Secondary Blooming and Mottling in an Intravaginal Drug Release Product

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Abstract

This study was undertaken to determine the root cause of secondary blooming (surface progesterone) and mottling (translucent regions) in the CIDR insert.

A stability study on CIDR inserts found that the alternative supplier silicone (Dow Corning silicone is used in normal manufacture) did not exhibit either secondary blooming or mottling, in agreement with previous work. There was no difference in 'in vitro' drug release rate between CIDR inserts made from the two silicone feedstocks. This silicone was found to have a higher ratio of low molecular weight cyclic silicone compounds to straight chain silicone compounds (Measured through detected ion ratios in the leachate by ESMS). The exact relationship between blooming and secondary mottling and this discovery remains unknown.

Results from this research found that part B of the Dow Corning silicone was not responsible for causing secondary blooming and mottling in contrast to previous research. ESMS and GCMS results found that the Dow Corning part B had a more complex composition than of the samples from Dow Corning part A and alternative supplier silicone part A and part B.

It was found that addition of a silicone crosslinker into the Dow Corning silicone, increased mottling, but not secondary blooming. Increasing the mixing of progesterone and liquid silicone decreased mottling (via a static mixer), but did not increase surface progesterone.

The results from two studies into the packing of CIDR inserts immediately after manufacture were contradictory as one found a decrease in mottling from packing in line, while the other found no change in mottling. However there was no difference observed in surface progesterone levels between the packing conditions. Studies on the length of cure were also found to have no predictable effect on mottling.

Abstract

Studies on arresting the cooling cured silicone above, between and below the melting points of the α and β progesterone polymorphs found that pausing cooling after cure at ~135 ^{o}C resulted in decreased secondary blooming and mottling, and that pausing after cure at ~125 ^{o}C exhibited less secondary blooming.

Neither DSC nor XRD was able to determine a polymorphic bias in progesterone cooled by this cooling process (β progesterone polymorph was in all but one case (that had an added reduction in normal cooling rate)). Studies (XRD and DSC) on the cooling rate of progesterone found that only with very slow cooling rates was the α progesterone polymorph formed. XRD studies also revealed that the mottled regions of CIDR inserts exhibit the β progesterone polymorph, whereas the α progesterone polymorph was detected in non-mottled regions. The mottled regions were found to continue to exhibit the same polymorph after removal of surface progesterone, whereas the non-mottled regions gave no detectable XRD peak intensity.

The study found useful information to assist in the determination of the root cause of secondary blooming and mottling in CIDR inserts. However the root cause remains unknown.

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This thesis is dedicated to two very important people in my life, my father the late Peter John Waugh (1953 to 1995) and to the formerly dead Lord Jesus Christ.

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List of abbreviations and terms

A - Initial amount of drug impregnated in the matrix (by unit volume).

Alternative Supplier Silicone - An alternative supplier of silicone that cannot be revealed in this thesis for reasons of commercial confidentiality. Comes as two liquid parts (A and B) that when mixed together and heated will result in the formation of silicone rubber.

Amorphous - Non-crystalline material. Will not give a spectra in XRD.

ATR FTIR - Attenuated Total Reflectance Fourier Transform Infar-red spectroscopy.

AU - Absorbance Units.

Blooming - Progesterone on the surface of the CIDR insert. Defined further in Chapter One. See initial blooming and secondary blooming.

Bulk Density - The volume taken up by a specific mass of powdered progesterone.

cGMP -current Good Manufacturing Practice. Required for manufacture of pharmaceutical products.

CAS - Chemical Abstracts Service. A CAS number is a unique number used to define different chemicals.

CIDR insert- Controlled Internal Drug Release. CIDR is a registered Trademark of InterAg NZ. The term is used by both customers and manufacturing staff at DEC Manufacturing NZ Ltd, to denote the CIDR insert.

CIDR 330 insert - A CIDR insert with 330 mg of progesterone and used in sheep and goats.

CIDR 1380 insert - A CIDR insert with 1380 mg of progesterone used in cattle.

CIDR 1900 insert - A CIDR insert with 1900 mg of progesterone used in cattle, with a thicker skin than the CIDR 1380 insert.

Cooling Oven - An oven set to a specific temperature (greater than ambient temperatures), into which samples were placed for a specific period of time. Samples would have been at a higher temperature before placement into the oven (see Chapter 4).

C_p - Solubility of the progesterone in the polymer

DEC International NZ Ltd. - The parent company that owns InterAg (who developed the CIDR insert) and DEC Manufacturing (that manufacture the CIDR insert). Based in Hamilton, New Zealand.

DEC Manufacturing - DEC (Manufacturing) NZ Ltd, owed by DEC International NZ Ltd, manufactures and tests CIDR inserts before sale.

D_p - Diffusion Co-efficient of progesterone in the polymer.

Drug - In this thesis unless stated otherwise refers to progesterone.

Drug Release - The rate of progesterone release from a CIDR insert. Measured in units of $\mu g/cm^2/\sqrt{t}$. In this thesis this refers to the rate of progesterone released from the CIDR insert or sample unless stated otherwise.

DS - Diluted Sample.

DSC - Differential Scanning Calorimetry.

E - Undiluted extract.

EIMS - Electron Impact Mass Spectrometry.

E08105 - A batch of CIDR 330 inserts made in August 2004 using the current Dow Corning Q7-4840 silicone.

E08106 - A batch of CIDR 330 inserts made in August 2004 using the alternative supplier silicone.

E09559 - Liquid Silicone mixed with progesterone from this CIDR insert batch was used to manufacture slabs.

E09338 - Liquid Silicone mixed with progesterone from this CIDR insert batch was used to manufacture slabs.

ESMS - Electrospray Mass Spectrometry (see Chapter Two).

Elastomeric- Rubber like material.

Enantiotropic - Park (Park et. al., 2003) defines enantiotropic as "where a transition point exists below the melting point (above and below this different polymorphic forms are stable) and this transition is reversible. The free energy and solubility curves of an enantriotropic system cross before the melting point and are equal at the crossing point" (see Chapter One).

Flash - Silicone that squeezes between the two sides of the tool, creating a thin film of cured silicone.

GC - Gas Chromatography.

GCMS - Gas Chromatography Mass Spectrometry.

Gravimetric Dilution - Sample is diluted by adding a known weight of solvent to a known weight of the sample.

HPLC - High Performance Liquid Chromatography.

ICH - International Conference on Harmonisation. See www.ich.org.

Initial Blooming – Blooming that occurs shortly after manufacture.

LCMS - Liquid Chromatography Mass Spectrometry.

Monotropic - Park (Park et. al., 2003) defines monotropic as "where only one polymorph is stable below the melting point, the free energy and solubility curves do not cross and there is no reversible transition between the polymorphic forms below the melting point" (see Chapter one).

Mottling -Translucent areas on a CIDR insert.

NICAR - Non-conformance Investigation, Corrective Action Report. Used at DEC Manufacturing to document deviations from normal practice.

NMR - Nuclear Magnetic Resonance Spectroscopy.

Oestrus - The period of sexual receptivity in animals.

ppm - Parts Per Million.

Packing in line - Where a CIDR insert is packaged immediately after being moulded.

Polymorph - Where a material has a different crystal lattice from another material, but possess the same chemical composition. Progesterone has five polymorphs (see Chapter One).

Q - Mass of drug released by unit area of CIDR insert.

Q7-4840 - See Silastic® Q7-4840.

QC - Quality Control.

Residue silicone – Liquid silicone that has been mixed with progesterone for intended use in manufacturing, but has not been mixed with the other part of the batch. Parts A and B are separate.

RH - Relative Humidity.

Rosette - A group of CIDR inserts moulded from the same shot of silicone, held together through the nylon spine runners.

rpm - Revolutions Per Minute.

RR0XX - Abbreviation for Research Report, XX refers to the number of the report. These were undertaken at DEC Manufacturing. The term is used in the references of this thesis.

Reserve Samples - Samples of CIDR inserts from each batch held in reserve to assist with any future investigations into product quality. Often used in research investigations. Reserve samples are held for at least twice the length of the expiry batch date.

SDA - Simple Denatured Alcohol.

Secondary Blooming - Occurs after the initial blooming, in the form of progesterone powder, crystals and flakes. See initial blooming, and blooming.

SEM - Scanning Electron Microscopy (see Chapter Two).

Sprew - A rod shaped piece of silicone that is left after moulding. Connects the point of injection into the tool to the actual sample being moulded. The spew is normally discarded after use.

Shot - The volume of silicone injected into the moulding tool.

Silastic® Q7-4840 - Made by Dow Corning and is the silicone used in the manufacture of most of the CIDR inserts at DEC Manufacturing. Comes as two liquid parts (A and B) that when mixed together and heated will result in the formation of silicone rubber.

Slab - A sample of silicone that has been cured and contains progesterone (normally 10 % w/w). Like a CIDR insert skin.

STAB - Stability. Abbreviation used at DEC Manufacturing to number various stability jobs. (see references).

Std. Dev. - Standard Deviation.

STPXXX - Standard Test Procedure. DEC Manufacturing acronym relating to the standard test procedures used in the quality control laboratory. The XXX refers to the number of the test. STP011 is used to determine progesterone

content in CIDR inserts, STP048 or STP055 is used to determine progesterone drug release rate.

TIC - Total Ion Chromatogram. Total ion current (peak intensities of all peaks detected) versus time. Provides no specific information on a particular peak.

Tool - The mould used to make CIDR inserts.

USP - United States Pharmocopeia.

UV - Ultra Violet.

$\mathbf{V_{Ethanol\ in\ Container}}$ -Volume of ethanol in the extraction container.

$\mathbf{W_{Diluting\ Ethanol}}$ - Weight of the diluting ethanol.

$\mathbf{W_{Extract}}$ - Weight of the extract.

XRD - X-ray Diffraction (see Chapter Two).

1.0 Introduction

1.1 Oestrus and the CIDR insert

Oestrus (InterAg, 1994) is a period when animals exhibit sexual activity or heat and can undergo insemination. A Cow exhibits sexual receptivity with restless behaviour, allowing herself to be mounted, mounting other animals, bellowing, and having her vulva sniffed by other cows (Gordon, 2004). In sheep the animal will associate with the ram, and can withdraw from the flock (Gordon, 2004). The oestrus cycle is a complex relationship between a range of hormones in the animal including progesterone and oestradiol (InterAg, 1994). Figure 1.1 shows the blood hormone concentrations during the oestrus cycle in cattle. Oestrus occurs at day zero in the cycle, with ovulation occurring at day one (InterAg, 1994).

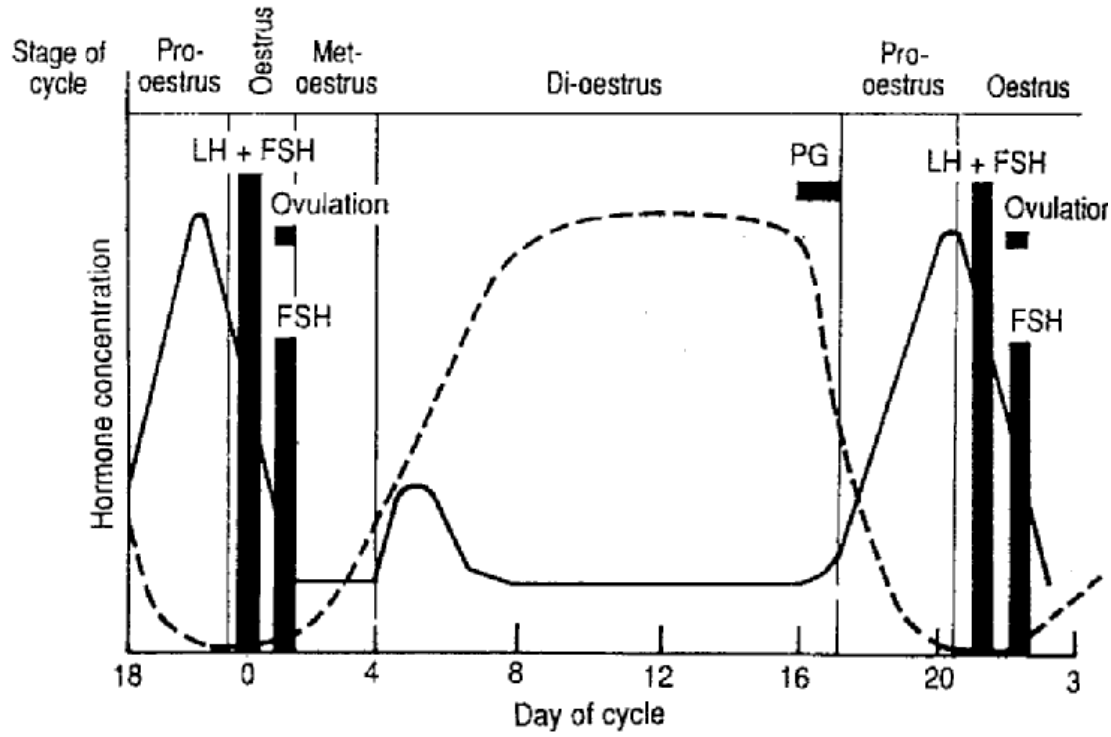


Figure 1.1 Oestrus cycle blood plasma hormone concentrations. LH = Luteinising Hormone, FSH = Follicle Simulating Hormone, PG = Prostaglandin F2α (InterAg, 2004).

There is a decline in the blood plasma progesterone concentration before oestrus occurs (see Figure 1.1). Pro-oestrus occurs during this stage, with an increase in oestradiol levels, which cause the behavioural changes during heat (InterAg, 1994). During this phase (InterAg, 1994) the increasing concentration of oestradiol increases the amount of luteinising hormone, which results in further increases of the oestradiol level. The surge in luteinising hormone results in ovulation (InterAg, 1994).

Progesterone treatment can be used to control the oestrus cycle, as the progesterone suppresses the luteinising hormone and hence prevents oestrus and ovulation (Gordon, 2004). Once the progesterone treatment ceases the decline in progesterone simulates again the natural conditions prior to the onset of oestrus (InterAg, 1994).

Controlling the Oestrus cycle provides many benefits (Gordon, 2004) including assisting in embryo transfer programs, keeping births within a specified time period, and reducing the labour required to detect oestrus. The first commercial intravaginal oestrus control in cattle has been available to the farmer since the 1970's in the form of the PRID (Progesterone Releasing Internal Device) (Gordon, 2004). There are three progesterone releasing intravaginal devices available on the New Zealand market (Rennie, 2005), the Cu-mate, the PRID and the CIDR insert (pronounced SEE-dur, abbreviation for Controlled Internal Drug Release). This thesis focuses on the CIDR insert.

The CIDR insert is a single use device, used to deliver progesterone intravaginaly to cows, sheep and goats in order to provide control over the oestrus cycle, through the controlled release of progesterone. The CIDR insert is manufactured by DEC Manufacturing NZ Ltd, based in Hamilton, New Zealand and is sold by Pfizer as a veterinary pharmaceutical product in a number of countries including Canada, the USA and the UK.

The CIDR insert was initially inserted for a 12 day period in cattle (Rathbone, 2002), however this period reduces fertility, the current program only uses a seven day insertion period. To improve fertility of the CIDR insert application

(Burggraaf, 2006c) an injection of Prostaglandin F2α is given to the animal one day before CIDR insert removal and one day after removal an injection of oestradiol benozoate is given to the animal.

The basic design of the CIDR insert is a T shaped device (see Figure 1.2) that consists of a nylon spine, over which an elastic silicone skin is injection moulded. The two arms of the 'T' or "wings" can be folded together in order to facilitate insertion into the vaginal cavity and also to promote retention after insertion. A tail is attached to the base of the CIDR insert to enable removal from the vagina after treatment. The skin (consisting of an elastic silicone rubber) serves as a matrix for the active ingredient, progesterone.

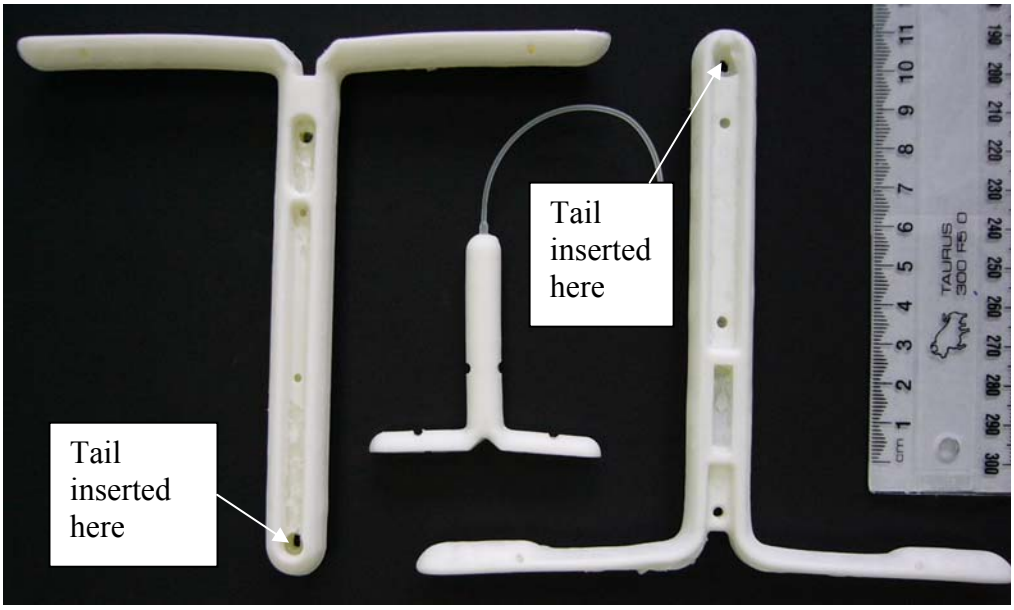


Figure 1.2 CIDR inserts. CIDR 1900 insert (left), CIDR 330 insert (middle), and CIDR 1380 insert (right). The tails of the CIDR 1380 insert and the CIDR 1900 insert are not shown.

DEC Manufacturing, currently manufactures three types of CIDR inserts for sale. These are the CIDR 1900, CIDR 1380 and the CIDR 330 inserts. Examples of all three types of CIDR inserts are shown in Figure 1.2. Both the CIDR 1900 and the CIDR 1380 inserts are intended for use in cattle, while the CIDR 330 insert is designed for use in sheep and goats. The CIDR 1380 insert has a progesterone load of 1380 mg, compared with the CIDR 1900 insert, which has 1900 mg of progesterone. The CIDR 1380 insert was designed to have reduced progesterone

load while having the same 'in vivo' effect as the CIDR 1900 insert (Rathbone, 2002). Both the CIDR 1380 insert and the CIDR 1900 insert have a skin drug loading of 10 % w/w of progesterone. The CIDR 330 insert has a skin drug loading of 9 % w/w of progesterone. The CIDR 330 insert is the smallest CIDR insert with 330 mg of progesterone and has a nylon tail incorporated as part of the spine. This is different from the CIDR 1380 and the CIDR 1900 inserts, as these inserts have a tail installed after manufacture of the main device. Work by Rathbone et. al. (Rathbone, 2002), found the 'in vivo' drug release rate in cattle depended on surface area of the insert and the insert drug loading.

The CIDR insert was developed by InterAg (currently owned by DEC International NZ Ltd.) in the 1980's with the CIDR 330 insert entering the market in 1985 (Epps, 2006), and the CIDR 1900 insert entering the market in 1987 (Epps, 2006). The cattle CIDR 1900 insert was further developed in the 1990's to make the CIDR 1380 insert (Rathbone, 2002), which entered the market in 1998 (Epps, 2006). This is a more cost effective design as it reduces the mass of progesterone used in the device, while retaining the same biological performance.

Before moulding, the nylon spine is annealed in water to provide strength and flexibility (Burggraaf, 2005c). Before the spine is placed into the tool (mould see Figure 1.3) the spine is allowed to drip-dry, during which small amounts of white material drip off the spine. The residue has been determined by XRD as a nylon residue (Wong, 2003i).

The progesterone is held in the silicone skin of the CIDR insert, which covers the spine. Silicone is supplied by Dow Corning as a viscous liquid in two parts (labelled as part A and part B). Micronized progesterone is mixed separately into each part of the liquid silicone. The two silicone parts (mixed with progesterone) are mixed together using a static mixer (a method of in-line mixing without moving parts), before being injected into the tool and then cured (vulcanisation) at a temperature higher than 150 ^{o}C between 20 to 50 seconds depending on the insert type. The moulded CIDR insert is removed from the tool, bench cooled before the tail is added and packaged into either a paper foil lined bag (CIDR 1900 and CIDR 330 inserts) or a polyethylene bag (CIDR 1380 insert).

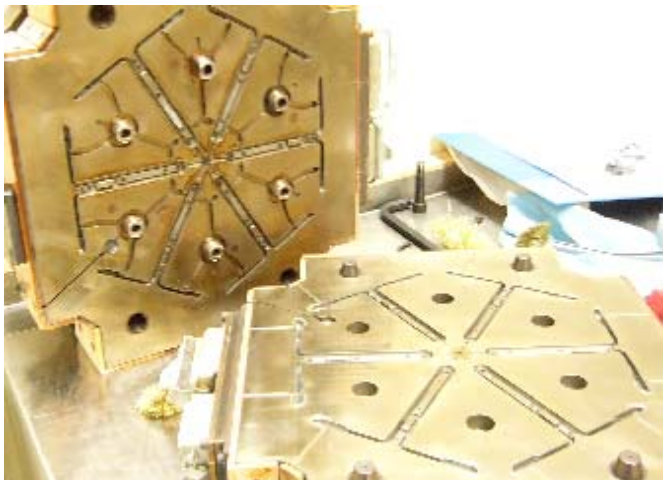


Figure 1.3 The two sides of the tool for the CIDR 1380 insert.

All manufacturing and laboratory testing activities in the manufacture of CIDR inserts are strictly controlled in accordance with current Good Manufacturing Practice (cGMP). This is to ensure that all CIDR inserts sold are fit for their intended purpose and to allow traceability of all actions. Research activities into CIDR insert production must ensure that product quality is unaffected.

1.2 Mottling and blooming

The phenomena of mottling and blooming occurs on the silicone skin after the curing. Blooming is defined as any surface progesterone. Mottling is defined as translucency on the CIDR insert surface (Figure 1.4). The aim of this thesis is to study the phenomena of blooming and mottling with the aim of either minimising or stopping it.

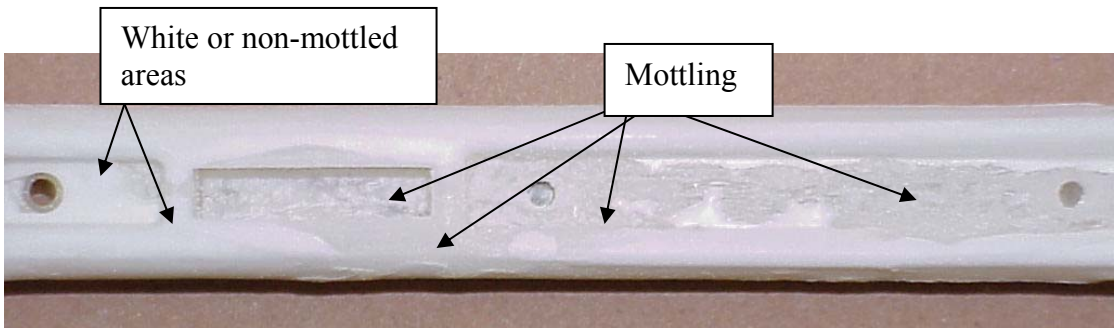


Figure 1.4. Mottling on a CIDR insert. (Rathbone & Ogle, 2000).

The normal colour of a CIDR insert is white and this is caused by the dispersion of progesterone in the silicone matrix, (silicone rubber without progesterone is clear). Translucent regions (non-white) are defined as mottling. This is illustrated in Figure 1.4, which shows the white regions of a CIDR insert and the translucent (mottled) areas. Mottling is measured as a % area of the device that is translucent; the method of doing so is discussed in Chapter Three. Mottling (Rathbone & Ogle, 2000) occurs over one to two weeks after manufacture of a CIDR insert, with areas of mottling becoming more translucent with time. Mottling (Rathbone & Ogle, 2000) can cover up to 50 to 60 % of a CIDR insert.

After curing, progesterone starts to migrate to the surface as white spots that eventually cover the surface as shown in Figures 1.5 and 1.6 (Reardon, 2004b). This is defined as initial blooming. Anything that touches the slab shortly after manufacture causes initial blooming to occur (Reardon, 2004b). Over the next two to three days, secondary blooming occurs (see Figure 1.7) on the CIDR insert (Reardon, 2004b). Secondary blooming involves further migration of progesterone to the surface and the formation of crystals, flakes and microcrystalline powders of progesterone on the insert (Rathbone & Ogle, 2000). Figure 1.8 shows an SEM image of crystals formed on a CIDR surface one month after manufacture. Migration of progesterone to the surface of the CIDR insert has been found to occur for two to three months after manufacture of the CIDR insert before finally ceasing (STAB001, 1999-2004).

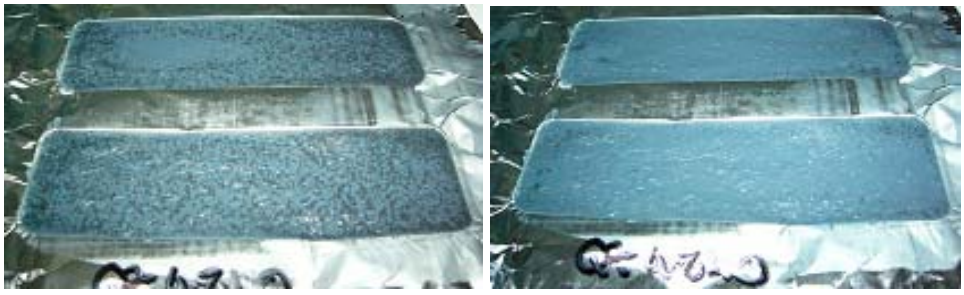


Figure 1.5 (left) and Figure 1.6 (right) Slabs of Silicone showing the initial blooming on slabs made with Dow Corning Q7-4840 silicone, black dye added for visualisation purposes. Figure 1.5 photograph taken 20 minutes after manufacture. Figure 1.6 photograph taken 30 minutes after manufacture. (Reardon, 2004b).

Figure 1.7 Slabs of Dow Corning Q7-4840 exhibiting characteristic secondary blooming. Slabs made at the same time as slabs in Figures 1.5 and 1.6. Photograph taken two months after manufacture. Black dye added to aid visualisation of blooming on the sample surface. (Reardon, 2004b).

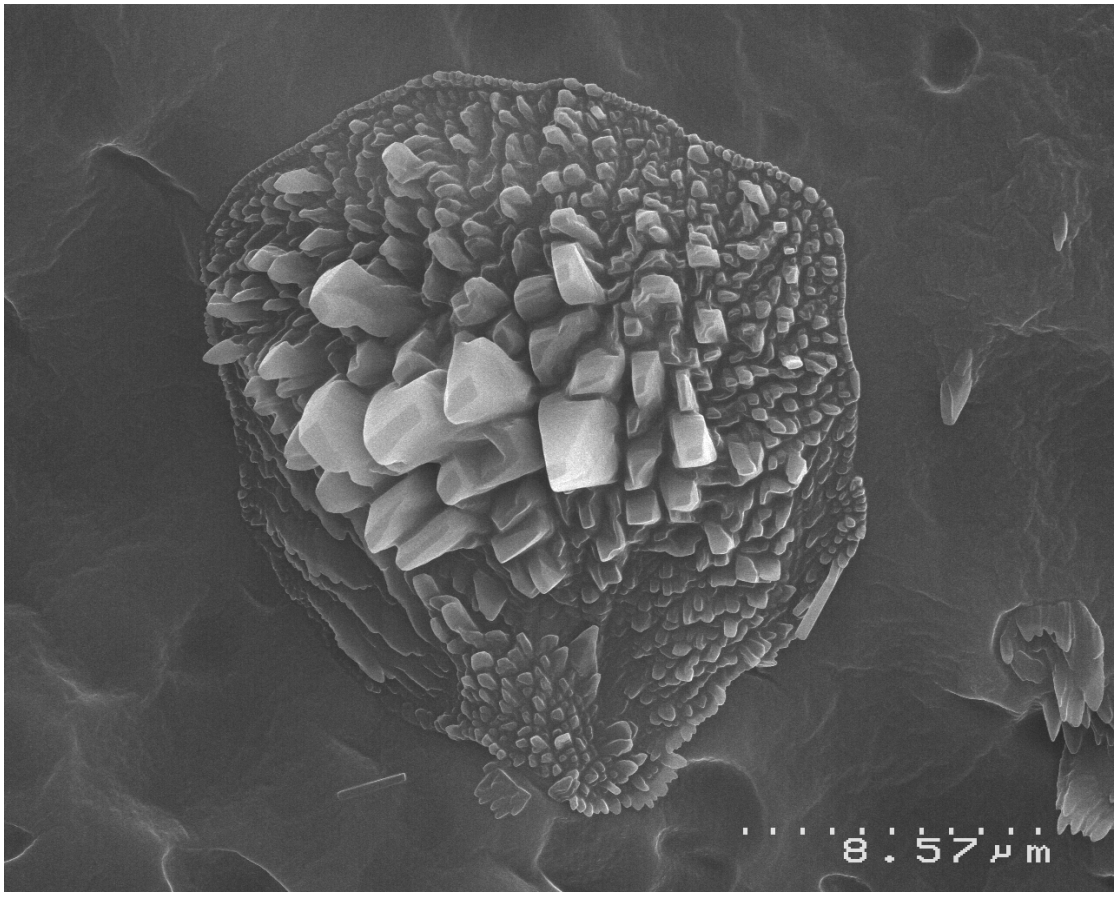


Figure 1.8 Progesterone crystals on the surface of a CIDR insert as observed using a SEM.

Rathbone and Ogle (Rathbone & Ogle, 2000) found that mottled areas on a CIDR insert have significantly reduced drug loading (ranging from 4.89 to 7.61 % w/w) compared to the white (non-mottled) regions on the CIDR insert. Rathbone and

Ogle (Rathbone & Ogle, 2000) also analysed the % progesterone loading versus depth profile in mottled regions on the CIDR insert and found a wide range of variation in progesterone loading (from 14.29 % to 5.01 % w/w), which contrasted with the depth profiles from a white region (non-mottled) that had drug loads similar to that in the manufactured insert.

Analysis of mottled areas (Wong, 2003e) using SEM and XRD has found that crystalline progesterone changes from being an amorphous white powder to a large clear crystal, which gives the mottled sections their translucent appearance. Wong (Wong, 2003e) hypothesised that a CIDR insert with increased permeability (diffusion) would have increased mottling.

1.2.1 Techniques used to determine the level of surface progesterone (blooming)

An accurate assessment of blooming by visual examination is widely prone to errors from analyst interpretation, and furthermore handling the insert (to observe the blooming) dislodges progesterone from the insert and hence the observation is unreliable. A number of techniques have been explored to determine the level of surface progesterone on CIDR inserts. Wong (Wong, 2003d) and Ogle (Ogle, 1999) have both worked on separate techniques to determine the level of surface progesterone on CIDR inserts. There is also a method that is used as part of stability studies at DEC manufacturing.

Wong (Wong, 2003d) placed CIDR inserts into a 1 L container in a shaking water bath at 20 ^{o}C containing a release medium of 62.5 % ethanol : 37.5 % (v/v) water and then sampled at fixed intervals. The shaking water bath was operating throughout the test. Wong (Wong, 2003d) noted that over 20 minutes it was possible to differentiate between old (mottled and bloomed) and new (fresh) CIDR inserts. Ogle's method using CIDR 1380 inserts (Ogle, 1999), involved rinsing the surface of the CIDR insert with 45 to 50 ml of ethanol. The ethanol was then made up to 100 mL in a volumetric flask. The concentration of progesterone was determined using UV spectrophotometry thus providing a measure of surface progesterone.

The DEC Manufacturing QC drug release rate test (STP048 for CIDR 1380 inserts, and STP055 for CIDR 330 inserts) can be used to provide an estimate of surface progesterone levels. The drug release rate for a insert is calculated from the slope of the plot of the mass of progesterone released per unit area ($\mu g/cm^2$) versus $\sqrt{t}$ (t = time) to get the drug release rate of ($\mu g/cm^2/\sqrt{t}$). A discussion of the drug release rate test is in Chapters Two and Three. The mass of progesterone released after one hour into a release media of 62.5 % ethanol : 37.5 % (v/v) water at 37 ^oC, can be used to gain a measure of the surface progesterone. The mass of progesterone released after t = 1 hour comprises of surface progesterone and progesterone released from the silicone matrix. Hence this method allows differentiation between CIDR inserts with different amounts of surface progesterone, but is unable to determine the mass of progesterone on the surface of the CIDR insert. Standard DEC Manufacturing QC drug release tests take 20 hours to collect samples, resulting in a test that is far longer than either the method of Ogle (Ogle, 1999) or Wong (Wong, 2003d).

1.2.2 The effect of secondary blooming and mottling

Secondary blooming and mottling pose both aesthetic problems and 'in vitro' drug release rate problems. Secondary blooming and mottling may reduce the 'in vitro' drug release rate in the CIDR insert (Wong, 2003e) (NICAR FL320, 2005) (Wong, 2003j). The sale of CIDR inserts in many markets requires drug release rate tests to pass market specification (some markets do not require a drug release rate test). Secondary blooming and mottling is an aesthetic issue as the visual appearance of the CIDR insert is impaired.

Studies undertaken by DEC Manufacturing (STAB001**,** 1999-2004) have shown that there is little difference in the 'in vivo' blood plasma progesterone levels, between freshly manufactured inserts and inserts that exhibited mottling and secondary blooming. The progesterone blood plasma levels from a stability study (STAB001**,** 1999-2004) are shown in Figure 1.9.

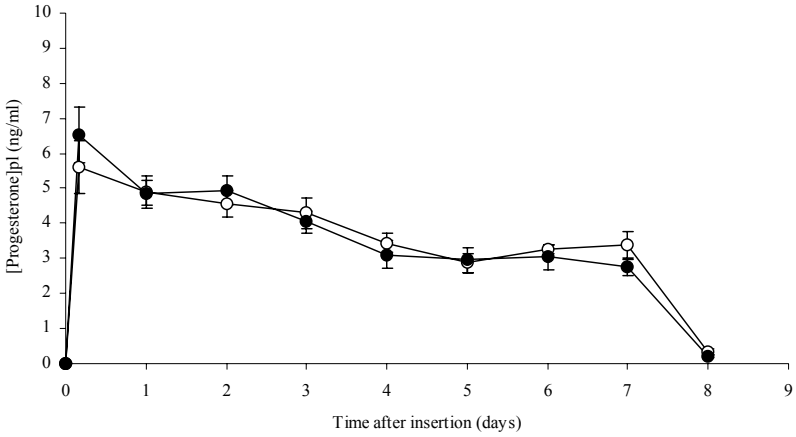


Figure 1.9 Average 'in vivo' plasma progesterone levels (ng/mL) in cattle CIDR inserts from batch 9328. o = Freshly manufactured CIDR inserts, • = CIDR inserts that had been stored for one year at 40 °C and 75 %RH. Blood samples taken from animals at t = 2 hours, and daily for eight days. (STAB001, 1999-2004).

It was initially thought (Rathbone, 2005) that the initial burst of progesterone in the blood plasma upon insertion of the CIDR insert (caused by the increased surface progesterone, see Figure 1.9), was beneficial. However current experience is that the burst neither contributes to nor compromises the effectiveness of the CIDR insert (Rathbone, 2005).

Analysis by DEC Manufacturing (NICAR FL320, 2005) comparing the drug release rates, versus % mottling, and secondary blooming (analysing amount of progesterone released after t = 1 hour in a 'in vitro' drug release rate test) on 14 CIDR inserts, found a number of trends. CIDR inserts with high levels of secondary blooming have high levels of mottling (NICAR FL320, 2005), and CIDR inserts with a high level of mottling tend to have a lower drug release rate (NICAR FL320, 2005). CIDR inserts with low drug release rates are found to have higher levels of surface progesterone (NICAR FL320, 2005). Drug release rates and the surface progesterone were determined using Hanson Dissolution apparatus.

If the drug release rate is plotted versus % mottling, then it is observed (Figure 1.10) (NICAR FL320, 2005) that the higher the % mottling, the lower the drug release rate. It is observed in Figure 1.10 that $R^2 = 0.6809$ indicating that while a trend may exist, there is significant scatter in the data.

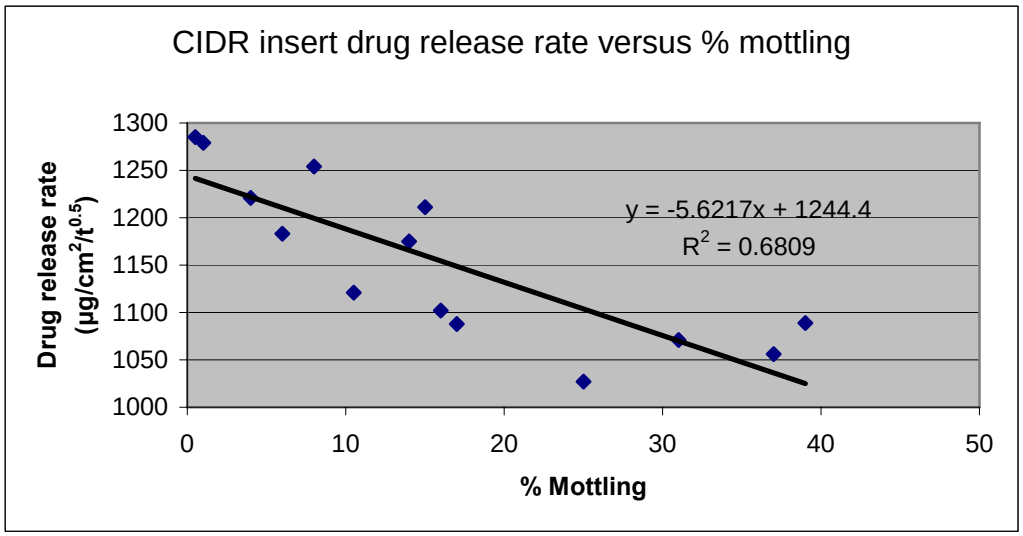


Figure 1.10 % Mottling versus CIDR 1380 insert drug release rate (NICAR FL320, 2005).

