CHAPTER FOUR

4 PLASMA GRAFTED MICROCAPSULES

4.1 INTRODUCTION

Microcapsules can be used to protect the encapsulated material from the environmental conditions or to release the active agent in a sustained and controlled manner into the surrounding medium. Using microcapsules as drug carriers have several advantages including large specific interfacial area, high selectivity, maximal loading ratio, and most importantly stability. Many polymers can be used to fabricate carriers for controlled release, but only a few can be used to form microcapsules.

This chapter describes the research done to fabricate microcapsules using interfacial polymerisation techniques. Argon plasma treatment was then used to graft acrylic acid to these microcapsules. The objective was to develop a functional microcapsule that responds to changes of pH. The function and physical properties of the microcapsules were then determined. The influence of processing parameters on particle size distribution, morphology of the microcapsule walls, and model drug molecules release were investigated.

The interfacial polymerisation technique was chosen because it is one of the simplest *in situ* polymerisation techniques and almost always produces a microcapsule other than a microsphere. The method involves condensation

polymerisation between two complementary monomers, each soluble in one phase of a two-phase dispersion system. The rate that the contents are released from such microcapsules depends largely on the structure of the microcapsule formed, which has been influenced by the conditions used in the preparation. Therefore, it would be useful to study the release behaviour of model drugs and how the structure of the microcapsule influences drug release.

4.2 FABRICATING MICROCAPSULE AND MORPHOLOGICAL ANALYSES

Microcapsules were prepared from water-in-oil emulsions using an interfacial polymerisation technique (Section 3.3.1). The aqueous phase of this emulsion contained monomers with primary and tertiary amino groups and the organic phase, which was a mixture of chloroform and cyclohexane, contained terephthaloyl dichloride as a monomer. Chloroform was added to the oil phase to enhance amine transfer to the organic phase. A microcapsule shell, which was insoluble in both water and the solvent mixture, then formed at the interface. Various ratios of two monomers altered the morphological properties and thus permeability of the resultant polymer microcapsule. Interfacial polymerisation offers an excellent array of possibilities for microencapsulation purposes. Particle size, porosity, stability of produced microcapsule, and influence of processing parameters were determined.

4.2.1 Morphology and size

All samples of polyamide microcapsules fabricated in this study were observed using a field emission scanning electron microscope (SEM), one of the most used techniques for characterising microcapsule morphology. Typical polyamide microcapsules prepared from interfacial polymerisation had a hollow core-shell structure and a porous shell with a smooth external surface and a rough internal surface (Figure 4.1). The microcapsules had an average diameter of 28 μm (Figure 4.2).

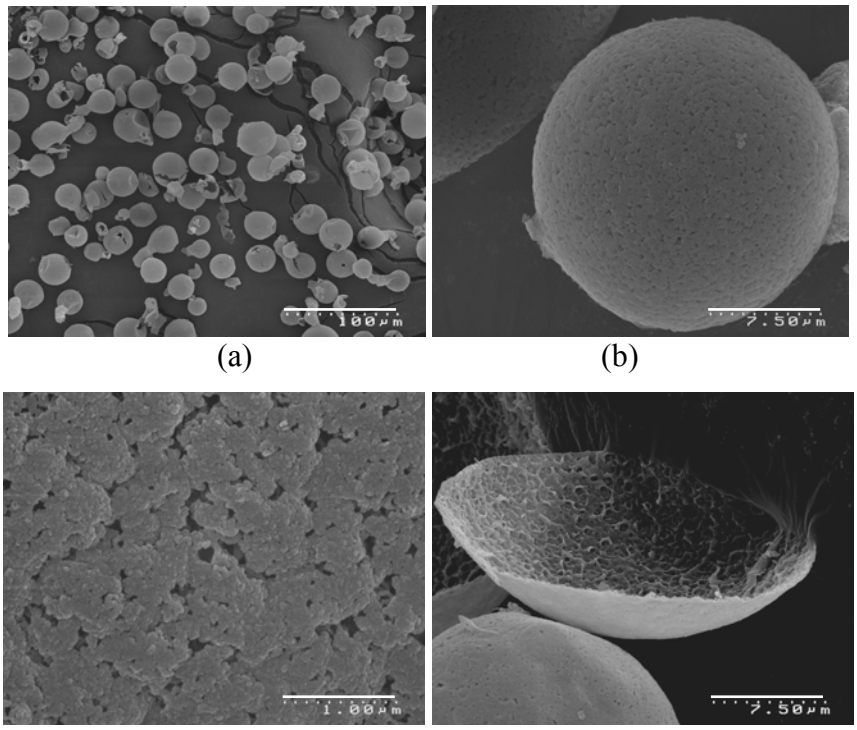


Figure 4.1 SEM images of polyamide microcapsules: (a) microcapsule appearance, (b) single capsule, (c) external surface, (d) internal surface

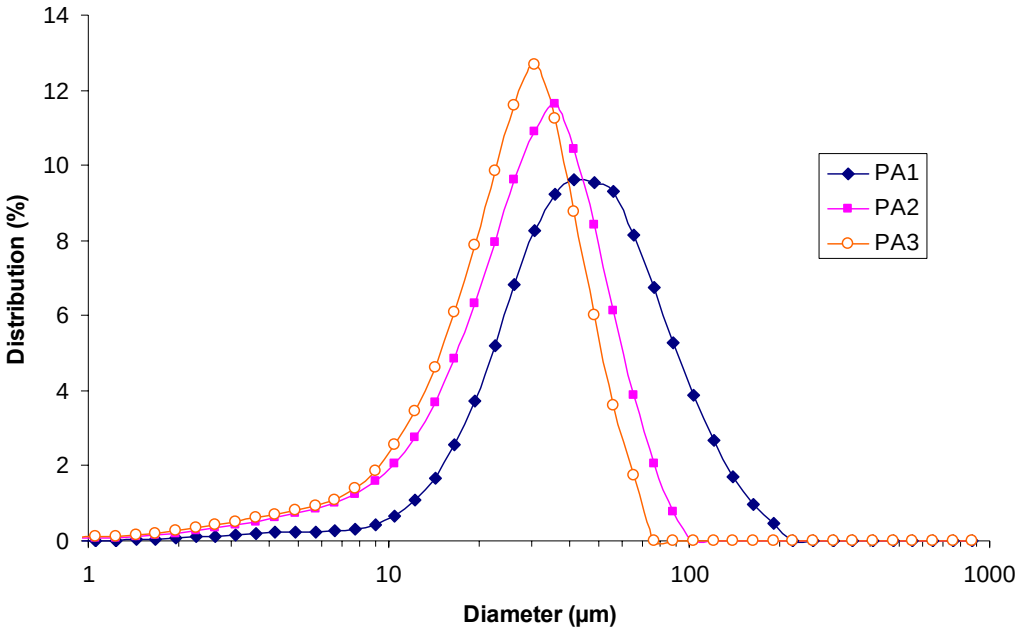
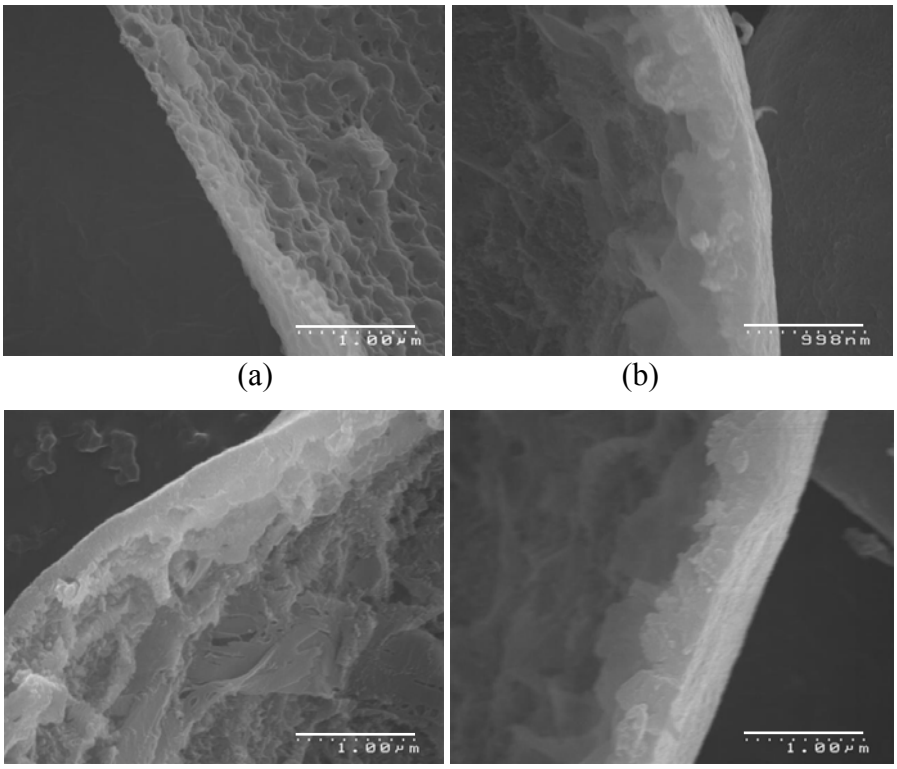


Figure 4.2 Particle size distribution of polyamide microcapsules (n=3)

Microcapsule shell thickness slightly increased from about 0.2 to about 0.3 µm with increasing polymerisation time (Figure 4.3). It was assumed that the limited solubility of the acid monomer (TDC) limited concentration of the monomer

solution. Therefore, increasing reaction time may increase only the number of microcapsules that could be made rather than affecting capsule wall thickness. The TDC concentration in this study was approximately 0.03 mol/L, which is only one-third the 0.1 mol/L used by Chu *et al*. (2001) for the same method.



