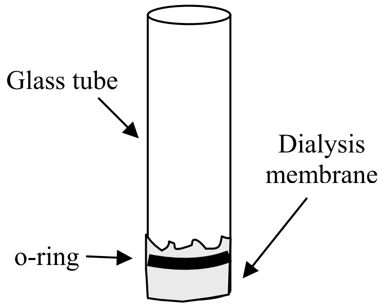


Figure 3.7b – The Glass Tube Held in the Vessel of the Modified Dissolution Apparatus

A drug concentration of 0.8% (w/w) was used in the ISGM system. The UV spectrum of propranolol had absorption maxima at 214 and 290 nm (Section 4.4e). Many researchers have reported using 290 nm for monitoring the drug to ensure minimal interference from any other UV absorbing material that may be present (Guyot and Fawaz, 2000; Namdeo and Jain, 2002; Taylan *et al*., 1996; Huang *et al*., 2004). However with this study a dialysis membrane was used which prevented the polymers from diffusing into the dissolution test medium (this was verified as described in Section 4.5). The molecular weight cut-off for the dialysis membrane is 14 000, therefore only the drug and propylene glycol will be able to diffuse out. Propylene glycol and HPMC have no UV absorption peaks in the region of interest however polycarbophil does (Section 4.4e) but because it is retained within the glass tube, the 214 nm wavelength was able to be used to monitor the drug concentration without interference. This was verified by monitoring the absorbance values at 214 nm of the dissolution test medium using a formulation without drug to ensure absorbance did not change with time (so indicating the polymer was not diffusing through the dialysis membrane and into the solution contributing to the absorbance). Even though the analytical wavelength of 214 nm is in a region where selectivity and specificity may be comprised, the UV absorbance did not register any change over time and therefore drug levels were able to be monitored at 214 nm with confidence. The advantage of utilising 214 nm was that the molar absorptivity coefficient of this UV absorption peak for propranolol was higher than for the other propranolol-associated absorbance at 290 nm. This hence allowed lower concentrations of drug to be detected in the dissolution test medium.

The majority of experiments were conducted using a stirring rate of 100 RPM using 500 mL of water (the dissolution test medium) with the glass tube placed at a fixed position of 1 cm from the lid of the vessel. A stirring rate of 100 RPM was chosen as it was the ideal speed to ensure the drug concentration at the release interface was zero at a faster rate. Speeds greater than 100 RPM resulted in an agitated dissolution test medium. The 1 cm position ensured the o-ring was located above the water preventing water from leaking into the glass tube.

Unless otherwise stated the final formulation was analysed in the *in vitro* trials i.e. a uniformly dispersed suspension (assessed by sight) of 10% (w/w) HPMC (hydrophilic gelling agent) and 0.8% (w/w) propranolol (the model drug) in the continuous phase comprising of propylene glycol (the non-aqueous solvent) and 0.67% (w/w) polycarbophil (viscosity inducing agent).

The following trials detailed in 3.5b *i* to 3.5b *x* were conducted to determine whether these parameters had an effect on the drug release behaviour.

i. Reproducibility of Release Trials Conducted on the Modified Dissolution Apparatus

Initial trials involved determining whether the method was reproducible. The experiment was run using 6 vessels and repeated on 3 different days to ascertain the intra- and inter-day reproducibility. Reproducibility studies were undertaken to ensure results were reproducible so that any differences which may occur will not be due to inter- or intra-run variations.

2 g of the formulation was drawn into a syringe and then injected into the glass tube (closed off at the end by the dialysis membrane). After injection, stirring was commenced at 100 RPM.

ii. Effect of the Hydrophilic Gelling Agent

A run was conducted where the formulation did not contain the hydrophilic gelling agent. This was to determine whether the absence of the gelling agent in the formulation would result in an altered release profile. The absence of the gelling agent should obviously give full release at a faster rate than with the gelling agent. The formulation was injected into each of the 6 glass tubes. The stirrer was maintained at 100 RPM.

iii. Effect of Volume of Water in Tube

Water was added to the glass tube prior to addition of the formulation. Upon injecting the formulation into the glass tube, the formulation gelled. The experiment was conducted to determine whether premature gelling of the formulation prior to release had an effect on the drug release rate. 0, 0.5 and 2.5

mL of water was added via syringe for each run. Each run consisted of 6 samples. The stirrer was maintained at 100 RPM.

iv. Effect of Tube Height

The height at which the glass tube was placed in the vessel of the modified dissolution apparatus was altered from 1.0 cm (measured from the lid of the vessel) to 0.7 cm to determine whether the distance between the stirrer and the diffusion site (end of the glass tube) had an effect on the drug release rate. 6 samples were analysed and the stirrer was maintained at a speed of 100 RPM.

v. Stirring Rate

The effect of the stirring rate on the release profile was investigated. The stirring rate was altered from 100 RPM to 50 and 150 RPM. Each trial involved 6 samples.

vi. Effect of pH of Dissolution Test Medium

The effect of altering the pH of the dissolution test medium was investigated as the pH of the system can affect the extent of drug release (as discussed in Section 4.5b *vi.*). A dissolution test medium at pH 1 was prepared by simply using 0.1 mol L^{-1} hydrochloric acid in place of the water. Dissolution test medium adjusted to pH 4.5 were prepared by the use of a solution which was 0.05 mol L^{-1} in sodium dihydrogen phosphate (May & Baker, Australia) to which 0.1 mol L^{-1} sodium hydroxide was added dropwise to bring the solution to pH 4.5.

Each trial comprised 6 samples. The stirrer was maintained at 100 RPM.

vii. HPMC (Gelling Agent) Concentration

The HPMC concentration in the formulation was altered from 10% (w/w) to 5 and 25% (w/w) to assess the effect on the release rate of the drug. Each run consisted of 6 samples and the stirrer was maintained at a speed of 100 RPM.

viii. Polycarbophil (Viscosity Inducing Agent) Concentration

The concentration of polycarbophil in the formulation was altered from 0.67% (w/w) to 0.5 and 1% (w/w). Each run consisted of 6 samples and the stirrer was maintained at 100 RPM.

ix. HPMC Viscosity Grade

A different viscosity grade of HPMC (HPMC 90SH (Shin-Etsu Chemical Co. Ltd, Japan) instead of HPMC 60SH was used in the formulation to determine if there was any influence on drug release. 6 samples were analysed and the system was stirred at 100 RPM.

x. Different Gelling Agents

The effect of using a gelling agent other than HPMC in the formulation was also studied out of scientific interest (as discussed in Section 4.5b *x*). Three additional gelling agents from the initial screening process (Section 3.2), PEO 303, sodium CMC and xanthan gum were selected. The formulation was prepared using 10% (w/w) of the gelling agent. Each run consisted of 6 samples and the stirrer was maintained at 100 RPM.

3.5c Modelling of the Drug Release Data

Modelling of the drug release data obtained from the experiments described in Section 3.5b was attempted using the Higuchi and the power law relationships as discussed in Section 4.5c. Modelling was used as a means of comparing the effect of various parameters on the release rate. Microsoft Excel software (Microsoft Corporation, 2002) was used to conduct the modelling studies.

3.6 Stability Assessment of the Final Product

The stability of the final formulation was assessed visually and by using an HPLC-based protocol. The purpose of the stability trial, carried out under accelerated conditions, was to determine how the quantity of the drug changed over time (refer to Section 4.6 for a discussion on the need for stability studies).

3.6a Drug Stability

i. Method Development

The stability of the drug was monitored using a Nova-Pak C18 column (8 x 100 mm) (Waters, USA) for reasons as discussed in Section 4.6a *i.* The column was held in a RCM (radial compression module) 8 x 10 module (Waters, USA). The module was used to apply and maintain radial compression along the circumference of the flexible Radial-Pak® cartridge. The pressure moulded the cartridge wall around the column packing decreasing the interstitial spaces within the column bed. Consequently the more homogeneous packed bed structure resulted in increased cartridge efficiency and eliminated the formation of voids. Methanol was injected into the column holder to maintain the pressure at 17 MPa. The mobile phase (USP, 1994) was prepared by dissolving 1 g of sodium dodecyl sulphate in 36 mL of 0.15 mol L^{-1} phosphoric acid (Merck, Germany) in a glass vessel. To this glass vessel, 180 mL of HPLC grade methanol (Scharlau, Spain) and HPLC grade acetonitrile (Burdick and Jackson, USA) in addition to 104 mL of water were added to produce a final volume of 500 mL. The mobile phase was filtered (0.2 μm) (Nalgene Filtration Products, Nalge Nunc International, USA). The flow rate of the mobile phase was maintained at 1 mL min^{-1}. A guard column was used to protect the column (Nova-Pak, 4 μm, 60 Å, C18 Guard-Pak Insert). 20 μL of the sample was injected into the HPLC using a Hamilton syringe (Hamilton, USA). The Waters 996 PDA (Photo Diode Array) detector (Waters, USA) was able to scan wavelengths from 200 - 410 nm. The PDA detector was used as propranolol has a UV chromophore thus allowing UV to be used as a mode of detection.

A diode array detector is made up of many photosensitive diodes adjacently placed and insulated from each other in the form of a multilayer sandwich. The output from each diode is scanned, stored and processed by the computer. The diode array detector monitors the light which has passed through a liquid sensor cell. The light source such as a deuterium or xenon lamp is focussed by an achromatic lens through the sample cell and the light is dispersed by a holographic grating onto the surface of the diode array. Each diode will receive light of a slightly different wavelength (Beesley *et al*., 2001).

The output from any diode can be selected to give a chromatogram using the wavelength which fell on that particular diode. The advantage of using this detector is that analysis can be carried out simultaneously on a range of wavelengths.

The process of method development involved assessment of parameters such as the linearity of measured peak areas, the influence of forced drug degradation on specificity and robustness in the solvent composition used in the protocol.

Linearity and Calibration

The linearity of the method assesses the ability within a concentration range to obtain results which are proportional to the peak area (International Conference on Harmonisation, 2005; Center for Drug Evaluation and Research, 2000). Pure drug standards were prepared by dissolving propranolol in methanol to give concentrations of 0.25, 1, 5, 10, 15 and 20 mg mL^{-1}. 20 µL of each standard was injected into the HPLC. The peak area measured for the propranolol drug samples were plotted against the concentration of propranolol in the standards to obtain a calibration curve. The data was analysed to determine whether a linear relationship existed between the peak area and concentration over the expected range of concentrations that would arise in analyses of actual samples.

Forced Drug Degradation Study

This study was conducted to investigate the specificity of the HPLC method, i.e. its ability of detecting the analyte in the presence of other components such as degradants from the propranolol (Rao *et al*., 2005).

Forced degradation of propranolol was carried out by subjecting a 10`mg mL^{-1} starting concentration of propranolol to the following extreme conditions for 24 hours (except for the heat degradation experiment which was carried out for only 1 hour):

- For the alkali-induced degradation, addition of 1 mol L^{-1} NaOH to the 10 mg mL^{-1} propranolol solution in a glass vial to give effectively a concentration of 5 mg mL^{-1} (propranolol).

- For the acid-induced degradation, addition of 1 mol L^{-1} HCl was added to 10 mg mL^{-1} propranolol solution in a glass vial to give a concentration of 5 mg mL^{-1} (propranolol).

- For oxidatively induced degradation, addition of 3% H_2O_2 was added to 10 mg mL^{-1} propranolol solution in a glass vial to give a concentration of 5 mg mL^{-1} (propranolol).

- For thermally induced degradation, 0.2 g of propranolol was heated in a contherm stability oven for one hour at 105 °C.

- For photoinduced degradation, a 5 mg mL^{-1} solution of propranolol in water was placed in front of a UV light source at 254 nm for 24 hours (Uwai *et al.*, 2005). The broad distribution range of wavelengths on either side of the wavelength source centred at 254 nm most likely would have intensities included at 214 and 290 nm (the absorption maxima for propranolol). A 5 mg mL^{-1} solution of propranolol in water subjected to open light for 3 months was also analysed.

Robustness

The robustness of the method towards solvent composition variables in the HPLC protocol was determined by changing the following components in the mobile phase used:

- Acetonitrile concentration from 36% to 66%

- Methanol concentration from 36% to 66%
- Phosphoric acid concentration from 0.15 mol L^{-1} to 0.30 mol L^{-1}

ii. *The Final Method*

The final procedure decided upon for conducting a drug stability study (as a result of conducting the method development study) is given in Table 3.10.

Table 3.10 – HPLC Method for the Analysis of Propranolol

Parameter	**Description**
Column	Nova-Pak C18 column (8 x 100 mm)
Guard Column	Nova-Pak, 4 µm, 60 Å, C18 Guard-Pak Insert
Mobile Phase	The mobile phase (USP, 1994) was prepared by dissolving 1 g of sodium dodecyl sulphate in 36 mL of 0.15 mol L^{-1} phosphoric acid (Merck, Germany) in a glass vessel. To this glass vessel, 180 mL of HPLC grade methanol (Scharlau, Spain) and HPLC grade acetonitrile (Burdick and Jackson, USA) as well as 104 mL of water were added to give a final volume of 500 mL.
Flow Rate	1 mL min^{-1}
Temperature	The temperature was not varied during the method development as the column was not heated.
Injection Volume	20 µL
Detector	PDA 200 – 410 nm

iii. *Method Validation*

Method validation was conducted by assessing the linearity, accuracy, precision, limit of quantitation, limit of detection, robustness and specificity. The object of method validation was to demonstrate that the method was suitable for its intended purpose (International Conference on Harmonisation, 2005; Center for Drug Evaluation and Research, 2000).

Linearity

Pure drug standards were prepared by dissolving propranolol in methanol to give concentrations of 0.25, 1, 5, 10, 15 and 20 mg mL^{-1}. 20 µL of each standard was injected into the HPLC. The data was analysed to determine whether a linear relationship existed between the peak area and concentration over the expected range of concentrations in the analysis.

Accuracy

The accuracy of the method shows the closeness of agreement between the reference value and the experimentally determined value on the instrumentation used following the decided protocol (International Conference on Harmonisation, 2005; Center for Drug Evaluation and Research, 2000). To assess the accuracy of the method, 3 samples of 2 mg mL^{-1}, 3 samples of 9 mg mL^{-1} and 3 samples of 17 mg mL^{-1} propranolol dissolved in methanol were passed through the column to determine whether the concentrations from the calibration curve matched the actual concentration of the prepared samples.

Precision

Precision can be determined on 3 levels – repeatability, intermediate precision and reproducibility. Repeatability assesses the precision of the method over a short period of time under the same operating conditions. It can also be referred to as intra-assay precision. Intermediate precision expresses the precision under conditions such as different days of analysis or different equipment. These are conducted in the same laboratory. Reproducibility is the measure of precision in different laboratories (International Conference on Harmonisation, 2005; Center for Drug Evaluation and Research, 2000).

In this study the repeatability and intermediate precision were assessed. In terms of repeatability, the %RSD (relative standard deviation) was calculated using 6 individual samples of pure drug with a concentration of 0.5 mg mL^{-1} and 6 individual samples of pure drug with a concentration of 5 mg mL^{-1}.

The intermediate precision was assessed by preparing calibration curves for concentrations of propranolol of 0.25, 1, 5, 10, 15 and 20 mg mL^{-1} (dissolved in methanol) on 3 different days.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD is the lowest concentration which can be detected and LOQ is the lowest concentration which can be quantitatively determined (International Conference on Harmonisation, 2005). The LOD and LOQ were calculated in Section 4.6 (Chapter 4). To verify the theoretically derived value calculated from these equations, a 0.040 and 0.060 mg mL^{-1} sample of propranolol dissolved in methanol was passed through the column to determine if these concentrations could be detected.

iv. Extraction of the Drug from the Formulation

Initially extraction of the drug from the formulation was assessed using the size exclusion KS-803 sugar column (300 x 8 mm) (Phenomenex, USA). The formulation was diluted with water in the ratio 1:5 (formulation:water). This dilution was sufficient to ensure the formulation was no longer viscous as a result of addition of water. 20 µL of sample was injected into the HPLC (Waters, USA) via a Hamilton (Hamilton, USA) syringe using a mobile phase of water at a flow rate of 1 mL min^{-1}. The column was heated to a temperature of 30°C. The Waters 2410 RI (refractive index) detector (Waters, USA) and Waters 996 PDA detector were both used to detect the drug.

Intra-sample variation (for 4 samples) and variation in retention time for differing concentrations of propranolol (0.5, 1, 5, 10, 15 and 20 mg mL^{-1}) were also assessed using the above described procedure with the KS-803 column.

Due to the issues involving the use of the KS-803 column (see Section 4.6a *iv* in Chapter 4 for a discussion of these) drug extraction from the formulation was attempted using the Nova-Pak C18 column (8 x 100 mm).

Extraction of the drug from the final product was conducted by diluting the final product in a ratio of 4:1 (methanol:final product). However, diluting the final product with methanol resulted in the HPMC precipitating out. Consequently the vessel containing the methanol diluted final product was centrifuged (Heraeus, Sepatech, Germany) at 4000 RPM for 5 minutes to collect the HPMC. 20 µL of the supernatant was then injected into the HPLC.

The efficiency of the drug extraction process from the final product was determined by addition of methanol to a formulation with a known concentration of drug (5 mg mL^{-1}). 20 µL of the supernatant (containing the dissolved drug) was passed through the HPLC. The peak area obtained was used to determine the concentration of propranolol in the supernatant using the calibration curve. The concentration interpolated from the calibration curve was compared to the known concentration to ascertain the efficiency of the extraction process. This analysis was conducted in triplicate. The experiment was also repeated for a formulation containing only 1 mg mL^{-1} propranolol.

v. Accelerated Stability Study of the Drug in the ISGM System

Samples of the final product were stored in conditions of 40°C / 75% relative humidity for a period of 4 weeks. The temperature of the heated room was maintained by a thermostat. The temperature and humidity was monitored using a digital thermometer (Tecpel, Taiwan). Each week a sample was removed, methanol was added to the sample (as described in Section 3.6a i*v*) and then 20 µL of the supernatant was introduced to the HPLC to measure the peak areas. The concentration of drug remaining in the solution prepared after this treatment was determined by interpolation from the calibration curve.

This experiment was conducted in triplicate on each occasion.

3.6b Appearance of the Final Product

The appearance of the formulation subjected to accelerated conditions (40°C / 75% relative humidity) was assessed over a period of 4 weeks. The experiment was conducted in triplicate. Each week the appearance of the formulation was visually assessed and the observations recorded. The presence/absence of the following features that may have appeared in the final product were assessed by careful observation:

- Air bubbles
- Colour
- Caking
- Particulate Matter

The air bubbles and colour of the final product were assessed visually. Each vial containing the formulation under observation was also set up such that a paper ruler was attached on the side (as described in the screening methodology (Section 3.2a)). If any caking events were to occur in the final product, the height of the "caked material" could then be measured using the paper ruler. The observation of particulate matter may be not be feasible due to the difficulty of differentiating the suspended gelling agent from any particulate matter which may form.

CHAPTER FOUR

Results and Discussion

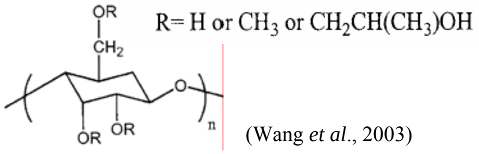
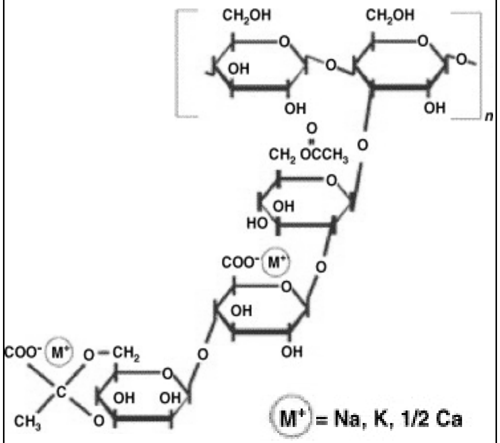
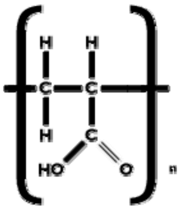
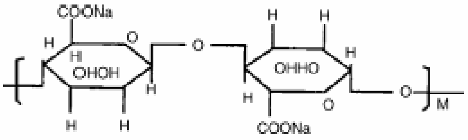
The overarching goal of this research was to convert the conceptual technology described in Bunt *et al.*'s (2003) patent into a viable formulation. In order to achieve this a systematic and thorough investigation had to be carried out to identify the key components which led to a workable system and a system of potential commercial value. Chapter 3 has described the experimental details of this investigation which involved a cursory biocompatibility study for identifying possible formulation components (i.e., hydrophilic polymer and non-aqueous solvents) that caused the least or no irritation to tissues and also a screening/sedimentation study for determining how to formulate the ISGM system into a chemically and physically stable product. Chapter 3 then described how the chosen drug, propranolol, was incorporated into the formulated ISGM system to give the "final formulation" consisting of drug plus ISGM system and then how the "final formulation" was characterised and assessed in terms of its performance as a drug delivery vehicle by conducting *in vitro* drug release experiments using propranolol.

4.1 Structure, Properties and Biocompatibility of the Hydrophilic Polymers and Non-Aqueous Solvents Used in the Study

4.1a Structure and Properties

The structure and properties of the hydrophilic polymers and non-aqueous solvents used in this study are summarised in Tables 4.1 and 4.2.

Table 4.1 - Hydrophilic Polymer Properties

Polymer	**Structure**	**Properties**		
		State (20ºC)	Bulk Density (g mL^{-1}) (20ºC)	Colour
HPMC	 (Wang *et al.*, 2003)	Powder	0.313	White
Xanthan Gum	 (Rosalam and England; 2006)	Powder	0.620	Brown
PEO 303, N12 and N10	$HO-[CH_2-CH_2-O]_n-H$ (Berg *et al.*, 2006)	Powder	0.469 (303) 0.451 (N10) 0.496 (N12)	White
Polycarbophil	 (Noveon, 2005)	Powder	0.174	White
Sodium Alginate	 (Peng *et al.*, 2006)	Powder	0.382	Orange-Brown

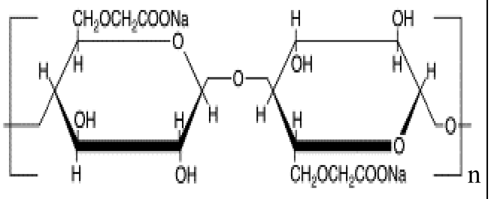
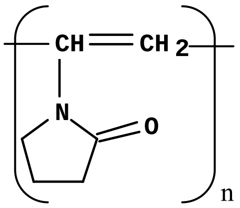
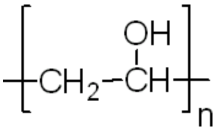
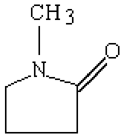
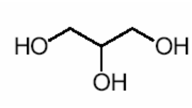
Polymer	Structure	Properties		
		State (20ºC)	Bulk Density (g mL^{-1}) (20ºC)	Colour
Sodium CMC	 (Guo *et al.*, 1998)	Powder	0.430	White
PVP	 (Nierle, 2006)	Powder	0.241	White
PVA	 $-[CH_2-CH(OH)]_n-$ (Porter, 1998)	Powder	0.484	White

Table 4.2 – Non-Aqueous Solvent Properties

Non-Aqueous Solvent Name	Structure	Properties					Pharmaceutical Uses
		Density (25ºC)	Molec. Wt.	State (20ºC)	Colour	Boiling Point	
Propylene Glycol	$CH_3\text{-}CHOH\text{-}CH_2OH$ (Sengwa, 2003)	1.04	76.09	Liquid	Colourless	188°C	Used as a humectant Solvent in many oral, injectable and topical formulations (Smolinske *et al.*, 1987)
PEG 400	$HO-[CH_2-CH_2-O]_n-H$ (Nandi *et al.*, 2003)	1.12	400	Liquid	Colourless	250°C	Ointment Bases Suppository Bases Capsules Tablet Binders (Hermansky *et al.*, 1995)

Non-Aqueous Solvent Name	Structure	Properties					Pharmaceutical Uses
		Density (25°C)	Molec. Wt.	State (20°C)	Colour	Boiling Point	
Ethanol	CH_3-CH_2-OH (Pilling *et al.*, 2007)	0.789	46.1	Liquid	Colourless	79°C	Manufacturing of pharmaceutical products Mouthwash Cough and cold medicines
NMP	 (Carnerup *et al.*, 2005)	1.026	99.1	Liquid	Colourless	202°C	Solvent in pharmaceutical preparations (Koizumi *et al.*, 2004)
Glycerin	 (Pouilloux *et al.*, 1999)	1.258	92.1	Liquid	Colourless	290°C	Excipient and formulation aid

According to the patent awarded to Bunt *et al.* (2003), any or all of these materials could be used to produce the ISGM system. However, formulation of the ISGM system requires consideration of the biocompatibility of the ingredients with the tissues with which they will come into contact since the introduction of a xenobiotic to a body may elicit an immunological response. Consequently, the materials and their degradation products (if any) used in attempts to formulate a stable product needed to be proven safe (Fournier *et al.*, 2003). Therefore biocompatibility studies were conducted to determine the nature and extent of the immunological response to the materials listed in Tables 4.1 and 4.2.

4.1b Biocompatibility

Biocompatibility has been described as “the ability of a material to perform with an appropriate host response in a specific application” (Williams, 1987). There are many types of biocompatibility tests that can be conducted to determine the nature and extent of any immunological response such as the Draize eye test (Lambert *et al.*, 1993; Balls *et al.*, 1995; Cormier *et al.*, 1996), the low volume eye test (Cormier *et al.*, 1996; Lambert *et al.*, 1993; Calvin, 1992; Bruner *et al.*,

1992) and the HET-CAM test (Luepke and Kemper, 1986; Dannhardt *et al*., 1996; Balls *et al*., 1995; Dahl, 1999; Schlage *et al*., 1999).

The HET-CAM test (hen's egg test – chorioallantoic membrane) is often used as a *quasi-in vitro* test to assess the irritation potential of the substance to be tested. The CAM or chorioallantoic membrane is a highly vascularised membrane which surrounds the developing chick embryo. The HET-CAM test is described as a cross between *in vitro* and *in vivo* testing (Luepke and Kemper, 1986) and has the advantage that the CAM has the ability to simulate the pharmacokinetic profile of the substance tested which is not the case with *in vitro* assay techniques (Dannhardt *et al*., 1996). The HET-CAM test is seen as a more humane alternative to the Draize eye test for assessment of irritation, where the effects on the blood vessels of the CAM are comparable to the irritation caused in the eye by the same test substance (Balls *et al*., 1995). It has even been suggested that the HET-CAM test is more sensitive than the Draize eye test as the CAM (HET-CAM test) is thinner than the mucous membrane of the eye (Draize eye test). In addition, the HET-CAM is less expensive as well as the advantage of its complete lack of involvement of the use of live laboratory animals (Dahl, 1999).

After application of the test substance to the CAM, the specifically categorised irritant reactions such as haemorrhage, coagulation and lysis must be observed for. Haemorrhage refers to bleeding of the CAM vessels and this can either be punctuate (i.e. manifesting multiple bleeding points) or diffuse (i.e. bleeding generally over the entire CAM). A lysis event refers to damage in endothelial vessel structure leading to disappearance or "whitening" (due to blood loss) of these vessels in the CAM. A coagulation event indicates blood clotting which can be either intra-vascular or extra-vascular. In the case of intra-vascular coagulation, the blood flow in the vessels will decrease and eventually stop as a result of thrombosis, resulting in "tree" branches and increased vessel diameter. For extra-vascular coagulation, in contrast, precipitation of CAM proteins is the cause as it leads to increased opacity with the membrane becoming completely white so that the CAM takes on a mesh-like porous appearance. A hyperaemia event is characterised by the swelling of small blood vessels resulting in an increased blood flow.

The biocompatibility studies performed in this research were conducted on each of the possible formulation candidates listed in Tables 3.1 and 3.2 using the HET-CAM test. The following presents the results of this study together with a discussion.

The ideal approach for direct comparison of all materials tested is obviously to conduct all HET-CAM experiments at the same time. In this study, however, the volume of the incubation oven limited the realisation of this ideal as the attempted incubation of as many as 60 eggs in this small oven cavity resulted in a low 25% success rate of embryo development due to overcrowding-induced contamination. This thus necessitated the execution of multiple experiments or 'tests' to assess each of the polymers and solvents used in the studies. Each experimental run hence involved incubating only 20 eggs at a time. Because these experiments had to be carried out on numerous occasions, it was necessary to employ positive and negative controls (refer to Section 3.1b for details of the controls) in each experimental run. The use of 20 eggs in each experimental run resulted in a success rate of embryo development of approximately 70%. The chance of cross contamination was successfully minimised by using an approach of spraying and washing all holders and sleeves with 70% isopropyl alcohol between runs.

Figures 4.1a - 4.1d illustrate the observations given by the HET-CAM test in the case of application of the non-aqueous solvent NMP. When applied to the CAM over a period of 5 minutes, the blood vessels in the CAM go initially from the time zero appearance of being discrete and intact (Figure 4.1a) to the appearance shown in Fig. 4.1b where haemorrhaging and hyperaemia occurs after only a 30 second contact time. Exposure to the NMP for longer times is observed to cause coagulation of the blood (Figure 4.1c) which progressively gets worse with continued exposure (Figure 4.1d).

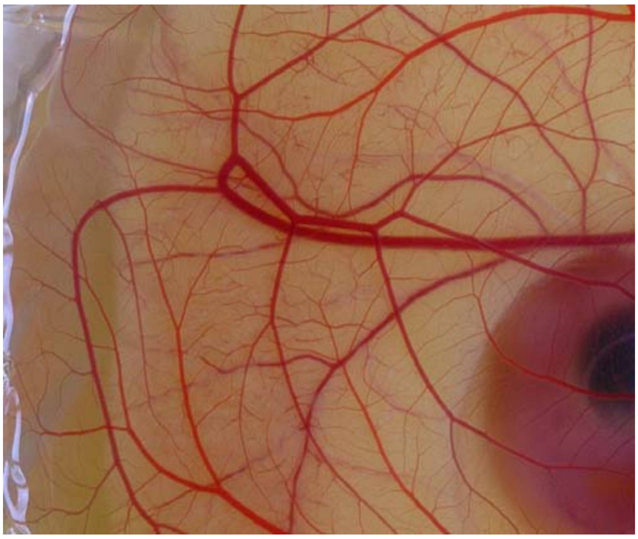


Figure 4.1a – CAM Appearance at Time Zero
Before Addition of NMP

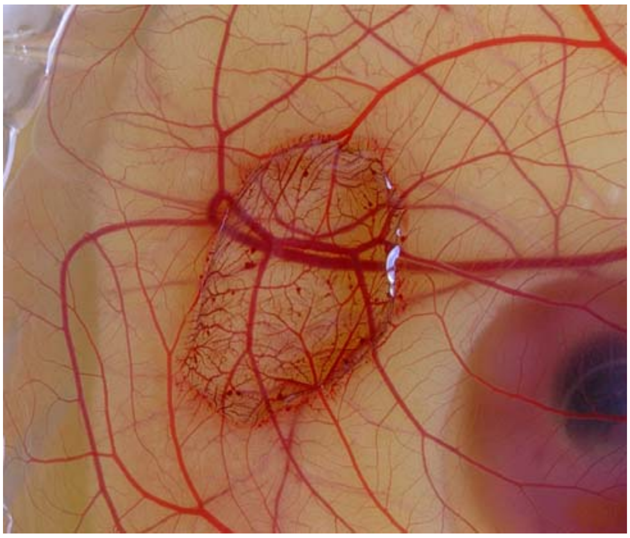


Figure 4.1b – CAM Appearance at 30 Seconds
After Addition of NMP

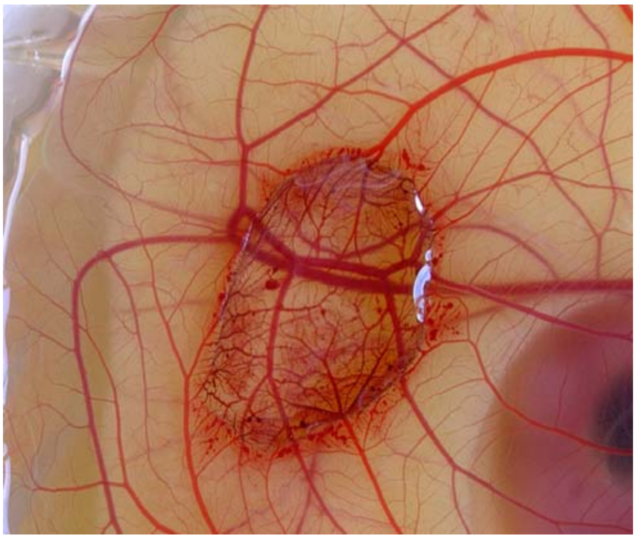


Figure 4.1c – CAM Appearance at 2 Minutes After Addition of NMP

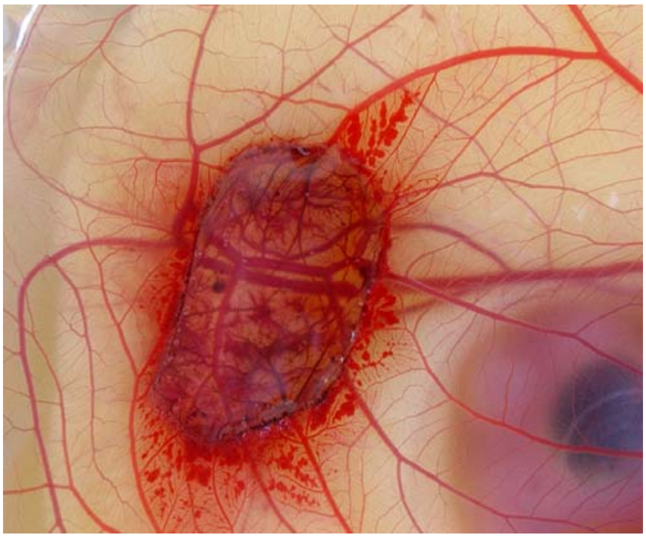


Figure 4.1d – CAM Appearance at 5 Minutes after Addition of NMP

These reactions were observed to occur to differing degrees with the different materials tested in this HET-CAM study. The irritancy and severity of reaction potential for each material assessed is shown in Table 4.3 (see Section 3.1b in Chapter 3 for criteria on how scores were assigned).

Table 4.3 - Irritancy Potential of Each Test Material in the HET-CAM Tests for Non-Aqueous Solvents and Polymers Under Consideration

Test Material		**Average Score**	**Irritancy Potential**
Non-Aqueous Solvent	NMP	18.5	Strong
	Ethanol	12	Strong
	PEG 400	5	Moderate
	Glycerin	5	Moderate
	Propylene glycol	3.5	Slight
Polymer*	PEO 303	0	Practically None
	PEO N10	0	Practically None
	PEO N12	0	Practically None
	Polycarbophil	0	Practically None
	PVP	0	Practically None
	Sodium Alginate	0	Practically None
	Sodium CMC	0	Practically None
	HPMC	0	Practically None
	PVA	0	Practically None
	Xanthan Gum	0	Practically None

*Results were the same regardless of which application technique was adopted.

From Table 4.3 it can be seen that of all the non-aqueous solvents tested, ethanol and NMP gave strong reactions according to the scoring system reported by Schlage *et al.* (1999). In contrast, glycerin and PEG 400 produced moderate effects. Propylene glycol produced a very weak effect. None of the solid polymers used elicited any immunological response of note (Table 4.3).

Biocompatibility studies from previous work on similar materials were located for comparison although it should be noted that the literature-sourced biocompatibility studies of the tested materials did not use the HET-CAM test. Al-Shamkhani *et al*. (1991) evaluated the biocompatibility of xanthan gum and found that it showed good haemocompatibility. Xanthan gum was also tested for toxicity in the stomachs of rats and mice where it was found that toxic effects did not occur despite the administration of an extremely high dose which caused an actual obstruction of the gastrointestinal tract in the animals tested (Diao *et al*., 1990). Smyth *et al*. (1970) found larger doses (oral dosage form) of high molecular weight PEO (approximately 4 000 000) had no immediate detectable effect. However 90 days after administration minor kidney and liver effects in the rat were noted. (A discussion on the clearance of a high molecular weight polymer is given in Section 4.3d). A study on alginates revealed that the compounds did not accumulate in any major organs. However, some evidence of *in vivo* degradation has been reported (Al-Shamkhani *et al*., 1991). Sodium alginate is widely used in pharmaceutical formulations (Kubo *et al*., 2003). Zhang *et al*. (2004) reported PVA has low toxicity and no irritation. Rodwell *et al*. (2003) studied the effect of PVA on fertility, embryonic and growth development in rats. No effect was found in the female reproductive performance, pup survival, growth and organ weight. Warne and Schifko (1999) conducted a study on the toxicity of laundry detergents and their components on a freshwater cladaoceran which included sodium CMC. Sodium CMC was found to be among one of the most toxic components. However it contributed little to the overall toxicity of the detergent due to its low concentration in the powder. The biocompatibility of HPMC has been assessed in the anterior chamber of the rabbit's eye. Ehrich *et al*. (1990) found no toxic effects and that the HPMC was well tolerated. The Food and Drug Administration (FDA) allows the use of HPMC as a direct and indirect food additive (for specific uses) (Organic Materials Review Institute for the USDA National Organic Program, 2002). HPMC is generally recognised as safe (GRAS), through scientific procedures, as an ingredient for use in food in general (including meat products) (Food and Drug Administration, 2006). Polycarbophil is listed by the FDA as safe and effective as an over-the-counter bulk laxative and antidiarrheal (Food and Drug Administration, 1990). Danhof (1982) reported polycarbophil use in laxatives as being non-toxic and did not interfere with digestion or absorption and did not cause gastrointestinal irritation (at daily doses of 4-5 g in adults). Hornof and

Bernkop-Schnurch (2002) used polycarbophil-cysteine conjugates to enhance the corneal permeability for ocular delivery systems. The extent of corneal damage was assessed using a corneal hydration level test and it was found corneal tissues were not damaged. Higa *et al.*, (1999) conducted a biocompatibility study for PVP wound dressings. Their study showed no cell toxicity.

The results of the HET-CAM study carried out in this investigation found the polymers did not elicit an immunological response. The majority of the polymers in the literature are described as being relatively biocompatible. Based on the result of the HET-CAM study (which in most cases was in agreement with the literature), none of the hydrophilic polymers investigated were eliminated.

In contrast to the hydrophilic polymers which did not cause irritation in the HET-CAM test, the non-aqueous solvents produced an immunological response ranging from slight to severe. The literature-sourced findings gave various results. The effect of PEG 400 on rats was monitored (Hermansky *et al.* 1995) over a 13 week period. A modest decrease in food consumption and body weight in rats which were administered mid (2.5 mL kg^{-1} day^{-1}) and high level doses of PEG 400 (5 mL kg^{-1} day^{-1}), was noted. This was attributed to the presence of relatively large amounts of PEG 400 in the intestinal tract (which would have had an increased "satiety" effect in the rats) and not a result of direct toxicity effect from the PEG 400 (Hermansky *et al.* 1995). In a study by Montaguti *et al.* (1994), the acute intravenous toxicity of certain solvents including PEG 400 on mice was investigated. The mice were given doses of PEG 400 ranging from 2 – 8 mL kg^{-1}. While the lowest dose of 2 mL kg^{-1} was found to be safe, the highest dose of 8 mL kg^{-1} resulted in approximately half the mice dying. Montaguti's study also reported that PEG 400 resulted in a body weight decrease in one of the mice strains. However, despite the observation of these apparently adverse effects, they still conclude by recommending the use of PEG 400 as a solvent. As a general rule, polyethylene glycols such as PEG 400 are considered inert and of low toxicity (Chadwick *et al.*, 1977; Klugmann *et al.*, 1984; Swinyard and Pathak, 1980). This claim has been verified in several species including humans (Chadwick *et al.*, 1977; Principe, 1968, Shaffer *et al.*, 1950; Smyth *et al.*, 1955). Cutaneous injection of PEG 400 for 13 weeks at 2.5 mg kg^{-1} was well tolerated.

Increasing the residence time from 13 weeks to 104 weeks was still well tolerated (Gad *et al*., 2006). Subcutaneous injection of PEG 400 (up to 10 mL – 10 times the human dose) caused no permanent damage. The reactions observed were blanching of the skin and scab formation in 48 hours. PEG 400 did not give a foreign body reaction in animals. The removal of PEG from the injection site (in dogs) was rapid as the PEG diffused freely into the surrounding tissue (Spiegel and Noseworthy, 1963). PEG 400 is generally regarded as being one of the safest non-aqueous solvents (Gad *et al*., 2006). The HET-CAM investigation carried out in this study has shown PEG 400 to have a moderate effect where the average cumulated score was found to be 5 (Table 4.3). PEG 400 was therefore investigated further as a possible non-aqueous solvent for the ISGM system.

Subcutaneous injection of ethanol caused pain followed by anaesthesia (Spiegel and Noseworthy, 1963). Injections in or close to the nerves are made to cause anaesthesia to treat severe pain. If injections of ethanol occurred near or in the nerves, then neuritis and nerve degeneration may occur. Cutaneous administration of ethanol into the mouse for 13 weeks was well tolerated for a 70% solution (100 μL/animal/day) (Gad *et al*., 2006). Wali and Hayter (1987) investigated the pharmacological and toxic side effects of ethanol in rats. They found that low concentrations of ethanol had no toxic side effects but high concentrations of ethanol resulted in toxic side effects in all the tissues studied especially the liver and the lung. This was further supported by Joshi *et al*. (1998) who reported the use of high concentrations of ethanol in sustained release injectables was a drawback due to local intolerance. This was in agreement with the findings of the HET-CAM test where ethanol had a cumulative score of 12, indicating a severe immunological response. The toxicity of ethanol will therefore depend on its concentration. As the ISGM technology being developed in this study is a non-aqueous system, water diluted forms of ethanol cannot be used. Though the HET-CAM test found ethanol to have a severe effect, ethanol was still investigated as a possible non-aqueous solvent as the extent of reaction in the HET-CAM test was lower than that of NMP (12 compared to 18.5 for NMP). If ethanol is considered as one of the final possible non-aqueous solvents to use in the ISGM system, its biocompatibility results will be considered before choosing the ideal non-aqueous solvent.

Guchok (1986) found administration of glycerin during an early postnatal period was not very toxic to rats. Subcutaneous injection of glycerin into the rat and mouse at a dose of 10 mg kg^{-1} was well tolerated (Gad *et al.*, 2006). Glycerin has low toxicity in oral formulations as no deleterious effects were observed in humans dosed with 110 g of glycerin per day for 50 days. There is, however, some controversy regarding the use of glycerin in parenteral formulations as administration of glycerin to animals resulted in hemoglobinurea, hypotension, central nervous disturbances and weight loss (Spiegel and Noseworthy, 1963). Sloviter found no such toxic effects of glycerin administered intravenously to both humans and mice and attributed the previously observed toxicities to the osmotic disturbances caused by injecting high concentrations of glycerin (Sloviter *et al.*, 1957; Sloviter, 1958). Parenteral administration of large doses of glycerin can affect the central nervous system resulting in effects such as paralytic symptoms. The blood corpuscles were found to be laked which is thought to be due to the osmotic effect. Glycerin was found to remain at the injection site unabsorbed and in high concentrations and it was thought the corpuscles were laked during their passage through this area (Spiegel and Noseworthy, 1963). In this investigation the cumulative score for glycerin was 5 (Table 4.3) which represents a moderate irritation potential. Though the literature indicates some issues in the use of glycerin as a non-aqueous solvent, glycerin was still investigated as a possible non-aqueous solvent as the HET-CAM test resulted in only a moderate irritation potential.

The European regulatory authorities (The European Agency for the Evaluation of Medical Products, EMEA) have critically judged the use of NMP in parenteral pharmaceutical technologies. NMP is regarded as a Class 2 solvent (Matschke *et al.*, 2002). Class 2 solvents have significant toxicity such as neurotoxicity, non-genotoxic carcinogenicity or birth defects (International Conference on Harmonisation, 2000). Despite this NMP still seems to be used in several delivery systems. Injectable implants containing only NMP as a solvent were tested in Rhesus monkeys (Royals *et al.*, 1999). Results showed only a mild local tissue response and inflammatory effects such as swelling, redness or irritation were not observed. However NMP is not permitted for use in dogs and cats due to observed pain reaction during application followed by local inflammatory effects (Matschke *et al.*, 2002). Such results question the safety of NMP as a solvent for parenteral products. The findings of the literature correlated with the findings of this investigation where NMP was found to cause a severe immunological response. The cumulative score of NMP in the HET-CAM test was calculated to be 18.5 (Table 4.3) out of a maximum 21 thus indicating the severity of the reaction. Consequently NMP was removed as a possible solvent choice for the ISGM system.

Propylene glycol is the most common organic solvent used in commercially available formulations (Gad *et al.*, 2006). Propylene glycol has been considered as safe to use in commercial formulations of foods, drugs and cosmetics (Center for the Evaluation of Risks to Human Reproduction, 2004). Propylene glycol is FDA approved in the use of food, tobacco and pharmaceutical products as an inert ingredient (Food and Drug Administration, 1982). In the pharmaceutical industry propylene glycol is used as a glycogenic in ruminants, co-solvents for drugs, stabiliser for vitamins and ointments for medical applications (Center for the Evaluation of Risks to Human Reproduction, 2004).

Propylene glycol is GRAS in the direct addition to foods. Such substances are also permitted as packaging materials provided the substances are used in amounts not to exceed that required to accomplish their intended physical or technical effects (Food and Drug Administration, 1982). Propylene glycol is also used as a humectant in pet food products. However it is not used in cat food. Propylene glycol causes a red blood cell disorder in cats called Heinz disorder (Dzanis, 2001). Consequently propylene glycol was removed from cat food products by the FDA in 1996 (Food and Drug Administration, 1996). Subcutaneous administration of propylene glycol into rats for 4 weeks was well tolerated (dosage of 2.5 mL/kg). Cutaneous administration of propylene glycol into a minipig for 26 weeks at the same dose was also well tolerated (Gad *et al.*, 2006). The intramuscular minimum fatal dose of propylene glycol for rats and rabbits were 14.7 and 9.7 g kg^{-1} respectively. Mice are more sensitive to propylene glycol and consequently have a lower tolerance. The HET-CAM investigation found propylene glycol to have a slight irritation potential with a cumulative score of only 3.5 (Table 4.3). According to the HET-CAM test, propylene glycol was the most biocompatible of the non-aqueous solvents and therefore would be the ideal solvent to use for the ISGM system based on these results.

This discussion includes aspects of systemic toxicology of formulation components. The object of this review was to determine what effect and whether there are any implications of using the materials considered for the ISGM system. Consequently one can gain an understanding of the issues, if any, involved with the material to be used. Correlation of the literature findings and those in this research using the HET-CAM methods suggest that all the materials thus far investigated in the study (with the exception of NMP) could be used in the final formulation as they should be safe for injection into animals. A review of the literature showed there was some controversy over the use of NMP. The findings from this study showed NMP caused a severe immunological response and was consequently removed as a possible non-aqueous solvent choice for the technology. Furthermore as mentioned earlier in this section, Matschke *et al.* (2002) reported the use of NMP is not allowable for cats or dogs due to observed pain reaction during application followed by local inflammatory effects. Consequently the delivery of propranolol to dogs using the ISGM technology developed in this study would not be able to use NMP as a non-aqueous solvent.

As a result of the HET-CAM study, NMP was not investigated any further as a possible non-aqueous solvent due to the severe reaction observed in the HET-CAM test. Ethanol also produced a strong immunological response in the HET-CAM test and there is some debate in the literature on the use of ethanol. As mentioned earlier in this section Joshi *et al.* (1998) reported the use of high concentrations of ethanol in sustained release injectables was a drawback due to local intolerance. In contrast, cutaneous administration of ethanol into the mouse for 13 weeks was well tolerated (100 µL/animal/day) (Gad *et al.*, 2006), but, this was for a concentration of 70% ethanol. As the technology being developed in this study is a non-aqueous system, water diluted forms of ethanol cannot be used. Ethanol was still investigated as a possible non-aqueous solvent as the extent of reaction in the HET-CAM test was lower than that of NMP (12 compared to 18.5 for NMP). Therefore, if ethanol is considered as one of the final possible non-aqueous solvents to use in the technology, its biocompatibility results will be considered before choosing the ideal non-aqueous solvent.

The next stage of this investigation involved analysing the sedimentation behaviour of the hydrophilic polymers (PVP, PVA, HPMC, PEO 303, PEO N12, PEO N10, xanthan gum, polycarbophil, sodium CMC and sodium alginate) in the non-aqueous solvents (ethanol, glycerin, PEG 400 and propylene glycol).

4.2 Formulation and Manufacture of the ISGM System

4.2a Sedimentation Behaviour of Hydrophilic Polymers in Non-Aqueous Solvents

The polymers, PVP and PVA dissolved completely in all the test solvents investigated in this study (see Section 3.2a in Chapter 3) giving clear solutions. In terms of our working definition for our ISGM system, this is undesirable as the hydrophilic gelling agent needs to exist in a *suspended* state in the non-aqueous solvent as described in Section 2.6.

When polycarbophil was added to all the test solvents, it exhibited a very different behaviour in that much of the powder that was added to the non-aqueous solvent clumped in the medium and the polymer appeared to become partially gel forming.

The remaining hydrophilic polymers (HPMC, PEO 303, PEO N12, PEO N10, xanthan gum, sodium CMC, and sodium alginate), which were initially homogeneously dispersed as discrete particles throughout the pure non-aqueous solvents, were observed to sediment to the bottom of the vials very rapidly (within 60 minutes they had completely settled). In one solvent (ethanol), the polymers sedimented rapidly within a few minutes. Upon standing, the sedimented particles were observed to compact or "cake" into a compact mass that was not easily redispersed by shaking. This phenomenon has been commonly observed (Burgess, 2005; Mysels, 1959; Shaw, 1970; Cartensen, 1995).

All suspensions are susceptible to some kind of physical instability such as sedimentation of the particulate matter over time by gravity so leading to a non-uniformly distributed system (Burgess, 2005; Yoshida *et al.*, 2004). Not only sedimentation but also "creaming" processes can occur where the dispersed particulate matter can rise to the top. Creaming occurs when the density of the particles is less than the density of the solvent while sedimentation occurs when the density of the particles is higher than the density of the solvent (Burgess, 2005).

Flocculation events occur when aggregates or more appropriately “flocs” build up from particles which come together due to weak attractions between them. Redispersion of the flocs should result in no changes to the particle size or size distribution as one is simply breaking up loosely held masses of particles (i.e. no discrete growth of a particle diameter has occurred). Therefore in flocculated suspensions, the particles are loosely held together by electrostatic forces and the suspension will exist as a loosely packed network of particles (Burgess, 2005; Ansel and Popovich, 1990). As a result, caking of the sediment is not expected to occur so that the system should be easy to redisperse by relatively non-aggressive methods.

The velocity, v, of sedimentation for a uniform collection of spherical particles can be predicted by Stokes Law (Burgess, 2005; Ansel and Popovich, 1990):

$$v = \frac{\mathrm{d}^2(\rho_1 - \rho_2)\mathrm{g}}{18\eta_0} \tag{4.1}$$

where v = the sedimentation velocity
d = the diameter of the suspended particles
ρ_1 = the density of the suspended particles
ρ_2 = the density of the medium
g = the acceleration of gravity
η_o = the viscosity of the external phase

When a suspension settles rapidly v, the sedimentation velocity, is relatively large. As shown in the equation above (Equation 4.1), v is directly proportional to $\Delta\rho$ (the difference in density between the solid and liquid i.e. ρ_1 - ρ_2) and inversely proportional to the viscosity of the suspending liquid. Therefore to decrease v, $\Delta\rho$ needs to be reduced or the viscosity of the suspending liquid has to be increased. To reduce the difference in density between the solid and liquid (i.e. reduce $\Delta\rho$) the density of the liquid is manipulated by addition of solvents such as sucrose, sorbitol, and glycerin to make the liquid medium more dense. Alternatively the viscosity can be increased (as viscosity is inversely proportional to v as mentioned above) by addition of a viscosity inducing agent. This is the basic underlying principle for improving the dispersion properties of the ISGM system in this study.

At this stage of the screening study, the decision was made not to consider the following polymers for any further development work (in their role as the dispersed gelling polymer) of the ISGM system:

1. PVA and PVP as these polymers clearly dissolved in each of the solvents, and the criteria for the ISGM system under study demand that they be *suspended as particles*.

2. Polycarbophil since this gelled when incorporated into each of the non-aqueous solvents. (Though polycarbophil was not investigated further as a dispersed gelling agent, the polymer was considered as a viscosity inducing agent in Section 4.2c).

4.2b Effect of Glycerin on Sedimentation Rate of Hydrophilic Polymers

Glycerin was one candidate used to manipulate the viscosity and density of the continuous phase. Density as shown in the Stokes Law (Equation 4.1) is indirectly related to the viscosity. Glycerin alters the density of the continuous phase by reducing the difference in density between the solid and liquid (i.e. reduce $\Delta\rho$) making the liquid medium more dense (and more viscous). This has the effect of reducing the sedimentation velocity of the suspended particulate matter.

Figures 4.2 – 4.4 show the sedimentation profiles for the polymer sodium CMC in the different non-aqueous solvents investigated in the study (PEG 400, propylene glycol and ethanol) each containing varying amounts of glycerin in the continuous phase. The graphs for all the remaining polymers showed similar trends and are documented in the appendix (A1 – A18).