If the plot (NICAR FL320, 2005) of surface progesterone measured as the mass released after one hour in a drug release test versus % mottling is analysed (Figure 1.11) it is observed that the higher the level of surface progesterone the higher the level of % mottling on a CIDR insert. Figure 1.11 indicates a R^2 value approaching 1, indicating that there is a definite relationship between mottling and secondary blooming. Work by Wong (Wong, 2003j) shows similar results but $R^2 = 0.8736$. The scatter in Figure 1.10 could be the result of dislodgement of progesterone from the surface of the device before testing.

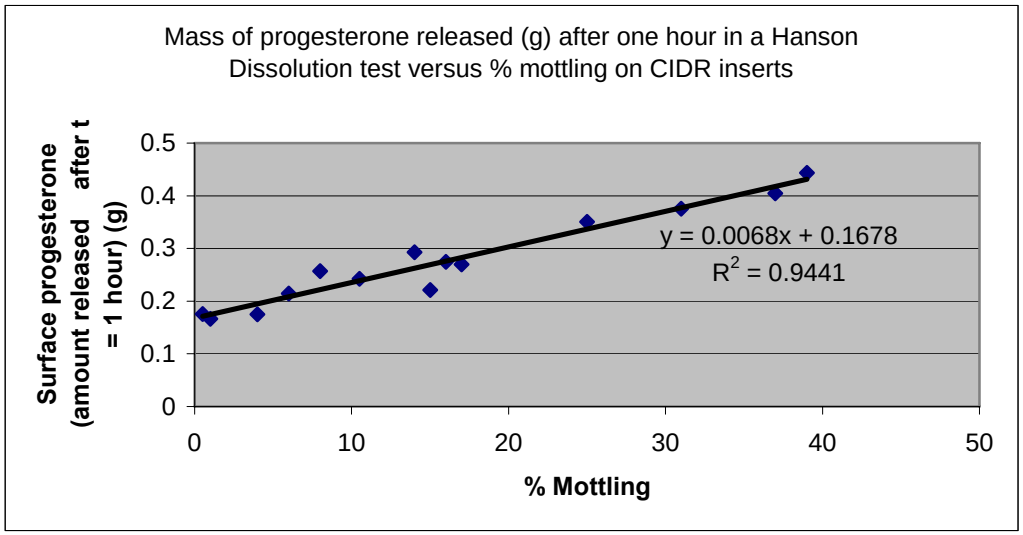


Figure 1.11 Amount of progesterone released after one hour versus % mottling on CIDR 1380 inserts (NICAR FL320, 2005).

Mottled sections of a CIDR insert can release (Wong, 2002a) approximately 70 to 75% of the progesterone load into release media of 62.5 % ethanol: 37.5 % water (v/v) at 37 ^{o}C in the first hour of a drug release test, whereas white (non-mottled) regions from the same CIDR insert can release approximately 15 % of their load in the first hour under the same conditions.

If an analysis (NICAR FL320, 2005) is undertaken comparing the drug release rate on the CIDR inserts versus the surface progesterone on the same CIDR inserts, it is found that CIDR inserts with lower drug release rates tend to have higher levels of surface progesterone (see Figure 1.12). However it should be noted that $R^2 = 0.7181$ in Figure 1.12 and there is a lot of scatter. This could be caused by dislodgement of progesterone from the surface of the device before testing affecting the drug release rate (The mass released at t = 1 hour).

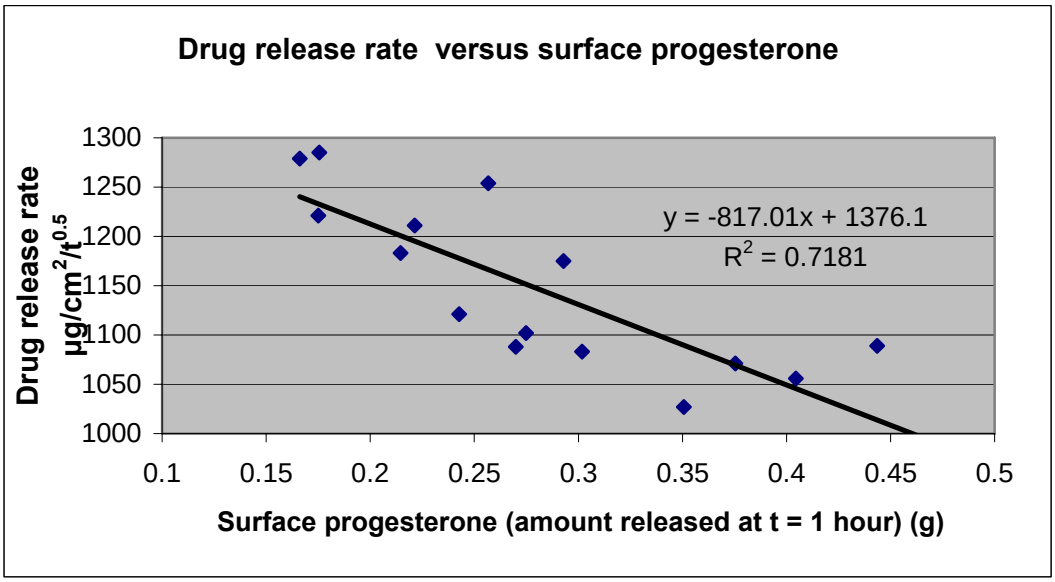


Figure 1.12 Surface progesterone versus drug release rate. Sample size of 16 CIDR 1380 inserts. (NICAR FL320, 2005).

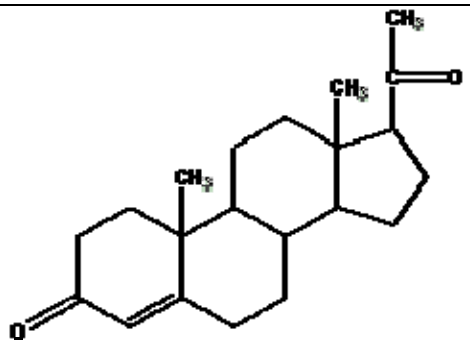
Work by Wong (Wong, 2003e) (Wong, 2003j) had similar results as compared to the results from NICAR FL320 (NICAR FL320, 2005), showing that the higher mass of progesterone released after one hour from a drug release rate test, resulted in lower drug release, and that higher % mottling resulted in an increase in mass of drug released after one hour in a drug release rate test (Wong, 2003j).

From these studies is clear that there is a relationship between secondary blooming and the 'in vitro' drug release rate. However current experience is that the burst neither contributes to nor compromises the 'in vivo' effectiveness of the CIDR insert (Rathbone, 2005). The progesterone on the surface creates a burst effect at the start of the 'in vitro' drug release test. This lowers the slope of the graph used to calculate drug release rate (see Figure 1.12) because the t = 1 hour time point for a highly bloomed CIDR insert will be higher than a CIDR insert with less secondary blooming and so reducing the slope of the graph used to calculate drug release rate. It is also clear from Figure 1.11 that there is a relationship between the % mottling and secondary blooming, with higher % mottling resulting in higher levels of surface progesterone.

There is also a relationship (Wong, 2003e) between the progesterone content (total mass of progesterone in the CIDR insert) and % mottling for stored stability samples. The higher the % mottling on a CIDR insert, the less progesterone the CIDR insert will contain (Wong, 2003e). This can be explained through progesterone migration to the spine and surface of the CIDR insert and dislodging of progesterone from the surface to give the impression of progesterone loss over time. The review noted the linear regression results gave a slope of -0.0017 and $R^2 = 0.616$ (Wong, 2003e) indicating a wide degree of scatter, which would be expected due to the uncontrolled nature of progesterone dislodgement.

1.3 Progesterone

Progesterone is a steroid and is the sole drug used in the CIDR insert. The basic details of progesterone are listed in Table 1.1.

Table 1.1. Basic information on progesterone (Diosynth MSDS, 2003).	
Substance Class	Progestogens
Structure	 Pregn-4-ene-3,20-dione Formula: $C_{21}H_{30}0_2$ Molecular weight: 314.46 $gmol^{-1}$ CAS Number: 57-83-0
LD_{50} oral rat	>6400 mg/kg (applies to medroxy progesterone acetate)
Routes of human exposure	Harmful by inhalation, contact with skin and through swallowing. Also absorbed though mucous membranes.
Effects of overexposure	In males reversible gynaecomastia (enlargement of a man's breasts). May also affect libido and reduce glucose tolerance. In females there is an effect on menstruation and fertility and may cause amenorrhoea (absence of a menstrual period).

1.3.1 Progesterone polymorphism

Progesterone exists in five polymorphic forms (Kuhnert-Brandstätter et. al., 1965). The most common polymorphic forms of progesterone are the α (Form I) and β (Form II) polymorphs. Other progesterone polymorphs exist, but are more rare. These are summarised in Table 1.2.

Table 1.2 The polymorphic forms of progesterone. (Kuhnert-Brandstätter et. al., 1965) (Muramatsu, et. al. 1979).		
Progesterone polymorphic Form	**Melting point (^{o}C)**	**Comments on polymorphic properties and formation**
α (Form I)	131 ^{o}C (Kuhnert-Brandstätter, et. al., 1965), 128 ^{o}C (Muramatsu et. al., 1979)	The melt of the α progesterone polymorph solidifies as a glass that acts in the same manner as the molten form of the β polymorph (Kuhnert-Brandstätter, et. al., 1965). The only way to obtain the α polymorph from the melt is to seed it with a crystal of the α polymorph (Kuhnert-Brandstätter, et. al., 1965). The α polymorph is the thermodynamically stable form (Wang et al., 2000).
β (Form II)	123 ^{o}C (Kuhnert-Brandstätter, et. al., 1965), 122 ^{o}C (Muramatsu et. al., 1979)	The melt of the β progesterone polymorph solidifies to a glass (Kuhnert-Brandstätter, et. al., 1965). The β polymorphic form forms in the melt at room temperature at 60 ^{o}C (Kuhnert-Brandstätter, et. al., 1965). When β progesterone polymorph forms in the melt any other polymorphic forms present are converted to the β polymorphic form (Kuhnert-Brandstätter, et. al., 1965). If the β polymorphic from does not form in the melt, then the melting points of Forms III to V are able to be determined (Kuhnert-Brandstätter, et. al., 1965). The α polymorph will never spontaneously form on the reheat of the super cooled melt of the β polymorph (Kuhnert-Brandstätter, et. al., 1965).

Form III	111 °C (Kuhnert-Brandstätter, et. al., 1965)	Enantiotrope of Form IV (Kuhnert-Brandstätter, et. al., 1965). Form III is the polymorph of progesterone most commonly formed upon the melting of the β progesterone polymorph (Kuhnert-Brandstätter, et. al., 1965).
Form IV	106 °C (Kuhnert-Brandstätter, et. al., 1965)	Enantiotrope of Form III (Kuhnert-Brandstätter, et. al., 1965).
Form V	100 °C (Kuhnert-Brandstätter, et. al., 1965)	Kuhnert-Brandstätter et. al. (Kuhnert-Brandstätter, et. al., 1965) notes that this form appears rarely. This form converts slowly to Forms III and IV.

Muramatsu et. al. (Muramatsu, et. al., 1979) discovered that holding the progesterone at a constant temperature of 85 °C ('equilibrium melting') in air, nitrogen or helium resulted in the formation of the β progesterone polymorph. However in a vacuum the α progesterone polymorph was formed. Both polymorphs were stable at room temperature for several months (Muramatsu, et. al. 1979). High temperatures also cause the β progesterone polymorph to convert to the α progesterone polymorph (Muramatsu, et. al. 1979).

DSC (Muramatsu, et. al., 1979) cooling of molten progesterone, at rates between –1 and –10 °C/minute, resulted in super cooling of the sample, with crystallisation occurring during the reheating step. Crystallisation temperatures ranged from, 37 to 120 °C depending on the experimental conditions, but regardless of solidification conditions the β progesterone polymorph was formed (Muramatsu, et. al. 1979). Muramatsu et. al. (Muramatsu, et. al. 1979) found that cooling between –1 to 10 °C/minute resulted in progesterone becoming supercooled, crystallisation would then occur on the subsequent reheating process with crystallisation temperatures from 37 to ~120 °C. Muramatsu et. al. (Muramatsu, et. al. 1979) also found that re-crystallisation in an organic solvent such as benzene,

ether, ethanol, acetone or a binary combinations of these solvents always resulted in the α polymorphic form. Muramatsu et. al. (Muramatsu, et. al. 1979), did not provide an explanation of this phenomena.

Muramatsu et. al., (Muramatsu, et. al., 1979) determined the unit cell values for both the α and β progesterone polymorphs. These are listed in Table 1.3. The differences in properties between the α and β progesterone polymorphs arises from dissimilar modes of molecular packing, not from differences in the molecular conformation (Muramatsu et. al., 1979).

Table 1.3 Lattice Constraints of α and β polymorphic forms of progesterone with an orthorhombic crystalline system. (Muramatsu et. al., 1978).

Å	**α progesterone polymorph**	**β progesterone polymorph**
a	12.62	12.65
b	13.84	22.65
c	10.38	6.38
Total volume ($Å^3$) (2 d.p.)	1813.00	1828.01

The α and β polymorphs of progesterone are monotrophs (Wang et al. 2000) and the α polymorph is the thermodynamically stable form (Wang et al., 2000). The β progesterone polymorphic is unstable (Muramatsu, et. al. 1979) and at 99 ± 1 ^oC the β progesterone polymorph converts to the α progesterone polymorph. Muramatsu et. al. (Muramatsu, et. al. 1979) discovered that after 484 hours that the DSC peak area between the α and β progesterone polymorphs was approximately equal.

Progesterone is very soluble in ethanol (Rathbone, et. al.), with a solubility of 101.29 mg/mL but in water the solubility is only 0.01 mg/mL (Rathbone, et. al.). Wang et. al. (Wang et al., 2000) analysed the polymorphic transition of progesterone from the β polymorph to the α polymorph in slurried water between 5 to 45 ^oC, through observing the shift in the carbonyl stretching peak between 1662 cm^{-1} (α progesterone polymorph) and 1667 cm^{-1} (β progesterone polymorph) in the Raman spectrum. It was found that the rate of transformation from the

β polymorph to the α polymorph increased exponentially with temperature (Wang et al., 2000). The phase transformations observed by Wang et. al. (Wang et. al., 2000) were consistent with Ostwald's law of stages.

When molten progesterone cools (Legendre, et. al., 2003) there is competition between the formation of the α and β polymorphs of progesterone. If cooling rates are slow then the α polymorph forms first (Legendre, et. al., 2003). However formation of the β polymorph can also occur (Legendre, et. al., 2003). If the cooling rate is high then the range of crystallisation of the β polymorph is reached and hence the system will be comprised of the β progesterone polymorph (Legendre, et. al., 2003).

Similar results to the work by Legendre et. al. (Legendre, et. al., 2003) were observed in work undertaken by Duclos et. al. (Duclos, et. al., 1991) who found that progesterone cooled in the presence of a PEG 6000 co-melt at -1 ^{o}C/minute, the α polymorph was observed, whereas progesterone quenched cooled (cooling at > -10 ^{o}C/minute) in the presence of a saccharose distearate co-melt, the β progesterone polymorph was observed.

1.4 Polydimethylsiloxane (silicone rubber)

Silicone rubber (polydimethylsiloxane) is used as the matrix in the CIDR insert. The polydimethylsiloxane repeat unit is shown in Figure 1.13 and consists of a silicone oxygen chain, which has two methyl groups attached to the silicon atom. The mass of the repeat unit is 74 Da.

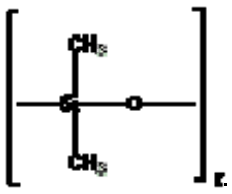


Figure 1.13 The polydimethylsiloxane repeat unit.

Silicone rubber has been used since 1943 when silicone rubber was used in insulating sparkplugs in aircraft (Braley, 1968), and in 1953 the first production of

silicone for use in medical applications occurred (Braley, 1968). Medical grade silicone rubber is a polymer that contains no toxic components that would result in inflammatory reactions in tissue (Braley, 1968). Silicone has a low degree of toxicity (Kern, et. al., 1949), however many silicone rubbers contain additives that are not suitable for the human body such as plasticizers, and catalysts which can make some silicone rubber products unsuitable for pharmaceutical use (Braley, 1968). Monomeric precursors of silicone can be toxic such as tetraethyl-ortho-silicate (Kasper, et. al., 1937) and alkylchlorosilanes (Rowe, et. al., 1948). Silicone fluids however are of very low toxicity, even after feeding to rats for one month (Rowe, et. al., 1948). However the Dow Corning MSDS's of parts A and B Dow Corning Q7-4840 liquid silicone (used in the manufacture of CIDR inserts) states that the liquid silicone of Dow Corning Silastic® Q7-4840 is not classified as hazardous by Worksafe Australia (Dow Corning Australia Pty Ltd., 2004a) (Dow Corning Australia Pty Ltd., 2004b).

In chemical terms, a silicone rubber consists of (Rochow, 1987) a high molecular weight methyl silicone gum (2,000,000 to 5,000,000 $gmol^{-1}$), a reinforcing filler to give strength and a vulcanising agent (to provide crosslinking upon cure). Curing can occur through a number of mechanisms (Rochow, 1987), such as a peroxide catalyst like benzoyl peroxide (as illustrated in Figure 1.14), or the reaction of Si-H groups with $Si\text{-}CH{=}CH_3$ (added to the methyl silicone chain) to form a $SiCH_2CH_2Si$ crosslink between the silicone chains. Reinforcing silica (SiO_2) is used, which has similar bond distances and angles to the silicone chain allowing the silicone chain to bind to the surface, because the surface has been rendered anhydrous by the fuming process (Rochow, 1987). Fuming silica replaces the hydrophilic silanol groups (Si-OH) on the surface of the silica, by converting these groups to hydrophobic organosilicon groups (for example $Si\text{-}O\text{-}Si(CH_3)_3$)) (Cochrane & Lin, 1985). The rigid particles of silica provide strength to the silicone polymer (Rochow, 1987). The manufacture of silicone rubber is outlined in Figure 1.14. Work by Mazan et. al, (Mazan, et. al., 1992) found that the diffusion coefficient for progesterone in polydimethylsiloxane decreases with increasing silica loading.

Silica Rock is heated in a furnace to make silicon.

$$SiO_2 + C \rightarrow Si + 2CO$$

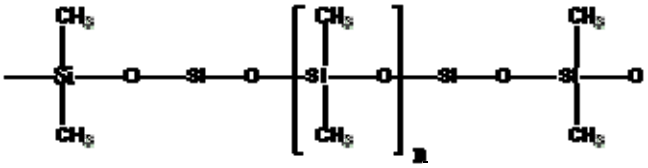
Silicon is reacted with methyl chloride.

$$Si + CH_3Cl \rightarrow SiCl(CH_3)_2$$

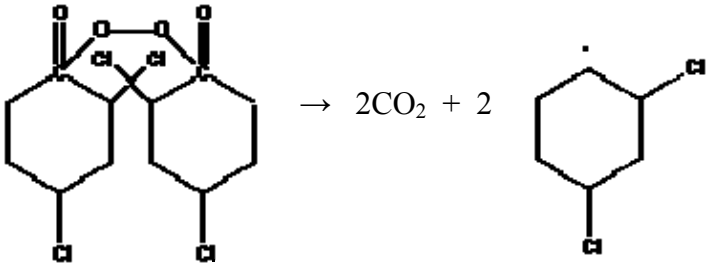
Dimethyl dichlorosilane is reacted with water.

$$SiCl_2(CH_3)_2 + H_2O \rightarrow Si(OH)_2(CH)_3 + 2HCl$$

Dimethyl dihydrosilane will immediately condense to a polymer, as it is extremely unstable.



Heat curing with radical agents such as dicholorbenoyl peroxide can create silicone rubbers.



Silicone crosslinking occurs as the free radical extracts H· from a silicone methyl group and then the two radicals combine resulting in the polymer chains linking.

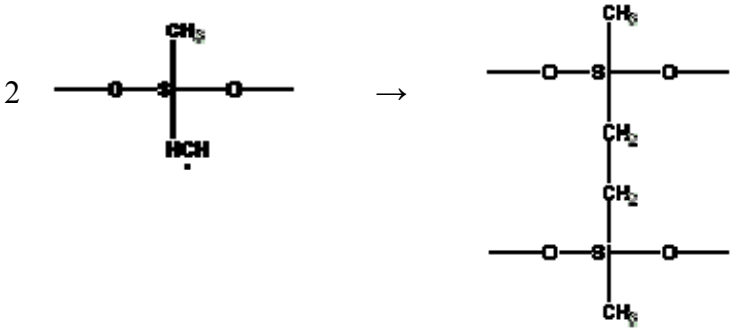


Figure 1.14 The synthesis of silicone polymer (Colas, 2001) (Rochow, 1987) (Braley, 1968) (Braley, 1970).

1.4.1 Solubility of progesterone in silicone rubber

Work by Bernabei et. al. (Bernabei et. al., 1982) analysed the solubility of different polymorphic forms of progesterone in silicone rubber (Silastic 382). Bernabei et. al. (Bernabei et. al., 1982) reported the solubility of progesterone in silicone rubber at 37 ^{o}C as 0.639 mg/mL for the α polymorphic form and 0.789 mg/mL for the β polymorphic form. They also reported that the solubility at melting point of progesterone was 6.35 mg/mL for the α polymorphic form and 7.77 mg/mL for the β polymorphic form. Bernabie et. al, (Bernabei et. al., 1982) also found that the polymorphs were stable at room temperature, in water at 37 ^{o}C and during polymerisation.

Analysis of progesterone solubility in Elastosil 3003/40 made by Wacker (Taghizadeh, et. al., 2003) reported a solubility of 0.53 mg/mL at 37 ^{o}C. This does show some difference from the values obtained by Bernabei et. al. (Bernabei et. al., 1982), however this would possibly be due to the differences in the silicone rubber from different manufacturers.

1.4.2 Silastic® Q7-4840 (Dow Corning Q7-4840)

Silastic® Q7-4840 made by Dow Corning is the silicone currently used in the manufacture of most CIDR inserts. The material is supplied in two parts A and B that are mixed prior to use. Once the parts are mixed the material will remain useable for 24 hours at room temperature (Dow Corning, 1990). Cure is achieved through a platinum catalysed reaction (Dow Corning, 2005). A photo of part B showing the general appearance of the starting material is shown in Figure 1.15. Both parts are identical in appearance.

Figure 1.15 Dow Corning Q7-4840 liquid silicone (part B). The part A is identical in appearance.

Dow Corning Q7-4840 silicone cures by application of heat, raising the temperature to 140 ^{o}C quickly results in a tough elestomeric (rubber like substance) material (Dow Corning, 1990). Once cured, the resulting rubber consists of a crosslinked methyl-vinyl and dimethyl siloxane copolymers reinforced with silica (contained in the two parts of A and B) (Dow Corning, 2005). After cure no peroxides or peroxide by-products are present and volatile compounds evolved post cure are water vapour and polydimethylsiloxane fluids (low molecular weight) (Dow Corning, 2005). Cure may be inhibited by trace amounts of nitrogen oxide, sulphur, organotin, and amines compounds as well as carbon monoxide (Dow Corning, 1990). Dow Corning Silastic® Q7-4840 is manufactured to cGMP requirements (Dow Corning, 2005).

The composition of Dow Corning Q7-4840 is outlined in US patent 4,162,243 (Dow Corning, 1990), which is summarised in Table 1.4.

Table 1.4 Composition of Dow Corning Silastic® Q7-4840 as specified by US Patent 4,162,243 (Lee et. al., 1979).		
Compound	**Details**	**Parts by weight**
Triorganosiloxy endblocked polydimethylsiloxane fluid.	Triorganosiloxy units consist of dimethylvinylsiloxy and methylphenylvinylsiloxy. Major molecular weight of the fluid ranges from 68,000 to 135,000 $gmol^{-1}$.	100
Reinforcing amorphous silica.	Surface area greater than 100 m^2g^{-1}, and the surface of the silica has silicon atoms which are bonded to organosiloxane groups.	20 to 60
Organohydrogensiloxane fluid.	Added in such amounts to provide from one to three silicone bonded hydrogen atoms for every vinyl radical from the triorganosiloxy endblocked polydimethylsiloxane fluid and amorphous silica.	-
Platinum catalyst.	5 to 50 parts by weight for every million parts of polydimethylsiloxane fluid.	-
3,5 dimethyl-1-hexyn-3-ol.	Inhibitor for the platinum catalyst.	Varies depending on the shelf life.

Lee et. al. (Lee et. al., 1979) notes that the platinum catalyst along with the organohydrogensiloxane fluid and the polydimethylsiloxane fluid will cure at room temperature. To prevent the action of the catalyst Lee et. al. (Lee et. al., 1979) recommends the use of an inhibitor. Dow Corning advises that Dow Corning Q7-4840 has an 18 month usable life, if stored unopened in ambient conditions (Dow Corning, 2005).

The two parts of Dow Corning Q7-4840 silicone both contain the silica and the polydimethylsiloxane fluid (Lee et. al., 1979). One part would have the platinum catalyst, while the other would have the organohydrogensiloxane (Lee et. al., 1979). It should be stressed that the patent will only provide a general guide to the composition of Dow Corning Q7-4840, as is common for companies to write patents to give broad coverage and preserve commercial secrets.

1.5 Studies into the cause of mottling and secondary blooming

In order to determine a likely cause of mottling and secondary blooming a large number of studies have been undertaken by a number of researchers, of which the most notable are Reardon, and Wong. These studies analysed factors including progesterone polymorphism, the differences between Dow Corning Q7-4840 silicone and the alternative supplier silicone, variations in manufacturing conditions, and variations in the cooling rate of the CIDR insert after cure.

While some of these studies were conducted on CIDR inserts a number of studies used a silicone/progesterone mix containing a black dye, which was then cured in an oven and observed over time to monitor secondary blooming (these samples are called slabs). The black dye facilitated visualisation of secondary blooming.

1.5.1 Alternative supplier silicone

An alternative supplier silicone feedstock (Wong, 2003e) (Wong, 2003a) (Reardon, 2004b) has been found to not exhibit secondary blooming and mottling. The name of this alternative silicone supplier cannot be revealed in this thesis for commercial confidentiality reasons, and will henceforth be referred to as the ‘alternative supplier silicone’. The alternative supplier silicone consists of two parts of heat cured rubber, like the Dow Corning Q7-4840 silicone.

Work by Reardon (Reardon, 2004b) found that both the current and alternative supplier silicones formed an initial surface layer of progesterone after cure (initial blooming). However the rate of formation of this layer for the alternative supplier silicone was found to be approximately four times less than Dow Corning

Q7-4840 silicone (Reardon, 2004b). Furthermore the alternative supplier silicone did not exhibit secondary mottling, whereas the Dow Corning Q7-4840 silicone started exhibiting this after only an hour (Reardon, 2004b). Reardon's work also found that part B of the Dow Corning liquid silicone was the part that was causing the secondary blooming (Reardon, 2004b).

Work by Reardon (Reardon, 2004a) comparing drug release rates of CIDR inserts made with either the alternative supplier silicone or Dow Corning Q7-4840 silicone, found that while both types of silicone were within drug release rate specifications, the alternative supplier silicone appears to have a slightly higher drug release rate. Reardon hypothesised that this could have been caused by lower levels of surface progesterone on the CIDR inserts, or different cure times between the different types of silicone.

SEM analysis (Bourke, 2004) on a slab made with alternative supplier silicone showed after 14 days that there were some small crystal structures present on the surface, and bubbles in the silicone sample were unpopulated with crystals. This contrasted with Dow Corning Q7-4840 samples that had crystal deposits on the CIDR insert surface and air bubbles inside the sample filled with crystals (Bourke, 2004).

1.5.2 Analysis of compositional differences between Dow Corning Q7-4840 and the alternative supplier silicone

It is known that the alternative supplier silicone does not cause secondary blooming and mottling (Reardon, 2004b), whereas the Dow Corning Q7-4840 silicone does exhibit mottling and secondary blooming (Reardon, 2004b). A number of studies have been undertaken to analyse the different silicones with the aim of finding differences in the feedstocks, which may have an effect on secondary blooming and mottling.

Work undertaken by Dow Corning (Siang, 2003) on low molecular weight polymers by GC analysis on a sample of the alternative supplier silicone and the Dow Corning Q7-4840 silicone found that there was little difference in the

concentration of low molecular weight polymers between the samples. It was however noted by Siang (Siang, 2003) that there was little historical data on the level of low molecular weight polymers in Dow Corning batches and hence it was too early to determine if this was a cause of mottling and secondary blooming.

X-ray Fluorescence (Wong, 2003f) was undertaken on both part A and part B from the Dow Corning Q7-4840 silicone and the alternative supplier silicone, by SpectraChem Analytical Ltd. in Lower Hutt, New Zealand. A summary of these results is shown in Table 1.5. It was found that the alternative supplier silicone had a lower level of platinum present. Also the alternative supplier's silicone formulation of part A had sodium, potassium and calcium present with a combined total of 0.034 % w/w and that the Dow Corning part B had nickel present at 0.002 % w/w.

Table 1.5 Elemental analysis of both parts A and B for both Dow Corning Q7-4840 and alternative supplier silicones. (LLD = 0.001%) (Analysis undertaken by SpectraChem Analytical Ltd. in Lower Hutt, New Zealand) (Wong, 2003f).

	Silicon (% w/w)	**Copper (% w/w)**	**Platinum (% w/w)**
Alternative supplier silicone part A.	41.71	0.002	0.003
Alternative supplier silicone part B.	42.01	0.001	0.002
Dow Corning Q7-4840 part A.	42.12	0.002	0.007
Dow Corning Q7-4840 part B.	44.69	0.002	0.007

Headspace analysis has been undertaken (NS432, 2003) on both parts of the alternative supplier's silicone and two different batches of the Dow Corning, one from a silicone batch that mottled badly and another from a silicone batch that mottled to a lesser degree. Samples were tested according to purge and trap GCMS USEPA method 8260 at Hill Laboratories. The differences of note are

shown in Table 1.6. These show that the alternative supplier silicone has a high level of 2-butanone and toluene compared to the Dow Corning silicone.

Table 1.6 Results from purge and trap on alternative supplier silicone and Dow Corning Q7-4840 silicone. (NS432, 2003) (Wong, 2003a).

(ppm)	**Dow Corning batch 0001129367**		**Dow Corning batch 0001053180**		**Alternative supplier silicone**	
	Part A	**Part B**	**Part A**	**Part B**	**Part A**	**Part B**
2-butanone	<1	<1	<1	12	13	9
Toluene	0.3	1.1	<0.2	2.8	74.7	69.1

In order to assess the effect of 2-butanone and toluene on secondary blooming, some spike studies were undertaken by Wong (Wong, 2003a). A spike study is a study where an amount of material not normally present in an uncured slab mix, is added to determine if there is any effect on secondary blooming and mottling in the resulting slab. Initial observations (Wong, 2003a) of the slabs made with a spike (one to ten drops per slab) of 2-butanone or toluene, showed that toluene spikes did not have any effect in secondary blooming, while the 2-butanone reduced the secondary blooming (at higher concentrations only).

^{29}Si NMR analysis (Bourke, 2004) has been undertaken on liquid silicone (parts A and B) dissolved in dichloromethane, using silicone from Dow Corning Q7-4840 liquid silicone and the alternative supplier liquid silicone. Bourke (Bourke, 2004) found that both parts of silicone for both the alternative supplier silicone and Dow Corning Q7-4840 silicone had similar spectra with a single peak in the region of -20.01 to -19.98 ppm, which corresponds to silicon compounds of the R_2SiO_2 type.

1.5.3 Process studies

A range of studies have been undertaken into exploring the effect of changes in the manufacturing environment with respect to secondary blooming and mottling. A minor change in manufacturing conditions is far easier to implement than the

effort required introducing new raw materials into an established and licensed production process.

Analysis was undertaken (Wong, 2003e) to determine if there was a trend between the month of manufacture and the % mottling. Results (Wong, 2003e) showed that there was little relationship (see Figure 1.16) between the month of manufacture except for the months between August 2000 and July 2001 that also corresponded with a period of poor manufacturing performance. It was concluded (Wong, 2003e) that mottling was "an inherent part of the current product and process".

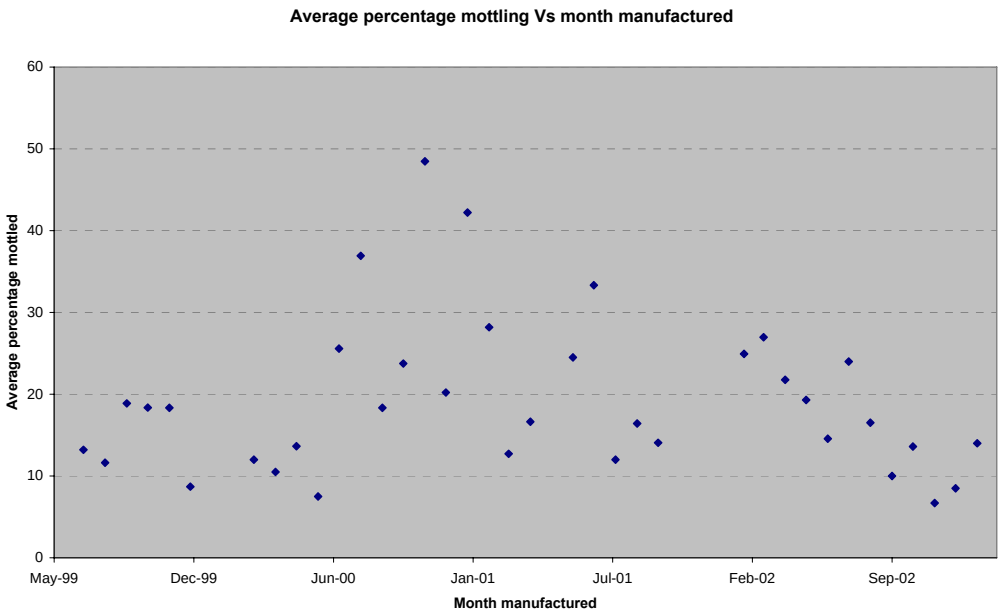


Figure 1.16 Average % mottling versus the month of CIDR manufacture in reserve samples. (Wong, 2003e).

Work by Reardon (Reardon, 2003) also investigated the effect of manufacturing cleaners of Pegasol (3440) and a bench cleaner made of ethanol, water and glycerol on the % mottling, and concluded that mottling was not caused by the cleaning products used.

Work undertaken by Rathbone and Ogle (Rathbone & Ogle, 2000) found that a higher cure temperature (analysed between 130 and 210 ^{o}C) resulted in an increase in the amount of surface progesterone. They also discovered that a cure

temperature of 120 ^{o}C using Wacker silicone was completely eliminated mottling and secondary blooming (Rathbone, & Ogle, 2000). At this temperature the progesterone would not have melted as the β progesterone polymorph melts at 123 ^{o}C (Kuhnert-Brandstätter et. al., 1965). However manufacturing at 120 ^{o}C would mean that cure times for the CIDR insert would significantly increase and this would increase the manufacturing cost of a CIDR insert, because of increased production time per device.

Freezing is commonly used to increase the physical stability and shelf life for pharmaceutical products. Freezing (Wong, 2003e) (Reardon, 2003a) has been found to retard the formation of secondary blooming and mottling. However when the CIDR insert is returned to room temperature mottling resumes.

Vacuum packing of CIDR 1380 inserts with silica (to absorb water) resulted in a decrease in average mottling by 9 % (Wong, 2003d). When the experiment was repeated, with dry and non-annealed spines, it was found that the amount of mottling was even less than when the CIDR inserts that had been vacuum packed (Wong, 2003k). The packing isolated the CIDR inserts from the atmospheric conditions. Wong noted that part of the effect could be due to the CIDR inserts being packed before they cooled down.

Work by Rathbone and Ogle (Rathbone & Ogle, 2000) found that the temperature and humidity conditions of storage did not appear to have a strong influence on the amount of progesterone microcrystalline powder on the CIDR insert surface, however they noted that it is unknown if temperature and humidity conditions will have any effect on progesterone crystals and flakes on the surface of a CIDR insert. Rathbone and Ogle (Rathbone & Ogle, 2000) also found that mottling increases with temperature and humidity and this result is in agreement with the work by Wong (Wong, 2003d).

In manufacturing, progesterone and liquid silicone (either part A or part B) are mixed together then placed under vacuum to remove air bubbles in the mixed progesterone and silicone. Samples that have been evacuated (Wong, 2003) appear to mottle less, however there was seen a need to conduct further work into

this area. Bourke (Bourke, 2004) noted that silicone slabs contained variable amounts of air bubbles compared with CIDR inserts. These air bubbles as noted earlier (Section 1.5.1) were filled with crystals. Bourke (Bourke, 2004) speculated that either the bubbles helped reduce mottling as extensive crystallisation within the silicone would impede the process or conversely mottling would be increased by gas dissolved in the matrix lifting the progesterone to the surface of the matrix.