(a) (b)

(c) (d)

Figure 4.3 Effect of reaction times on thickness of polyamide microcapsule: (a) 30, (b) 60, (c) 120, (d) 180 minutes

4.2.2 Specific surface area and porosity

Various methods have been developed to characterize porous structure but intrusive methods and adsorption methods are the most widely used. In this study, microcapsules porosity was determined by adsorption and desorption of nitrogen gas within the microcapsule pores, and expressed as percentage of fraction volume:

$$\text{Porosity (\%)} = \frac{V_v}{V_t} \times 100$$

where V_v is the volume of void-space and V_t is the total or bulk volume of microcapsules, including the solid and void components. Raw data are in the Appendix. Specific surface area is calculated by the Brunauer-Emmett-Teller (BET) theory (Brunauer *et.al*., 1938). This theory is used to relate physical

adsorption of inert gas molecules on a solid surface to indicate specific surface area of a material. Pore size distribution is obtained by plotting adsorbed pore volume with pore radius. The typical pore size distribution of polyamide microcapsule (Figure 4.4) showed that most pores have a radius of 10–35 nm.

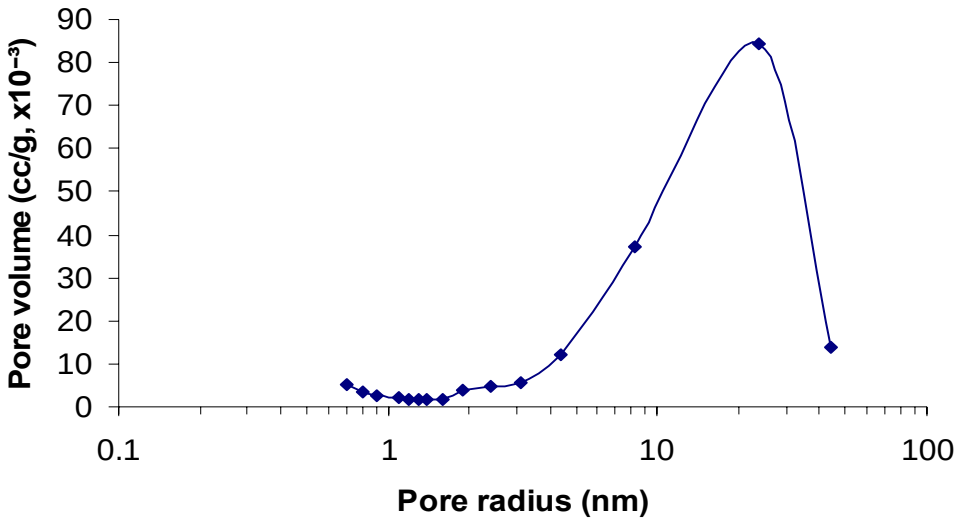


Figure 4.4 Pore size distribution of polyamide microcapsules

4.2.3 Stability and rigidity

To investigate stability, samples of prepared microcapsule were stored in various media for a month and visual observations and measurements made on microcapsule breakdown or degradation. The size distribution of microcapsules stored in solutions of various pH values (Figure 4.5) and in organic solvents such as ethanol, acetone, benzene, and chloroform did not change over one month, indicating the particles were stable. The aromatic polyamides are highly insoluble materials and were not affected by the storage medium.

There is no ASTM method for testing microcapsule strength. To examine mechanical strength, samples of prepared microcapsules were stirred at various magnetic stirring rates for 2 hours. Particle size analysis (Figure 4.6) showed that microcapsule size distribution was not affected at low stirring rate. Some small 0.1–1 μm in diameter particles appeared under medium and high stirring rates, indicating some microcapsules had been mechanically broken.

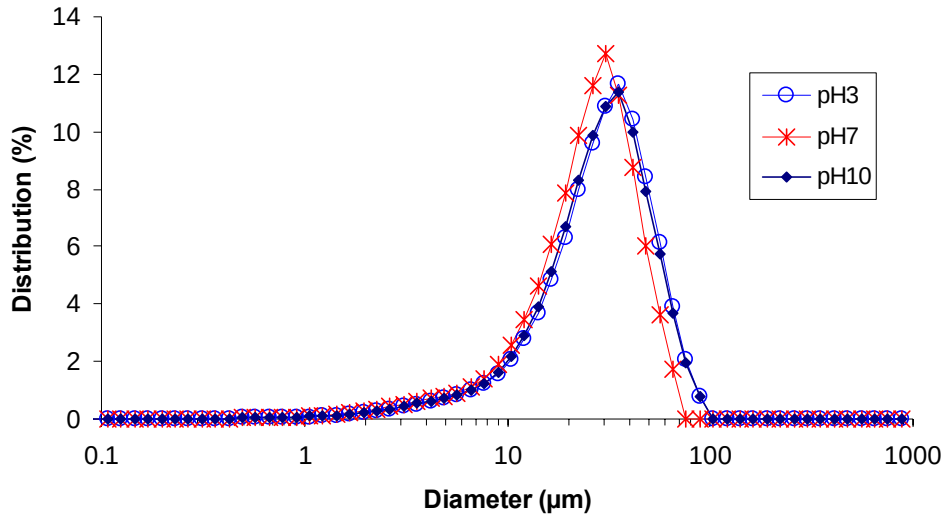


Figure 4.5 Effect of solution pH on particle size distribution of microcapsules stored for one month (n=3)

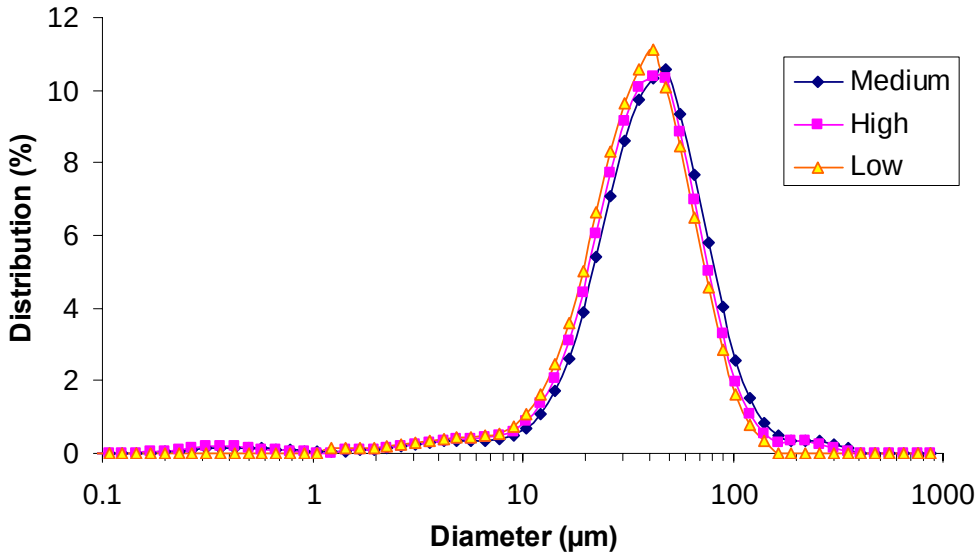


Figure 4.6 Effect of magnetic stirring rate on microcapsule stability (n=3)

4.2.4 Influence of process parameters

Several factors may influence the properties of microcapsules formed by interfacial polymerisation. Mathiowitz and Cohen (1989) found that the chemical nature of the amine monomer(s) and the conditions for the polycondensation reaction affected chemical composition and porosity of the microcapsule membrane, degree of polymer cross-linking and, eventually, distribution of charged groups in the membrane made from polyterephthalamide particles.

This section describes the influence of stirring speed and ratio of monomers on microcapsules properties.

4.2.4.1 Effect of stirring speed on microcapsule size

Microcapsule sizes were mainly controlled by process conditions during the emulsification process, with microcapsule size being controlled by droplet size in the emulsion. Smaller-sized microcapsules were obtained when emulsions were prepared at higher stirring rate (Figure 4.7). Thus, increasing stirring rate from 600 rpm to 800 rpm and then to 1000 rpm decreased average diameter from 40 µm to 27 µm and finally 20 µm. However, the monomer ratio did not affect microcapsule size, indicating that size was influenced by the emulsification parameters rather than by monomer composition.