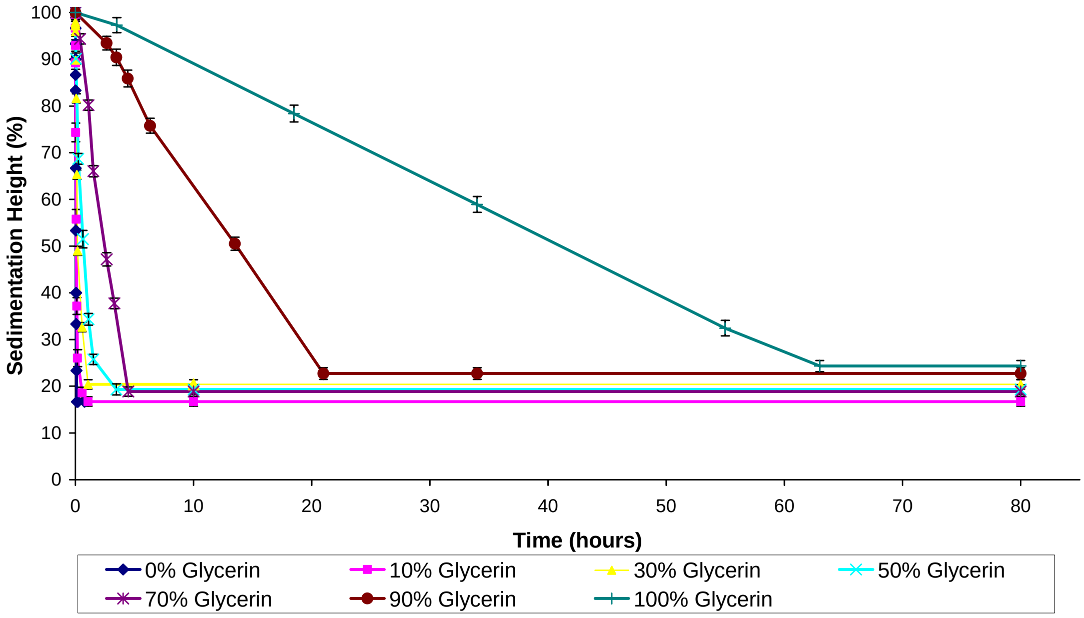


Figure 4.2 - Comparison of Sedimentation Profiles for Sodium CMC at each Glycerin Concentration in Alcohol (n=2)

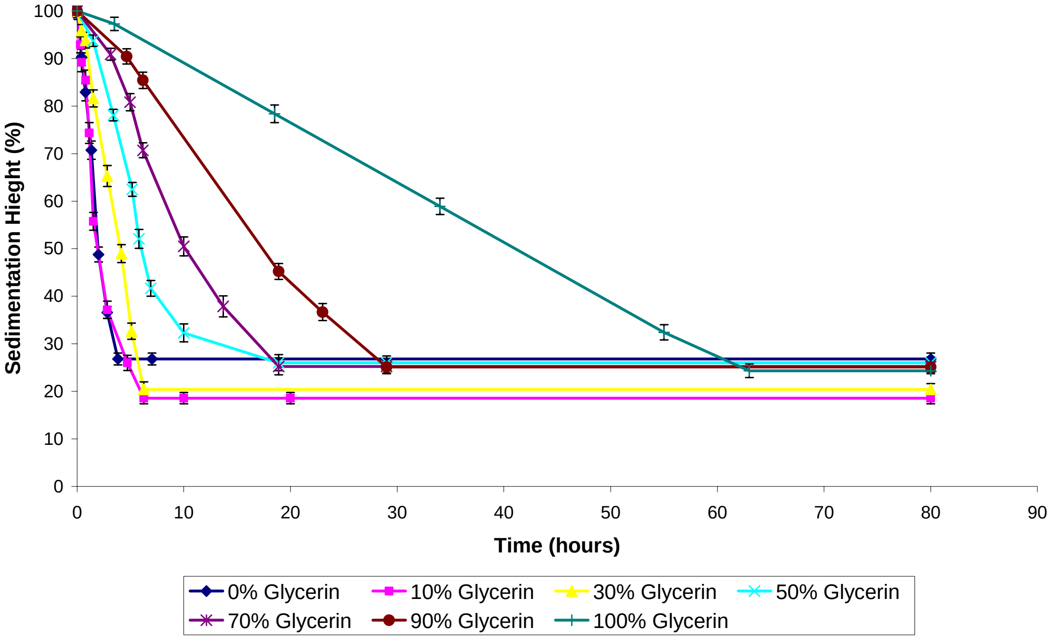


Figure 4.3 – Comparison of Sedimentation Profiles for Sodium CMC at each Glycerin Concentration in PEG 400 (n=2)

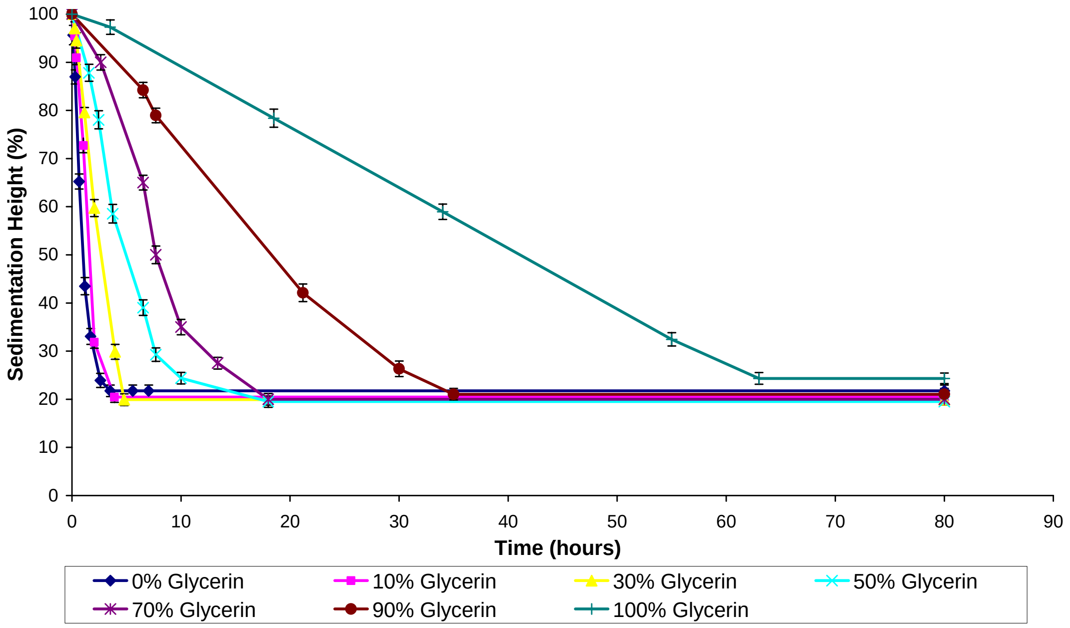


Figure 4.4 – Comparison of Sedimentation Profiles for Sodium CMC at each Glycerin Concentration in Propylene Glycol (n=2)

The experimental errors for these procedures were calculated as the relative standard deviation in the percentage sedimentation height of the replicates (where each experiment was carried out in duplicate).

Figures 4.2 – 4.4 show that for sodium CMC (10% w/w) in each of the non-aqueous phases that were investigated (PEG 400, propylene glycol and alcohol), as the glycerin concentration in the medium increased, the rate of sedimentation of the polymer decreased. In all cases the pure non-aqueous solvent containing sodium CMC exhibited the fastest rate of sedimentation and 100% glycerin resulted in the slowest rate of sedimentation of the polymer particles. The effect is plainly ascribable therefore to the addition of glycerin to the non-aqueous solvent which increases the solution density and viscosity and hence decreases the sedimentation rate.

Figures 4.2 – 4.4 show that the sodium CMC reached a final sedimentation height of 20 - 30%. This was observed for all the other polymers except HPMC which exhibited a much higher sedimentation height of approximately 65% in all solvent systems (Refer to A1 – A3 in the Appendix). HPMC had the smallest bulk density of the investigated hydrophilic polymers (Table 4.1). The smaller the bulk density, the greater the inter-spatial voids between the particles (as explained in Section 4.2h) Therefore the greater sediment height observed for the HPMC system can be attributed to the larger inter-spatial voids between the particles.

PEO N12, PEO N10 and PEO 303 exhibited similar sedimentation behaviour (Appendix A4 – A12). Therefore there was no need to continue investigating all three PEOs. PEO N10 and PEO N12 were therefore eliminated from the screening process. In addition sodium alginate was also removed from the screening process. Sodium alginate was available as a powder that had a wide ranging particle size (from large particles to very fine ones). All the polymers were sieved to give a particle size of less than 100 μm. However, despite this, there was still a range of particle sizes (an analysis of the polymer particle sizes was conducted on the polymers at a later stage of the analysis as discussed in Section 4.2h). Consequently, when the polymer was dispersed throughout the non-aqueous solvent the larger particles sedimented quickly. According to Stoke's Law (Equation 4.1), the larger the particle diameter the faster the velocity of sedimentation. Consequently, the very fine particles, which will have a smaller diameter, will have a slower sedimentation velocity and therefore remain suspended longer producing a very diffuse layer. The diffuse layer caused difficulties in determining the sediment height. This was compounded by the orange-brown colour of the polymer. Consequently sodium alginate was removed as a possible gelling agent.

The above study has shown that the use of glycerin to stabilise a suspension (i.e., retard sedimentation of the polymer particles) was only partially successful and required the use of a high concentration of glycerin to achieve the desired effect. A complication noted in the systems investigated was that although the polymer remained dispersed for longer in the solvent when high concentrations of glycerin were incorporated into the non-aqueous solvent, most of the continuous phases (i.e. non-aqueous solvent plus glycerin) were observed to gel over time. This was postulated to be due to the absorption of atmospheric moisture by virtue of the hygroscopic nature of the glycerin which was sufficient to initiate the gelation of the continuous phase. This postulation was supported by the observation that gelling was only observed in the systems containing high concentrations of glycerin (50 - 100% (w/w) glycerin). The occurrence of this gelling phenomenon was obviously not desirable from a practical point of view as the ISGM system involves being injected *as a relatively lower viscosity liquid containing dispersed particles* and the occurrence of gelling may result in a preparation that is too thick to be injected using conventional administration techniques such as hypodermic syringes.

An interesting observation made during this part of the study was that, as mentioned earlier in this section, the sedimentation height of HPMC was greater than the other polymers. This suggested that HPMC may be a more favourable polymer to use compared to the other hydrophilic polymers as the greater sedimentation height should lead to a decreased chance of caking (as it is perceived to be more greatly dispersed). Ideally a sedimented suspension should be able to be redispersed with minimum effort as caking (due to the close proximity of the sedimented particles) of the particles may bring about permanent coalescence. This would be favourable from a storage point and a “shelf life” point of view as often a common situation is that pharmaceuticals when manufactured are not necessarily used immediately. These sedimentation observations with respect to the HPMC (A1-A3 in the Appendix) thus suggested that formulations containing this polymer would be easier to redisperse than the others and this was the general observation made in this work.

The next step in the formulation development involved addition of a solid polymer as a viscosity inducing agent to modify the viscosity of the vehicle in order to retard the sedimentation rate of the polymer particles.

At this stage of the study it was decided the following polymers would not be studied further as a result of the observations made during these studies:

1. PEO N12 and PEO N10 were eliminated as screening three different types of PEO was deemed unnecessary because the sedimentation profiles of each of the three different molecular weight PEO polymers were similar.

2. Sodium alginate produced a very diffuse layer which caused difficulties in determining the sediment height. This was compounded by the orange-brown colour of the polymer.

4.2c Determination of the Potential Use of Polycarbophil as a Viscosity Inducing Agent in Non-Aqueous Solvents

In contrast to the formulation of suspensions in which the continuous phase is aqueous based (Ansel and Popovich, 1990; Cuna *et al*., 2000) the formulation for suspensions in which the continuous phase comprises a *non-aqueous solvent* is much less documented in the literature. Studies on the formulation of aqueous suspensions and the delay of sedimentation studies by added excipients are numerous in the literature. However, although polycarbophil was eliminated in Section 4.2a as a potentially useful hydrophilic polymer for inclusion in the ISGM system (since it partially gelled in each of the solvents tested), the fortuitous observation that it underwent this reaction to form a colloidal gel in each of the non-aqueous solvents tested was recognised as being a potentially valuable attribute to the system under development. Polycarbophil was therefore investigated from the point of view of its use as a viscosity-inducing agent in the non-aqueous solvents being considered in this study.

A preliminary (and qualitative) evaluation of polycarbophil as a viscosity inducing agent was thus carried out and involved manufacture by simple addition and manual shaking of various concentrations of polycarbophil (0.50, 1.00 and 1.25% w/w) in the non-aqueous solvent, propylene glycol. These preparations were visually examined for their ability to flow (a more quantitative viscosity analysis on the effect of polycarbophil concentration on the continuous phase viscosity is discussed in Section 4.2f). As expected, the flow properties of these dispersions did change as a function of polycarbophil concentration which confirmed the viscosity inducing role of the polycarbophil. Hence this component could be used in this role to retard the sedimentation of suspended particles. One problem that was observed during manufacturing was that not all the polycarbophil was dissolving in the non-aqueous solvent. Incomplete dissolution of the polycarbophil from sample to sample can pose problems in terms of varying concentrations. It was important that the concentration of this component in the non-aqueous solvent be accurately known for quantitative evaluation of its viscosity inducing effect. Complete dissolution would also be vital in any mass manufacture of this as it is essential to make a material with a consistent composition.

Therefore numerous manufacturing methods were investigated in order to achieve the complete dissolution of the polycarbophil in the ISGM system so that its property of being a viscosity inducing agent could be exploited for physically stabilising the polymer dispersion (either HPMC, PEO 303, sodium CMC or xanthan gum).

4.2d Manufacturing Methods Investigated for Incorporating the Polycarbophil Viscosity Inducing Agent into the Non-Aqueous Solvent

i. Magnetic Stirrer

The first manufacturing process investigated involved stirring the solvent using a simple magnetic “flea”. This method proved to be ineffectual as the polycarbophil did not mix into the non-aqueous solvent and clumps of polycarbophil were observed on the surface of the non-aqueous solvent and remained there despite the long mixing times (see Section 3.2d *i*). It was concluded that a faster, more vigorous stirring method was required as the polycarbophil was not being adequately mixed into the non-aqueous solvent.

ii. Overhead Stirrer

The overhead stirrer (three blade overhead stirrer at 2000 RPM) was considered a faster stirring method than the magnetic stirrer. It was observed that upon addition to the non-aqueous solvent, the polycarbophil was drawn into the vortex created by the agitation of the overhead stirrer paddle. This observation contrasted to the magnetic stirrer where the polycarbophil was not drawn into a vortex. Despite this improvement in the mixing method, the polycarbophil though no longer sitting in clumps on the solvent surface, did manifest itself as white clumps of polymer within the solvent.

iii. Overhead Stirrer Plus Heating

Since it is commonly known that increasing temperature aids dispersion of most materials, raising the temperature of the mixing solution was investigated in combination with the overhead stirrer. The suspension was thus heated to a temperature of 80°C whilst stirring, but observations of the resultant mixture revealed that this led to little improvement as clumps of polymer were still observed in the final mixture.

iv. Overhead Stirrer Followed by Placing on a Roller at 40°C

Noveon (2002) claimed that polymers such as polyacrylic acid (for example polycarbophil) take a long time to disperse throughout a non-aqueous solvent. The results of this study support this view, that complete dispersion of polycarbophil in propylene glycol would require a lengthy mixing process if a simple mixing apparatus is used. It was obvious that mixing beyond that subjected by the overhead stirrer needed to be carried out. Therefore further investigations involved placing a suspension obtained from previous mixing (using an overhead stirrer) onto a roller in a hot room maintained at 40°C (by a thermostat as described in Section 3.2d *iv*). The roller was used to continuously stir the suspension in an attempt to increase dispersion of and hence help dissolve the polycarbophil in the propylene glycol. Initial results had suggested that this helped but only to the extent that fewer and smaller sized clumps of unsolvated polycarbophil were observed in the propylene glycol.

Noveon (2002) reported clumps of particles may form and the surface of these clumps solvate to form a layer, leaving a dry interior. In the methods used to date in this study (magnetic stirrer, overhead stirrer, overhead stirrer plus heating and overhead stirrer followed by placing on a roller at 40°C) the formation of the polycarbophil clumps may be due to the agglomeration of particles that were only wetted on the outside without adequate penetration of solvent to solvate the presumably dry interior of the clumps. The mixing time required to remove these globules will thus be dictated by the slow diffusion of the solvent through the solvated layer to the dry interior. If we were to rely on this mechanism of solvation whilst using the present “gentle” methods of stirring, the time to achieve this penetration would be impractically long. Noveon (2002) suggested that outright prevention of the clumping process will reduce the mixing time required to effect complete solution of the polymer in the solvent of choice, hence the mixing methodology was altered with this aim in mind.

v. Mortar and Pestle

This mixing method involved the use of a mortar and pestle to give a more intimate contact between the solvent and the solid particles (see Section 3.2d *v*). Propylene glycol and polycarbophil were ground for 2 hours continuously (except where the solvent/polymer mixture was left to stand intermittently for 5 minutes to allow the gel to thicken). The results from this suggested that the clumping of particles was overcome, but then a new phenomenon was noted. The phenomenon had been reported previously (Charles Ross and Son Company, 2005; Noveon, 1993) and is referred to as "fish-eyes". This is where the formed dispersion looks to the naked eye as being homogeneous and complete, however when the containing vessel is tipped onto its side, and the thickness of the liquid layer becomes thin on the glass, semi-transparent globules of undissolved polymer (hence the "fish eyes" appearance) can be seen (Figures 4.5a and 4.5b).

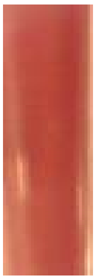


Figure 4.5a – Vial Containing the Prepared Continuous Phase (Against a Red Background)

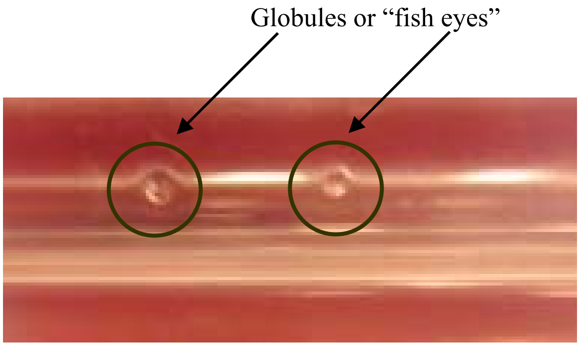


Figure 4.5b – Vial Tilted to its Side Revealing the Existence of Globules or "Fish Eyes" (Against a Red Background)

The fish eyes can also be detected using UV-Vis spectrophotometry as these will cause scattering of light in the same way as a turbid solution would. Alternatively a viscosity analysis of the continuous phase can be conducted. If fish eyes were present, the viscosity of the continuous phase will be affected to some extent. However, visual observation (as used in this study) is still an effective means of confirming the presence of fish eyes, as the undissolved polymer on the side of the tilted glass tube is very easy to see with the naked eye.

vi. Mortar and Pestle followed by Roller in 40°C Room

In a similar vein to the overhead stirrer/roller method, the polycarbophil and propylene glycol were ground together and the resulting polymer solvent mixture was placed on a roller in a 40°C hot room (see Section 3.2d *vi* in Chapter 3). This procedure merely served to decrease the size of the polycarbophil globules but not make them disappear from the medium. In addition, during the preparation of the gels by the mortar and pestle method, a change of weight was noted as shown in Table 4.4. The change in weight was attributed to the absorption of water by the solvent during the manufacturing process which was conducted in open air. Both the solvents and the polycarbophil are inherently hygroscopic substances and given the manufacturing process was conducted over a lengthy period of time, water absorption from the atmosphere occurred (consequently an investigation into the extent of water absorption by the ISGM technology was undertaken as described in Section 4.3a). It was concluded from this part of the study that a faster method of making the gels was required that minimised water absorption from the air.

Table 4.4 – Weight Increase During Manufacture of Continuous Phase Due to Water Absorption

Sample	Solvent (g)	PCP (g)	Total Wt. (g)	Actual Wt. (g)	Wt. Increase (g)
1	50.000	0.025	50.025	51.600	1.575
2	50.051	0.025	50.076	51.595	1.519

As was noted earlier, Noveon (2002) suggested that preventing clumping will reduce the mixing time required and recommended the use of a high speed mixing tool. The mortar and pestle, though providing a better degree of admixing of the non-aqueous solvent and the polycarbophil, caused the emergence of the "fish eye/globule" phenomenon. It was important to eliminate these globules, therefore, the next method investigated used an even more aggressive mixing approach (i.e. Silverson® homogeniser) which involved stronger (and faster) mechanical dispersion with cutting of the polycarbophil particles. It was also envisaged that this would destroy the globules and by virtue of the faster mixing regimen decrease mixing time and prevent/minimise water absorption from the air.

vii. Silverson® Homogeniser

The Silverson® homogeniser operates by rotating the rotor blades (within the workhead of the homogeniser) at high speeds causing a powerful suction action which draws the dispersion into the centre of the workhead. Inside the workhead the solvent/polymer dispersion is furthermore subjected to a milling action followed by an intense hydraulic shear as the materials are forced out through the slots of the head. Once expelled, the materials are projected radially towards the sides of the mixing vessel and fresh material is drawn into the workhead to maintain the mixing cycle.

Initial work on the Silverson® homogeniser (high speed dispersion tool) involved determining which head to use for the dispersing process. The general purpose disintegrating head is used in preparation of gels and suspensions. The square hole high shear screen head can be used to prepare emulsions and fine colloidal suspensions (see Figures 3.4, 3.5a and 3.5b)

Table 4.5 - Comparison of Different Heads Utilised in Manufacturing Process

Speed (RPM)	**Head**	
	General Purpose Disintegrating Head	**Square Hole High Shear Screen**
2 000	Large clumps of polycarbophil	Globules present
5 000	Clumps remain	Fewer globules than above
8 000	Fewer clumps but globules were present	Even fewer globules than above

As shown in Table 4.5 the square hole high shear screen was more effective at preventing globule formation than the general purpose disintegrating head (where the presence for globules was assessed visually). The general purpose disintegrating head had fewer and less sharp perforations, therefore it was harder to break down the polycarbophil. The square hole high shear screen, which has the ability to rapidly reduce the size of granular solids, did not lead to clump formation as the many sharp perforations broke down the polycarbophil granules. It was also apparent that the higher speeds utilised were more effective than the other mixing methods investigated thus far (Section 4.2d *i* – 4.2d *vi*). Consequently subsequent work was conducted using the square hole high shear screen head.

As indicated in the following table (Table 4.6) mixing for 2 hours decreased the amount of globules present. Longer time periods were not investigated as such time periods caused an increase in weight due to absorption of water (as with the mortar and pestle mixing method).

Table 4.6 - Observations from an Extended Study at Various Stirring Speeds Using the Square Hole High Shear Screen on the Silverson® Homogensier

Time (mins)	**Stirring Speed (RPM)**		
	2 000	5 000	8 000
5	Globules present	Globules present	Globules present
10	Same as above	Same as above	Same as above
15	Same as above	Globules present but smaller in size	Globules present but smaller in size
20	Same as above	Same as above	Same as above
25	Smaller number of globules present.	Smaller number of globules present.	Smaller number of globules present.
60	Less globules present.	Less globules present.	Mixing not conducted due to a high increase in temperature.
120	Less globules present.	Very little globules present.	Mixing not conducted due to a high increase in temperature.

The introduction of the polymer to the solvent was the vital stage in the mixing process as it is desirable to disperse the polycarbophil rapidly before the semi-solvated and difficult-to-disperse clumps or globules can form. Unfortunately as shown in Table 4.6 above, the polymer was still not satisfactorily dissolved in the non-aqueous solvent even at the much higher mixing speed as globules still remained despite the use of this more mechanically aggressive stirring regimen. The next step was thus to advance to even more aggressive stirring methods which required the use of another dispersing tool, i.e. the *Ultra Turrax*®.

viii. Ultra Turrax®

The Ultra Turrax® operates similarly to the Silverson® but is capable of much faster speeds than the Silverson® (see Section 3.2d *vii* and 3.2d *viii*). Unlike the Silverson® homogeniser, an ice bath was not required as the temperature increase during the mixing operation was not significant. Preliminary investigations revealed that the method of introduction of the polymer to the solvent was critical as the polymer needed to be sprinkled into the small vortex which appeared during mixing. This ensured the polymer was instantly drawn into the dispersion tool and hence dispersed rapidly.

The use of the Ultra Turrax® thus gave a manufacturing process that resulted in fast and effective dispersion of the polycarbophil into the propylene glycol and produced a continuous phase with no polymer floating on the surface and most importantly no globules (or "fish-eyes") present. This was an important development in the steps to obtaining a workable ISGM system.

4.2e Characterisation of Potentially Useful Continuous Phases

Several continuous phases were now able to be manufactured using the Ultra Turrax® manufacturing method and then characterised. During the manufacture of the ethanol-based continuous phases it was found that this solvent was not a suitable non-aqueous solvent as the polycarbophil did not dissolve to form a gel but remained in permanent suspension. Not using ethanol any further also avoided potential biocompatibility issues that were highlighted in the HET-CAM studies as discussed in Section 4.1b. This left only two possible solvents to consider further i.e., propylene glycol and PEG 400.

i. Appearance of the Continuous Phase

Table 4.7 summarises information on the colour, clarity and presence of any globules for each of the continuous phase manufactured in propylene glycol and PEG 400. It was noted that after manufacturing the solvent/polycarbophil system, the PEG 400 system appeared turbid whereas the propylene glycol system was transparent. As a result of this observation a physical sedimentation study was carried out on each solvent/polycarbophil system to assess whether each system was stable over a 4 week period (see Figures 4.6 and 4.7).

Table 4.7 - Appearance of Continuous Phase After Manufacture on the Ultra Turrax®

Continuous Phase	**Observations**		
	Colour	Clarity	Globules
Propylene glycol and 0.5% w/w polycarbophil	Colourless	Transparent	Globules were not observed
PEG 400 and 0.5% w/w polycarbophil	White	Turbid	Globules were not observed

ii. Physical Stability

The propylene glycol/polycarbophil continuous phase was physically stable as no particulate matter was ever observable (Figure 4.6) over the 4 week period. Hence the continuous phase exhibited no sedimentation indicating that the polycarbophil had effectively dissolved in the propylene glycol.

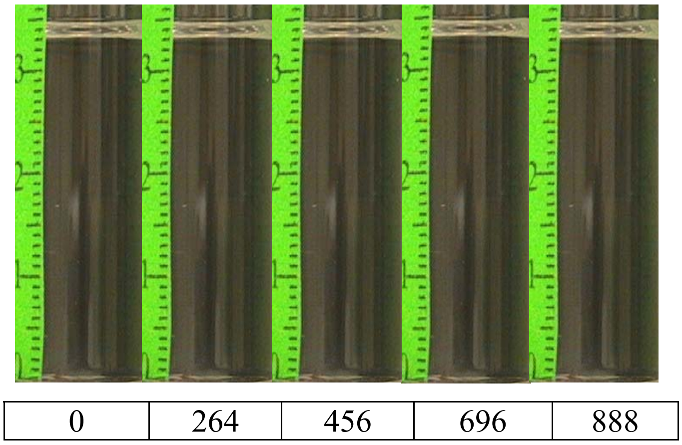
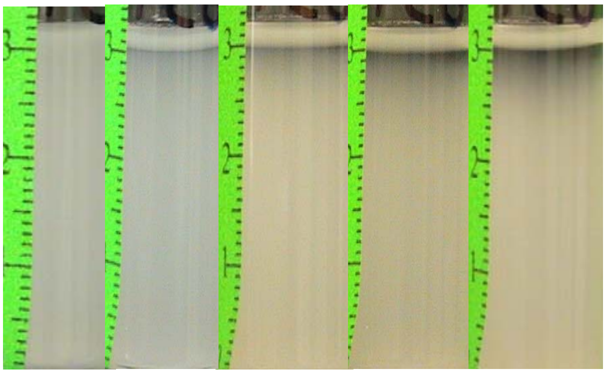


Figure 4.6 – 0.5% Polycarbophil in Propylene Glycol. Time is Shown in Hours.

In contrast the turbid PEG 400 system appeared to sediment over time (Figure 4.7) indicating the polycarbophil had produced a suspension that was not physically stable. This indicated that although the Ultra Turrax® had thoroughly dispersed the polycarbophil in the PEG 400, it had not dissolved in this solvent to produce a transparent continuous phase as had happened in the case of the propylene glycol.



0	264	456	696	888

Figure 4.7 – 0.5% Polycarbophil in PEG 400. Time is Shown in Hours.

To confirm whether or not heating of the PEG 400/polycarbophil system would clear the turbidity by facilitating the dissolution of the polycarbophil, mixing with an Ultra Turrax® followed by mechanical stirring at 70°C (using an overhead stirrer) for 30 minutes (following a procedure reported by Bonacucina *et al.* (2004) as described in Section 3.2e *ii*) was attempted. This did not clear the turbidity to produce a homogeneous transparent system. Consequently PEG 400 was eliminated as a possible solvent choice for the continuous phase.

At this stage of the research, only one non-aqueous solvent had been identified as being potentially useful in the ISGM system (i.e., propylene glycol). This was based on the fact that the hydrophilic polymer that was to be added to make the ISGM system had to be physically stable in the continuous phase. The research has shown (Section 4.2 c) that to achieve this polycarbophil needed to be added as a viscosity inducing agent. Propylene glycol proved to be the only non-aqueous solvent that gave a physically stable system (i.e., propylene glycol and the viscosity inducing agent polycarbophil) as the polycarbophil dissolved in the propylene glycol. The solubility of the polycarbophil in propylene glycol was greater than the 1% (w/w) investigated in this study. Greater concentrations of polycarbophil in propylene glycol were not investigated as concentrations greater than 1% (w/w) produced a solvent system which was very viscous and difficult to inject.

The biocompatibility study (Section 4.1b) showed that neat propylene glycol solvent gave low scores in the HET-CAM test so indicating minor reactions. As mentioned earlier (Section 4.1b) many of the reported toxicity studies involve the non-aqueous solvent in a diluted form and this is not representative of a practical situation when the non-aqueous solvents may be used in an undiluted form. Propylene glycol has been used in formulations in an undiluted form such as the propylene glycol based formulation of lorazepam (Ativan) which is administered intramuscularly (Strickley, 2004). Therefore propylene glycol was chosen as the non-aqueous solvent to use in the ISGM system as the biocompatibility and the physical stability results showed propylene glycol to be the most practical solvent to use.

The next stage of this research will involve a quantitative investigation of the effect of the concentration of the viscosity inducing agent (polycarbophil) on the viscosity of the vehicle (propylene glycol).

4.2f Determination of the Effect of Polycarbophil Concentrations on Viscosity of the Continuous Phase (Polycarbophil Plus Propylene Glycol)

The viscosity of the propylene glycol/polycarbophil continuous phase and the effect of the polycarbophil concentration were determined by using a S10 vibro-viscometer. Details of these measurements are described in Section 3.2f in Chapter 3. The concentrations investigated are given in Table 4.8.

Table 4.8 - Polycarbophil Concentrations (%) Used in the Continuous Phase

Solvent	**Polycarbophil Concentration (%)**			
Propylene Glycol	0.50	0.67	0.84	1.00

Prior to assessing the viscosity of the samples on the viscometer, a standard operating procedure had to be developed (see Appendix). The viscometer was very sensitive to changes in measurement conditions and therefore it was important to ensure readings were determined in the same manner from sample to sample and from day to day.

Temperature, as expected, had a dramatic effect on the viscosity. Therefore viscosity measurements were conducted at a fixed temperature of 25 ± 0.4°C. Within this range viscosity readings on the samples were stable.

The amount of liquid in the sample volume cup (10 mL) had to be kept constant to within small tolerance (± 2%). Any large variation in sample volume (i.e., by 1 mL) resulted in a 5% difference in the readings taken on identical samples.

The most critical parameter in which to obtain reproducible results was the level of the sensor plates. The sensor plates must be placed in the sample at the same position for each reading (this was achieved using a magnifying glass as described in Section 3.2f). If the sensor plate was lowered so the top portion of the halfway point was in line with the solvent level, a variation of 8 - 9% in the viscosity was observed compared to when the sensor plate was lowered to the bottom portion of the halfway point as shown in the following diagram (Figure 4.8).

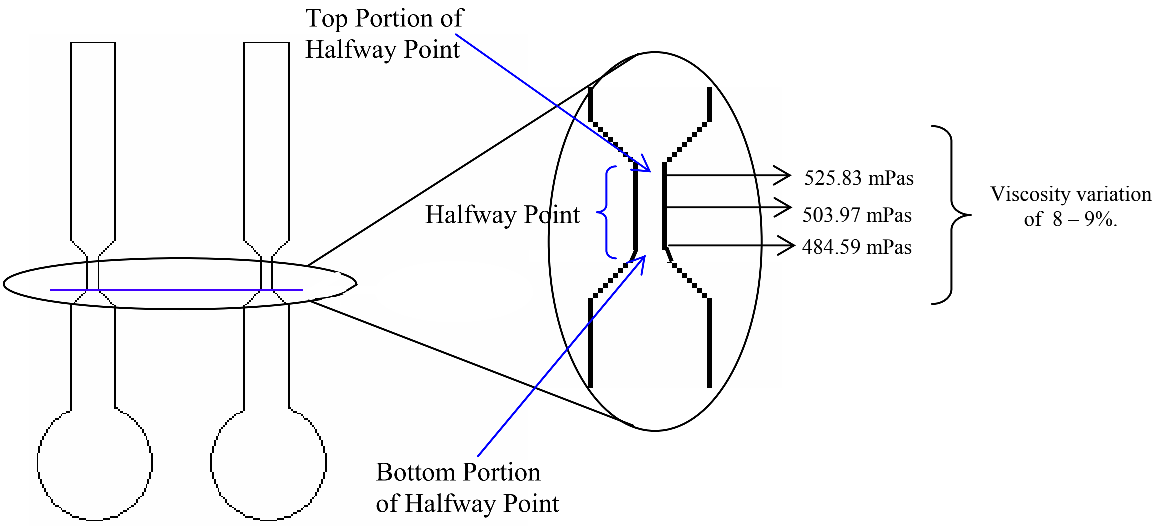


Figure 4.8 – Effect of Sensor Plate Position on Viscosity Reading

Samples of propylene glycol with concentrations of 0.5, 0.67, 0.84 and 1% (w/w) polycarbophil were manufactured using the Ultra Turrax® manufacturing method and their viscosities determined. A straight line relationship with a positive slope was found to exist between the viscosity of the continuous phase and the concentration of polycarbophil in the continuous phase (see Figure 4.9). Each viscosity measurement reported in the plot represents the average of 24 readings and the error was calculated as the standard deviation of these readings. The error is depicted in the following graphs (Figures 4.9 and 4.10).

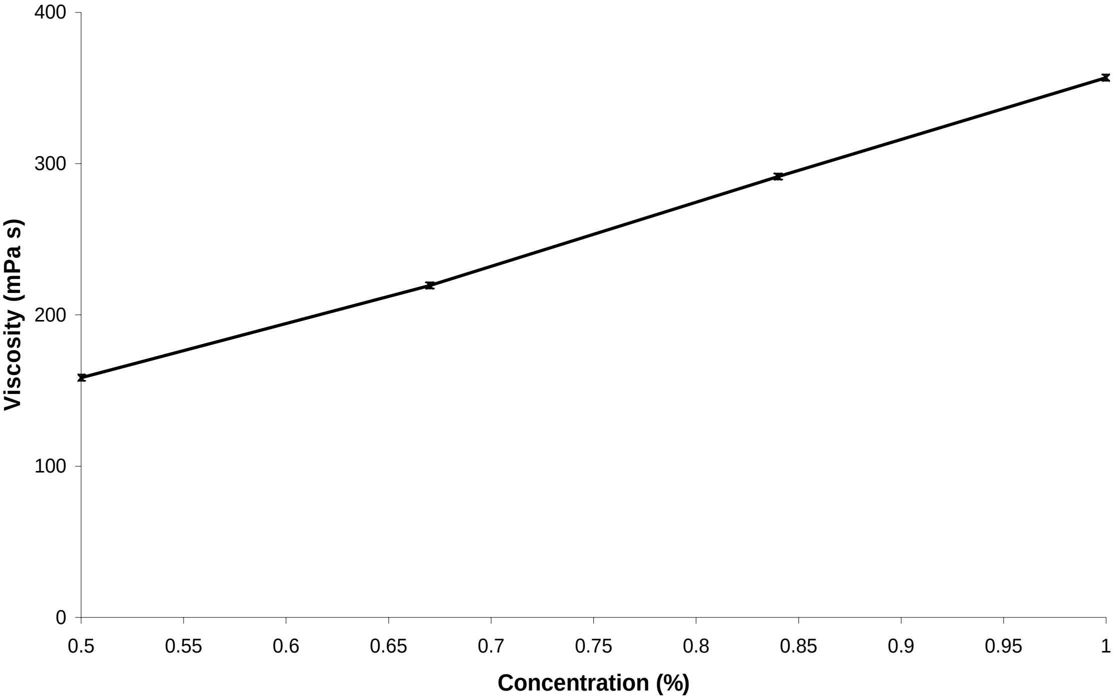


Figure 4.9 – Effect of Polycarbophil Concentration on Viscosity of Propylene Glycol

However, the viscosity of neat propylene glycol did not fall on the straight line of the graph above (Figure 4.9). The viscosity of propylene glycol was much lower than what the graph predicted (Figure 4.10). Propylene glycol is classed as a Newtonian fluid (Shekarriz and Sheen, 1998; Khomami and Su, 2000). The addition of polycarbophil to propylene glycol resulted in the solvent system displaying non-Newtonian behaviour. A Newtonian fluid is a fluid with constant viscosity at a given temperature regardless of the rate of shear. In contrast the viscosity of a non-Newtonian fluid changes with shear. This would explain why the viscosity of propylene glycol did not lie on the trend line of the graph (Figure 4.10) for the solvent systems containing polycarbophil. However this was not regarded as being a serious impediment to the use of polycarbophil in propylene glycol.

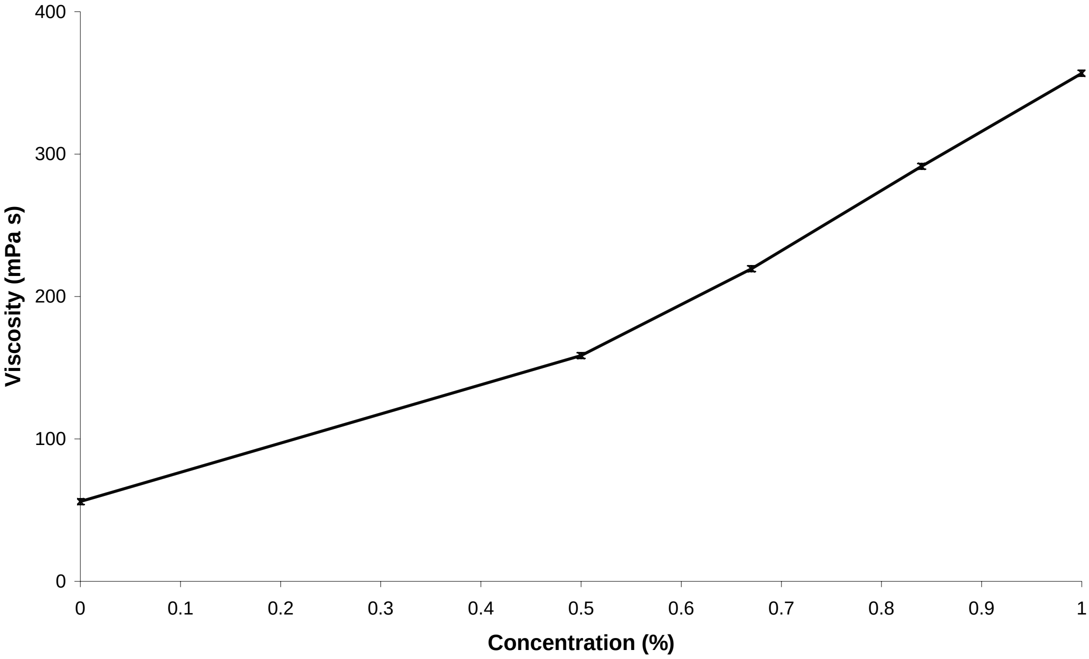


Figure 4.10 – Comparison of Viscosity of Pure Propylene Glycol to the Viscosity of the Continuous Phase (i.e. Comparison of Figure 4.9 to the Viscosity of Pure Propylene Glycol)

This study confirmed the function of the polycarbophil as a viscosity inducing agent when it is dissolved in the propylene glycol. Hence this continuous phase system can be used in the confidence that it should retard the sedimentation of any added hydrophilic polymer to produce a stable dispersion.

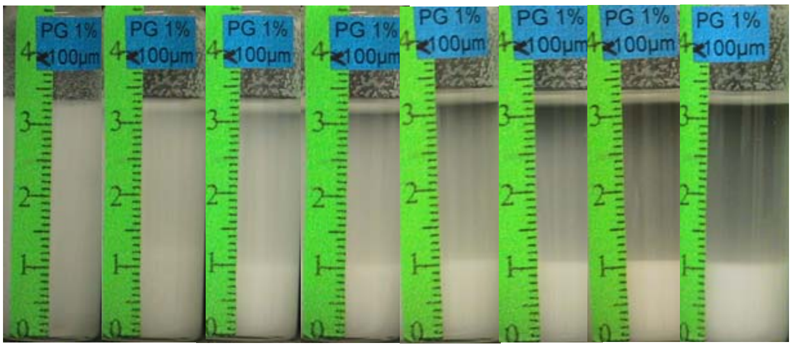
4.2g Incorporation of the Hydrophilic Polymer into the Continuous Phase

The next step in the formulation of the ISGM system was the incorporation of the insoluble and dispersed hydrophilic gelling agent, which, upon addition to tissues should gel to form the controlling matrix from which a drug can slowly be delivered. The manufacturing method described in the Methodology Section (Section 3.2g) was very successful at incorporating and uniformly dispersing the hydrophilic polymer into the continuous phase, as issues such as clumping or caking of the polymer did not occur.

4.2h Sedimentation Behaviour of the Hydrophilic Polymers in the Continuous Phase

Four hydrophilic gelling agents (xanthan gum, HPMC, PEO 303 and sodium CMC) were incorporated into the propylene glycol continuous phase containing different concentrations of polycarbophil viscosity inducing agent and left to stand in order to observe the sedimentation behaviour of the hydrophilic polymers. The aim of the study was to identify the optimum concentration of polycarbophil that prevented sedimentation of the dispersed, insoluble hydrophilic polymers, yet remained at a viscosity low enough to allow parenteral administration through a syringe needle.

Xanthan gum had to be eliminated as a candidate because this hydrophilic polymer appeared to be slowly dissolving over time in the continuous phase so increasing its viscosity. A continuous phase in such a *pre-gelled* state would be too thick to inject hence the xanthan gum polymer was unsuitable. Sodium CMC was also eliminated as a possible hydrophilic gelling agent because the hydrophilic sodium CMC polymer sedimented too rapidly despite the presence of the viscosity inducing agent. The two remaining polymers, HPMC and PEO 303 settled more slowly in contrast and were investigated further (Figures 4.11 and 4.12). (The screening process was conducted on all 4 concentrations of polycarbophil (0.5, 0.67, 0.84 and 1.0% w/w) for both HPMC and PEO 303. The Figures 4.11 and 4.12 are illustrative examples of the 0.67% w/w polycarbophil for HPMC and 0.5% w/w polycarbophil for PEO 303).



0	24	91	135	254	434	622	700

Figure 4.11 – PEO 303 Sedimentation over Time in the Continuous Phase Comprising Propylene Glycol and 1% (w/w) PCP. Time is Shown in Hours.

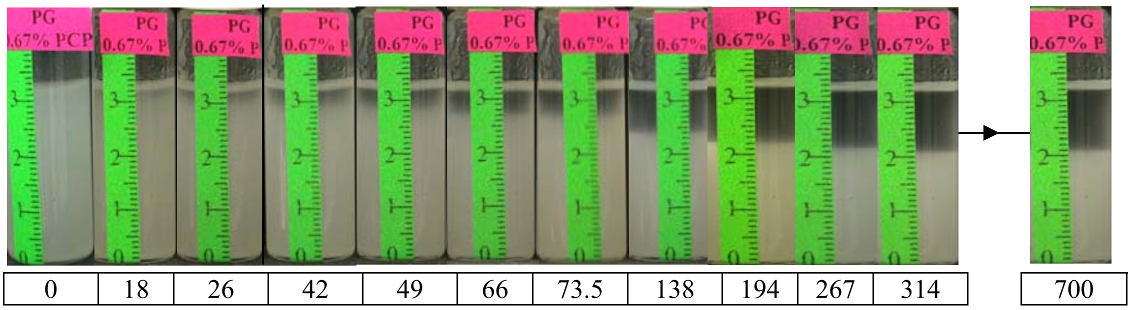


Figure 4.12 – HPMC Sedimentation over Time in the Continuous Phase Comprising of Propylene Glycol and 0.67% (w/w) PCP. Time is Shown in Hours.

The sedimentation analysis was carried out in duplicate. The displayed error bars (Figures 4.13-4.14) represent the relative standard deviation in the percentage sedimentation height of the replicates.

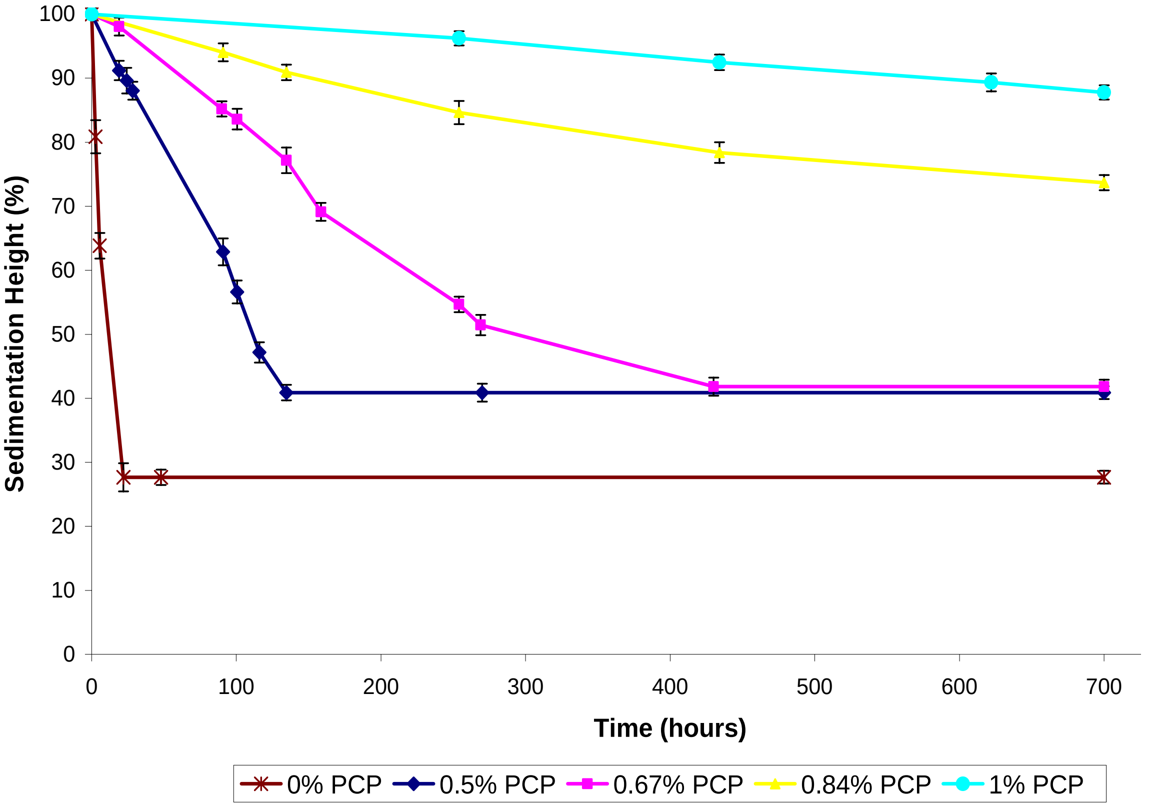


Figure 4.13 – Comparison of PEO 303 Sedimentation in Each Continuous Phase (n=2)

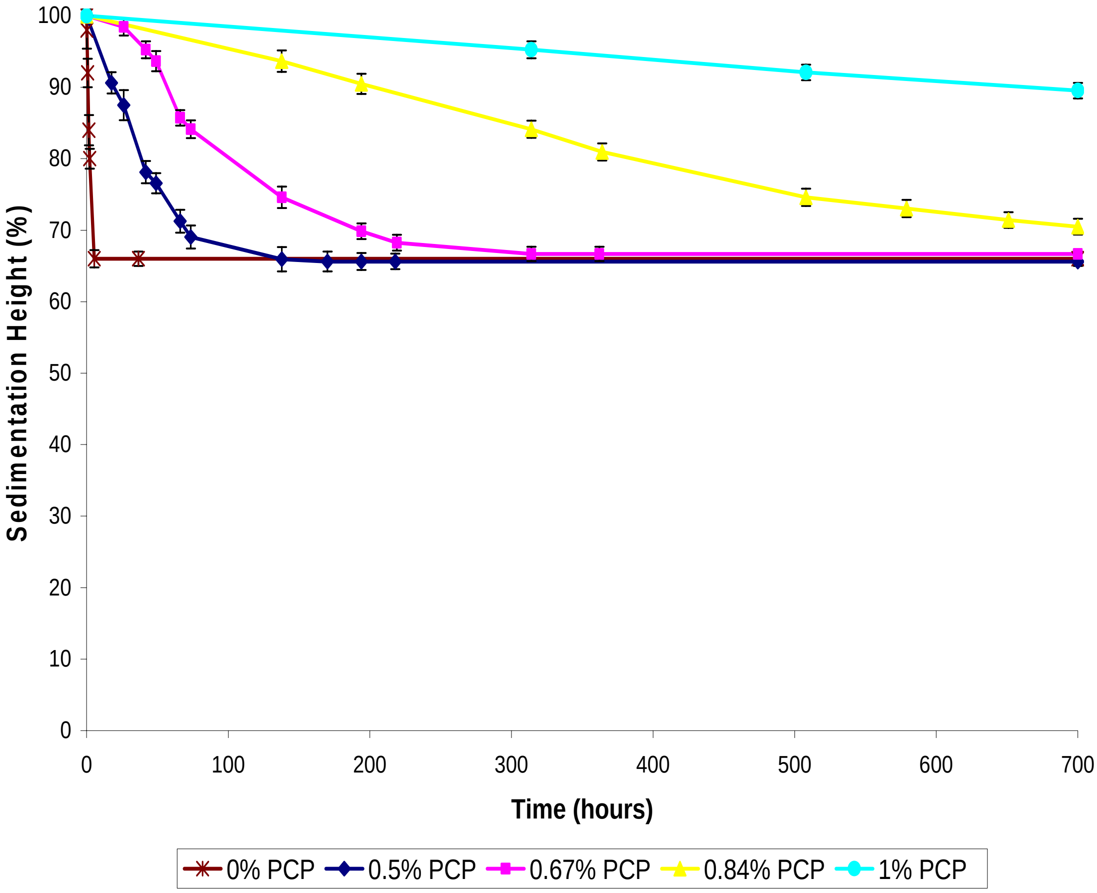


Figure 4.14 – Comparison of HPMC Sedimentation in Each Continuous Phase (n=2)

The addition of polycarbophil successfully retarded the sedimentation of both HPMC and PEO 303 (Figures 4.11 - 4.14). Addition of increasing amounts of polycarbophil decreased the rate of sedimentation of both hydrophilic polymers (Figures 4.13 and 4.14). HPMC proved to be the ideal polymer to use as the final sedimentation height was greater than that of PEO 303's. Unlike HPMC (Figure 4.12), PEO 303 produced a large diffuse region with no definitive line along which the polymer settled (Figure 4.11). A possible reason for the diffuse region can be attributed to the particle size range of the polymer. Despite the fact that the PEO 303 was sieved to give a particle size of less than 100 µm, the larger particles settled quicker than the smaller particles thus resulting in a diffuse region of smaller particles which remained suspended for a prolonged period.

As explained in Section 4.2a, according to Stokes Law (Equation 4.1), the larger the particle diameter, the faster the sedimentation velocity.

A particle size analysis between the two possible gelling agents was carried out using an optical digital microscope (Figures 4.15 and 4.16) (see Section 3.2h for experimental details). The error bars in Figures 4.15 and 4.16 represent the relative standard deviation.

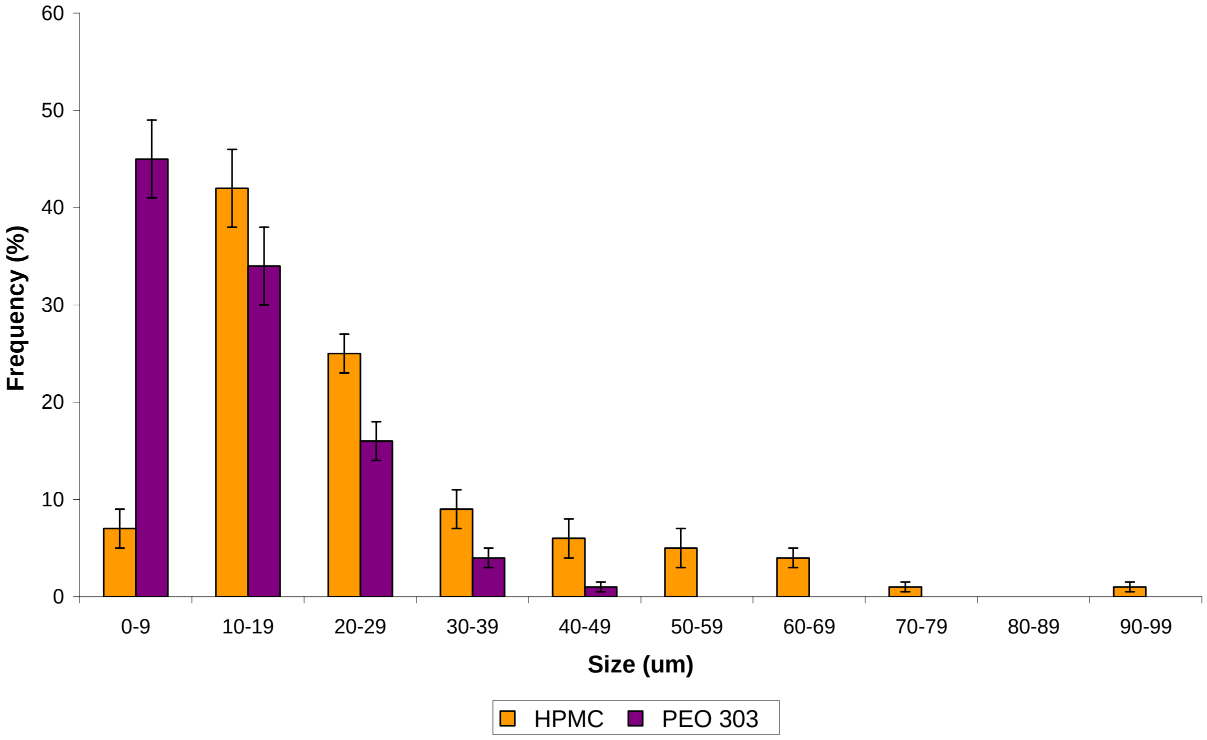


Figure 4.15 – Comparison of HPMC and PEO 303 Particle Size

HPMC has a broader particle size range than PEO 303 (Figure 4.15). This larger range did not cause a diffuse region in the sedimentation analysis (Figure 4.12). The very diffuse region seen in the sedimentation analysis for PEO 303 (Figure 4.11) must be due to the 0 - 9 µm particle size range, as HPMC in contrast has few particle sizes in that size range. This hypothesis was tested by conducting a particle size analysis of the PEO 303 in the disperse phase at the end of the 4 week sedimentation analysis.