Wong (Wong, 2002c) investigated the effects from centrifuging silicone to remove air bubbles from mixed silicone. It was found that drug release decreased when the silicone was centrifuged to remove the air bubbles. The samples did not exhibit any mottling. Analysis of the mass of surface progesterone released after one hour in a drug release test in Wong's (Wong, 2002c) work found that there was a higher mass of progesterone released for samples made with the non-centrifuged silicone compared with the samples made with the centrifugation silicone. The result is interesting as it is known that high secondary blooming leads to low drug release rates (Wong, 2003e), but Wong's results (Wong, 2002c) show the opposite result.

1.5.4 Raw material analysis

The manufacture of CIDR inserts requires many tonnes of progesterone and silicone every year. The raw material is provided as a number of batches. Inter batch differences between the batches of either progesterone or silicone could be a cause of secondary blooming and mottling and hence this was also an area of investigation by previous researchers.

Acetone (a residue from progesterone re-crystallisation) is known to inhibit curing of silicone. A number of studies have been undertaken into analysing the effects of acetone on mottling and secondary blooming by (Wong, 2003e) (Rades & McFetridge, 2002). While slabs made with added acetone do exhibit more mottling compared to control slabs (Wong, 2003e), current research has not found any consistent relationship between acetone residues with mottling and secondary blooming.

Analysis (Wong, 2003e) of the % mottling versus various batches of progesterone found that there were significant inter-batch and intra-batch variation (see Figure 1.17). Silicone batches exhibit a range of % mottling, with some batches exhibiting more mottling compared with others (Wong, 2003e).

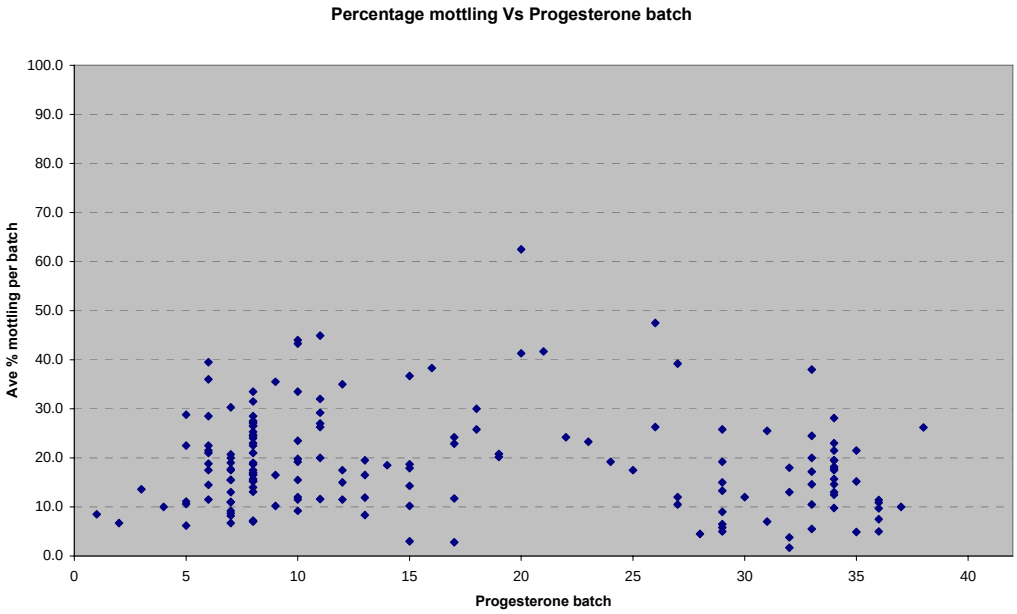


Figure 1.17 Average % mottling per CIDR insert batch versus progesterone batch from reserve samples (Wong, 2003e).

Work by Reardon (Reardon, 2004c) noted that slabs made with non-micronized progesterone had a grainy surface and that after four days there is secondary blooming on 20 % of the surface of the slabs. Historically DEC Manufacturing (Wong, 2003e) has made CIDR inserts with non-micronized progesterone. Larger particle size has been found to reduce secondary blooming. Unfortunately larger particle size has also been found to reduce drug release rate (Wong, 2003e).

1.5.5 Studies into progesterone polymorphism in the CIDR insert

A range of studies by has been undertaken into analysis of the polymorphism of progesterone in the silicone matrix. The aim of these studies was to determine if the polymorphic change of progesterone from the β polymorph to the thermodynamically stable α polymorph (Wang, et. al., 2000) is a root cause of the observed secondary blooming and mottling on the CIDR insert.

Bourke (Bourke, 2004) analysed slabs made with Dow Corning Q7-4840 silicone and the alternative supplier silicone using a range of techniques (ATR FTIR and XRD). It was found using ATR FTIR that the alternative supplier silicone showed the least amount of change over time and no peaks from progesterone were observed. Bourke noted that it was not possible to differentiate between the progesterone polymorphs using ATR FTIR. XRD analysis (Bourke, 2004) on slabs made with Dow Corning Q7-4840 silicone showed two broad humps (amorphous material from the silicone matrix) along with peaks patterns that predominantly resembled the β progesterone polymorph.

Research by Rades & McFetridge (Rades & McFetridge, 2003) on crystals scraped off CIDR inserts after storage were found to be the α progesterone polymorph (using XRD and DSC), which is the same polymorph as the progesterone feedstock. Rades and McFetridge analysed shavings from mottled and non-mottled sections of CIDR inserts with XRD and DSC. XRD detected the β progesterone polymorph in both mottled and non-mottled samples (Rades & McFetridge, 2003). However DSC analysis showed that both the α and β polymorphs were present in both the non-mottled and mottled CIDR inserts in contrast to other XRD results in the same study, which was noted by Rades and McFetridge (Rades & McFetridge, 2003).

Analysis of progesterone polymorphism (Rades & McFetridge, 2003) was undertaken on liquid silicone mixed with progesterone in a DSC on samples of a 1:1 mix of parts A and B and on each part individually (mixed with progesterone). The progesterone was in the α polymorph in all samples analysed. Rades and McFetridge (Rades & McFetridge, 2003) also found that directly after curing progesterone was in amorphous form. Analysis of heat flow during curing of the silicone led to the conclusion that DSC could not reliably determine if there were differences in curing behaviour, as the thermograms of part A, part B and the 1:1 mix of parts A and B were essentially the same (Rades & McFetridge, 2003).

1.5.6 Studies into the slow cooling of the CIDR insert

A number of studies have been undertaken into the effect of controlling the cooling rate of CIDR inserts. Controlled cooling has been found to have a significant effect on the amount of mottling on a CIDR insert. Work by Rathbone and Ogle (Rathbone and Ogle, 2000) has shown that curing at 120 ^{o}C (below the melting point of both the α and β polymorphs of progesterone (Kuhnert-Brandstätter et. al., 1965)) eliminated mottling and secondary blooming. Rathbone and Ogle also discovered that lower cure temperatures resulted in less secondary blooming but that curing at 130 ^{o}C did not totally eliminate secondary blooming and mottling.

A large number of studies into controlled cooling were undertaken by Reardon (Reardon, 2003a) to determine if controlling the cooling rate of a CIDR 1380 insert had any effect on mottling. Reardon (Reardon, 2003a) found that there was a decrease in % mottling with some of his cooling methods, however some CIDR inserts, which had a low level of mottling without slow cooling were found to exhibit an increase due to Reardon's cooling method. It was also found that slow cooling could increase the degradation of progesterone due to increased heat exposure during the process of slow cooling (Burggraaf, 2006b).

1.6 Aims of thesis

As discussed previously secondary blooming and mottling is of concern in terms of an 'in vitro' drug release rate and aesthetics for DEC Manufacturing, but does not appear to have any effect on the 'in vivo' effectiveness of the CIDR insert. DEC Manufacturing saw a need to have a dedicated study to gain an improved understanding of secondary blooming and mottling in CIDR inserts in order to make sense of the hitherto fragmented and piecemeal work done by the earlier researchers. The main objective of the research was to find a means of definitively minimising mottling and secondary blooming. Any remedy to the current problem must also be cheap and practical enough so that it could be implemented without any significant impact on the current manufacturing process.

2.0 Theory behind specialist instrumental techniques used in the thesis

2.1 Ultra Violet (UV) spectrophotometry

UV spectrophotometry involves analysing the absorption of radiation by UV chromophores in molecules at a specific wavelength λ (Progesterone absorbs at 239 nm and hence this is its analytical wavelength). The adsorption by a sample will be proportional to concentration in accordance with Beer's law (Equation 2.1) where c is concentration, A is absorbance, ε is the molar absorptivity and d is the cell thickness.

$$A = c\varepsilon d \qquad (2.1)$$

Beer's law is true so long as the solution is reasonably dilute, ($c \leq 0.1$ mol L^{-1}) as at higher concentrations, the plot of absorbance versus concentration deviates from non-linearity due to changes in ε (Kellner, et. al., 1998).

2.2 X-ray Diffraction (XRD)

Crystal lattices provide a diffraction grating for X-rays. In 1913 Bragg (Kellner, et. al., 1998) demonstrated that the when parallel diffracting waves (1', 2', 3' etc.) all have a path difference that is λ (2π) to the waves of the nearest neighbours, constructive interference will occur. The Bragg Equation is:

$$\lambda = 2\, d_{hkl} \sin\theta \qquad (2.2)$$

Where λ is the wavelength, θ is the Bragg angle and d_{hkl} is the interplanar difference as observed in Figure 2.1.

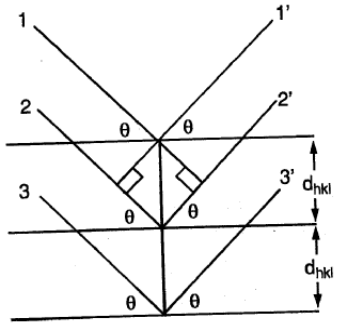


Figure 2.1. The Bragg Equation (Kellner et. al., 1998).

XRD can be used to analyse any crystalline sample, but amorphous samples give no diffractogram. When XRD is undertaken on powders (Kellner, et. al., 1998) all possible random orientations of the crystals should be exposed and hence upon scanning all possible peaks defined by Equation 2.2 are possible. In order to see all these peaks it is preferable that the analyte be a finely dispersed powder so allowing all possible crystal orientations. If this is not the case then the intensities of some peaks will be enhanced relative to peaks reported in reference XRD, due to the phenomena of preferential orientation of crystallites.

2.3 Electrospray Mass Spectrometry (ESMS)

ESMS is used to analyse positive and negatively charged ions from aqueous or mixed aqueous-organic solvents, with an analysable mass range from 100 to 50,000 Da. ESMS is used for molecules that are not amenable to vacuum phase EIMS (Electron Impact Mass Spectrometry). A schematic diagram of an ESMS interface is shown in Figure 2.2.

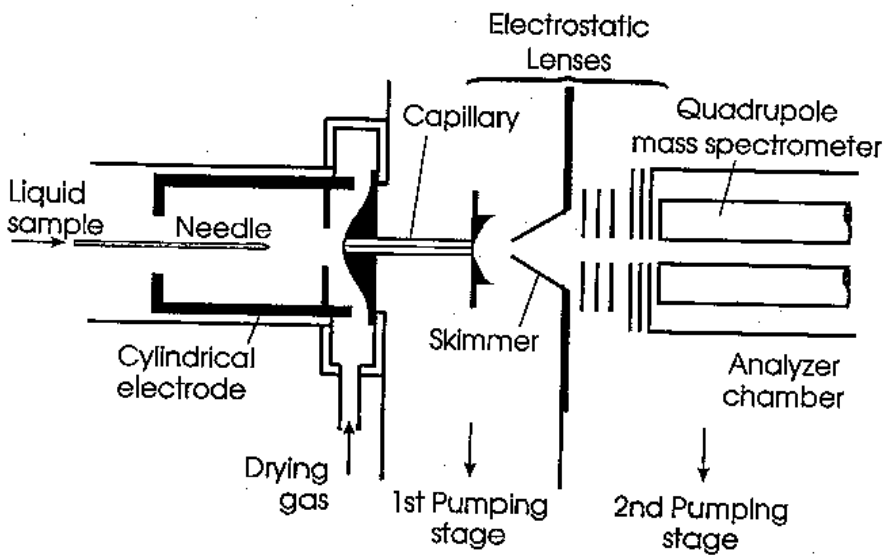


Figure 2.2 A schematic setup for an electrospray interface (Kellner et. al., 1998).

To ionize the analyte, the solution containing the analyte is sprayed through a fine capillary (Figure 2.2), which is maintained at a high voltage, into a chamber that is at atmospheric pressure. Simultaneously the sample is then dried by a stream of gas (eg. nitrogen) to give naked ions, or ion clusters (eg. $[M + Na]^+$, $[2M + Na]^+$ etc.) in the gas phase. The formation of these ions is thought either to be from a columbic explosion, or from an ion evaporation from the gas phase solvent (Henderson & McIndoe, 2003). In order to fragment the ions a cone voltage must be applied. The degree of fragmentation increases with an increasing cone voltage.

In ESMS, ionisation occurs in a solution under ambient pressures rather than in a vacuum, hence there is an increased potential of contamination occurring. It is well known that in ESMS, as opposed to EIMS there is a tendency for species from previous analyses (both organic molecules and ionic species such as KCl, LiCl etc.) to be temporarily adsorbed onto the lines in the ESMS system. The adsorption time can last from hours to days. In order to reliably differentiate artefact ions (contaminants) from genuine sample ions, it is necessary to analyse samples on different occasions. A quadrupole mass filter is used to filter ions by their m/z value before detection.

An ESMS spectra consists of ion intensities (on the y axis) versus the m/z value (x on the axis). For a peak to be observed in an ESMS spectra, the analyte must either be an ion, or in the case of neutral molecules bound to an ion such as H^+, Ag^+, or Na^+, to give for example $[M + H]^+$ or $[M + Ag]^+$ ions. Cluster ions are also observed, for example $[2M + Na]^+$, $[3M + Na]^+$ ions.

2.4 Gas Chromatography Mass Spectrometry (GCMS)

GCMS is widely used as a qualitative and quantitative analytical tool to analyse molecules in the gas phase. The sample is injected as either a liquid or a gas. Following injection the sample is volatilised (if a liquid) and travels through the column. A capillary column is used to separate different molecules in a gas, which are observed as peaks by the detector. The column is placed in an oven and during an experiment the oven temperature can be increased in order to volatilise liquid compounds.

GCMS can only detect molecules that are in the gas phase, hence non-volatile compounds are not detectable with GCMS. Electron impact is used to fragment molecules, and the fragments are able to provide structural information on the molecule because different molecules fragment in different ways. The detector consists of a quadropole mass filter, which filters ions by their mass to charge ratio (m/z).

A GCMS can operate in two modes, specific ion mode, in which case it detects only the intensity of ions at a particular m/z values, resulting in high sensitivity or in total ion mode where the mass filter scans for a wider range of ions. In this case the detection limit decreases, but the analyst is able to detect, all peaks within the specific ion range. When analysis is undertaken in total ion mode, the analyst is provided with a total ion chromatogram (TIC) that consists of the sum total of all peaks in the m/z detection range versus peak retention time.

2.5 Scanning Electron Microscopy (SEM)

SEM involves rastering the surface of a conductive sample with a beam of electrons in order to obtain (Fleger et. al., 1993) a high-resolution image of the surface of a sample, with magnification between 10 to 150,000x (Fleger et. al., 1993).

Electrons are generated in the microscope using a filament such as LaB_6 or tungsten (Kellner, et. al., 1998), then the electrons are accelerated to an energy range of 1 to 50 keV (Kellner, et. al., 1998). Electrostatic lenses are used to focus the beam of electrons onto the sample.

Non-conductive samples (Flegler, et. al., 1993) require a plasma applied conductive coating (10 to 30 nm thick) to allow the sample to conduct and hence prevent formation of a negative charge. Common coatings include gold and carbon (Fleger, et. al., 1993).

The secondary electrons are used to form the image. Secondary electrons (low energy) are generated by the interaction of the electron beam and inelastic interactions with the sample (Fleger et. al., 1993). Changes in the surface topography (Figure 2.3) of the sample mean that flat areas have less secondary electrons leaving the surface, but a section of sample that is at an angle to the electron beam, will release more secondary electrons, hence giving information on the surface topography (Fleger et. al., 1993). These secondary electrons will be detected and so form the image. Secondary electrons due to their low energy (10 eV (Kellner, et. al., 1998)) are unable to escape the sample from any significant depth and hence the imaging technique is surface specific only (Fleger et. al., 1993). In order to prevent inelastic collisions of secondary electrons with gas molecules, the instrument operates in a high vacuum.

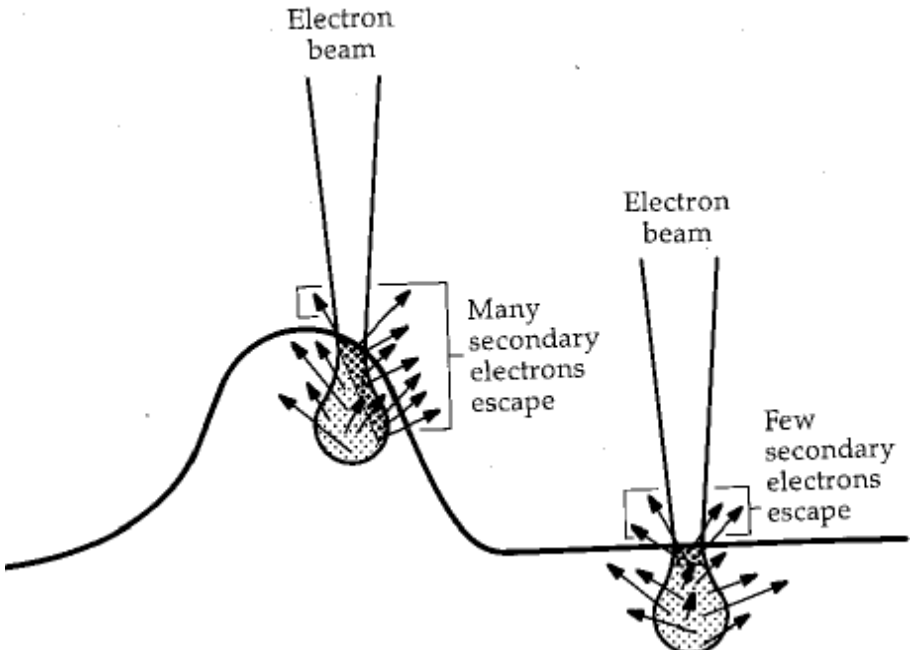


Figure 2.3 The interaction of an electron beam with a sample and how the secondary electrons escape depends on the surface topography. (Flegler et. al., 1993).

2.6 Differential Scanning Calorimetry (DSC)

The DSC setup consists of two identical pans, one containing a sample and the other being empty (used as a reference). A DSC setup is shown in Figure 2.4. Throughout a DSC experiment there is no temperature difference between the two pans. A DSC experiment measures the heat flow into the sample compared to the reference pan throughout the duration of the experiment. During an experiment the DSC follows a temperature program, which controls the rate of heating, the rate of cooling and the isothermal (hold at constant temperature) periods of the temperature program. The DSC technique is used to analyse materials for properties including the glass transition temperature, melting points, and crystallisation temperatures.

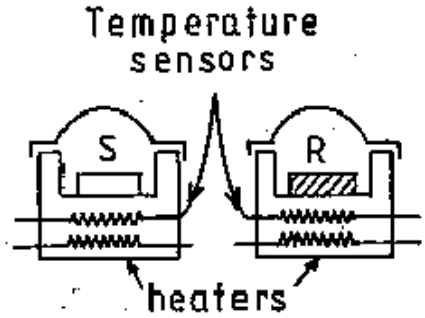


Figure 2.4 A DSC set-up. The sample will go in one pan while the other pan will be left empty as a reference. (Kellner et. al., 1998).

2.7 Rheology

Rheology is defined by Chambers Scientific and Technological Dictionary as, "the science of the flow of matter" (Chambers, 1991). Viscosity is the resistance by a fluid to shear forces and therefore flow (Chambers, 1991). At the same temperature a viscous fluid (e.g. silicone oil) will flow less easily compared to a fluid with lower viscosity (e.g. water).

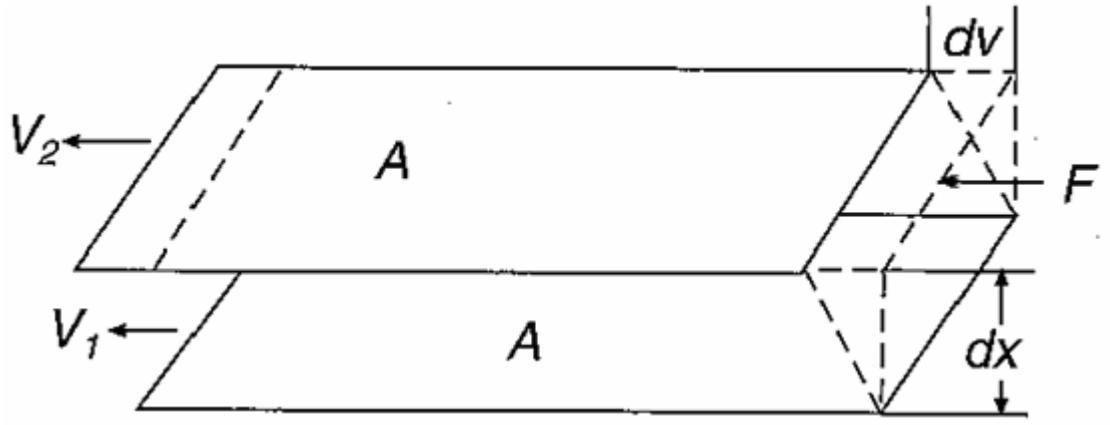


Figure 2.5 Neuton fluid model for viscosity showing the two theoretical plates. (Brookfield).

Newton defined viscosity using the fluid model to relate the ratio of shear stress and shear rate (Brookfield). Newton's model assumed two parallel plates of fluid with the same area moving at different velocities, and with a distance of separation of dx (Brookfield). Newton made the assumption that the force needed to maintain the difference in velocity was proportional to the velocity gradient

(difference in velocity through the fluid) (Brookfield). This is expressed in Equation 2.3 as:

$$\frac{F}{A} = \eta \frac{dv}{dx} \tag{2.3}$$

Where η is the viscosity, A is the areas of the fluid, F is the force applied on the plate (in dynes), and dv and dx are defined in Figure 2.5. The shear stress (τ) is defined in Equation 2.4 and τ has units of dynes/cm^2 or N/cm^2 (Brookfield).

$$\tau = \frac{F}{A} \tag{2.4}$$

The velocity gradient is defined in Equation 2.5 and γ has units of seconds^{-1} (Brookfield).

$$\gamma = \frac{dv}{dx} \tag{2.5}$$

η is defined by Newton as the ratio of the stress to flow gradient shown in Equation 2.6 (Brookfield):

$$\eta = \frac{\tau}{\gamma} \tag{2.6}$$

There is a range of units to describe the viscosity (Thibodeau, 2004) (Brookfield), outlined in Equations 2.7 to 2.9:

$$1 \text{ poise} = 1 \frac{\text{dyne} \,/\, \text{cm}^2}{\text{seconds}^{-1}} \tag{2.7}$$

1 poise = 100 centipoise (2.8)

1 Pascal second = 1000 centipoise (2.9)

The yield stress of a sample is the stress required to initiate flow (Brookfield), below which the sample will deform proportionally with stress and above the yield stress there would be unlimited deformation (Schramm, 2000). The stress is related to the deformation rate, with the viscosity being the relating factor (Schramm, 2000).

An R/S Soft Solids Tester as made by Brookfield Engineering is able to measure samples such as slurries, gels and suspensions (Brookfield Engineering, 2005). A R/S Soft Solids Tester is shown in Figure 2.6. The vane in the instrument can

be immersed into the sample while causing minimal disruption to the sample (Brookfield).

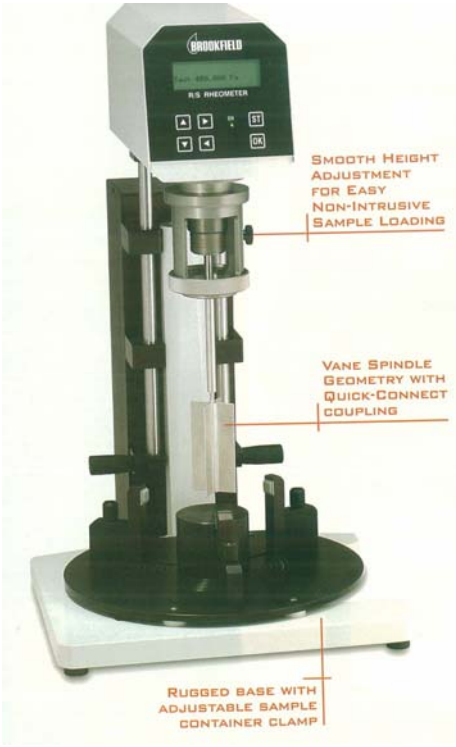


Figure 2.6 A R/S Brookfield Soft Solids Tester. A four winged vane is placed down into the sample as shown in Figure 2.7. (Brookfield, 2004).

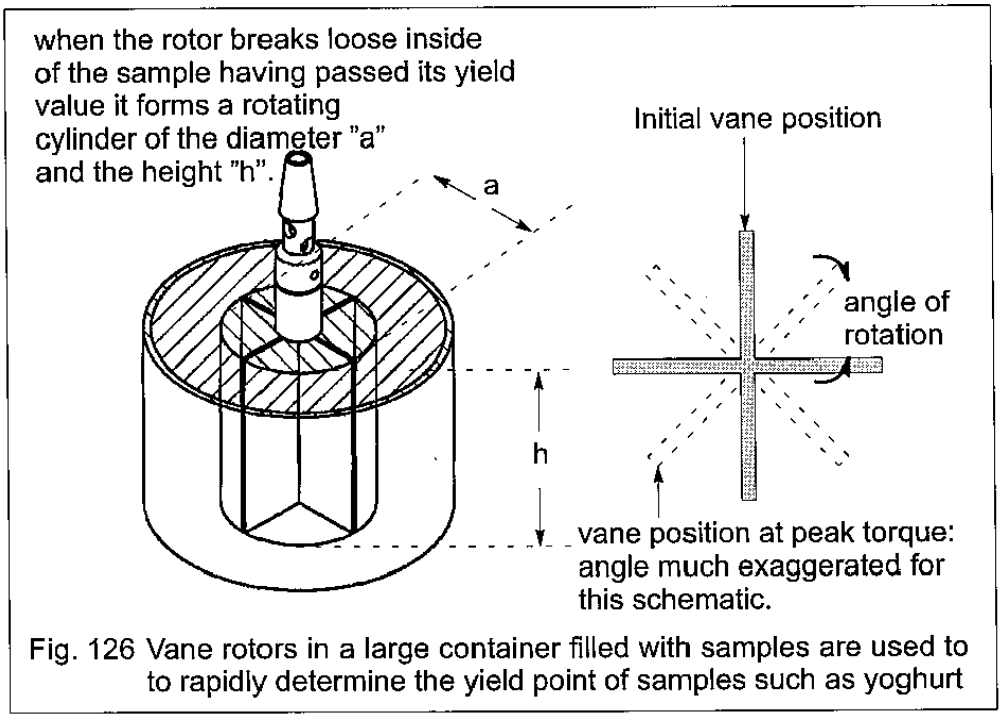


Figure 2.7 A vane in a sample. (Schramm, 2000).

The vane (Brookfield Engineering, a) can be turned at a constant rate or under a constant torque. If the vane is turning at low speeds, then the vane acts like a virtual cylinder (Brookfield). Upon application of torque to the vane (Schramm, 2000), a shearing stress acts on the cylinder that is formed by the vane, when the yield stress is reached the "vane rotor breaks loose in the sample" (Schramm, 2000) and forms a rotating cylinder (see Figure 2.7). It is possible to use a low rotational rate to obtain the yield stress when using a vane on a controlled rate viscometer, with the sheer stress plotted against time (Schramm, 2000). The yield stress is the calculated from the maximum torque recorded in a test converted to the yields stress through manufacturer's formula (Brookfield).

On the Brookfield R/S Soft Solids Tester the vane (Brookfield Engineering, b) is rotated at a constant rate, and stress or torque is measured versus strain or time. This enables determination of the modulus, yield strain and yield stress. This is demonstrated in Figure 2.8. Further information on the Brookfield R/S Soft Solids Tester is found in Appendix B.

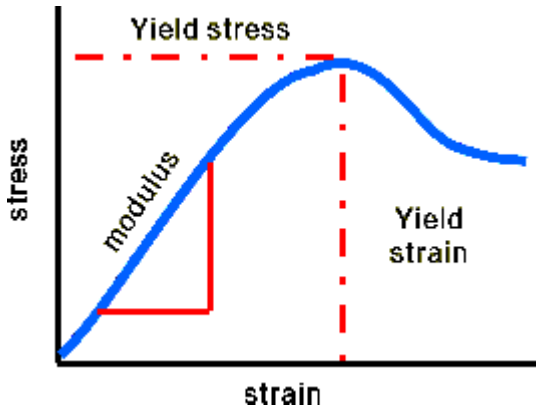


Figure 2.8 A plot of stress and strain from a typical constant rate yield test on a Brookfield R/S Soft Solids Tester. (Brookfield, 2005).

2.8 Dissolution (drug release rate)

Dissolution tests are required for many pharmaceutical products (Hanson Research Corporation, 1990). Drug release rates can be determined using a Hanson Dissolution apparatus, which collects samples of release media during the

course of the test. Samples collected are analysed to determine the concentration of drug released at a specific timepoint.

The CIDR insert is a controlled release drug delivery system, and drug release involves more steps compared to the release for example, of drug from a pill or capsule. Hence an outline of the theory of 'in vitro' drug release from the CIDR insert is provided to outline the steps of drug release. The CIDR insert can be defined as a dispersed matrix system, which is defined as a system where the drug is dispersed in the matrix that is a non-degradable polymer and where the drug is present in concentrations above the saturation solubility of the drug, but not at concentrations that would cause the formation of channels or pores in the matrix (Rathbone, et. al., 2000).

Drug release from a dispersed matrix system requires six assumptions (Rathbone, et al., 2000) in order to gain a better understanding of the processes.

1. A perfect sink is provided by the receptor fluids.
2. Drug is distributed uniformly throughout the matrix and the mass of drug present per unit areas is far higher than the solubility of the drug in the matrix.
3. Once drug release commences the polymer does not swell or shrink.
4. The dissolution rate of the drug in the matrix is rapid relative to the drug transport rate.
5. The thickness of the polymer is larger than the diameter of the drug particles.
6. The drug release rate-limiting step is diffusion through the polymer.

The drug release away from the polymer (Rathbone, et. al., 2000) occurs in a series of steps that are:

1. Dissolution by the drug particles into the matrix that surrounds the polymer.
2. Diffusion of the dissolved drug to the surface of the matrix from the region where dissolution occurred. (Assumed to be the rate-limiting step).
3. Drug at the surface of the matrix enters the surrounding environment.
4. Released drug leaves the polymer surface.

These steps result in a zone of depletion (Rathbone, et. al., 2000) that increases with time. The zone of depletion starts at the surface of the device and as time progresses moves further into the device (Rathbone, et. al., 2000). The increasing size of the zone of depletion results in a non-linear relationship of drug release versus time.

The Higuchi square-root-of-time model (Rathbone, et. al., 2000) has been developed to describe the drug release from a dispersed matrix system. The cumulative mass of drug released by unit area (Q) in a dispersed matrix is defined in Equation 2.10 as (Rathbone, et. al., 2000):

$$Q = [(2A - C_p)C_p D_p t]^{1/2} \quad (2.10)$$

In Equation 2.10, A is the initial amount of drug impregnated in the matrix (by unit volume), and the drug solubility in the polymer phase being C_p and the diffusion coefficient of the drug in the polymer phase being D_p. Since it is assumed that $A >> C_p$ (Rathbone, et. al., 2000) then Equation 2.10 can be reduced to Equation 2.11.

$$Q = [2AC_p D_p t]^{1/2} \quad (2.11)$$

Hence the drug release from a solid matrix can be defined as:

$$\frac{Q}{t^{1/2}} = [2AC_p D_p]^{1/2} \quad (2.12)$$

Now Equation 2.12 means that the drug release from the matrix is dependent upon the factors of time, drug content (Q), C_p and D_p (Rathbone, et. al., 2000). The slope of a plot of Q versus $t^{1/2}$ is linear and has a slope of $[2AC_pD_p]^{1/2}$ (Rathbone, et. al., 2000). Drug release tests can be undertaken with a Hanson Dissolution apparatus, which will automatically sample the release media at certain intervals while keeping the dissolution medium temperature constant. The method of obtaining the drug release rate is described in Chapter Three.

3.0 Materials and methods

3.1 Materials used

3.1.1 Solvents

Most ethanol used in this research was SDA (Simple Denatured Alcohol) grade consisting of absolute ethanol with ~2 % added methanol. SDA ethanol was supplied by Anchor Ethanol (NAA grade) and ExxonMobil (SDA-3A grade). AR grade ethanol was supplied by Biolab (Pronalys). All ethanol used was checked before release to the DEC Manufacturing cGMP laboratory, against a blank standard of ethanol at 239 nm ($\lambda_{max.}$ of progesterone in ethanol) to ensure that there was no component in the ethanol that could be mistaken for progesterone in UV spectrophotometry tests. Any ethanol that gave AU > 0.06 (at 239 nm) was not used.

3.1.2 Progesterone

All progesterone used in this study was USP grade micronised progesterone made by Diosynth, and Pfizer. Non-micronized progesterone from Pfizer (batch 33JFW) was also used.

3.1.3 Silicone

The silicone used was Dow Corning Silastic Q7-4840, a two-part heat cured silicone. This silicone is currently used in the manufacture of virtually all CIDR inserts at DEC Manufacturing. Silicone from an alternative manufacturer was also used in some experiments and similar to Dow Corning Q7-4840. The identity of the alternative supplier silicone will not be revealed in this thesis for reasons of commercial confidentiality.

3.1.4 Packaging

In this research two types of bags were used, both of which are used to package CIDR inserts for commercial sale. Paper-foil lined bags supplied by Detpak were used along with plastic polyethylene bags made by Chequer. The polyethylene bags are used to package CIDR 1380 inserts in normal production, whereas the paper-foil lined bags are used to package CIDR 1900 inserts. The polyethylene bags do not provide a total seal against moisture and hence storage in a humid environment does not necessarily mean that bag contents will be protected from moisture.

3.2 Laboratory methods

Some of the laboratory methods used as part of this research required cGMP training, these were CIDR drug release rate tests and progesterone content analysis. Trained DEC Manufacturing staff performed these tests as the time required to be fully trained in these techniques to cGMP standards would have taken too long.

3.2.1 UV Spectrophotometry

To determine concentration of progesterone in samples of ethanol a Beckman DU-600 spectrophotometer was used. Samples of were sampled through the use of a sipper and were analysed at a single λ of 239 nm. A blank sample was scanned at regular intervals (~ every ten scans) to ensure that the baseline was stable. Analysis was carried out at ambient laboratory temperatures (an air conditioned room).

The concentration of progesterone was determined using standards ranging between ~5 to ~30 μg/mL. Standards were made by gravimetrically (by weight) diluting a laboratory stock solution. The stock solution was made up by trained laboratory staff. On one occasion the samples were scanned using a Perkin Elmer instrument by a trained operator. In order to use the UV spectrophotometer in a cGMP laboratory, training was undertaken in its correct use.

3.2.2 Differential Scanning Calorimetry (DSC)

Samples were introduced into aluminium pans, and a cover was then placed over the sample. The sample along with a reference pan were analysed in a Perkin Elmer DSC 6 according using a variable heating, cooling and isothermal (hold at a specific temperature) temperature program (ranging from 26 to 190 ^{o}C). Samples were often (but not always) weighed before analysis allowing the calculation of peak areas.

3.2.3 Stability oven

Mottling and secondary blooming has been found to be influenced by the amount of moisture and temperature (Rathbone & Ogle, 2000). In order to promote secondary blooming and mottling in some samples a high humidity environment was simulated. This involved placing a tray of water inside a preset set oven at 29 ^{o}C ± 2 ^{o}C (see Figure 3.1). The oven was a Cotherm Scientific M180FHS. While the oven was in use, a data logger was often used to monitor temperature, and the water level was checked regularly to ensure a humid environment. If temperatures outside the oven went above 29 ^{o}C then a temperature spike was observed because the oven had no cooling system. This was not of great concern as the purpose of the oven was to promote maximum secondary blooming and mottling and a higher temperature would only have served to further that aim. The humidity was measured in December 2005 (using a Thermo-Hydro meter) and it was found that there was a maximum humidity over a four day period of 87 % RH, with the humidity before the removal of the gauge being 82 % RH.

Figure 3.1 The open stability oven. The white container at the bottom contains water used to provide humidity inside the oven.

A wide range of packing materials were used to hold samples in the oven. This included plastic ziplock bags (often with holes), unsealed foil lined bags, polyethylene bags and plastic bags. Progesterone was stored inside the oven in plastic containers with holes in the lids.

At one stage in the research the water container in the oven ran dry for approximately two months. While this is undesirable the majority of the mottling and secondary blooming occurs over the first few months after manufacture (STAB001, 1999-2004) (Rathbone & Ogle, 2000) and hence this should have minimal impact. Studies that were impacted by the lack of water in the oven are noted as they are outlined.

3.2.4 X-ray Diffraction (XRD)

X-ray Diffraction was undertaken on both progesterone powder and solid samples using a Philips High Performance X-ray Diffraction System. The scan axis was Gonio. The scan was a continuous scan, with a step size of 0.02 2θ and a step time of one second. The anode used was copper. The scan range varied but at the least ranged from 10 to 25 2θ.