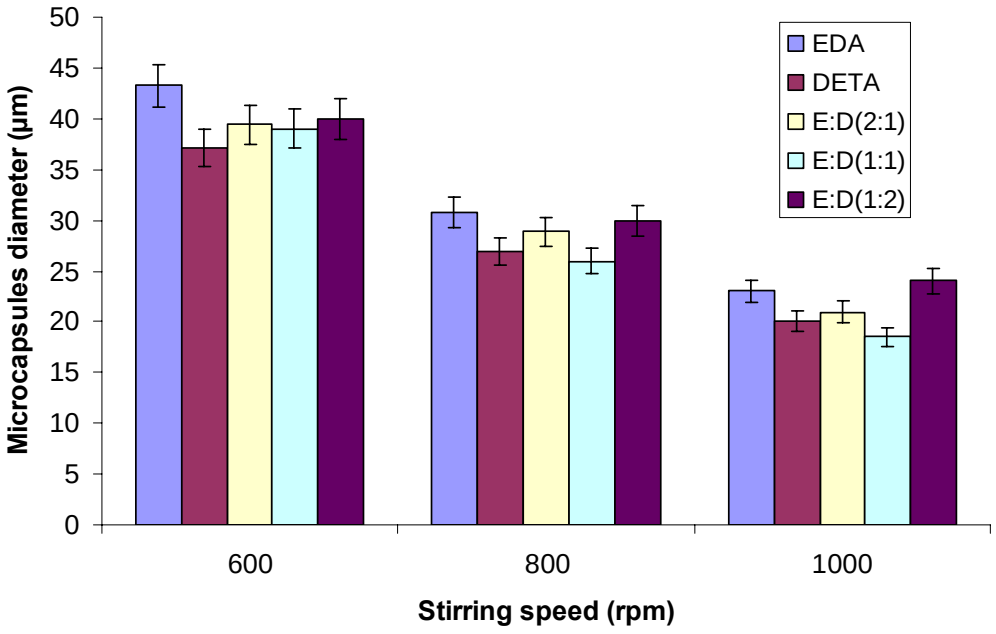


Figure 4.7 Effect of stirring speed during emulsification on microcapsule diameter (n=3)

4.2.4.2 Effect of monomer ratio on capsule surface morphology

To investigate the effect of polymerisation conditions on surface morphology, batches of microcapsules were prepared with different ratios of DETA to EDA in the aqueous phase. In all experiments, the oil-soluble monomer TDC was dissolved in a 2:1 mixture of benzene/xylene with the same ratio.

The SEM images (Figure 4.8) show the microcapsules were spherical and hollow. Those prepared with a 2:1 EDA to DETA had a porous external surface whereas those prepared with a 1:2 of EDA to DETA had a smooth, dense external surface. No pores were detected at 7000 × magnification (Figure 4.8 b2); their absence was assumed to be due to greater cross-linking during interfacial polymerisation.

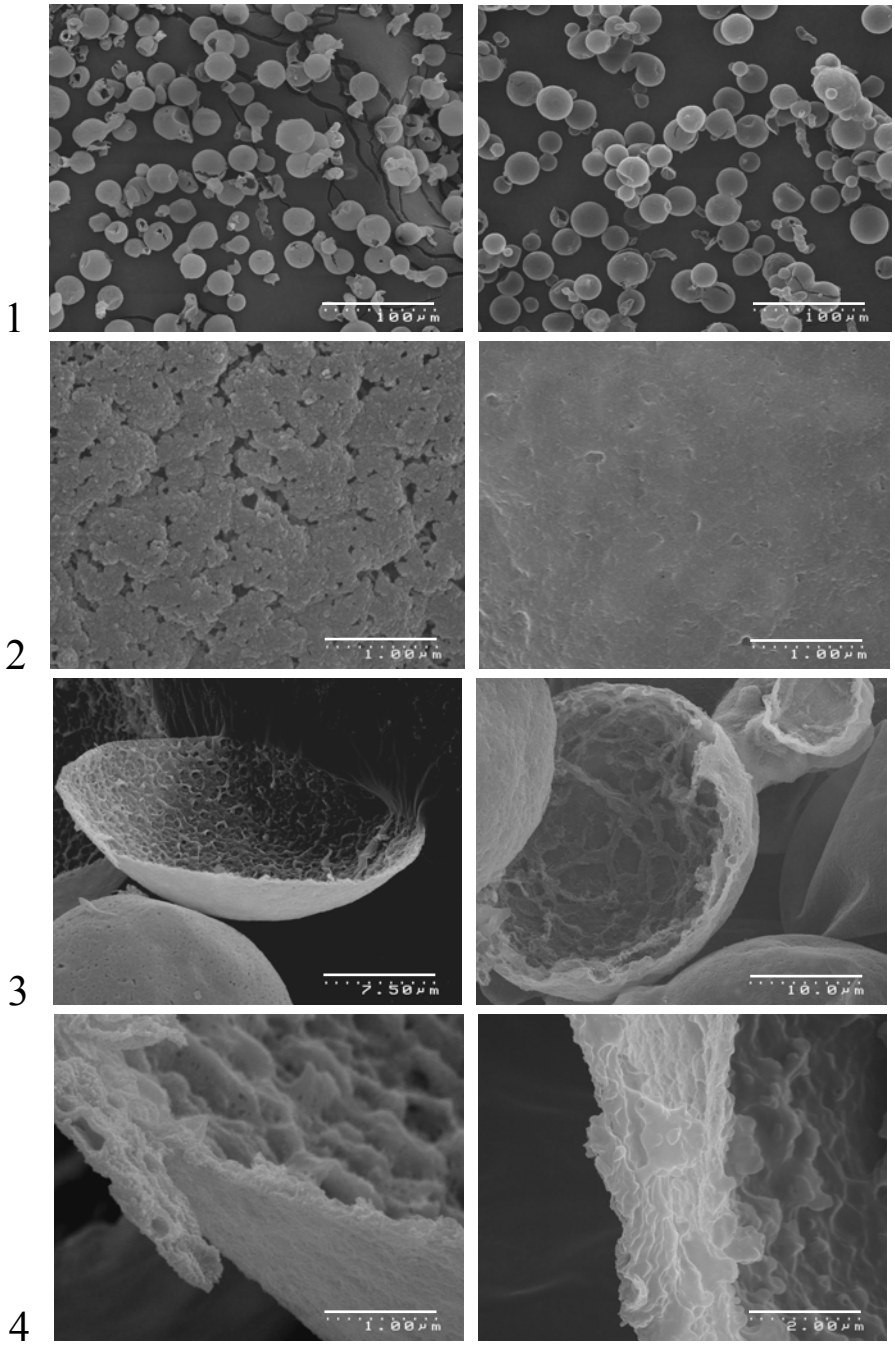


Figure 4.8 Effect of varying ratio of EDA:DETA on microcapsules appearance: column a - 2:1 ratio; column b - 1:2 ratio.

1. Microcapsules; 2. External surface; 3. Internal surface; 4. Cross-section

The collapsed appearance of some microcapsules indicates they had low mechanical strength. It is thought that the long linear polymer chains formed when EDA reacts with TDC lowered the degree of cross-linking (Figure 4.9). These trials showed that microcapsule shell porosity can be controlled by adjusting the ratio of amine monomers in reactions to form the microcapsule shell.

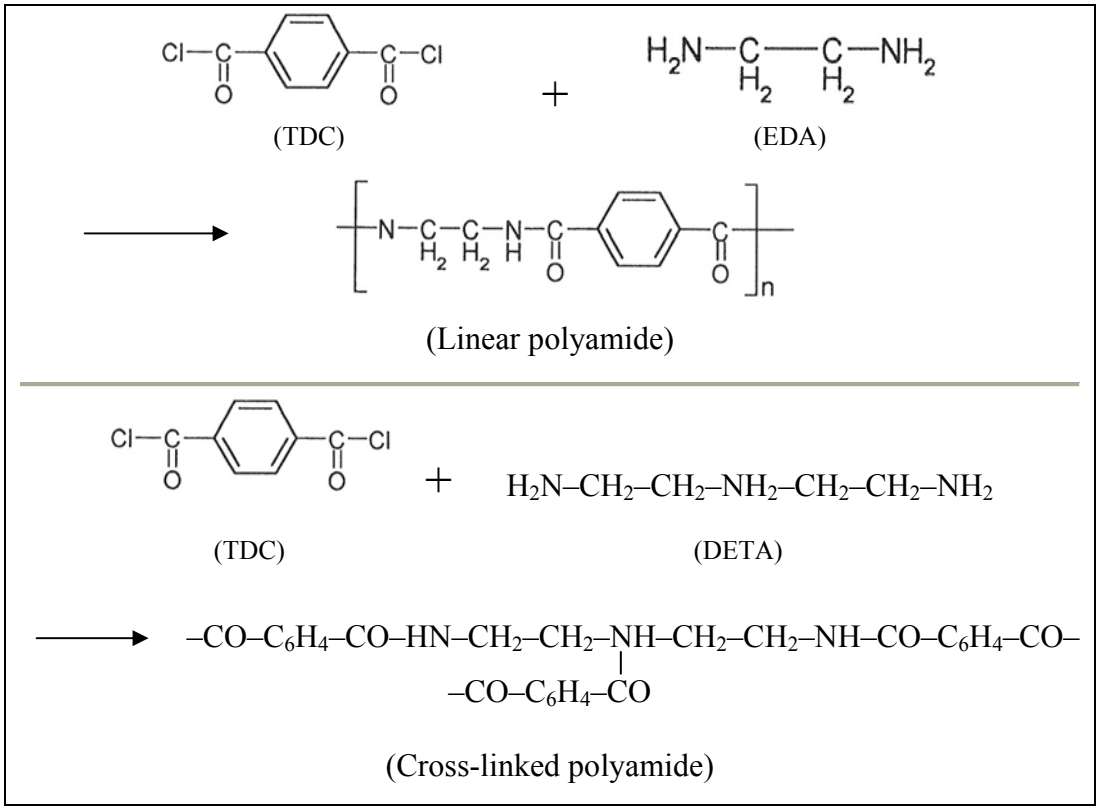


Figure 4.9 Effect of monomers on cross-linking degree during the polymerisation reaction