According to Figure 4.16 over 95% of the particles in the disperse phase were in the 0 - 9 µm range. Consequently the diffuse region of particles in the disperse phase must be due to the very fine particles. As mentioned previously (Section 4.2a), the very fine particles will be expected to sediment more slowly. The majority of the HPMC particles lie in the range of 10 – 19 µm and as a consequence of the particles being in this size range, no diffuse region was observed in the sedimentation studies.

Furthermore, the bulk densities of the polymers are in agreement with their sedimentation behaviour. HPMC has a bulk density of 0.31 (Vlachou *et al*., 2001) which is lower than the bulk density of PEO 303 of 0.473 (Picker-Freyer, 2006) (the bulk densities were in agreement with the calculated values in Table 4.1). Bulk density incorporates the inter-spatial voids between the particles. Consequently particles which are smaller in diameter will reduce the size of these voids and thus increase the bulk density. As shown in Figure 4.15 the majority of the HPMC particles were larger in size than the PEO particles. Consequently HPMC will have greater inter-spatial voids and hence the smaller bulk density. Therefore the greater sediment height observed for the HPMC system can be attributed to the greater inter-spatial voids between the particles.

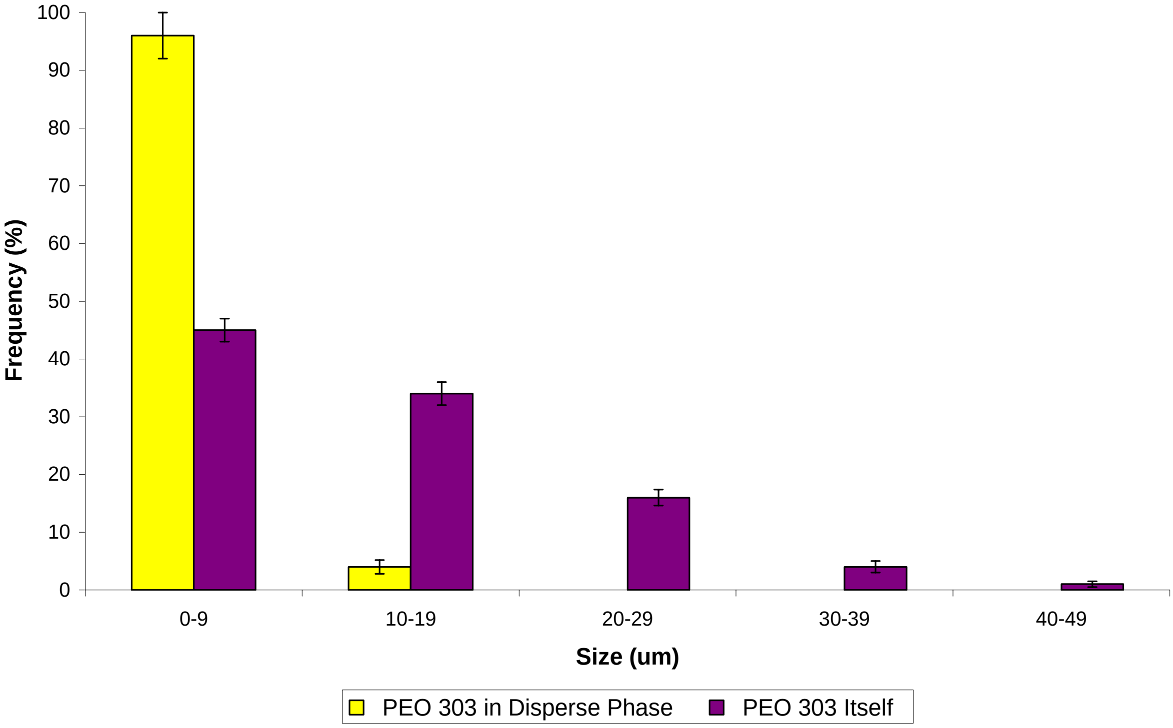


Figure 4.16 – Comparison of Particle Size of PEO 303 Itself and PEO 303 in the Disperse Phase After the 4 Week Sedimentation Analysis

The observations hence made about sedimentation behaviour for the two polymers coupled with the particle size analysis led to HPMC being chosen as the most appropriate hydrophilic gelling agent for further investigation in the remainder of this research.

The inclusion of the hydrophilic gelling agent HPMC to the ISGM system will result in gel formation upon addition of water. The swelling of HPMC is mainly due to the disruption of hydrogen bonds between the polymer chains. Water inserts itself into the hydrogen bonds between the polymer chains. As more water penetrates into the polymer the forces between the chains diminish. Consequently the chains gain rotational freedom and begin to occupy more space. Water continues to fill the voids between the polymer chains and then diffuses into the denser regions of the polymer. Water will continue to force additional chains apart in these denser regions (Fyfe and Blazek, 1997). Once the hydrogen bonds have completely been disrupted between the HPMC chains, a chain can disentangle and diffuse away into the water. Once disentangled, the polymer chain will become completely solvated.

In summary, the ideal excipients to use for the ISGM have been determined by a process of systematic experimentation and elimination after consideration of the observations made. The next step in the formulation process was to determine the ideal polycarbophil concentration to use in the ISGM system. As shown earlier in Figure 4.14, a polycarbophil concentration of either 0.67, 0.84 or 1% by weight in the propylene glycol could have been used as the continuous phase as all adequately retarded the sedimentation of HPMC (the 0.5% polycarbophil system was not as effective at retarding the sedimentation rate). The optimum percentage weight value of polycarbophil to use in the continuous phase was ultimately chosen after determining which of the ISGM preparations containing the 0.67, 0.84 and 1% by weight polycarbophil were able to have the suspended HPMC redisperse after 700 hours settling time. The redispersion test was executed by manually shaking the suspension. The 0.67% polycarbophil system proved the easiest to redisperse. The 0.84 and 1% polycarbophil suspensions required much effort to redisperse and consequently were eliminated as possible formulations. The 0.67% polycarbophil continuous phase was therefore chosen to use in the final formulation as it was the most facile of the three formulations to redisperse manually and exhibited an acceptable retardation of sedimentation rate of the hydrophilic polymer (a more extensive study on the redispersion ability of the ISGM system was also conducted and this is described in Section 4.3d).

In summary, as a result of conducting extensive formulation studies, the ideal excipients to use in the ISGM system were found. These were HPMC as the hydrophilic gelling agent, propylene glycol as the non-aqueous solvent and polycarbophil as the viscosity inducing agent.

4.3 Characterisation and Stability of the ISGM System

4.3a Water Absorption Studies on the ISGM System

Now that a definitive composition for the ISGM system had been established, further characterisation was carried out on the system to investigate stability when it is exposed to normal atmospheric conditions. In previous experiments (Section 4.2d *vi*) it was observed that during the manufacturing process of continuous phases with a mortar and pestle, moisture from the air resulted in an increase in weight. Because of this observation (which has implications in any potential use of this formulation in open air and what protection it might need) the effect of exposure of the ISGM system (i.e. 10% HPMC in the continuous phase comprising polycarbophil (0.67%) and propylene glycol) to the atmosphere was assessed.

The effect of HPMC concentration in the ISGM system on atmospheric water uptake by the ISGM system was determined by carrying out the water absorption experiments at different HPMC concentrations while maintaining the polycarbophil concentration at a constant value. However a parallel set of experiments in which the polycarbophil concentration was varied while holding the HPMC concentration constant was also carried out. The experimental details of the procedures followed are described in Section 3.3a in Chapter 3.

As shown in Figure 4.17 an increase in HPMC concentration in the ISGM system did not lead to an increase in water absorption but in fact led to a decrease. Placing a pure sample of HPMC in the humidity controlled oven showed that up to 400 hours at 60% relative humidity the HPMC did not increase in weight (Figure 4.18). The observed decrease in water uptake may therefore be due to the non-aqueous solvent propylene glycol. Increasing the concentration of the HPMC in the ISGM system meant the amount of propylene glycol in the ISGM system had decreased.

It was noted that after 400 hours it appeared as though the rate of water absorption of the ISGM system was about to plateau out (Figure 4.17). Therefore the humidity level was increased to 80% to determine whether a further increase in humidity would result in an increase in water absorption. Though such studies are not required in stability guidelines, this effect was investigated to determine whether an increased humidity level would result in an increased water intake. As shown in Figure 4.17 after 400 hours the water absorption increased substantially. Water absorption also increased slightly for the pure HPMC (Figure 4.18).

The percentage gain in weight shown in the following graphs (Figures 4.17 – 4.19) is that averaged over 3 samples and the error bars shown represent their 95% confidence interval. For clarity the errors bars are not shown on all the profiles.

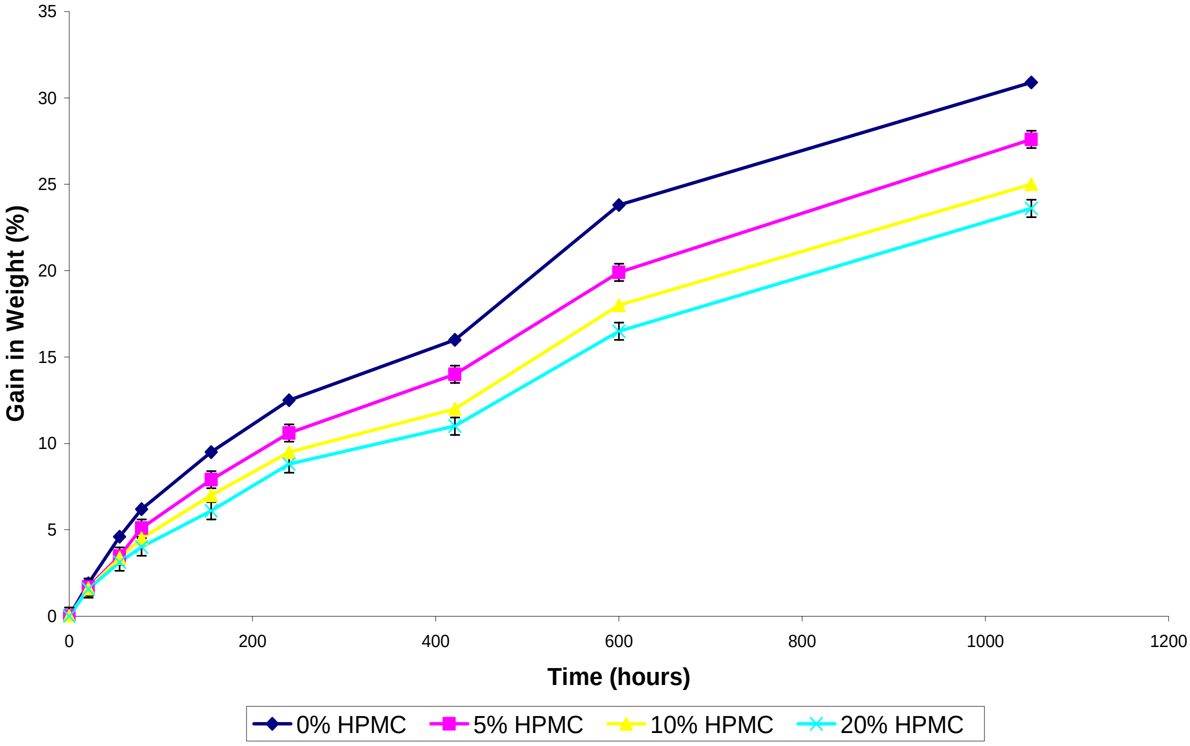


Figure 4.17 – Gain in Weight Due to Water Absorption for the ISGM System Containing Varying Concentrations of HPMC (n=3)

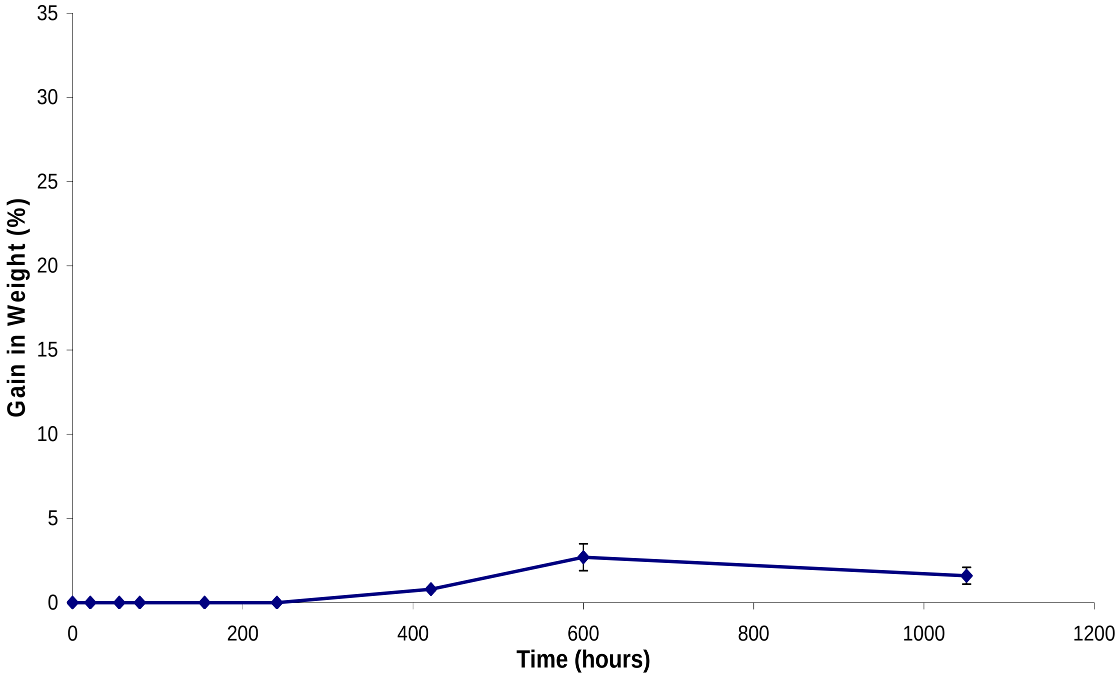


Figure 4.18 – Gain in Weight Due to Water Absorption for HPMC (n=3)

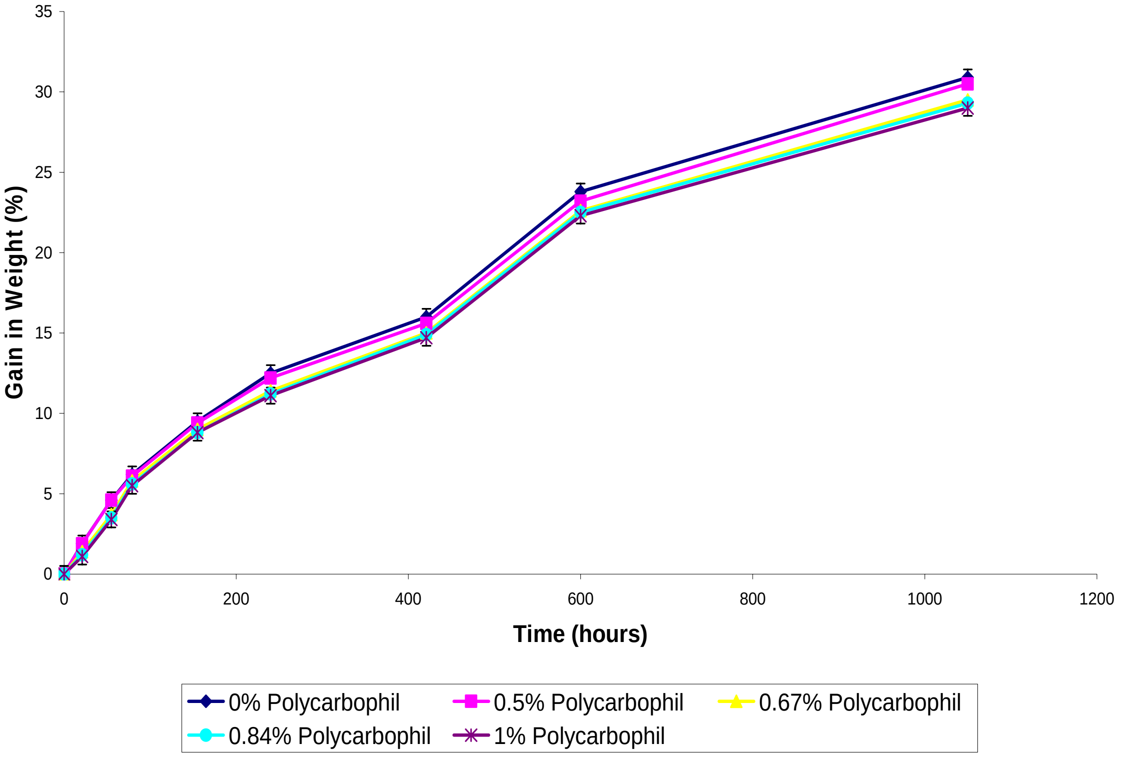


Figure 4.19 – Gain in Weight Due to Water Absorption for ISGM System Containing Various Concentrations of Polycarbophil (n=3)

Varying the amount of polycarbophil in the continuous phase (while keeping the HPMC concentration fixed at 10%) did not make a significant difference to the water absorption (Figure 4.19). Increasing the humidity level to 80% after 400 hours also resulted in an increase in water absorption (Figure 4.19). The humidity was increased to 80% to determine whether the increased humidity level resulted in an increase in water uptake. As explained earlier in this section, though such studies are not part of the stability guidelines, the increased humidity level was investigated for scientific interest. The exaggerated increase in the humidity conditions (80%) led to the expected result of an increase in water uptake.

Results of the studies where the amount of polycarbophil in the ISGM system was varied showed a marginal trend where an increase in the polycarbophil concentration resulted in a slightly lower water intake. These differences were not significantly different at the earlier time points (differences were within the 95% confidence interval as shown in Figure 4.19). However, the differences between the profiles became more significant at longer times. The propylene glycol, which is a hygroscopic solvent, must be the principal cause of the water absorption. This conclusion is supported by the fact that increasing the concentration of the HPMC or polycarbophil meant the amount of propylene glycol in the ISGM system had decreased. Consequently the lower propylene glycol concentration led to a decrease in water intake as seen in Figure 4.17.

The absorption of water upon standing is not a desirable attribute of the ISGM system as this will lead to gelling of the ingredients resulting in difficulty of injecting the formulation.

An additional experiment was conducted to check whether water absorption occurred in sealed vials (as described in Section 3.3a of Chapter 3). A weight increase did not occur indicating that sealed vials were effective in preventing moisture intake thus preventing gelling of the suspension.

It was concluded that any formulation must be stored in sealed containers and must not be exposed for long periods to the atmosphere. This was due to the inherent water absorbing nature of the product ingredients.

There are other methods to measure water intake such as the Karl Fischer titration but this did not prove necessary as the monitoring of the weight clearly showed the ISGM system was absorbing water from the atmosphere. Solvent evaporation was not an issue as the experiment was carried out at a temperature of 25ºC and given the boiling point of propylene glycol is much higher at 188ºC (Section 4.1a), its vapour pressure at 25ºC is expected to be very low.

4.3b Chemical Stability

i. Hydrophilic Polymer (HPMC)

A chemical stability study was conducted on the hydrophilic polymer, HPMC that formed part of the ISGM system. This method, which was HPLC-based, was not a stability indicating assay and therefore the method was not validated. The study was conducted to determine whether any changes in the HPMC's HPLC profile occurred as a result of possible degradation. If degradation was to occur, then changes to the peak height and geometry in the chromatogram would be observed. At the time of the study this means of assessment was deemed adequate to monitor the stability of HPMC. However, if the ISGM system is developed with the intention of creating a commercial product, then a validated stability indicating method would need to be developed for this component of the ISGM system.

Studying the potential for degradation of the polymer in the ISGM system is important as any degradation that may occur could compromise its role as a gelling agent in the ISGM system. For example degradation to smaller fragments may influence gelling properties and hence limit the useful shelf-life of the material. The chemical stability of the HPMC was monitored by size exclusion chromatography (SEC).

SEC is a standard chromatography technique used to determine molecular weights. Size exclusion works by separating the polymer molecule according to their hydrodynamic volume. The hydrodynamic volume can be correlated to the molar mass which is determined by calibration or by using molar mass sensitive detectors (Trathnigg, 2000).

In SEC, the column is packed with a material having a well-defined distribution of pores. These pores influence elution time of the molecules passing through them. Larger molecules elute first as they are too large to penetrate the pores and hence flow quickly through the SEC column. In contrast, the smaller molecules are able to penetrate these pores, consequently increasing the time required to exit the column as a diffusional process is then set up where the smaller molecule penetrate or exit the porous network.

In this investigation, initial polymer stability studies were conducted on an HPLC instrument using a T load column (as described in Section 3.3b *i*). This method did not give any peaks in the resulting chromatrogram. Numerous attempts were made to try and observe a peak where the mobile phase was altered and the sample concentration was altered but peaks were not detectable using the RI detector.

Another method was, therefore, investigated to try and detect a peak due to HPMC. There have been reports in the literature for using an ultrahydrogel column (Crowley *et al.*, 2002; Vijayalakshmi *et al.*, 2005) or TSK-Gel column (Wittgren *et al.*, 2005; Liu *et al.*, 2004) to analyse high molecular weight polymers. Therefore an ultrahydrogel 1000 column was purchased and used on the Waters HPLC system (Section 3.3b *i*).

A 0.1% (w/w) sample of HPMC was injected into the HPLC resulting in the detection of an asymmetrical peak at 7.4 minutes (Figure 4.20). Due to the shape of the peak, it was thought the peak must be due to the HPMC as this polymer has a broad molecular weight. An asymmetrical peak usually indicates a molecule with a broad molecular weight.

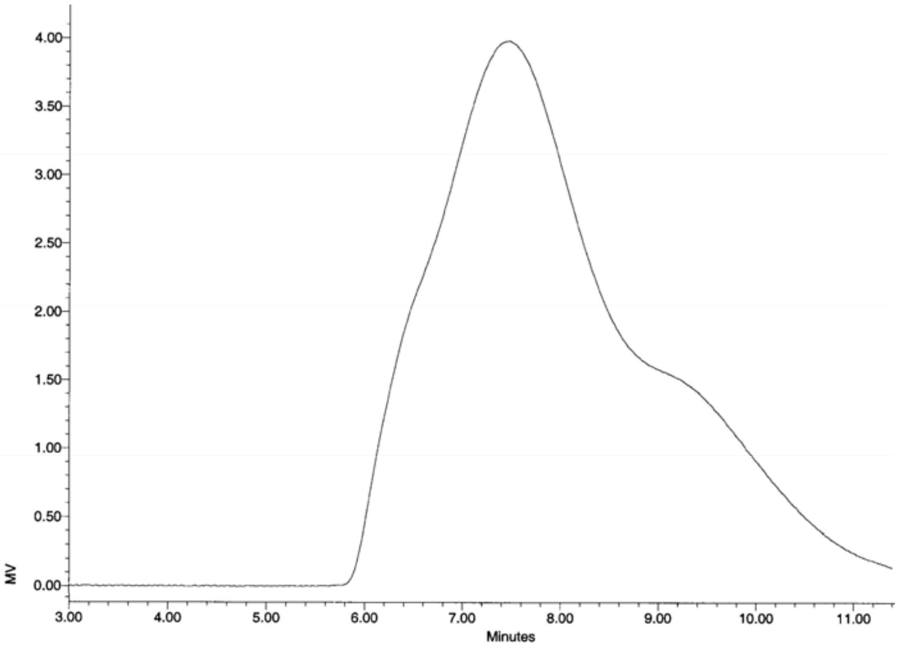


Figure 4.20 – Chromatogram of HPMC

Water (which was initially used to prepare the HPMC sample) itself was analysed and the peak at 7.4 minutes was no longer observable indicating the peak had to be HPMC. This was further confirmed by halving the HPMC concentration. The resulting spectrum gave a peak with the same retention time but a decreased peak intensity as expected.

Upon successfully identifying the SEC-based HPMC peak, stability studies of the hydrophilic polymer over time in accelerated conditions could then be conducted. To obtain an indication of how degradation of HPMC might appear in a chromatogram, a chromatogram of a sample of HPMC was run after it had been subjected to hydrolysis by addition of acid (see Section 3.3b *i* in Chapter 3 for details). The resultant chromatogram (Fig. 4.21 (a)) gave a peak which had decreased strongly in intensity relative to the peak detected from untreated HPMC (Fig. 4.21 (b)) indicating that hydrolysis and therefore degradation of the HPMC had occurred. The other major difference was that, though the peak remained asymmetrical, the geometry of the peak had altered. A more dominant shoulder peak had appeared prior to the main peak (Figure 4.21(a)) which is most likely due to the reduction in size of the overlying main peak.

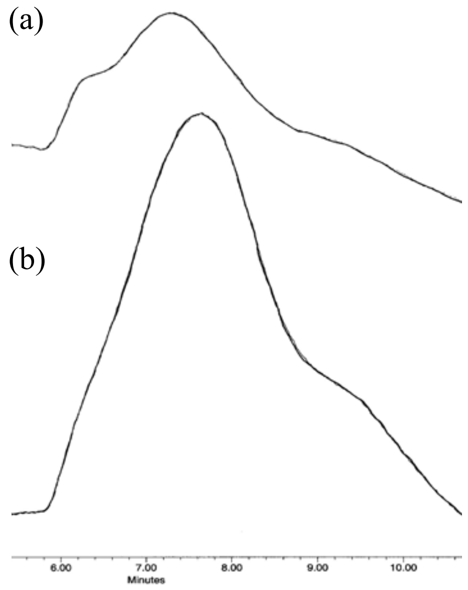


Figure 4.21 – Comparison of the Acid Treated HPMC Chromatogram (a) and the Untreated HPMC Chromatogram (b). Both Traces are on the Same Response Scale where the Response value for the Acid Treated HPMC Peak (a) is 4 mV and the Response Value of the Untreated HPMC Peak (b) is 0.9 mV.

The stability study which tested how stable the HPMC was on standing over time was conducted on samples of HPMC that were dispersed in propylene glycol and stored in a controlled room of 40°C / 75% relative humidity for a period of 9 months (see Section 3.3b *i* in Chapter 3 for a description of the experimental details) . Over this period, samples of the polymer dispersion withdrawn for SEC analysis showed no discernible change in the HPMC chromatogram peak profile taken at time zero and after periods of 3, 6 and 9 months. This is illustrated in Figure 4.22 which gives the SEC chromatogram of the HPMC/propylene glycol sample prior to storage (Fig. 4.22(a)) and the HPMC spectrum at the 9 month time point (Fig. 4.22(b)). The spectrum shows no easily detectable alterations in the intensity or shape of the peak had occurred.

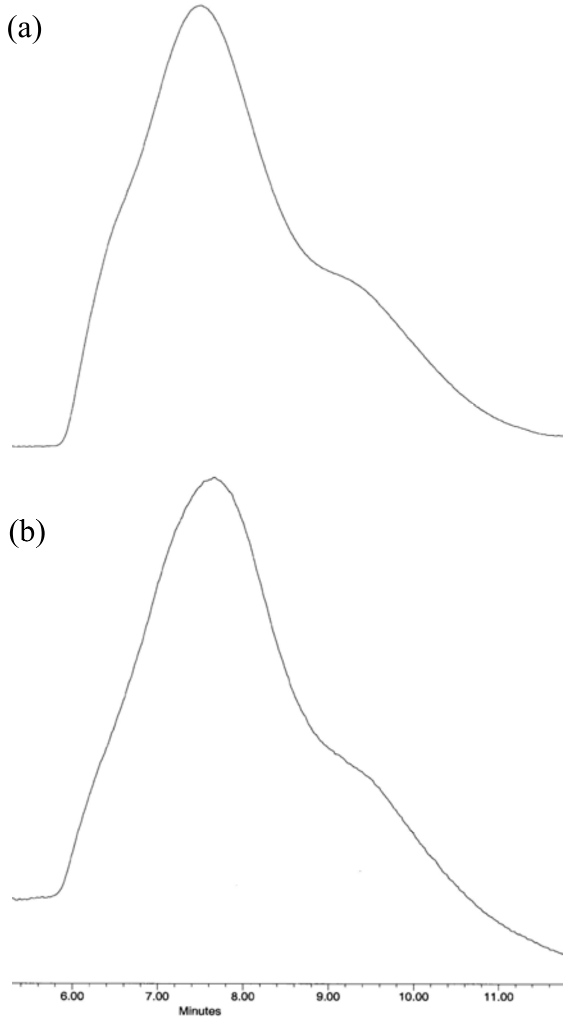


Figure 4.22 - Comparison of HPMC Chromatogram at Time 0 Months (a) and HPMC Chromatogram at 9 Months (b). Both Traces are on the Same Response Scale and the Response Value (i.e. Maximum Peak Height) of (a) and (b) is 4 mV.

The determination of the actual molecular weight of HPMC was subsequently attempted (see Section 3.3b *i* in Chapter 3). This was achieved by taking a series of compounds with similar molecular shape and density (i.e. hydrodynamic volume) which should give a sigmoidal relationship between the K_{av} value and the logarithm of the molecular weight. However, over a considerable molecular weight range this relationship should be linear. The calibration curve was constructed using dextran standards as dextran being a carbohydrate is expected to have the closest hydrodynamic volume (compared to other standards such as PEG) to HPMC which is a cellulose molecule. The K_{av} was calculated according to Equation 4.2 (Fee and Van Alstine, 2004; Amersham Pharmacia Biotech, 1998).

$$K_{av} = \frac{(V_e - V_o)}{(V_t - V_o)} \tag{4.2}$$

Where

K_{av} = Distribution coefficient

V_e = Volume of eluent

V_o = Void volume of the column

V_t = Total volume of the column

The V_o and V_t of the ultrahydrogel column were found to be 3 and 12.9 mL respectively. The V_t was the total volume of the column and the V_o was calculated as the time taken for a large molecular weight molecule to pass through the column (the large molecular weight molecule will be too large to travel through the pores and consequently travel straight through the void spaces in the column). The K_{av} values were calculated for each dextran standard and plotted against the natural logarithm of the molecular weight (Figure 4.23).

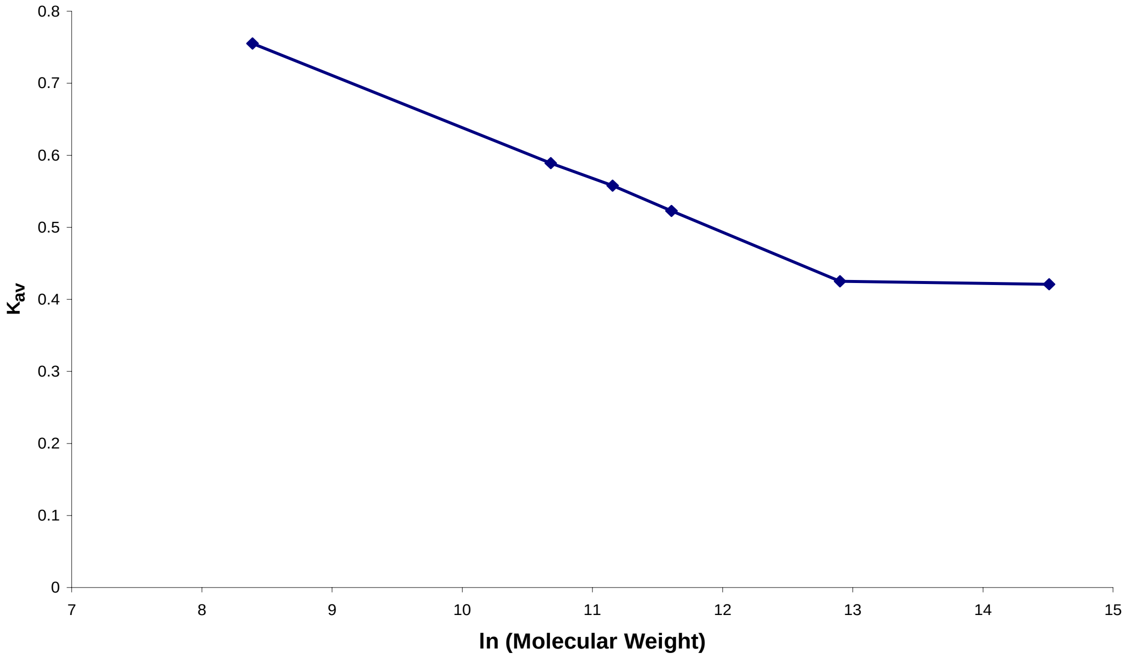


Figure 4.23 – Relationship Between K_{av} and ln(Molecular Weight) for Dextran Standards

The K_{av} value for HPMC was calculated to be 0.4609. According to the calibration curve in Figure 4.23, a K_{av} value of 0.4609 means HPMC should have a molecular weight of approximately 255 250. There are reported discrepancies in the molecular weight of HPMC. According to Hsaio (1985) HPMC has a molecular weight ranging from 20 000 to 140 000 (Hsiao, 1985). In contrast, Conte *et al.,* (1995) reported HPMC to have a molecular weight range of 1000 to 4000 000. The experimentally obtained value in this study (255 250) falls within the molecular weight range reported by Conte *et al*., (1995). The molecular weight of HPMC will depend on the extent of substitution and the intrinsic viscosity of the sample. The Mark-Houwink equation (Equation 4.3) is used to calculate the molecular weight of polymers using the intrinsic viscosity.

$$\eta = kM^{\alpha} \tag{4.3}$$

where η is the intrinsic viscosity, k and α are constants and M is the molecular weight. Therefore the intrinsic viscosity is dependant on the molecular weight of the polymer. The differences in the reported molecular weights of HPMC will depend on the viscosity and the degree of substitution (where a greater degree of substitution will give an increased viscosity).

As mentioned earlier in this section, the 9 month stability assessment of HPMC showed no apparent degradation and therefore no apparent change in the molecular weight.

ii. Continuous Phase

The chemical stability of the continuous phase was monitored by evaluation of viscosity. The viscosity of the system was not observed to change significantly over time indicating the polycarbophil remained stable at room temperature (20°C in sealed vials) in the propylene glycol (Figure 4.24). The error in each viscosity measurement was calculated as described in Section 4.2f.

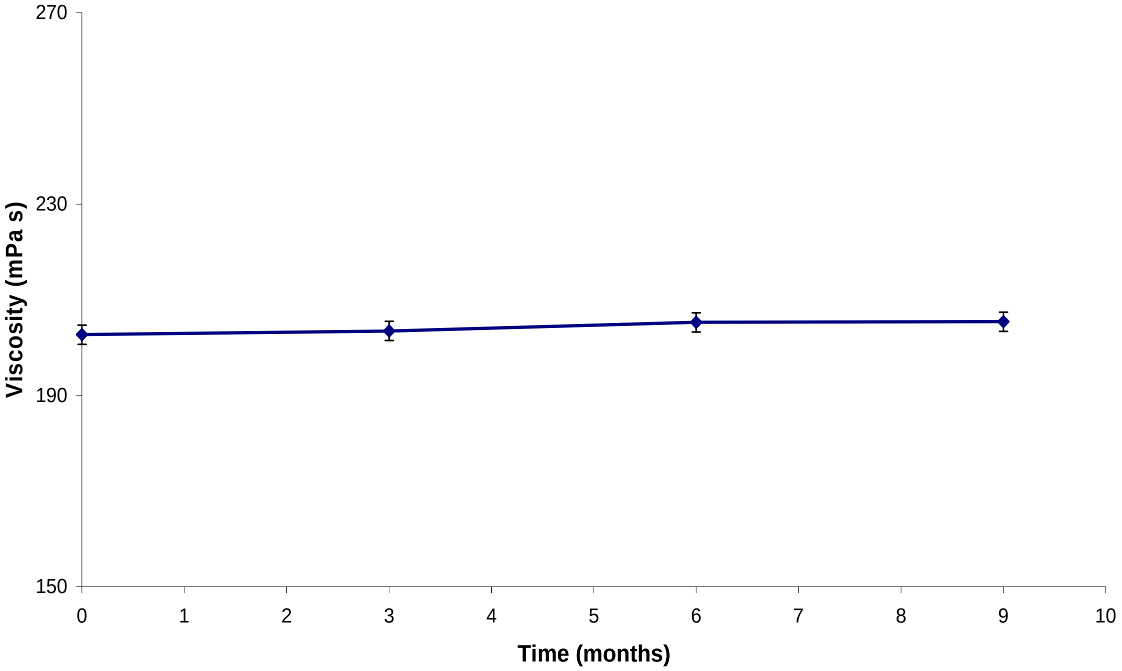


Figure 4.24 – The Viscosity of the Continuous Phase over a 9 Month Period

4.3c Ease of Redispersion of the ISGM System

Redispersion studies were conducted to evaluate the ease at which the (sedimented) ISGM system could be homogeneously dispersed after storage. The study was conducted over a period of 4 weeks where each week a sample was taken to assess its redispersibility.

In all cases the ISGM system had redispersed to give a uniformly dispersed suspension with minimal effort (Figure 4.25). The error bars on the graph represent ± 5 seconds where the time was observed on an electronic timer.

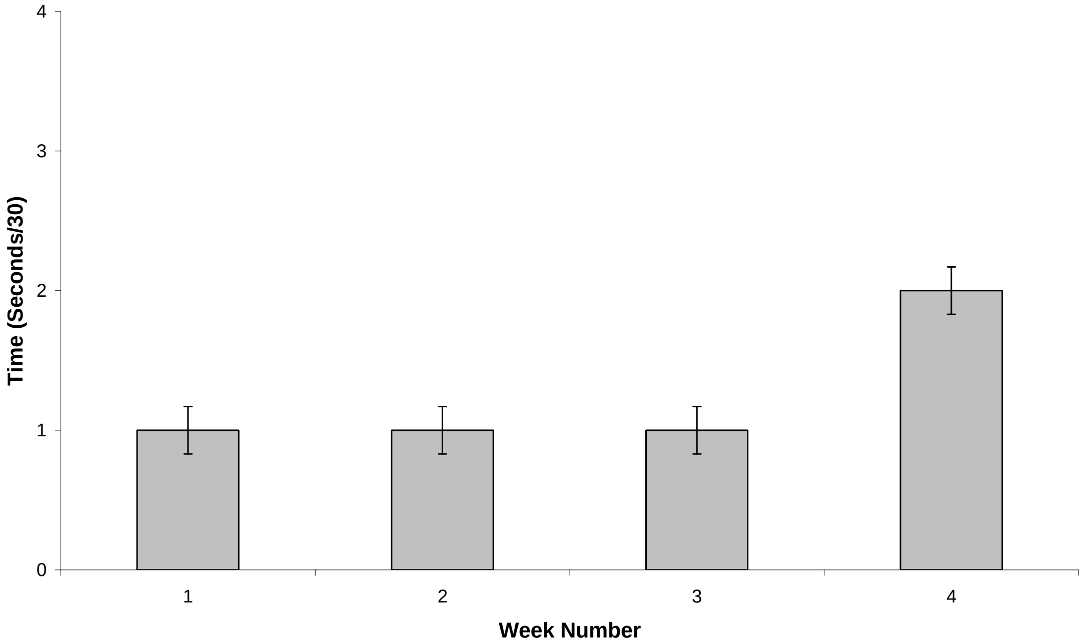


Figure 4.25 – The Ease of Redispersing the ISGM System after 1, 2, 3 and 4 Weeks of Storage.

The samples at week 1, 2 and 3 were fully redispersed within 30 seconds (see Section 3.3c in Chapter 3 for experimental details). The sample at week 4 had a sediment level of 0.1 cm. After 30 seconds the sediment level had decreased to 0.05 cm but had not yet fully redispersed. After the second 30 second block, the suspension had fully redispersed to its original form. Duro *et al.* (1998), conducted stability studies on their suspensions and found their systems varied in redispersion times from 0 to 35 minutes after 15 days where their systems consisted of aqueous suspensions of pyrantel pamoate with the inclusion of HPMC or sodium CMC as stabilisers. However, the ISGM system developed in this study did not require as much effort to redisperse as the suspension described in Duro *et al.*'s study. It is difficult to make a direct comparison between the two systems as they are not the same – the system studied in this investigation is non-aqueous where as the system studied by Duro *et al.* (1998) is an aqueous suspension. However there is a considerable difference in the ease of redispersion between the two systems. After 4 weeks of standing, the ISGM system developed in this study was fully redispersed after 1 minute which is a favourable attribute were it to be considered for further commercial development. This would also necessitate the addition of a proviso that the ISGM system be always shaken immediately prior to its use.

4.3d Appearance of the ISGM System After Administration into an Aqueous Environment

i. In Vitro Appearance of the ISGM System After Administration into an Aqueous Environment

The ISGM system (with a dye incorporated) was injected into a cellulose medium to observe how the formulation might form *in vivo* (see Section 3.3d in Chapter 3). Upon injection into the cellulose medium, the ISGM system gelled to form a sphere shaped depot as illustrated in Figure 4.26.

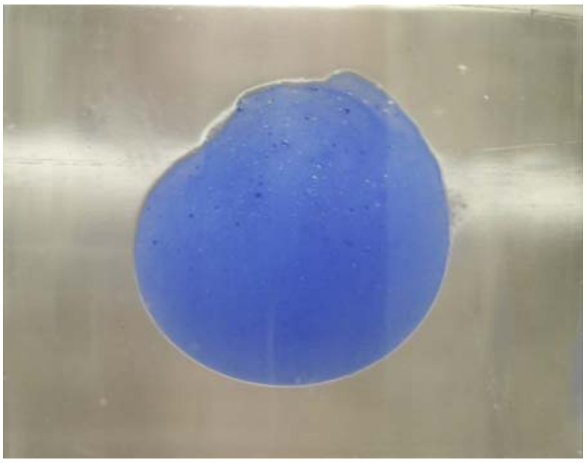
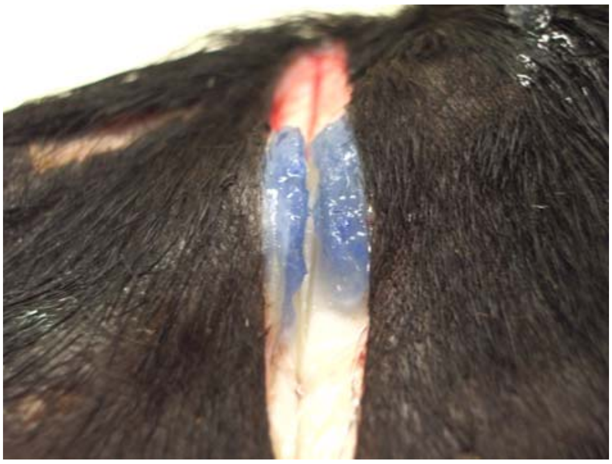


Figure 4.26 – Injection of the ISGM System (with a Blue Dye Incorporated) into a Cellulose Medium

ii. In Situ Appearance of the ISGM System After Administration into an Excised Calf Ear

The ISGM system was also injected into the ear of a dead calf at room temperature. The ISGM system was injected at a reasonably rapid rate over 20 seconds to avoid blocking of the needle during administration as a result of the polymer gelling. Water will unavoidably enter the needle as a result of injecting into tissue as some tissue will part resulting in some fluid going up the bore of the needle.

Incision of the injection site (6 hours after administration) on the carcass revealed the depot (sphere shaped) had gelled indicating water from the internal ear tissue had diffused into the formulation. The depot, itself, was then sliced open with a scalpel to determine the integrity of the depot contents. The depot was found to have remained intact in shape and showed the formulation had also gelled inside the body of the depot (Figure 4.27).



Figures 4.27 – Injection of Formulation into the Ear of a Dead Calf

iii. In Vivo Appearance of the ISGM System After Administration into a Rat

The ISGM system was also injected into a rat to determine if a gelled depot would form (Figures 4.28a – 4.28e). Injection via a 22 gauge needle was made into the area posterior to the scapulae (Figure 4.28a).

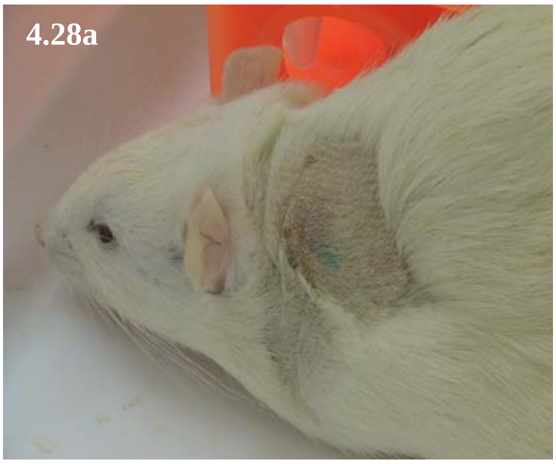


Figure 4.28a – The Injection Site in the Shaved Region of the Rat’s Shoulder

Figure 4.28b - Incision of the Rat Revealing the Injected Depot

Figure 4.28c – Further Opening of the Incision Site

Figure 4.28d – Removal of the Injected Depot

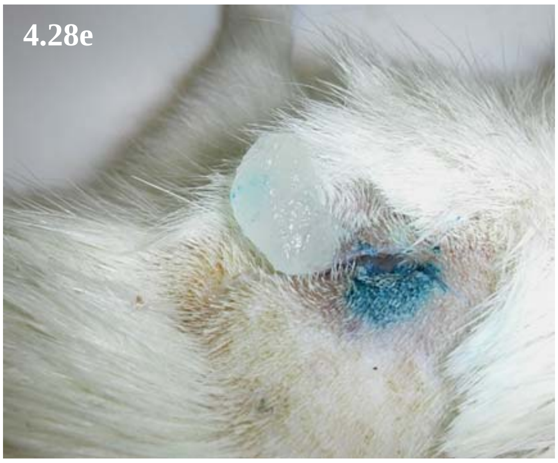


Figure 4.28e – The Depot Removed From the Injection Site

The rat was dissected (6 hours after injection) to observe and remove the depot (Figure 4.28b and 4.28c). 6 hours after injection, the depot had successfully gelled (semi-solid) and was easily removed from the body (Figure 4.28d and 4.28e). The histology report found mild diffuse inflammation at the injection site of the rat. Histology of injection sites almost always indicates inflammation. This can be due to the trauma of injection rather than the actual formulation (Baggot and Brown, 1998). The aim of the experiment was to determine if the depot would form *in vivo.* The results proved that the ISGM system successfully formed a gelled depot at the site of injection.

During this investigation it was also found that the injection of the ISGM system did not result in bleed back of the formulation through the injection tract (which is clearly shown in Figure 4.26).

The depot formed after injection appeared as though it would break down easily via a process of polymer chain disentanglement (as discussed in Section 4.2h) despite the presence of a high molecular weight polymer. During the *in vitro* trials (Section 4.5), the gel was left in the dissolution apparatus to determine how long it would take for the gel to break down. Four weeks after injecting the formulation into the dissolution apparatus, the depot appeared fluid in nature and was no longer viscous. Obviously the residence time of the depot can be manipulated by increasing the concentration of the hydrophilic gelling agent. Fernandez-Vigo *et al*., (1990) conducted studies to show the residence time of the HPMC may be able to be controlled in the vitreous cavity. Elimination of HPMC was established to occur by first order kinetics. HPMC with a molecular weight of 86 000 had a half life of 10.4 days while the higher molecular weight HPMC (of 120,000) had a half life of 38.4 days (in the vitreous cavity). This indicates therefore that a higher molecular weight polymer should give a longer residence time for the gelled depot. Nafee *et al*., (2004) conducted studies to determine the residence time of mucoadhesive tablets. A 180 mg mucoadhesive HPMC tablet had an *in vitro* residence time of 8 hours. The residence time of the HPMC depot injected intramuscularly or subcutaneously will not be the same (as the residence times reported by Nafee *et al.* (2004) and Fernandez-Vigo *et al.* (1990)) as different sites and different dosage forms may exhibit different residence times.

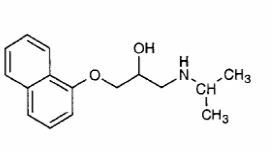
However, it gives an indication on the relative time frame involved and the effect of the molecular weight will be the same regardless of the site of administration. The residence time of a gelled depot can be manipulated by the amount and molecular weight of the hydrophilic gelling agent.

This study has shown that the ISGM system offers the potential as a controlled release bolus. In a cellulose medium, excised calf ears and in rats, the ISGM system was observed to form a gelled depot. These experiments demonstrated that the ISGM system was performing its physicochemical role of forming the depot so allowing the drug contained with it to be trapped thus acting as the retarding agent for its release. It was now important to test how good a retarding agent the depot would be by assessing its performance using the drug propranolol.

4.4 Formulation and Characterisation of the Final Product

As discussed in Section 3.4 of Chapter 3 the drug release performance of the ISGM system was assessed using propranolol HCl which has veterinary applications (Section 2.6). The physicochemical properties of propranolol are given in Table 4.9.

Table 4.9 - Physicochemical Properties of the Drug Propranolol

Drug	**Structure**	**Properties**					
		State (20°C)	Colour	Molecular Weight	Melting Point (°C)	pK_a (24°C)	Solubility
Propranolol HCl	 (Thevis *et al.,* 2001)	Powder	White	295.81	162-165	9.5	1 g in 20 mL of water

4.4a Incorporation of the Drug into the ISGM System

The propranolol was added as a solid to the propylene glycol and shaken by hand, followed by the addition of the hydrophilic polymer to create the ISGM system with drug incorporated into it. The solubility of the drug in the continuous phase was found to be 0.003 g mL^{-1} which was determined by continuous addition of drug to the continuous phase until a saturated solution of drug was obtained.

4.4b Differential Scanning Calorimetry (DSC) Analysis

DSC studies were conducted to determine whether interactions between the excipients of the formulation and the chosen drug existed. Such interactions are detected by appearance, disappearance, or shifting of endothermic or exothermic peaks and/or variations in the relevant enthalpy values. DSC measures the energy required to establish a near zero temperature difference between an inert reference material and the sample when subjected to identical temperature regimes in an environment heated or cooled at a controlled rate. The temperatures of the sample and reference are made identical by varying the power and the energy required to do this is a measure of the enthalpy (or heat capacity) changes in the sample relative to the reference. The difference in the heat flow to a sample and reference is monitored against time or temperature (Verma and Garg, 2005).

If interactions, as mentioned above, between the drug and the excipient exist, the stability and / or bioavailability of the drug may be altered i.e. there may be changes in the chemical nature, solubility and absorption and therapeutic response of the drug (Mura *et al*., 1995). This may affect the safety and/or efficiency of the drug in the formulation.

DSC is a rapid response technique which evaluates any physiochemical interactions between the drug and excipients and hence provides information on potential physical or chemical incompatibilities. Careful interpretation of DSC results is required to avoid misinterpretation of the data. To confirm whether any incompatibilities or otherwise do exist, DSC studies need to be used in conjunction with other analytical tools such as IR spectroscopy (Mura *et al*., 1998). Investigation of drug-excipient compatibility is very important but has no universal protocol available to conduct such investigations.

DSC studies were conducted on the drug itself and physical mixtures of the ISGM system excipients and the drug. The thermogram of the propranolol on its own showed a melting endotherm at 165°C. The melting point peak of the drug did not shift and peaks did not disappear or appear in the thermograms of the HPMC and propranolol (Figure 4.29) as well as polycarbophil and propranolol (Figure 4.30) indicating that interactions between these excipients and the drug did not appear to exist. The thermogram of HPMC showed a very low intensity broad peak at 55°C (Figure 4.29). This broad endotherm was attributed to moisture loss from HPMC (Ford, 1999). Interactions also did not appear to exist between propylene glycol and the drug as well as the final product itself.

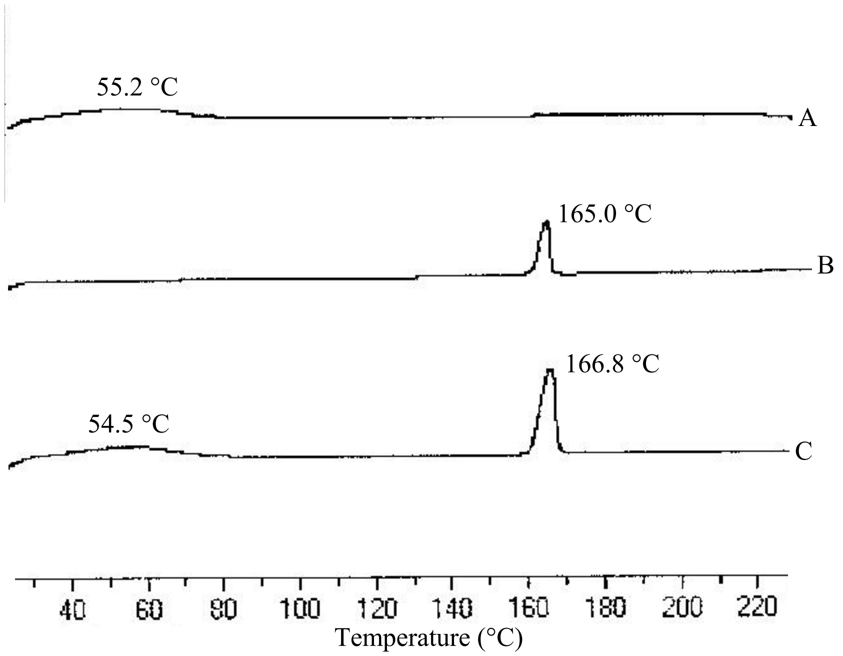


Figure 4.29 – DSC Thermogram of HPMC (A), Propranolol (B) and Propranolol + HPMC (C)

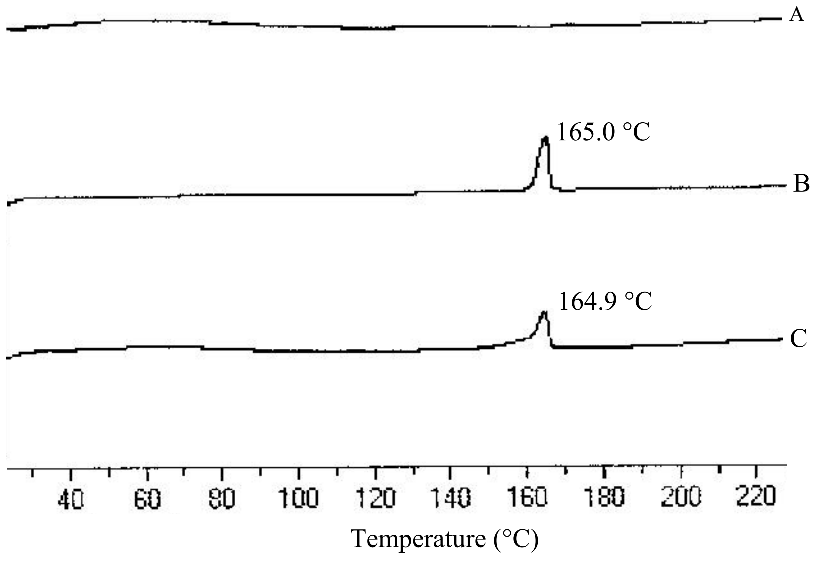


Figure 4.30 – DSC Thermogram of Polycarbophil (A), Propranolol (B) and Propranolol + Polycarbophil (C)

Contrary to this finding, the *in vitro* studies (to be discussed in Section 4.5) suggested interactions may exist as the formulation was not giving 100% release of the drug. The interaction must have occurred after water had diffused into the formulation. Therefore DSC studies were repeated on formulations that had been gelled by the addition of water.