Samples consisted of either progesterone powder, or silicone slabs. Silicone slabs were placed directly into the instrument sample holder and scanned without any preparation. Silicone slabs were often scanned without any markings being made on the slab, indicating the slabs position in the XRD sample holder, hence latter scans may have been done in a different location on the slab. Identification of the progesterone polymorph was undertaken by comparing samples with XRD spectra of the α and β progesterone polymorphs. The α progesterone polymorph was sourced from a standard manufacturer's batch of progesterone (all progesterone supplied to DEC Manufacturing is in the α progesterone polymorph), and the β progesterone polymorph was prepared by melting progesterone, and then analysing the crystallised melt under XRD. These standards were verified by comparison to a reference XRD spectrum published in the literature (Muramatsu, et. al., 1979) and shown in Figure 3.2.

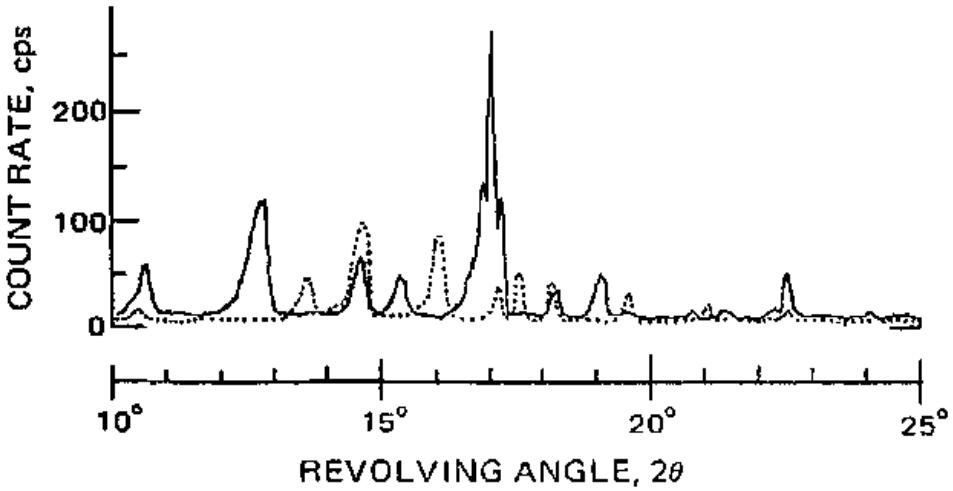


Figure 3.2 XRD diffractorgrams of the α (-) and β (·····) progesterone polymorphs.(Muramatsu, et. al., 1979).

3.2.5 Electro Spray Mass Spectrometry (ESMS)

Samples of either liquid silicone (~1 cm^3) or sections of cured silicone were partially dissolved in AR grade ethanol (~100 mL) then the ethanol was dissolved into methanol. In the case of cured silicone samples the ethanol does not dissolve the silicone matrix. NaCl was added as dilute solution to serve as an ionisation agent.

Samples were analysed on a Fisons VG Platform II in positive ion mode, with a cone voltage of + 45 V and at a room temperature of 21 ^{o}C. Samples were stored at ambient temperatures in glass capped bottles until required for analysis. Samples were analysed on several occasions in order to differentiate variable background peaks (dependant on previous samples passed through the instrument) from authentic sample peaks. The resolution of ESMS spectra in this thesis are typically ± 0.5 Da, and the calibration was typically ± 0.5 Da. The mass range analysed depended on the sample undergoing analysis. The minimum mass range was from m/z 400 to 1600.

3.2.6 Data processing

Data produced from experiments would often be analysed using Microsoft Excel spreadsheets. Excel spreadsheets were used in activities such as producing graphs, and calculating concentrations of progesterone from UV absorbance measurements and dilutions. An Excel spreadsheet, prepared by DEC Manufacturing was used to undertake statistical analyses between different sets of data. A copy of this spreadsheet is found in the CD. The spreadsheet used a p value of 0.05 in Student T tests.

3.2.7 Yield stress from the Brookfield R/S Soft Solids Tester

To gain information on the yield stress of uncured liquid silicone, a Brookfield R/S Soft Solids Tester was employed (SST2000) (see Figure 3.3). The test method involved introduction (lowering) of a V40-20 vane into the liquid silicone so that the vane was completely immersed. The height of the liquid silicone over the vane was not recorded, as it was not regarded as significant. Then a constant rate yield test was undertaken over 120 seconds, giving a plot of the yield stress versus time. The yield stress was calculated as the highest stress in the plot over the 120 seconds of the measurement period. Figure 3.4 shows a typical stress versus time plot.

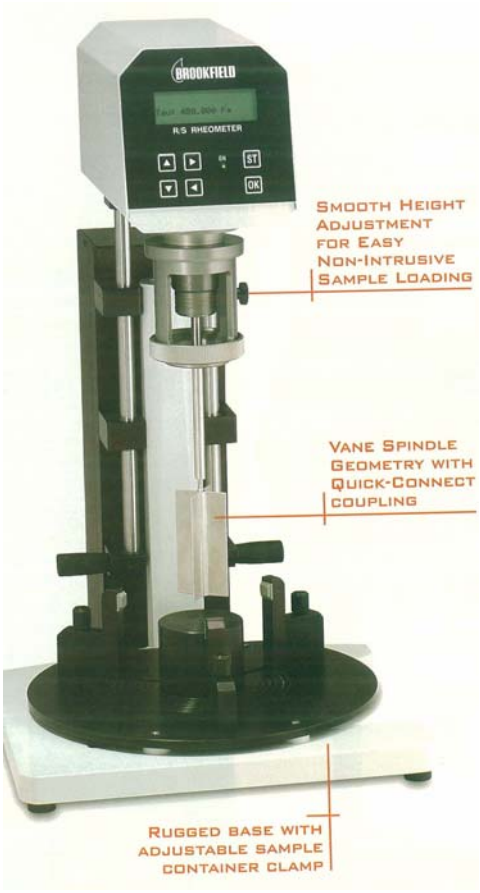


Figure 3.3 A R/S Brookfield Soft Solids Tester. (Brookfield, 2004).

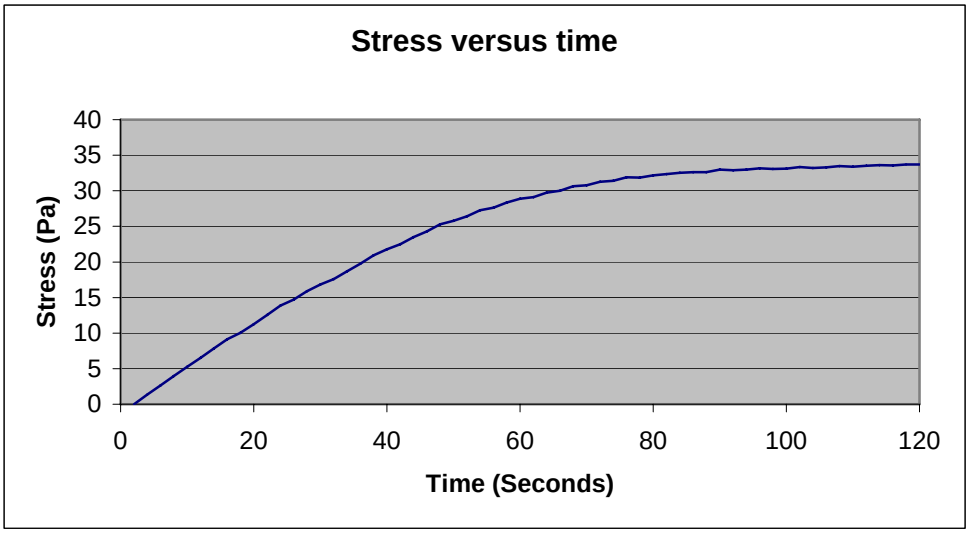


Figure 3.4 A typical stress versus time plot for a liquid silicone batch.

The silicone was not removed from its storage container during the testing, as this would have created air bubbles in the silicone. The storage container (Nexus Polypot) has a diameter of 97 mm. After each test the vane was wiped down with

tissues until it was clean before being used on the next sample. No solvents were used to clean the vane to prevent contamination of liquid silicone samples.

In order to gain reliable results a number of repeat measurements were undertaken. The results were then averaged and a standard deviation gained. The yield stress from the first measurement was always rejected as the yield stress increased after the first measurement, and hence the first measurement was too low.

Due to incorrect programming the software running the tester was set to run using a different vane (V80-40). This was discovered after most or all of the work had been done and hence this data can only afford comparisons between samples analysed on this instrument at the incorrect settings, without comparison with any externally derived results. The conclusions reached by this thesis are not affected by this issue as the results were not used as absolute values, but in a comparative manner.

3.2.8 Progesterone content extraction

In order to determine the percentage by weight of progesterone in a sample of silicone, a DEC standard testing method was used. Testing was undertaken by DEC Manufacturing staff in a cGMP laboratory using DEC testing method STP011 unless otherwise specified. The progesterone content method STP011 is based on the methods outlined by Macmillan et. al, (Macmillan, et. al., 1990) and Cleeff et. al, (Cleeff, et. al., 1992).

In test STP011 the CIDR insert skin is removed, weighed and then the progesterone in the silicone matrix is extracted by soxhlet extraction by ethanol for 16 hours. The ethanol was then made up to a known volume, diluted twice and analysed by UV spectrophotometry. A pre-calibrated Microsoft Excel spreadsheet (a spreadsheet with had each calculation periodically checked by hand) was used to determine the percentage weight of progesterone in each sample.

3.2.9 Progesterone drug release rate

DEC Manufacturing has a drug release rate test to determine the rate of progesterone release into 1100 ml of release media, consisting of 62.5 % ethanol : 37.5 % (v/v) water (double distilled) at a temperature between 36.5 ^{o}C to 37.5 ^{o}C. A picture of a Hanson Dissolution apparatus used to undertake the test is show in Figure 3.5. CIDR inserts were placed into specially designed holders that were spun at 100 rpm in the release media during the test. Samples (~ 1 mL) were taken at t = 1, 4, 9, 15 and 20 hours, diluted and analysed using UV spectrophotometry to determine progesterone concentration. All CIDR inserts that are tested for the purpose of sale are tested using AR grade ethanol rather than SDA grade ethanol, which is generally used in research tests.

Figure 3.5 A Hanson Dissolution apparatus in operation. The automated sample collection system is the apparatus to the left where the samples are collected in test tubes. Each CIDR insert is in an individual flask in a water bath.

The test method used varied on the type of the CIDR inserts being tested, with the CIDR 330 being tested using DEC test method STP055 and CIDR 1380 being

tested using DEC test method STP048. All drug release testing was undertaken by trained laboratory staff on calibrated Hanson Dissolution apparatus.

To obtain the drug release rate, the mass of progesterone released per area ($\mu g/cm^2$) (The surface area of CIDR 330 insert is 28 cm^2 and the surface area of CIDR 1380 insert is 123 cm^2) is plotted versus the square root of time (t in hours). This produces a linear graph, and the drug release is calculated from the slope of the graph and has units of $\mu g/cm^2/\sqrt{t}$. The CIDR 1380 insert drug release rate specifications for the USA market requires a drug release rate between 998 and 1736 $\mu g/cm^2/\sqrt{t}$ (STP048, 2003). A pre-calibrated Excel spreadsheet (a spreadsheet with had each calculation periodically checked by hand) was used to determine the drug release rate.

The test also provides information on the amount of progesterone released at each time point and this can be used as an estimate of the amount of progesterone on the surface of the CIDR insert by analysing the mass of progesterone released after one hour in the 20 hour drug release rate test.

3.2.10 Determination of the % mottling of a CIDR insert

The % mottling on a CIDR insert is the % of the area of the CIDR insert that is translucent (mottled). The method is based on DEC Manufacturing research method STP057. Figure 3.6 from STP057 outlines the method. The % mottling on a CIDR insert depends on the type of CIDR insert due to the differences in surface area between the different types of inserts. On a CIDR 1900 or CIDR 1380 insert 1 % mottling corresponds to an area of 11 mm x 11 mm from respective surface areas of 120 cm^2 and 123 cm^2. On a CIDR 330 insert 1 % mottling corresponds to an area of 5 mm x 5 mm from a device surface area of 28 cm^2.

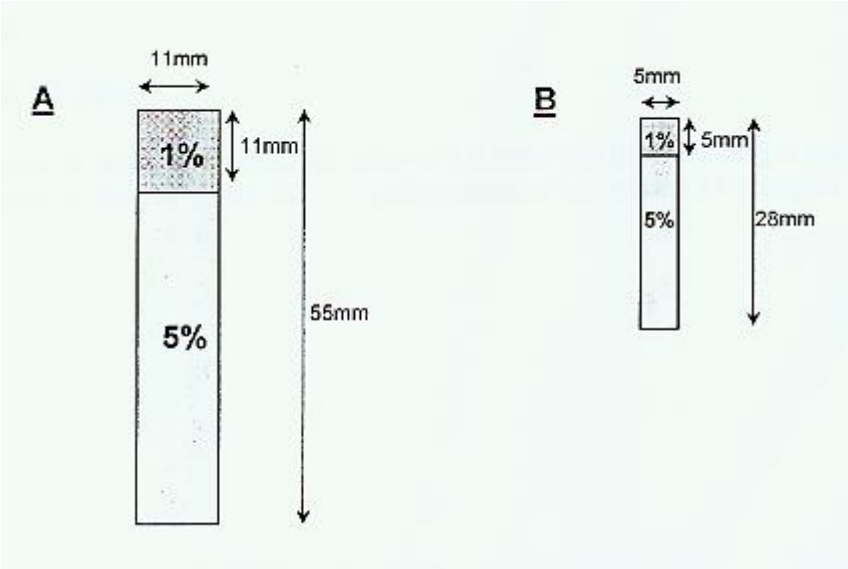


Figure 3.6 Diagram used to determine the % mottling on a CIDR inserts. The grey area refers to 1 % of the surface. Diagram A is used for the CIDR 1380 and 1900 inserts while diagram B is used for CIDR 330 inserts. (STP057, 2004).

The % mottling value must be regarded as a relative degree of mottling on a CIDR insert and the value considered with some degree of caution. This is because the areas are visually measured. Because of this there will be varying results.

3.2.11 Scanning Electron Microscopy (SEM)

Samples were coated with a thin layer of platinum using a Hitachi E-1030 Ion Sputter Coater. Samples were then analysed using a Hitachi S-4100 Field Emission Scanning Electron Microscope with X-ray analyser.

3.2.12 Surface progesterone analysis

3.1.12.1 Purpose of the surface progesterone method

In order to place a numerical value on the amount of surface progesterone on an insert, a technique was developed to measure the surface progesterone on a CIDR insert. This is desirable, as visual estimates of surface progesterone vary widely depending on a range of factors including, observation of progesterone on the

CIDR insert and the scale used. A discussion of the surface progesterone method development can be found in Appendix A.

3.2.12.2 Basic method

3.2.12.2.1 Extraction

A known volume of SDA ethanol is placed into a container, which is sitting in a sonicator (see Figure 3.7). Just before the sample is added the sonicator is turned on. Samples of ethanol are removed from the top of the container (by either a Oxford Macroset pipette or other means) at timed intervals after the insertion of the sample. Sampling times were two minutes and five minutes after the test started and a sample volume of 5 mL was commonly removed.

Figure 3.7 A CIDR insert in a container in the sonicator after the test. Except for the CIDR 330 inserts all samples were tested without the tail.

The samples are placed into a capped glass bottle, which are then further sealed with Parafilm®. Some samples were stored in the refrigerator to preserve stability of the progesterone extract. Upon removal from the refrigerator samples were allowed at least an hour to return to room temperature before analysis or dilution.

3.2.12.2.2 UV analysis

The extracted sample undergoes gravimetric dilution (dilution by weight) (on calibrated scales to three decimal places) with further ethanol to ensure that the diluted extract has a blanked absorbance at 239 nm between ~0.2 and ~1.6 AU.

The degree of dilution depended on the amount of surface progesterone dissolved off the CIDR insert.

Four standards were made up gravimetrically to approximately 5 µg/mL, 10 µg/mL, 20 µg/mL and 30 µ/mL of progesterone. A quality control standard of approximately 20 µg/mL of progesterone was also made using a different stock solution. To ensure standard quality, a R^2 value greater than 0.9999 was required and the recovery of the quality control standard had to be ± 2 % of the expected concentration. All ethanol used in making the standards and dilutions were from the same batch and drum. This is done to ensure that the background absorbance of the ethanol is kept constant.

Samples were then analysed on a UV spectrophotometer in accordance with DEC Manufacturing methods (See Section 3.2.1). A Microsoft Excel spreadsheet was used to calculate the slope and intercept of the standards (a plot of concentration of progesterone versus the absorbance) that was then used to calculate the concentration of the diluted samples (DS) in Equation 3.1.

$$[DS] = \frac{(\text{absorbance-intercept})}{\text{slope}} \qquad (3.1)$$

To gain the concentration of the undiluted extract (E) Equation 3.2 was used:

$$[E] = \frac{[DS]}{\frac{W_{Extract}}{W_{Extract} + W_{Diluting\ Ethanol}}} \qquad (3.2)$$

Where $W_{Extract}$ is the weight of the extract and $W_{Diluting\ Ethanol}$ is the weight of the diluting ethanol. To calculate the percentage amount of progesterone released Equation 3.3 was used;

$$\%\ \text{Progesterone Released (mg)} = \frac{[E]V_{Ethanol\ in\ Container}}{\frac{1000}{W_{\text{Progesterone in CIDR(mg)}}}} \qquad (3.3)$$

Where $V_{Ethanol\ in\ Container}$ is the volume of ethanol in the extraction container. The amount of progesterone in a CIDR was taken to be the stated label claim, of 1380 mg for a CIDR 1380 insert, 1900 mg for a CIDR 1900 insert, and 330 mg for a CIDR 330 insert.

3.2.12.3 Variation in surface progesterone technique

Due to the wide range of samples analysed the technique was altered to suit the size and type of the sample. CIDR 1900 and CIDR 1380 inserts were analysed in 1 L of ethanol that was in a 1.2 L polystyrene Clickclack container (see Figure 3.7).

Due to the small sample size slabs and CIDR 330 inserts were analysed in a 100 mL Techno-plas container made from polystyrene. Slabs were analysed with 110 mL of ethanol whereas CIDR 330 inserts were analysed with 100 mL of ethanol. To ensure that the container did not float in the water, lead sinkers were placed in the bottom of the container (see Figure 3.8). On CIDR 330 inserts the tail did stick out of the container however the tail was not removed as it assisted with sample handling.

Figure 3.8 CIDR 330 insert in container after test.

To ensure that the wings of the CIDR insert did not inhibit the quick insertion of the insert into the container, wire was used to bend the wings of all the CIDR inserts into an anchor configuration as clearly observed in Figure 3.8.

3.1.13.4 Visual appraisal of surface progesterone (powders and crystals)

Progesterone often appears as crystals or powder on the surface of a CIDR insert. Hence it is possible to undertake a visual approximation of the levels of

progesterone on the CIDR insert. This is useful as sometimes it is not possible to remove a sample for analysis (e.g. reserve samples), it also helps to give the analyst an understanding into the degree of mottling on the sample before undertaking surface progesterone analysis. Any such measurement remains an approximation and should be treated with a degree of caution due to errors such as:

- Analyst not observing white progesterone on the white surface of the CIDR insert.
- Scale of analysis changing between experiments.
- Subjective method.

3.2.14 Gas Chromatography Mass Spectrometry (GCMS)

Samples of liquid silicone (6 to 14 mg) were dissolved in dichloromethane (10 to 18 g). Typically 1 to 3 μL of the dichloromethane solution was manually injected onto a 20 m x 0.25 mm id Phenomenex Zebron ZB5 column installed with a Hewlett Packard (HP) 6890 GC interfaced to an HP 5973 mass selective detector. The injector and mass spectrometer interface were maintained at 250 ^{o}C and 280 ^{o}C respectively. The GC oven was temperature programmed from 50 ^{o}C (0.5 minutes initial hold) then ramped at 25 ^{o}C minutes^{-1} to 100 ^{o}C and then at 15 ^{o}C minutes^{-1} to 295 ^{o}C (ten minutes final hold) .The total run time was 25.5 minutes. Mass spectral data was acquired in total ion chromatogram mode over the m/z range of 42 to 600 Da.

Samples were prepared in glass bottles that had previously been cleaned by rinsing with water and detergent, followed by washing in a dishwasher, using a reverse osmosis water rinse.

3.3 Manufacturing methods

3.3.1 Oven cured slabs

Slabs of silicone and progesterone are made to test different theories on mottling and secondary blooming. Slabs are easier to make compared to CIDR inserts for a number of reasons:

- Manufacturing equipment is used for production purposes only.
- Uses less raw materials compared to CIDR manufacture.
- Some experiments such as spike tests (addition of material to liquid silicone) can not be done without causing an entire batch of CIDR inserts to be rejected.
- Does not require any cGMP approval to undertake.

In this method of manufacture, slabs were produced by laying out a mix of both parts of liquid silicone that had been mixed with progesterone onto tin foil. Mixing was done with a drill mounted stirrer for an approximate period of time, usually five minutes. Slabs were made with 10 % w/w of progesterone and equal parts of part A and part B silicone.

The tin foil was supported by a mould (see Figure 3.9) to give shape and support to the slabs, but the mould would be removed before curing. Once the silicone had been laid down the slabs were smoothed flat. The tin foil was then removed from the mould support and placed in the oven. During curing the temperature in the oven would be monitored for temperature fluctuations using a Fluke 52 thermometer (thermocouple). The oven used was either a Ronson Bench top oven or a Contherm oven. While slabs cooled, any area of the slab that was touched with anything would rapidly bloom. In order to easily observe surface progesterone a small amount of black dye was added to some mixes. This would give a grey to black colouring depending on the amount of dye added. After curing slabs would normally be left to cool on the bench before being packaged.

Images of slabs were collected using a scanner. To prevent equipment becoming contaminated with progesterone, slabs were scanned in clear plastic bags.

Figure 3.9 Mould used to support oven moulded slabs. When in use the mould would be covered in tin foil which, which would then be covered in liquid silicone mixed with progesterone.

3.3.2 Hand moulded slabs

Different oven cured slabs have approximately similar dimensions, and similar cure temperatures. However such slabs are not desirable for surface progesterone analysis and are best used for XRD analysis and visual observations. Hand moulded slabs however have fixed dimensions, and controlled cure temperatures, and are suitable for surface progesterone analysis.

A silicone and progesterone would be mixed together in the same manner as of oven cured slabs. The hand moulder is shown in Figure 3.10. An amount of mixed silicone was placed into the barrel (Figure 3.11) and inserted into the hand moulder with the top of the barrel set into a piston. The piston would then be lowered and hence silicone would be injected into the heated tool.

There was very little control on the amount of silicone being injected into the tool (see Figure 3.12). If too much silicone was injected then flash (silicone that squeezes in between the two parts of the mould) would form on the slabs produced. If not enough silicone was used the mould would only be half filled (short). A timer clock was used to ensure a minimum cure time was observed. All slabs were made using the tool used to make the slab in Figure 3.12. The flash and

surplus silicone would be removed before testing to leave a slab similar to the one shown in Figure 3.13.

Figure 3.10 The hand moulder. The tool comprises two parts, the movable top plate (right) is placed over base of the tool, and held down by compressed air clamps. The piston to push silicone down the barrel is the small white cylinder between the clamps. The tool is held at a constant temperature.

Figure 3.11 The barrel of the hand moulder. The tip (the cone on top of the barrel) would be inserted into the back of the tool. The barrel is hollow and contains the silicone progesterone mix, which is added at the opposite end to the tip (not shown).

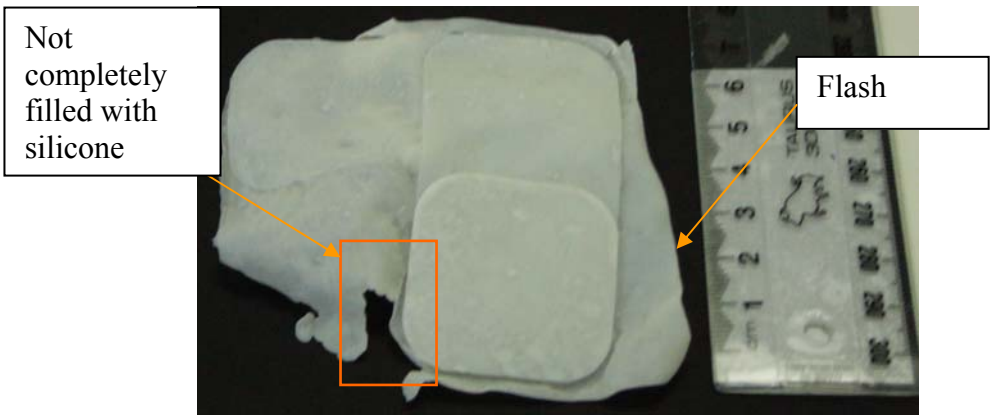


Figure 3.12 Slab produced by the hand moulder. In this slab the shot is too small and hence the tool was not completely filled with silicone. The flash is clearly visible.

Figure 3.13 Slab produced by the hand moulder after removal of flash and surplus silicone. Most slabs used in this research were in this form. Surplus silicone was often used in progesterone content analysis.

3.3.3 Manufacturing of CIDR inserts

CIDR inserts used in this study were always made by cGMP trained staff at DEC Manufacturing using standard injection moulders used to make CIDR inserts for local and international sale. All the processes of manufacturing CIDR inserts are controlled under DEC Manufacturing quality control systems, which comply with the rigorous United States Food and Drug Administration standards, along with the standards required in the various international markets that the CIDR insert are sold in.

The tool for the CIDR 1380 inserts makes six inserts with each shot of silicone (a rosette), whereas a CIDR 330 insert tool makes four inserts and a CIDR 1900 insert tool makes three inserts. A CIDR insert has no identifying marks to determine the batch it is from, however the tool has cavity numbers to determine the location in the tool an insert was made in.

A CIDR insert is made by mixing progesterone separately to each part of liquid silicone. A picture of this is shown in Figure 3.14. The silicone is then placed under vacuum to remove air, and introduced into the injection moulder. As the silicone flows to the tool it is mixed in line using a static mixer (a fixed piece of equipment that mixes the two parts of liquid silicone) before being injected into the tool by use of a screw.

Before each shot (injection of silicone into the tool) can commence the operator places the nylon spine into the tool, upon which the machine tool closes, and known volume of the mixed silicone is added to the closed tool. The tool is heated throughout the cure process curing the silicone, after cure the tool is opened and the inserts are removed and bench cooled.

Figure 3.14 A pail of liquid silicone mixed with progesterone. This is from part B. The part A is identical.

The cure time for a CIDR insert depends on the silicone batch (Jackman, 2006), with CIDR 1900 inserts requiring a cure period of 30 to 50 seconds (Jackman, 2006), whereas CIDR 1380 inserts have a cure period of 20 to 40 seconds (Jackman, 2006). CIDR 330 inserts have an average cure time of 35 seconds (Murray, 2005).

3.3.3.1 Reserve samples

As part of pharmaceutical requirements, each batch of CIDR inserts manufactured has a small number kept back at the factory as a reserve in the event that retesting is required. The number of samples retained depends on the end market of the CIDR insert. Reserve samples are stored in their packaging in a temperature monitored warehouse at the factory in Hamilton, New Zealand. Access to the samples is controlled in accordance with pharmaceutical regulations.

3.3.3.2 CIDR insert batch numbers

Each batch of CIDR inserts is given a unique batch number which consists of a letter signifying the year of manufacture, two numbers specifying the month, a number specifying the type of CIDR insert, and two numbers to differentiate the batch from other batches in that month (within its own CIDR insert type).

For example batch E08106 was made in August 2004, consists of CIDR 330 inserts and was the sixth batch of CIDR 330 inserts made in August. Some CIDR insert batch numbers used in this thesis (for example 9329319) refer to batch numbers that were used under an older batch numbering system.

4.0 Fundamental studies

As discussed in the Chapter One, progesterone exists in five polymorphic forms, of which the α and β polymorphs are the most commonly observed. The α polymorph has a melting point of 131 ^{o}C and the β polymorph a melting point of 122 ^{o}C (Kuhnert-Brandstätter et. al., 1965)**,** with the α polymorph being the more thermodynamically stable form (Wang et. al., 2000). Melting of either the α or the β polymorph results in the β polymorph being formed upon resolidification (Muramatsu et. al., 1979).

Work by Rathbone and Ogle (Rathbone and Ogle, 2000) has shown that curing at 120 ^{o}C (below the melting point of both the α and β polymorphs of progesterone) eliminated mottling and secondary blooming. Work by Reardon (Reardon, 2003a) showed that controlled cooling could prevent mottling. It is proposed that the controlled cooling affects the polymorph of progesterone formed (Legendre et. al., 2003) (Duclos et. al., 1991) and hence helps to influence the prevention of secondary blooming and mottling. The aim of the research detailed in this Chapter was to investigate how progesterone polymorphism is influenced by different manufacturing conditions, to see if any insights into secondary blooming and mottling can be obtained.

4.1 X-ray Diffraction (XRD) investigations into polymorphism of progesterone

4.1.1 XRD investigation into slabs made with different feedstock silicones

Slabs were made with different feedstock silicones, and some slabs were also made with increased progesterone loading (30 % w/w) to determine if these conditions cause a difference in progesterone polymorphism. Slabs were also studied to determine if there was any change in progesterone polymorphism after manufacture. All studies into slab polymorphism were undertaken using XRD. XRD analysis determines if crystalline material is present on the surface of slabs.

The maximum depth that the XRD is able to sample to is unknown but is reasoned to be in the order of microns.

4.1.1.1 Progesterone polymorphism after manufacture

The alternative supplier silicone does not exhibit secondary blooming or mottling (Reardon, 2004b). It is important to have an understanding of the effect of the different silicone feedstocks on the polymorphism of progesterone. To investigate this slabs were made with Dow Corning Q7-4840 silicone (batch 0001815030) and the alternative supplier silicone, both sets of slabs used progesterone from batch 37JHF. Slabs were cured for 60 seconds between 175 ^{o}C to 193 ^{o}C. Four slabs made with the different silicone feedstock were scanned using XRD at t = 0, 1, 3, days and in some cases t = 7 days after manufacture. Slabs were stored in the laboratory. Figures 4.1 to 4.4 shows the XRD diffractograms from each slab analysed over the duration of the experiment. It is unknown if the same areas on the slabs were scanned each time.

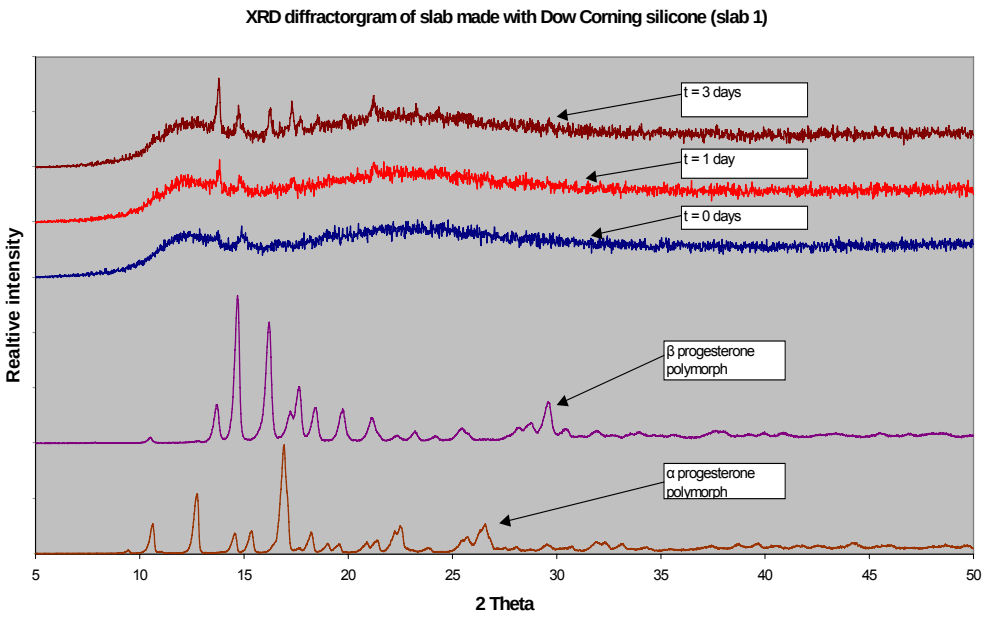


Figure 4.1 XRD diffractogram of slab 1 made with Dow Corning silicone over three days after manufacture.

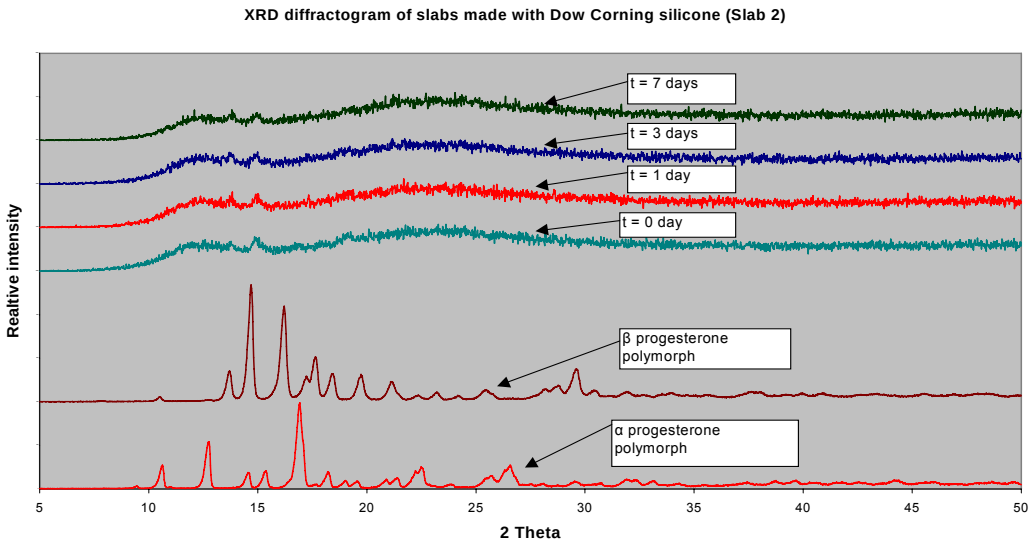


Figure 4.2 XRD diffractogram of slab 2 made with Dow Corning silicone over seven days after manufacture.

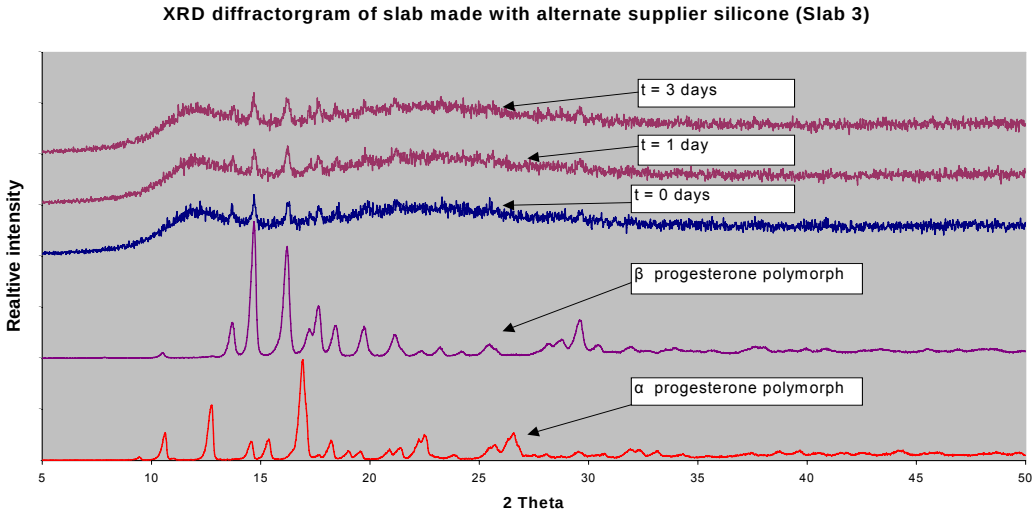


Figure 4.3 XRD diffractogram of slab 3 made with alternative suppler silicone over three days after manufacture.

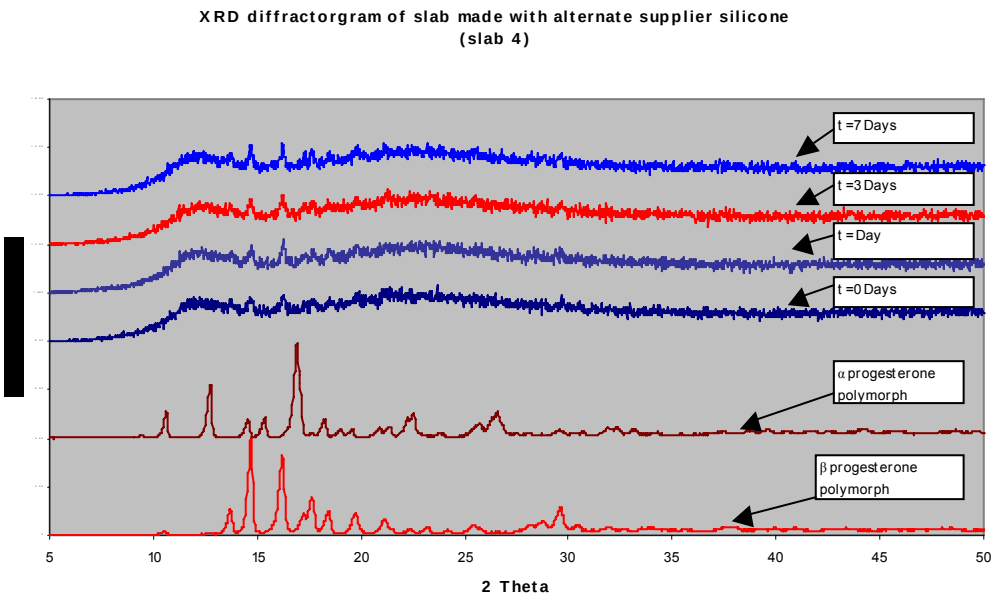


Figure 4.4 XRD diffractogram of slab 4 made with alternative supplier silicone undertaken over seven days after manufacture.