Theoretically the molecular and morphological properties of the polymer membrane shell can also be affected by initial monomer concentration. Membrane shell mechanical strength is very dependent on molecular weight of the polymer, which is determined by the extent of polymerisation. Higher monomer concentrations produce more rigid and stable microcapsules with thicker polymer membrane shell. Increasing the ratio of DETA to EDA improved microcapsule strength because the polyterephthalamide polymer produced higher cross-linking density. On the other hand, low ratio of DETA to EDA produced more porous but less rigid microcapsules. Microcapsules produced with EDA only tended to be brittle (Figure 4.10). Similar observations were reported previously (Mathiowitz and Cohen, 1989)

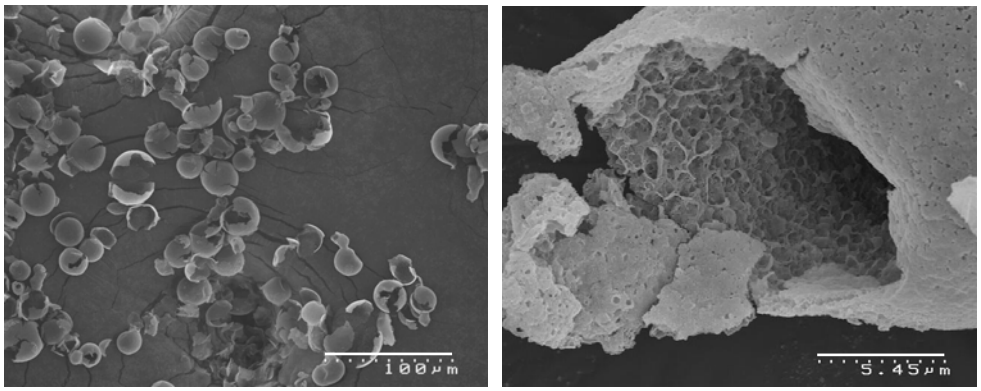


Figure 4.10 Fragility of polyamide microcapsule

The microcapsules were thoroughly washed using a centrifugation-decantation procedure. These microcapsules were well-dispersed, free-flowing fine particles although some aggregations could be seen under the microscope. Two methods were found to reduce aggregation: (a) washing the microcapsules with 30% ethanol to remove residual organic solvent inside the microcapsule before freeze-drying, and carrying out the washing steps at low (~4°C) temperatures; or (b) dialysing the microcapsules against deionised water for two days.

4.3 FUNCTIONALISED MICROCAPSULES

Controlled drug release can be achieved by using a pH-triggered release. One method to produce functional groups that will react when pH of the environment changes is to introduce carboxyl groups by graft polymerisation of acrylic acid onto the polymer surface. Polyacrylic acid (PAA) has been commonly used and investigated for a variety of material applications where the environmental responsive is needed (Kost, 1999). Also, PAA is on the GRAS non-oral list allowing it to be used for human and animal applications. This section describes functionalising the microcapsule using plasma-induced graft polymerisation.

4.3.1 Plasma-induced grafting

Plasma-induced graft polymerisation is an attractive way of modifying the surface chemistry and morphology of polymeric materials. Plasma treatment only affects a few nanometres on the surface and has little influence on the bulk properties of

the material. It has been widely used to functionalise the surface of materials for improving biocompatibility (Ikata, 1994).

The process for generating the plasma used in this thesis differs from a normal DC glow. The heated tungsten filaments provided thermally emitted electrons, which were then accelerated into the main chamber body. The negative glow of the discharge was confined to a small diameter Pyrex tube while the main positive column of the glow filled the main chamber body and provided the working plasma. These conditions were optimised by adjusting the pressure. Stable operating conditions were obtained when the plasma discharge current was below 4 amps and the DC voltage drop was less than 400 V.

4.3.2 Determining carboxyl groups

Two methods, chemical titration and Fourier transform infrared (FT-IR), were used to determine the concentration of carboxyl groups grafted to the microcapsules. The FT-IR is convenient but results can be very variable so it was used only as a qualitative indicator.

The number of carboxylic acid groups was determined by measuring total carboxylic acid group capacity by back titration. The extent of grafted polyacrylic acid can be described as the percentage of grafted carboxylic acid groups over microcapsule weight:

$$Extent\ of\ graft\ (\%) = \frac{Content\ of\ grafted\ carboxylic\ acid\ groups\ (mmol)}{Total\ weight\ of\ microcapsules\ (g)} \times 100$$

The KBr FT-IR spectra of grafted microcapsules had absorption peaks at 1708 and 803 cm^{-1}, which are attributed to C=O stretching vibration of carbonyl group, indicating acrylic acid has grafted onto the microcapsules (Figure 4.11, top and middle). Ungrafted microcapsules did not have those peaks. These data confirmed plasma-induced graft polymerisation of polyacrylic acid on the microcapsules.

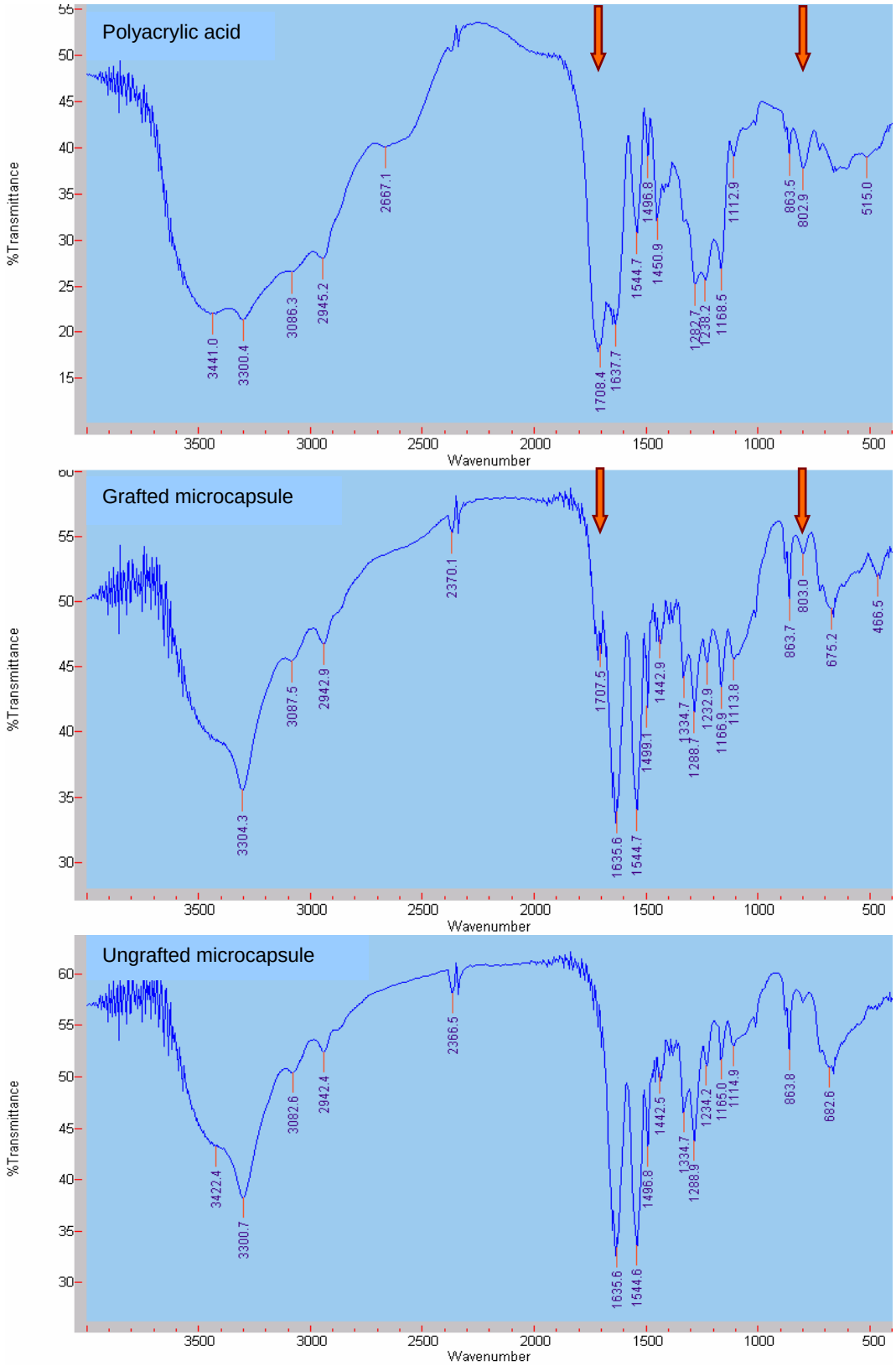


Figure 4.11 FT-IR of polyacrylic acid (top), grafted (middle) and ungrafted (bottom) polyamide microcapsules

Plasma treatment time, polymerisation reaction time, and monomer concentration affect the extent of grafting. Maximum of grafting was reached on 5 to 6 hours of polymerisation reaction with 30% AAc at 50°C (Figure 4.12). Similar trends were shown for various plasma treatment times (30, 60, and 90 sec.) and a maximum of 0.56 mmol/g of grafting was produced in 90 seconds of plasma treatment. The effect of monomer concentration will be discussed in Section 4.4.4.