The resulting DSC curve no longer revealed a melting endotherm at 165°C (propranolol melting point peak) but an endotherm at 146.1°C (Figure 4.31) was present indicating some form of interaction was occurring. To verify the water was responsible for the interaction the experiment was repeated where pH buffers of 1 and 4.5 were added instead of water to give a gelled depot. The release profile of the drug using pH 1 and 4.5 buffers as the dissolution test medium resulted in 100% release (see Section 4.5b *vi*.). Consequently anomalies in the DSC thermogram should not occur. This was found to be the case as the propranolol peak was observed for both pH 1 and 4.5 systems. The introduction of water to the formulation resulted in an interaction between the drug and a formulation excipient. A detailed discussion of the effect of pH is given in Section 4.5b *vi*. The gel without the drug was also analysed. The peaks at 165 or 146.1°C were not observed indicating the interaction did involve the drug.

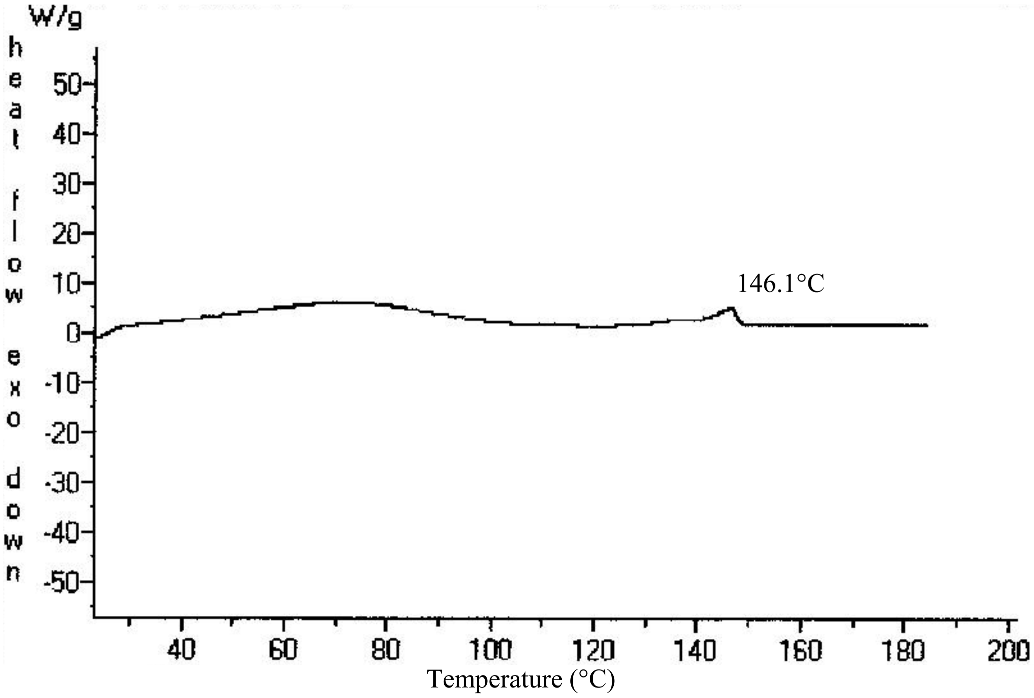
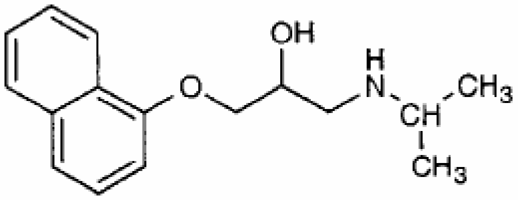


Figure 4.31 – DSC Thermogram of Gelled Formulation

Interactions between propranolol and anionic polymers such as polyacrylic acids (for example polycarbophil) can occur (Taylan *et al*., 1996; Wang *et al*., 2003) (This is discussed in more detail in Section 4.5b *vi*.). Therefore an interaction could be occurring between polycarbophil and propranolol. Propranolol is effectively a cationic drug when introduced as the hydrochloride salt in which the propranolol itself would exist as the conjugate acid, propranolol-H^+. Consequently there may be a propranolol-H^+-anionic polymer binding interaction. DSC studies alone cannot confirm this interaction and so careful interpretation of DSC results is required to avoid misinterpretation. Use of another analytical technique such as IR spectroscopy is necessary to provide further evidence to refute or support DSC observations. Consequently the observed interaction was verified by conducting an infra-red scan of the gelled ISGM to determine if any new absorption bands due to a possible polycarbophil-propranolol complex appear (see Section 4.4c).

4.4c Infra-Red (IR) Analysis

An IR study was conducted to determine whether a possible propranolol/polycarbophil interaction existed (as discussed in Section 4.4b) in the ISGM as suggested by DSC studies. The IR spectrum of the pure drug recorded as a KBr disk is shown in Figure 4.32 and the band assignments described in Table 4.10.



Propranolol Structure (Thevis *et al.,* 2001)

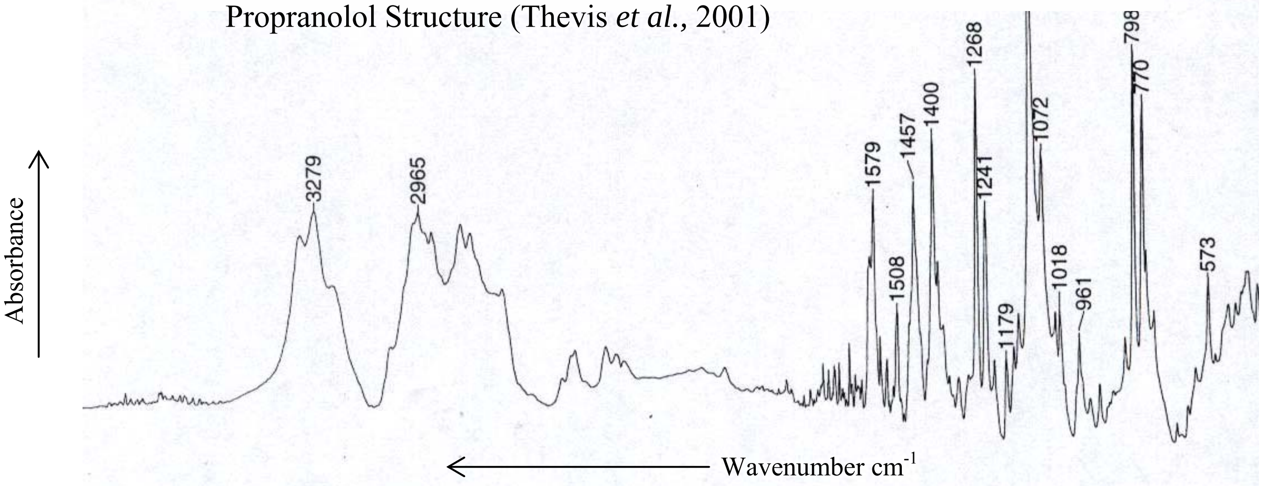


Figure 4.32 – IR Spectrum of Propranolol

Table 4.10 – Key for Figure 4.30 Identifying the Peaks in the IR Spectrum of Propranolol

Frequency	Assignment
3330 - 2500	Broad and strong band due to OH stretches as well as N-H (and C-H stretches.
1400 – 1600	Aromatic C=C stretch
1080 – 1360	C-N stretch
1150 - 1080	C-O stretch
900 - 670	Aromatic character due to out-of-plane proton-carbon bending.

If the drug and polycarbophil exhibit an interaction then the functional groups in the IR spectrum of the combined two species may reveal the emergence of new bands or alterations in wavenumber position or broadening when compared to the spectra of the pure polymer and the drug (Takka, 2003).

The IR spectrum of the gelled formulation was compared to the spectrum of the pure drug (Figure 4.33a) and polycarbophil (Figure 4.33b) (and also the spectrum of the formulation without the drug). A new but weak absorption band was found at approximately 1557 cm^{-1} (Figure 4.33c) which is thought to be a result of salt formation. The formation of a salt can occur by addition of an amine group to the carboxylic acid (Takka, 2003; Taylan *et al.*, 1996).

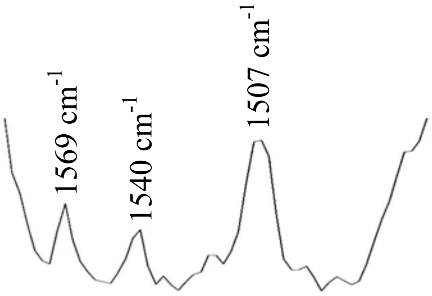


Figure 4.33a – The Propranolol Spectrum Showing the Absence of the Peak at 1557 cm $^{-1}$

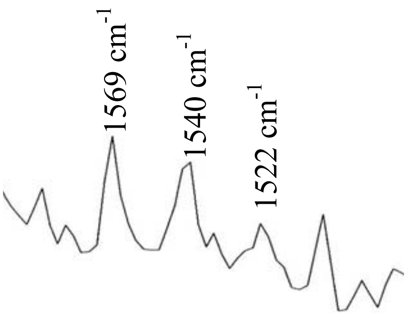


Figure 4.33b – The Polycarbophil Spectrum Showing the Absence of the Peak at 1557 cm $^{-1}$

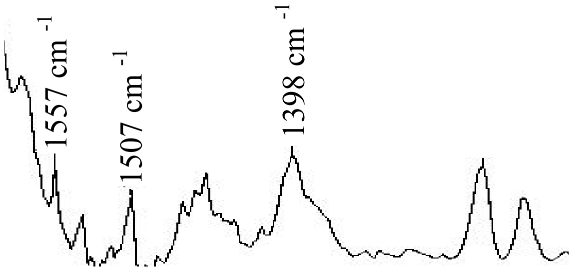


Figure 4.33c – New Absorption Band at 1557 cm $^{-1}$ Possibly Due to the Propranolol / Polycarbophil Complex

Although the observed band was weak, the IR study has given some ancillary evidence to the DSC studies for showing an interaction between polycarbophil and propranolol.

4.4d Electrospray Mass Spectrometry (ESMS) Analysis

Propranolol was also analysed on an electrospray mass spectrometer as one other possible means of characterising the drug. This technique was also used in the degradation studies (Section 4.6a). The propranolol electrospray mass spectrum is shown in Figure 4.34.

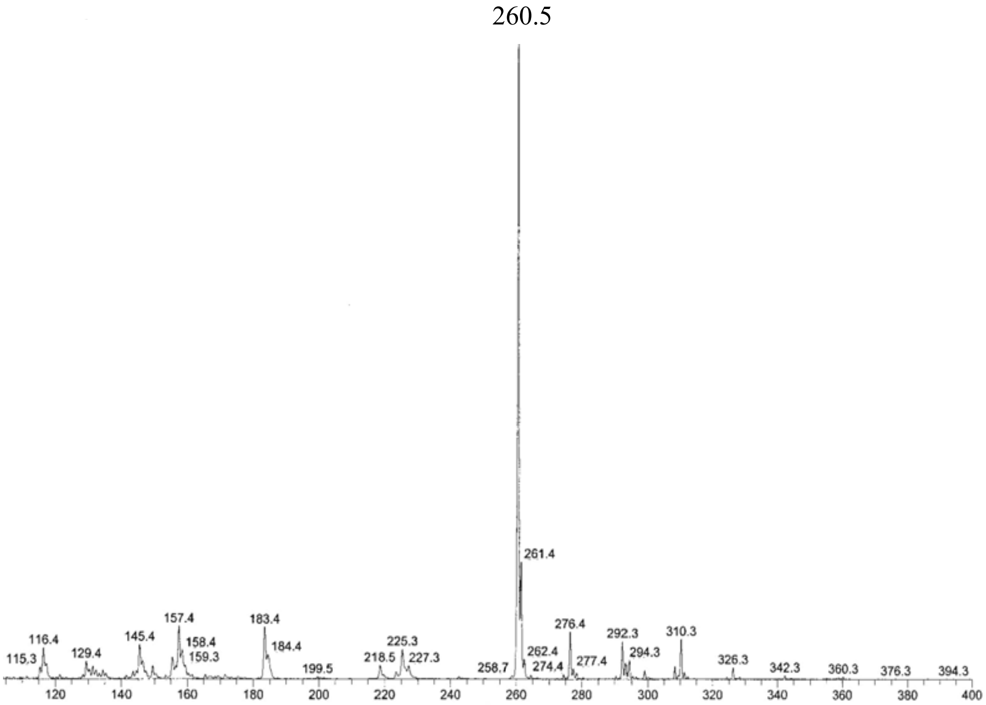


Figure 4.34 – Electrospray Mass Spectrum of Propranolol

The spectrum was in agreement with the previously reported propranolol spectrum where the characteristic peaks of 116 (Figure 4.35), 157, 183 and 260 (Figure 4.36) m/z were observed. The peak at 260 m/z was the $[M + H]^+$ species. The peak at 183 m/z is the result of elimination of water (18 amu (atomic mass units)) followed by elimination of the isopropyl amine group (59 amu), which gives a total loss of 77 amu, resulting in the structure $[M + H]^+ - 77$ (Figure 4.37).

However a different structure for the 183 peak has also been proposed by Upthagrove *et al.* (1999). The structure at 183 m/z could be a result of intra-molecular rearrangement through a 6-membered transition state, resulting in the addition of the 3-carbon portion of the side chain to the 2'-position located on the naphthalene ring (Figure 4.38). Therefore it is possible the product ions of propranolol can arise from dissociation of an ether oxygen-protonated species rather than protonation of the amine nitrogen.

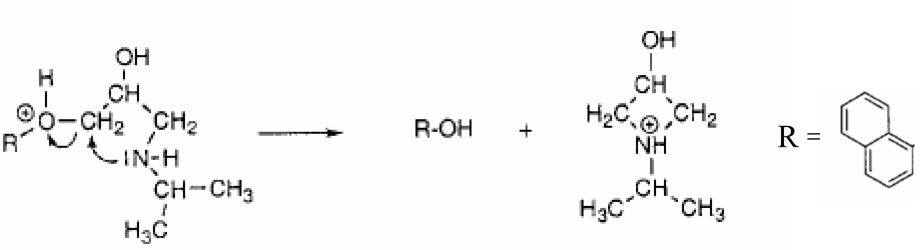


Figure 4.35 – [M – ROH]$^+$. This Structure is Assigned to the Peak at 116 m/z (Thevis *et al.*, 2001)

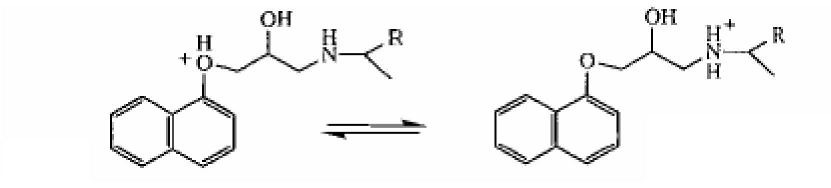


Figure 4.36 –The Structures for [M + H]$^+$ at 260 m/z (Upthagrove *et al.*, 2001)

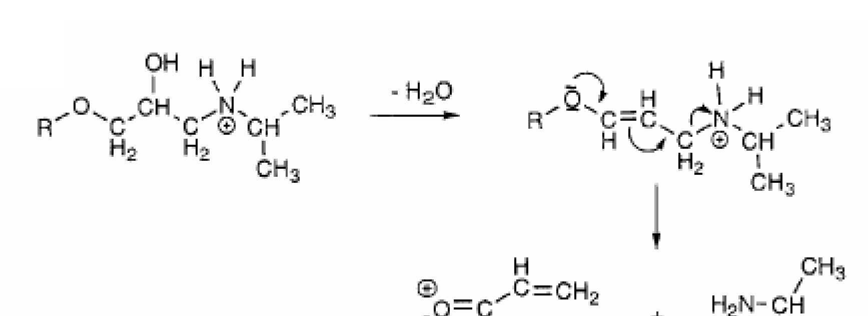


Figure 4.37 – [M + H]$^+$ - 77 amu. This is the Proposed Structure for the Peak at 183 m/z (Thevis *et al.*, 2001)

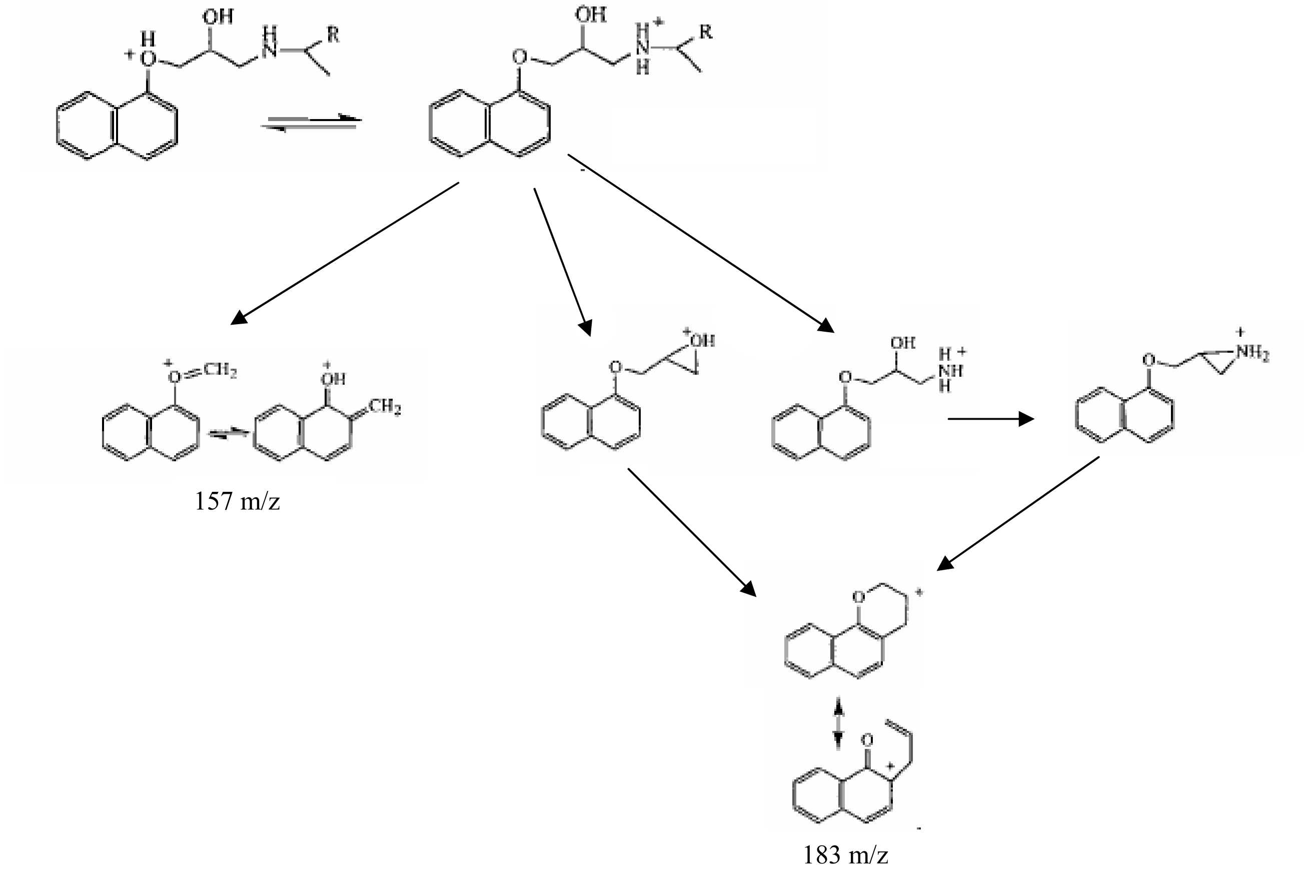


Figure 4.38 – Upthagrove *et al*. (1999) Proposed Structure for the Species at 183 m/z

4.4e Ultra-Violet (UV) Analysis

UV analysis was conducted to characterise the excipients of the final formulation. Propranolol (Figure 4.39) has absorbance maxima at 214 and 290 nm. The data from this investigation was used for the *in vitro* drug release studies (Section 4.5).

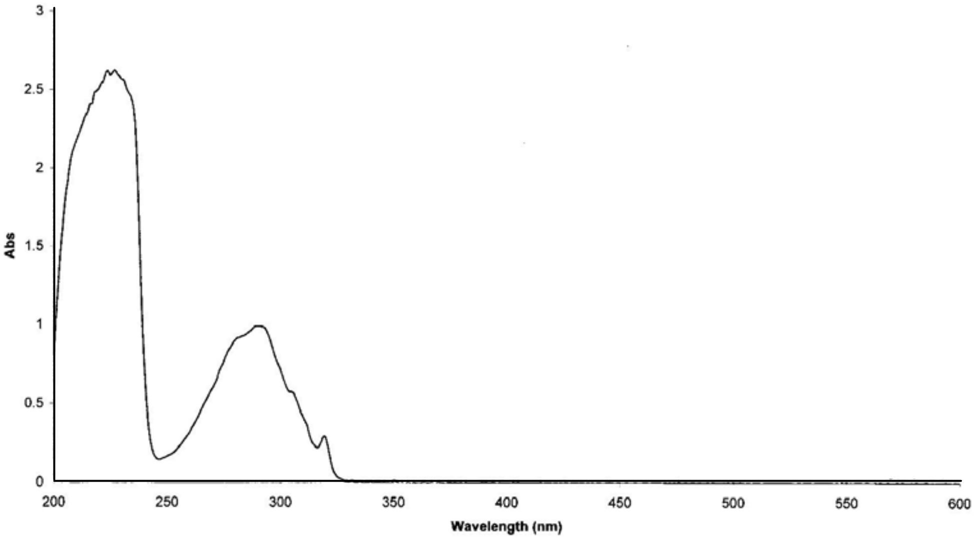


Figure 4.39 – UV Spectrum of Propranolol

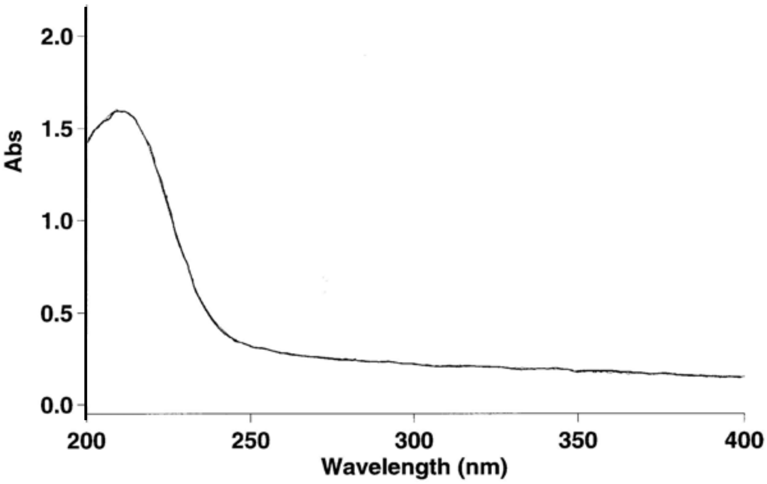


Figure 4.40 – UV Spectrum of Polycarbophil

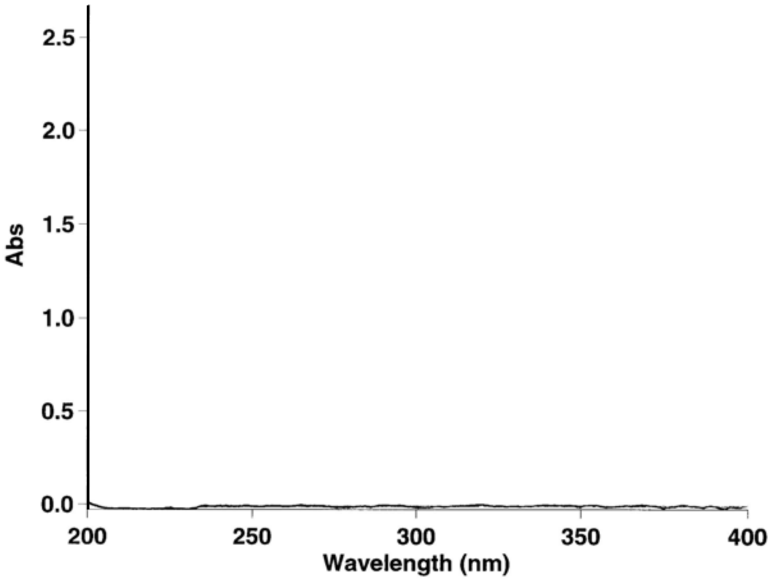


Figure 4.41 – UV Spectrum of Propylene Glycol

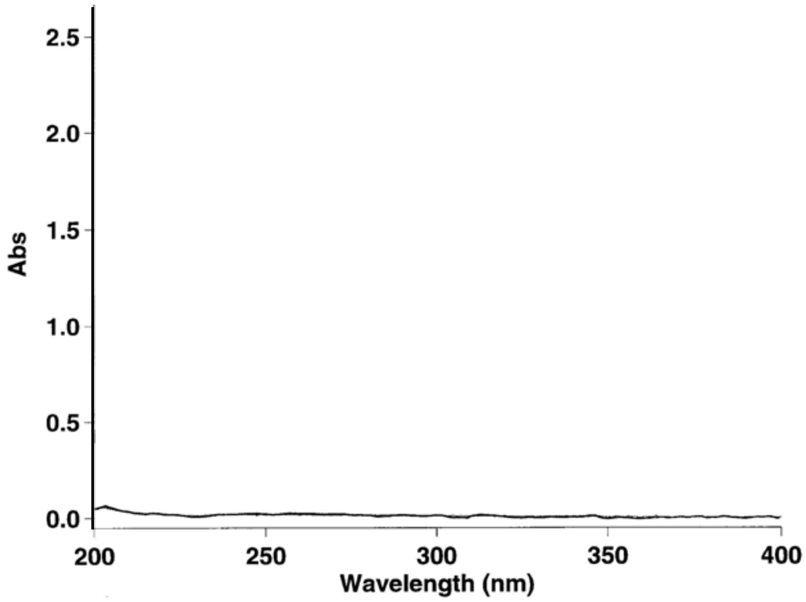


Figure 4.42 – UV Spectrum of HPMC

The polycarbophil also exhibited UV absorbance as shown in the spectrum (Figure 4.40). Propylene glycol (Figure 4.41) and HPMC (Figure 4.42), in contrast, had no UV absorbance in the region of interest.

4.5 Drug Release Studies on the Final Product

An *in vitro* study is one which is conducted in isolation from a living organism, literally *"in glass"* the term implies. The biological processes and reactions are carried out in simulated *in vivo* conditions such as in synthetic mixtures of cell components. An *in vivo* study is one which is conducted within a living organism (Godman and Payne, 1981).

The ideal aim of an *in vitro* test is to achieve a 1:1 *in vitro* : *in vivo* correlation. The attainment of the 1:1 ratio is important as it serves as a quality control procedure during the manufacturing of the product. Such tests are employed to assess lot-to-lot variability and product shelf life (Clark *et al*., 2005; Baxter, *et al*., 2005; Morita *et al*., 2003; Ouriemchi *et al*., 1995; Peltonen *et al*., 2003; Qureshi and Shabnam, 2001; Schliecker *et al*., 2004; Schmidt and Glombitza, 1995; Siewert *et al*., 2002; Souliman, 2006).

In the early 1960's disintegration tests were the only official means of conducting *in vitro* experiments. The tests were used to predict *in vivo* release and assessment of product performance. Despite the disintegration test being only indirectly related to drug bioavailability (Cohen *et al*., 1990) it was still the preferred choice for pharmaceutical industries as the test was inexpensive and quick.

Due to the advancement of research and modernisation of technology dissolution tests are the preferred choice of *in vitro* testing. Dissolution is described as the process by which a solid substance enters a solvent to give a solution i.e. the solid dissolves in the solvent. Upon administration, a dosage form undergoes dissolution into body tissue or other media and the drug will ultimately be absorbed into the systemic circulation (Banakar, 1992).

Dissolution tests are recognised as an official *in vitro* test in the United States Pharmacopoeia (USP, 1994). When conducting dissolution studies, several parameters such as geometry, dimensions and materials must be controlled to obtain reliable results. The release profile is obtained by measuring the absorbance of the drug over time (in the case where UV spectrophotometry is used). The concentration of the drug released is then calculated using the absorbance values. The dissolution tests have been successfully applied to conventional dosage forms.

It is very difficult to completely simulate *in vivo* conditions *in vitro* (due to factors such as the presence of enzymes). This is especially the case for controlled release formulations because they are designed for prolonged release and variables in *in vivo* conditions such as the presence and nature of food in the gastrointestinal tract, time of day and dosage administered may affect the drug release. For example, Vidgren *et al.* (1991) found an oral dosage form could reach the intestine in 10 minutes on an empty stomach but in a fed stomach, the times were 119 - 285 minutes depending on the size of the meal consumed (Davis *et al.*, 1984).

The aim of conducting *in vitro* drug release studies in this research was to assess whether the ISGM system fulfilled its expected role of being a controlled release agent using the drug.

4.5a Drug Release Studies Conducted on the Hanson Dissolution Apparatus

Drug release studies were conducted on the final product (the ISGM system and the drug propranolol) using the Hanson Dissolution apparatus. Initial work involved determining the ideal method of introducing the final product to the dissolution apparatus and ascertaining the reproducibility of the system.

i. Mode of Formulation Introduction to the Hanson Dissolution Apparatus

When the formulation was introduced from above the vessel, the milky white suspension (the formulation) was instantly dispersed into the dissolution test medium by the paddle and eventually settled onto the bottom of the vessel. Injection from the top also resulted in a much higher initial concentration of drug (Figure 4.43) which can be attributed to the greater surface area of the dispersed formulation. The greater surface area allows more of the drug to be released compared to the depot introduced at the bottom of the vessel which has a much smaller surface area. Injection of the formulation onto the bottom of the vessel produced a globular shaped depot. Therefore the ideal method of introducing the formulation is by injection at the bottom of the vessel, avoiding contact with the paddle. There was a visible effect of the paddle on the depot, as the top of the depot appeared to be disturbed by shear from the rotating paddle (for both modes of introducing the formulation to the vessel).

The errors shown in the following graphs (Figures 4.43 – 4.56) represent the standard deviation of the percentage release as calculated from the 6 replicates.

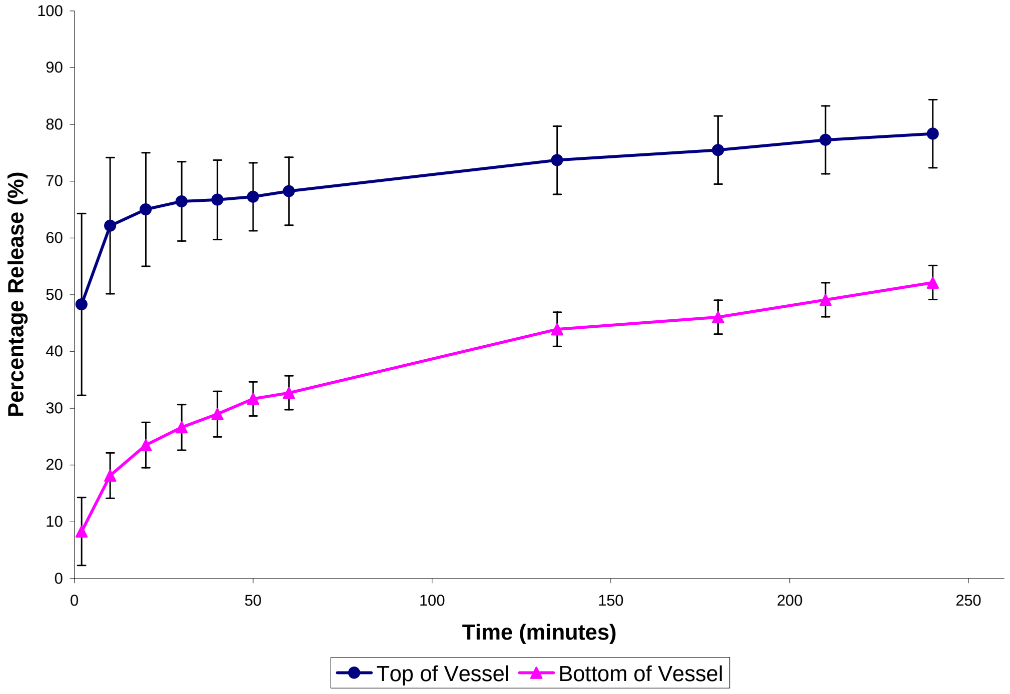


Figure 4.43– Effect of Introducing the Formulation at the Top or Bottom of Vessel on the Drug Release Profile (n=6)

ii. *Effect of the Hydrophilic Gelling Agent*

These experiments were conducted to show the role of the hydrophilic gelling agent in controlling the release of drug into the receptor medium. The omission of the hydrophilic gelling agent from the formulation caused instantaneous release of the drug whereas inclusion of the gelling agent gave a much slower release profile indicating the gel was retarding the release of the drug (Figure 4.44). Upon injection of the formulation without the hydrophilic gelling agent, the suspension spun with the rotating blade and within 20 minutes had completely dispersed and dissolved. This was reflected in the spectrophotometric results where within 10 minutes complete release of the drug had occurred (Figure 4.44). In contrast the formulation with the gelling agent exhibited sustained release and had not reached 60% release after even 2 hours (Figure 4.44).

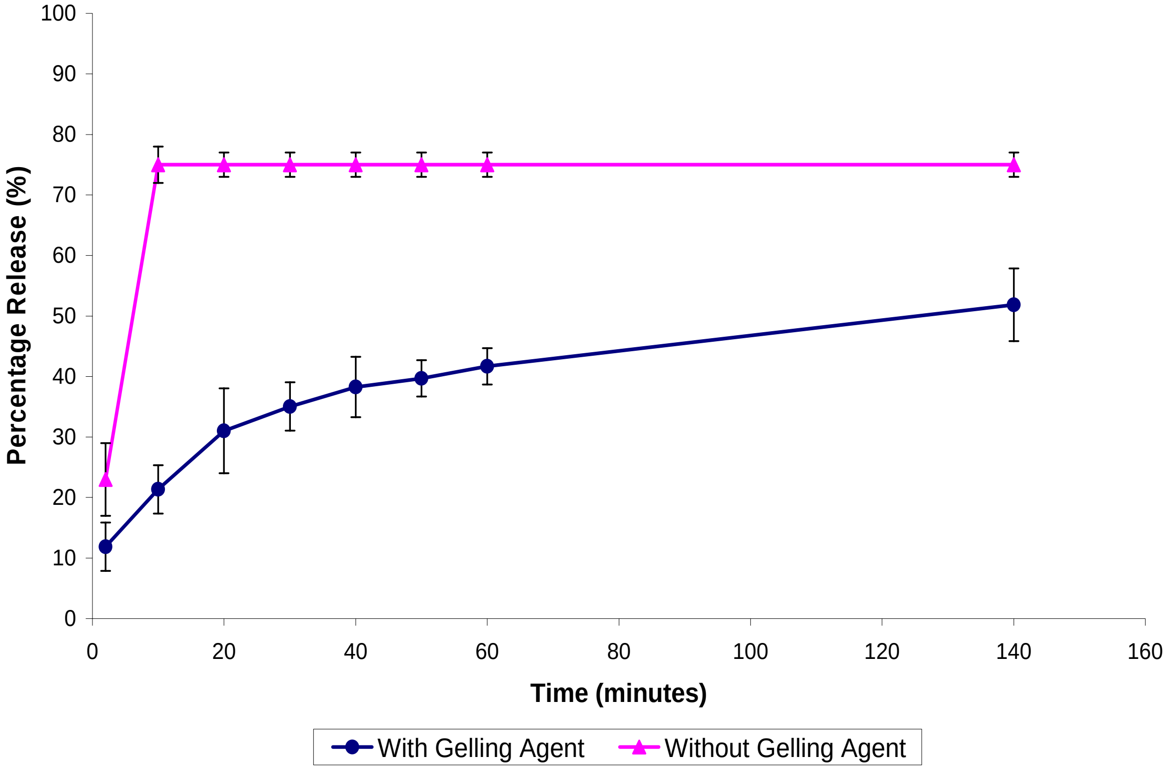


Figure 4.44 – Effect of Hydrophilic Gelling Agent on Drug Release Rate (n=6)

During the run it was found the drug release did not reach the expected 100%. This was found to be the case for each run where the release profile reached a maximum of approximately 80% (Figure 4.44). It was thus determined that the 100% release was not obtained due to some form of drug interaction in the formulation. It is possible the propranolol which is a cationic drug may have formed a complex with the polycarbophil as this is an anionic polymer. However as described in Section 4.4b, initial DSC results had indicated interactions between the drug and polymer did not exist. The DSC experiment was repeated but with addition of water to the system to determine if the presence of water in the formulation was responsible for the interaction. The introduction of water proved to have an effect (Section 4.4b) indicating an interaction between the polycarbophil and the drug may have occurred. To ensure the maximum release obtained was not due to an artefact of incomplete incorporation of the correct amount of drug, a drug release experiment was conducted without the polycarbophil in the continuous phase. In this case, 100% release of the drug was observed. A DSC analysis of the formulation (where 100% release was not observed) remaining in the glass tube at the end of the *in vitro* release experiment was carried out. The peak at 146°C was found indicating an interaction may be occurring and thus why full release of the drug was not observed.

The effect of changing the gelling agent to an anionic polymer (sodium CMC and xanthan gum) was also investigated as discussed in Section 4.5b *x*.

iii. Basket Method (USP Apparatus I)

The basket method was investigated (see Section 3.5a *iii* in Chapter 3 for experimental details) but proved not ideal as introduction of the formulation into the basket posed problems. This was because the basket must be held in the dissolution test medium when injecting the formulation to allow the formulation to gel upon contact with water. However, this was not successful as the formulation did not gel instantaneously upon contact with the dissolution test medium and consequently the formulation ran out through the pores in the basket and slowly settled towards the bottom of the vessel. Upon stirring, the formulation became dispersed and resembled the dissolution profile of the formulation when it was injected from above the vessel (Figure 4.43). The basket method also proved disadvantageous in regard to agitation of the gel depot. As the basket was spinning around, the gel depot was also spinning around thus increasing chances of disturbing the depot. This caused the pores in the basket to become clogged with the gelling agent ultimately affecting the diffusion of the drug from the gel system. As a result, the release profile using the basket was variable (Figure 4.45). It was thus abandoned as a viable method.

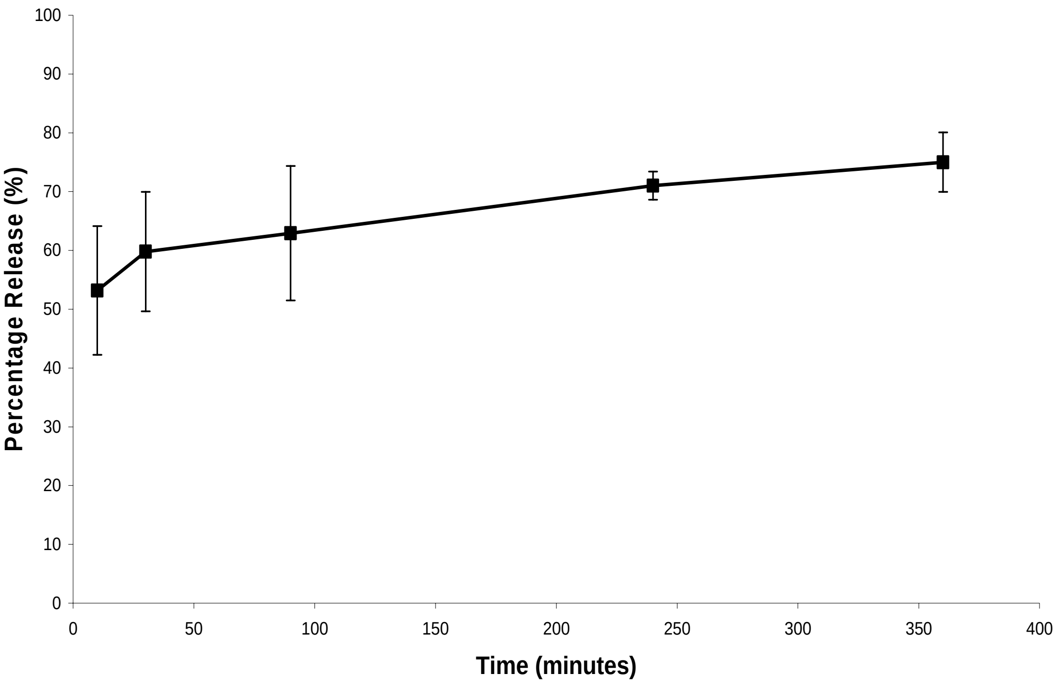


Figure 4.45 – Drug Release Experiment Conducted using the Basket Method (n=6)

The rotating cylinder (Apparatus 6) is used for the assessment of drug release from transdermal patches (USP, 1994). The difference between this apparatus and the basket is that the basket is replaced with a steel cylinder. With a rotating cylinder, the adhesive side of the transdermal patch is placed on a piece of cuprophan (membrane). The system is then attached onto the exterior of the cylinder which is then placed into the dissolution test medium. This method would not allow the system to have a constant surface area and the system would be disturbed by the rotating motion of the cylinder.

iv. Reproducibility of Release Trials Conducted on the Hanson Dissolution Apparatus

As a result of the above trials using the Hanson dissolution apparatus, it was obvious that this approach of investigating *in vitro* release was proving unsuccessful as results were not reproducible (where each run involved 6 replicates to check the consistency of the results). There was a significant observed variation for intra-day trials (Figure 4.46). Injecting the formulation directly into the vessel was not regarded as an ideal method to conduct *in vitro* trials as the propensity for the ISGM system to behave unpredictably is far greater due to the actions of the stirrer causing varying surface area of the depot generated.

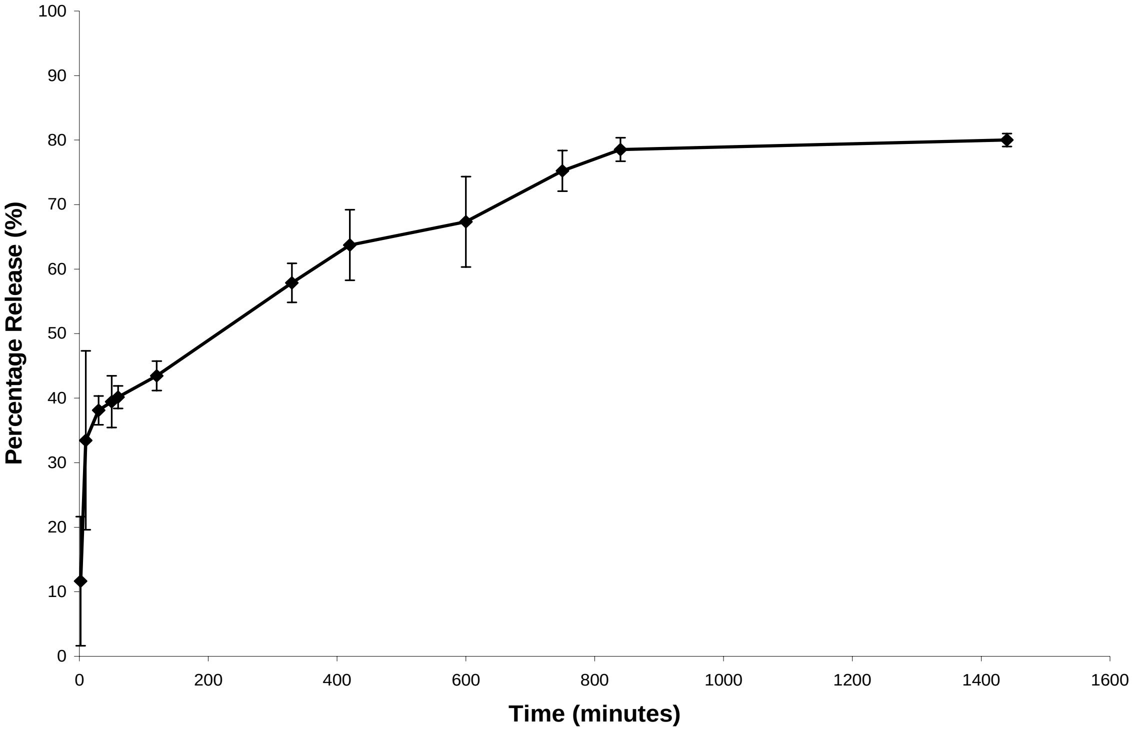


Figure 4.46 – Intra-Day Reproducibility using the Hanson Dissolution Apparatus (Mean Curve of 6 Samples)

There are numerous reports in the literature indicating the considerable level of variability and unpredictability in dissolution profiles where a stirrer has been used (McCarthy *et al.*, 2004; Kukura *et al.*, 2004; Cox *et al.*, 1982; McCormick, 1995; Moore *et al.*, 1995). The mass transfer in a stirred system will be dependent on the fluid flow and hydrodynamics.

With dissolution testing many solid dosage forms will disintegrate into smaller fragments and particles. These particles will continually decrease in size during the course of the dissolution, thus changing the surface area from which the drug can diffuse.

Cammarn and Sakr (2000) reported differing dissolution rates with differing size of the dosage form. They attributed this to the increased linear velocity around the dosage form and an increased cross-sectional area blocking the flow. Therefore the shape of the dosage form (i.e. length, thickness and width) will affect the dissolution profile. Injection of the formulation did not give consistent shapes, adding to the variability of the release profile.

The uncontrolled variability in the dissolution test may also be a result of the hydrodynamic factors for example, fluctuations in flow introduce random variations in factors such as de-agglomeration of particles and mass transfer (Kukura *et al*., 2004).

A different method of conducting *in vitro* trials was thus required. Any new method should ensure the surface area of the exposed depot to the dissolution test medium remains fixed over the period of the release experiment. The injected formulation must also remain isolated from the stirrer to prevent spurious hydrodynamic factors influencing the release.

4.5b Drug Release Studies Conducted on the Modified Dissolution Apparatus

As mentioned in Section 4.5a, the Hanson Dissolution Apparatus was not an ideal means of carry out drug release experiments. There was variation in the results as a result of the depot being exposed to the effects of the stirrer. Furthermore, there were issues regarding the surface area of the depot, where the surface area did not remain fixed during the release experiment as a result of swelling.

A new means of carrying out drug release studies was therefore required. Initially dialysis membranes with clips on either end were investigated (but not used any further). The formulation was injected into a dialysis bag and the ends of the bag were sealed off with plastic clips. The dialysis bag was then placed into the dissolution test medium. In this case, though the surface area of release remained fixed, there was no space to allow the gel to expand as a result of swelling. Therefore this approach was not ideal.

A dialysis membrane enclosed by a plastic frame was also investigated. This provided sufficient room for the gel to swell, but, had the drawback that the surface area did not remain fixed. This approach was also abandoned.

Clearly the dialysis membrane would be an ideal means to carry out release as it has the potential to maintain a fixed surface area. However, accommodation for swelling is needed so that the depot can expand. Therefore a novel means of carrying out release was developed by having a glass tube with a dialysis membrane at one end. The injected formulation would have a fixed release interface and at the same time the glass tube would allow for swelling in the axial direction only.

As a result, in this part of the study *in vitro* drug release studies were conducted on a modified dissolution apparatus (see Figures 3.7a and 3.7b in Chapter 3). The modified dissolution apparatus allowed the formulation to be "protected" from the stirrer (paddle) and the apparatus also ensured the drug diffusion site had a fixed surface area.

i. Reproducibility of Release Trials Conducted on the Modified Dissolution Apparatus

Both intra- and inter-day trials produced reproducible release profiles (Figures 4.47 and 4.48) over the 6 replicates for each of the 3 runs separate runs. 100% release of the drug was not observed (as discussed in Section 4.5a *ii*).

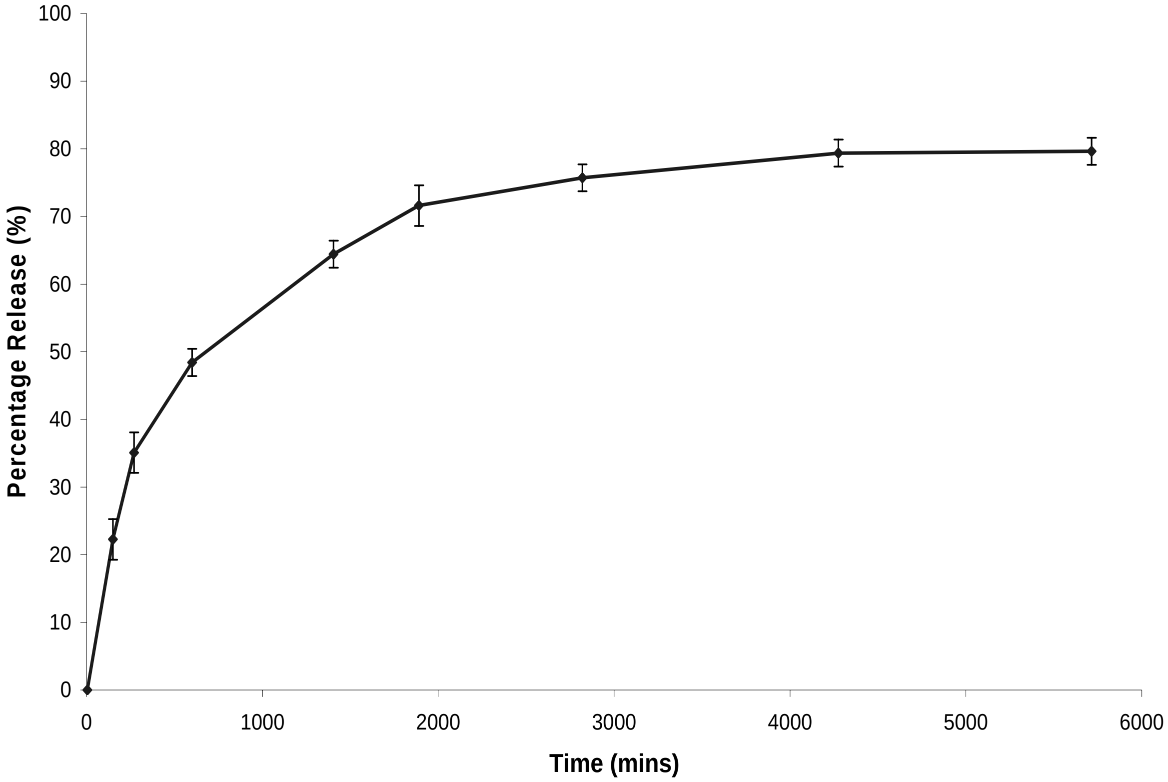


Figure 4.47– Intra-Day Reproducibility using the Modified Dissolution Apparatus (Mean Curve of 6 Samples)

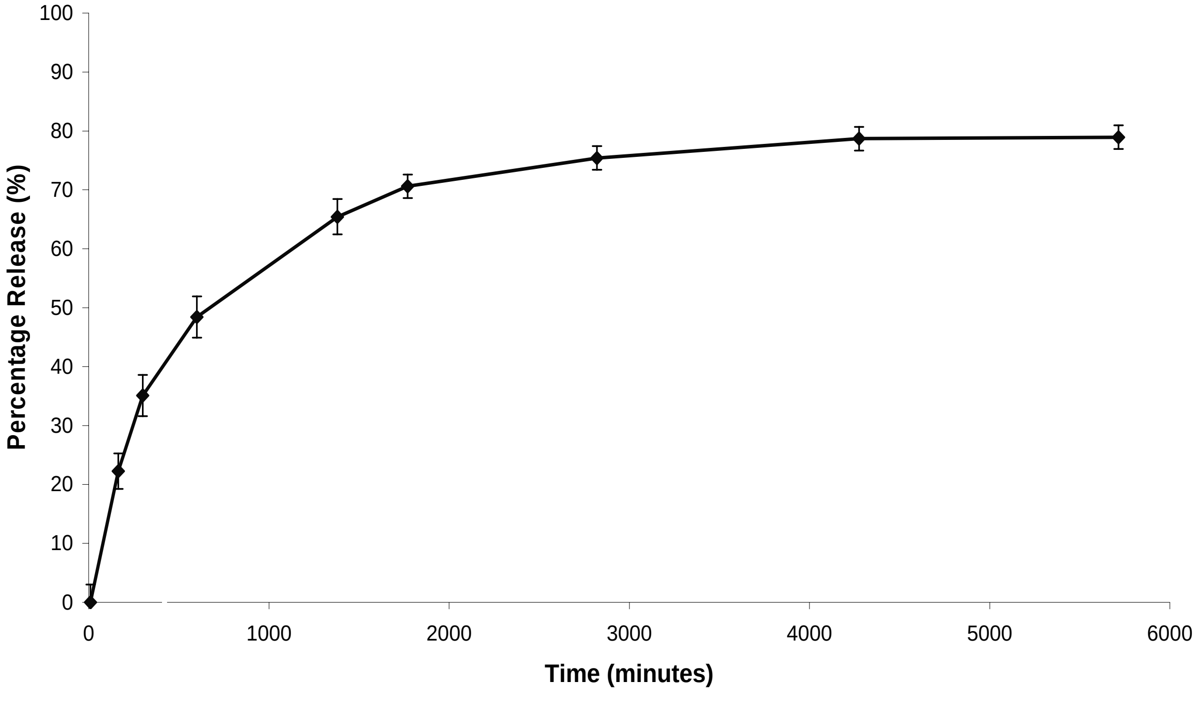


Figure 4.48 – Inter-Day Reproducibility using the Modified Dissolution Apparatus (Mean Curve of 6 Samples)

ii. Effect of the Hydrophilic Gelling Agent

As expected the presence of the gelling agent markedly reduced the drug release rate (as described in Section 4.5a *ii*) (Figure 4.49).