Figures 4.1 to 4.4 clearly show that all but one (Slab 2 made with Dow Corning silicone (Figure 4.2)) of the slabs exhibited the β progesterone polymorph three days after manufacture. No slabs were found to be exhibiting α progesterone polymorph. The signal on slab 2 appears to be a very weak β progesterone polymorph. Work by Bourke (Bourke, 2004) found that slabs made with Dow Corning silicone exhibited what appeared to be the β progesterone polymorph 14 days after manufacture, Bourke also noted some peaks appeared to be from the α progesterone polymorph (Bourke, 2004).

It is known that slabs made with the alternative supplier silicone do undergo initial blooming (Reardon, 2004b), which explains the detection of progesterone on the slab surface by XRD. Furthermore SEM results in Chapter Six show progesterone crystal formations on the surface of CIDR 330 inserts made with the alternative supplier silicone and SEM results from Bourke (Bourke, 2004) also concur with this result.

In Figure 4.1 (slab 1 made with Dow Corning silicone) and in Figure 4.4 (slab 4 made with alternative suppler silicone) the progesterone peaks increase in intensity over time after manufacture, but this is not observed in Figure 4.3 (slab 3

made with alternative supplier silicone), which indicates that the time difference between manufacture and scanning does not have an impact on the intensity of the progesterone XRD peaks or is caused by different scanning locations. The lack of crystals of progesterone on the slab 2 made with the Dow Corning silicone is explained by the fact that secondary blooming does not always occur on inserts in a predictable manner. It could also be possible that the rate of secondary blooming may vary across the specimens.

4.1.1.2 XRD scans on slabs over five months after manufacture of the slab

In order to assess the long term effect of progesterone polymorphism in silicone slabs, samples were made using Dow Corning silicone from batch 0001549541 and the alternative supplier silicone using progesterone from Pfizer batch 37JHF. Slabs were cured for 60 seconds between 144 ^{o}C to 172 ^{o}C. XRD analysis was undertaken on one slab made with each feedstock during the term of the experiment (5 months). The area of analysis varied between different scans. Figure 4.5 and 4.6 shows the results over the length of the study.

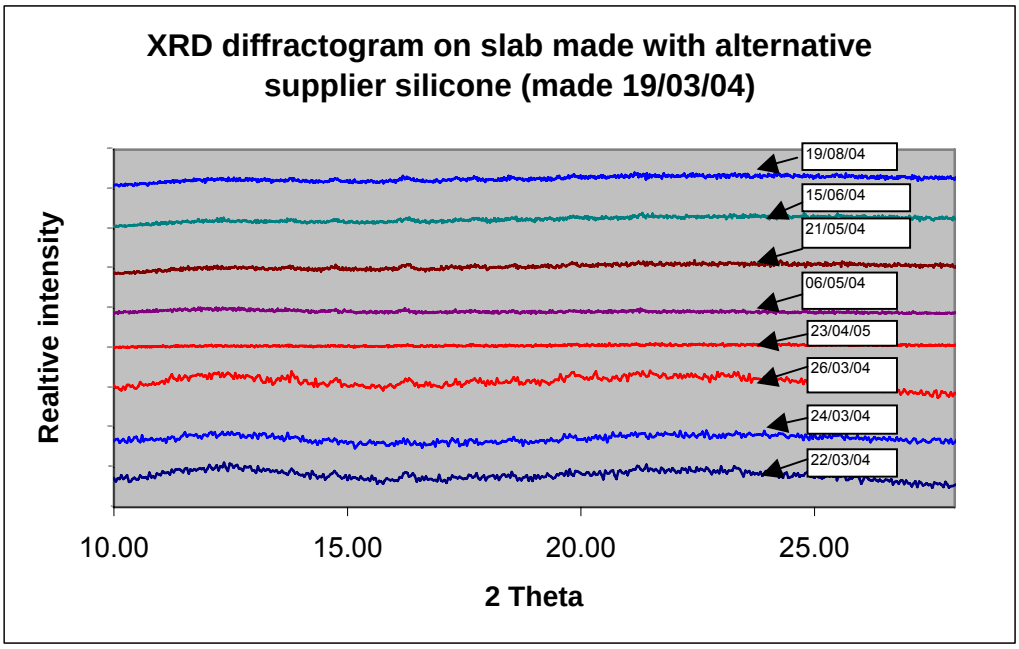


Figure 4.5 XRD diffractogram of slab made with alternative supplier silicone. Made 19th of March 2004. Date in format of day/month/year.

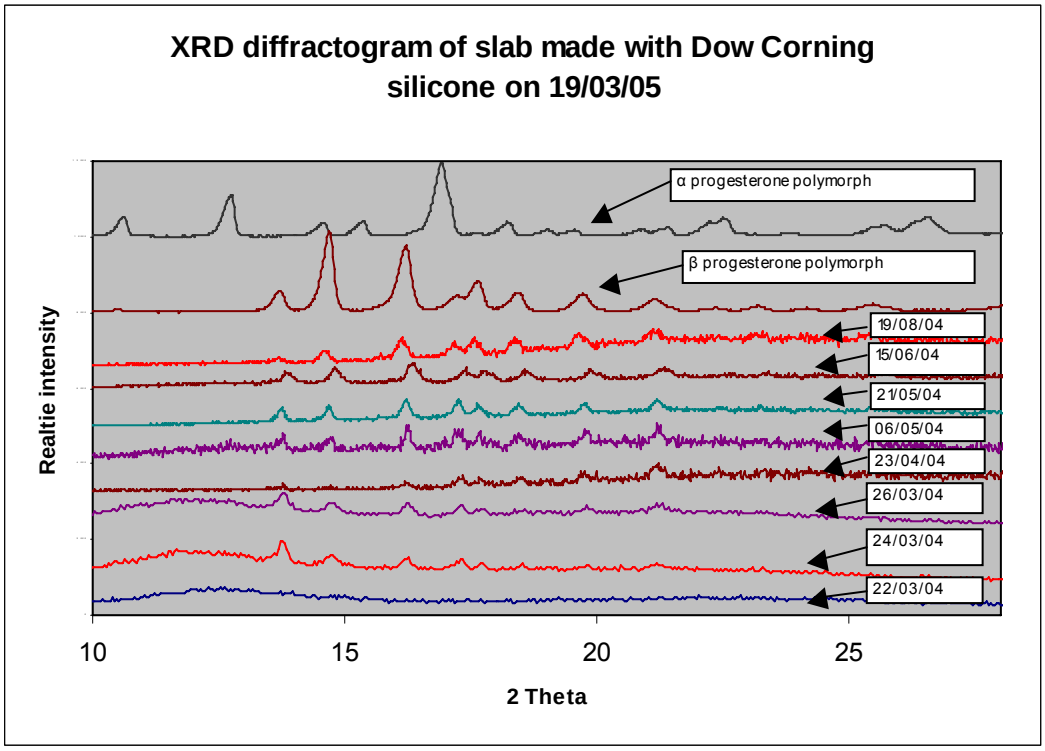


Figure 4.6 XRD diffractogram of slab made with Dow Corning silicone. Made 19th of March 2004. Date in format of day/month/year.

From Figure 4.5 it is clear that there are no progesterone polymorphs detected on the sample made with the alternative supplier silicone. This result does not necessarily imply that the progesterone is not present as previous results in this Chapter show otherwise, but that the progesterone could be in an amorphous form. Figure 4.6 of the slabs made with the Dow Corning silicone show the β progesterone polymorph on the surface, which appears within a few days of manufacture and persists for five months after manufacture. Variations in the intensity of the peaks over the course of the study are most likely to have been caused by different areas of the slabs being scanned.

The lack of crystalline material on the slab made with the alternative supplier silicone should not be taken as evidence that all slabs made with the alternative supplier silicone do not have any surface progesterone as Reardon (Reardon, 2004b) found that there was initial blooming on slabs made with the alternative supplier silicone, furthermore SEM results from Chapter Six show that there are regions covered in crystals on the surface of a CIDR insert made with the alternative supplier silicone as well as regions with less crystal coverage.

It was also found that the XRD diffractograms of both sets of slabs made with Dow Corning silicone and the alternative supplier silicone taken on the 22, 24th and 26th of March 2004, had a sudden increase in intensity outside of the 10 ^{o} to 28 ^{o} 2θ regions shown in Figures 4.5 and 4.6. An example of this is shown in Figure 4.7. This phenomena was not observed in other XRD experiments undertaken as part of this experiment, however an XRD work undertaken by Bourke (Bourke, 2004) did show this phenomena.

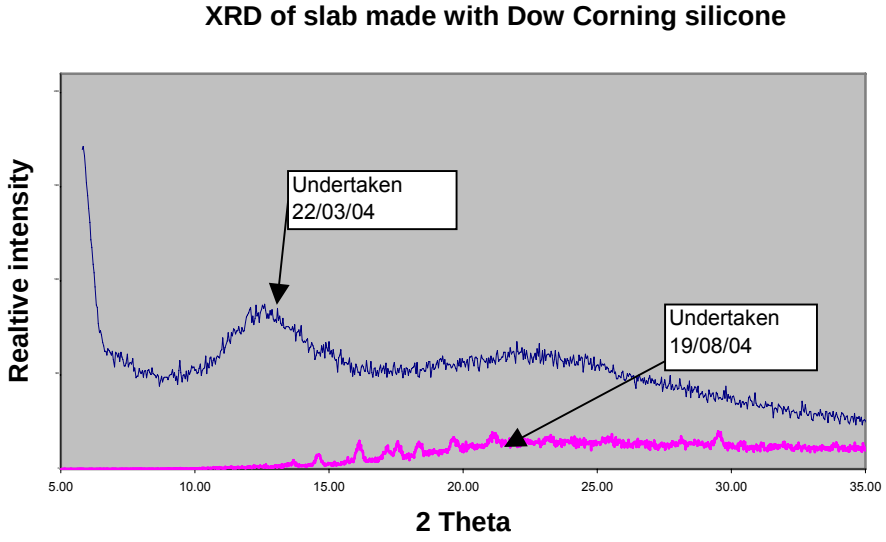


Figure 4.7 XRD diffractogram of slab made with Dow Corning silicone showing the variation in intensity between scans undertaken five months apart on the same sample. Peaks in the 19/08/04 scan are from progesterone polymorphism.

4.1.1.3 XRD on slabs made with 30 % w/w of progesterone

CIDR 1380 and 1900 inserts are made with 10 % w/w progesterone. CIDR inserts are not made with higher drug loadings as work by Macmillan et. al, (Macmillan, et. al., 1990) found that increasing CIDR drug loadings increased the 'in vivo' drug release rate, but did so at a diminishing rate, so that a CIDR 1900 insert with 10 % w/w progesterone had a similar blood plasma level to a CIDR insert with 13.3 % w/w progesterone. The effect on secondary blooming with an increased drug loading is unknown. To investigate the effect of increasing the drug loading on progesterone polymorphism, slabs were made with 30 % w/w progesterone using Dow Corning silicone (batch 0001854557) and the alternative supplier

silicone. Progesterone from batch 15KDY was used. Slabs were scanned after manufacture and at least one month after manufacture. The area of the slab that was scanned was fixed between scans. XRD diffractograms are shown in Figures 4.8 to 4.11.

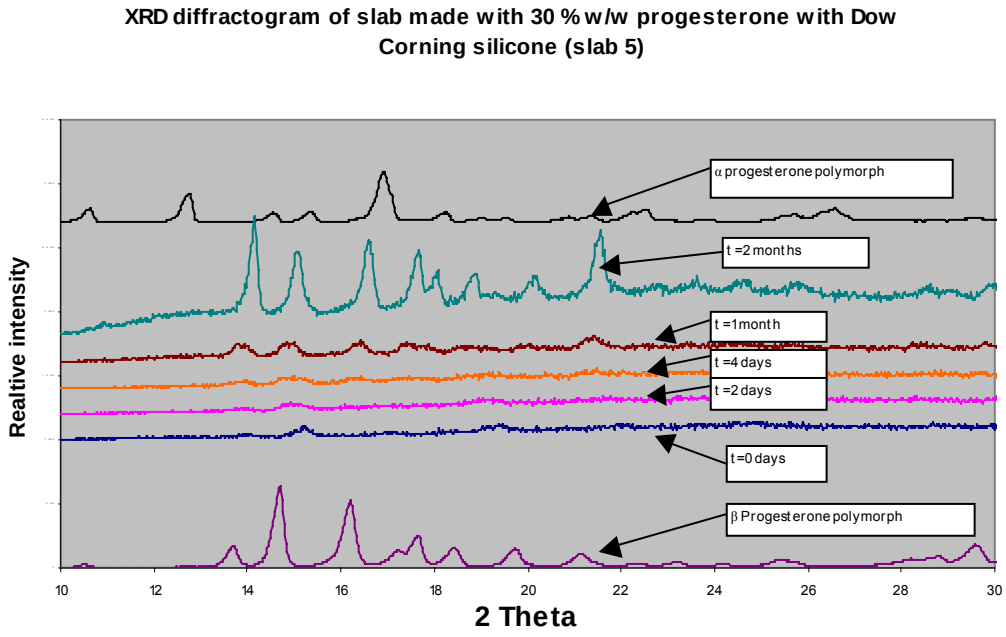


Figure 4.8 XRD diffractogram of slab 5 made with Dow Corning silicone with 30 % w/w progesterone.

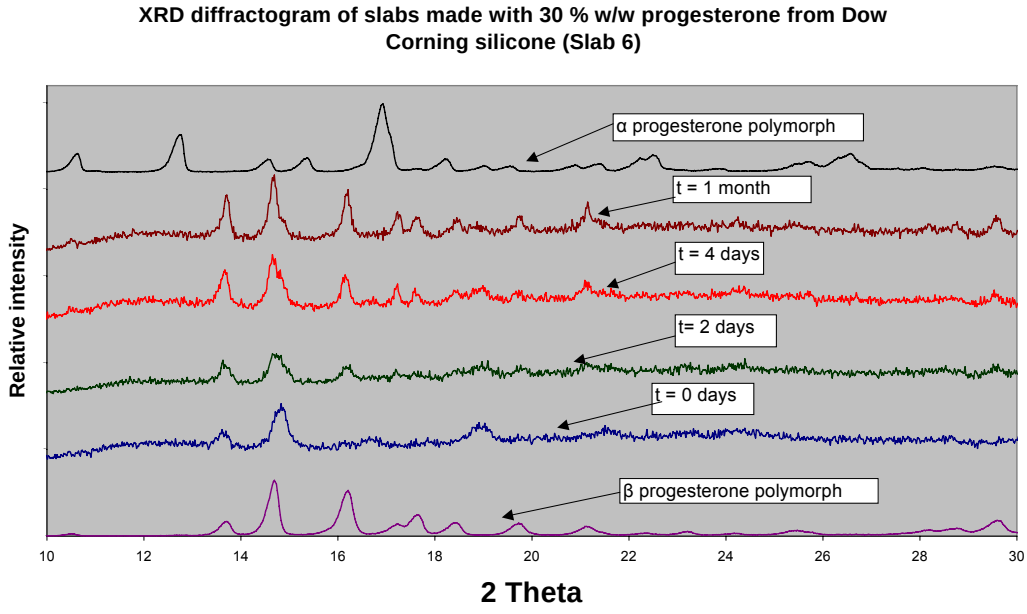


Figure 4.9 XRD diffractogram of slab 6 made with Dow Corning silicone with 30 % w/w progesterone.

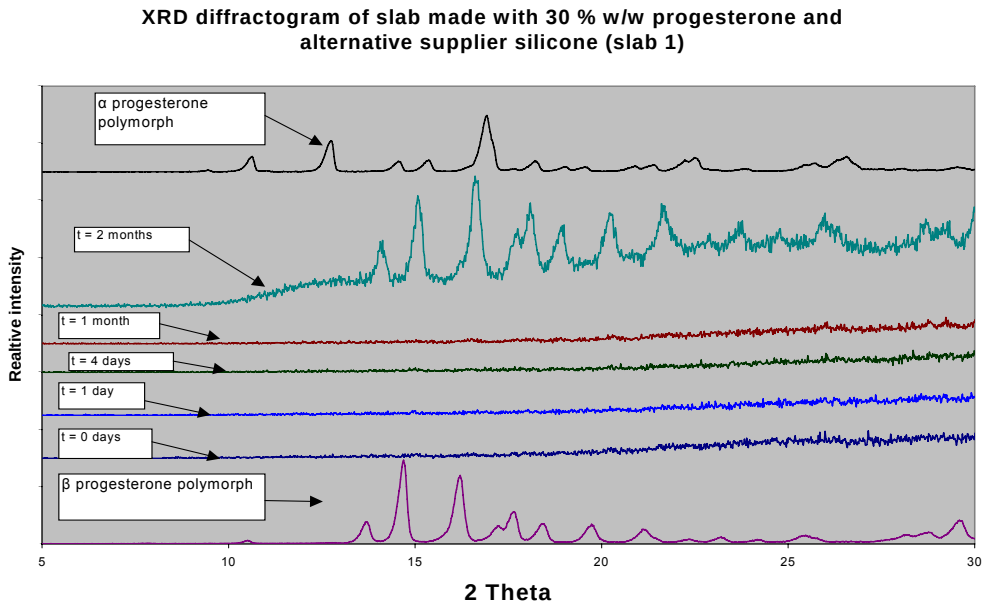


Figure 4.10 XRD diffractogram of slab 7 made with alternative supplier silicone containing 30 % w/w progesterone.

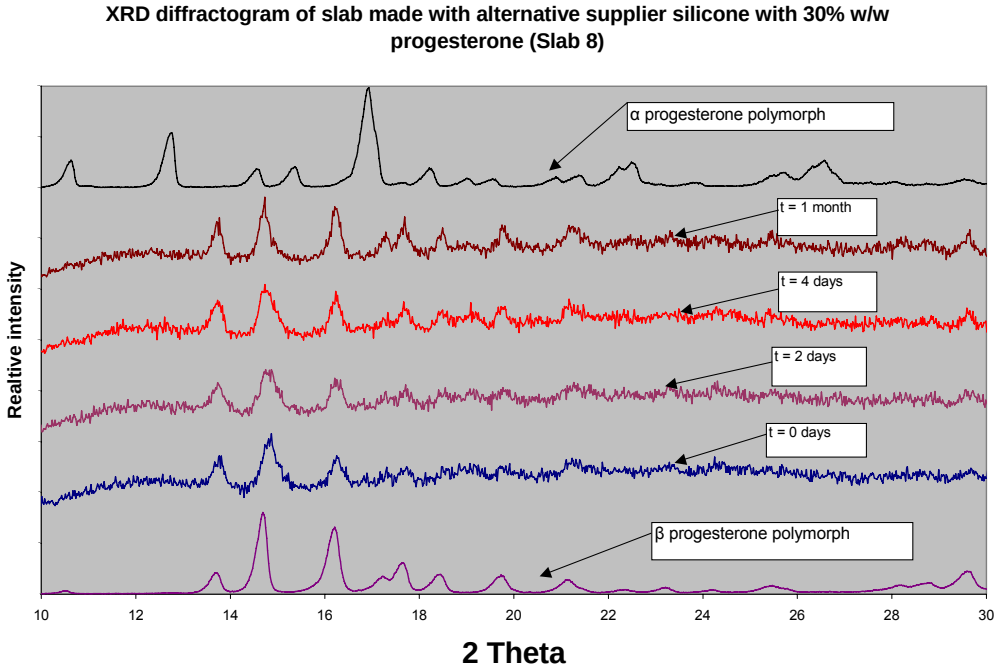


Figure 4.11 Scan of slab 8 made with alternative supplier silicone containing 30 % w/w progesterone.

From Figure 4.8 to 4.11 it is clear that slabs made with 30 % w/w progesterone exhibited different surface XRD diffractograms. Slabs 5, 6, 7 and 8 all exhibit the

β progesterone polymorph (Figures 4.8, 4.9, 4.10 and 4.11). Some slabs exhibited signal immediately (slabs 6 and 8) while other slabs (slabs 5 and 7) took one month before a signal was observed. There appears to be no difference in progesterone polymorphism due to the increased drug loading.

4.1.2 XRD scans on mottled and non-mottled CIDR insert regions

As progesterone exists in two common polymorphic forms (α and β), it is useful to know the particular polymorphic form that exists on mottled and non-mottled sections of CIDR inserts. To investigate this, XRD scans were undertaken on mottled and non-mottled sections of CIDR inserts. The samples were then wiped with ethanol to remove surface progesterone, and reanalysed. All samples scanned were CIDR 1380 insert's that were at least two years old. The level of surface progesterone on the samples is unknown unless stated. The removal of the progesterone allows a determination of the polymorphic form of progesterone below the surface of the device without interference from signals associated with surface progesterone.

XRD analysis found the α progesterone polymorph on the white non-mottled regions of the CIDR insert (Figure 4.12) whereas the mottled (progesterone depleted) regions showed the β progesterone polymorph (Figure 4.13). After wiping the mottled regions continued to exhibit the β progesterone polymorph, and the white non-mottled regions ceased to give signal. A summary of the XRD diffractograms and the results are summarised in Table 4.1. No polymorphic transformation will have occurred to create the β progesterone polymorph during the process of wiping with ethanol, as Muramatsu et. al., (Muramatsu et. al., 1979) found that re-crystallisation of progesterone in ethanol would produce the α progesterone polymorph.

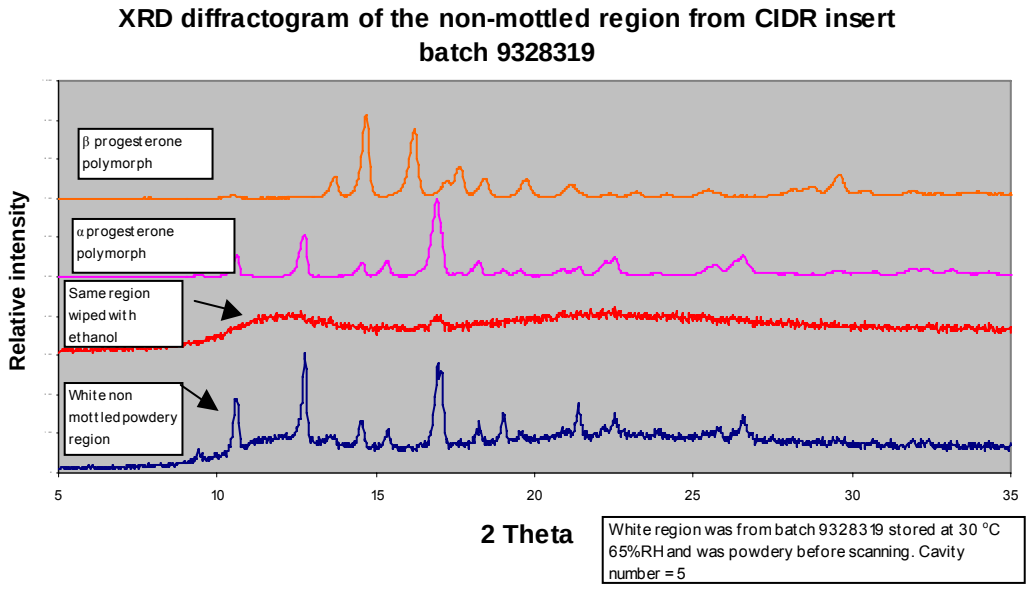


Figure 4.12 XRD diffractogram of a white non-mottled region of CIDR insert before and after wiping with ethanol.

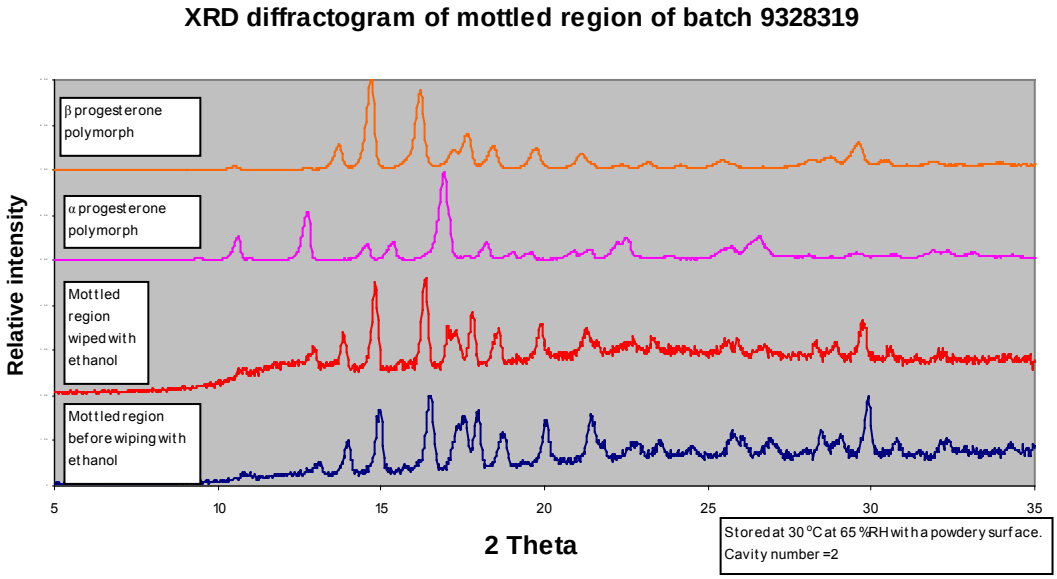


Figure 4.13 XRD diffractogram of a mottled region of CIDR insert before and after wiping with ethanol.

Table 4.1 Summary of XRD diffractograms on mottled and non-mottled regions of CIDR inserts.				
Sample	**Section**	**Polymorph initially exhibited**	**Polymorph exhibited after ethanol wiping.**	**Comments**
B06301 – cavity number 4 scanned 24/09/04	Mottled	β polymorph	β polymorph	-
B06301 – cavity number 1 scanned 24/09/04	White region	α polymorph	None determined (Loss of signal)	-
Batch 9328319 cavity number 2 scanned 11/10/04	Mottled region – powdery surface	β polymorph	β polymorph	Stored at 30 ^{o}C and 65 % RH See Figure 4.13.
Batch 9328319 cavity number 5	White powdery surface	α polymorph	One peak – possibly an artefact from α polymorph.	Stored at 30 ^{o}C and 65 % RH See Figure 4.12.
Batch 9330319	Partly mottled sample	β polymorph	β polymorph	Stored at 30 ^{o}C and 65 % RH.

From Table 4.1 it is clear that the mottled regions exhibit the β progesterone polymorph, which is thermodynamically less stable than the α progesterone polymorph (Wang et. al., 2000). The mottled regions are progesterone depleted (Rathbone & Ogle, 2000) whereas the white non-mottled regions are not. However it is interesting that removal of the surface progesterone from the non-

mottled regions results in a loss of signal (Figure 4.12) despite the fact that these regions are known to contain a higher drug loading compared to mottled regions (Rathbone & Ogle, 2000). It is possible that the progesterone is in an amorphous form in the white regions, whereas in the mottled regions the progesterone has crystallised.

DSC work by Rades and McFetridge found both the α and β progesterone polymorphs in the mottled and non-mottled regions (Rades, & McFetridge, 2003), however they found that XRD detected the β progesterone polymorph but not the α progesterone polymorph. Rades & McFetridge (Rades & McFetrige, 2003) also found that the powder on the surface of the insert was the α progesterone polymorph. It is possible that the α progesterone polymorph detected in the non-mottled regions is from this powder.

The detection of the β progesterone polymorph in mottled regions of the CIDR insert agrees with the XRD results from Rades & McFetridge (Rades & McFetridge, 2003). Furthermore, the detection of the β progesterone polymorph after wiping down with ethanol to remove surface progesterone would be expected from Rades and McFetridge's DSC results, which would analyse the whole sample rather than just the surface. It should be noted however that Rades & McFetridge (Rades & McFetridge, 2003) also noted the existence of the α progesterone polymorph in these regions (by DSC), which could indicate that the α progesterone polymorph is not found near the surface of mottled regions (that would be detected by XRD). The surface regions of the CIDR insert would cool faster compared to areas inside the CIDR insert. This would result in the production of the β progesterone polymorph (Legendre, et. al., 2003), whereas sections of the CIDR insert close to the spine would cool slower and form the α progesterone polymorph (Legendre, et. al., 2003). This however does not explain the appearance of the β progesterone polymorph on the surface of the CIDR insert in the mottled areas of the CIDR insert. Since the mottled areas have a lower concentration of progesterone (Rathbone, & Ogle, 2000) it could also be possible that the progesterone in these regions is migrating to the surface as the α progesterone polymorph leaving the β progesterone polymorph in the mottled regions.

It could be reasoned that the differences in progesterone polymorphism between mottled and non-mottled areas of the CIDR insert is from one polymorph being denser that the other polymorph, which would indicate that either a mottled or non mottled region of the CIDR insert favoured denser progesterone polymorph. Muramatsu et. al. (Muramatsu et. al., 1979) discovered that there was very little difference in the unit cells volumes of the two progesterone polymorphs (α and β progesterone polymorphs), but that the differences in polymorphism occurred from dissimilar modes of packing. Hence a hypothesis based on unit cell volume differences is not supported.

4.2 The effect of slow cooling on progesterone polymorphism

4.2.1 Slow cooling of progesterone in an oven

Work by Legendre, et. al. (Legendre, et. al., 2003) and Duclos et. al, (Duclos, et. al., 1991) found that slow cooling of progesterone would result in the formation of the α progesterone polymorph, whereas rapid cooling would form the β progesterone polymorph. Reardon (Reardon, 2003a) found that slow cooling reduced mottling.

In order to investigate the effect of slow cooling progesterone at different rates, a sample of Diosynth progesterone (batch L0024354) was melted in a Contherm Digital Series oven at 150 ^{o}C. The sample was then cooled at ~ - 0.9 ^{o}C/minute until the temperature reached 107 ^{o}C after 45 minutes. Then the temperature was held at approximately 100 ^{o}C for 20 minutes and was then allowed to cool further until solid. The sample (called the stock) was in a tin foil cup and crystallised, before another progesterone sample (from the same material crystallised, which was being used for other purposes).

The stock progesterone sample was then ground using a mortar and pestle into a powder and underwent XRD analysis. The results are shown in Figure 4.14 and these indicate that α progesterone polymorph was formed instead of the

β progesterone polymorph (which is exhibited on freshly made CIDR inserts and is formed when progesterone is melted (Muramatsu, et. al, 1979)). Legendre et. al, (Legendre et. al., 2003) and Duclos et. al, (Duclos et. al., 1991) determined that if the cooling rate was low the α polymorph would form rather than the β progesterone polymorph.

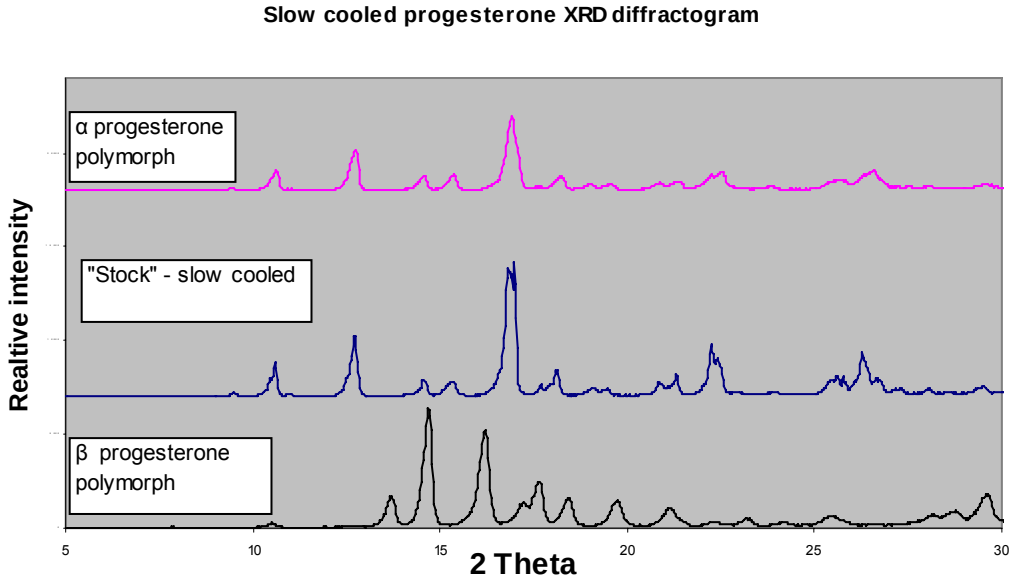


Figure 4.14 XRD diffractogram of progesterone that was allowed to slowly cool from 150 to 100 ^{o}C over 20 minutes.

4.2.2 DSC experiments on progesterone that was slow cooled (without a pause in cooling)

XRD can be used to detect progesterone polymorphism, however use of a DSC instead of XRD can both detect the progesterone polymorph and undertake the heating and cooling steps far more accurately, using less progesterone and with no operator involvement after the start of the experiment. However with a DSC method it is not possible to examine the melt during cooling to determine if crystallisation has occurred before the next reheating step.

Samples of progesterone were analysed under different cooling rates. Samples were Pfizer progesterone batch 79HWH and Diosynth Progesterone batch L00024354. Some repeat analysis was undertaken. The experimental heating and cooling steps are shown in Table 4.2, while Table 4.3 lists the set up conditions

and results from these experiments. Figure 4.15 shows a typical DSC diffractogram upon reheating from the sample cooled at –50 ^{o}C/minute.

Table 4.2 Experimental method used on fast and slow cooling of progesterone.
1. Heat at 35 ^{o}C /minute to 115 ^{o}C.
2. Heat at 4 ^{o}C/minute to 145 ^{o}C.
3. Hold for 2 minutes 145 ^{o}C.
4. **Cooling steps which vary depending on the method (see Table 4.3)**
5. Hold for 20 minutes at 26 ^{o}C.
6. Heat at 20 ^{o}C/minute to 95 ^{o}C.
7. Heat at 5 ^{o}C/minute to 140 ^{o}C.

Table 4.3 DSC experimental set up and results for different cooling routes of progesterone.

Test and progesterone batch	**Cooling Steps**	**Peaks on 1st heating (^{o}C)**	**Peaks on 2nd heating (^{o}C)**	**Heating Endotherm**
Progesterone slow cooling (79HWH).	1. Cool at -1 ^{o}C/minute to 80 ^{o}C. 2. Cool at -20 ^{o}C/minute to 26 ^{o}C.	131.0 ^{o}C	123.4 ^{o}C	51.4 ^{o}C
Progesterone slow cooling (8.3 mg) (Diosynth).	Same as above	132.9 ^{o}C	125 ^{o}C (area of 76.8 J/g)	56.0 ^{o}C (area -50.9 J/g)
Progesterone – 20 ^{o}C/minute cooling (79HWH).	1. Cool at -20 ^{o}C/minute to 26 ^{o}C.	131.7 oC	124.6 ^{o}C	57.3 ^{o}C

Progesterone – 30 °C/minute cooling (79HWH).	1. Cool at -30 °C/minute to 120 °C. 2. Cool at -5 °C/minute to 26 °C.	131.6 °C	107.2 °C and 109.7 °C	Observed but the temperature was not measured due to loss of data.
Progesterone – 50 °C/minute cooling (3.9 mg) (79HWH).	1. Cool at -50 °C/minute to 26 °C.	132.1 °C	124.8 °C (area of 73.3 J/g)	58.3 °C
Progesterone – 50 °C/minute cooling (8.9 mg) (Diosynth).	Same as above.	132.9 °C (area 85.6 J/g)	126.7 °C (area 77.6 J/g)	58.2 °C (area -55.6 J/g)

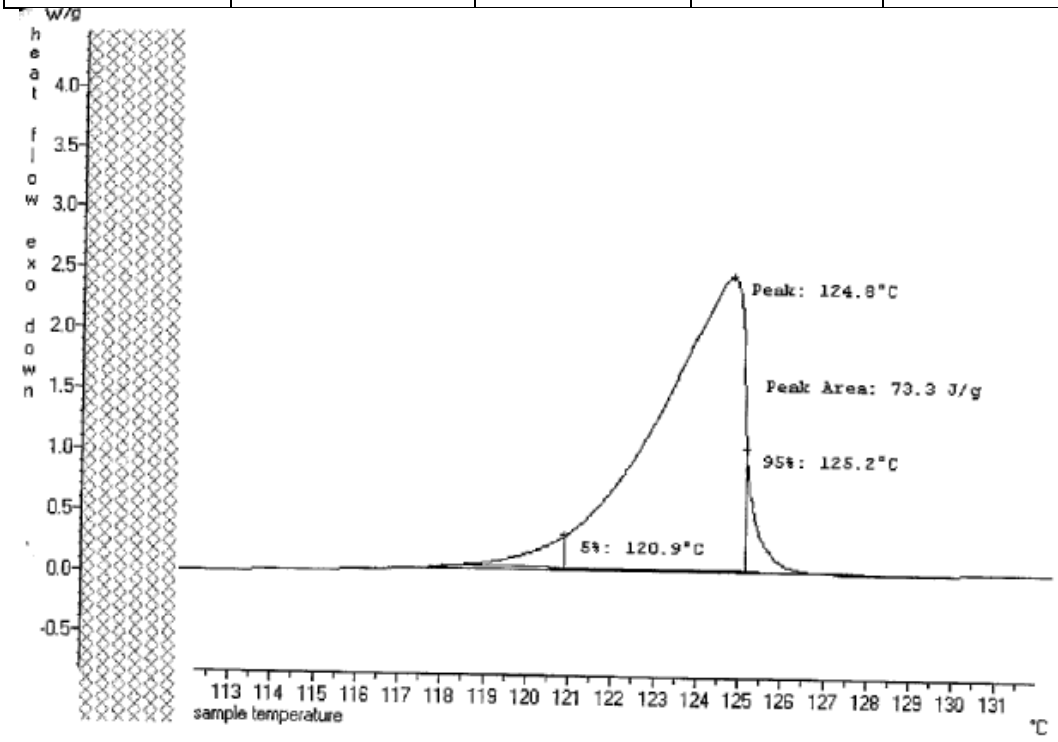


Figure 4.15 Spectra from the DSC of a sample of progesterone quickly cooled at - 50 °C/minute then reheated. See Table 4.3. Spectra of the sample upon reheating.