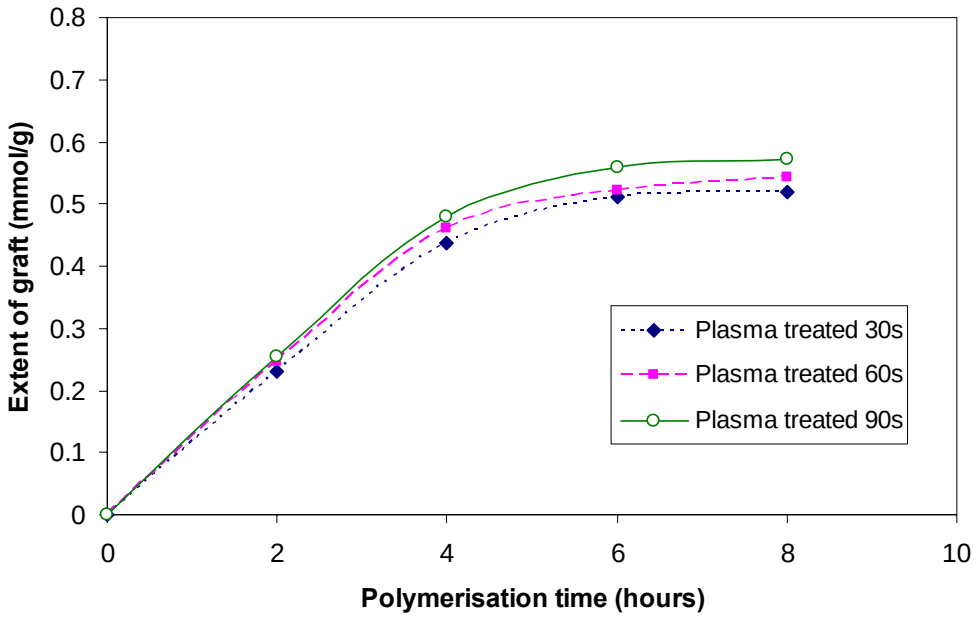


Figure 4.12 Effect of plasma treatment time on extent of grafting (n=3)

Solvent degassing also influences graft polymerisation. Helium purging was used to degas in initial graft polymerisation experiments but no carboxyl groups could be detected on the resultant polyamide microcapsules. It was assumed the degassing method affected some excited species from plasma and inhibited graft polymerisation.

Three solvent degassing methods commonly used in chemical graft polymerisation were investigated:

1. Purging with inert gas, which is the least effective method, but can be used when large amounts of solvent need to be degassed.
2. Sonicating, which also degases the solvent.
3. Freeze-pump-thaw, which is the most effective method for solvent degassing. This was used in subsequent experiments.

4.4 RELEASE STUDIES

There are many release studies on microspheres made from biodegradable polymers such as PLA, PLGA but only two reports (Mathiowitz, 1989; Chu *et al*., 2004) on release from polyamide microcapsule were found. This section describes the release of two model drugs from PAA-grafted polyamide microcapsules and the factors that influenced the release rates.

4.4.1 Loading capacity

Drug content encapsulated in microcapsules can be described as:

$$\text{Loading capacity (LC)} = \frac{\Delta D}{W_m}$$

Or $$\text{Encapsulation efficiency (EE)} = \frac{\Delta D}{D_T}$$

where W_m is the weight of the microcapsules,

ΔD is the amount of loaded drug (D_T minus the amount of unloaded drug),

D_T is the total amount of drug employed.

However, it is difficult to measure precisely the internal volume of microcapsules because internal surfaces were not morphologically uniform and there is no accurate method to determine thickness and density of the microcapsule. Calculations of microcapsule volume were based on wall thickness observed on SEM micrographs and the density obtained from BET analysis (Appendix).

4.4.2 Model drug molecules release studies

To validate pH reversibility in the manufactured grafted microcapsules, model drug release experiments were done (Section 3.6) using vitamin B_{12} and cytochrome *c*, both of which are commonly used in drug release studies. Vitamin B_{12} has a low molecular weight and cytochrome *c* has a medium molecular weight similar to many other proteins (Table 4.1). The red associated with vitamin B_{12} and the reddish-brown of cytochrome *c* allows visual confirmation of their release. Cytochrome *c* release from biodegradable polyesters microspheres is faster than other model proteins (Kissel *et al*., 1996). The first commercially-

available microparticle-based veterinary product launched in New Zealand was used to deliver vitamin B_{12} (Rathbone and Martinez, 2002).

Table 4.1 Physicochemical properties of model drugs.

Compound	Molecular weight (Da)	Radius (nm)	p*Ka*	Aqueous diffusivity $D_0 \times 10^6$ (cm^2/s)
Vitamin B_{12}	1355	0.87	neutral	3.79
Cytochrome *c*	12327	1.88	10.6	1.75

4.4.2.1 Vitamin B_{12}:

Data from the release study indicate the grafted microcapsules had different release rates at pH 2 and 7 (Figure 4.13). No contents were released from the microcapsules at pH 7 because pores on the microcapsule surface remain closed. At pH 2, un-ionised carboxyl groups in the grafted PAA chain collapse and pores on the surface open and allow contents to diffuse into the surrounding medium.

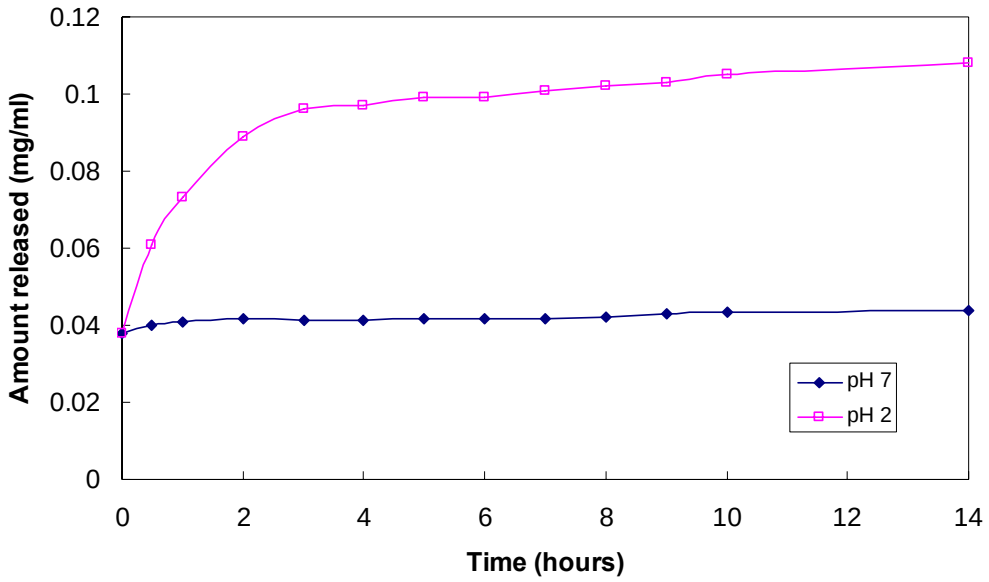


Figure 4.13 Effect of pH on release of vitamin B_{12} from PAA-grafted polyamide microcapsules (n=3)

Vitamin B_{12} was released very slowly when the pH was 2 (0 to 2 hours and 4 to 7 hours, Figure 4.14). However, B_{12} was rapidly released between 2 and 4 h, after the pH had been switched to pH 2. The change in release rate continued over several pH switches (line at the top of Figure 4.14).

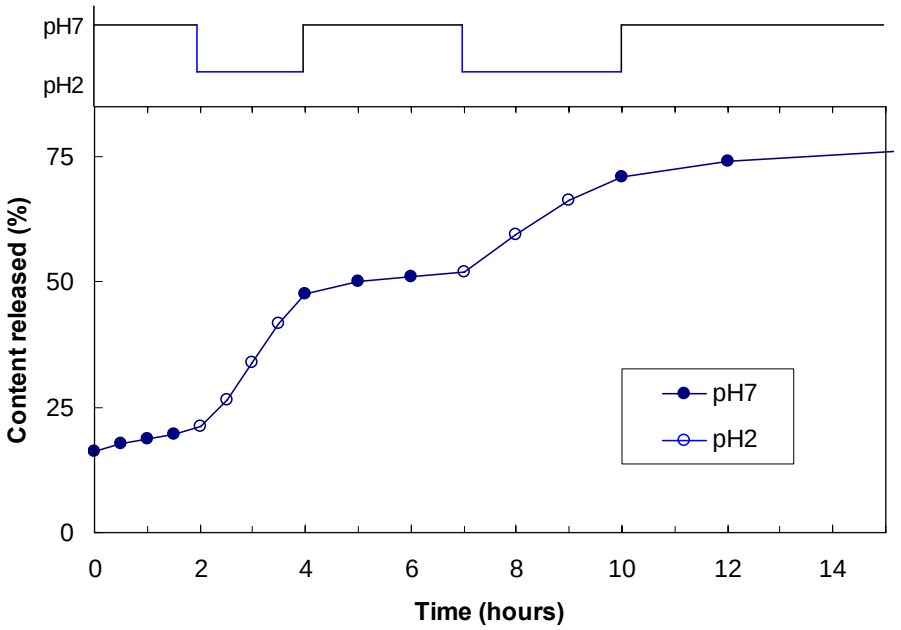


Figure 4.14 Effect of changing pH on release of vitamin B_{12} from PAA-grafted polyamide microcapsules (n=3)

4.4.2.2 Cytochrome *c*:

Release rate of cytochrome *c* was affected when the pH of the media the grafted microcapsules were in was switched between 2 and 7 (Figure 4.15), indicating that the pH change triggered an on-off release profile. A maximum of about 75% of the vitamin B_{12} and about 65% of the cytochrome *c* was be released from the grafted microcapsules for the five changes in pH.