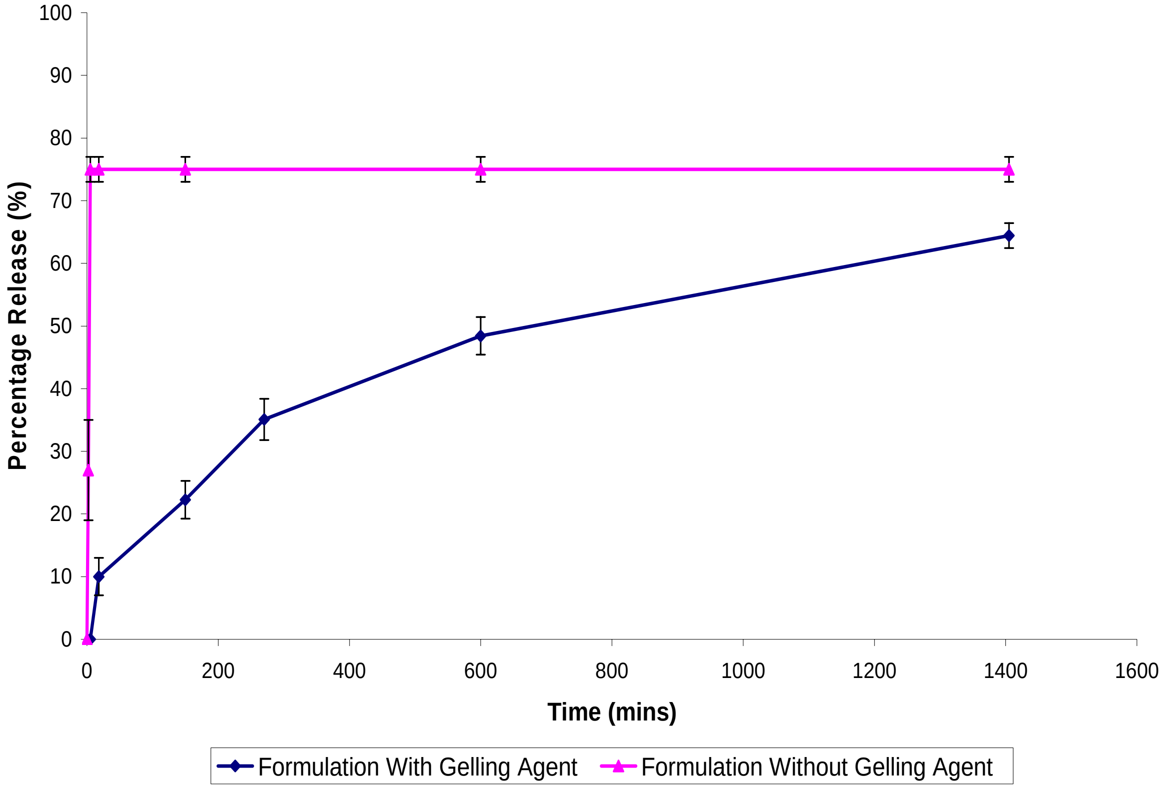


Figure 4.49 – The Effect of the Hydrophilic Gelling Agent on the Drug Release Profile (n=6)

It was at this stage after developing the new modified dissolution apparatus and confirming its inter- and intra-day reproducibility with respect to drug release to a satisfactory standard, that subsequent release experiments were carried out for approximately 600 minutes. This was chosen because at this stage, approximately 50% of the drug had been released (Figure 4.48). This was considered adequate to define the profile and allow comparisons between different profiles run under different conditions. This time period was also ideal for the modelling of the release data conducted in Section 4.5c as modelling of release is generally conducted up to 60% release (Nam *et al*., 2004; Perez-Marcos *et al*., 1996).

iii. Effect of Volume of Water in Tube

The addition of water to the glass tube prior to formulation introduction was investigated to determine whether the formulation in a pre-gelled state had any effect on drug release rates. In the case of a depot where water was not introduced, the gel progressively formed as water diffused into the depot from the dissolution test medium. It was apparent that the more water that was added, the slower rate of diffusion of the drug within the gel to the surface from which it can be released (Figure 4.50).

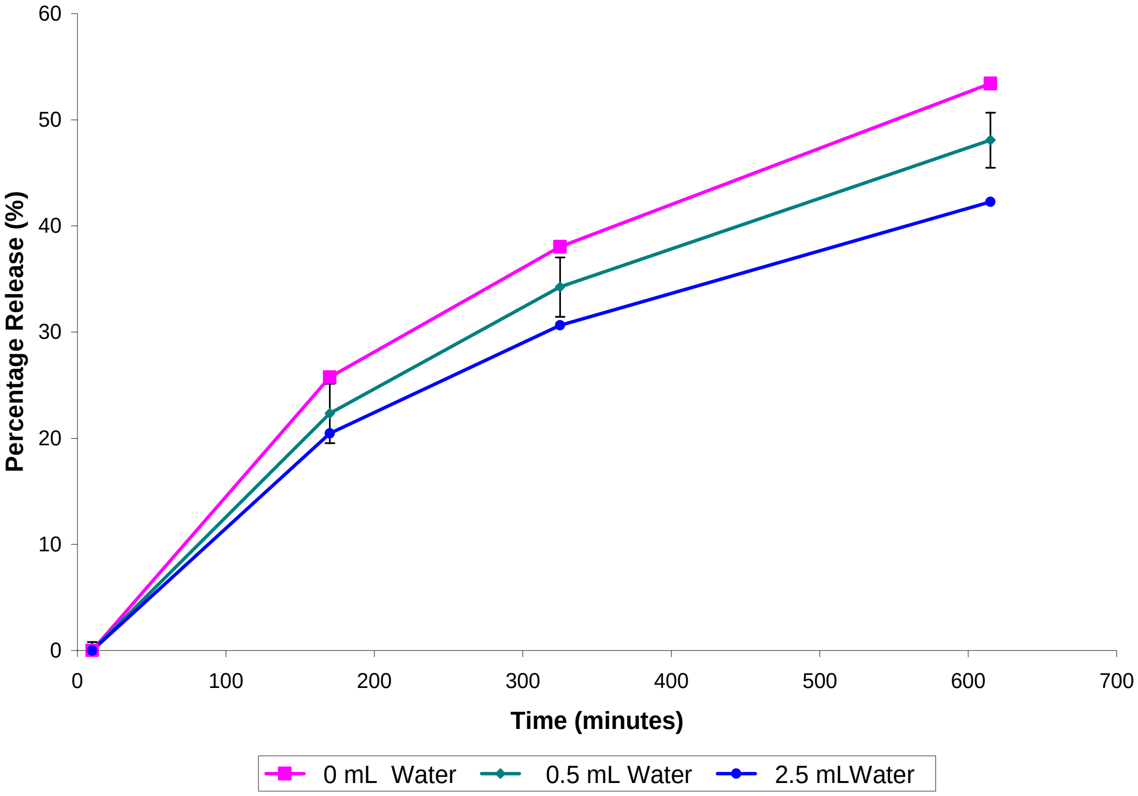


Figure 4.50 – Effect of Volume of Water in Tube on Drug Release (n = 6)

The addition of water prior to release resulted in the depot having a larger volume than that for the depot where water was not added before release. Consequently, the drug had a greater distance to travel before it reached the diffusion site (i.e. the dialysis membrane). Therefore the primary difference between the two systems was the volume of the depot during the release of the drug. The more water added to the system prior to release, the greater the volume of the depot, the further the drug had to diffuse to reach the release interface, and the slower rate of diffusion of the drug within the gel to the surface from which it can be released.

It is also apparent in the release profiles that drug release was not instantaneous. The observed lag time may be due to the time taken for the water to diffuse into the formulation and for the gelling agent to hydrate.

iv. Effect of Tube Height

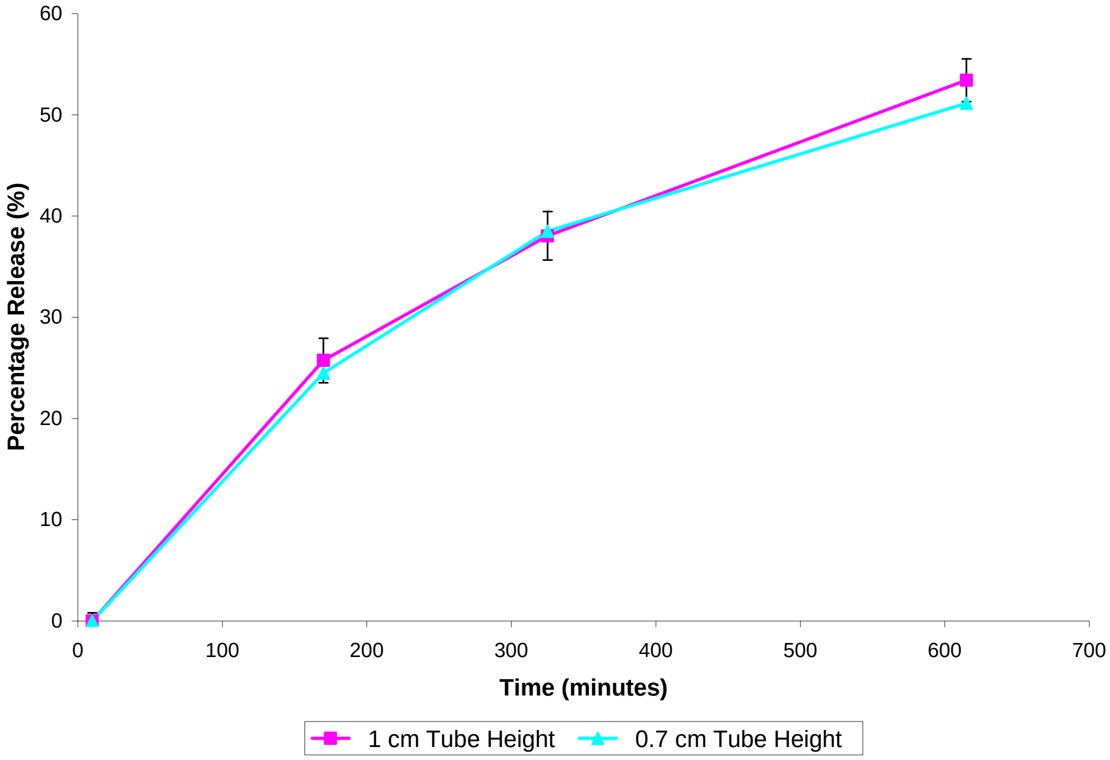


Figure 4.51 – Effect of Tube Height on Drug Release (n = 6)

Altering the height of the tube containing the formulation was investigated to determine whether a change in the distance between the stirrer and the diffusion surface affected the drug release rate. Ideally, the distance should not affect the release as the stirrer is merely employed to homogenise the test system and thus the position of the donor chamber should not matter. This was observed to be the case (Figure 4.51) as the slope of the release curves was constant with height of the tube.

Another means of assessing the "closeness" or similarity of the dissolution profiles is by calculating the difference factor between the two curves (Moore and Flanner, 1996). This method calculates a difference factor f_1 (Equation 4.4) and similarity factor f_2 (Equation 4.5) to compare the dissolution profiles.

The difference factor f_1 represents the percentage error between the two curves. If the percentage error is calculated to be zero then the two dissolution profiles can be said to be equal. The dissolution profiles can be said to be similar if f_1 is in the range of 0-15.

$$f_1 = \frac{100\sum_{t=1}^{n}\left|R_t - T_t\right|}{\sum_{t=1}^{n} R_t} \tag{4.4}$$

where

f1 = difference factor

n = number of points

R_t = reference assay at time point t

T_t = test assay at time point t

The similarity factor f_2 is the logarithmic transformation of the sum of the squared error and represents the similarity between the two curves. If f_2 is found to be 100 then the two dissolution profiles can be said to be equal. For two dissolution profiles to be similar, the f_2 value must be in the range of 50 - 100.

$$f_2 = 50\log\left[\frac{100}{\sqrt{1+\frac{1}{n}\sum_{t=1}^{n}\left(R_t - T_t\right)^2}}\right] \tag{4.5}$$

where

f_2 = similarity factor

n = number of points

R_t = reference assay at time point t

T_t = test assay at time point t

Consequently though a graphical representation of the dissolution profiles may show how similar the curves are, it can cause problems in regards to quantitating the closeness. Therefore, this comparative mathematical tool, allows one to comment on the similarity between the curves.

As mentioned earlier in this section, the effect of the height of the tube did not appear to have an effect on the release profile (Figure 4.51). This was tested using the mathematical model to calculate the difference factor f_1 and the similarity factor f_2 (Table 4.11) (the reference assay was the 0.7 cm tube height).

Table 4.11 - f_1 and f_2 Values for Effect of Tube Height

f_1	f_2
3.38	89.16

Consequently, because the difference factor f_1 is close to 0 and the similarity factor is in the accepted range, the two dissolution profiles for the effect of tube height can be said to be similar.

v. Stirring Rate

The effect of stirring speed on the release rate of propranolol is shown in Figure 4.52. Increasing the stirring speed from 50 to 100 to 150 RPM resulted in slightly faster drug release as expected. Moore *et al.* (1995) investigated various stirring speeds from 20 – 80 RPM and found increasing stirring rates gave a faster release rate. They attributed the faster release to the thickness of the boundary layer at the surface of the gel which had formed due to the accumulation of disentangled micelles. The thickness of this boundary layer decreased with increasing stirring speeds and the disentangled micelles accumulating at the gel surface would be removed faster at the higher stirring speeds. The drug was thus released at a faster rate. In the current case the propranolol system is enclosed by a membrane but there will be a static boundary layer on the dissolution test medium side of the dialysis membrane leading to non-zero drug concentrations at the interface.

Increased stirring rates may “thin” this boundary layer and therefore increase the release rate as observed.

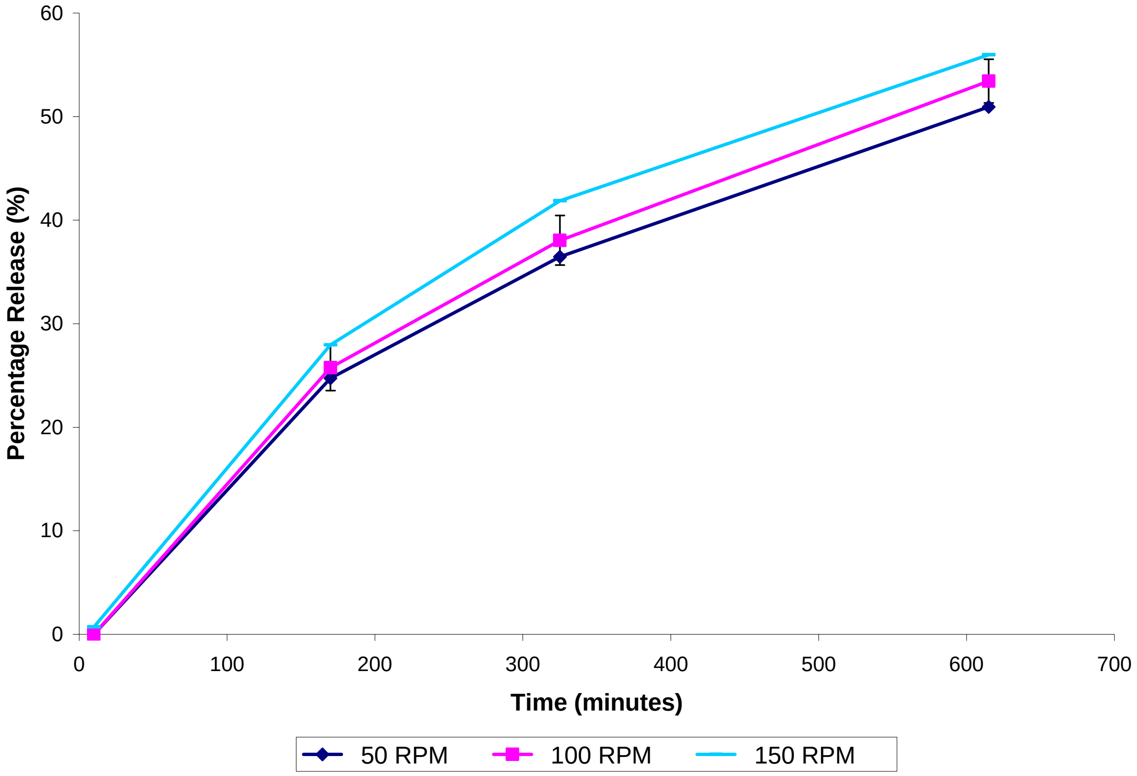


Figure 4.52 – Effect of Stirring Rate on Drug Release (n = 6)

The similarity and difference factors between profiles (the reference assay was 100 RPM) were calculated using Equations 4.4 and 4.5 and the values are shown in Table 4.12.

Table 4.12 - f_1 and f_2 Values for Effect of Stirring Rate

Stirring Rate	**f_1**	**f_2**
50 RPM	4.32	86.67
150 RPM	7.94	77.92

The dissolution profiles for each stirring rate can be said to be similar. However, it can be seen that the increased stirring rate of 150 RPM resulted in the profile being less similar than the profile at 50 RPM (but can still be regarded as similar as the values are within the ranges specified in Section 4.5b *iv*).

vi. Effect of pH of the Dissolution Test Medium

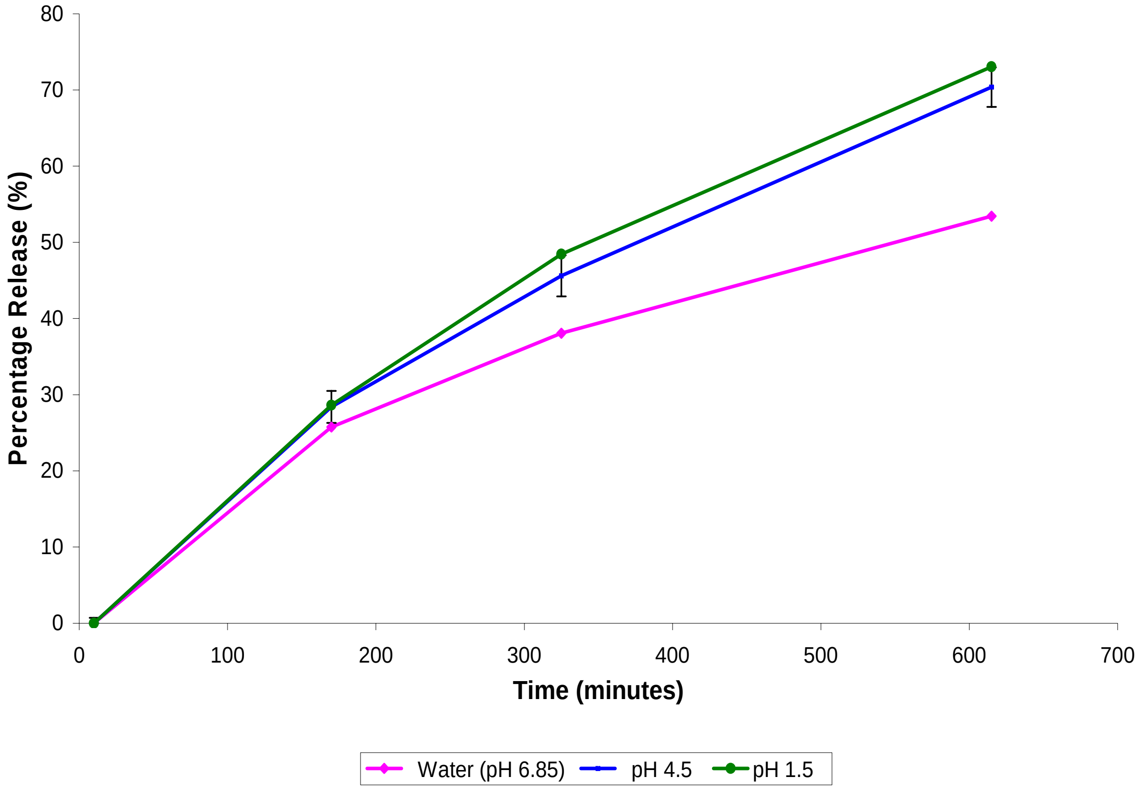


Figure 4.53 – Effect of pH of Dissolution Test Medium on Drug Release (n = 6)

An effect on drug release was observed when the pH of the dissolution test medium was altered (Figure 4.53). The constituents of the final formulation which may be affected by the pH are the drug propranolol and the viscosity inducing agent polycarbophil, so, pH may play a decisive role in the drug release capability of the formulation as both possess acid-base properties. Upon investigation of the pH effect, it was found the lower pH resulted in faster release. It has been reported (Taylan *et al*., 1996; Wang *et al*., 2003) that interactions between propranolol and anionic polymers such as polyacrylic acids (for example polycarbophil) can occur. HPMC is not affected by pH (Perez-Marcos *et al*., 1996). The propylene glycol should not affect drug release as it is a non-ionic compound. Hence, the change in release rate is likely due to the polycarbophil and/or drug. Polymers such as polycarbophil which have a carboxylic group are affected by the pH (Wang *et al*., 2003).

For instance, at pH 1 the polycarbophil would almost totally be non-ionised as the pKa of polycarbophil is 4.3 (Bologna and Levine, 2000). The polycarbophil will therefore not hydrate and release of the drug will be almost completely controlled by the HPMC (the compact nature of the dosage form may also retard the release rate). Full release of the drug should be however observed as the polycarbophil being in its non-ionised form will not interact with the drug as it does in the ISGM system and this was observed to be the case.

The Henderson-Hasselbalch equation (Equation 4.6) is used to calculate the pH and also the extent of ionisation (Martinez and Amidon, 2002; Driscoll, 2003; Ansel and Popovich, 1990). The Henderson-Hasselbalch equation was thus rearranged to calculate the percentage ionised (Equation 4.7).

$$\mathrm{pH} = \mathrm{pKa} + \log \frac{[\mathrm{salt}]}{[\mathrm{acid}]} \tag{4.6}$$

$$\mathrm{pH} = \mathrm{pKa} - \log \frac{[\mathrm{acid}]}{[\mathrm{salt}]}$$

$$\mathrm{pKa} - \mathrm{pH} = \log \frac{[\mathrm{acid}]}{[\mathrm{salt}]}$$

$$10^{(\mathrm{pKa}-\mathrm{pH})} = \frac{[\mathrm{acid}]}{[\mathrm{salt}]}$$

$$[\mathrm{salt}]10^{(\mathrm{pKa}-\mathrm{pH})} = [\mathrm{acid}]$$

$$\text{Fraction of } [\mathrm{salt}] + [\mathrm{acid}] = 1$$

$$[\mathrm{salt}] = 1 - [\mathrm{acid}] \qquad\qquad [\mathrm{acid}] = 1 - [\mathrm{salt}]$$

$$(1 - [\mathrm{acid}])10^{(\mathrm{pKa}-\mathrm{pH})} = [\mathrm{acid}] \qquad\qquad [\mathrm{salt}]10^{(\mathrm{pKa}-\mathrm{pH})} = 1 - [\mathrm{salt}]$$

$$10^{(\mathrm{pKa}-\mathrm{pH})} - [\mathrm{acid}]10^{(\mathrm{pKa}-\mathrm{pH})} = [\mathrm{acid}] \qquad\qquad [\mathrm{salt}]10^{(\mathrm{pKa}-\mathrm{pH})} + [\mathrm{salt}] = 1$$

$$[\mathrm{acid}](1 + 10^{(\mathrm{pKa}-\mathrm{pH})}) = 10^{(\mathrm{pKa}-\mathrm{pH})} \qquad\qquad [\mathrm{salt}](10^{(\mathrm{pKa}-\mathrm{pH})} + 1) = 1$$

$$[\mathrm{acid}] = \frac{10^{(\mathrm{pKa}-\mathrm{pH})}}{1 + 10^{(\mathrm{pKa}-\mathrm{pH})}} \qquad\qquad [\mathrm{salt}] = \frac{1}{1 + 10^{(\mathrm{pKa}-\mathrm{pH})}}$$

$$\%\,\text{Ionised} = \frac{[\text{salt}]}{[\text{acid}] + [\text{salt}]}$$

$$\%\,\text{Ionised} = \frac{\frac{1}{1+10^{(\text{pKa}-\text{pH})}}}{\frac{10^{(\text{pKa}-\text{pH})}}{1+10^{(\text{pKa}-\text{pH})}} + \frac{1}{1+10^{(\text{pKa}-\text{pH})}}}$$

$$\%\,\text{Ionised} = \frac{\frac{1}{1+10^{(\text{pKa}-\text{pH})}}}{\frac{10^{(\text{pKa}-\text{pH})}+1}{1+10^{(\text{pKa}-\text{pH})}}}$$

$$\%\,\text{Ionised} = \frac{\frac{1}{1+10^{(\text{pKa}-\text{pH})}}}{1}$$

$$\%\,\text{Ionised} = \frac{1}{1+10^{(\text{pKa}-\text{pH})}} \qquad (4.7)$$

It should be noted that this formula when used to calculate the percentage of ionisation for an acid, is strictly only applicable to monoprotic acids. Polycarbophil is a polymer with more than one carboxylic group and each of these groups are separated by a methylene group. Therefore polycarbophil can be described as a polyprotic acid. As mentioned earlier in this section, the pKa of polycarbophil was reported to be 4.3 (Bologna and Levine, 2000). However this is only an estimate and not an accurate value due to the polycarbophil being polyprotic. The calculation of the pKa for a polyprotic acid is complex as when one carboxylic group comes off, it will affect the pKa of the remaining protonated groups on the polymer. Consequently each time a carboxyl group is removed, the pKa will change. Therefore the weakness of this equation is that it can only be taken at best as an estimation of the percentage ionised.

At pH 4.5 approximately 60% of the polycarbophil will be ionised (calculated according to Equation 4.7). Therefore there will be some interaction between the drug and polycarbophil. The pH 4.5 dissolution test medium was created using phosphate buffer. It has been reported (Mitchell *et al.*, 1990; Perez-Marcos *et al.*,

1996) the presence of phosphate ions can retard the hydration of HPMC leading to an increased release rate. This factor may explain why the release rate of the drug at 1 and 4.5 were closer than expected due to the additional effect of phosphate.

When distilled water was employed as the dissolution test medium, the polycarbophil will be 99% ionised. Therefore the polycarbophil will hydrate and retard the release of the drug as well as interacting with the drug.

The formation of complexes between cationic drugs and anionic polymers is documented (Wang *et al.*, 2003). Propranolol can form an anionic-cationic complex with polyacrylic acid affecting the drug release from the matrix (Perez-Marcos *et al.*, 1994; Perez-Marcos *et al.*, 1996; Wang *et al.*, 2003) as explained in Section 4.4b. This interaction would explain the observation of the inability of the formulation to release the total amount of the drug. This was confirmed by conducting a DSC analysis of the formulation remaining in the glass tube at the end of the *in vitro* release experiment (Section 4.4b). A peak at 146°C was found indicating there may be an interaction occurring.

Propranolol is a weakly basic drug with a pKa of 9.5 (see Table 4.9). Although the dissolution of the drug into the distilled water (pH = 6.85) will lead to the dissociation of some propranolol-H+ into free propranolol and H+ which would reduce the pH (measured pH = 6.5), the dissociation is negligible so that the equilibrium will lie mainly to the left, i.e. the drug will exist mainly in its protonated form when dissolved in the distilled water.

The graphical representation of the dissimilarity of the dissolution profiles (the reference assay was pH 6.85) was verified by calculating the similarity and difference factors using Equations 4.4 and 4.5.

Table 4.13 - f_1 and f_2 Values for Effect of pH on the Dissolution Test Medium

pH	**f_1**	**f_2**
4.5	23.18	49.98
1	28.07	47.46

The release profiles at each pH were different from each other especially for the case where a pH 1 dissolution test medium was used. This was also reflected in the f_1 and f_2 values (Table 4.13) which indicated the profiles were dissimilar from each other - more so in the case for the pH 1 dissolution test medium.

vii. HPMC (Gelling Agent) Concentration

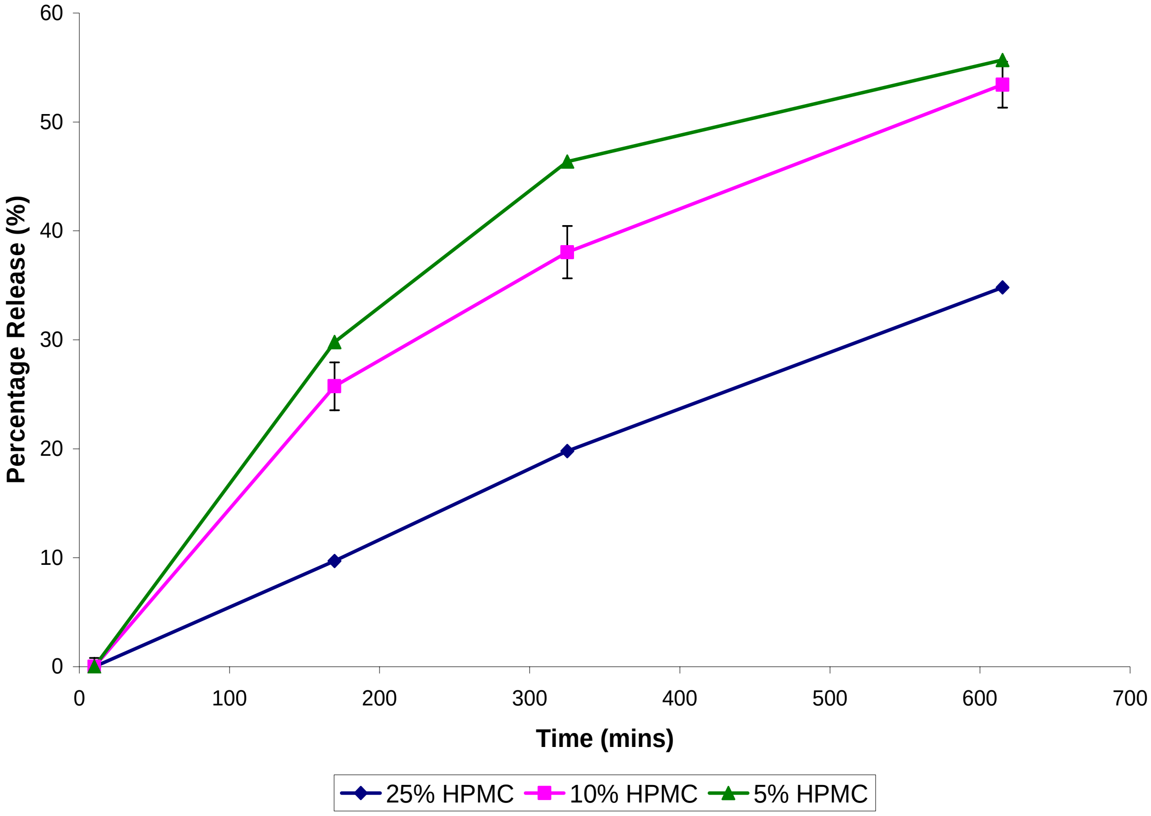


Figure 4.54 – Effect of HPMC Concentration on the Drug Release Profile (n = 6)

Increasing the hydrophilic gelling agent concentration should decrease the drug release rate (Reza *et al*., 2003; Tapia-Albarran and Villafuerta-Robles, 2004) which was proven to be the case as shown in Figure 4.54. A higher HPMC concentration resulted in a thicker gel slowing the diffusion of the drug. The appearance of the suspension prior to injection and at the end of experiments was recorded as shown in the Table 4.14.

Table 4.14 – Effect of HPMC Concentration on the State of the Formulation Prior to Injection and at Completion of the Experiment

HPMC Concentration (% w/w)	**Observation**	
	Prior to Injection	**Completion of Experiment**
5	Very fluid	Watery depot
10	Fluid	Gelled depot
25	Thick. Barely fluid.	A thick unmoveable depot

At the completion of each experiment the depot was poured out to observe its fluidity. The 5% HPMC formulation poured out with ease much like water. This indicated a very weak gel had formed, consistent with the fast release of the drug from this system. With 10% HPMC, the depot was more viscous, with a slower release rate. The 25% HPMC formulation could not be poured as it was very thick and had a much slower release rate than the 5% HPMC formulation.

The Moore and Flanner Equations (Equations 4.4 and 4.5) were used to calculate the difference and similarity factors (Table 4.15) (the reference assay was 10% gelling agent concentration).

Table 4.15 - f_1 and f_2 Values for Effect of Gelling Agent Concentration

Gelling Agent (HPMC) Concentration	**f_1**	**f_2**
5%	12.48	65.66
25%	45.14	40.71

In the case of the formulation where a 5% gelling agent was used, the release profile can be said to be just within the range of being similar. The difference factor f_1 was found to be 12.48 (accepted range 0-15% - as discussed in Section 4.5b *iv*) and the similarity factor f_2 65.66 (accepted range 50 - 100). The difference is more apparent in the graphical representation of the profiles (Figure 4.54). When the gelling agent concentration was increased to 25%, it resulted in an f_1 value of 45.14 and an f_2 value of 40.71 (Table 4.15). This strongly indicated that the two release profiles were indeed very different.

viii. Polycarbophil (Viscosity Inducing Agent) Concentration

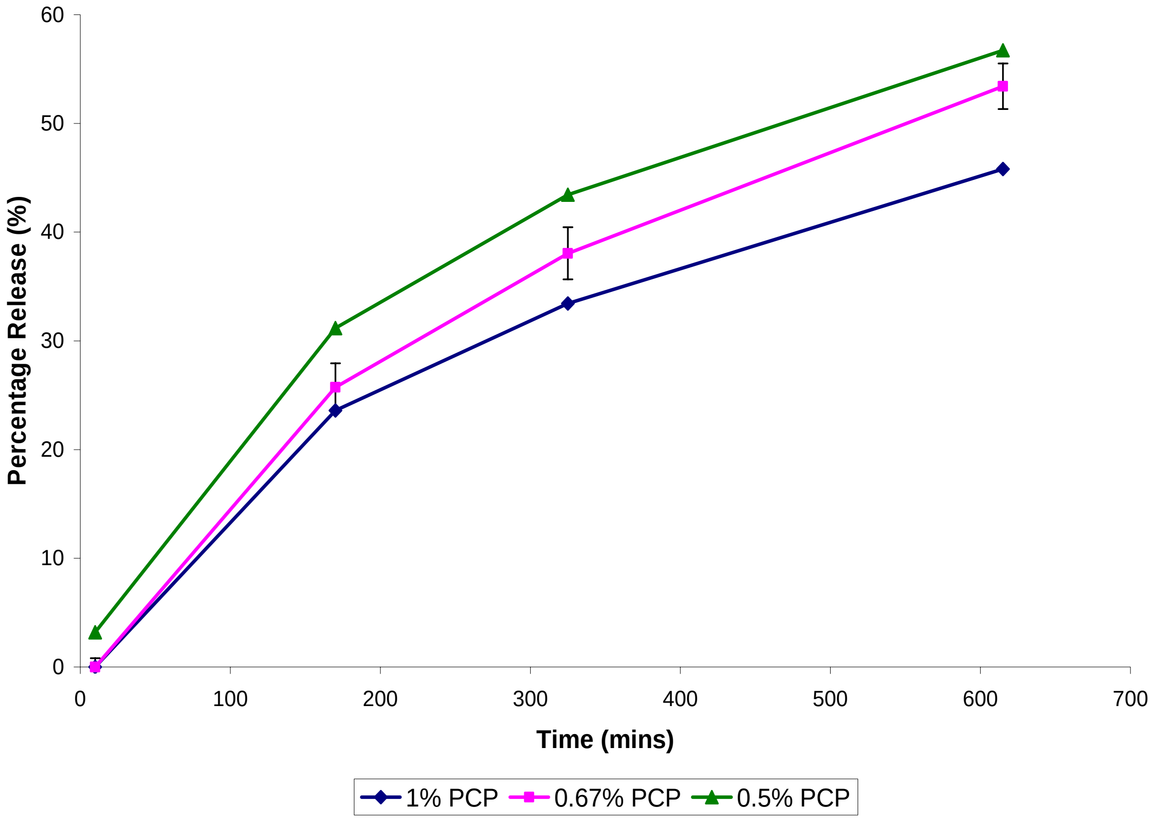


Figure 4.55 – Effect of Polycarbophil Concentration on the Drug Release Profile (n = 6)

As expected, increasing the polycarbophil concentration resulted in a slower rate of release of the drug (Figure 4.55). Polycarbophil is a hydrophilic polymer and therefore will also thicken in the presence of water. Therefore increasing amounts of polycarbophil will result in a more viscous gel (due to hydration of the hydrophilic polymer) thereby decreasing the drug release rate. In the case of the formulation containing 0.5% polycarbophil, a lag time was not observed as a less viscous gel had formed.

The similarity and difference factors were similar (just within the accepted range discussed in Section 4.5b *iv*) to one another between the release profiles (Equations 4.4 and 4.5 and Table 4.16) (the reference assay was 0.67%

polycarbophil). The differences between the profiles were more apparent in the graphical representation (Figure 4.55).

Table 4.16 - f1 and f_2 Values for Effect of PCP Concentration

PCP Concentration	**f_1**	**f_2**
0.5%	14.78	67.00
1%	12.26	66.46

ix. *HPMC Viscosity Grade*

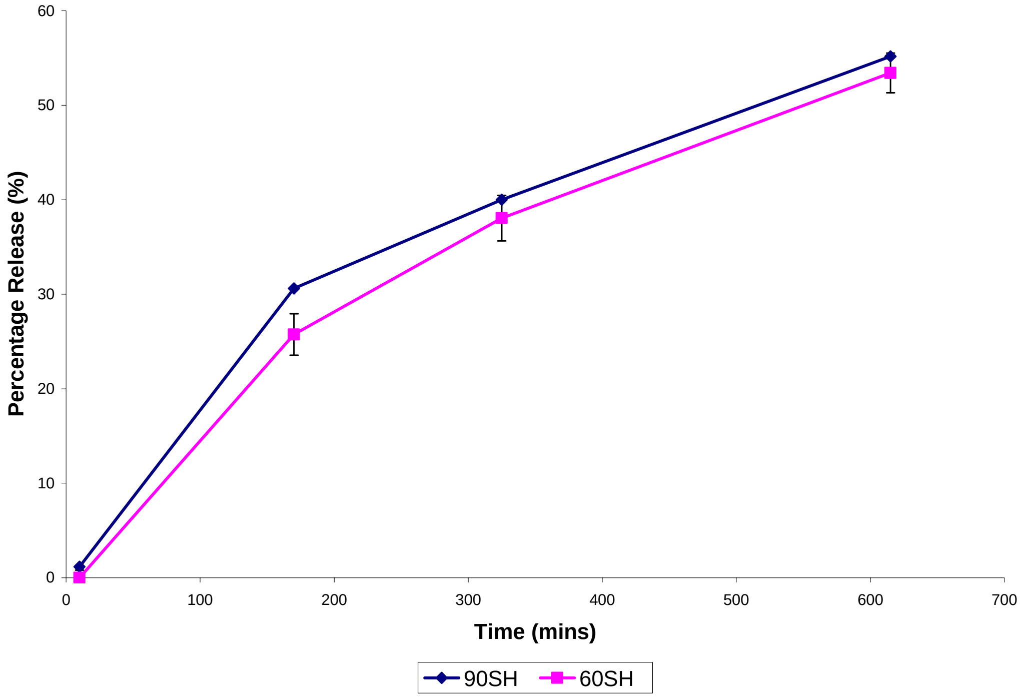


Figure 4.56 – Effect of HPMC Grade on the Drug Release Profile (n = 6)

The effect of the viscosity grade of the HPMC on the drug release rate was investigated. HPMC 90SH has a higher viscosity than HPMC 60SH (for an equivalent amount of material dispersed in the formulation which gels). The graph (Figure 4.56) shows the higher viscosity grade HPMC resulted in a marginally higher drug release (though the increase was close to the margin of error), contrary to what was predicted, as the high viscosity grade HPMC should give a slower release. Upon initial contact with water, HPMC 90SH should be more viscous than HPMC 60SH thus producing a thicker gel and hindering the release of the drug. However as shown in the profile above (Figure 4.56) this was not the case. Huang *et al.* (2004) conducted a full release profile on 3 viscosity grades of HPMC: 4 000, 15 000 and 30 000 (in HPMC matrices tablets). Their results were in agreement with the findings of this study where the initial release was faster for the higher viscosity grade. They attributed the faster release of the drug in the higher viscosity grade (during the initial release process) to the lack of ease (compared to the low viscosity grade) that the higher viscosity grade spread over the matrix surface. This had the effect of leading to a brief period of enhanced dissolution until the higher viscosity grade HPMC began to have a more widespread effect on viscosity. They also found that as the release progressed the lower viscosity grade HPMC ultimately gave a faster release. The slower release for the higher viscosity grade would be due to the more viscous, thicker and stronger hydrogel structure that eventually formed (Huang *et al.*, 2004; Zuleger and Lippold, 2001). Therefore the higher viscosity grade HPMC initially gives a faster release but with time slows down as the strong hydrogel network forms to retard the drug release. In Figure 4.56, it can be seen that the slope of the release curve for 90SH drops more than 60SH after approximately 170 minutes indicating with time the release rate for 90SH may decrease to below that of 60SH.

Table 4.17 - f1 and f_2 Values for Effect of HPMC Viscosity Grade

f_1	**f_2**
8.34	76.12

The release profile (Figure 4.56) is shown to be similar as shown in the results of the calculated values of f_1 and f_2 (Table 4.17) using Equations 4.4 and 4.5 (the reference assay was 60SH).

x. Different Gelling Agents

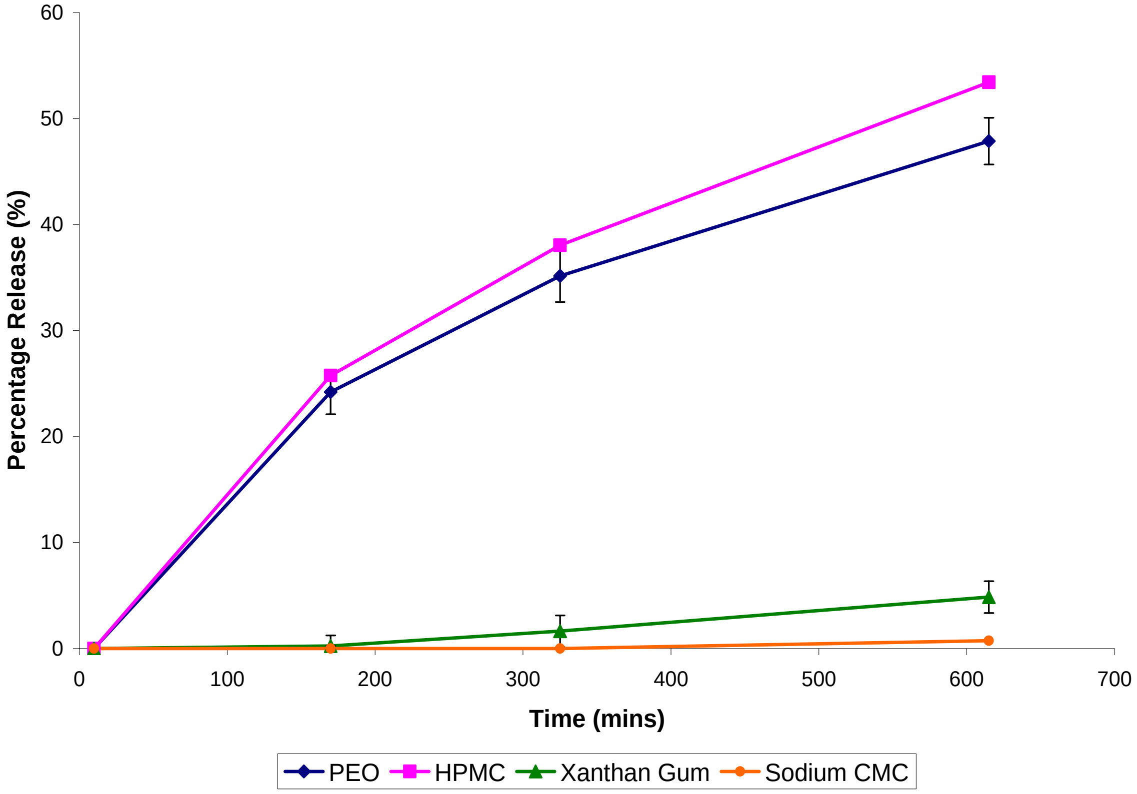


Figure 4.57 – Effect of the Type of Hydrophilic Gelling Agent in the Final Product on the Drug Release Profile (n = 6)

Drug release studies were conducted on a formulation containing a different hydrophilic gelling agent (all at the same concentration of 10%) to observe how the release profile would change. It was observed earlier (Section 4.5a *ii*) full release of the drug did not occur as the propranolol was interacting with the anionic polycarbophil (viscosity inducing agent). The gelling agent was altered to determine whether the presence of an anionic gelling agent would have the same effect (sodium CMC and xanthan gum) (PEO 303 was also investigated as an additional non-ionic polymer). As shown in Figure 4.57, the different gelling agents produced markedly different release profiles. PEO 303 released the drug at a similar rate at the start to the formulation containing HPMC, but as time proceeded, the release rate decreased. Xanthan gum and sodium CMC were markedly different. 10% drug had not been released when over 50% of the drug had been released in the formulation containing HPMC. The drug may have been interacting with the gelling agents xanthan gum and sodium CMC. Xanthan gum and sodium CMC are both anionic polymers. As mentioned earlier (Section 4.4b) propranolol, a cationic drug, appeared to exhibit a binding interaction with anionic polymers. The amount of gelling agent in the formulation was 10% which was much higher than the 0.67% of the anionic polycarbophil (the viscosity inducing agent) which resulted in a maximum of 80% drug release. Because the gelling agent is present at a higher concentration than the polycarbophil (viscosity inducing agent), the interaction between the propranolol and anionic gelling agent will be markedly greater. This is apparent in the release profile (Figure 4.57) as the anionic polymers xanthan gum and sodium CMC gave slower release profiles.

Table 4.18 - f_1 and f_2 Values for Effect of Gelling Agent

Gelling Agent	**f_1**	**f_2**
PEO	8.53	73.57
Xanthan Gum	94.26	24.12
Sodium CMC	99.97	22.82

The difference and similarity factors were calculated (Table 4.18) according to Equations 4.4 and 4.5 (the reference assay was HPMC as the gelling agent). PEO had a similar release profile to HPMC, but the release profiles for the gelling agents xanthan gum and sodium CMC were markedly different. This was also very much evident in the release profile (Figure 4.57). As already mentioned in this section, this vast difference was most likely due to the polymers being anionic.

The *in vitro* drug release tests have shown that the ISGM system developed in this study performed its intended role as it retarded the release rate of propranolol into the receptor medium. The *in vitro* tests constituted a means of assessing the effect of various factors on the release rate of propranolol but it is important to note that they are not indicative of the drug release behaviour *in vivo*. Indeed, the *in vitro* drug release experiment imposed restrictions which would not be the case *in vivo*. For instance, the effect of swelling was minimised as it was constrained to occur only in the axial direction. Were this formulation to be injected into the body, both radial and axial swelling of the depot can realistically be expected. The release interface may also be different from the *in vitro* situation as drug release would not necessarily occur from a single interface with a fixed surface area (as was the case in the experimental set-up). Another point of difference between *in vitro* and *in vivo* release trials is that the release experiments *in vitro* were conducted using water as the dissolution test medium. The release of drug *in vivo* would not occur into a "pure" water medium as there are tissues and other fluids which would affect the drug release as well as polymer dissolution/gelling into these tissues. Distilled water has been used as a dissolution test medium for release experiments (Miranda *et al.*, 2006; Anderson *et al.*, 2001; Lee and Peppas, 1987; Korsmeyer *et al.*, 1983; Grassi *et al.*, 2004; Colombo *et al.*, 1997; Moore *et al.*, 2000). In general, *in vitro* drug release experiments functioned as a comparative tool to observe how various factors affected the release rate of propranolol. It can be said that as a result of conducting the *in vitro* drug release experiments, that the ISGM system functioned as intended in that it interacted with water resulting in a gelled depot which retarded the release rate of propranolol.

4.5c Modelling of the Drug Release Data

Mathematical modelling of the drug release data was conducted to determine if a relationship between the release rate and the tested parameter existed. The modelling algorithms incorporate many assumptions/parameters that are made or set for the system under study and serve to allow predictions on how the system changes its release behaviour in response to these changes in parameters. As such they constitute an *in silico* approach to understanding drug release better.

The development of mathematical models has included factors such as swelling and erosion into the model. The inclusion of such factors can make the mathematical modelling more complex as the mathematical equations involved are altered to take into account any such complications.

In systems where swelling of the drug delivery matrix occurs, there are two major factors which control the drug release rate. The first factor is the rate of aqueous medium infiltration into the matrix followed by a relaxation process, i.e. hydration, gelation or swelling. The second factor is the rate of erosion of the matrix. Consequently, there are two fronts – a swelling front (glassy polymer /

gel interface) and an eroding front (gel/medium interface). The term front indicates the position in the matrix where the physical conditions change sharply (Colombo *et al*., 2000). The distance between the gelling and swelling front depends on the relative rates at which these fronts move in relation to each other (Tahara *et al*., 1995). There are many mathematical models proposed to describe such systems: (Peppas and Sahlin, 1989; Ford *et al*., 1991; Ritger and Peppas, 1987b; Harland *et al*., 1988; Lee and Peppas, 1987; Korsmeyer *et al*., 1983). A discussion on examples of modelling approaches is given below.

Grassi *et al*., (2000) proposed a new model to describe drug release from an ensemble of swellable cross-linked polymer particles. The model takes into account factors such as particle size distribution and the visco-elastic properties of the polymer-penetrant system among many others. The detailed description of the mechanism and phenomena, involved the use of many parameters. Certain parameters were estimated due to the practical difficulty in determining their actual values. The model proposed in their study was able to describe the drug release kinetics from delivery systems containing cross-linked polymer particles.

Wu *et al*., (2005) developed a model to describe the transport phenomenon of a water soluble drug from a swellable and dissoluble tablet. The model considers factors such as swelling of the matrix, water penetration and concentration dependent water and drug diffusion and polymer dissolution. A parameter not incorporated into the model was the diffusion coefficient which is related to the microstructure of diffusion components and the polymer in a system. The experimental results were found to be in agreement with Wu's model, despite not including the parameter of the diffusion coefficient.

Siepmann *et al*., (1999) developed a model combining diffusion, swelling and dissolution mechanisms. The model was a 2-dimensional approach which takes into account axial and radial swelling.

Another means of modelling the data is to simplify the experiment to minimise the effects of certain parameters. Grassi *et al*., (2004) minimised the effects of swelling from HPMC tablets by coating the tablet with an impermeable layer so that swelling occurred from one area only. Consequently solvent penetration and drug release was constrained to occur in one direction only (Colombo *et al*., 1990; Colombo *et al*., 1996).

An issue concerning modelling of *in situ* systems is the gels which form can often have irregular geometries which are difficult to predict prior to injection. The modelling to take into account such phenomena thus becomes more complex as the irregular geometry can lead to a non-uniform drug distribution in the gel (Lin and Metters, 2006).

Therefore there are various means of modelling release data. As discussed earlier in this section, a very comprehensive approach can be taken where many parameters are considered such as in the study conducted by Grassi *et al*., (2000). The study by Wu *et al*., (2005) showed that despite not including all the parameters such as the dissolution coefficient, results were still in agreement with the model. Therefore it is not always critical to include all the possible parameters in the model.

In studies where both simplified and comprehensive models were used to analyse the data, it was found the comprehensive models gave a very good fit to the release data. However, the simplified models when used anyway were still found to be in good agreement with the experimental data. Therefore, simplified models such as the Higuchi equation are still able to be used with the confidence that that they will provide important information on the systems under study (Faisant *et al*., 2003).

In the modelling study carried out on the ISGM system developed in this research, the release experiment was deliberately modified to simplify the modelling process. Swelling was minimised by ensuring swelling only occurred upwards (i.e axially). Any radial swelling that might also have occurred was prevented due to the constraints of the glass tube that the ISGM system was resident in. In addition, an erosion event of the polymer into the dissolution test medium could not occur as the polymer would not be able to diffuse through the dialysis membrane (Section 4.5b). The surface area of the drug release interface would thus remain fixed. This more simplified experimental scenario for carrying out release experiments meant that a less complex approach of modelling the data could then be undertaken by using the Higuchi Equation and the power law. These modelling equations were deemed appropriate as they are the frequently used models (Sung *et al.*, 1996; Schliecker *et al.*, 2004; Miranda *et al.*, 2006; Moore *et al.*, 2000; Tahara *et al.*, 1995).

Drug release rate data were thus modelled first using the widely applied Higuchi equation (Higuchi, 1961) (Equation 4.8a) for release from a matrix solution (Siepmann and Peppas, 2001).

$$\frac{M_t}{M_\infty} = k\sqrt{t} \tag{4.8a}$$

M_t = absolute cumulative amount of drug released at time t

M_∞ = absolute cumulative amount of drug released at time ∞

k = constant which incorporates the design variables of the system

t = time

When modelling data using the Higuchi equation the following assumptions must be considered, (Siepmann and Peppas, 2001):

- The initial drug concentration in the system is higher than the solubility of the drug.

- The suspended drug particles must be smaller in diameter than the thickness of the system.

- The drug diffusivity is constant.

- Perfect sink conditions (i.e., the volume of medium at least 3 times greater than that required to form a saturated solution of a drug) are maintained.

- The mathematical analysis assumes a 1-dimensional diffusion.

The gel depot in this study has the shape of a cylinder but because release of the drug is only occurring at one end of the cylinder, the geometry of the release interface is essentially a slab. Consequently, release can be regarded as 1-dimensional diffusion. It should be noted that this is a major simplification from the expected *in vivo* process, as in such a case, the gel depot would be 3-dimensional and swell in all directions.