Results from Table 4.3 show that despite the differences in the rate of cooling, all but one progesterone sample exhibited the β progesterone polymorph upon reheating. The exception being the sample cooled at –30 ^{o}C/minute, which had peaks at 107.2 and 109.7 ^{o}C on second reheating. These peaks would be from the melting of the progesterone polymorphic Forms III and IV respectively. Kuhnert-Brandstätter et. al., (Kuhnert-Brandstätter et. al., 1965) noted that the appearance of the β progesterone polymorph in the melt resulted in all other polymorphic forms of progesterone being converted to the β progesterone polymorph. The appearance of these peaks could be due to the two stage cooling process where the β progesterone polymorph has not yet formed, or it could be related to random phenomena. Kuhnert-Brandstätter et. al. (Kuhnert-Brandstätter et. al., 1965) notes that in the melt of the β progesterone polymorph, crystals of the β progesterone polymorph do not always appear allowing, other polymorphs to be detected.

Overall the result is different from the one that would be expected. Legendre et. al. (Legendre et. al., 2003) and Duclos et. al. (Duclos et. al., 1991), noted that if the cooling rate was low, the progesterone α polymorph would form. This is not observed in the slow cooled sample. Possible causes could be due to the fact that the sample did not have enough time to crystallise after cooling. However the appearance of the β progesterone polymorph in the samples that were quickly cooled is in line with the work of Legendre et. al. and Duclos et. al..

It is also observed that all the samples had an endotherm upon reheating at ~50 to 60 ^{o}C (see Figure 4.16). This would be from the crystallisation of progesterone and agrees with the DSC work by Muramatsu et. al, (Muramatsu, et. al. 1979) who found that when cooling molten progesterone at rates between –1 to –10 ^{o}C/minute supercooling of the sample resulted, with crystallisation occurring during the reheating step. Crystallisation temperatures ranged from, 37 to 120 ^{o}C depending on the experimental conditions, but regardless of conditions the β progesterone polymorph was formed.

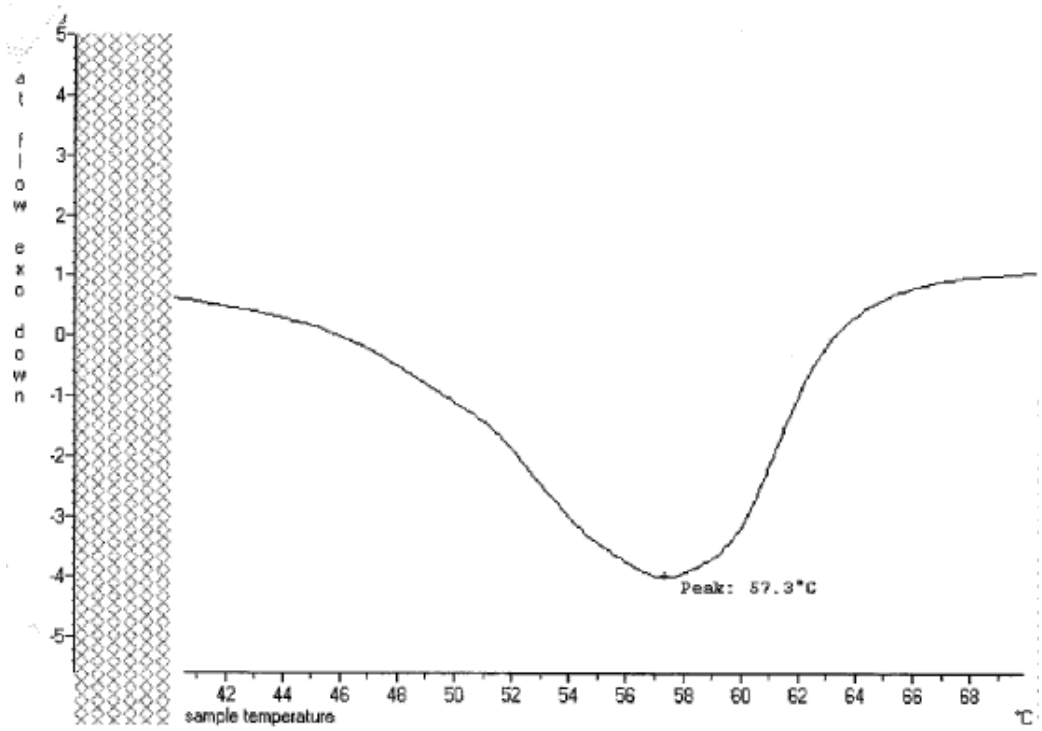


Figure 4.16 Crystallisation peak from DSC scan of sample of progesterone that was cooled at –20 ^{o}C. Spectra is of the crystallisation peak during reheating.

4.3 The effect of slow cooling on secondary blooming and mottling in slabs

Work by Reardon (Reardon, 2003a) has shown that mottling is inhibited if the CIDR insert is slow cooled after manufacture. The α and β polymorphs of progesterone have different melting points (α = 131 ^{o}C and β =123 ^{o}C (Kuhnert-Brandstätter et. al., 1965)). It was suggested by Reardon that if post-cure samples were held at temperatures that were either, above, between or below the two polymorphic melting points that blooming and mottling may be inhibited. Hence a study was undertaken to determine if samples cooled under these conditions had different levels of secondary blooming and mottling compared with controls.

Slabs were cured and cooled in two Contherm ovens. A cure time of 120 seconds was used, and the temperature in the curing oven (as recorded from the oven temperature read out) would reach ~175 ^{o}C during curing. Immediately after curing slabs were placed into the cooling oven, set at a temperature above, below or between the melting points of the α and β progesterone polymorphs. The temperature in the cooling oven was monitored with a Fluke 52 thermometer

(thermocouple) recording the temperature on the shelf on which the slabs were placed. The length of time that slabs were placed in the cooling oven varied and after their removal, slabs were left to cool on a laboratory bench. Control slabs were left to cool on the laboratory bench immediately after cure, rather than being placed in the cooling oven. The control slabs were cured simultaneously with the slabs being analysed.

Table 4.4 lists the various conditions under which the slabs were made. The temperature in the cooling oven at zero minutes is excluded, as opening the oven door (to place samples in the oven) will decrease the oven temperature. Slabs from lots 21 to 24 were manufactured on the same day and initial results from the manufacture of these slabs suggested that a cooling halt above the α progesterone polymorph melting point would have the greatest effect in reducing secondary blooming and mottling, so the remaining slabs were made with halt temperatures above the α progesterone polymorph melting point.

Table 4.4 Slabs manufactured under different cooling conditions.

Lot and slab colour	**Cooling oven temperature range (^{o}C) (temperature at t = 0 seconds excluded)**	**Time in Cooling oven (minutes)**	**Number of slabs in cooling oven**	**Number of Control slabs (cooled on bench with out placement into cooling oven)**
Lot 21 - Black	132 ^{o}C - 136.7 ^{o}C	10	6	2
Lot 22 - Black	121.7 ^{o}C - 126.1 ^{o}C	10	6	2
Lot 23 - Black	110.6 ^{o}C - 113.2 ^{o}C	10	6	2
Lot 24 - Black	124.6 ^{o}C - 127.0 ^{o}C	3	6	4
Lot 25 - White	131.9 ^{o}C - 135.7 ^{o}C	10	6	2
Lot 26 - Black	136.4 ^{o}C -137.0 ^{o}C	10	6	2
Lot 27 - Black	133.6 ^{o}C - 135.4 ^{o}C	4	6	2
Lot 28 - Black	130.5 ^{o}C - 135.1 ^{o}C	2	6	2

Slabs from lots 21 to 24 were made from Pfizer progesterone from batch 24JAF and silicone from Dow Corning Q7-4840 batch 0001854557. Slabs from lots 25 to

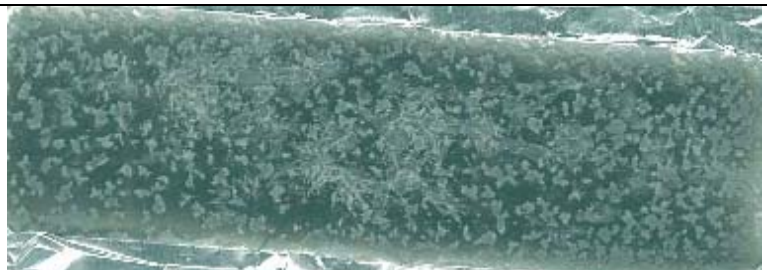
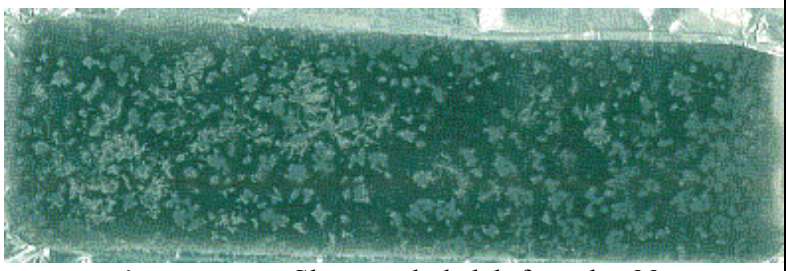
28 were made from Diosynth progesterone from batch L00024354, and silicone from Dow Corning Q7-4840 batch 0001689247.

Degradation of progesterone occurs if the progesterone is heated for a long period of time and results in discolouration of slabs (yellowing). Colour tests were undertaken on white slabs from lot 25 and it was found that the slabs complied with DEC Manufacturing's CIDR insert colour test (STP021), indicating that progesterone degradation had not occurred.

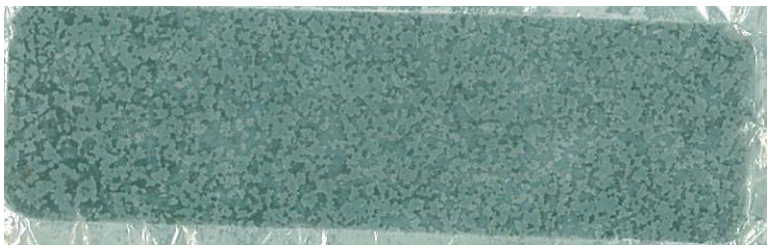
Figure 4.17 describes the differences between the control slabs and the slabs that underwent controlled cooling under various conditions as described in Table 4.4. Slabs made were not placed into the stability oven.

Figure 4.17 Visual observations of slabs made with various cooling conditions ~15 months after manufacture. Slabs shown are representative.

Lot	**Maximum cooling oven temperature (^{o}C) and cooling time (minutes)**	**Slab observations (not to scale)**
Lot 21	136.7 ^{o}C (ten minutes)	 **Figure 4.17.1** Control Slab from lot 21 (bench cooled).  **Figure 4.17.2** Slow cooled slab from lot 21. A portion of the sample between the pen lines was analysed with XRD (see Section 4.3.1).

		While both sets of slabs exhibit secondary blooming, there is far more secondary blooming on the control (Figure 4.16) slabs compared with the slow cooled slabs (Figure 4.17). 'Island formations' also exist on the surface of the slabs.
Lot 22	126.1 ^{o}C (ten minutes)	 **Figure 4.17.3** Control slab from lot 22 (bench cooled).  **Figure 4.17.4** Slow cooled slab from lot 22. Both sets of slabs exhibit mottling on the surface, however there is more secondary blooming on the control slabs (Figure 4.19) compared with the slow cooled slabs (Figure 4.20). 'Islands formations' also exist on the surface of the slabs.
Lot 23	113.2 ^{o}C (ten minutes)	No difference observed between slabs of control and slow cooled slabs. Secondary blooming and mottling on all slabs.
Lot 24	127.0 ^{o}C (three minutes)	No difference observed between slabs of control and slow cooled slabs. Secondary blooming and mottling on all slabs.

Lot 25	135.7 °C (ten minutes)	Control slabs  **Figure 4.17.5.1** Control Slab used in this test scanned 15 months after manufacture (bench cooled). Tin foil removed.  **Figure 4.17.5.2** Control Slab used in this test scanned 15 months after manufacture (bench cooled). Scanned with tin foil attached. Slow cooled slabs  **Figure 4.17.6.1** Slab from lot 25 that underwent slow cooling. Tin foil removed.  **Figure 4.17.6.2** Slab from lot 25 that underwent slow cooling. Scanned with tin foil. Removal of the tin foil from the slab showed that the control slab (Figure 4.16.5.1) had translucency (mottling), whereas the slow cooled slab (Figure 4.15.6.1) possessed

		far less translucency and is basically white. It is noted that the slab (slow cooled) does possess the 'island formation', which is observed with far greater clarity on the control slab.
Lot 26	137.0 ^{o}C (ten minutes)	 **Figure 4.17.7:** Slab from lot 26 that had been slow cooled.  **Figure 4.17.8:** Slab from lot 26 control (bench cooled). The control (Figure 4.16.8) slabs have more secondary blooming, crystals, and appears to be 'whiter' that the slow cooled slabs (Figure 4.16.7), which no less secondary blooming. Both slabs show 'island formations'.
Lot 27	135.4 ^{o}C (four minutes)	No difference observed between slabs of control and slow cooled slabs. Mottling but no secondary blooming observed on all slabs
Lot 28	135.1 ^{o}C (two minutes)	No difference observed between slabs of control and slow cooled slabs. Mottling but no secondary blooming observed on all slabs

It is evident from Figure 4.17 that the cooling conditions do have an effect on the level of secondary blooming and mottling, with slabs that were cooled for ten minutes above the melting point of α progesterone having less secondary blooming and mottling compared with the controls. It is also of note that the slabs that were slow cooled between the melting point of the α and β progesterone polymorphs (lot 22) also exhibited less secondary blooming.

4.3.1 XRD analysis of slabs that were slow cooled

Slow cooled slabs from lots 21 and 26 were analysed using XRD. Figure 4.17.2 shows the region that was scanned on the slab from lot 21 and Figure 4.18 shows the area scanned by XRD for lot 26. Scans were undertaken less than a month after the manufacture of the slabs. The scans are shown in Figure 4.19.

Figure 4.18 Slab from lot 26 that had been slow cooled. A portion of the area between the pen lines as illustrated was scanned using XRD. The white region to the top of the slab between the pen lines is a region of the mould that was not filled with silicone.

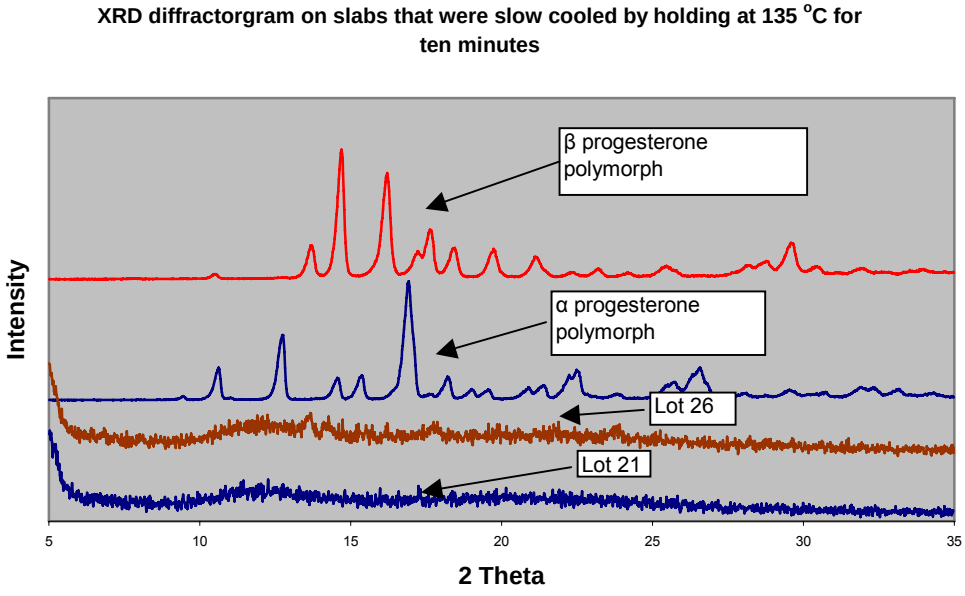


Figure 4.19 XRD diffractogram of slabs that were slow cooled at by holding at 135 °C for ten minutes.

Both diffractograms show an amorphous sample. Lot 26 had two possible peaks at 13.36 ° and 23.91 ° 2θ (Figure 4.26). The lack of signal is due to the low levels of crystalline surface progesterone on the slabs (secondary blooming). This result

indicates that if any progesterone is present it must be either be amorphous, dissolved in the silicone or present below the surface of the silicone rubber (hence not detectable by XRD).

4.3.2 Slow cooling on progesterone

Since holding the temperature of slabs at ~135 ^{o}C for ten minutes has been found to have an effect on the level of secondary blooming and mottling (see Section 4.2.1), a sample of progesterone was analysed using the same cooling method to see if any polymorphic changes occurred in the progesterone.

Progesterone from Diosynth batch L00024354 was melted in a Contherm oven at ~136 ^{o}C, before being removed from the oven and left to cool on the bench. The laboratory was dusty on the day of the experiment, and dust may provide a site of nucleation for progesterone crystallisation. The sample did reach a temperature of ~140 ^{o}C in the oven requiring the oven door to be opened to decrease oven temperature (at around eight minutes). The oven temperature was 136.2 ^{o}C at the time of sample removal. The sample was analysed using XRD and the results are shown in Figure 4.20.

A further sample of progesterone from Diosynth batch L00024354 was melted in the Contherm oven at ~ 140 ^{o}C. The oven was turned off and allowed to cool. After 50 minutes of cooling the oven temperature was 103.3 ^{o}C and no crystallisation had occurred when the door was opened. At 60 minutes (95.1 ^{o}C) the sample was removed and showed some crystallisation. The results from this experiment are shown in Figure 4.20.

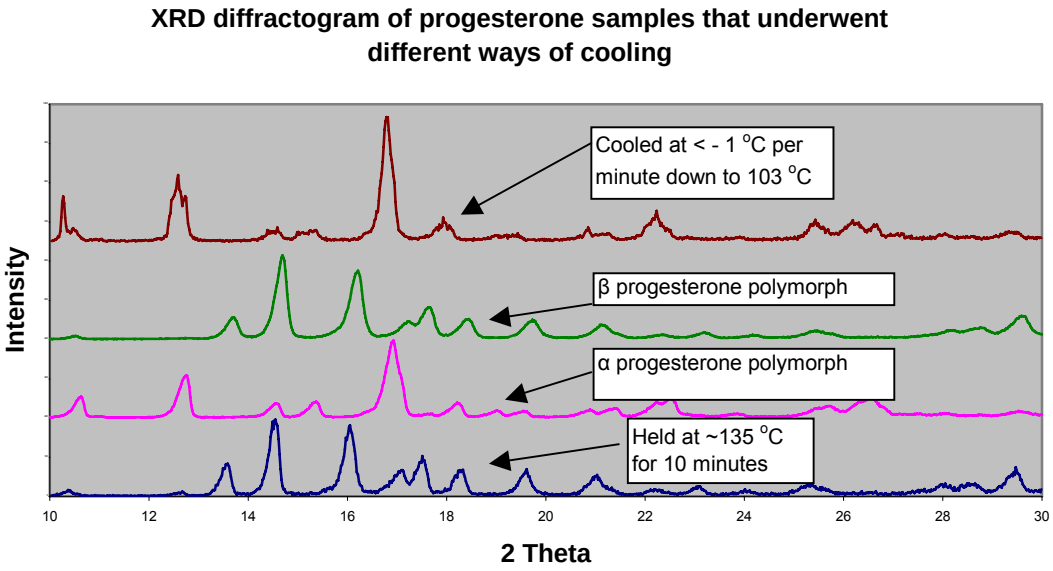


Figure 4.20: XRD diffractogram of samples of progesterone that had undergone different slow cooling routines.

From Figure 4.20 it is clear that the progesterone that underwent slow cooling at < -1 °C /minute exhibited the α progesterone polymorph whereas the sample held at 10 °C exhibited the β progesterone polymorph. This result agrees with the results from Duclos et. al. (Duclos et. al., 1991), and the results in Section 4.2.1. Legendre et. al, (Legendre et. al., 2003) predicted that a slow rate of cooling forms the α progesterone polymorph. It is noted that holding the sample of progesterone at 135 °C formed the β progesterone polymorph, indicating that halting the cooling rate does not have an effect on progesterone polymorphism.

4.3.3 DSC controlled slow cooling experiments (with cooling pause at specified temperatures)

In order to gain a further understanding on progesterone under slow cooling with a halt at different temperatures, samples of progesterone were placed into a DSC and then run through a melt and controlled cooling cycle before being reheated to determine the polymorph of progesterone formed. Table 4.5 describes the basic temperature program. Progesterone was used from Pfizer batches 79HWH and 24JAF and Diosynth progesterone from batch L00024354. The machine setup

conditions and results are shown in Table 4.6. Samples were analysed twice. Figure 4.21 shows a typical DSC spectra upon the reheating of a sample that had been previously melted, cooled to 125 ^{o}C, held at that temperature, cooled to 26 ^{o}C and then reheated.

Table 4.5 Slow cooling (with a cooling halt) temperature program. Results are in Table 4.6.
1. Heat at 5 ^{o}C /minute to 190 ^{o}C.
2. Hold for 2 minutes at 190 ^{o}C.
3. Cool at –5 ^{o}C /minute to target temperature (that cooling is halted at).
4. Hold for ten minutes at 26 ^{o}C.
5. Cool at -5 ^{o}C/minute from target temperature to 26 ^{o}C.
6. Hold at 26 ^{o}C for ten minutes.
7. Heat at 5 ^{o}C /minute to 150 ^{o}C.

Table 4.6 DSC experimental set up and results for progesterone samples undergoing slow cooling with a cooling halt. Refer to Table 4.5 for the temperature program.

Test	**Progesterone used**	**Peaks on 1st heating (^{o}C)**	**Peaks on 2nd heating (^{o}C)**	**Exotherm peaks on reheating**
Hold at 120 ^{o}C	Pfizer 79HWH	132.3 ^{o}C	106.3 ^{o}C, 109.5 ^{o}C	59.1 ^{o}C, 62.1 ^{o}C
	Pfizer 24JAF 1.8 mg	130.8 ^{o}C	105.6 ^{o}C, 108.6 ^{o}C	72.0 ^{o}C (area -41.5 J/g)
Hold at 125 ^{o}C	Pfizer 79HWH	131.1 ^{o}C	105.7 ^{o}C, 108.5 ^{o}C (See Figure 4.20)	Observed, between 60 to 70 ^{o}C.
	Pfizer 24JAF 2.6 mg	131.4 ^{o}C (area 84.3 J/g)	105.5 ^{o}C, 109.2 ^{o}C	54.2 ^{o}C (area –38.6 J/g)

Hold at 130 °C	Pfizer 79HWH	131.6 °C	108.9 °C	Observed ~ 90 °C
	Pfizer 24JAF 6.4 mg	132.9 °C (area 86.7 J/g)	106.5 °C, 109.2 °C	63.2 °C (area 63.2 °C)
Hold at 135 °C	Pfizer 24JAF 1.1 mg	130.2 °C (area 77.6 J/g)	105.0 °C, 108.5 °C	59.0 °C (area –34.3 J/g)
	Diosynth L00024354 2.5 mg	131.4 °C (area 84.9 J/g)	108.0 °C, 110.1 °C	60.4 °C (area –44.0 J/g)

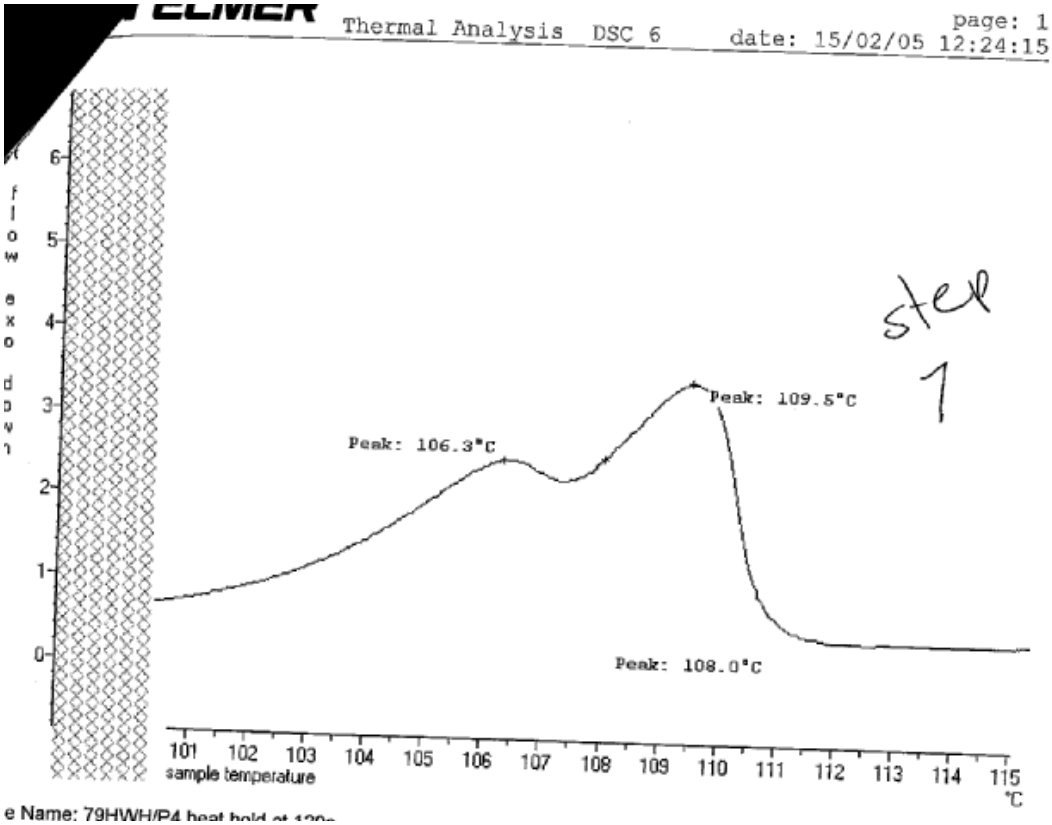


Figure 4.21 DSC analysis of progesterone that had been control cooled and then reheated. Figure shows the exotherm upon the reheat.

It is clear that the progesterone analysed was in the α progesterone polymorphic form as this is observed in the initial melt (see Table 4.6) in all samples analysed.

It is also clear that the samples gave similar melting peaks upon reheating, except for the 1 st sample that was held at 130 °C (Pfizer batch 79 HWH). It is known that the melting point of Form IV progesterone polymorph is 106 °C (Kuhnert-Brandstätter et. al., 1965) and for Form III is 111 °C (Kuhnert-Brandstätter et. al., 1965) so it most possible that the progesterone peaks observed on the second reheating are from these polymorphic forms. Kuhnert-Brandstätter et. al. (Kuhnert-Brandstätter et. al., 1965) notes that the melt of the α progesterone polymorph acts in the same manner as the β progesterone polymorph, and that in the melt of the β progesterone polymorph, crystals of the β progesterone polymorph do not always appear.

It was thought that the ten minutes hold time at 26 °C was too short as the step may have had an effect on polymorphism, as the progesterone would have not had enough time to crystallise. In order to investigate this fully a 1.9 mg sample of Pfizer progesterone (batch 24JAF) was analysed in the same manner as the 'Hold at 135 °C sample' however the hold time at 26 °C after cooling (step 6 in Table 4.5) was increased to 60 minutes. It was found that there was no difference in progesterone polymorphs produced in this sample from the two samples previously analysed, which had a cooling halt at 135 °C and a shorter hold time. However this sample did possess an exotherm upon reheating with conjoined peaks at 57.5 °C and 60.2 °C, which was observed in other progesterone samples tested in Table 4.7. The variations in the exotherm peak is in line with work by Muramatsu et. al, (Muramatsu, et. al. 1979) who found that there was a range of progesterone crystallisation temperatures upon reheating.

In Sections 4.2.1 and 4.3.2 it was found that a slow rate of cooling (-1°C/minute) did produce the α progesterone polymorph. However DSC work in Section 4.2.2 did not. An experiment was set up to determine if the addition of a halt in cooling at 135 °C would have an effect on the polymorph of progesterone produced in samples of progesterone from Pfizer batch 24JAF. The temperature program in Table 4.7 was used.

Table 4.7 Temperature program for samples of progesterone that were slow cooled.

1. Heat to 145 ^{o}C at 5 ^{o}C/minute.
2. Hold for two minutes at 145 ^{o}C.
3. Cooling to 26 ^{o}C steps (see Table 4.9).
4. Hold for ten minutes at 26 ^{o}C.
5. Heat to 145 ^{o}C at 5 ^{o}C/minute.

Table 4.8 shows that one sample of progesterone cooled at –1 ^{o}C/minute with a halt at 135 ^{o}C for ten minutes did produce both the α and the β progesterone polymorphs upon reheating, although this effect was not reproducible. Legendre et. al, (Legendre, et. al., 2003) notes that DSC is not well suited to gain good reproductions of results due to the small cell volume. The exotherm peaks are found during the cooling step indicating that the progesterone has undergone a crystallisation during the cooling. Exotherms were observed upon reheating in the samples that had a halt in cooling at 135 ^{o}C (see Table 4.8). The progesterone samples in Table 4.1 in Section 4.1.2 that underwent slow cooling without a ten minute halt at 135 ^{o}C also had the endotherm peak upon reheating, despite having a longer cooling time. The appearance of the α progesterone polymorph agrees with the work by Legendre et. al. (Legendre et. al., 2003) and Duclos et. al. (Duclos et. al., 1991).

Table 4.8 DSC experimental set up and results for progesterone samples undergoing slow cooling with a halt in cooling at 135 ^{o}C for ten minutes. Refer to Table 4.7 for the temperature program.

Test	**Cooling Steps**	**Peaks on 1st heating (^{o}C)**	**Peaks on 2nd heating (^{o}C)**	**Exotherm peaks (^{o}C)**
Cooling with a halt at 135 ^{o}C for ten minutes.	1. Cool at –1 ^{o}C/minute to 135 ^{o}C. 2. Isothermal for ten minutes. 3. Cool at –1 ^{o}C/minute to 26 ^{o}C.	133.1 ^{o}C (area 86.8 ^{o}C)	125.1 ^{o}C (73.5 J/g)	During cooling double peak at 44.6 ^{o}C and 42.7 ^{o}C.
		133.5 ^{o}C (area 89.0 J/g)	125.6 ^{o}C (area 63.4 J/g), 131.1 ^{o}C (area 13.6 J/g)	During cooling peak at 55.0 ^{o}C.
Cooling without a halt at 135 ^{o}C for ten minutes.	1. Cool at –1 ^{o}C/minute to 26 ^{o}C.	132.5 ^{o}C (area 83.0 J/g)	125.2 ^{o}C (area 72.8 J/g).	During cooling at 43.6 ^{o}C, 41.0 ^{o}C.

It can be concluded that only with a low cooling rate would the α progesterone polymorph form, and even in such circumstances it is affected by a degree of non-reproducibility. It has been observed in the XRD work that the slabs exhibit the β progesterone polymorph, and hence slow cooling (in the manner undertaken in this experiment) does not affect polymorphism per se.

4.4 Progesterone polymorphic transformations in the stability oven

Samples of the α and β polymorphs of progesterone, were placed into the stability oven to determine if there would be any polymorphic change over time. Work by Wang et. al. (Wang et al., 2000) noted that progesterone in slurried water does change polymorphic from the β progesterone polymorph to the α progesterone

polymorphic form. Samples analysed consisted of one β polymorph sample that was made of melted progesterone, micronized progesterone from a Diosynth batch (L00024354) (α polymorph), and non-micronized progesterone from Pfizer batch 33JFW (also α polymorph). Samples were placed into the stability oven on the 24/12/04 and analysed by XRD on the 28/02/05. It should be noted that during that time samples were removed from the stability oven for approximately two days.

As can be observed in Figure 4.22 there is no change in the polymorphism of either the α or the β polymorphic samples. Hence it can be concluded that polymorphic change does not occur in progesterone in the stability oven. The α progesterone polymeric particles from Pfizer batch 33JFW do not undergo any polymorphic transformation despite the larger particle size.

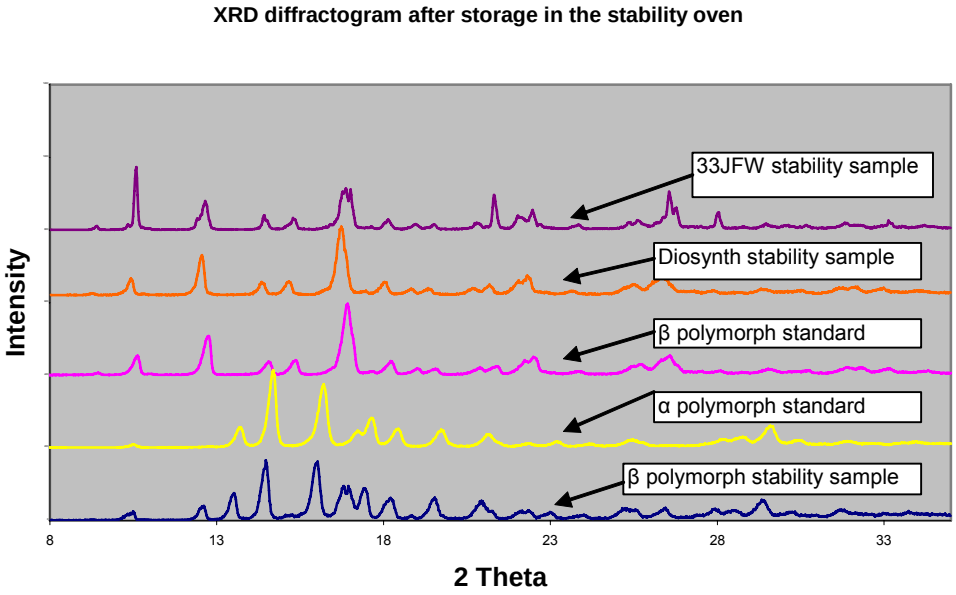


Figure 4.22 XRD diffractogram on samples of progesterone stored in the stability oven.

This result is in line with previous work of Muramatsu et. al. (Muramatsu et. al., 1979) who noted that both the α and β progesterone polymorphs were stable at room temperature and no polymorphic transformation occurred over the course of several months. Since the stability oven was not operating at 100 % RH there would have been no condensation on the progesterone, hence possibly allowing a

mechanism for polymorphic transformation of the type studied by Wang et. al. (Wang, et. al., 2000).

Further, work by Bernabei et. al. (Bernabei et. al., 1982) found that the polymorphic forms of progesterone mixed in polydiemthylsiloxane were stable in water at 37 ^{o}C. However it is known that rate of progesterone polymorphic transformation does increase with temperature as shown by Wang et. al. (Wang, et al., 2000).

Furthermore this work and work by Rades & McFetridge (Rades & McFetridge, 2004) have shown that both polymorphs exist on the CIDR insert and that it is possible the cured silicone has an effect on the progesterone polymorphic transformation by providing insulation decreasing the cooling rate and hence forming the α progesterone polymorph (Legendre, et. al., 2003).

4.5 Discussions on controlled cooling and progesterone polymorphism

In Section 4.1 it is found that there is a difference in progesterone polymorphism between mottled and non-mottled regions on CIDR inserts, with the mottled regions having the β progesterone polymorph, which persists after wiping down of the sample with ethanol. In contrast the white (non-mottled) regions exhibit the α progesterone polymorph, which does not persist after the sample is wiped with ethanol. This indicates that the β progesterone polymorph is found below the surface (as it can not be wiped off the device) in the mottled regions.
Rades & McFetridge (Rades & McFetridge, 2003) analysed mottled and non-mottled regions with DSC and found that both the α and β progesterone polymorphs were present.

XRD results show no crystalline progesterone on the wiped non-mottled regions of the CIDR insert, resulting in a number of possible conclusions. It is known that the white non-mottled regions contain desired levels of progesterone (Rathbone & Ogle, 2000), and $C_p << A$ (Rathbone, et. al., 2000). It can be concluded that on the white regions of the CIDR insert the progesterone is present in an amorphous form or dissolved in the silicone to the depth the XRD is able to sample.

In Section 4.2 it is found that slow cooling produces the α progesterone polymorph. However DSC experiments generally produce the β progesterone polymorph. This could be caused by the isothermal time (length of time the sample was held at 26 ^{o}C before undergoing a second heating to determine the polymorph of progesterone formed by the slow cooling) at 26 ^{o}C being too short and so not allowing enough time for crystallisation at 26 ^{o}C.

In Section 4.3, it is found that slow cooling on slabs held at 135 ^{o}C for ten minutes after curing reduces secondary blooming and mottling. Progesterone samples that underwent slow cooling by DSC in the same manner (as well as being held between, and below the melting points of the α and β progesterone polymorphs) did not change the progesterone polymorph produced. XRD spectra on slabs that were slow cooled and held at 135 ^{o}C did not show any progesterone polymorphs, which indicates that the progesterone could be amorphous.