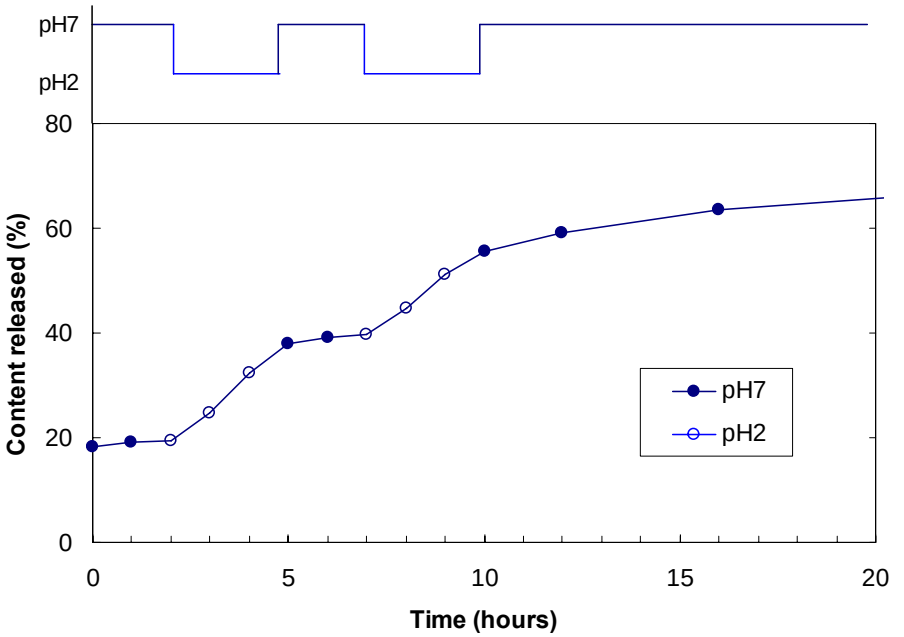


Figure 4.15 Effect of changing pH on release of cytochrome *c* from PAA-grafted polyamide microcapsules (n=3)

4.4.3 Effect of pH on release

Several factors including pH, capsule size, microcapsule porosity, extent of grafting, and molecular weight of loaded contents influence release of contents from porous microcapsules. The effect of pH is discussed in this section and other factors are discussed in Section 4.4.4.

To demonstrate how pH influences release of vitamin B_{12} from PAA-grafted polyamide microcapsules, release experiments were done at various pH values. Contents were not released when the pH was between 7 and 5.5 but did occur between pH 5 and 3.5 (Figure 4.16 and Figure 4.17). Full release occurred when the pH was below 3.

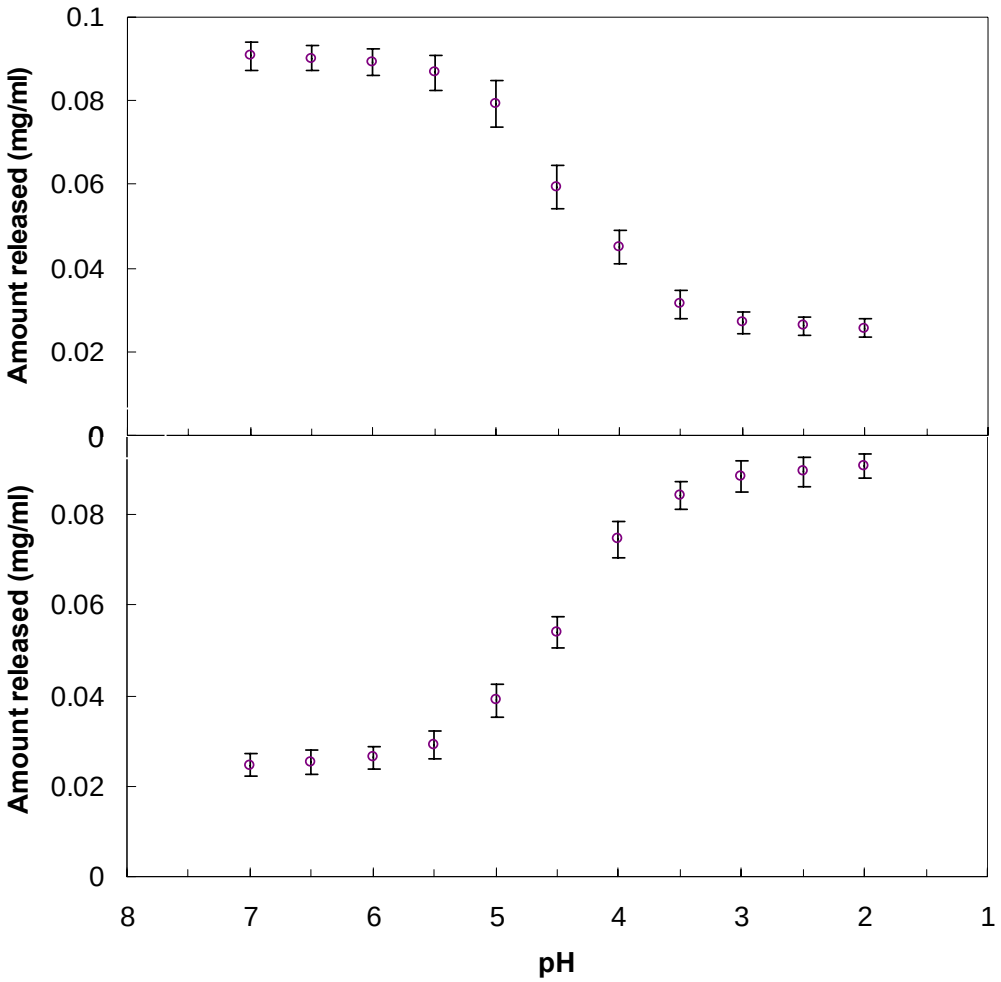


Figure 4.16 Effect of changing pH either from 2 to 7 (top) or from 7 to 2 (bottom) on contents release from PAA-grafted polyamide microcapsules

Polymers such as PAA are originally coiled neutral molecules with pendant alkyl side chains and weakly charged negative groups (e.g. carboxylic acids). They exhibit reversible expansion of the chain conformation above their p*Ka* and contraction as pH is lowered. The change from a charged extended polymer chain to a collapsed uncharged coil structure is sometimes called hyper-coiling behaviour (Tonge and Tighe, 2001) and enables the polymer to act as a simple switch between an 'on' and 'off' state in response to changes in the environmental pH. By substituting either weakly cationic or anionic pendant groups onto a polymer backbone, the polymer can be made to respond to pH changes.

Hysteresis occurred when changing pH between 2 and 7. Curves fitted to the data obtained from Figure 4.16 indicate a lag for release when pH was switched from 2 to 7 compared with data for switching pH from 7 to 2 (Figure 4.17). It was assumed that dissociation of the carboxylic acid group, which extends the grafted polymer chain, closes the pore and retards release of the contents. However, a short time is required to get full extension in polymer chain when the pH is changed the other way.

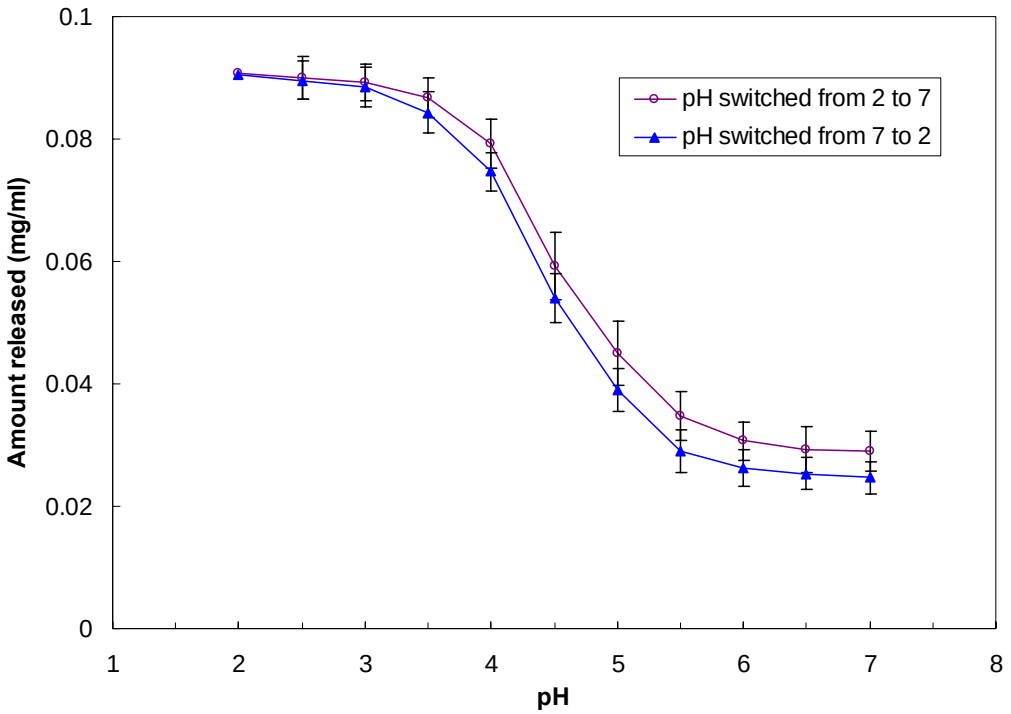


Figure 4.17 Hysteresis for release of vitamin B_{12} from PAA-grafted microcapsules when the pH is switched between 2 and 7 (n=3)