- Swelling or dissolution of the polymer is also assumed to be negligible. However in the case of the ISGM system being modelled, this is the main problem involved in applying the Higuchi equation to model the data. Swelling of the polymer in the ISGM system is not negligible during the release experiment; it increases in size as a result of swelling. However due to the fact that swelling occurs only in the axial direction (because of the experimental constraints) and does not alter the surface area of the release interface, the impact of swelling is perhaps not as critical as might be expected. Therefore the Higuchi equation was used to model the data.

Some of the assumptions listed above are not necessarily valid for most controlled drug delivery systems which incorporate HPMC (Siepmann and Peppas, 2001). This is because with the Higuchi equation, a linear plot of the fraction of drug released against the square root of time may be extract which superficially would signify a diffusion controlled system. However, any modelling results should be viewed with caution even if such a straight line relationship is found. The Higuchi equation assumes constant geometries during drug release and constant diffusivities of the drug and water. HPMC swells considerably and the diffusion co-efficient of the drug and water are strongly concentration dependent (Siepmann *et al.*, 1999) and HPMC also dissolves (Siepmann and Peppas, 2001). Therefore an apparent straight line can still result from the superpositioning of various effects and is not necessarily going to be an indication that the release behaviour is diffusion controlled. Because swelling was not regarded to affect the release interface area (as the surface area remained fixed as described in Section 4.5b) the Higuchi equation (Equation 4.8a) was used to model the release data.

The percentage release versus square root of time plot (Figures 4.58 – 4.65) produced straight line graphs for the majority of parameters tested. The errors were calculated as described earlier (Section 4.5a *i*.). For clarity the errors bars are not shown on all the profiles.

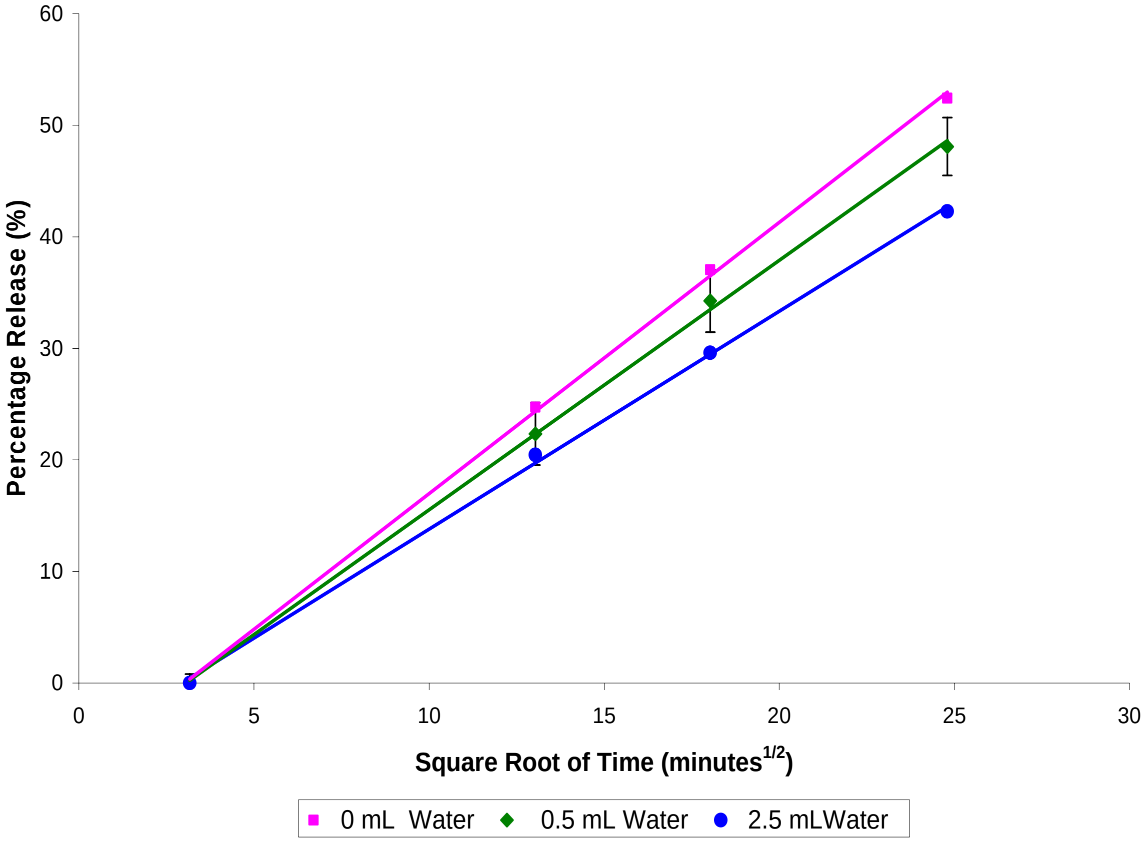


Figure 4.58 – Effect of Height of Water in Tube on Drug Release Rate (n=6)

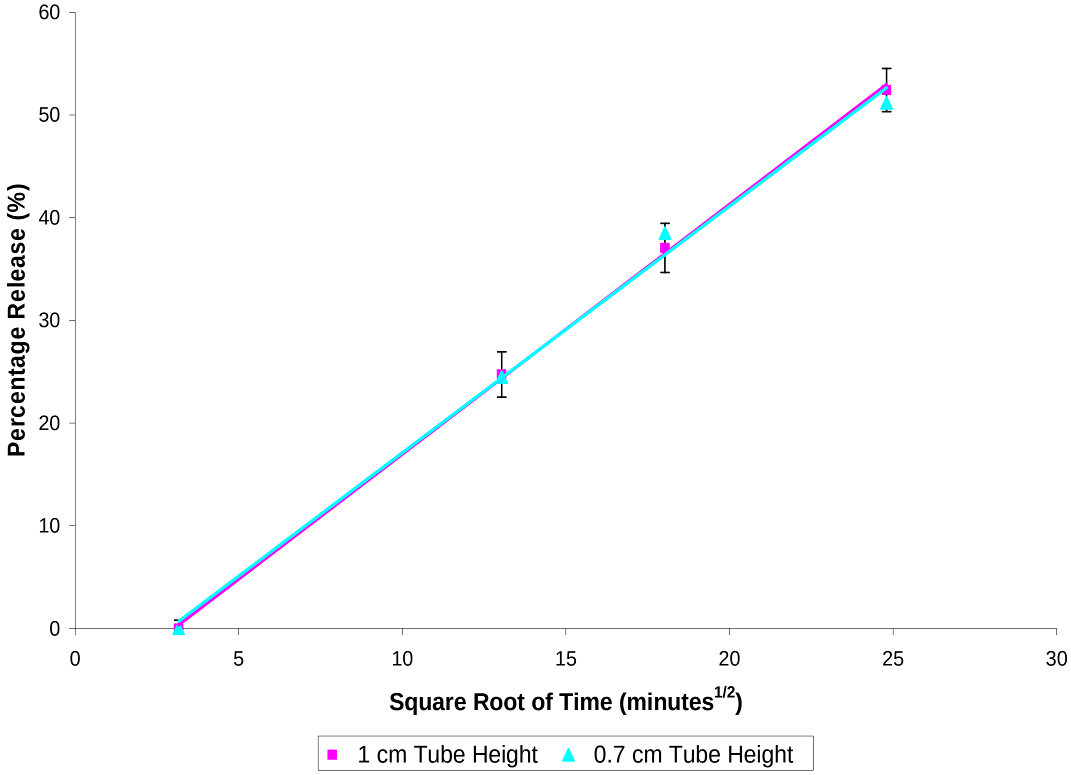


Figure 4.59 – Effect of Tube Height on Drug Release Rate (n=6)

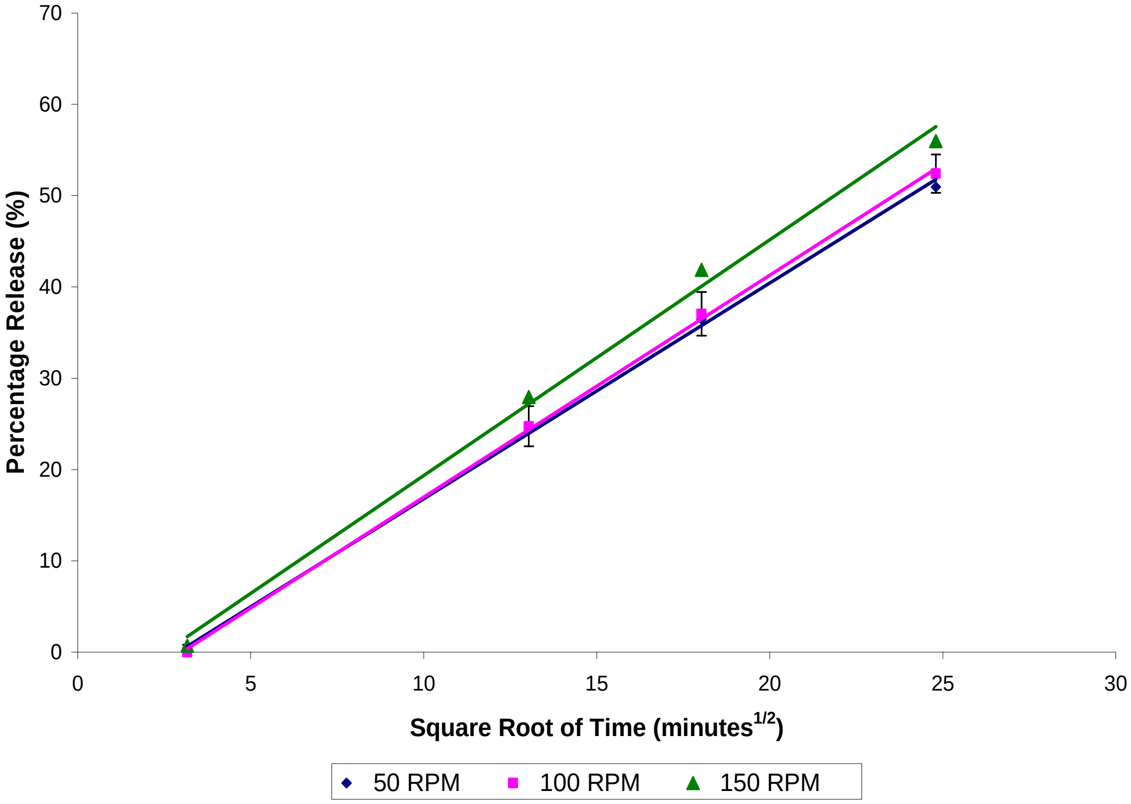


Figure 4.60 – Effect of Stirring Rate on Drug Release Rate (n=6)

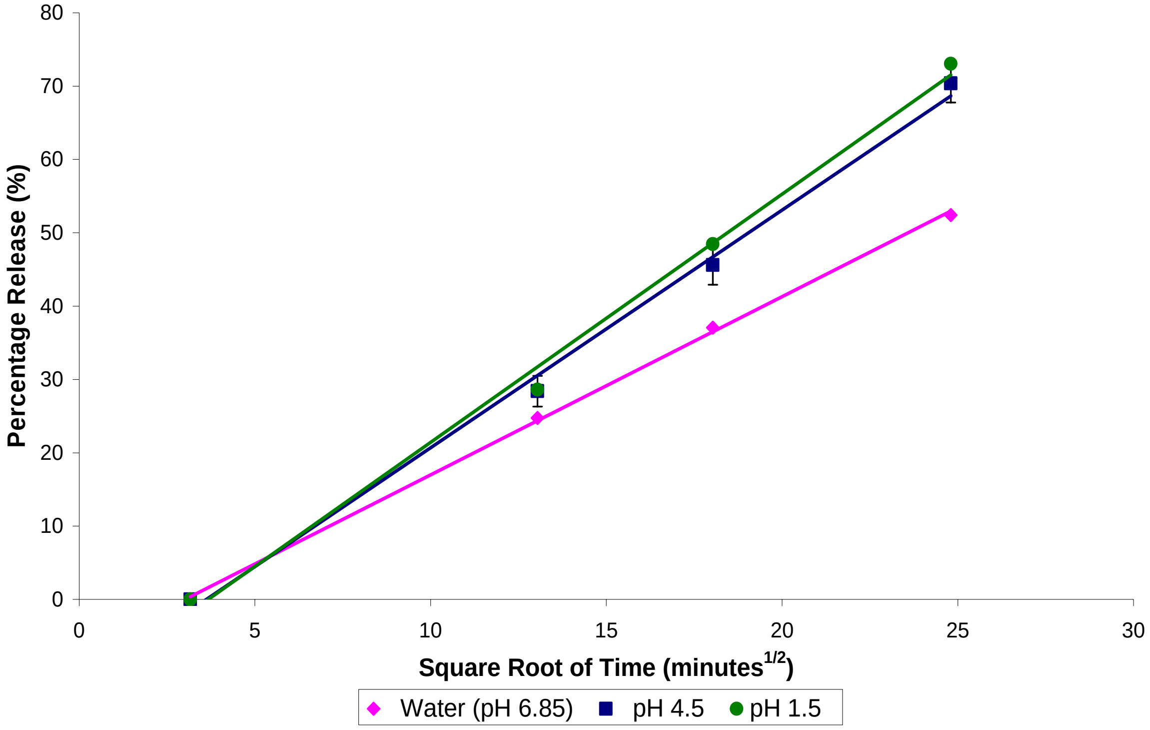


Figure 4.61 – Effect of pH of Dissolution Test Medium on Drug Release Rate (n=6)

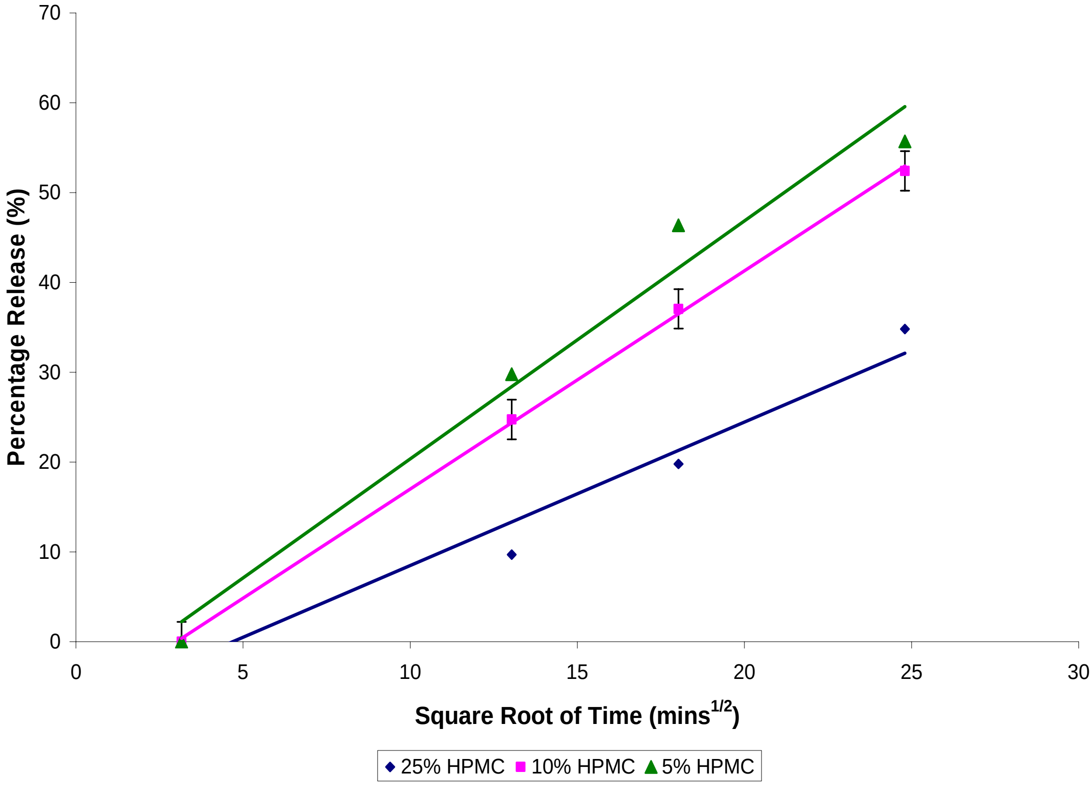


Figure 4.62 – The Effect of HPMC Concentration on Drug Release Rate (n=6)

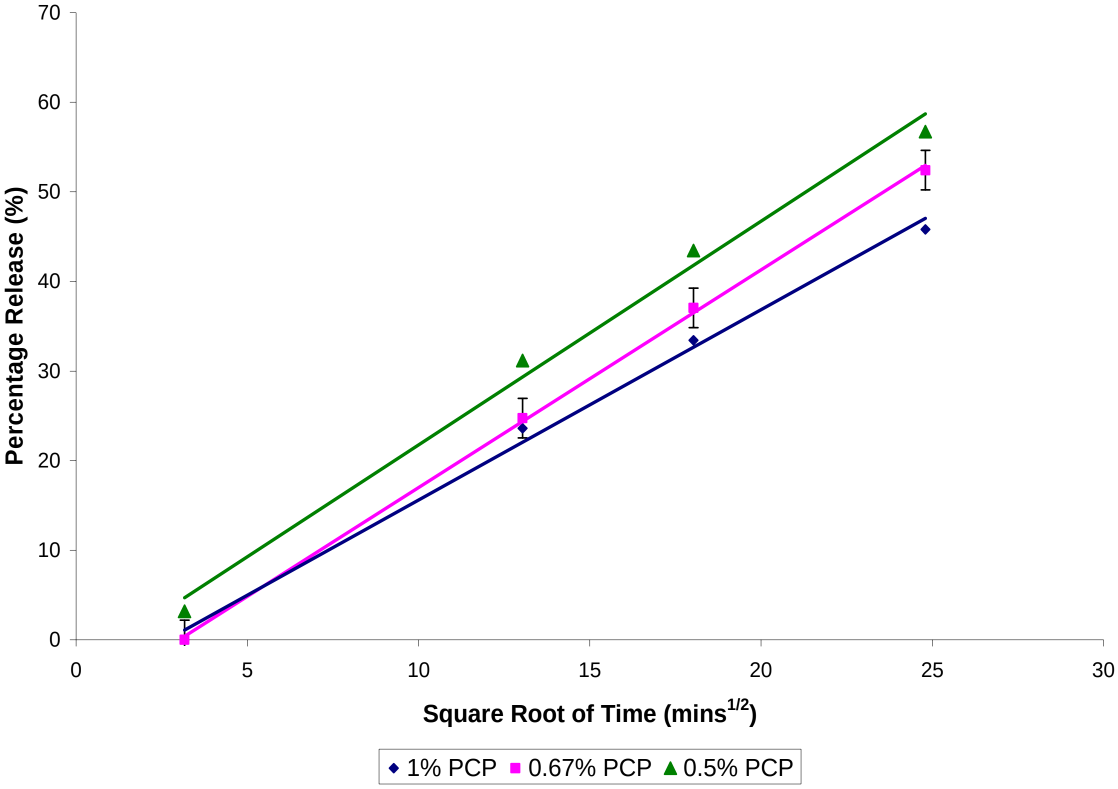


Figure 4.63 – Effect of Polycarbophil Concentration on Drug Release Rate (n=6)

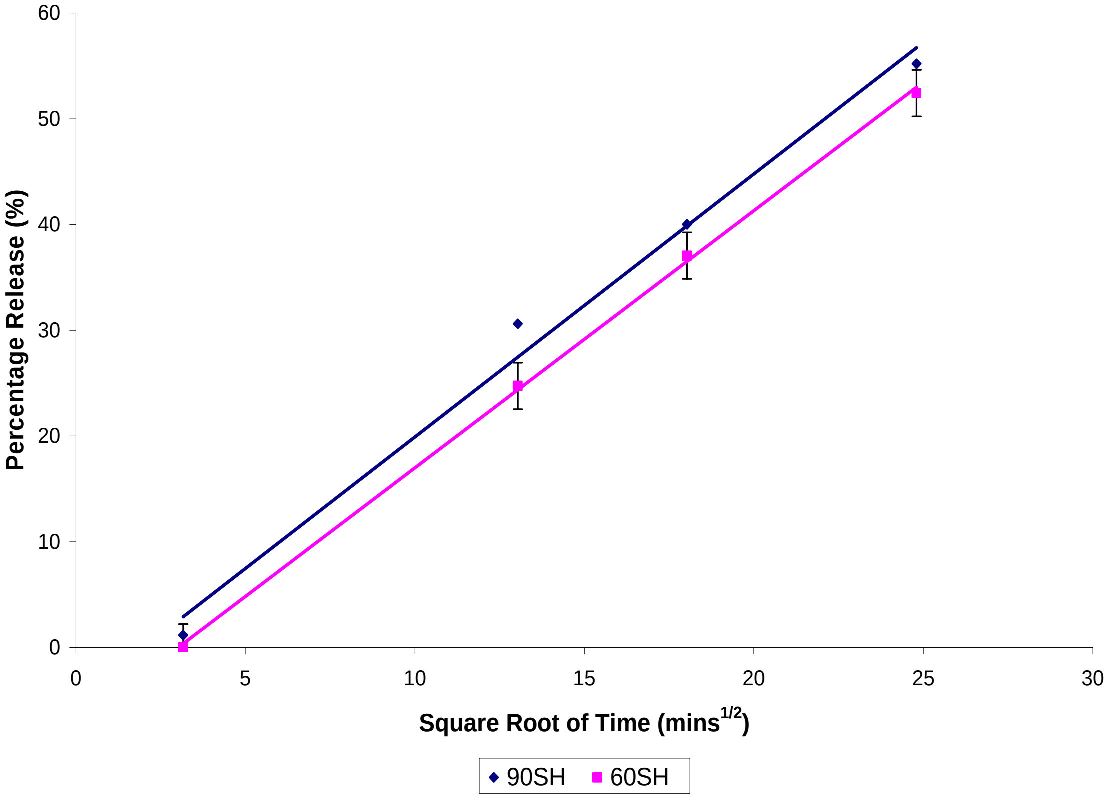


Figure 4.64 – Effect of HPMC Viscosity Grade on Drug Release Rate (n=6)

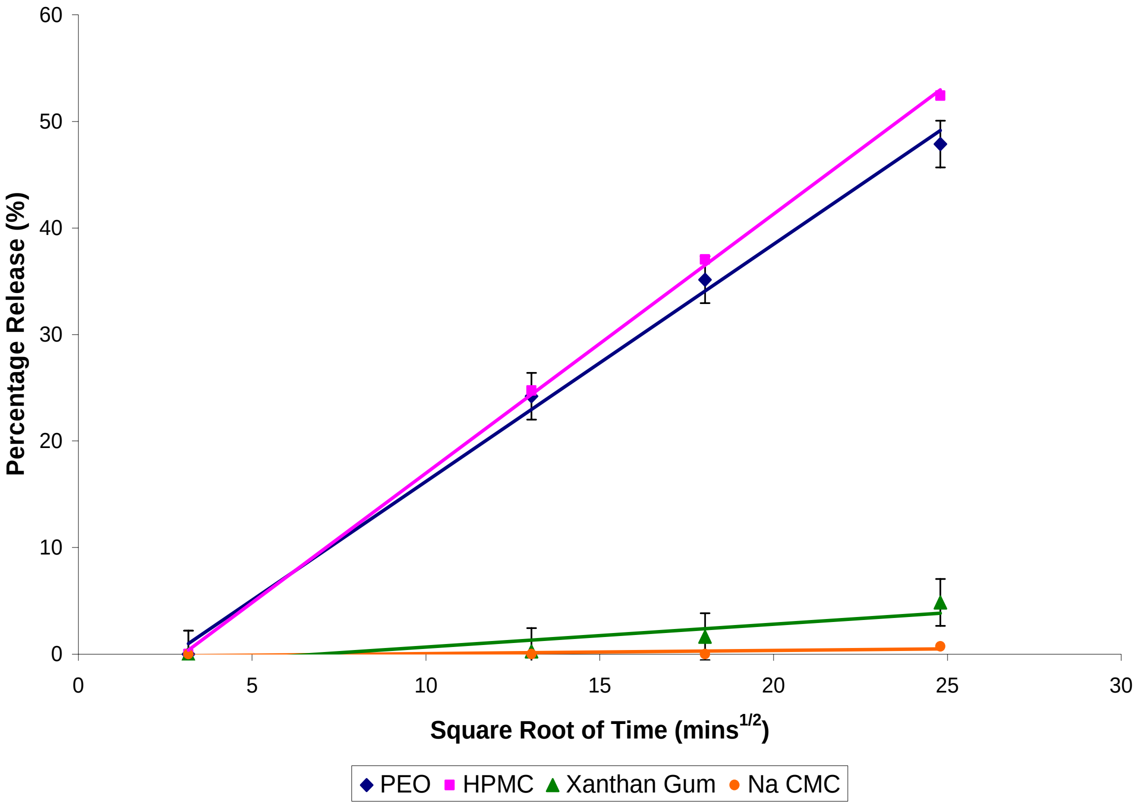


Figure 4.65 – Effect of Hydrophilic Gelling Agent on Drug Release Rate (n=6)

Figures 4.58 – 4.65 were consistent with modelling by the Higuchi equation up to 50% release. The value of k (release rate constant) was determined from each plot by calculating the gradient. As previously mentioned in this section the Higuchi equation is modelled using data up to 60% release. Swelling of the gel depot occurred throughout the duration of this release analysis period. Therefore a different mechanism of drug release (such as polymer dissolution) post gelling may have occurred after the 60% release period (after this time period the Higuchi equation was no longer linear). Consequently the time points chosen for the modelling analysis were appropriate. It was observed after a period of 4 weeks that the gel had degraded to a fluid (Section 4.3d *iii*). Therefore once the swelling had ceased it is likely that the drug release mechanism would have predominately been structural breakdown. Therefore, because swelling occurred throughout the duration of the release analysis period, a single mechanism rather than a change in mechanism was assumed to occur during the experiments.

The value of k was used to illustrate the significant difference the gelling agent had on the release rate.

Table 4.19 - Effect of Gelling Agent on the Value of k

Parameter	**k (% min$^{-0.5}$)**	**Standard Error in the Calculated Parameter (% min$^{-0.5}$)**
No Gelling Agent	8.35	$\pm$ 0.33
Gelling Agent	2.49	$\pm$0.31

As shown in Table 4.19 it is apparent that the presence of the gelling agent greatly reduces the value of k by a factor of 3.35. The standard error (calculated using regressional analysis) shows the k values are significantly different.

The values of k were also used to determine if a relationship between k and each parameter existed.

$$\frac{M_t}{A} = \sqrt{D(2c_0 - c_s)c_s t} \qquad \text{for } c_0 > c_s \tag{4.8b}$$

The original form of the Higuchi equation (Equation 4.8b) can be rearranged to the commonly used form (Equation 4.8a). As mentioned earlier in this section, k is a constant which reflects the design variables of the system. Therefore the value of k will incorporate parameters such as the surface of the release interface (A), the drug diffusivity (D) and drug solubility in the polymer (c_S).

The value of k for each concentration of HPMC was determined from their respective graphs (Figure 4.62). A plot of k against the HPMC concentration (Figure 4.66) revealed a linear relationship with an R^2 value of 1. Hence, the release rate of any system containing between 5 and 25% HPMC can be predicted by interpolation from the straight line illustrated in Figure 4.66 (for systems using the specific combinations of propranolol and excipients in this formulation). A linear relationship also existed between k and the stirring rate (Figure 4.67). Therefore the release rate of a system for a particular stirring rate can also be predicted from the plot. This result suggests that there is a mass transfer limitation on the sink side of the device. When the stirring rate is increased, the effect of the boundary layer is reduced so affecting k. As mentioned earlier in this section, k depends on the design variables of the system.

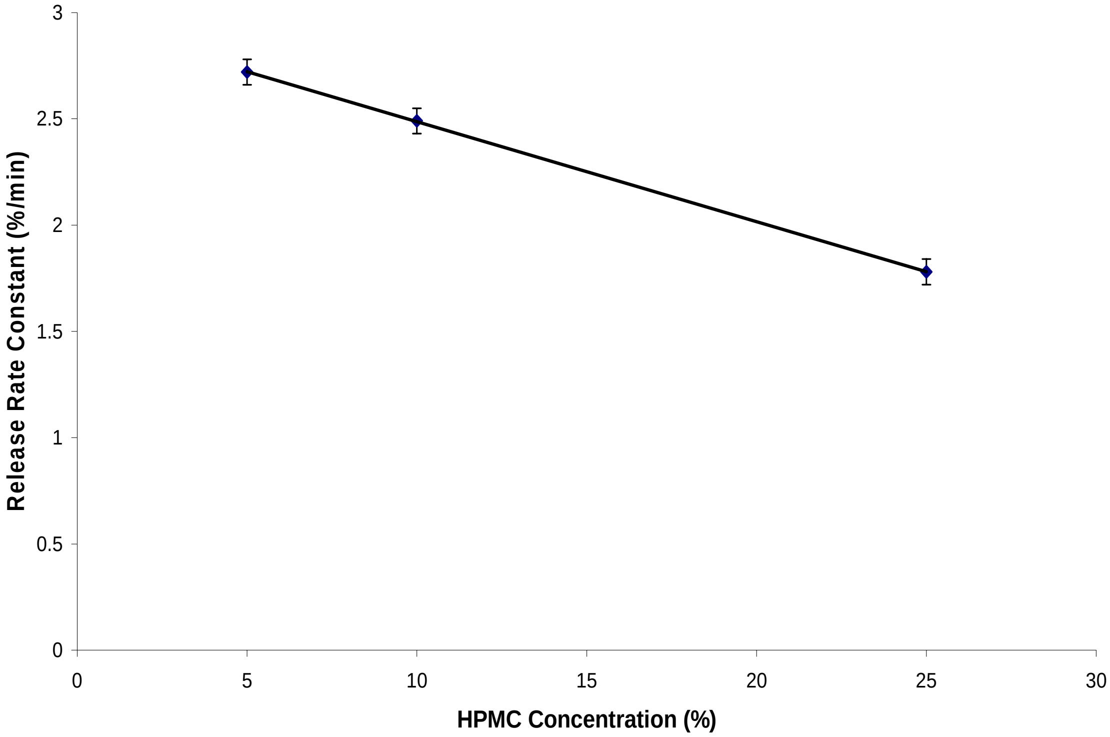


Figure 4.66 – Comparison of the Release Rate Constant and HPMC Concentration. The Graph Shows a Trend Line Running Through the Data Points.

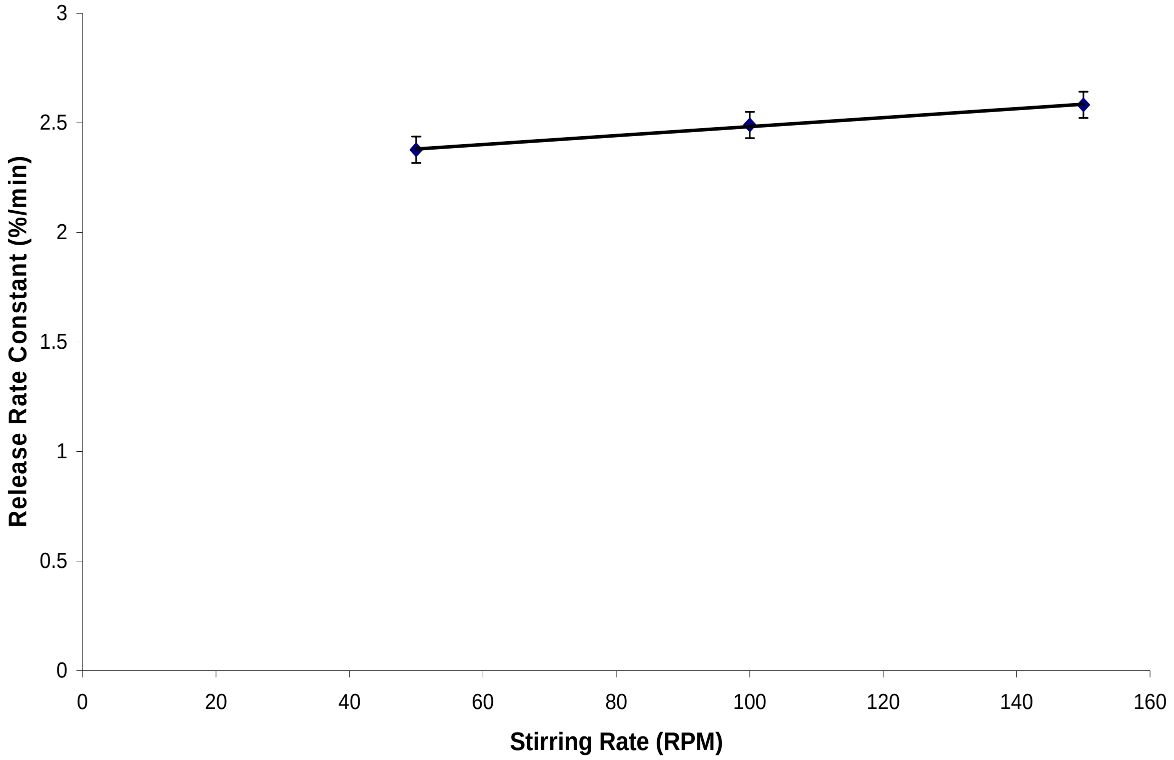


Figure 4.67 – Comparison of the Release Rate Constant and Stirring Rate. The Graph Shows a Trend Line Running Through the Data Points.

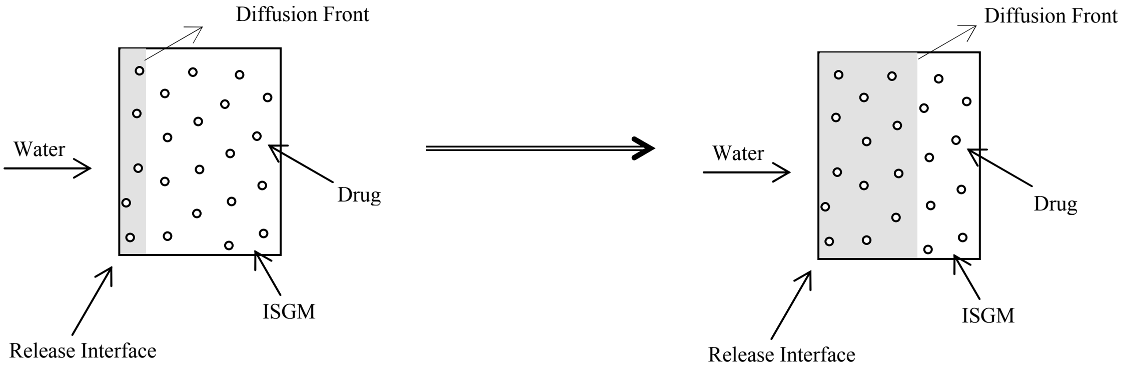


Figure 4.68 - Schematic of Drug Release From the ISGM System

Figure 4.68 depicts the release of drug from the ISGM system. When the drug is dissolved in the matrix, (i.e. drug concentration is less than the solubility of the drug in the continuous phase) the diffusion rate of the drug will not necessarily be constant. In contrast, when the drug is homogeneously dispersed throughout the ISGM system (i.e. drug concentration is greater than the solubility of the drug in the continuous phase), the drug will exist both in solution and as discrete solid particles. The drug release process will involve dissolution of the drug into the ISGM system surrounding the drug particle and then partitioning of the drug into the aqueous environment. In such a case the diffusion rate of the drug will be constant and a linear concentration gradient is maintained in the diffusion region.

In this system the initial drug concentration is greater than the solubility of the drug in the continuous phase (0.003 g mL^{-1}). The drug release mechanism will therefore tend to follow that of the dispersed system.

As the Higuchi equation assumes diffusion is the rate limiting step, it cannot give conclusive evidence of the mechanism of release. The more general power law (Korsmeyer *et al.*, 1983) (Equation 4.9) was therefore used to explore the release rate more fully.

$$\frac{M_t}{M_\infty} = kt^n \tag{4.9}$$

M_t = absolute cumulative amount of drug released at time t

M_∞ = absolute cumulative amount of drug released at time ∞

k = constant which incorporates the structural and geometric characteristics of the device

n = release exponent indicating mechanism of release

t = time

Equation 4.9 is identical to the Higuchi equation if n = 0.5. When n = 0.5, it indicates an internal (gel-side) diffusion controlled release mechanism (in accordance with the Higuchi equation) and when n = 1 it is indicative of a swelling controlled drug release. Values of n which lie between 0.5 and 1 are referred to as anomalous transport. Anomalous transport is the superpositioning of both diffusion controlled and swelling controlled release. However these values of n are only valid for matrices assumed to have a slab geometry. This fact is not always taken into consideration and consequently leads to misinterpretation of results. Different values of n have been derived for cylinders (Table 4.20) and spheres (Ritger and Peppas, 1987a; Ritger and Peppas, 1987b). The gel depot in this study is cylindrical in shape. According to Ritger and Peppas, one dimensional release from a cylinder can only occur if the cylinder is long and thin, where release from the ends of the cylinder is therefore negligible as the majority of release will occur radially not axially. In such a geometry, release can be regarded as one dimensional as the release rate will be constant over the whole radial surface.

Table 4.20 – Values of n for Cylinders

n value	**Release Mechanism**
0.45	Diffusion Controlled (Fickian)
0.45-0.89	Anomalous Transport
0.89	Case 2 Transport (swelling controlled)

The gel depot in this study has the shape of a cylinder, but, because release of the drug is only occurring at one end of the cylinder, the geometry of the release interface is essentially a slab (as described earlier in this section). Consequently, the values of n obtained from the experimental data should be compared to the value of n for a slab (Table 4.21).

Table 4.21 – Values of n for Slabs

n value	**Release Mechanism**
0.5	Diffusion Controlled (Fickian)
0.5-1.0	Anomalous Transport
1.0	Case 2 Transport (swelling controlled)

The effect of swelling in this system was limited, where radial swelling did not occur (due to constraints of the glass tube). Swelling only occurred in one direction (axial). Therefore the swelling in this system would not affect the surface area of the release interface but would affect the concentration of the release component in the gel by increasing the volume of the depot. Therefore swelling will affect the release component concentration over time but not the release area.

$\ln \frac{M_t}{M_\infty}$ was plotted against $\ln t$ for each experiment to determine if a straight line relationship existed. A straight line graph would indicate the power law modelled the data adequately.

The Final Formulation

The final formulation gave a straight line graph (Figure 4.69) with an R^2 value of 0.9987. Therefore the power law (as stated in Equation 4.9) appears to successfully model the release data. The value of n from this profile was found to be 0.6. The 0.6 value indicates the formulation exhibits an anomalous transport type release mechanism - not strictly diffusion controlled as thought with the Higuchi equation but close to it. This seems plausible because diffusion alone cannot dictate the release, given the observation that the HPMC in the system swells. The swelling in this regards will only affect release in terms of the greater distance the drug must diffuse to reach the release interface.

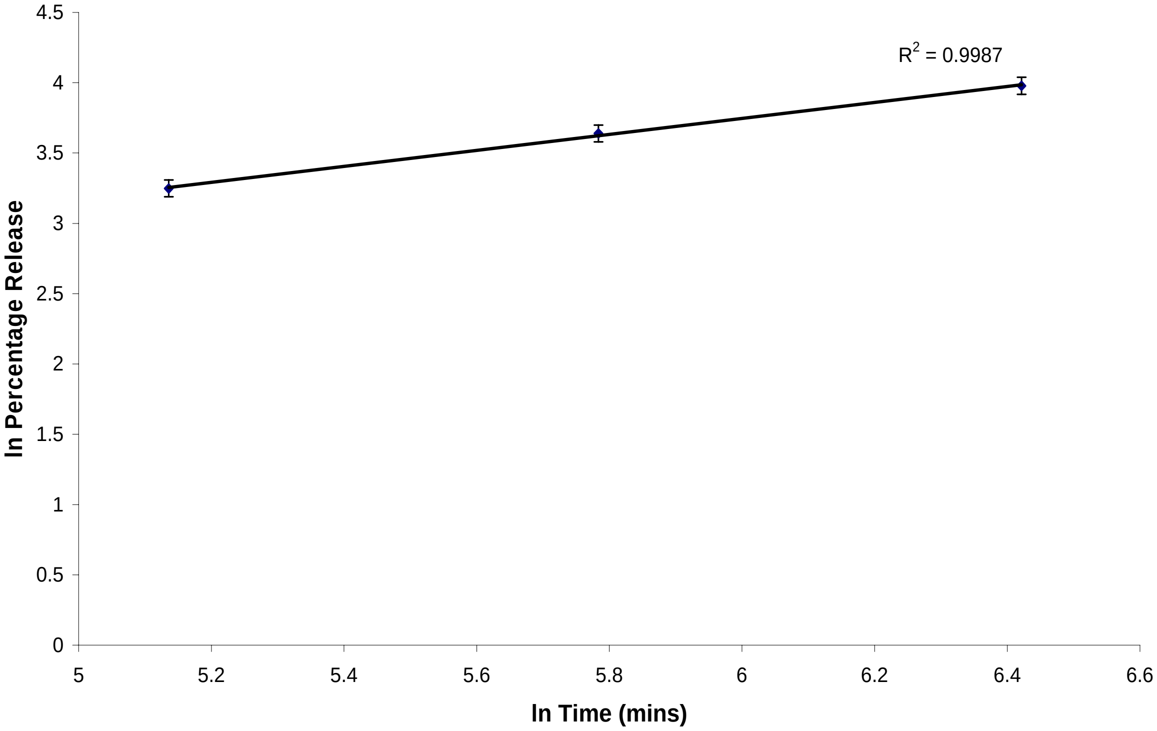


Figure 4.69 – Modelling of the Final Formulation using the Power Law. The Graph Shows a Trend Line Running Through the Data Points.

HPMC Concentration

The 5% HPMC system gave a value of n of 0.5 indicating a diffusion controlled release mechanism. Because there is less polymer in the formulation, the swelling of the HPMC has less of an effect on the release mechanism. Increasing the HPMC concentration to 25% resulted in a larger n value of 1.0. This value indicates a swelling controlled mechanism, consistent with the high degree of swelling of HPMC with water.

Polycarbophil Concentration

Decreasing the polycarbophil concentration to 0.5% caused a marginal deviation from a straight line (where the R^2 value decreased from 0.9987 (for the formulation containing 0.67% polycarbophil) to 0.9924 (for the formulation containing 0.5% polycarbophil)). Increasing the polycarbophil concentration to 1.0% still gave a straight line. However the value of n was found to be 1.9, much higher than the 1.0 value indicating a swelling controlled mechanism (Table 4.21). It is uncertain what the cause of the high value of n is. Obviously factors other than swelling and diffusion are contributing to the release mechanism. It is possible distortion of the matrix is occurring which may explain this deviation from expected behaviour. In the case where n is greater than 1, there may be a late stage acceleration in the permeation rate (this has also been referred to as Super-case II transport) (Tanchak and Barrett, 2004).

pH

Though altering the pH gave a completely different release profile (Figure 4.53), the power law still produced a straight line graph. Decreasing the pH from 6.85 to 4.5 and 1 gave straight lines with an n value of 0.7.

Discussion

Siepmann and Peppas noted that the application of the power law to HPMC systems can only give a limited insight into the exact release mechanisms (Siepmann and Peppas, 2001). The Higuchi equation and power law assume constant geometries during drug release and constant diffusivities of the drug and water. HPMC swells considerably and the diffusion co-efficient of the drug and water are strongly concentration dependent (Siepmann *et al*., 1999) and HPMC also dissolves (Siepmann and Peppas, 2001). In the case of the Higuchi equation an apparent straight line can still result from the superposition of various effects and is not necessarily diffusion controlled (as mentioned earlier in this section).

The more comprehensive power law has provided information indicating diffusion was not the only mechanism involved in the release of the drug. Much research has been conducted on HPMC based drug-release systems (Rekhi *et al.*, 1999; Colombo *et al.*, 1999; Grassi *et al.*, 2004; Siepmann and Peppas, 2001). The subsequent modelling has led to varied values of n where some claim the system to be predominately diffusion controlled (Rekhi *et al.*, 1999), whereas others have found n to be anomalous transport from a swellable matrix (Colombo *et al.*, 1999). Consequently a range of n values for HPMC systems was obtained suggesting a wide range of possible drug release mechanisms. In this study the radial swelling was prevented (as the glass tube prevented the gel expanding radially), however axial swelling still occurred. Though swelling did not affect the release interface, the axial swelling would have increased the distance which the drug would have to diffuse and therefore may contribute to the release mechanism. This would explain why the power law indicated a swelling type mechanism for drug release in this system.

The entire percentage release versus square root of time profile will not be linear as the thickness of the diffusional path in the drug depletion zone increases with time (Figure 4.70) (Chien, 1980) and this diffusional path also increases as the axial swelling occurs. In the case of the final formulation, the release profile gave a constant release up to approximately 50%. This was considered adequate to define the profile and allow comparisons between different profiles (as described previously in Section 4.5b) as release up to 60% is usually used to model the data (Ritger and Peppas, 1987a, Nam *et al.*, 2004; Perez-Marcos *et al.*, 1996).

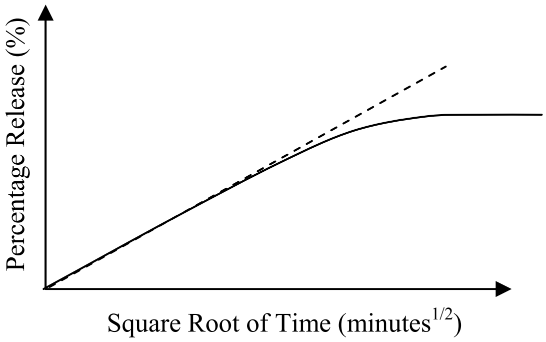


Figure 4.70 – How an Actual Release Profile Will Deviate From the Ideal Release (Chien, 1980).

As a result of the modelling, the formulation allows the release rate to be calculated. A release experiment was conducted to determine if k and n remained constant for a formulation with a different initial drug concentration.

The initial drug concentration was reduced by a third. The values of n and k were found to be 0.57 ($\pm$ 0.02) and 1.72 ($\pm$ 0.27) respectively (Figure 4.71). The value of n remained constant but k changed slightly (Table 4.22).

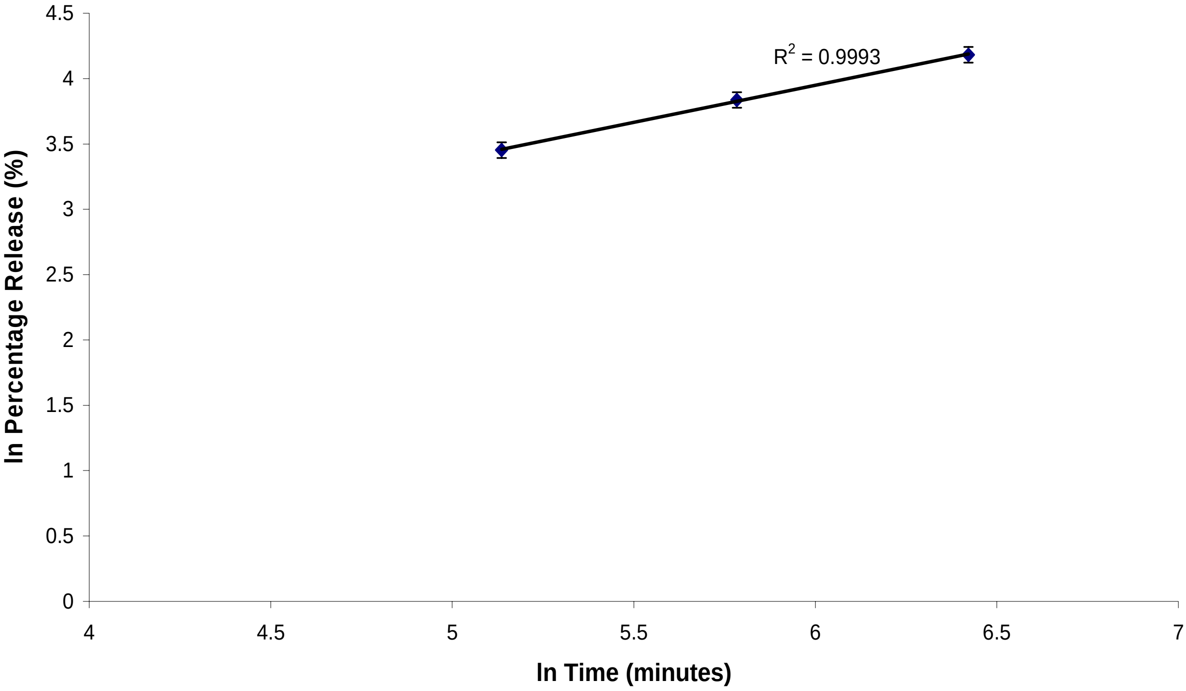


Figure 4.71 – Formulation Containing a Lower Drug Concentration

Table 4.22 - Comparison of n and k

Parameter	**The Final Formulation**	**Formulation With Reduced Drug Concentration**	**Standard Error in the Calculated Parameter**
n	0.57	0.57	± 0.02
k	1.40	1.72	± 0.27

The value of n should remain constant as the geometry of the system and all other factors remained unchanged. Consequently, the release mechanism should not change and this was in agreement with findings (Table 4.22). Designing a formulation to give a drug release over a desired time period for example 10 days may be possible as the difference in the k values were not significantly different as the values overlapped within the error range (Table 4.22). The error in k was calculated using a linear regression analysis program (Microsoft Excel-based) and was found to be ± 0.27. The value of k is a constant which incorporates the structural and geometric characteristics of the device (Siepmann and Peppas, 2001). The possible variation in k could be a result of the swelling in the system. Altering the drug load of a system, will affect the water absorbing capacity (if the drug is water soluble). Therefore the driving force for drug release (i.e. concentration gradient) will be affected, which in turn may affect the release rate of the drug, thus altering k.

4.6 Stability Assessment of the Final Product

4.6a Drug Stability

According to the US-FDA (United States – Food and Drug Administration), a stability indicating assay is a validated quantitative analytical method which can detect changes in the chemical, physical or microbiological properties of the drug substance and drug product over time. The assay is so specific that the contents of the active ingredient, degradation products and other components can be accurately measured without interference (Bakshi and Singh, 2002).

Bakshi and Singh (2002) conducted a review of the literature and found that very few methods which claimed to be stability indicating actually fitted the definition of a stability indicating assay given above. Some methods did not even include forced drug degradation studies.

Titrimetric, spectrophotometric and chromatographic techniques have all been used in stability studies. Spectrophotometric and titrimetric methods are inexpensive and simple but not always sensitive or selective. Consequently very few reports exist in the literature on their use today. However, there are some reports on the use of derivative spectrophotometry (Dabbene *et al*., 1994; Forsyth and Ip, 1994; Walash *et al*., 1994; Khamis *et al*., 1993).

In contrast, chromatographic techniques are the main stability analysis method. Chromatographic methods can separate multiple components, are more accurate and more sensitive.

Drug stability analysis is vital in pharmaceutical studies as increased degradation will decrease the potency of the drug and may also result in compounds which could give undesirable pharmaceutical side-effects (Andrisano *et al*., 1999).

i. *Method Development*

There are many reported methods in the analysis of propranolol by HPLC (Modamio *et al*., 1996; El-Saharty 2003; Venkatesh *et al*., 2007; Botterblom *et al*., 1993). The variations in the mobile phases are given in Table 4.23.

Table 4.23 – Reported Mobile Phases for Analysis of Propranolol

Mobile Phase	**Reference**
Acetonitrile and phosphate buffer with 0.2% (w/v) triethylamine.	Modamio *et al*., (1996).
0.02 M potassium dihydrogen phosphate and acetonitrile (80:20 v/v) adjusted to pH 4.5	El-Saharty (2003).
Ammonium acetate and acetonitrile 30:70 (v/v)	Venkatesh *et al*., (2007).
Acetonitrile and sodium dihydrogenphosphate – triethylamine (35:65:0.1 v/v)	Botterblom *et al*., (1993).
1 g of sodium dodecyl sulphate in 36 mL of 0.15 mol L^{-1} phosphoric acid, 180 mL of methanol, 180 mL of acetonitrile and 104 mL of water to give a final volume of 500 mL.	USP (1994).

However many of these methods include simultaneous detection of propranolol with other betablockers. This study involves the analysis of only one active agent - propranolol, therefore the USP method was adopted for HPLC analysis. Development and validation of the HPLC method was conducted on the pure drug. Extracting the drug from the formulation involved addition of methanol. This is discussed in further detail in Section 4.6a *iv*.

A peak was observed in the chromatogram for propranolol (see Section 3.6a *i* for experimental details). To verify the peak was due to propranolol, another sample of propranolol was passed through the column at a lower concentration. The corresponding peak signal decreased. To further confirm the observed peak was propranolol, the UV spectrum of the peak was analysed. The UV spectrum of the observed peak matched the UV spectrum of propranolol thereby verifying the detected peak was propranolol (Figures 4.72 and 4.73).