In Section 4.4 it is found that the α and β progesterone polymorphs are stable in a humid environment, (between 82 to 87 % RH) which eliminates atmospheric water being able to change the progesterone polymorph. The results may have been different if the oven was at 100 % RH, as condensation would have occurred on the progesterone particles, providing a medium for polymorphic transformation as Wang et. al. (Wang et al., 2000) found that the progesterone polymorph changed in slurried water. It is known that curing gives off formaldehyde (Burggraaf, 2006a), it is possible that the formaldehyde may serve as a medium for polymorphic change.

The surface of the CIDR insert would cool faster compared to areas inside the insert, which are insulated. This difference in cooling rate could have an effect on the progesterone polymorph formed. It has been found that mottled regions of the CIDR insert have the β progesterone polymorph, which persists after wiping with ethanol. Rades & McFetridge (Rades & McFetridge, 2003) analysed mottled and non-mottled regions with DSC and found that both the α and β progesterone polymorphs were present. However XRD is not able to scan the bulk sample and only analyses the surface. Since the surface would cool faster compared to the

remainder of the CIDR insert this would indicate that the β progesterone forms on the surface, due to slow cooling in accordance with the work by Legendre et. al, (Legendre, et. al., 2003). However work by Bourke (Bourke, 2004) using XRD thought that both the α and β progesterone polymorphs were present on the surface of slabs. Further, this hypothesis would not explain the lack of signal on the wiped white regions of the CIDR insert, which would be expected to cool at similar rates to the mottled areas of the CIDR insert. It could also be possible that the mottled areas are cooling faster than the rest of the slab.

In conclusion it is unlikely that progesterone polymorphism per se is the cause of mottling and secondary blooming in CIDR inserts. It is also observed that there are some discrepancies in the formation of progesterone polymorphs formed in various tests. Legendre et. al, (Legendre, et. al., 2003) note that the small size of a DSC pan gives different results compared to a large cell, making it hard to observe perfect reproduction of results. However it should be cautioned that the studies undertaken in thesis were not done in a silicone matrix, which would be closer to the real condition of the CIDR insert. Also very slow cooling was able to produce the α progesterone polymorph, which could play a factor. Finally no slabs were made with the micronized β progesterone polymorph, which would provide supporting evidence of the phenomena.

5.0 Chemical analysis of leachates from CIDR inserts and liquid silicone

Since the Dow Corning Q7-4840 silicone exhibits mottling and secondary blooming while the alternative supplier silicone does not (Reardon, 2004b), it is important to gain an understanding of the differences in these two feedstocks. Such information can then be used as a basis for further work into the causes of secondary blooming and mottling.

Analysis of leachates and dissolved materials was undertaken with ESMS (leachates) and GCMS (dissolved material in dichloromethane).

Interpretation of ESMS data requires consideration of both the calibration accuracy and the reproducibility (precision) of the ion data. For example the ESMS spectra of progesterone has an ion at m/z 337.6. This is consistent with the identification of this ion from a $[M + Na]^+$ cluster ion (nominal mass 314.5 + 23 = 337.5, with the exact mass of progesterone being 314.46 $gmol^{-1}$) taking into account both the calibration (± 0.5 Da) and precision (± 0.5 Da) errors, which can combine to give a total error of ± 1.0 Da as noted in Chapter Three.

5.1 Analysis of leachates from CIDR inserts by Eectrospray Mass Spectroscopy (ESMS)

As discussed in the Introduction there are differences between Dow Corning Q7-4840 silicone and the alternative supplier silicone. The purpose of these experiments was to analyse differences in the leachates between the different silicone feedstocks by ESMS with the underlying aim of determining the root cause of secondary blooming and mottling.

In order to explore the differences in leachates from CIDR inserts made with the alternative supplier silicone (batch E08106) and CIDR inserts made with Dow Corning Q7-4840 silicone (batch E08015) were extracted in ethanol and the

leachates were analysed using ESMS with a cone voltage of + 45 V. CIDR 330 inserts made with the respective silicone feedstocks were extracted in AR grade ethanol. Samples had been stored in the stability oven (see Chapter Three) for at least nine months or longer.

ESMS results can be contaminated by previously analysed samples. Hence it is important that samples are analysed at different times to ensure that peaks can be differentiated from spurious background peaks. One sample from batch E08105 (Dow Corning silicone made CIDR insert) and one sample from batch E08106 (alternative supplier silicone made CIDR insert) were analysed on the same day. This was then repeated with freshly made samples on another day.

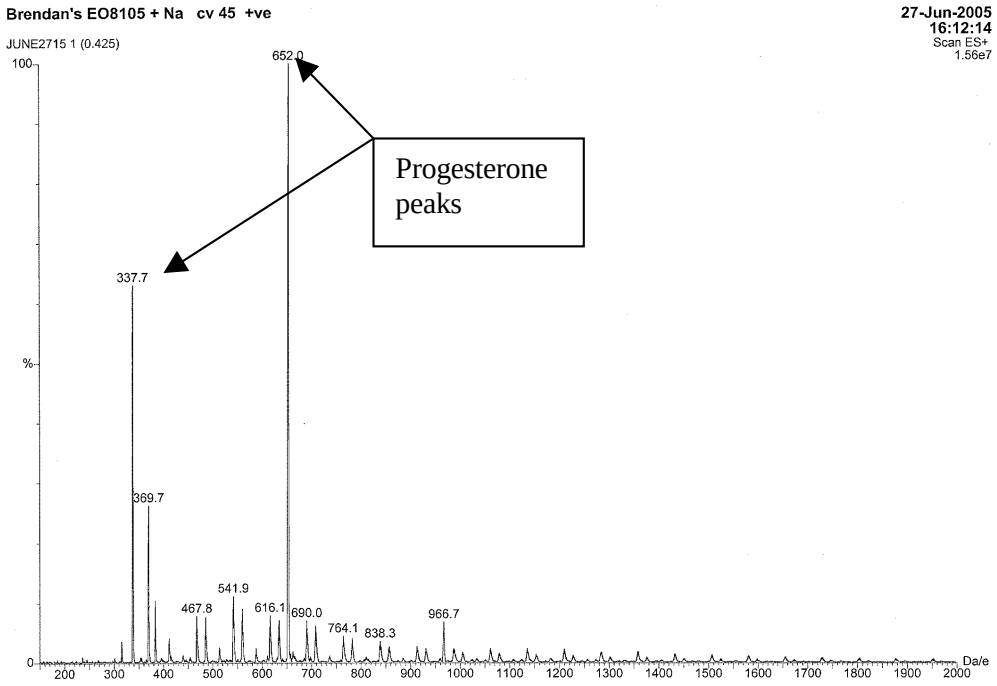


Figure 5.1 ESMS spectra from E08105 leachate. (Dow Corning Q7-4840 silicone). The progesterone peaks are from $[M + Na]^+$ and $[2M + Na]^+$.

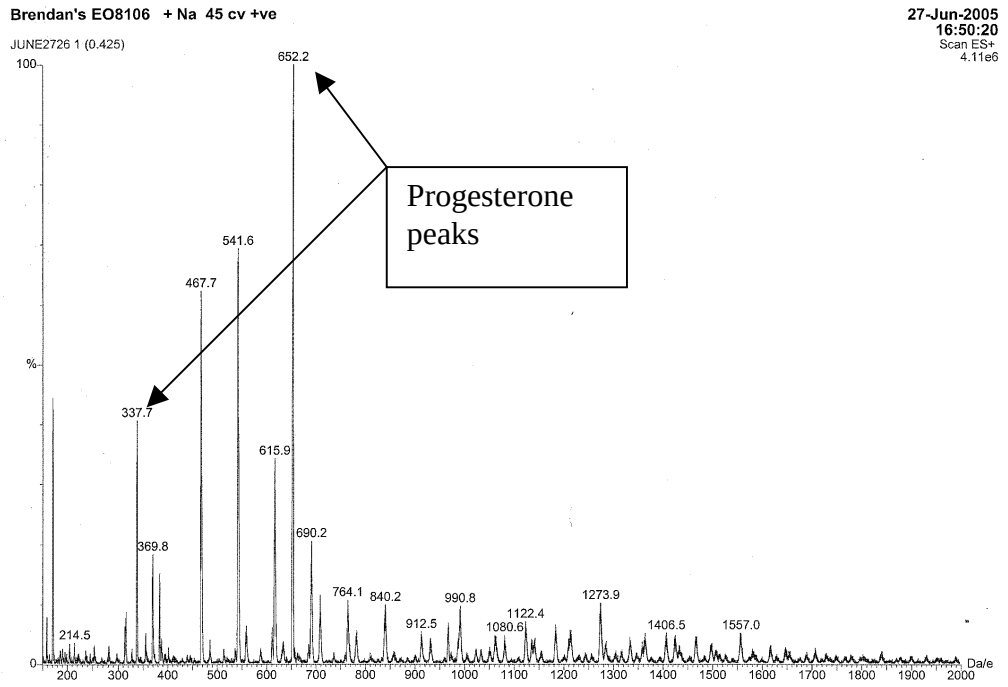


Figure 5.2 ESMS spectra from E08106 leachate. (Alternative supplier silicone) The progesterone peaks are from $[M + Na]^+$ and $[2M + Na]^+$.

The ESMS spectra of the samples (see Figure 5.1 and 5.2) analysed show evidence for three major species. These were progesterone, cyclic silicone polymers and straight chain silicone polymers. Ions attributable to the three species are listed in Tables 5.1 and 5.2. There are also a few peaks that cannot be assigned.

Table 5.1 Major peaks detected in ESMS of the Dow Corning CIDR insert leachate. See Figure 5.1.

Compound or class of compounds	**Major ions observed (m/z)**
Progesterone	315.4 $[M + H]^+$, 337.6 $[M + Na]^+$, 652.0 $[2M + Na]^+$, 966.7 $[3M + Na]^+$
Cyclic silicone compounds (similar to Figure 5.4).	467.5, 541.7,615.9, 690.0, 764.2, series with a repeat unit of m/z 74 continues to m/z 2000 but getting progressively weaker.
Straight chain silicone compound of type Figure 5.5.	485.7, 559.7, 633.7, 708.1, series with a repeat unit of m/z 74 continues to m/z 2000 but getting progressively weaker.

Unknown	369.6, 383.7

Table 5.2 Major peaks detected in ESMS of the alternative supplier silicone CIDR insert leachate. See Figure 5.2.

Compound or class of compounds	**Major ions observed (m/z)**
Progesterone	337.4, 651.9, 315.6, 966.6
Cyclic silicone compounds (similar to Figure 5.4).	467.5, 541.6, 615.7, 685.9, 764.1, above m/z 800 the series becomes less clear
Straight chain silicone compound of type Figure 5.5.	Detected.
Unknown.	990.3, 383.6
Notes.	Above m/z 800 the spectra becomes complex with peaks not showing a regular repeat unit of 74 between the peaks.

Three progesterone (molecular mass of 314.46 $gmol^{-1}$) peaks are detected at m/z ~337.6 m/z ($[M + Na]^+$), m/z 652.0 ($[2M + Na]^+$), in addition, a minor peak at m/z 966.7 corresponding to ($[3M + Na]^+$) is also observed.

Cyclic silicone compounds are observed. These peaks have a separation of m/z 74, which corresponds to a polymer with a repeat unit of 74 Da. The polydimethylsiloxane repeat unit is shown in Figure 5.3.

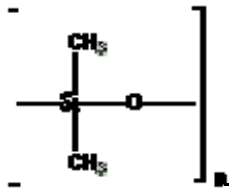


Figure 5.3 Polydimethylsiloxane repeat unit. Mass of 74 Da.

If 23 Da is subtracted (for a Na^+ ion), and the result divided by 74 (the repeat unit of the molecule) a whole number answer is obtained (n), which is a multiple of

74. This implies a cyclic silicone compound similar to the ones shown in Figure 5.4 with n repeat units of 74 Da.

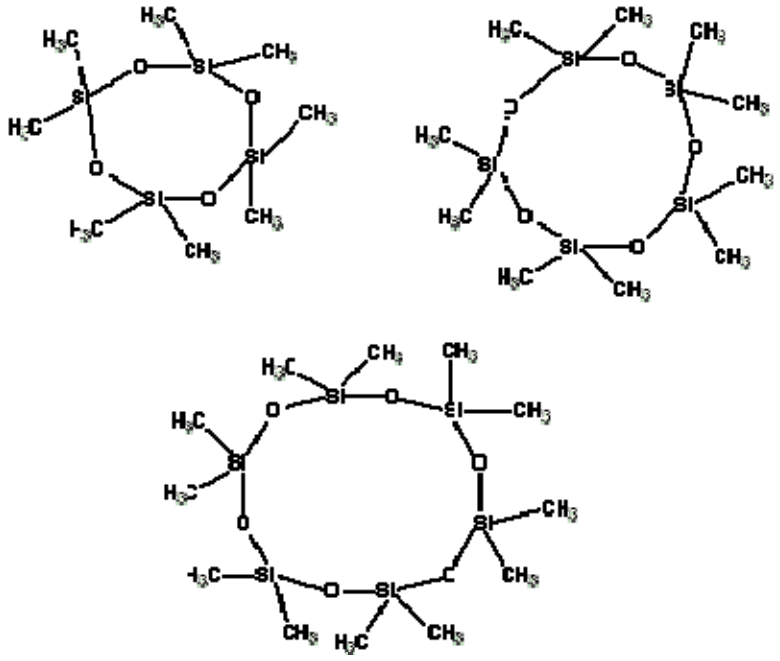


Figure 5.4 Examples of cyclic silicone compounds, octamethylcyclotetrasiloxane (n = 4, top left), decamethylcyclopentasiloxane (n = 5 top right) and a cyclic silicone with n = 6 repeat units (bottom).

These rings range from n = 5 repeat units or greater with the most common repeat unit being n = 6 to 7. It is noted that the alternative silicone supplier leachate appears to have more extractable cyclic silicone compounds compared with the Dow Corning leachate samples.

In addition to cyclic silicones, a series of straight chain silicone polymers is also observed in the Dow Corning Q7-4840 silicone leachate. While these are observed in the spectra of the alternative supplier silicone leachate, they are of much weaker intensity compared with the other peaks. These have the basic formula of:

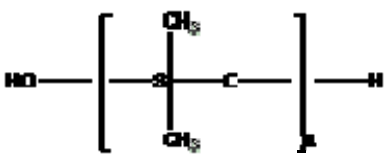


Figure 5.5 Straight polydimethylsiloxane chain.

These peaks are distinguished from the cyclic silicone peaks because they are m/z 18 higher than a cyclic silicone peak. A mass of 18 Da corresponds to H_2O and hence implies that there is an OH attached to one end of the polymer chain and an H attached to the other, as is shown in Figure 5.5. These peaks like the cyclic silicone rings also have a Na^+ ion attached from the ionisation agent. The peaks range from a repeat unit of n = 5 with the highest level of repeat units being n = 7.

The decrease in peak intensity of higher molecular weight cyclic silicones is not attributable to ring entrapment in the silicone matrix (i.e. polydimethylsiloxane chain passing through the cyclic ring, hence preventing ring leaching), as work by Carlson et. al. (Carlson et. al., 1986) found that silicone compounds with $n_n < 38$ will not be trapped in the matrix. It should be noted that other factors related to the low abundance of higher molecular weight polymers could include, solubility and the ability of the ESMS to detect the higher molecular weight compounds.

As all CIDR 330 inserts are made with the same amount of progesterone and since samples were prepared in a similar fashion it is possible to make approximate judgements on the amount of leachable material in the spectra. This was done through measuring the height of all the peaks between m/z 300 to 400 that were from the progesterone ions, cyclic silicone compound ions and the straight chain silicone compounds ions. The heights of all the peaks of the same type of compound in the m/z 300 to 400 region were then added together along with the peak height of $[2M + Na]^+$ (progesterone m/z 652.0) and used to form a ratio between the three classes of compounds measured. As a ratio is unitless, this can then be used to make comparisons between the two types of silicone analysed. Absolute concentrations of components cannot be determined using this method, as this would require information on the response factor, the concentration of one of the compounds, the exact ethanol volume used and the exact sample weight. The ratios for the samples analysed are shown in Table 5.3, 5.4 and summarised in Figure 5.6.

Table 5.3 Peak height ratios for Dow Corning Q7-4840 silicone (2 d.p.).			
Ion ratio	**Sample 1**	**Sample 2**	**Average**
Cyclic silicone polymer ions / Straight chain silicone polymer ions	1.08	0.88	0.98
Cyclic silicone polymer ions / Progesterone	0.24	0.14	0.19
Straight chain polymer ions / Progesterone	0.22	0.16	0.19

Table 5.4 Peak height ratios for alternative supplier silicone (2 d.p.).			
Ion ration	**Sample 1**	**Sample 2**	**Average**
Cyclic silicone polymer ions / Straight chain silicone polymer ions.	7.07	6.88	6.97
Cyclic silicone polymer ions / Progesterone.	1.34	0.44	0.89
Straight chain polymer ions / Progesterone.	0.19	0.06	0.13

Fresh samples were analysed on different days with different samples and as can be observed in Tables 5.3 and 5.4 the ratios between the different samples are similar. As a result the ratios obtained can be considered reliable.

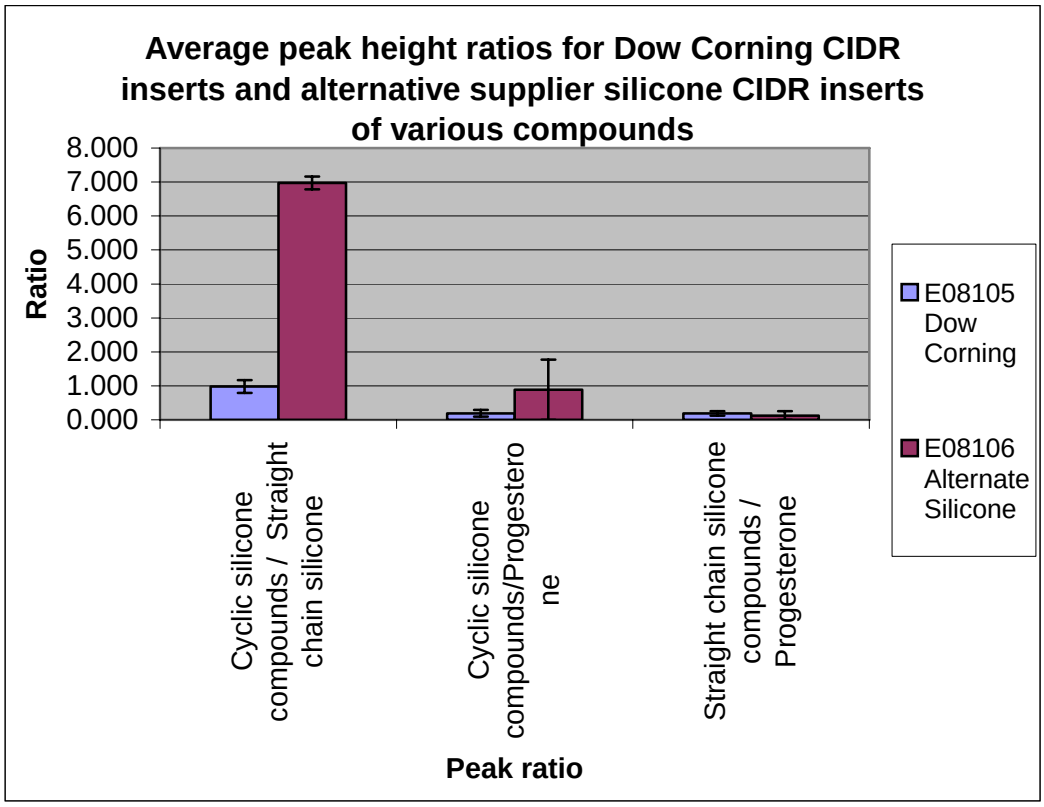


Figure 5.6 Peak height ratios for CIDR insert leachates made from different feedstocks. Error bars are the 95 % confidence interval.

From Figure 5.6 there is a clear statistical difference in the ratio of cyclic versus straight chain silicone polymers leachates detected by ESMS for the CIDR inserts made with the different feedstocks. It is found from Figure 5.6 that the Dow Corning Q7-4840 CIDR insert samples have far more straight chain polymers compared with the alternative silicone supplier samples.

Student T tests show insignificant difference between the two silicone feedstocks, i.e., the ratio of cyclic compounds to progesterone and the ratio of straight chain compounds to progesterone. This difference contrasts with the previous result, indicating no difference in ion ratios between the two silicone feedstocks for both cyclic and linear silicones with respect to the progesterone, however a difference in the ion ratio of cyclic to straight chain silicone compounds between the two feedstocks.

Comparing the results from this experiment to work by Heiner et. al. (Heiner et. al., 2003), who analysed the leaching of silicone into salt water. Heiner et. al,

(Heiner et. al., 2003) found that samples of a platinum catalysed silicone Elastosil® L43003/70 (made by Wacker), did not leach any organic compounds as analysed by GCMS (Heiner et. al., 2003). Hence it is possible that the analysed samples in this investigation (as previously discussed) may not have any extractable organics. However since Heiner et. al, used a water extraction medium, silicone fluids would not be soluble in this medium. Since the composition of the Wacker silicone will be different from Dow Corning Q7-4840 silicone, it is not possible to directly compare the results from this work with the work by Heiner et. al.

It is important to consider the work undertaken by Siang (Siang, 2003) from Dow Corning, who found that samples of cured silicone (Dow Corning Q7-4840 and alternative supplier silicone) had similar levels of low molecular weight polymers after extraction with n-hexane.

Dow Corning provided a bottle of 6-3570 polymer to use as a silicone crosslinker. The MSDS (Dow Corning, 2002) of the polymer notes that it contains between one to five % by weight of octamethylcyclotetrasiloxane, (n = 4). However octamethylcyclotetrasiloxane was not detected in the leachates from CIDR inserts made with either feedstock. It would be possible that the 6-3570 polymer could also contain low concentrations of cyclic silicone compounds with n > 4 repeat units.

Cyclic silicones are a by-product of the polymerization of silicone oils (Martín-gil et. al., 1997). Cyclic silicones do not have a role in the cure of Dow Corning Q7-4840 silicone (Inman, 2006), hence it is unlikely that the cyclic silicones play a role in the cure of the alternative supplier silicone. It is possible that progesterone is more soluble in the cyclic silicone oils inside the matrix and since the ion ratio (with respect to straight chain silicones) for this compound is higher in the leachate from alternative supplier silicone CIDR inserts, the progesterone is less prone to migrate to the surface. It is also possible that the cyclic silicones are an impediment to either progesterone dissolving into the silicone matrix, or the cyclic silicones impede the diffusion of silicone to the surface of the insert. The steps of drug dissolving into the insert and diffusion are steps in the mechanism of

'in vitro' drug release in the CIDR insert (Rathbone, et. al., 2000), with diffusion being assumed to be the rate determining step. However drug release rate results from Chapter Six (Figure 6.3) note that there is no statistical difference from the drug release rate between two different feedstocks.

5.2 Analysis of leachate from mottled and non-mottled sections of CIDR insert

In a further dimension to the previous ESMS study on cured silicones, analyses were undertaken on mottled and non-mottled CIDR inserts in order to determine if there was any difference in leachate, which could provide useful information on possible causes of secondary blooming and mottling.

Mottled and non-mottled sections of CIDR 1380 inserts were analysed from the following batches:

- Batch 9330319 (A CIDR 1380 insert formerly from a stability study and had been stored for a few years at 30 ^{o}C and 65 % RH).
- Batch B06301.
- Batch 9329319 (A CIDR 1380 insert formerly from a stability study and had been stored for a few years at 30 ^{o}C and 65 % RH).

Samples were analysed at a cone voltage of + 45 V. Samples were analysed once, with the samples from batch 9329319 being analysed on a different day from the other samples (which were analysed on the same day).

Comparison of the spectra of the leachate from batch 9330319 shows a difference between mottled and non-mottled sections of CIDR inserts, with non-mottled sections possessing silicone peaks of greater intensity (Figure 5.7) compared to the mottled sections of the CIDR insert (Figure 5.8).

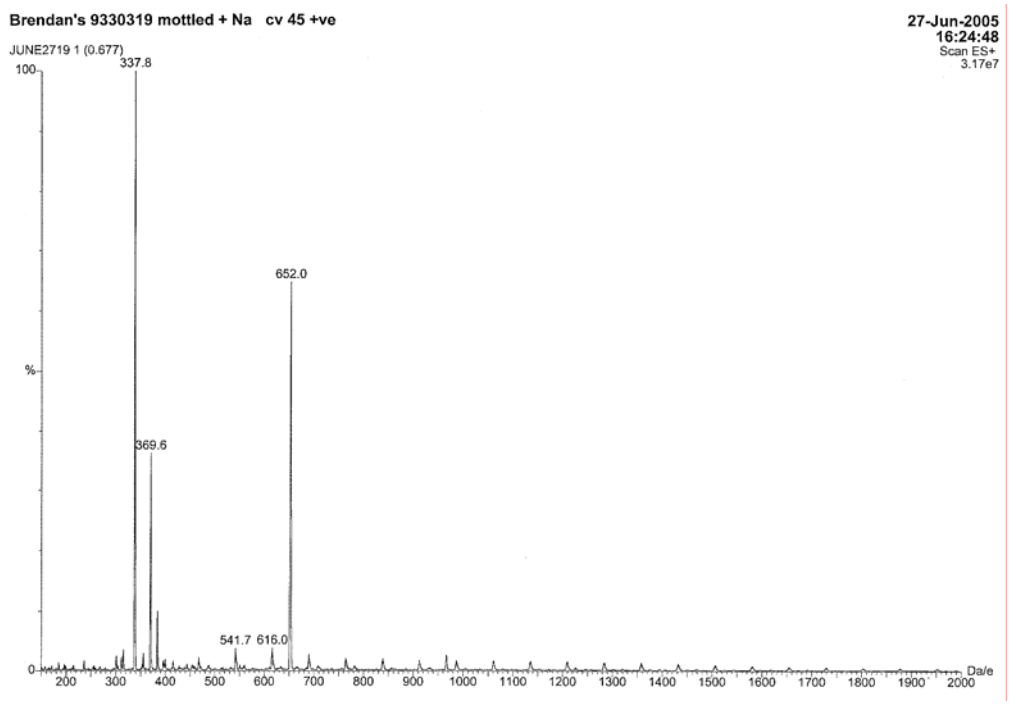


Figure 5.7 ESMS spectra of leachate from mottled section of CIDR insert from batch 9330319.

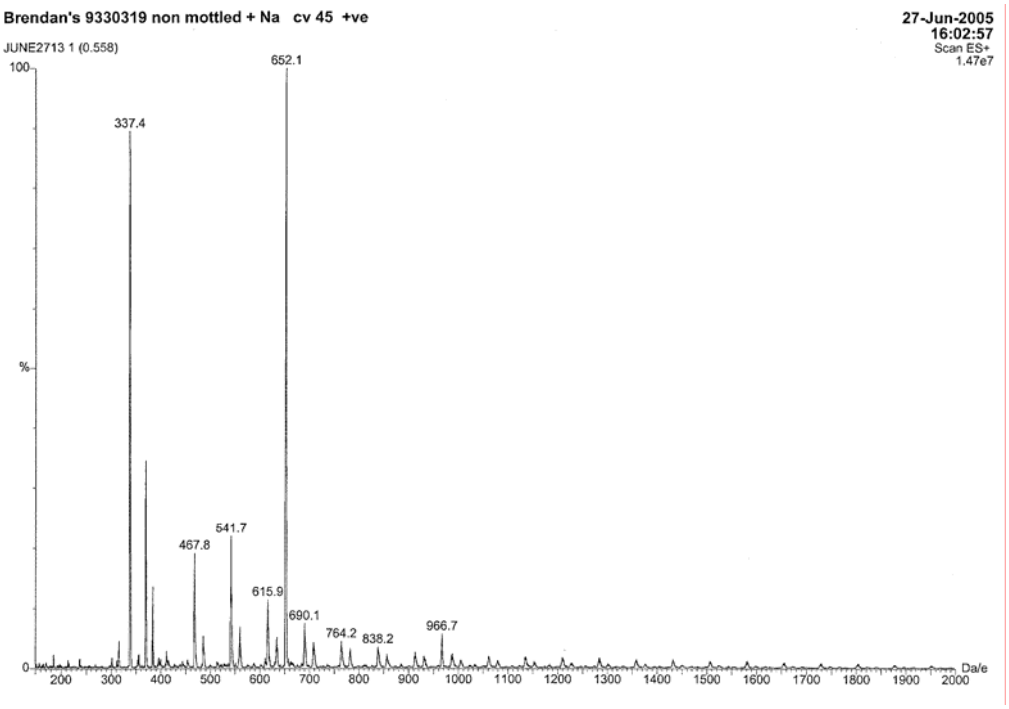


Figure 5.8 ESMS spectra of leachate from non-mottled section of CIDR insert from batch 9330319.

It should be noted that the leachate of samples from batches B06301 (Figures 5.9 and 5.10) and 9329319 do appear to be similar in intensity. However the response

factor for progesterone, cyclic silicones, and straight chain silicones is unknown. Further mottled sections of CIDR inserts have reduced drug loading (Rathbone & Ogle, 2000). The mass of sample and volume of ethanol used to make up each sample is also unknown. For these reasons it is not possible to reach any conclusion on the results from this Section.

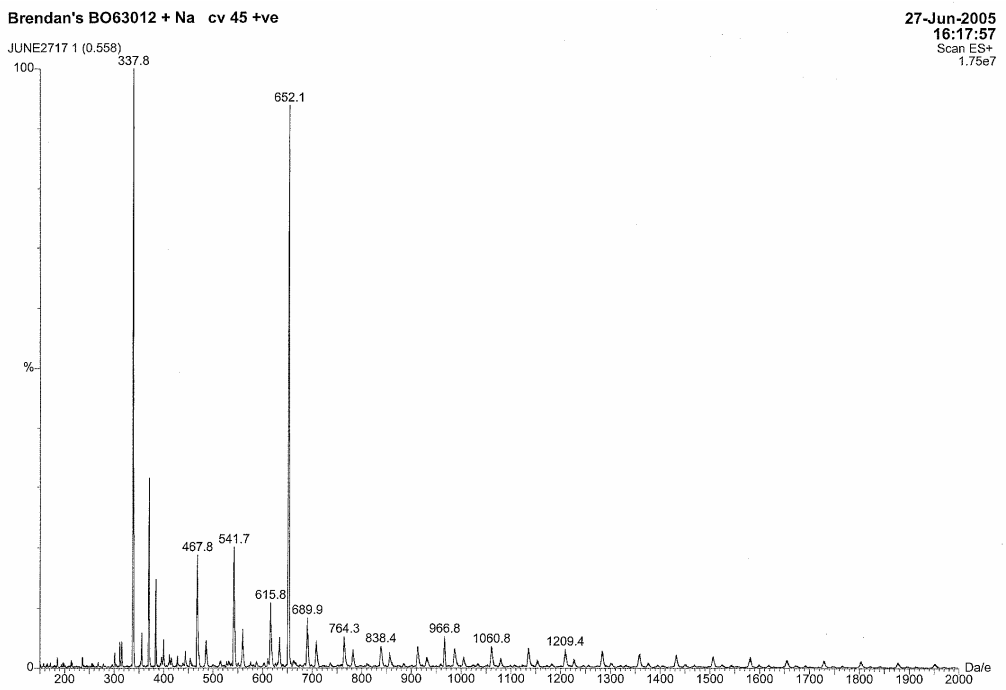


Figure 5.9 ESMS spectra of leachate from mottled section of CIDR insert from batch B06301.

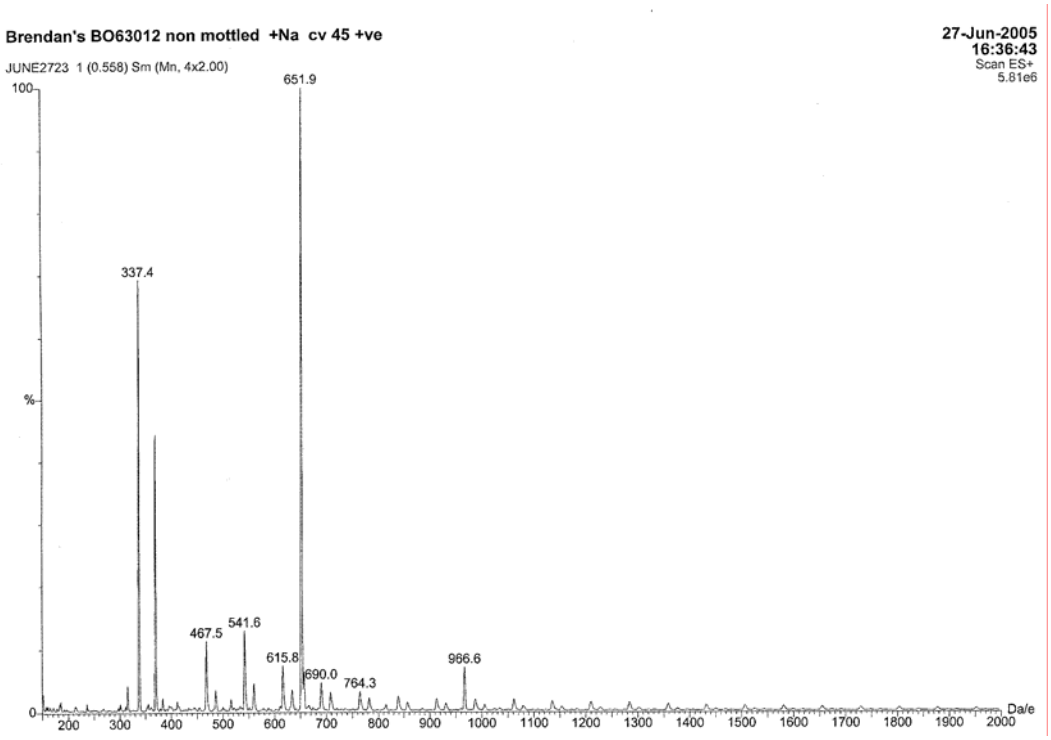


Figure 5.10 ESMS spectra of leachate from non-mottled section of CIDR insert from batch B06301.

5.3 ESMS analysis of extracts from liquid silicone from the alternative supplier and Dow Corning Q7-4840 silicones (i.e. parts A and B)

As discussed in the Introduction it is known that there are differences in the composition of the different liquid silicones. It was thought that analysis of the liquid silicone leachate *prior* to curing would provide information on the differences in liquid silicone feedstocks, which could be used to gain insights into the causes of mottling and secondary blooming.

Liquid silicone used was from Dow Corning Q7-4840 silicone batch 0001854557, and alternative supplier silicone provided by the manufacturer. The alternative supplier silicone manufacturer also provided a part B mix that had additional crosslinker added. Samples from parts A and B of liquid silicone were placed into AR ethanol, diluted in methanol with NaCl added. Samples were then analysed using ESMS with a cone voltage of + 45 V. In order to assess reproducibility, samples were rerun on different weeks. Both feedstocks exhibited cyclic and straight chain silicone compounds.

The spectra from the Dow Corning Q7-4840 liquid silicone extracts (parts A and B) are shown in Figures 5.11 and 5.12. The spectra for the Dow Corning Q7-4840 part B liquid silicone shows a more complex array of ESMS components than the spectra of the part A liquid silicone. This indicates that the part B liquid silicone has a more complicated matrix compared to part A. The major peaks detected in the Dow Corning Q7-4840 liquid silicone samples are summarised in Table 5.5.

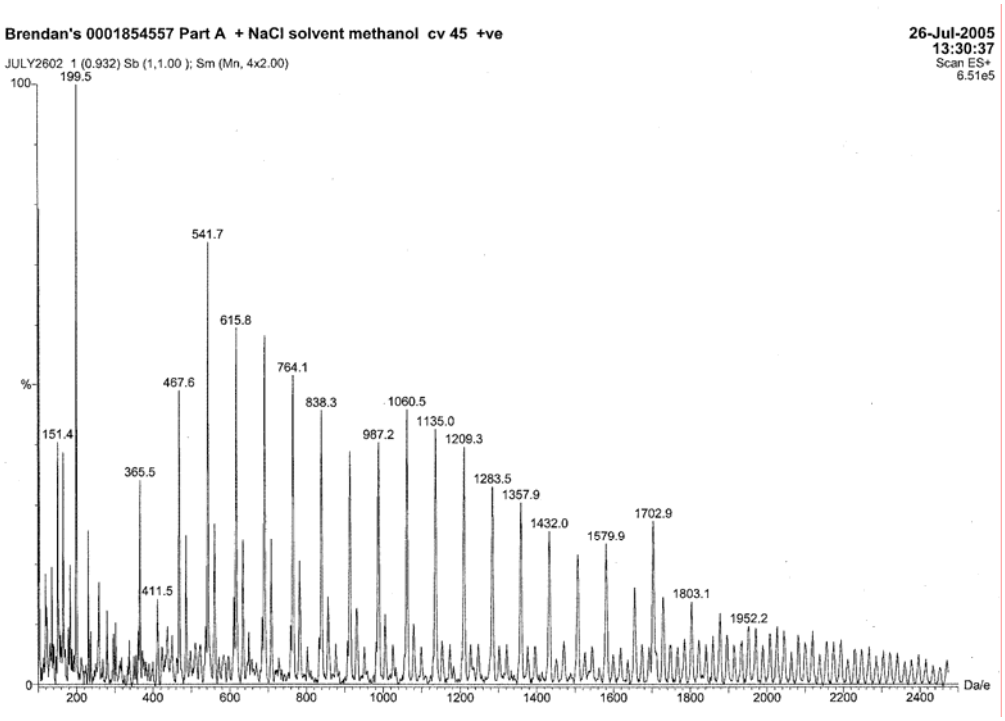


Figure 5.11 Part A extract from liquid silicone from Dow Corning Q7-4840.