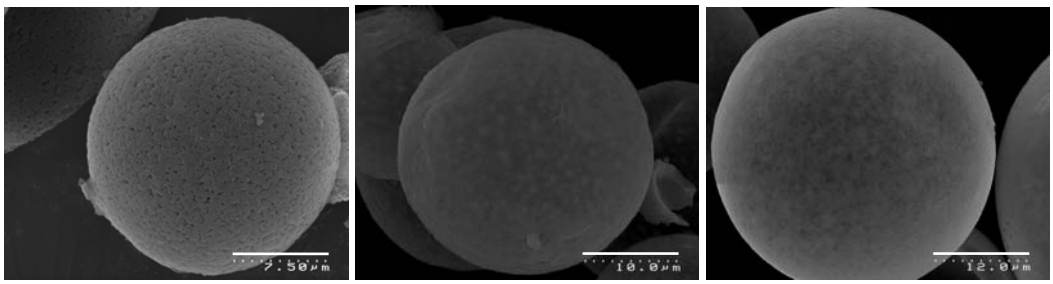
In most microencapsulation applications, even when the aim is to isolate the core from its surroundings, the wall must be ruptured to release the contents. This can be achieved by melting the wall or dissolving it under particular conditions (a method used for enteric drug coatings). In other systems, the wall is broken by solvent action, enzyme attack, chemical reaction, hydrolysis, or slow disintegration. The current study attempted to develop a novel technology. By incorporating pH-sensitive carboxyl groups, the microcapsule can be targeted for various biological environments or to specific sites. Lynn *et al*. (2001) studied release from microspheres at intercellular pH value using a poly(β-amino ester), which was stable over pH 7.0–7.4 but dissolved readily below pH 6.5. Release at pH 7.4 was very slow but full and immediate release occurred at pH 5.1. Another study (Lorenzo-Lamosa *et al*., 1998) with a chitosan microcore coated with poly(methacrylic acid-co-methylmethacrylate) achieved controlled release only after the pH-sensitive coating was dissolved. The current study demonstrated that the pH sensitivity of the PAA-grafted system (Figure 4.14 and Figure 4.15) was similar to the pH-sensitive coating dissolved system. The results indicated that contents loaded inside the grafted microcapsule released at pH values between ~5 and 3.5, which means the chain configuration of PAA is a function of p*Ka* of the polymer.

4.4.4 Factors affecting release rate

This section describes the influence of factors such as capsule size, porosity, extent of graft, and molecular weight of the loading contents on release rate of model drugs from grafted microcapsule.

4.4.4.1 Capsule size

Current knowledge for controlled release of drugs involve: (1) diffusion, (2) solvent activation, or (3) chemical reactions (Langer, 1990; Brannon-Peppas, 1997). Release rate of the microcapsules contents decreased with increasing particle sizes (Figure 4.18) and it was assumed the release mechanism is primarily controlled by diffusion. Bigger particles have lower specific surface area and fewer diffusion pathways, which will decrease release rates.



(a) 21 µm (b) 28 µm (c) 35 µm

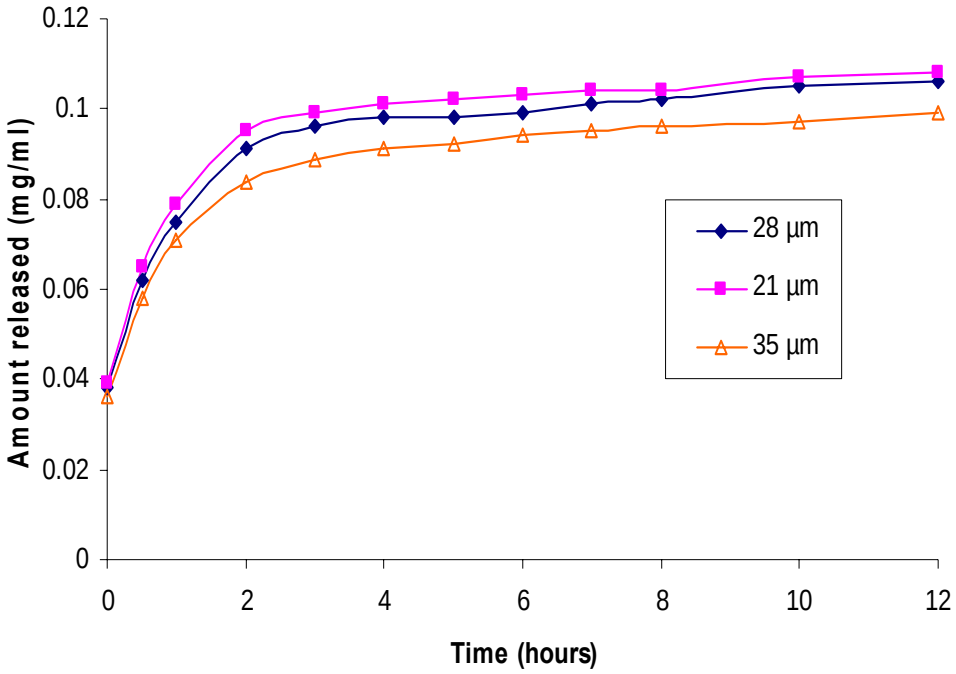
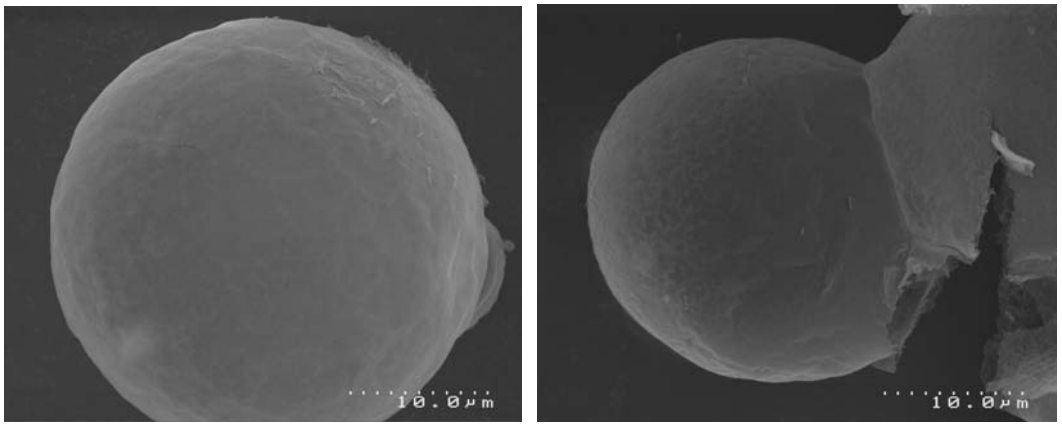


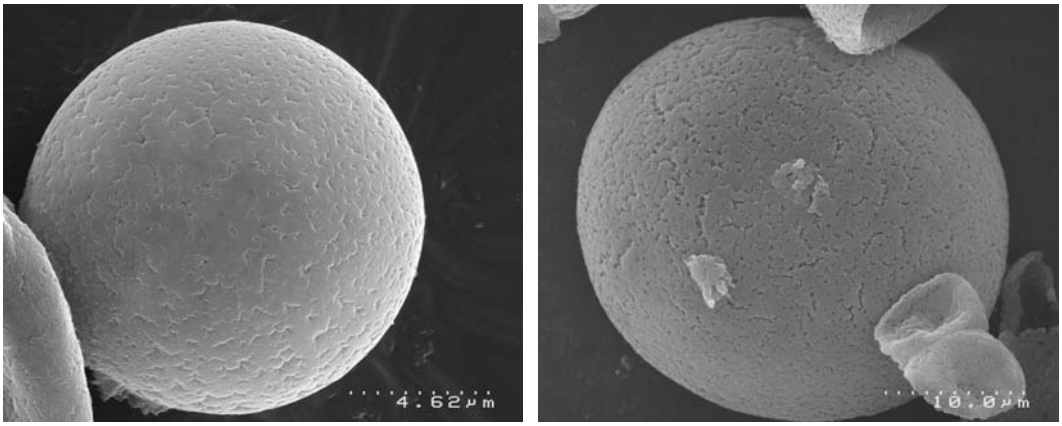
Figure 4.18 Effect of capsule size on vitamin B_{12} release from PAA-grafted microcapsules (n=3); (a), (b) and (c) average diameter of microcapsules

4.4.4.2 Porosity

Any drug near the surface will diffuse from the microcapsule first, causing a rapid initial release ("burst" release). Microcapsule morphology depends on the rate of polymer precipitation and solvent removal at the interface. Cross-sectional images of microcapsule (Figure 4.8) before the release study showed that the internal phase of the microcapsule is monomer ratio dependent and a core-shell structure was observed. Porosity has an important effect on drug release characteristics and is related to the initial burst effect. The porosity increased with increasing monomer ratio in EDA/DETA. The release rate increased with bigger microcapsule pore sizes (Figure 4.19).