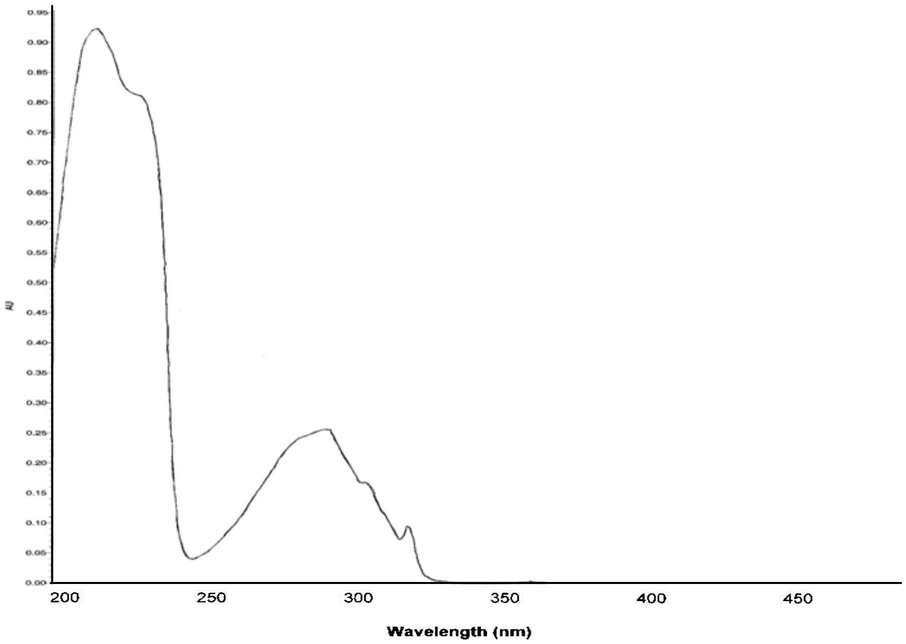


Figure 4.72 – UV Spectrum of the Observed Peak in the HPLC

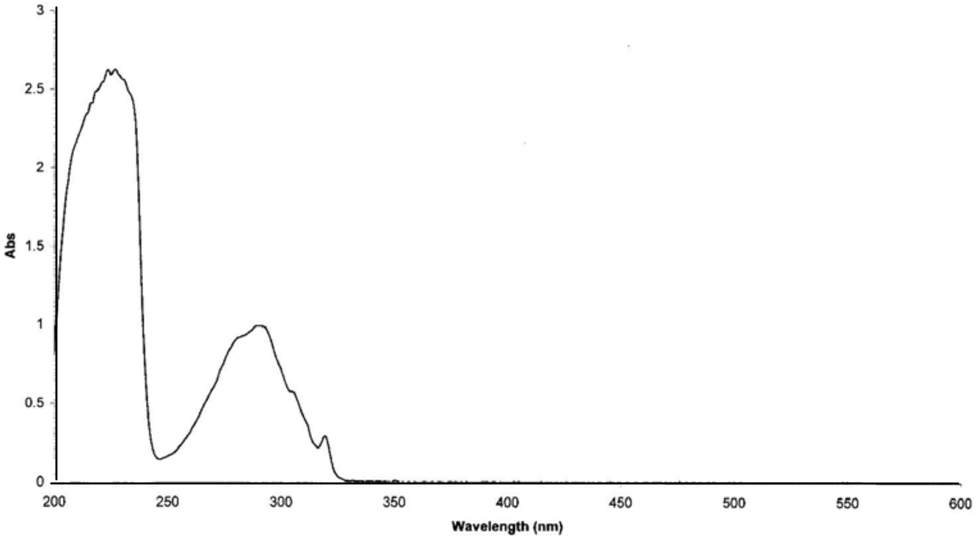


Figure 4.73 – UV Spectrum of Propranolol

After confirming the detected peak was propranolol, method development was conducted by carrying out robustness, drug degradation and linearity studies.

Linearity and Calibration

A straight line relationship was found for 6 concentrations (see Section 3.6a *i* for experimental details) of propranolol indicating the peak area was proportional to the concentration (Figure 4.74). The error bars displayed in Figure 4.74 represents the relative standard deviation.

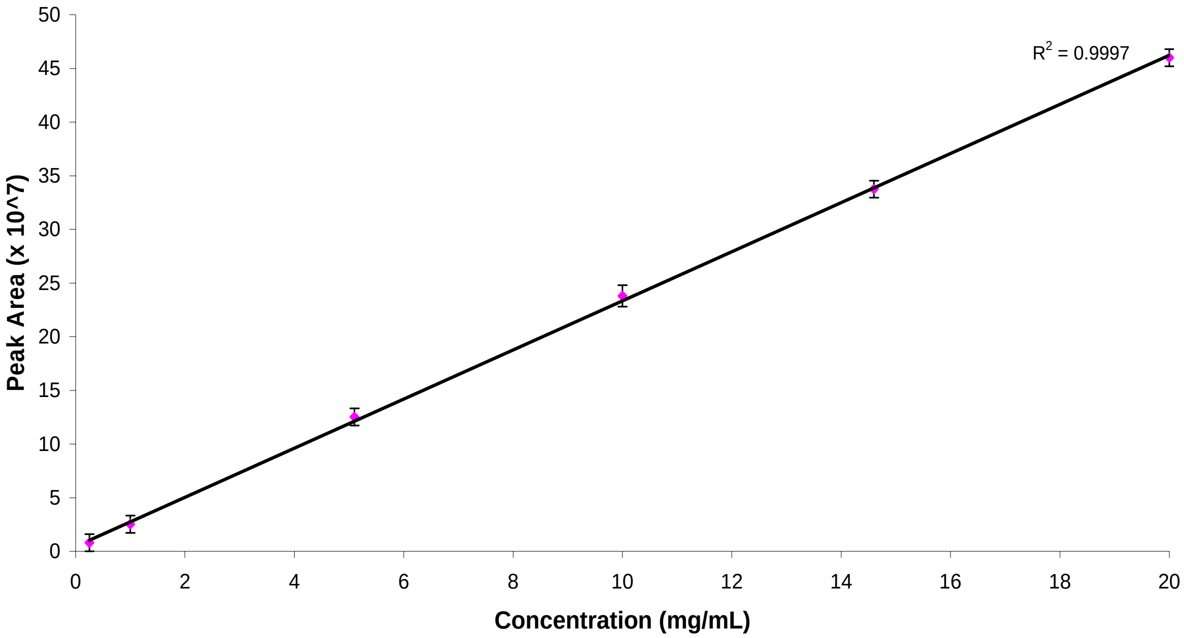


Figure 4.74 – Peak Area versus Concentration for 6 Concentrations of Propranolol

The method development has shown to give a linear response between peak area and propranolol concentration. The R^2 value should be greater than or equal to 0.999 (Center for Drug Evaluation and Research, 2000) and this was found to be the case (Figure 4.74) with the experimental value being 0.9997.

The next stage in the method development involved assessing the specificity to ensure degradation peaks do not interfere with the peak of interest.

Forced Drug Degradation Study

A forced drug degradation study was conducted to assess the ability of the analytical procedure to accurately and quantitatively measure the concentration of the analyte in the presence of any degradants. Degradation studies of propranolol have been conducted (Uwai *et al.*, 2005; Salomies, 1987; Sortino *et al.*, 2002; Modamio *et al.*, 1996).

Modamio *et al.*, (1996) conducted an accelerated drug stability of propranolol by subjecting the drug in a water bath at temperatures of 60, 70 and 90ºC. The analysis was conducted by measuring the percentage of unaltered concentration of the drug. Their study found no apparent degradation.

The degradation studies carried out in this research were based on those described in the literature (Bakshi and Singh, 2004; Marin and Barbas, 2004). In each degradation method (see Section 3.6a *i* for experimental details), no degradation peaks were found. The amount of drug remaining after degradation was calculated according to Equation 4.10 (based on the equation given by Ceschel *et al.*, 2003):

$$\%\ \text{Drug Remaining} = \frac{\text{Initial Drug Amount} - \text{Amount of Drug After Degradation}}{\text{Initial Drug Amount}} \times 100 \qquad (4.10)$$

The amount of drug remaining after each degradation treatment carried out it this study is illustrated in Figure 4.75. The error bars on the graph represent the relative standard deviation.

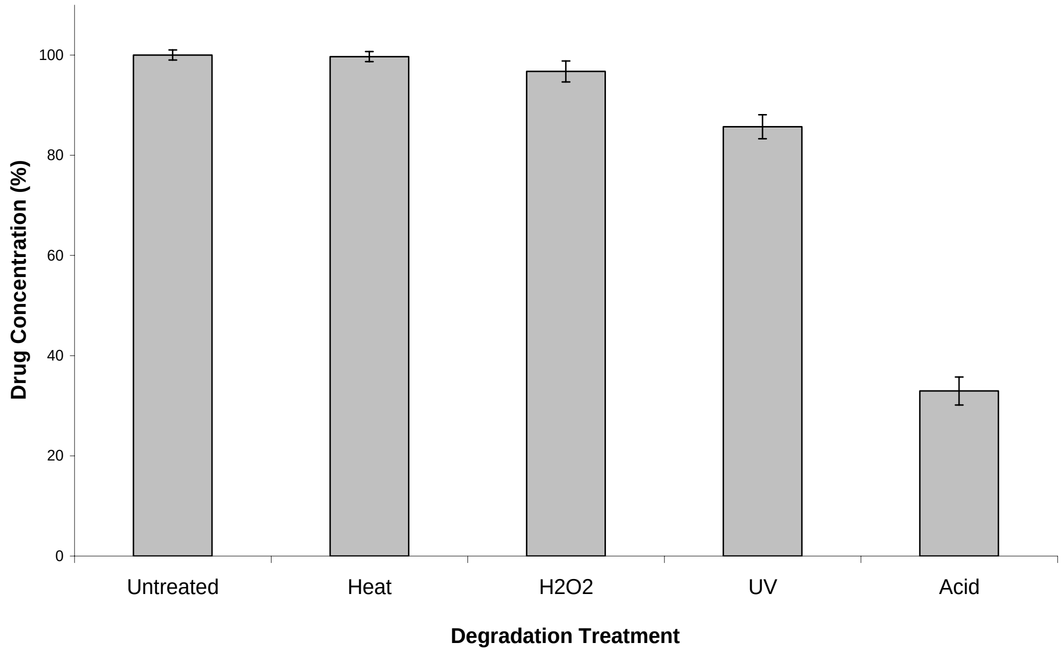


Figure 4.75 – Amount of Drug Remaining After Each Degradation Treatment

Addition of the base to the propranolol resulted in precipitation most likely caused by deprotonation of the propranolol hydrochloride so decreasing its solubility in the medium. The supernatant of the propranolol sample was separated from the precipitate and passed through the electrospray mass spectrometer. The ESMS spectrum revealed the dominant propranolol peak (at 260 m/z) was no longer present (Figure 4.76) indicating all the propranolol had precipitated leaving no drug in the supernatant.

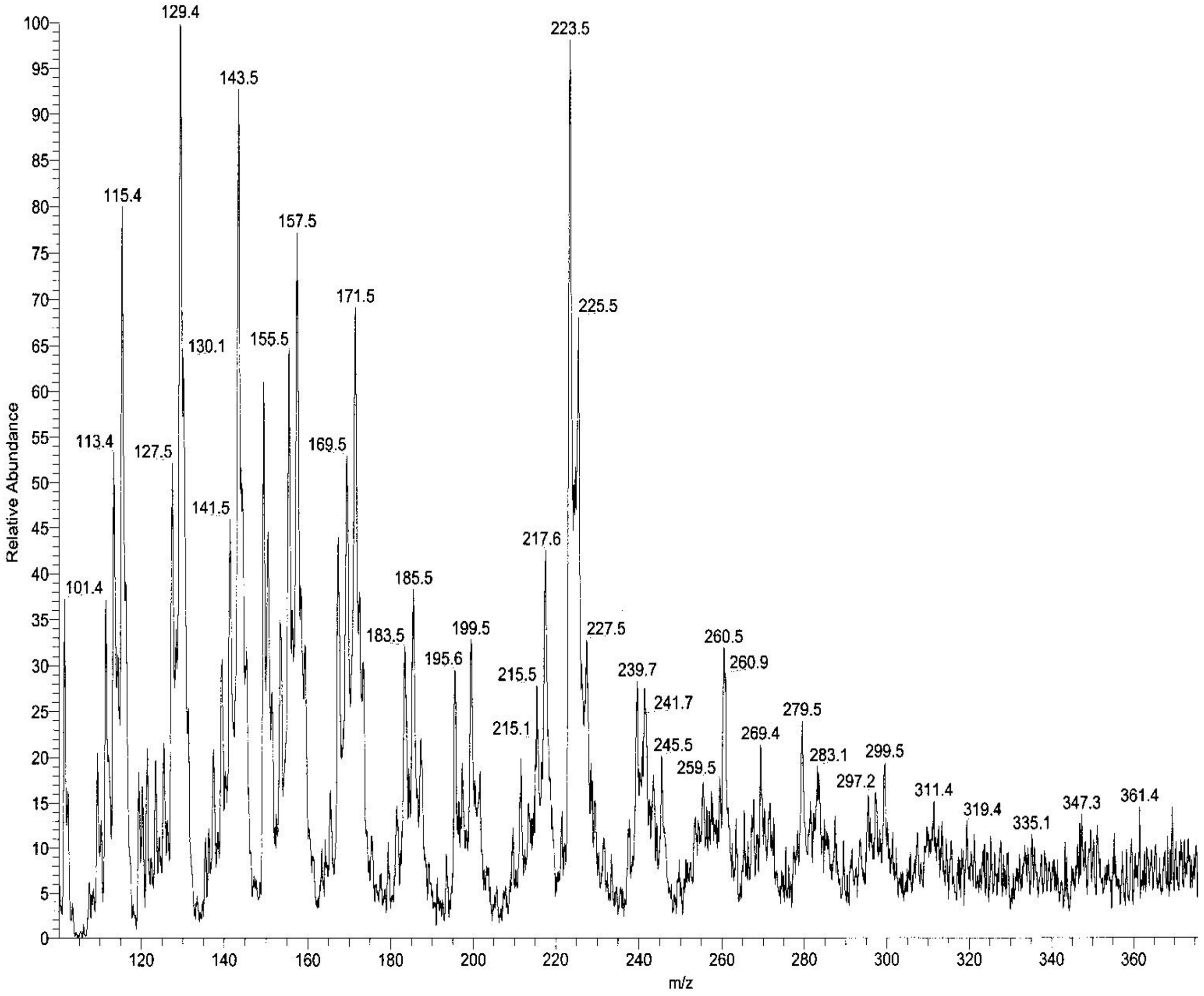


Figure 4.76 – Mass Spectrum of Propranolol Subjected to Basic Conditions

Addition of acid resulted in some precipitation, which would explain why much of the drug had degraded. This could be due to degradation by acid hydrolysis at the ether linkage in the propranolol.

Placing the drug in front of a UV source (254 nm) resulted in the drug changing from a colourless solution to a light orange colour. A colour change was also reported by Uwai *et al.*, (2005) where their work was conducted on propranolol tablets. Propranolol is known to be photosensitive. The HPLC did not show the presence of any degradation peaks. However the HPLC showed that some form of degradation had occurred as the amount of drug remaining had decreased (Figure 4.75). However, because the HPLC showed some form of degradation had occurred and the propranolol solution had changed to an orange colour, degradation or some other reaction had obviously occurred. Given the HPLC analysis was conducted on a PDA detector, degradation peaks were screened for under the wavelength range of 200 - 410 nm. However, degradation peaks were not observed. If any peaks due to degradation were present, they may have eluted in the solvent front. This would occur if the degradant was much more polar than the compound of interest. Alternatively, the degradant may be less polar than the analyte and therefore be retained by the column.

Propranolol is known to be a photosensitising agent and light unstable (Uwai *et al.*, 2005). The instability is a result of the napthalene skeleton which is a UV chromophore (Uwai *et al.*, 2005). Uwai *et al.* (2005) conducted TLC analysis and found photo-degradation products of propranolol after 10 days of UV treatment. However structural analysis proved difficult as the rate of photoproduct formation from pharmaceutical preparations was too slow to isolate enough degradation products. Therefore concentration of the degradation products may be an issue. This would explain why degradation peaks in the HPLC or ESMS were not observed.

Napthalene, 1,4-napthoquinone (Salomies, 1987), 6-hydroxy-1,4-napthoquinone (Sortino *et al.*, 2002), 1-napthol, N-acetylpropranolol and N-formylpropranolol (Uwai *et al.*, 2005) have all been reported as photodegradation products of propranolol. Each study found different photodegradation products. Uwai *et al.*

(2005) attributed the difference to the conditions which the propranolol was irradiated. Therefore propranolol does degrade in UV light but the products formed depend on the treatment method.

In the investigation reported in this thesis, heat and H_2O_2 treatment did not result in degradation as shown in Figure 4.75. Though degradation peaks were not detected, the HPLC analysis indicated (by means of monitoring the intensity of the propranolol peak) that some form of degradation had occurred because the amount of drug remaining in the sample decreased from its original concentration. The assay itself would not have contributed to a change of response in the HPLC as the degradation study was conducted in triplicate and the results were the same for each sample.

A stability indicating assay is an assay which is a validated quantitative procedure that can detect changes with time in the pertinent properties of the drug substance and product. The assay has the ability to accurately measure the active ingredient without interference from the factors such as the degradation by-products. In this study, peaks identifiable as degradation products were not detected. Degradation peaks were searched for under the wavelength range of 200 - 410 nm, but degradation peaks were not found. If any peaks due to degradation were present, they may have eluted in the solvent front or may have been retained by the column as mentioned earlier in this section. Alternatively they may have occurred in a higher wavelength range if they possessed absorptions in the visible region of the spectrum. The stability assay described in this section is not a true stability indicating assay in terms of the definition given in this discussion as degradation peaks were not detected. However, it can be argued that because there were no degradation peaks detected, there will be no interference with the peak of interest. Consequently, in this regard it can be claimed that the method is stability indicating. Therefore, drug stability studies can still be conducted using the method described in this section, as the peak of interest is void of interference and the drug remaining can be quantified. It is also possible the retention times of any degradation products may possess visible absorption maxima and hence occur outside the range of wavelengths investigated (200 – 410 nm). In such a case, the main propranolol peak could still be used as a means of quantifying the concentration of the drug as it would not have any interference from other species.

Robustness

The robustness of the method (see Section 3.6a *i*) was tested by determining the effect of varying the mobile phase composition on the retention time of a 5 mg mL^{-1} sample of propranolol. The results of this investigation are shown in Tables 4.24 – 4.26.

Table 4.24 – Effect of Acetonitrile Concentration in the Mobile Phase on the Retention Time

Mobile Phase	**Retention Time (mins)**
Mobile phase (containing 36% acetonitrile)	8.045
Mobile phase (containing 66% acetonitrile)	8.049

Varying the amount of acetonitrile in the mobile phase did not affect the retention time of the drug (Table 4.24). The retention times had a small relative standard deviation of 0.1%.

Table 4.25 - Effect of Methanol Concentration in the Mobile Phase on the Retention Time

Mobile Phase	**Retention Time (mins)**
Mobile phase (containing 36% methanol)	8.045
Mobile phase (containing 66% methanol)	6.546

Varying the amount of methanol in the mobile phase resulted in a change in the retention time of the drug (Table 4.25). This was also found to be the case when the strength of acid in the mobile phase was altered (Table 4.26).

Table 4.26 - Effect of Phosphoric Acid Concentration in the Mobile Phase on the Retention Time

Mobile Phase	**Retention Time (mins)**
Mobile phase (containing 0.15 mol L^{-1} phosphoric acid)	8.045
Mobile phase (containing 0.30 mol L^{-1} phosphoric acid	6.645

Therefore in the HPLC method employed for the drug stability studies, the retention time of the drug was not affected by the amount of acetonitrile in the mobile phase. In contrast the retention time of propranolol was affected by the amount of methanol and the strength of phosphoric acid in the mobile phase. There are few reports in the literature on such observed occurrences. The mobile phases in the HPLC analysis of beta blockers often use methanol or acetonitrile as a means of controlling the retention (Basci *et al.*, 1998). The polarity of the mobile phase can have a major effect on the chromatographic behaviour of the solutes. Basci *et al.* (1998) found increased concentrations of acetonitrile in phosphate buffer reduced the capacity factor of beta blockers (including propranolol) due to the decreased polarity of the mobile phase. The capacity factor was calculated according to Equation 4.11 (Basci *et al.*, 1998):

$$k' = \frac{(t_R - t_0)}{t_0} \tag{4.11}$$

where

k' = the capacity factor

t_0 = retention time of the unresolved peak

t_R = retention time of the solute

Therefore because the capacity factor is dependant on the retention time, a change in capacity factor indicated a change in the retention time. Though acetonitrile affected the retention time in Basci *et al.*'s study it did not affect the retention time in this study. Consequently the experiment varying the acetonitrile concentration was repeated to verify the findings of this study. Once more the retention time did not change with a change in concentration of acetonitrile.

Another possible cause of the shift in retention time of the propranolol with altering concentrations of methanol may be due to hydrogen bonding between the amine group of propranolol and the hydroxide group of methanol. Such an interaction will not occur between acetonitrile and propranolol.

A buffer is also commonly employed to control the ionic strength and pH of the mobile phase. Therefore altering the acid concentration in the mobile phase will change the pH of the mobile phase. Results showed (Table 4.27) that altering the acid concentration resulted in a change in retention time. Basic drugs such as propranolol are positively charged at low pH due to the amine group which becomes protonated. The pH of the mobile phase was altered from 3.20 to 2.03. This shift in pH may have altered the extent of protonation thus affecting the retention time of propranolol. In theory, at a lower pH the propranolol will be more protonated. A more protonated substance will have a stronger affinity for the mobile phase and therefore should have a faster retention time. This was found to be the case as decreasing the pH lead to a decrease in the retention time from 8.045 to 6.045 minutes (Table 4.26).

ii. *The Final Method*

As a result of conducting the method development study (Section 4.6a *i*), the drug stability will be assessed using the following method (Table 4.27):

Table 4.27 – The Final Method used in the Analysis of Propranolol

Parameter	Description
Column	Nova-Pak C18 column (8 x 100 mm)
Guard Column	Nova-Pak, 4 µm, 60 Å, C18 Guard-Pak Insert
Mobile Phase	Prepared according to USP (1994) by dissolving 1 g of sodium dodecyl sulphate in 36 mL of 0.15 mol L^{-1} phosphoric acid (Merck, Germany) in a glass vessel. To this glass vessel, add 180 mL of HPLC grade methanol (Scharlau, Spain) and HPLC grade acetonitrile (Burdick and Jackson, USA) as well as 104 mL of water to give a final volume of 500 mL.
Flow Rate	1 mL min^{-1}
Temperature	The temperature was not varied during the development as the column was not heated.
Injection Volume	20 µL
Detector	PDA 200 – 410 nm

Upon finding the ideal method to analyse the drug, the method was then validated. It is necessary to validate the HPLC method to ensure it is accurate and reproducible over the concentration range which the analyte will be analysed.

iii. Method Validation

Method validation was conducted by assessing the linearity, accuracy, precision, limit of quantitation, limit of detection, robustness and specificity.

Linearity

A linear relationship was found between concentration and peak area (Figure 4.77). The R^2 value was 0.9996 which is above the 0.999 value required as mentioned earlier in the linearity test described in the method development section (Section 4.6a *i*).

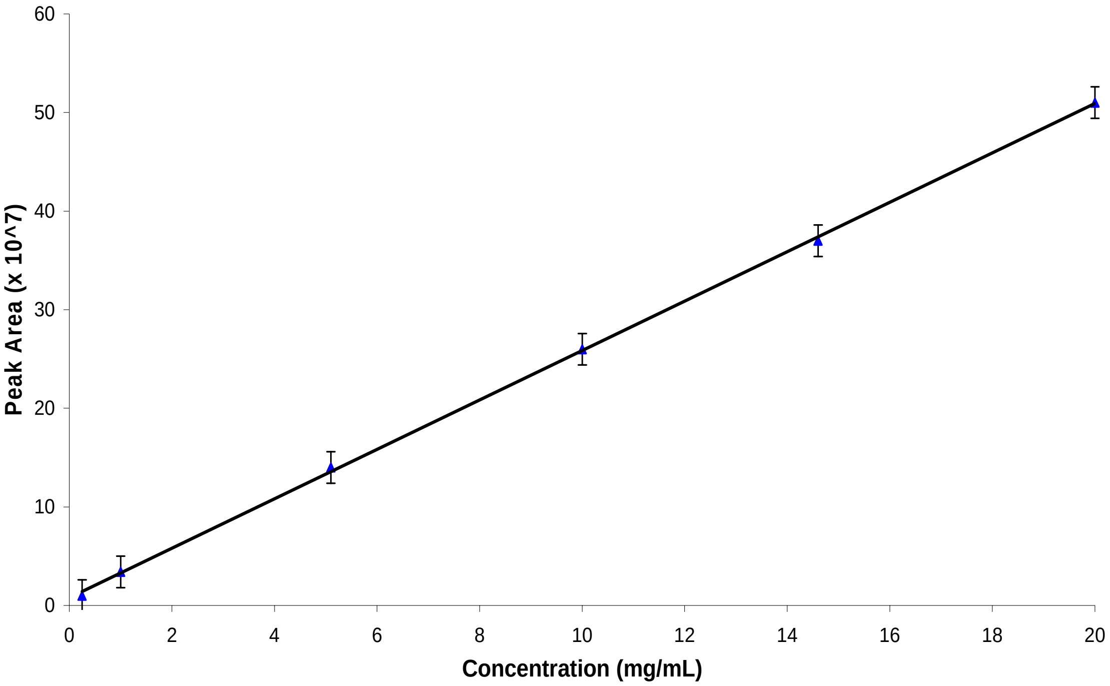


Figure 4.77 – Method Validation to Assess the Linearity of the Final Method

Accuracy

The accuracy of the method was tested by running samples of propranolol with known concentrations of 2, 9 and 17 mg mL^{-1} (in triplicate) to determine if the peak area gave the correct concentration. The method was accurate as the area of the peak from the graph (the graph plotted when testing the linearity, Figure 4.77) corresponded to the actual concentrations of 2, 9 and 17 mg mL^{-1} (within the percentage relative standard deviation as shown in Table 4.28).

Table 4.28 – Comparison of the Reference Concentration and the Average Experimental Concentration for Propranolol

Reference Concentration (mg mL^{-1})	**Average Concentration (mg mL^{-1}) (Determined from Calibration Curve)**	**% Relative Standard Deviation**
2	1.992	0.42
9	8.998	0.16
17	17.006	0.22

Precision

Repeatability: 6 samples with a concentration of 0.5 mg mL^{-1} and 6 samples with a concentration of 5 mg mL^{-1} had a relative standard deviation of 0.48 and 0.62% which were well within the 10% confirming the good precision of the method (Rao *et al.,* 2005).

Intermediate Precision: Figure 4.78 shows the analytical method was reliable in that the method provided similar calibration curves on 3 different days which agreed within error (for propranolol concentrations of 0.25, 1, 5, 10, 15 and 20 mg mL^{-1} as described in Section 3.6a *iii*). The error bars displayed in Figure 4.78 represents the relative standard deviation.

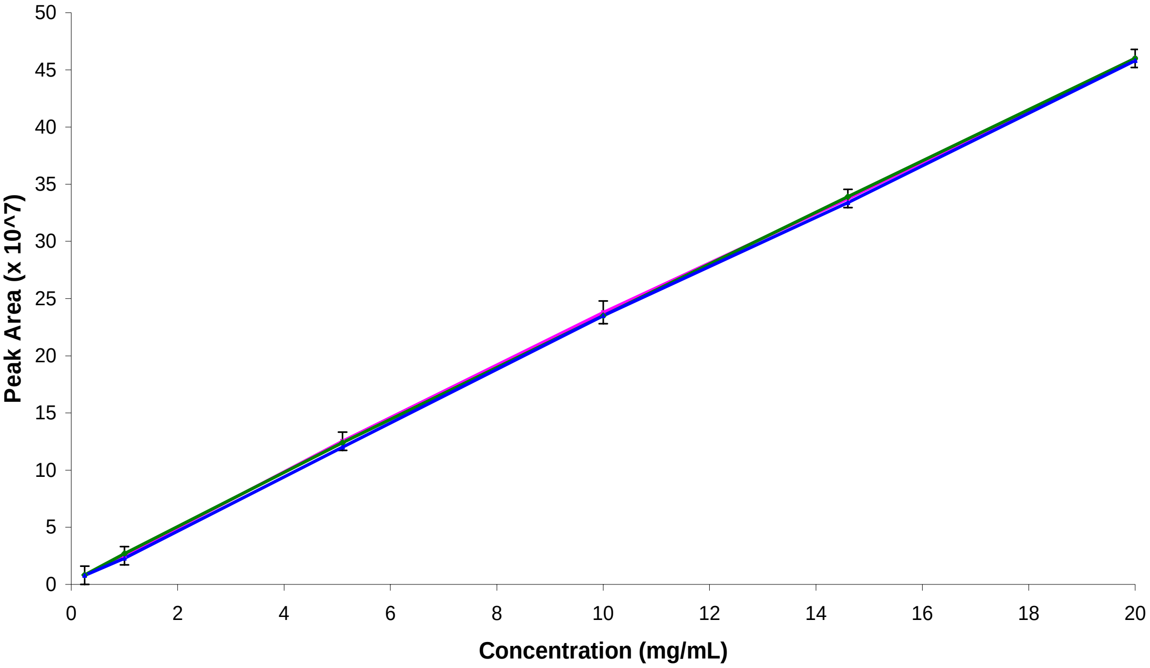


Figure 4.78 – Intermediate Precision of the Final HPLC Method for Propranolol

LOD and LOQ

The LOD was calculated according to Equation 4.12:

$$\text{LOD} = 3.3\ \text{SD/S} \tag{4.12}$$

where SD is the standard deviation of the response and S refers to the slope of the calibration curve. The LOD was found to be 0.0481 mg mL^{-1} which is the lowest concentration of propranolol that can be detected. The lowest concentration which can be quantitatively detected (i.e. LOQ) was found according to Equation 4.13:

$$\text{LOQ} = 10\text{SD/S} \tag{4.13}$$

The LOQ was calculated to be 0.145 mg mL^{-1}. Therefore the concentration of drug to use in the stability test should be above this concentration.

To verify the limit of detection, a sample with a concentration of 0.040 mg mL^{-1} was injected into the column. A peak was not detected. A more concentrated sample of 0.055 mg mL^{-1} was injected into the column, resulting in the detection of a peak. Therefore the calculated limit of detection (LOD) value of 0.0481 mg mL^{-1} is in agreement with the experimental findings. This limit of detection is quite high. It is uncertain as to the cause of this high value.

iv. Extraction of the Drug from the Final Formulation

In this initial study, extraction of the drug from the formulation was attempted by size exclusion chromatography (SEC) using the KS-803 sugar column. The formulation consists of the continuous phase (propylene glycol and polycarbophil), the hydrophilic gelling agent (HPMC) and the drug (propranolol). In order to extract the drug, the initial approach was to dilute the formulation to the extent where the HPMC was no longer viscous (as it is a hydrophilic gelling agent) so that the formulation could be introduced to the HPLC. The formulation components would then be separated according to size which was the basis of choosing the size exclusion column. Therefore, the size exclusion method could be a means of extracting the drug from the formulation and then introducing the extracted drug into the C18 column using the developed and validated method described in Sections 4.6a *i* and 4.6a *iii*.

Injection of the final product into the column, would result in the large molecular weight polymers HPMC and polycarbophil eluting at the start (due to their large size) leaving the low molecular weight propylene glycol and propranolol. Propylene glycol and propranolol would be identified by the detectors, where the PDA (photo diode array) and RI (refractive index) detectors were both used (the detectors were placed in series). Initial results showed 2 peaks in the RI chromatogram and only 1 peak in the PDA chromatogram. Propylene glycol does not have UV absorbance peaks in the region of interest, therefore the peak present in the RI chromatogram which did not have a corresponding peak in the UV chromatogram was due to propylene glycol. The peak observed in both the RI and UV chromatogram was therefore due to propranolol. This was further confirmed by the molecular weights of propranolol and propylene glycol. Propylene glycol being smaller than propranolol should have a longer retention time and this was observed to be the case. Hence, this size exclusion column was able to separate the drug from the excipients of the formulation. However, this approach was not used further as a problem arose concerning the retention time of the drug. During the development process, it was found there was intra-sample (Table 4.29) variation in the peak area. It was also found that the retention time of the drug increased with increasing concentration (Table 4.30).

Table 4.29 - Intra-Sample Variation for a 5 mg mL^{-1} Propranolol Sample using the KS-803 Column

Sample Number	**Peak Area**	**Retention Time (mins)**
1a	244200	6.334
1b	129034	6.337
1c	237423	6.337
1d	214700	6.335

The effect of intra-sample variation on a further concentration of propranolol (15 mg mL^{-1}) was also assessed. There was once more variation in the peak area.

Table 4.30 - Change in Retention Time for Varying Concentrations of Propranolol using the KS-803 Column

Propranolol Concentration (mg mL^{-1})	**Retention Time (mins)**
0.5	5.942
1	6.042
5	6.333
10	6.503
20	6.723

Table 4.31 - Extent of Peak Area Variation for 5 mg mL^{-1} Propranolol (Intra-Sample)

	Intra-Sample Analysis
Mean	206339.3
Standard Deviation	53058.6
% Relative Standard Deviation (%RSD)	25.7

Table 4.32 - Extent of Retention Time Variation for Different Concentrations of Propranolol

	Retention Time for the Different Concentrations of Propranolol
Mean	6.309
Standard Deviation	0.322
% Relative Standard Deviation (%RSD)	5.1

As shown in Table 4.31, the extent of variation in for the intra-sample analysis was very high (%RSD of 25%) hence the data was highly variable and a cause for concern. A change in the method was thus required. In the case where the concentration of the propranolol was varied, the variation in the retention time was not that high (%RSD of only 5.1% - Table 4.32). However, the cause of the increased retention time with increasing concentration was not known. Various factors were tested such as decreasing the injection volume to ensure the column was not being overloaded, ensuring the injector was adequately purged, determining whether the pressure fluctuated during the HPLC run and whether there were any leaks in the system. However, altering the injection volume did not have an effect. The pressure did not fluctuate during the run and there were no leaks in the system. The shifting in retention time may be a result of the mobile phase. No other reports in the literature have indicated the use of water on its own as a mobile phase for propranolol. Consequently, an acidified mobile phase was used. As the KS-803 can only have water as the mobile phase, a reverse phase C18 column was used.

As a result of the peak shifting and variations in the peak area, the size exclusion method of extracting the drug was not continued. The peak shifting and the variations in the peak area meant the extraction method would not have been efficient. Consequently, a new means of extracting the drug was investigated.

Addition of methanol to the formulation resulted in the hydrophilic gelling agent HPMC dropping out of solution. This observation was used to develop a new method of extracting the drug using the C18 column.

Extraction of the drug from the formulation involved addition of methanol which resulted in the HPMC dropping out of solution. The sample was then centrifuged to collect the HPMC, leaving the drug (which is soluble in methanol) in solution with the rest of the propylene glycol/drug mix. The supernatant was injected into the HPLC and a peak due to propranolol was observed.

The extraction efficiency was determined by addition of methanol to a formulation with a known concentration of drug. The supernatant (containing the dissolved drug) was passed through the HPLC. The peak area gave a concentration off the calibration curve which matched the true concentration of the drug in the formulation. This was repeated with 3 other samples to ensure the result was reproducible. The results were reproducible with a relative standard deviation of 0.8%. This was repeated for a drug concentration of 1 mg mL^{-1} and results were reproducible with a relative standard deviation of 0.97%.

v. Accelerated Stability Study of the Drug in the Formulation

Upon validation of the HPLC method, a stability assessment of the drug in the formulation was conducted. Extraction of the drug from the formulation involved addition of methanol which resulted in the HPMC dropping out of solution. The sample was then centrifuged to collect the HPMC, leaving the drug (which is soluble in methanol) in solution.

Upon finding a method to extract the drug from the formulation (as described in Section 3.6a *v*), samples (triplicate) of the final product with a known amount of drug were placed in accelerated conditions (40°C / 75% relative humidity) and each week samples were taken to monitor the drug level.

A 4 week accelerated study of the final product was carried out. A 4 week time period was chosen as the delivery of propranolol using the developed technology will be packaged as a two component system (as described in Section 2.6) where the dry powder is packaged separately along with the liquid for use at the time of reconstitution. Consequently, a 4 week time period is a sufficient length of time for the accelerated stability study.

Over the 4 week accelerated study it was found the level of drug remained constant within the calculated error of 0.8% for each of the 3 samples (Figure 4.79).

The peak area gave a concentration from the calibration curve which correlated to the actual concentration in the final product of 5 mg mL^{-1}.

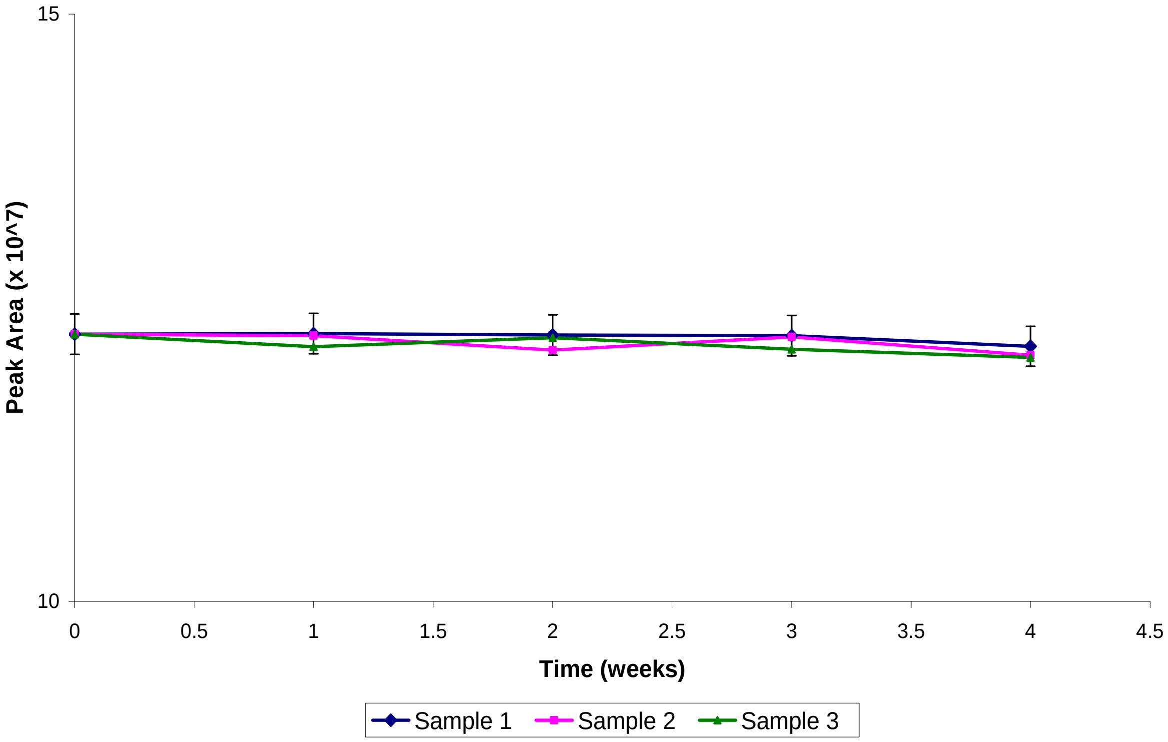


Figure 4.79 – Peak Area of Propranolol Over Time

As a result of conducting an accelerated stability study of the final product in accelerated conditions, it can be concluded that the final product is stable over the 4 week period studied as the drug level remained unchanged indicating degradation had not occurred.

4.6b Appearance of the Final Product

The appearance of the final product stored in accelerated conditions was recorded each week for 4 weeks (Table 4.33).

Table 4.33 - Appearance of Formulation Over Time After Storage in Accelerated Conditions

Week	Observations*			
	Colour	Caking	Air Bubbles	Particulate Matter
0	White	No caking present	No air bubbles observed	The presence of particulate matter was not measurable for this system due to the two particulate materials suspended in the final product (the drug and the hydrophilic polymer).
1	White	No caking present	No air bubbles observed	
2	White	No caking present	No air bubbles observed	
3	White	Slight caking with a sediment height of 0.05 cm which was readily dispersed.	No air bubbles observed	
4	White	Slight caking with a sediment height of 0.1 cm which was redispersed with minimal shaking.	No air bubbles observed	

* The observations for each of the 3 samples were identical

The stability studies have shown effectively that the drug remained stable in the final formulation over the 4 week period as little degradation of the drug had occurred. There was also no visible degradation of the formulation over time in the accelerated conditions employed. Previous reports state that systems able to withstand degradation under accelerated conditions should be stable under normal storage conditions (Davis, 1987). Therefore it can be concluded that the HPMC/propylene glycol/polycarbophil ISGM formulation should also be stable under normal (i.e. ambient) storage conditions for at least 4 weeks.

CHAPTER FIVE

Summary and Conclusions

5.1 Summary of Findings

This thesis reports the *ab initio* formulation and characterisation work surrounding a recently described new and novel *in situ* gelling matrix (ISGM) system that has been patented by Bunt *et al*. in 2003. Converting the conceptual ideas outlined in the patent into a practical technology was the main aim of the research. This was because the patent gave only a broad description of what the ISGM could incorporate, i.e. a suspension of almost *any* hydrophilic polymer in *any* non-aqueous solvent. As a result, extensive formulation studies had to be carried out to find the ideal excipients to use in the ISGM system.

Initial studies involved conducting a biocompatibility study based on the HET-CAM test to determine whether any of the possible candidates for the technology exhibited an immunological response. NMP was found to cause a severe reaction in the HET-CAM test and was hence removed from the non-aqueous solvent candidate list for the ISGM system. The remaining non-aqueous solvents and polymers were subsequently carried forward to the screening process.

The screening process involved addition of various hydrophilic polymers to non-aqueous solvents. This was found to result in rapid sedimentation of the hydrophilic polymer, which was not ideal from a usage point of view. A physically stable suspension should remain suspended for as long a period as possible at least to allow administration. Furthermore, rapid sedimentation may lead to caking of the settled particles, which is not desirable as redispersal (the simplest temporary remedy for a sedimented system) would not be completely feasible.

Hence, in order to retard the sedimentation rate, the physicochemical properties of the non-aqueous solvent were modified by addition of a density inducing or viscosity inducing agent. The density/viscosity of the non-aqueous solvent was increased initially by addition of various amounts of glycerin and as this occurred, a decreased sedimentation rate was observed. However, despite the success in retarding the sedimentation rate, ISGM systems with high glycerin content tended to become pre-gelled because of the hygroscopic nature of glycerin, which led to moisture uptake during handling, causing the hydrophilic polymer to gel. Gel formation in a formulation prior to injection is undesirable as it would be too thick to flow through a syringe. As a result of this outcome, viscosity modification of the non-aqueous solvent by addition of a solid polymer was considered as a means of retarding the sedimentation rate.

The viscosity of the non-aqueous solvent was manipulated by addition of a viscosity inducing agent, polycarbophil. An initial inspection of the manufactured continuous phase revealed the existence of clumps of polycarbophil. Such clumps are undesirable in a final product as properties of the system including the viscosity will vary. Consequently numerous manufacturing methods were attempted to avoid or prevent the formation of these globules. After attempting many methods to overcome this problem, a solution was found by utilising a high speed dispersion tool called the Ultra Turrax®. The Ultra Turrax® was found to be an effective tool which produced a small vortex into which to sprinkle the polycarbophil and this prevented globule formation by efficient and rapid dispersion into the medium.

As a result of this extensive study, the ideal constituents to use in the formulation were found. Investigations into the biocompatibility, the ideal manufacturing method (to prevent the formation of globules of polycarbophil) and physical sedimentation analysis were carried out to find the ideal excipients for the formulation. This was a significant result as there is little information on the stabilisation of suspended matter in non-aqueous systems (compared to aqueous systems) for pharmaceutical applications.

After a careful and systematically thorough investigation, the excipients for the ISGM system were ultimately determined. The final formulation consisted of the hydrophilic gelling agent HPMC (10%), the continuous phase (comprising of the viscosity inducing agent polycarbophil (0.67%) and the non-aqueous solvent propylene glycol) and the drug propranolol. The time for HPMC to fully sediment was increased from 6 hours in propylene glycol to approximately 314 hours by addition of polycarbophil (0.67%). As a result of this study the sedimentation rate of the hydrophilic gelling agent has been significantly decreased to give a physically stable suspension which was still able to be redispersed after 700 hours.

The ISGM system was assessed by carrying out characterisation studies. Water absorption studies were conducted to show the formulation (if open to the atmosphere) would absorb moisture. Studies were conducted in a controlled environment. Water absorption did occur due to the hygroscopic propylene glycol. Therefore it is essential to seal the vial containing the formulation to ensure the suspension does not gel and adversely affect the injectability of the formulation.

The chemical stability of the hydrophilic polymer and the continuous phase was determined. The stability of the hydrophilic polymer was assessed by size exclusion chromatography. Over a period of 9 months the HPMC did not appear to degrade, indicating the polymer was fairly stable. Therefore the HPMC should not limit the shelf life of the final formulation.

The stability of the continuous phase was assessed by viscosity. Over a period of 9 months the viscosity of the continuous phase remained constant within experimental error and therefore can be regarded as chemically stable.

The ease of redispersing the ISGM system was also assessed. The vials containing the ISGM system were stored at room temperature. Redispersion was achieved by controlled rotation of the vial. After 4 weeks, the ISGM system could be redispersed within 60 seconds indicating the ISGM system was still physically stable and that caking (which can prevent complete redispersion) had not occurred.

To determine how the ISGM system would appear when injected, the ISGM system was introduced via a syringe into a cellulose medium. The ISGM system gelled to form an intact bulb. Injection into the ear of a dead calf also led to a gelled depot. Consequently the ISGM system was injected into a live rat. Dissection of the rat showed the ISGM system had gelled and was easily removed after 6 hours. This experiment showed the ISGM system functioned adequately where injecting a suspension of the hydrophilic gelling agent in the continuous phase resulted in an intact gel in the body. The aim of the study was to find an ISGM system that would be capable of achieving this.

To ensure incompatibilities between the drug and the excipients of the formulation did not exist, DSC studies were conducted. The formulation itself showed no interactions with the drug but addition of water to give a gelled formulation resulted in shifting of the melting point peak from 165°C to 146.1°C. This interaction was thought to be a propranolol-anionic polymer binding interaction. IR studies also showed a possible interaction as a new absorption band was found at *ca*. 1557 cm^{-1}. The complex formation may be a result of the bonding between the amine group of propranolol and the carboxyl group of polycarbophil.

Mass spectroscopy analysis was conducted to analyse the drug propranolol and UV analysis was conducted on the constituents of the final formulation. The UV analysis also determined which wavelength to use to carry out the *in vitro* drug release studies.

The feasibility of the ISGM system was assessed using the drug propranolol. Propranolol was chosen as the model drug as this ISGM technology will provide an ideal means of delivering propranolol where factors such as the first pass effect, skin permeation and short plasma half life are no longer an issue.

The initial *in vitro* drug release studies were conducted on the Hanson dissolution apparatus. The Hanson dissolution apparatus was not an ideal means of conducting drug release studies as the USP dissolution test was designed for tablets not gel depot technology, as studied in this project. Results were not reproducible as the formulation was exposed to the stirrer and a constant surface area of the injected depot was not maintained. As a result the USP protocol needed to be modified to accommodate the different technology.

The development of an *in vitro* experiment to obtain a reproducible release profile was difficult. Many methods were attempted before development of the modified release apparatus. The modified dissolution apparatus proved successful in obtaining a drug release profile for the controlled release formulation.

During the release experiment, it was found that 100% drug release was not obtained. A combination of DSC and IR studies led to the finding that it was likely an interaction between the drug and polycarbophil was occurring which was preventing 100% drug release.

The effect of parameters such as stirring rate, pH of release assessment media, omission of gelling agent, gelling agent concentration, distance between stirrer and diffusion site and polycarbophil concentration on the drug release characteristics of the ISGM system were assessed separately using the modified dissolution apparatus.

The drug release results showed the gelling agent greatly reduced the release rate of the drug. Of the gelling agent concentrations tested, the ISGM system with the 10% weight loading of HPMC was found to perform the best. The ISGM system containing 5% (w/w) HPMC did not form a strong enough gel to hinder release for an appropriate time. ISGM systems containing too much hydrophilic gelling agent such as 25% (w/w) HPMC were found to be too thickly gelled and although the drug release was reduced in line with expectations, the formulation was no longer injectable with a hypodermic syringe due to its lack of fluidity.

An anionic constituent of the formulation (polycarbophil) was found to be interacting with the drug and the extent of the interaction appeared to depend on the pH of the media.

As a result of conducting the *in vitro* drug release experiments, the ISGM system proved to be feasible as the release rate of propranolol was retarded upon formation of a gelled depot.

Modelling of the release data was carried out to determine if a relationship between drug release and each parameter existed. The initial modelling approach involved using the Higuchi equation. The Higuchi equation gave a straight line relationship (up to 50%) indicating the fraction of drug released was proportional to the square root of time. Modelling was also conducted using a more general power law relationship. The power law indicated the release mechanism of the drug was anomalous transport, i.e. the release mechanism was a combination of swelling-controlled and diffusion-controlled release.

The modelling showed that n did not change when altering the amount of drug in the formulation. This was ideal as the release mechanism will remain constant.

The final aspect of this research involved evaluating the stability of the final formulation. The stability of the drug in the formulation was monitored by HPLC. The main issue with the stability assessment was that degradation peaks were not detected during the development of the stability method. If degradation products did exist, they may have eluted with the solvent front or been retained in the column. The absence of degradation peaks meant that there was no interference with the peak of interest, thus allowing the stability of the drug to be investigated by monitoring the drug level in the formulation. The 4-week accelerated stability indicated drug degradation had not occurred as the drug level in the formulation remained unchanged.

The appearance of the formulation stored in the accelerated “ageing” conditions was also monitored. Visible signs of degradation such as a change in colour were not observed, indicating the formulation had apparently not degraded (over the 4-week study). Therefore as a result of conducting a stability analysis of the final formulation, it can be concluded that the formulation should be stable under normal storage conditions (for at least 4 weeks), which are less severe than the “accelerated storage conditions”.

This study has shown that the ISGM system as a concept is technically workable with the optimised set of constituents determined in this study as these were found to retard the release rate of propranolol. Therefore there is much potential for this ISGM system concept from a practical point of view and it should be investigated further. These future studies should not be restricted to the delivery of propranolol. Other drugs such as hydrophobic or non-ionic drugs should also be investigated. Hydrophobic drugs will have a different release mechanism than propranolol (which is a hydrophilic drug) where instead of diffusion, the release mechanism may be dictated by break down of the polymer. Non-ionic drugs would be advantageous in this system as the drug would not interact with the anionic viscosity inducing agent polycarbophil so that 100% release may be realised.

Though this study has shown that the ISGM system is practically workable, it is not known whether the technology will be able to deliver propranolol at an appropriate rate to be useful. Assessment of the appropriate rate would require *in vivo* studies (which have been recommended as future studies). The *in vitro* drug release experiment was used as a comparative tool to assess how different parameters would affect the drug release rate. The *in vitro* release experiments were not indicative of the drug release behaviour *in vivo* as certain restrictions such as the surface area of the release interface and swelling of the system were enforced. This study has shown that injection of the formulation results in the formation of a gelled depot *in vivo*. However, further, more prolonged studies into the *in vivo* release behaviour need to be conducted before determining whether the ISGM system will release the drug at a rate that is useful and whether it can be well tolerated by the body. This will include studies into the appropriate amount of drug and gelling agent to incorporate into the formulation to achieve the desired release of the drug over the required treatment time.

In closing, formulation challenges were met and a critical assessment of the chemical and physical stability of the product was made. The research successfully converted the ISGM system from a concept delivery system with little supporting experimental investigation into a practical technology that is worthy of further investigation and development as an animal health product.

Further investigation of the ISGM system should include:

- *Mechanistic Studies of the ISGM System*

  Carry out mechanistic, rheological and physicochemical studies of the ISGM system to identify how certain formulation variables influence depot formation. This could provide information on how to manipulate the ISGM system when incorporating different bioactive agents.

- *Biocompatibility of the Final Formulation in Live Animals*

  The degree of tissue site reactions and inflammatory responses (such as swelling and redness), if any, should be assessed *in vivo.*

- *In Vivo Trials*

  In vivo trials should initially be conducted on small animals where the drug level in the blood is monitored over time. The trial should then move on to larger animals to examine the effect of scale.

- *Incorporation of Different Bioactive Agents*

  Various bioactive agents (other than the hydrophilic drug used in this study) such as other hydrophilic drugs, hydrophobic drugs, proteins and peptides (though these latter two agents would carry special challenges due to their greater degree of metabolism) should be investigated as possible agents to use in this formulation. If the formulation is able to deliver a wide array of bioactive agents then it can be applied to the treatment of many diseases.

5.2 Conclusions

The research achieved several aims. These included:

- Research of a broad definition of an ISGM system and its conversion from a conceptual idea into a practical system

- Demonstrating that all ingredients of the ISGM system were biocompatible.

- Successfully formulating the continuous phase into which both the hydrophilic polymer and drug could be incorporated.

- Successfully assessing the resultant suspensions to demonstrate their physical and chemical stability.

- Demonstrating the ISGM technology worked when injected into an aqueous environment as a gelled depot formed.

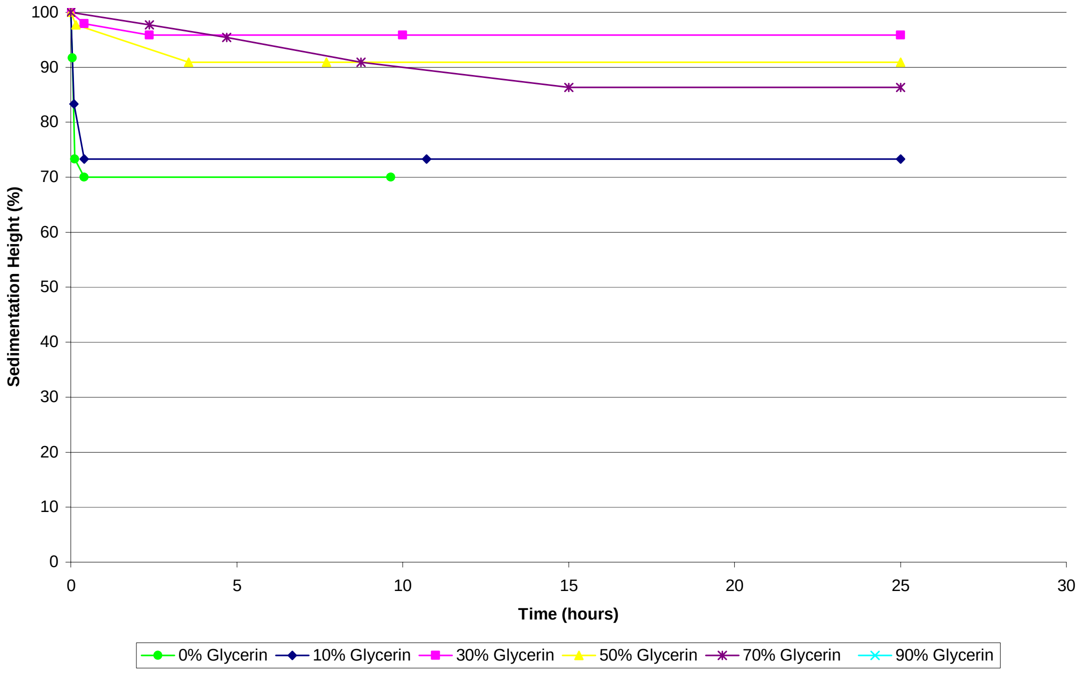
The significant contributions of this research to science were:

- The research identified a potentially useful polymer with which to modify the viscosity of a non-aqueous solvent and demonstrated that it could be readily manufactured and used to retard sedimentation of a range of polymers and drugs. This was a significant achievement, given the fact that the literature is sparse with respect to the reporting of compounds which increase the viscosity of non-aqueous solvents.