(a) 3.2 nm (b) 6.5 nm



(c) 9.1 nm (d) 11.6 nm

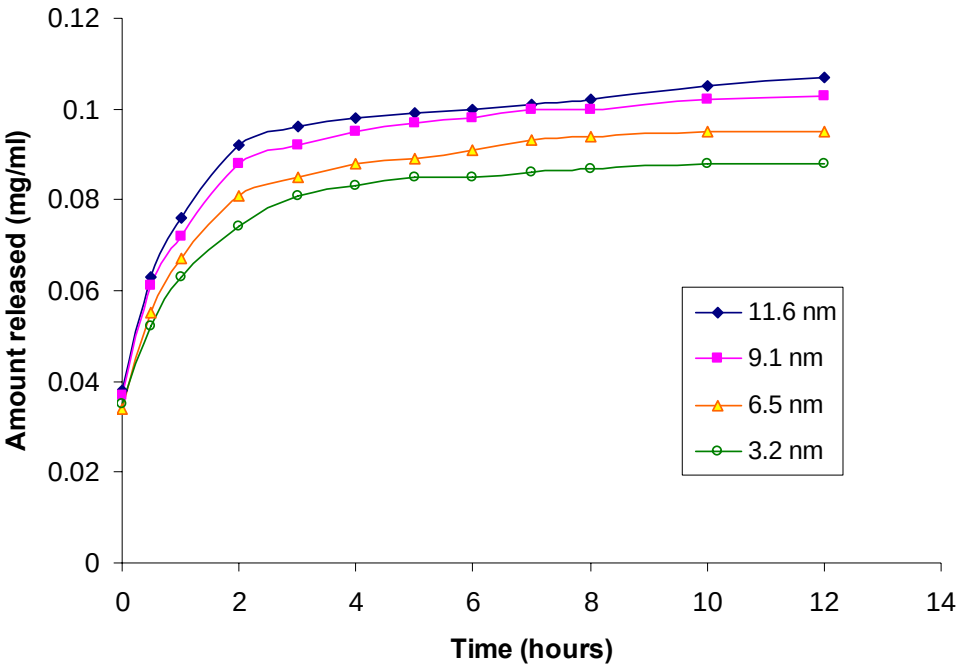


Figure 4.19 Effect of microcapsule pore size on vitamin B_{12} release from PAA-grafted polyamide microcapsules (n=3);

(a), (b), (c) and (d) pore sizes of microcapsules

4.4.4.3 Extent of graft

Grafting monomer concentration affected microcapsule properties and extent of grafting. Releases of vitamin B_{12} from the microcapsules made with 5–30% acrylic acid in graft polymerisation were investigated. All other processing conditions were kept constant (Section 3.6). The release rates of the content increased with monomer concentration up to 20% (Figure 4.20). However, the release rate decreased with further increase in monomer concentration. This was assumed to be due to over-grafting of PAA on the microcapsules surface, which caused some pores to remain closed.

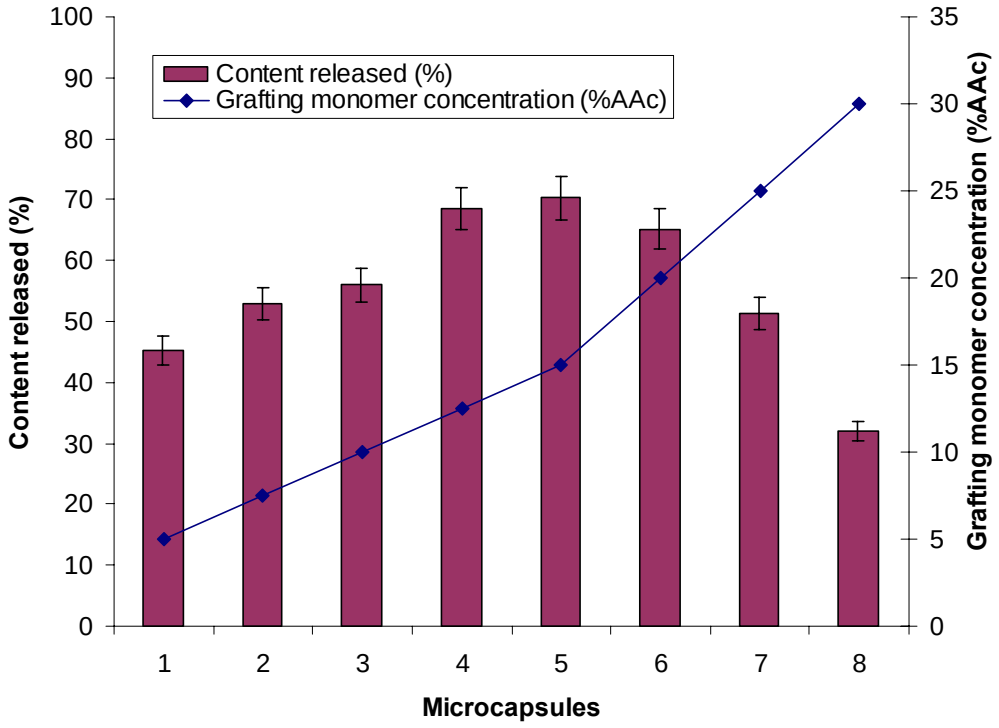


Figure 4.20 Effect of graft extent on vitamin B_{12} release from PAA-grafted polyamide microcapsules at pH 2 overnight (n=3)

4.4.4.4 Types of model drugs

The release rate of two types of model drugs (vitamin B_{12} and cytochrome *c*) from PAA-grafted microcapsules was very similar (Figure 4.21), indicating the type of model drug did not significantly affect release rate even though they have different molecular weights.

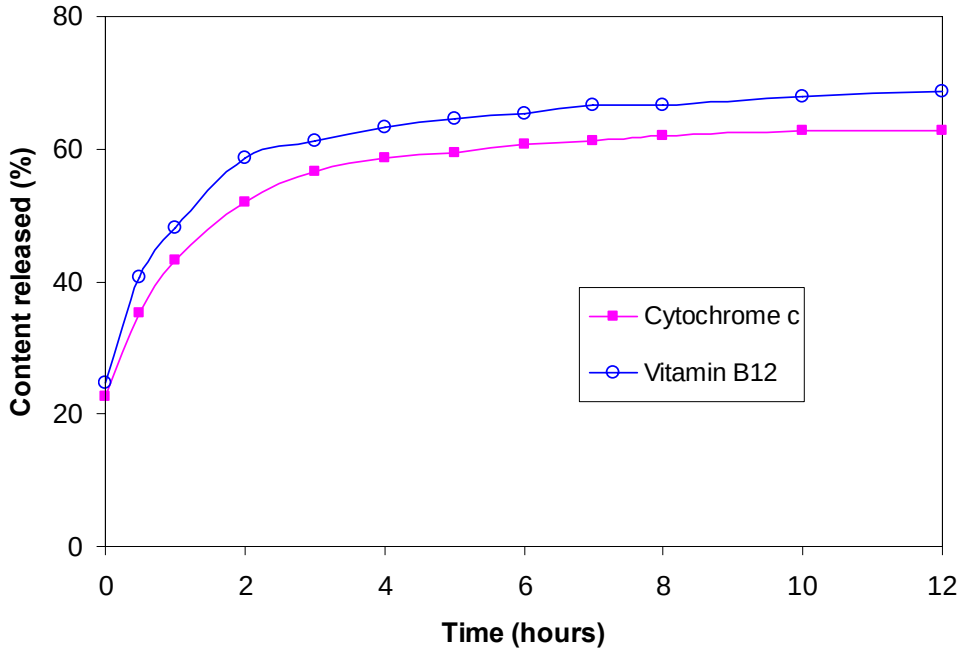


Figure 4.21 Effect of types of model drugs on release from PAA-grafted polyamide microcapsules (n=3)

4.5 CONCLUSIONS

This chapter describes the research on fabricating polyamide microcapsules using interfacial polymerisation techniques. Prepared microcapsules had a hollow core-shell structure and a porous shell with a smooth external surface and a rough internal surface and an average diameter of 28 μm. An argon plasma treatment was developed to graft acrylic acid to these microcapsules. The function and physical properties of the microcapsules were then determined.

The influence of processing parameters on particle size distribution, morphology of the microcapsule walls, and model drug molecules release were investigated. Average diameter of the microcapsules decreased with increase in stirring rate. Microcapsules shell porosity can be controlled by adjusting the ratio of the amine monomers in the reactions to form the microcapsule shell. The effect of two types of model drugs (vitamin B_{12} and cytochrome *c*) on release from PAA-grafted microcapsules was investigated. The results indicated that the size of the model drug did not significantly affect release rate. Contents were not released over the pH 7 to 5.5 but did occur between pH 5 and 3.5. Full release occurred when pH

was below 3. Thus, the microcapsules manufactured demonstrated a pH-triggered on-off release profile.

Results in this chapter demonstrated that pH-responsive functional microcapsules were successfully manufactured by interfacial polymerisation with plasma-induced grafting. The investigation was a proof of concept on the feasibility of developing a pH-responsive drug delivery system. The process was further developed and scaled up by other researchers in the NERF project team (Kelton, 2008).