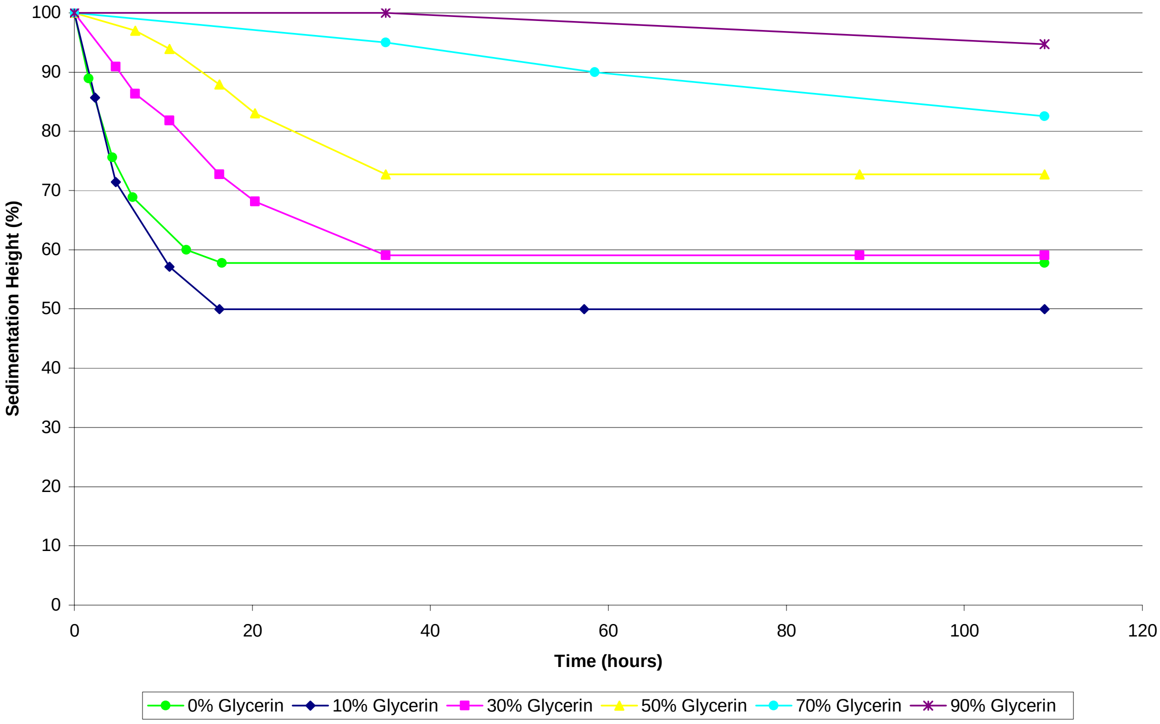
- The research successfully developed a novel method for determining the *in vitro* drug release of *in situ* forming gel technologies. This was a particularly significant achievement since the difficulty in developing an *in vitro* method for this type of controlled release delivery system is generally acknowledged as being very difficult. This contribution is again supported by the fact that the literature is sparse on reported methods.

- This research has successfully converted a novel idea for a technology that had been broadly described in the patent literature (Bunt *et al*., 2003) into a practical system. This was a technological achievement since there is little literature regarding the formulation on non-aqueous suspensions. The studies conducted in this research have shown that the ISGM technology is feasible, whereby introduction of the formulation to an aqueous environment resulted in a gelled depot which retarded the release rate of the drug, propranolol. Consequently this research has found that the ISGM technology has much potential and should be further investigated to develop it into a commercial product for the veterinary field.

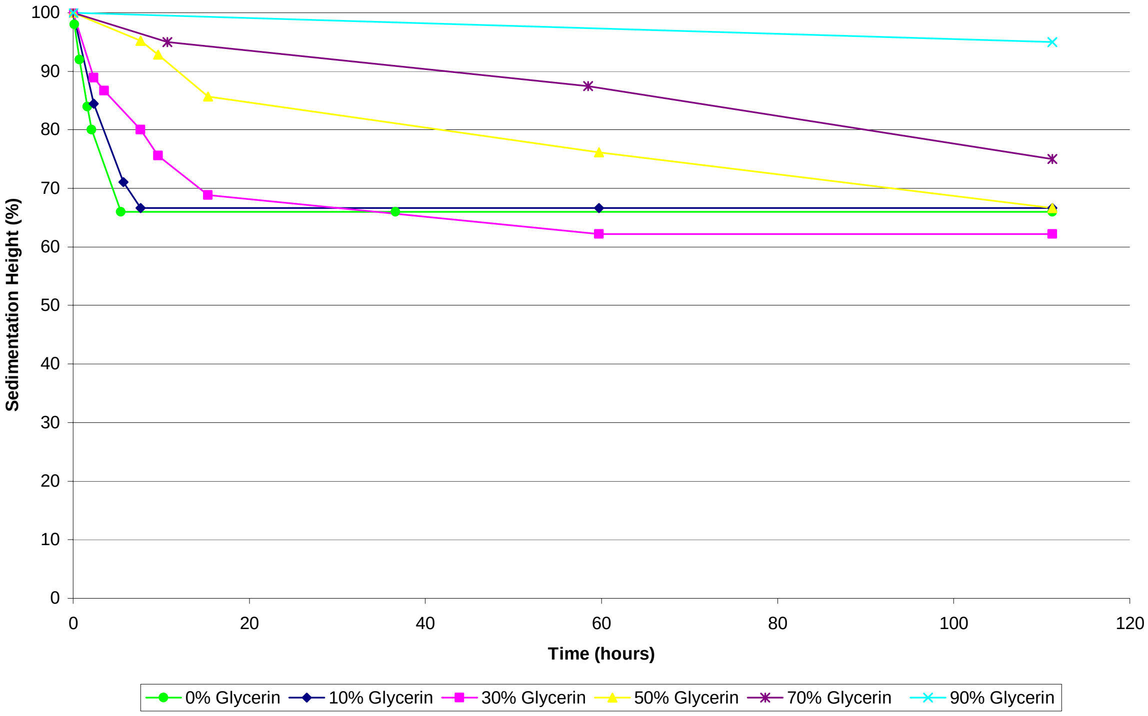
Appendix



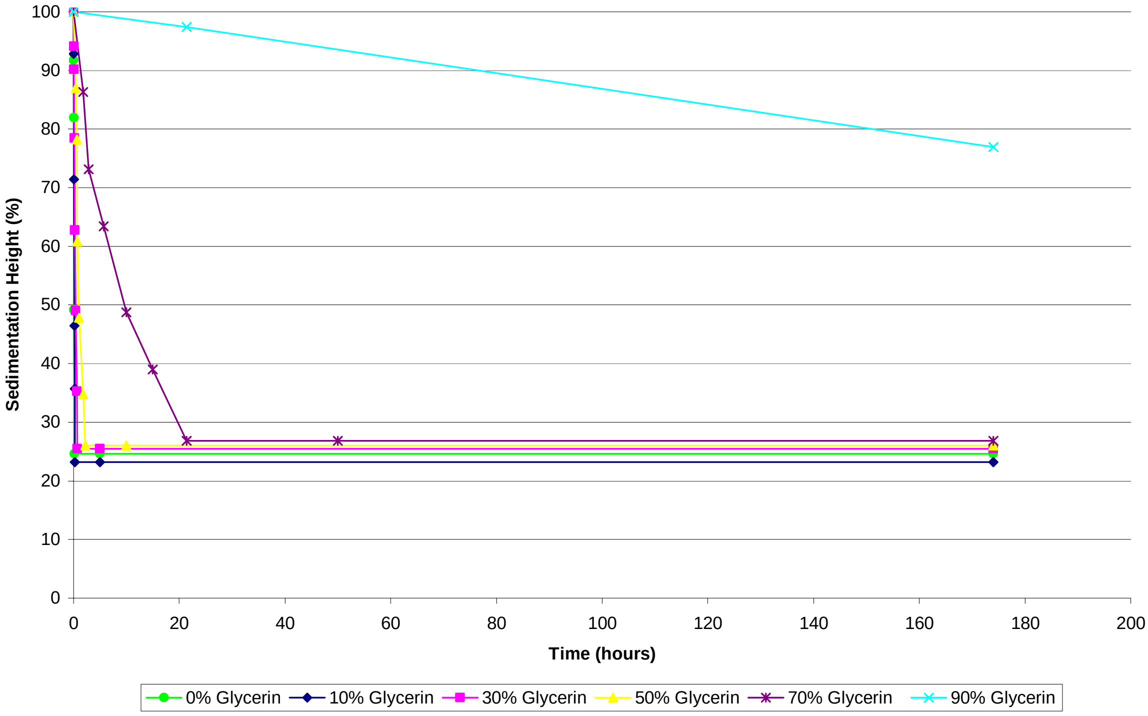
A1 – Comparison of Sedimentation Profiles for HPMC at each Glycerin Concentration in Alcohol (n=2)



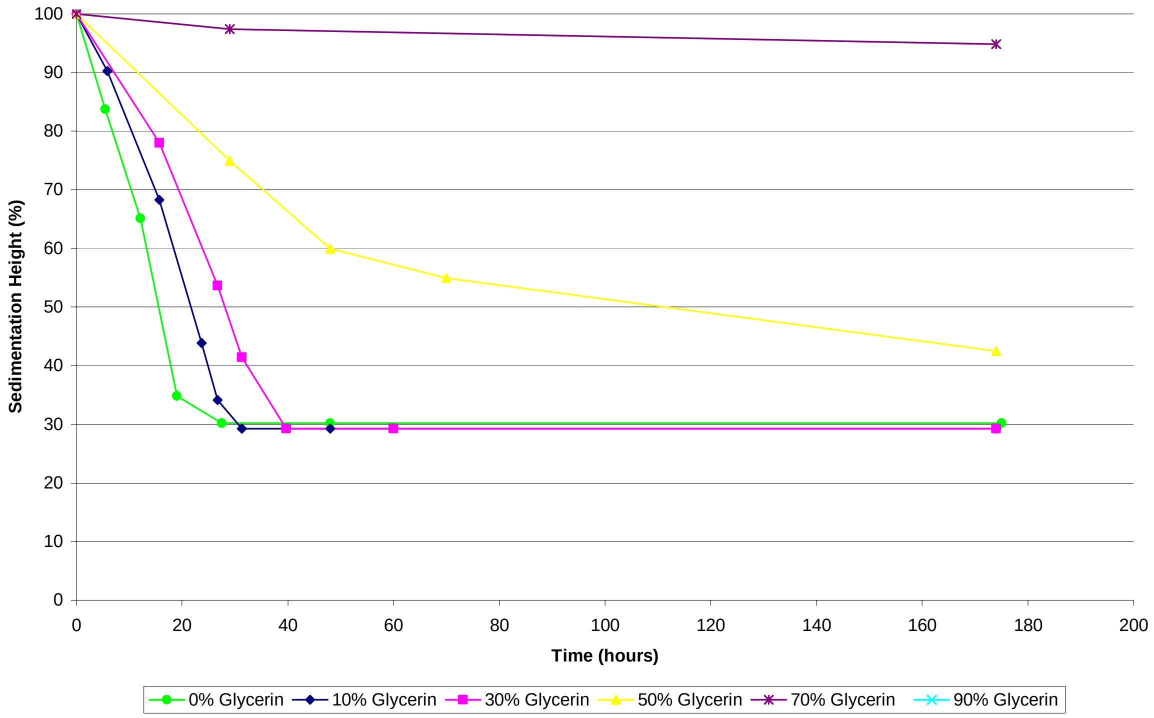
A2 – Comparison of Sedimentation Profiles for HPMC at each Glycerin Concentration in Polyethylene Glycol 400 (n=2)



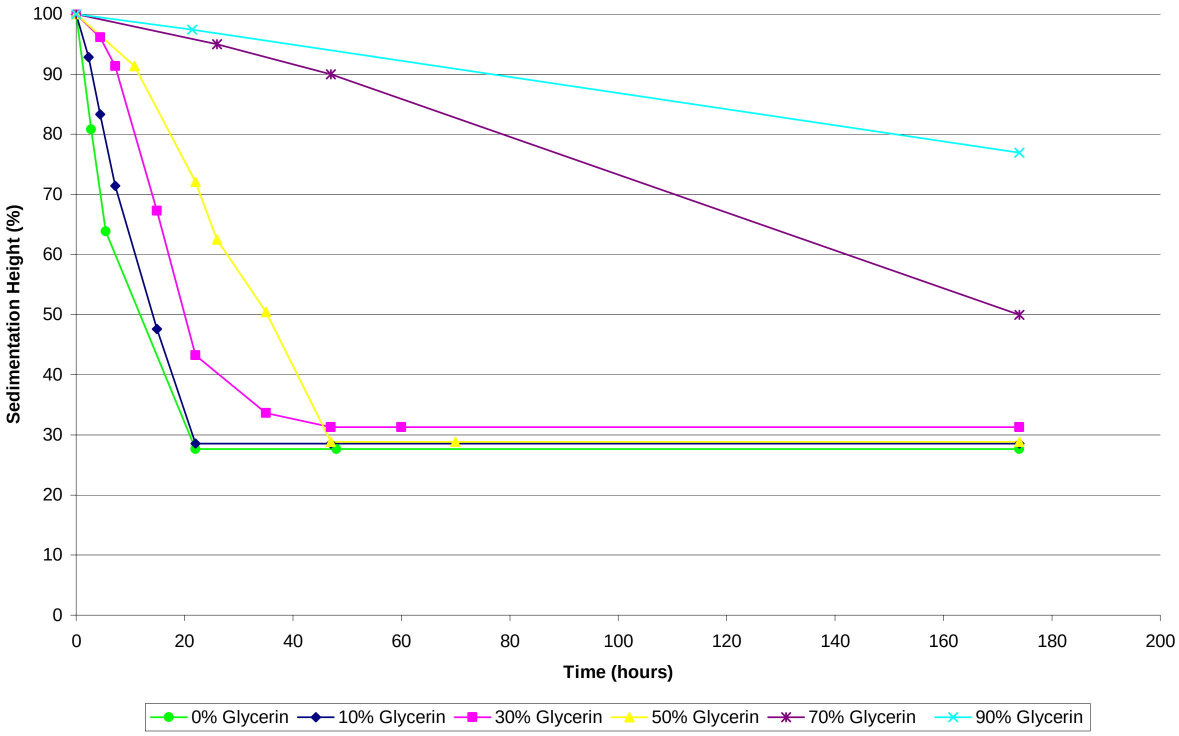
A3 – Comparison of Sedimentation Profiles for HPMC at each Glycerin Concentration in Propylene Glycol (n=2)



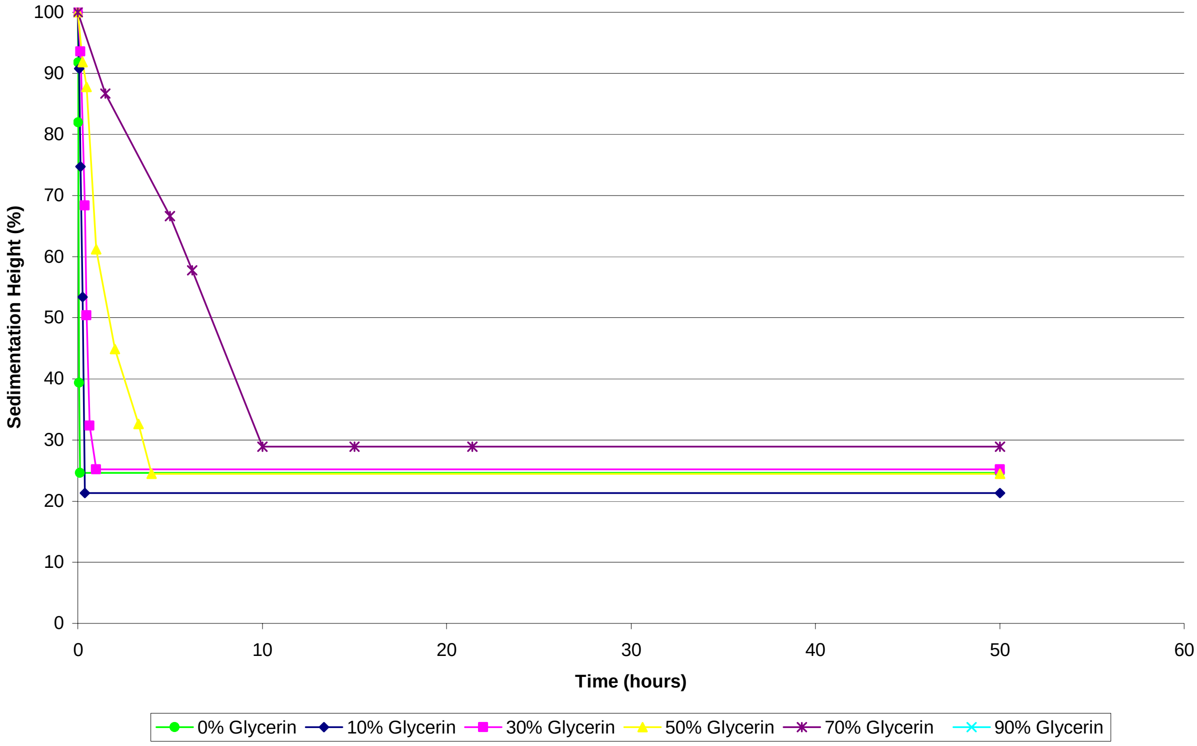
A4 – Comparison of Sedimentation Profiles for PEO 303 at each Glycerin Concentration in Alcohol (n=2)



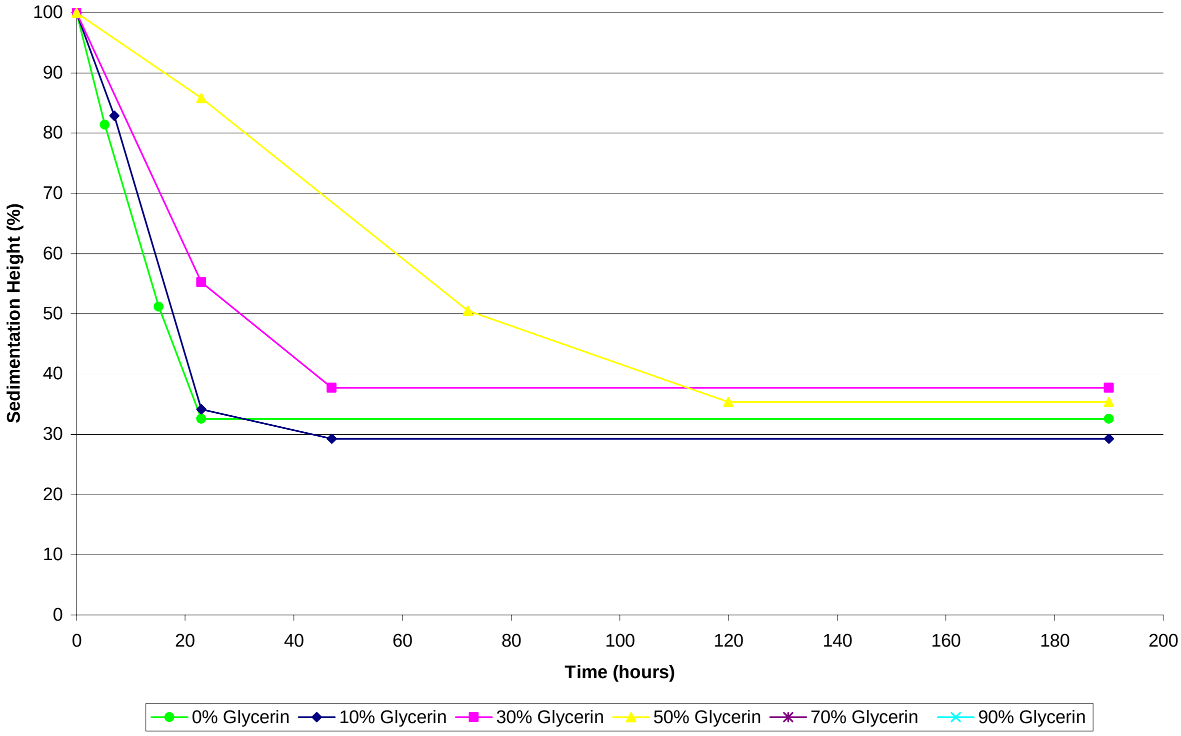
A5 – Comparison of Sedimentation Profiles for PEO 303 at each Glycerin Concentration in Polyethylene Glycol 400 (n=2)



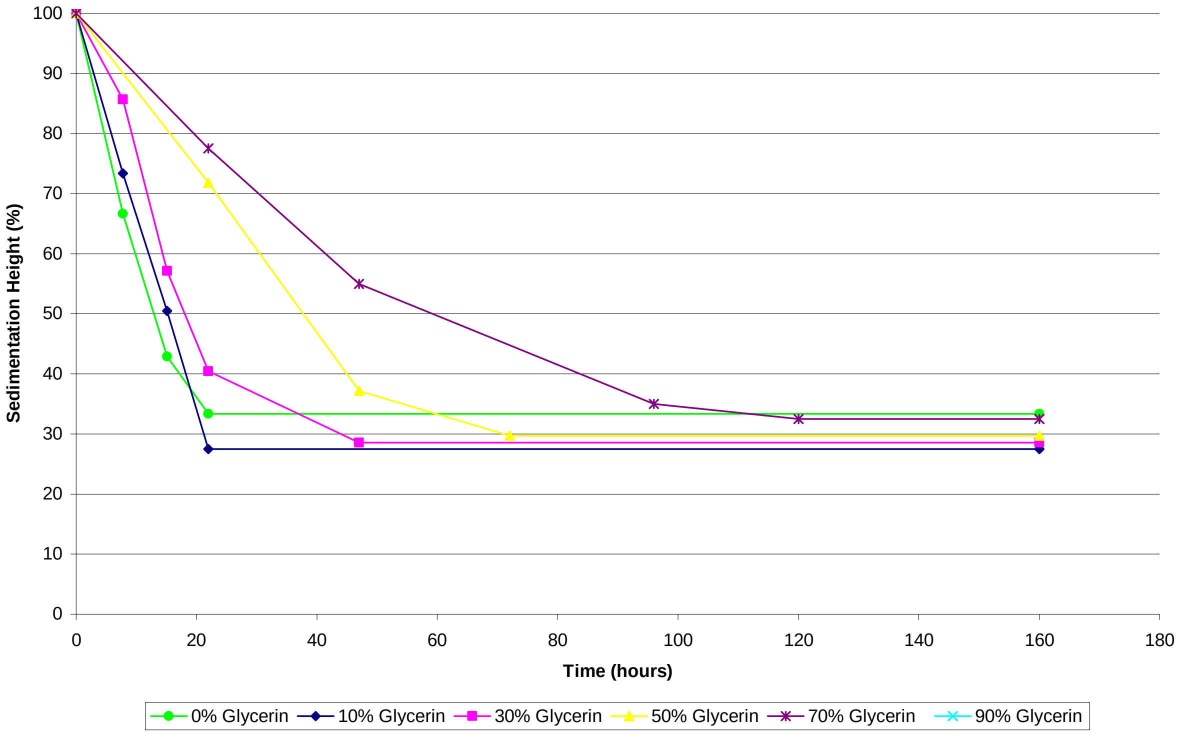
A6 – Comparison of Sedimentation Profiles for PEO 303 at each Glycerin Concentration in Propylene Glycol (n=2)



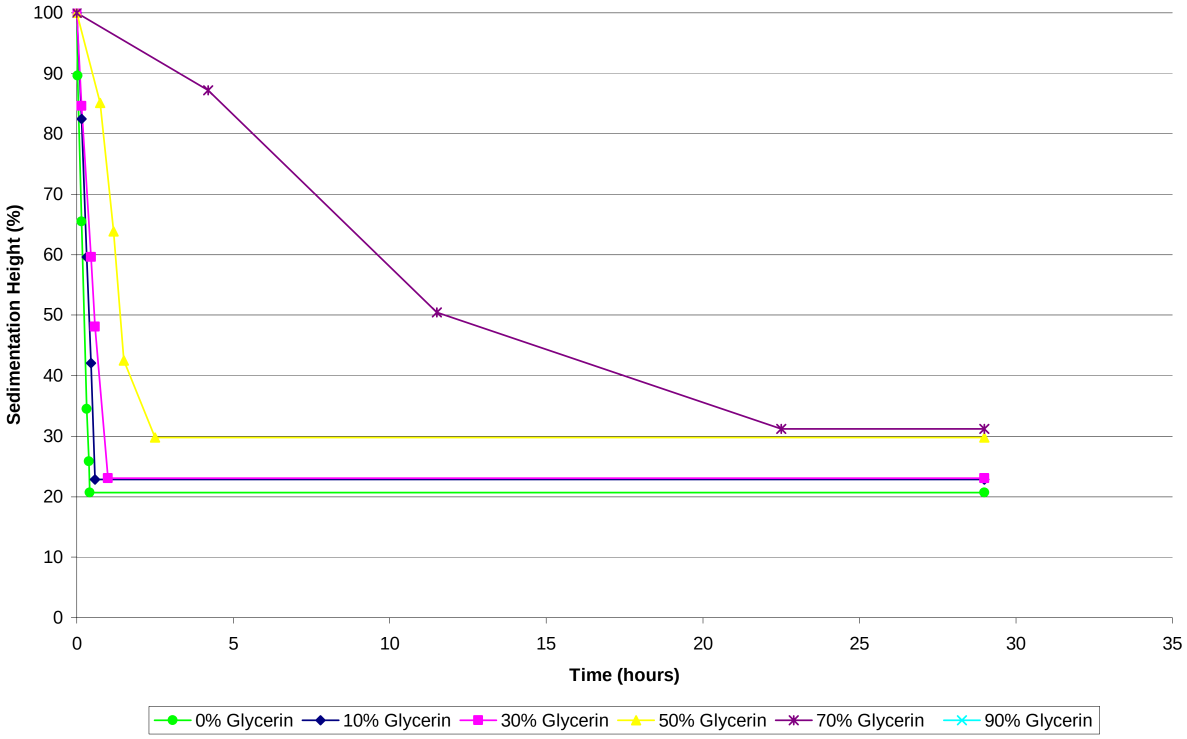
A7 – Comparison of Sedimentation Profiles for PEO N10 at each Glycerin Concentration in Alcohol (n=2)



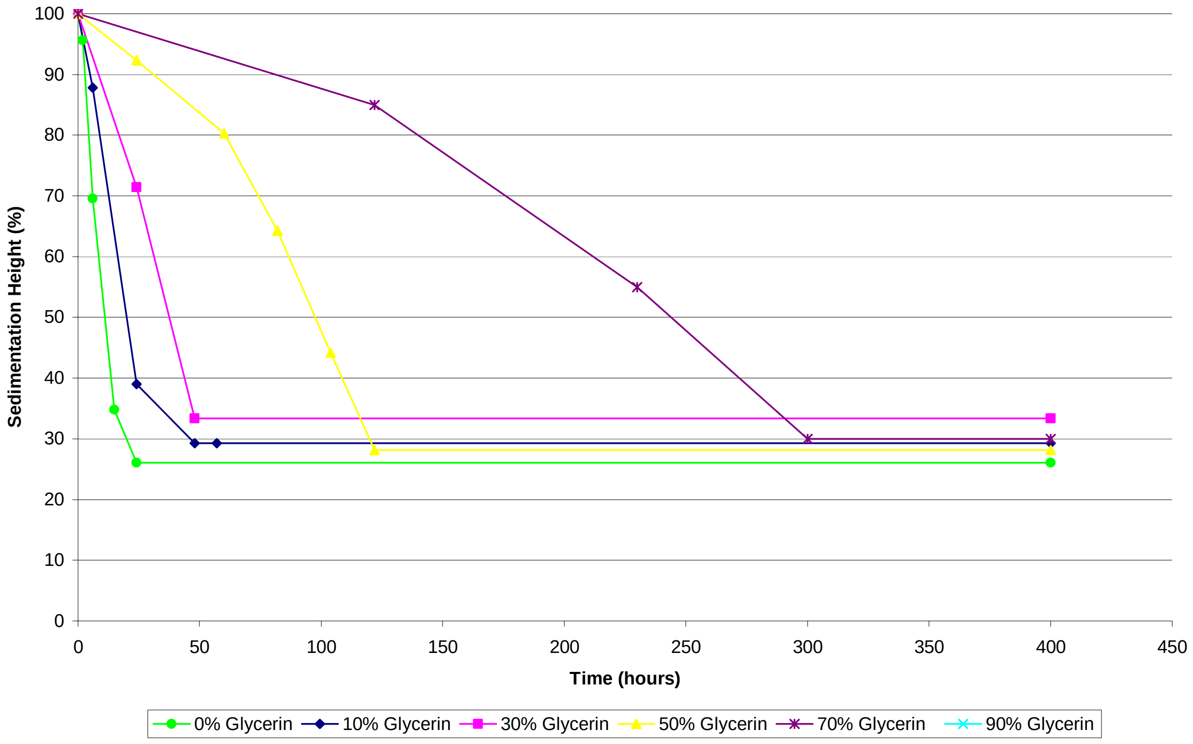
A8 – Comparison of Sedimentation Profiles for PEO N10 at each Glycerin Concentration in Polyethylene Glycol 400 (n=2)



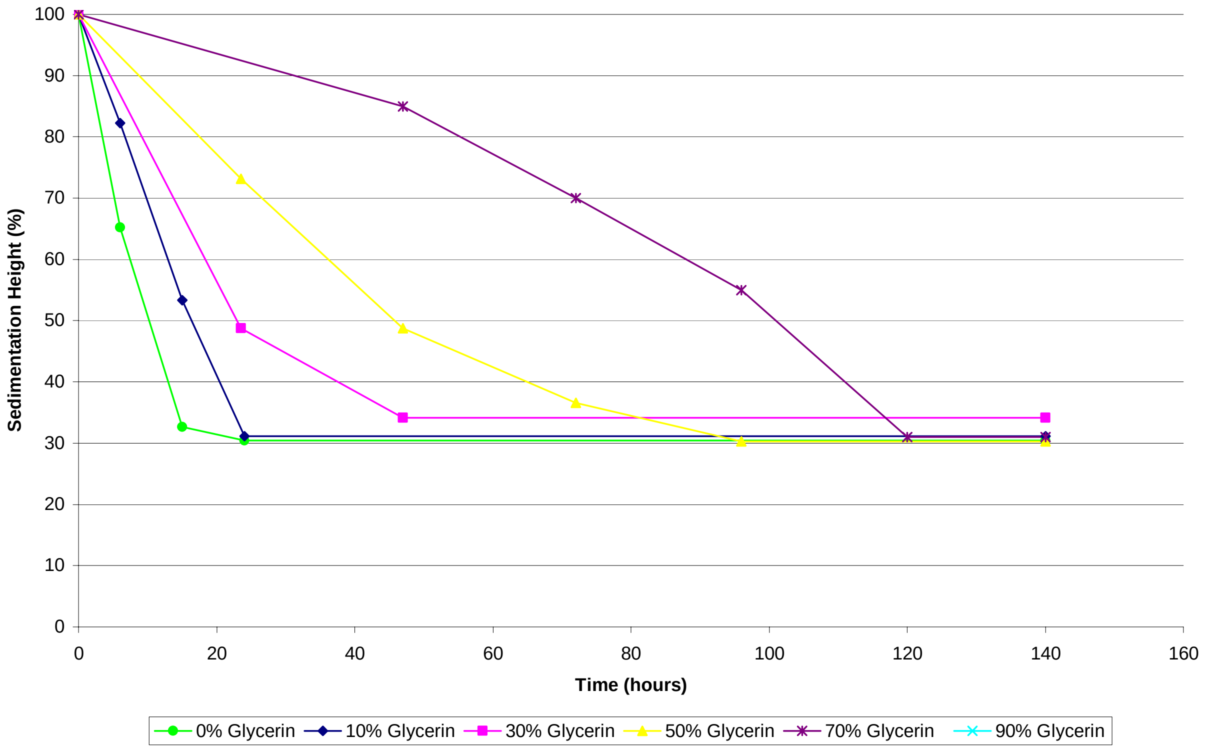
A9 – Comparison of Sedimentation Profiles for PEO N10 at each Glycerin Concentration in Propylene Glycol (n=2)



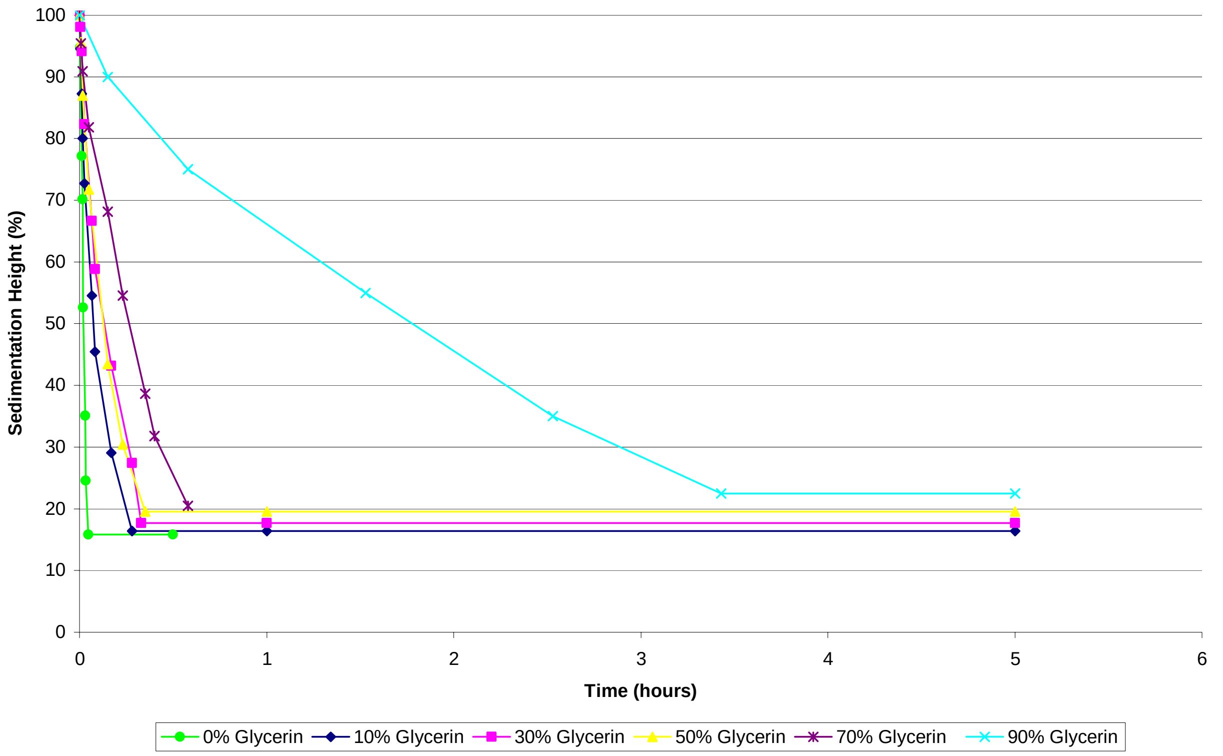
A10 – Comparison of Sedimentation Profiles for PEO N12 at each Glycerin Concentration in Alcohol (n=2)



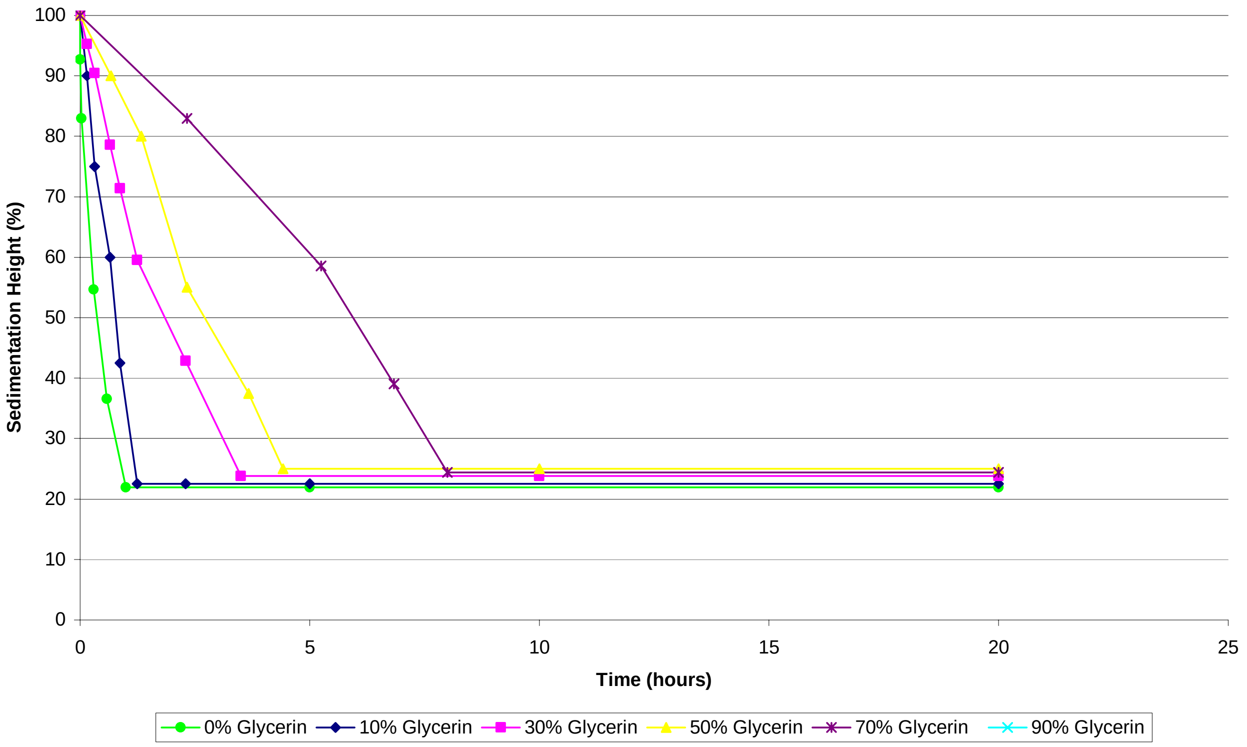
A11 – Comparison of Sedimentation Profiles for PEO N12 at each Glycerin Concentration in Polyethylene Glycol 400 (n=2)



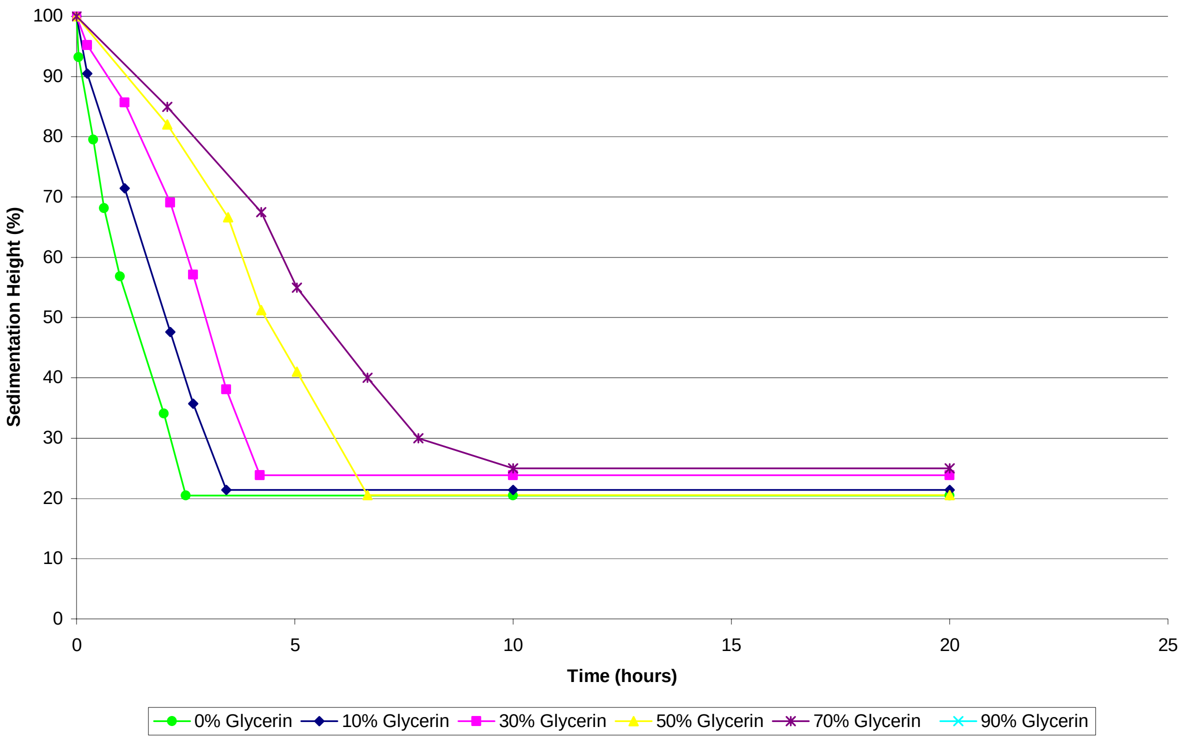
A12 – Comparison of Sedimentation Profiles for PEO N12 at each Glycerin Concentration in Propylene Glycol (n=2)



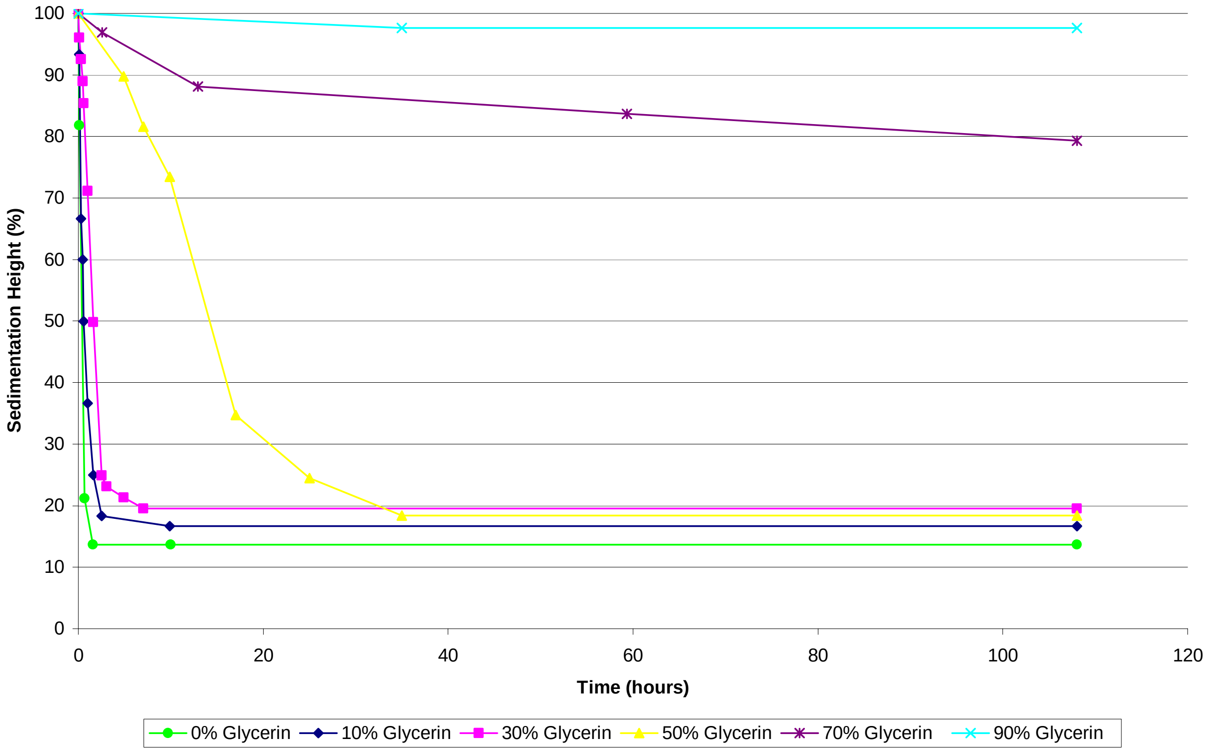
A13 – Comparison of Sedimentation Profiles for Sodium Alginate at each Glycerin Concentration in Alcohol (n=2)



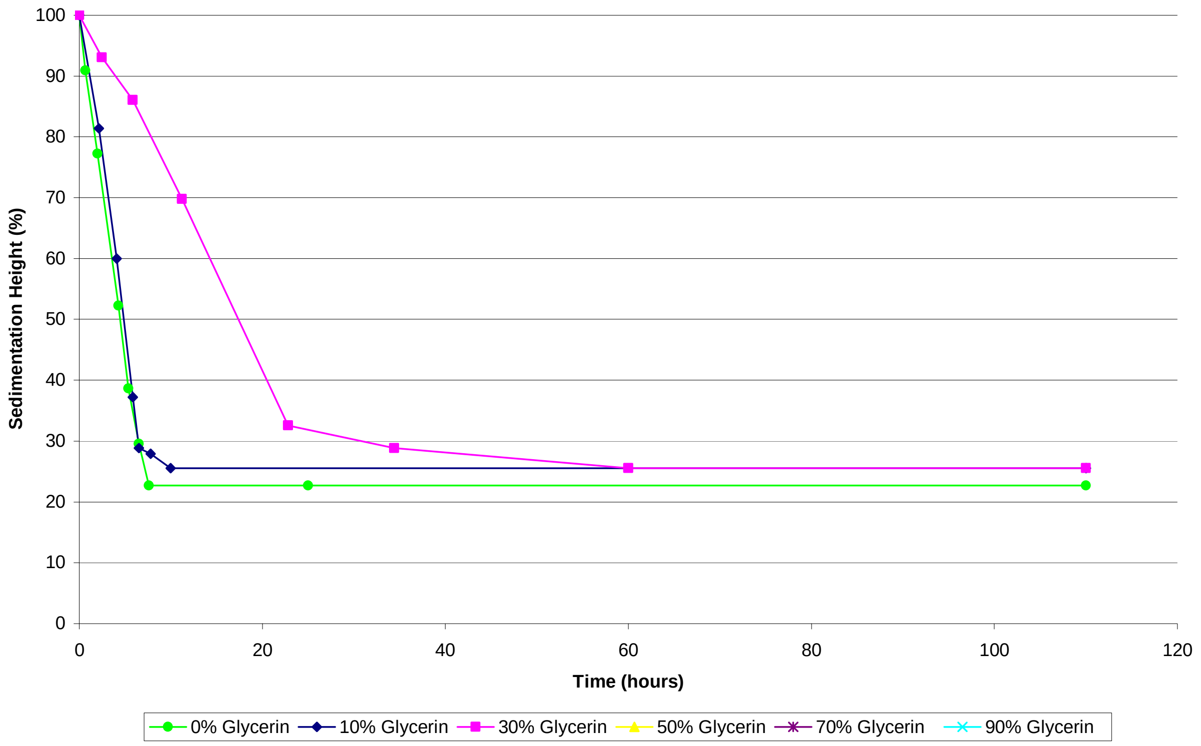
A14 – Comparison of Sedimentation Profiles for Sodium Alginate at each Glycerin Concentration in Polyethylene Glycol 400 (n=2)



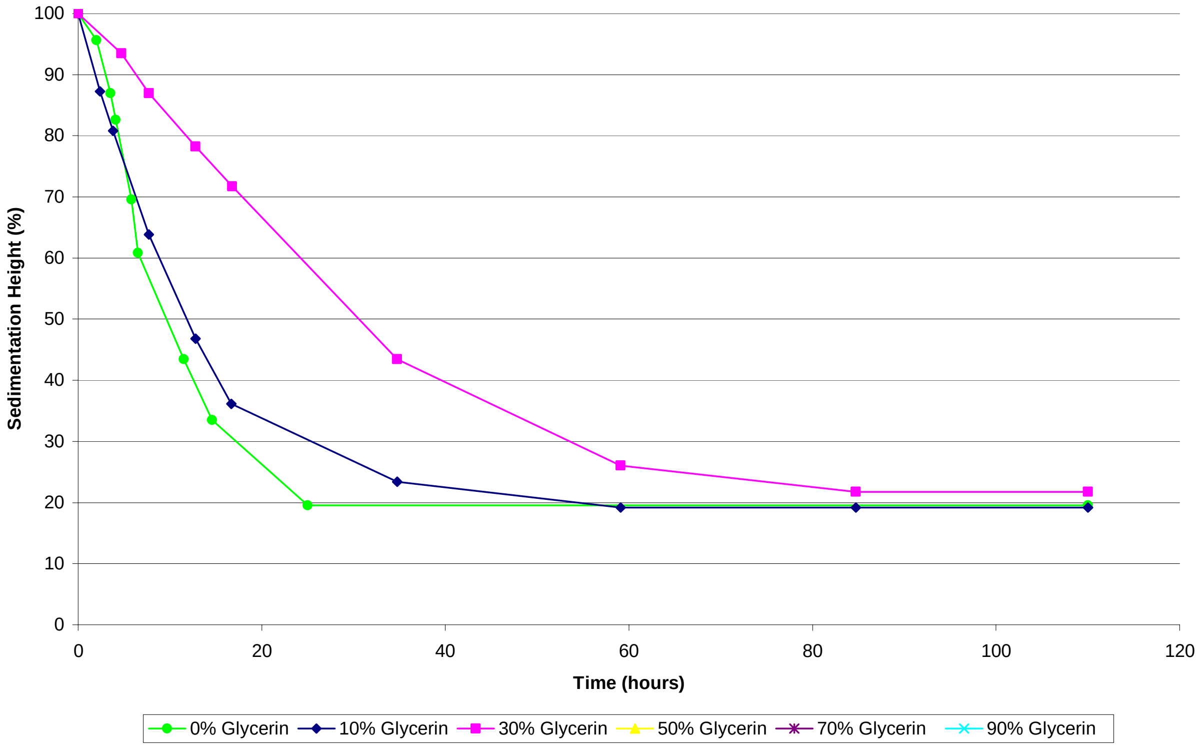
A15 – Comparison of Sedimentation Profiles for Sodium Alginate at each Glycerin Concentration in Propylene Glycol (n=2)



A16 – Comparison of Sedimentation Profiles for Xanthan Gum at each Glycerin Concentration in Alcohol (n=2)



A17 – Comparison of Sedimentation Profiles for Xanthan Gum at each Glycerin Concentration in Polyethylene Glycol 400 (n=2)



A18 – Comparison of Sedimentation Profiles for Xanthan Gum at each Glycerin Concentration in Propylene Glycol (n=2)

S10 Vibro-Viscometer Standard Operating Procedure

The following method describes how to carry out measurements on the S10 Vibro-viscometer.

1. The small sample cup is placed in the large sample holder. The test sample is then poured in to the 10 mL mark as close as possible. Addition of sample by weight is not required. Once the sample has been added, a lid is placed on top.

2. Before conducting any measurements ensure the air conditioner is switched off.

3. Ensure the temperature of the room is between 18 and 21 °C. If necessary heat/cool the room for a short time beforehand.

4. 16 mL of water is added to the water jacket which is sitting on the platform below the sensor plates of the viscometer.

5. The water bath should be continuously running 24 hours a day. Do not switch it off.

6. Ensure the water bath has been set to the required temperature.

7. Push the on/off button to turn the viscometer on.

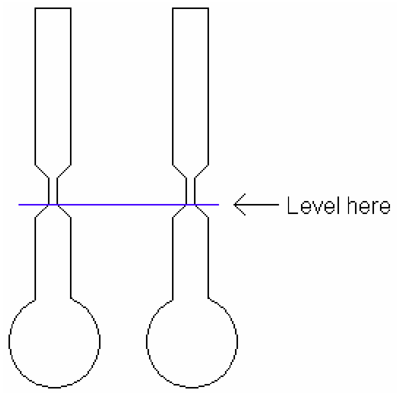
8. Ensure the protector (metal covering around sensor plates) is lowered to prevent accidental bumping of the sensor plates.

9. Place the sample holder in the water jacket.

10. On top of the lid there are two screw holes – one at the front and one at the back. There is only one white screw and this should be placed in the back hole leaving the front one empty.

11. Raise the protector. Lower the sensor plates into the sample by releasing the lever on the side and then while holding the top unit squeeze the two flaps at the back and lower the sensor plates until the sensor plates are about 1 cm from the sample cup. Secure the top unit by locking the lever.

12. Place the magnifying glass in front of the sample cup. Then raise the platform by turning the knob on the right hand side until the probe is covered over the half way point as shown in the diagram below. Use the bottom of the meniscus for leveling.



13. Wrap tissue around the sensor plates to prevent contact with air.

14. Open the RsVisco program on the computer by double clicking the icon on the desktop. Make sure *Auto stop* is not selected.

15. Click the *start* button and allow the sample to equilibrate for 30-45 minutes or until the temperature is 25.0 °C.

16. Click on *stop*. Then click on *file* and select *N*ew. A window will appear asking if you want to clear the data. Click *Yes*. Type in the required parameters i.e. auto stop on, a 2 minute run time with 5 second sampling. Click the start button.

17. When the sample run is complete remove tissue, lower the platform to the bottom. To obtain another reading of the same sample repeat steps 11 - 12. Then click start to commence a new run.

18. Repeat step 14 as many times as required (5 times recommended).

19. To save the data, click on File and select Save. Type in the appropriate name of your file and click Save.

20. To introduce a new sample, lower the platform, raise the sensor plates by unlocking the lever as mentioned earlier and squeeze flaps, raise the top unit and then tighten the lever once more.

21. Clean sensor plates and temperature probe by wiping with a tissue wetted with alcohol. Wipe carefully – try to avoid movement of sensor pates and temperature probe. Repeat once more. Once cleaned lower the protector back into place.

22. Unscrew the white cap and remove sample holder.

23. Add the new sample holder with the fresh sample. Repeat steps 9 – 12. Open a new file for the new data by clicking on File and selecting New.

24. Enter the required parameters and repeat steps 17 -19.

25. Once the user has completed using the viscometer repeat steps 20 - 22.

26. To remove water from the water jacket remove the water jacket and pour out the water.

27. Push the on/off button on the viscometer.

28. Close the program but do not shut down the computer.

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