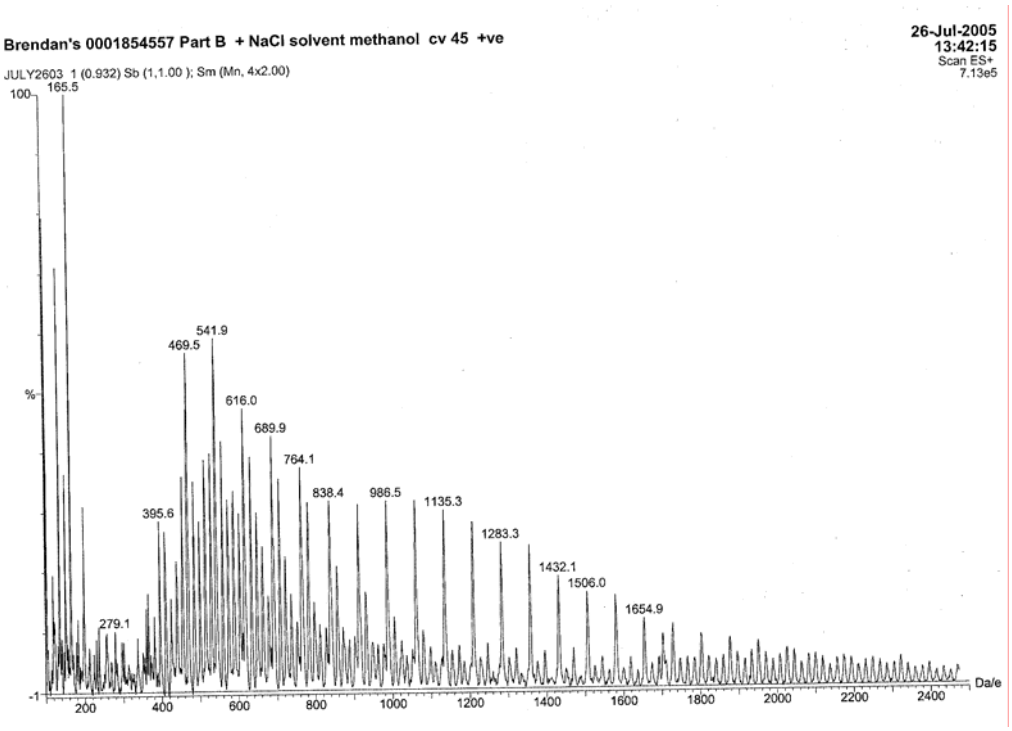


Figure 5.12 ESMS spectra of from part B Dow Corning Q7-4840 liquid silicone extract.

Table 5.5 Major peaks detected by ESMS of the Dow Corning Q7-4840 silicone batch 0001854557.

Compound or class of compounds	**Major ions observed (m/z)**	
	Part A	**Part B**
Cyclic silicone compounds (similar to Figure 5.4).	467.6, 541.7, 615.8, 689.9, 764.1, 838.3, 912.6, 987.2, 1060.5, 1135.0, 1209.3, 1283.5, 1357.9, 1432.0, 1506.6, 1579.9, 1654.8, 1728.8, 1803.1, 1877.7, 1952.2, 2026.3 and sequence continues with repeat units of 74 Da up to n = 33.	Very similar to part A.

Straight chain Silicone compound of type Figure 5.5.	411.5, 485.7, 559.7, 633.9, 708.1, 782.2, 856.3, 930.7, 1004.8, 1079.6, 1153.5 1227.5, 1301.5 At higher masses m/z ~ 1000 these peaks are quite weak.	Similar to part A.
Unassigned peaks	1247.3, 1321.3, 1395.9, 1470.5, 1543.3, 1618.0 1702.9. (see discussion latter in the document). Peaks less than m/z ~400. Between m/z 1600 and 2500 a there is a noticeable cluster of peaks appearing with an interval of m/z 74.	Peaks less than m/z ~400. Between m/z 1600 and 2500 there is a noticeable cluster of peaks appearing with an interval of 74 m/z. There is also a cluster of peaks around the 400 m/z to 700 region.

From Table 5.5 it is clear that the cyclic silicones up to n = 33 repeat units are detected in both parts A and B of the Dow Corning liquid silicone. For part A the straight chain silicone compounds are detected up to n = 17 repeat units (Figure 5.11) and for part B up to n = 13 repeat units (Figure 5.12).

It is notable that the abundance of the cyclic and straight chain polymers decreases with increasing mass. This may be caused by the solubility of the silicone in the ethanol or through a decrease in abundance. Lee et. al. (Lee, et. al., 1979) notes that the molecular weight of the Triorganosiloxy endblocked polydimethylsiloxane fluid ranges from 68,000 to 135,000 $gmol^{-1}$, which indicates that the straight chain polymers should be present in increasing abundance. Another possible cause of the declining abundance could also be a reduction in detection by ESMS of the higher molecular weight polymers.

ESMS spectra of the extracts from the alternative supplier liquid silicone are shown in Figures 5.13 and 5.14. Table 5.6 lists a summary of the major peaks observed in the spectra of the extracts.

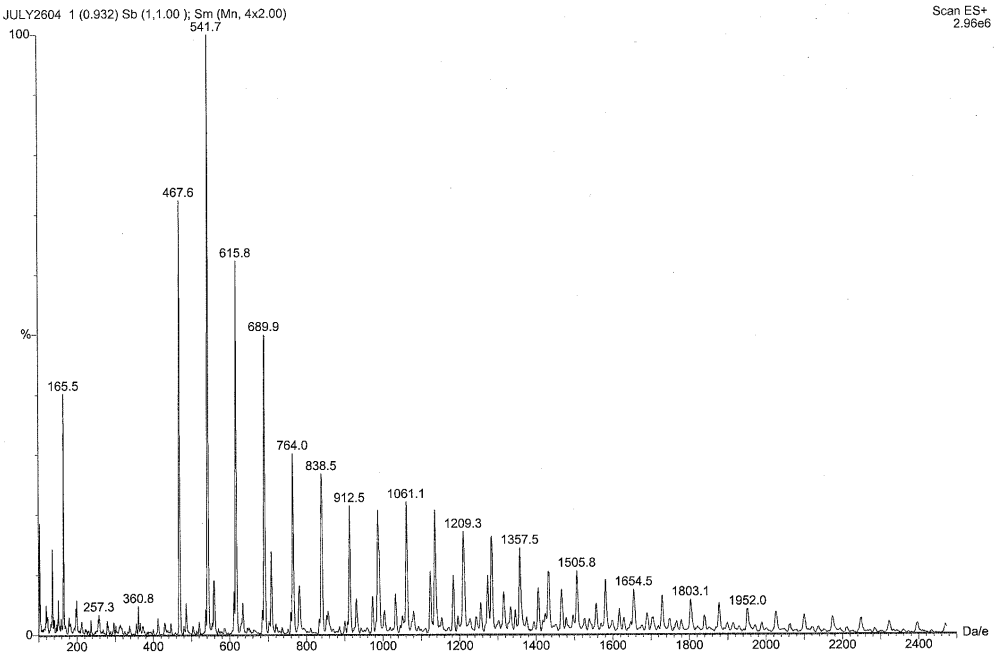


Figure 5.13 Part A extract from liquid silicone of the alternative supplier silicone.

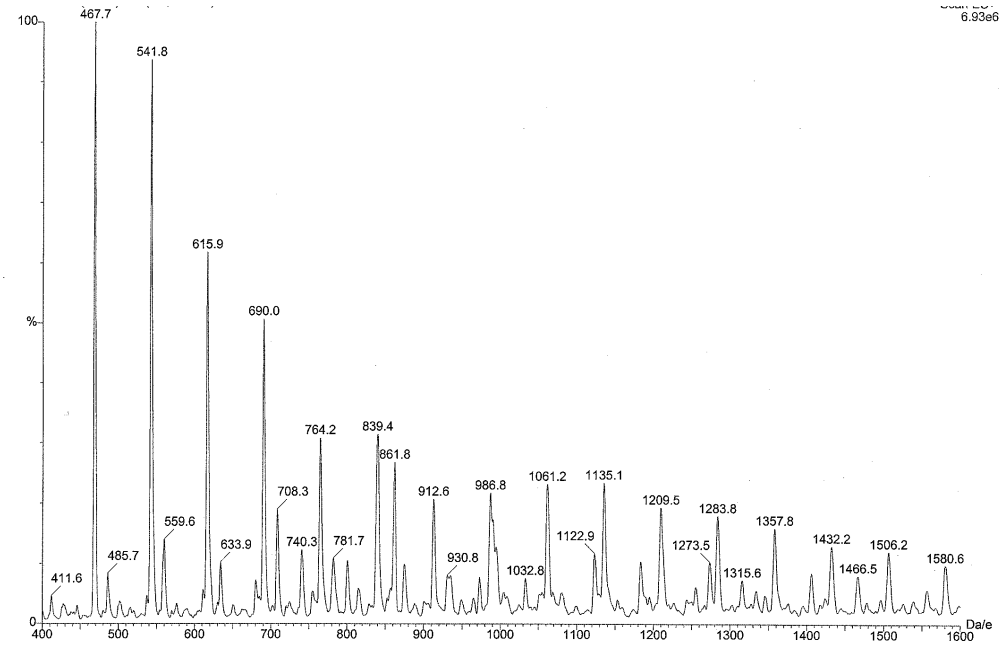


Figure 5.14 ESMS of alternative supplier liquid silicone part B extract. Peak at m/z 861.8 is a contaminant.

Table 5.6 Major peaks detected by ESMS of the alternative supplier liquid silicone extract.

Compound or class of compounds.	**Major ions observed (m/z)**		
	Part A	**Part B**	**Part B with four times extra crosslinker**
Cyclic silicone compound of the type shown in Figure 5.4	467.6, and repeats at 74 Da intervals up to 2469.7.	Very similar to part A.	467.6, and repeats at 74 Da intervals
Straight chain silicone compound of type shown in Figure 5.5.	411.6, 485.7, 633.9, 708.1, 781.9, Weak compared to the cyclic silicone peaks.	Very similar to part A.	485.7, 559.6, 633.9
Unassigned peaks.	A background cluster observed around m/z 1000 to 1600. Peaks less than m/z ~ 400. The peaks in the cyclic species become weaker above 1952, with the largest mass observed being m/z 2469.7.	Peaks less than m/z ~400. The cyclic silicone peaks are observed to become weaker after m/z ~1900.	There is a background cluster around m/z ~600 to 1200. Cyclic peaks are observed to be quite weak around m/z 1800 and at higher values of m/z.

Observation of the spectra from the alternative supplier liquid silicone extracts (Table 5.6 and Figures 5.13 and 5.14) show that cyclic silicone polymers were detected up to n = 33 repeat units, in both parts.

In order to gain an understanding of concentration differences in leached cyclic and straight chain silicones from the two samples, the heights of the peaks can be measured and a ratio of cyclic peak height to straight chain peak height can be gained. This is the same method as discussed earlier in Section 5.1 however the analysis was done on all cyclic and straight chain peaks from m/z 400 to 800. The results are shown in Figure 5.15.

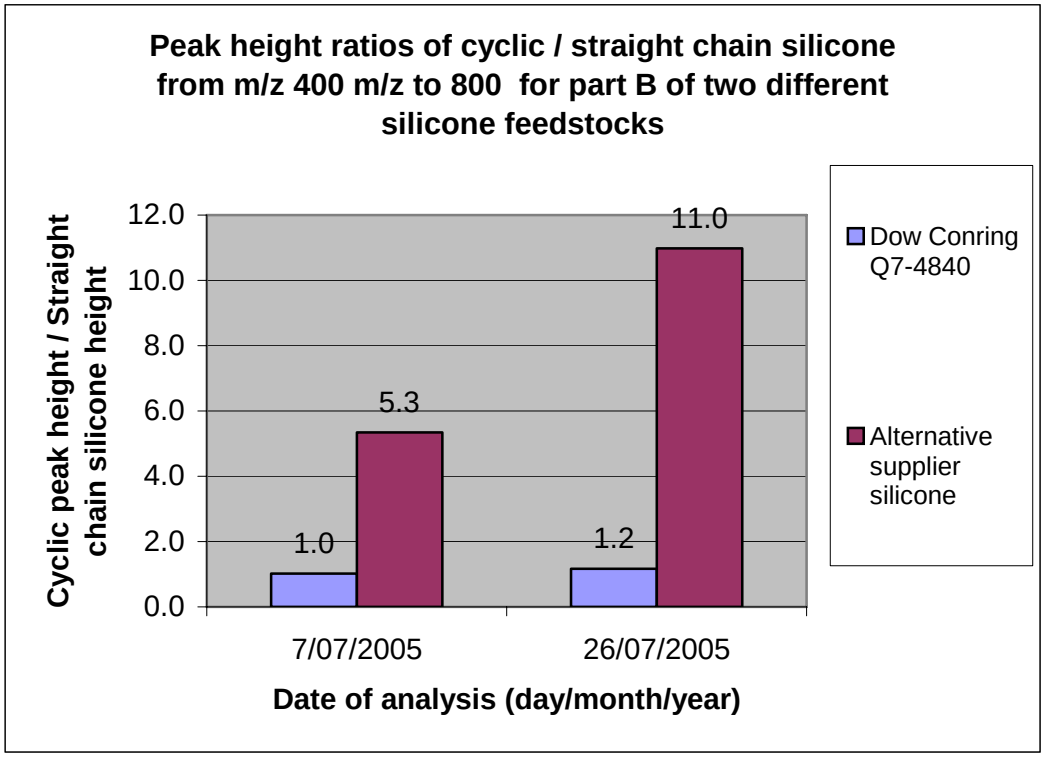


Figure 5.15 Peak height ratios for part B for different liquid silicones leachates analysed by ESMS.

Figure 5.15 clearly shows that there is a clear difference in the amount of leached straight chain silicone between the part B of the Dow Corning Q7-4840 silicone and the alternative supplier silicone extracts, with the alternative supplier silicone clearly showing that it has more cyclic silicone than straight chain silicone. This is the same trend observed from extract from cured silicone samples (see Figure 5.6) and hence it can be concluded that relative to the straight chain silicones there is more cyclic silicone polymers in the part B of the alternative silicone supplier.

Furthermore the part B extract of the Dow Corning Q7-4840 silicone extract possesses a more complex spectrum, compared to the ESMS of the alternative supplier silicone extract.

Analysis comparing the ESMS spectra of the part A liquid silicone from both feedstocks (Figures 5.11 and 5.13) shows that the part A of the alternative supplier silicone has fewer peaks and the majority of the peaks are from cyclic silicone compounds. This contrasts with the Dow Corning Q7-4840 part A liquid silicone extract that also contains straight chain silicone peaks as well as a large number of unassigned peaks from m/z 1600 to 2400.

The spectra of the alternative supplier silicone extract containing extra crosslinker is shown in Figure 5.16. There is little difference in this spectra, compared with the spectra for part B of the alternative supplier silicone. However the area from m/z ~600 to 950 shows a cluster of peaks, which is not observed in the alternative supplier silicone part B sample with normal levels of crosslinker. Figure 5.16 has some similarity with the spectra from the leachate of Dow Corning Q7-4840 silicone part B (Figure 5.12), but these peaks in Figure 5.16 are far less intense compared to the peaks in the Dow Corning Q7-4840 part B liquid silicone mass spectrum.

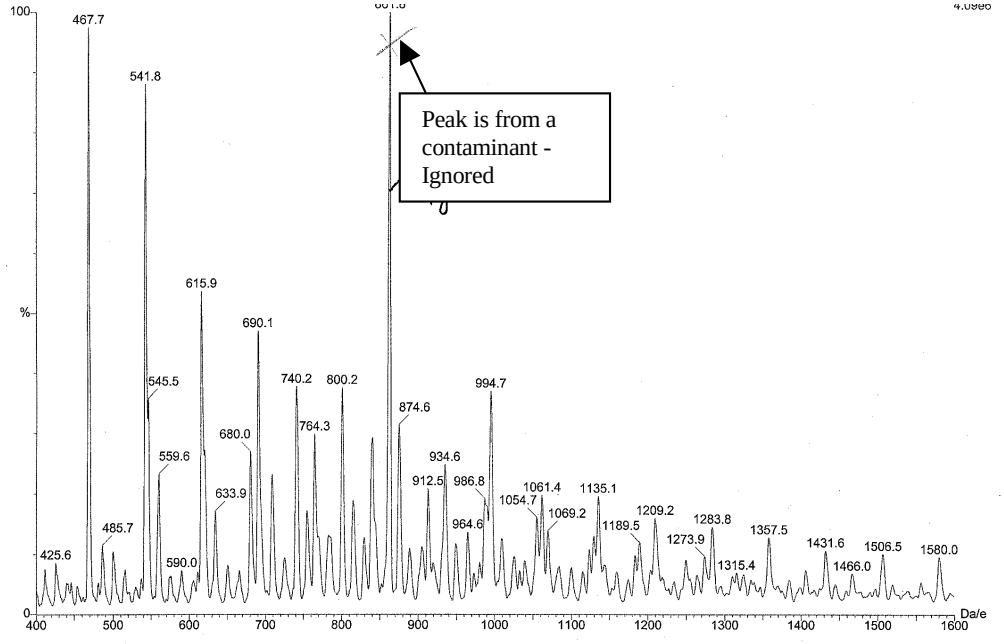


Figure 5.16 ESMS spectra of the leachate from alternative supplier silicone with extra crosslinker. The peak at 861.8 m/z is a contaminant from the background.

Reardon (Reardon, 2004) found that part B of the Dow Corning Q7-4840 silicone was responsible for secondary blooming. As observed in Figure 5.12 it is clear that there is a higher level of components in the part B Dow Corning Q7-4840 silicone compared to part A Dow Corning liquid silicone, or either part of the alternative supplier silicone. This could be a cause of blooming and mottling, however further work would need to be done to determine if this was the case. Chapter Six will investigate the effects on mottling and secondary blooming caused by the addition of extra crosslinker in the alternative supplier silicone.

It is also noted that the alternative supplier liquid silicone possesses a higher ratio of cyclic silicone compounds to straight chain silicone compounds, compared to the Dow Corning Q7-4840 liquid silicone. This result is in agreement with results in Section 5.1 on leachates from CIDR inserts. The cyclic silicones observed do not play a part in the curing of Dow Corning Q7-4840 silicone (Inman, 2006).

5.4 Analysis of liquid silicone using Gas Chromatography Mass Spectroscopy (GCMS)

GCMS was undertaken on Dow Corning Q7-4840 liquid silicone (batch 0001808115) and the alternative supplier liquid silicone. The method of analysis is discussed in detail in Chapter Three. Samples were analysed once except for part B of the Dow Corning Q7-4840 liquid silicone, which was analysed twice. It should be noted that the foil lid on the container used to make up the sample Dow Corning Q7-4840 liquid silicone part A, became detached and could be a source of error in the GCMS chromatogram due to leaching from plastic or glue.

The total ion chromatograms (TIC) for all samples analysed are shown in Figures 5.17 to 5.20. The rising baseline from ~14 minutes is from column bleed due to the increasing temperature of the column causing the stationary phase to decay.

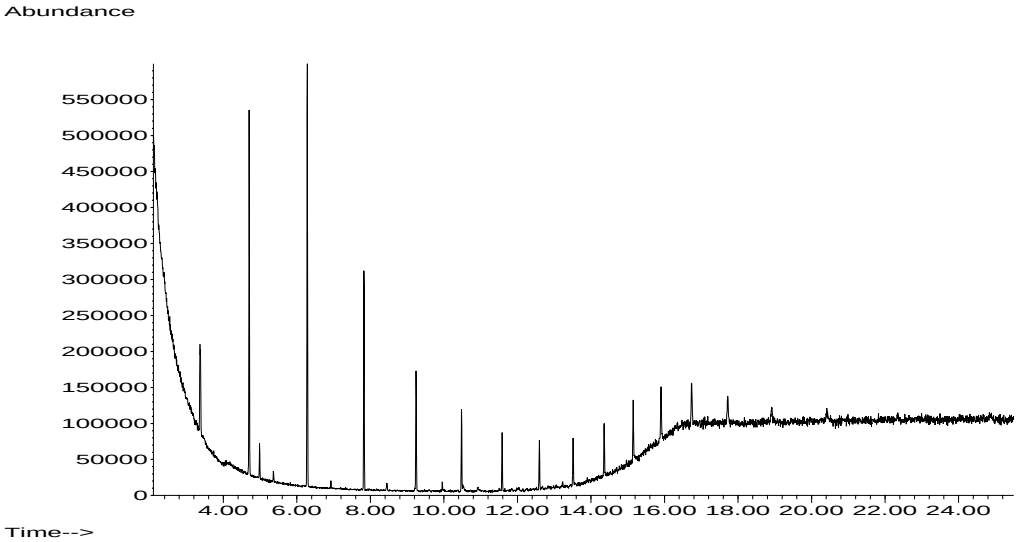


Figure 5.17 TIC for alternative supplier liquid silicone part A. X-axis scale is in minutes.

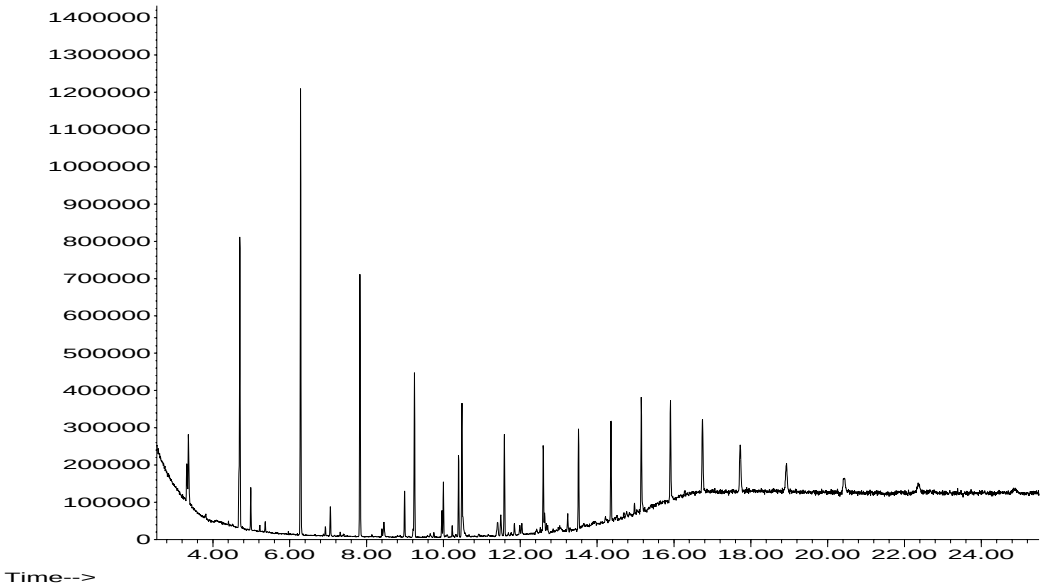


Figure 5.18 TIC for alternative supplier silicone liquid part B. Y-axis is abundance, X-axis scale is in minutes. No peaks eluted before 3.36 minutes.

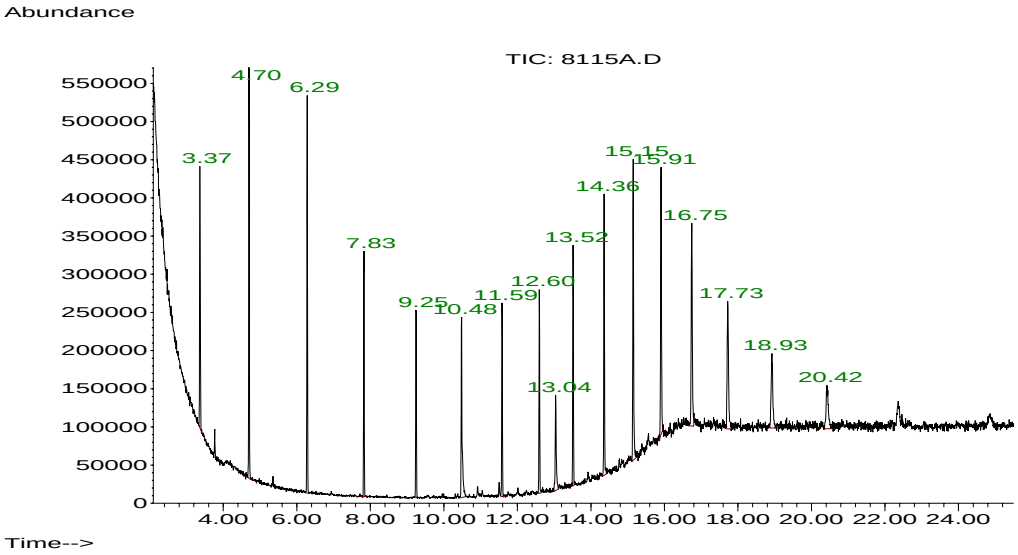


Figure 5.19 TIC of Dow Corning Q7-4840 liquid silicone part A. X-axis scale is in minutes.

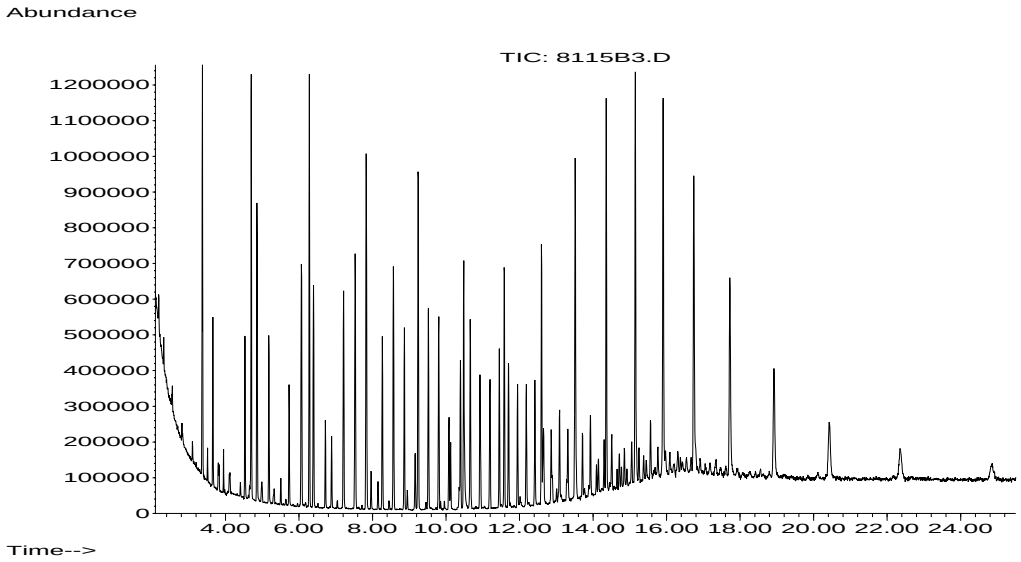


Figure 5.20 TIC of Dow Corning Q7-4840 liquid silicone part B. X axis scale is in minutes**.**

As can be observed from Figures 5.17 to 5.20 it is clear that the Dow Corning Q7-4840 part B liquid silicone is a more complex array of GCMS detectable components. compared to any other liquid silicone sample. It appears that there is little difference between the parts A and B of the alternative supplier silicone and the Dow Corning Q7-4840 liquid silicone part A. Similar retention times are observed for peaks in both parts of the alternative supplier silicone and the Dow Corning Q7-4840 part A.

In Section 5.3 it was observed that part B of the Dow Corning Q7-4840 liquid silicone has a larger number of peaks in ESMS compared with either the alternative supplier liquid silicone (parts A and B) or the part A Dow Corning Q7-4840 liquid silicone. Hence it is clear that part B, which causes secondary blooming (Reardon, 2004) has been shown to have an increased number of components as detected by ESMS.

The increased number of components in the Dow Corning Q74848 part B is interesting as part B causes secondary blooming (Reardon, 2004). These components may affect the binding of the polydimethylsiloxane chains to the fumed silica. Silicone rubber uses fumed silica as a support to provide strength (Rochow, 1987), and the SiO_2 particles possess similar bond angles and interatomic distances to the polydimethylsiloxane chains, this results in bonding between the silica and the polydimethylsiloxane (Rochow, 1987).

The silica is fumed by burning volatile silicone compounds, which coat the silicone particles and displace water on the surface of the silica particles (Rochow, 1987). As a result the polydimethylsiloxane chains bond to the silicone compounds on the silica particles. The extra components in the Dow Corning Q7-4840 part B liquid silicone may bind to the silica particles, this could affect the bonding of the silica particles to the polydimethylsiloxane chains, causing secondary blooming and mottling. Work by Mazan et. al, (Mazan, et. al., 1992) found that differences in the concentrations of low molecular weight unreactive polydimethylsiloxane oils (M_n = 1012) had a limited effect on the diffusion of progesterone, due to an effect on mesh size and mobility of progesterone (at high concentrations).

It should also be considered that the above hypothesis could be incorrect and the extra components cause mottling and secondary blooming through another pathway.

6.0 Studies into raw material variations

The manufacture of the CIDR insert requires the mixing two of parts of liquid silicone, and progesterone, which are then cured over the spine. Progesterone is supplied by Pfizer, whereas the silicone used is supplied by Dow Corning. Due to the large volumes of materials used to make CIDR inserts, a wide range of batches of progesterone and silicone have to be used. While each batch used must pass both DEC Manufacturing's raw materials tests and the suppliers QC tests, there will be variations between different batches of raw materials. This Chapter investigates if variations in raw materials including different silicone feedstocks and differences in progesterone can be related to secondary blooming and mottling.

6.1 Studies comparing CIDR inserts made with alternative supplier silicone versus Dow Corning Q7-4840 silicone

As discussed in the Introduction, it is known that the alternative supplier silicone does not exhibit secondary blooming and mottling (Reardon, 2004b). To further investigate this, a stability study was undertaken comparing the differences in CIDR 330 inserts made with Dow Corning Q7-4840 silicone and the alternative supplier silicone.

Fifty CIDR 330 inserts were taken from batch E08106 made with the alternative supplier silicone, and 50 CIDR 330 inserts were taken from batch E08105 made with Dow Corning Q7-4840 silicone (batch 0001923092). Both batches were made with Pfizer progesterone from batch 63KYW. Samples were placed in the stability oven in large unsealed plastic bags. The bags were wrapped around the CIDR inserts.

Tests were undertaken on the stability samples at periodic intervals to allow the observation of changes to the CIDR inserts over the duration of the experiment. Tests undertaken included SEM, surface progesterone, progesterone content analysis and drug release rate tests.

6.1.1 Surface progesterone on CIDR 330 inserts made with the alternative supplier silicone versus Dow Corning Q7-4840 silicone

During the study CIDR inserts were removed from the stability oven for surface progesterone analysis at a range of time points (t = 0, 2, 3, 4.5, and 5.5 months respectively, $n \geq 3$). The level of surface progesterone analysed by the method described in Chapter Three.

The results from the surface progesterone tests (two and five minute sampling points) are shown in Figures 6.1 and 6.2. It is clear that the average level of surface progesterone for the CIDR inserts made with Dow Corning Q7-4840 silicone (batch E08105) increased after two months in the stability oven and remaining constant at t = 3 months before increasing at t = 4.5 months. This is contrasted with CIDR 330 inserts made with the alternative supplier silicone (batch E08106), which had minimal changes in the average level of surface progesterone over the course of the study. These results concur with previous results by Reardon (Reardon, 2004b) who found that slabs made with the alternative supplier silicone did not exhibit secondary blooming as stated in Chapter One.

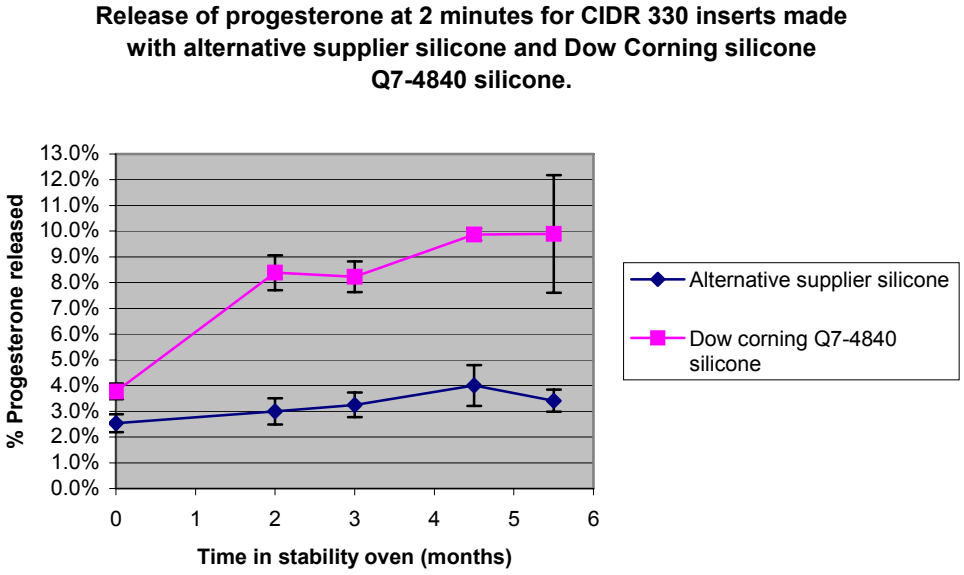


Figure 6.1 Surface progesterone analysis, average release at two minutes for CIDR 330 inserts made with alternative supplier silicone (batch E08106) and Dow Corning Q7-4840 silicone (batch E08105). Error bars are the 95 % confidence interval. n ≥ 3. % Progesterone released as % of label value (330 mg).

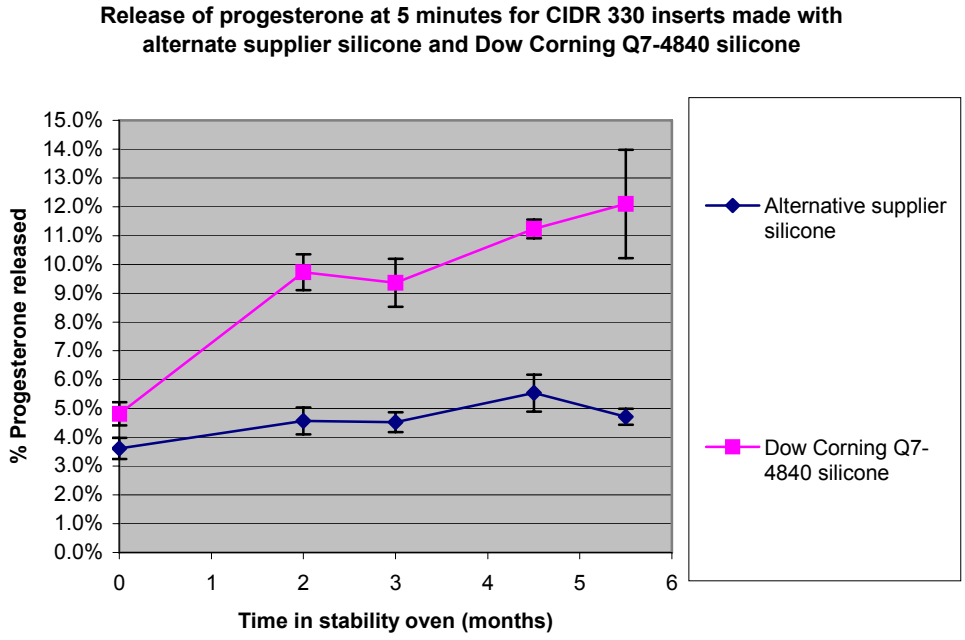


Figure 6.2 Surface progesterone analysis, average release at five minutes for CIDR 330 inserts made with alternative supplier silicone (batch E08106) and Dow Corning Q7-4840 silicone (batch E08105). Error bars are the 95 % confidence interval. n ≥ 3. % Progesterone released as % of label value (330 mg).

There is a large variation in the surface progesterone for CIDR 330 inserts made with Dow Corning Q7-4840 silicone at t = 5.5 months (Figures 6.1 and 6.2). It would be possible that some of the CIDR inserts analysed actually had lower surface progesterone levels, however visual observations of surface progesterone, undertaken before the start of the surface progesterone tests at the 5.5 month time point found that there was a high level of surface progesterone on all of the Dow Corning Q7-4840 CIDR inserts analysed, while the alternative silicone supplier CIDR inserts were recorded as having no surface progesterone.

It is also possible that either contamination or dilution occurred, which would result in the wide variation, however the blank sample did not show any sign of contamination. Furthermore samples were stored in capped glass bottles when not in use, which would further decrease the chance of contamination.
Other studies in this thesis that used surface progesterone analysis using the method developed for this thesis, often exhibited a wide range of surface progesterone levels. Furthermore this study found significant statistical differences in surface progesterone levels between the different silicone feedstocks, whereas other studies that the surface progesterone method developed in this thesis did not.

The most probable cause of this phenomena would be from dislodging of progesterone from the surface of some CIDR inserts before testing. Wong (Wong, 2003e) thought that the decreasing progesterone content values of CIDR inserts with increasing % mottling, is caused by dislodging of progesterone from the surface of CIDR inserts.

6.1.2 Drug release rate on CIDR 330 inserts made with Dow Corning Q7-4840 and alternative supplier silicones

As noted in the Introduction CIDR inserts with higher levels of mottling can have a lower drug release rate (NICAR FL320, 2005) (Wong, 2003j). Drug release rate tests are able to provide complementary information on the level of surface progesterone through the mass of progesterone released after one hour in a 20 hour drug release rate test. Hence CIDR inserts that had been stored in the

stability oven made from the alternative supplier silicone (batch E08106) and Dow Corning Q7-4840 silicone (batch E08105), were analysed using Hanson Dissolution apparatus to determine the drug release rate and surface progesterone levels.

CIDR inserts from batches E08105 (Dow Corning Q7-4840 silicone) and E08106 (alternative supplier silicone) were analysed at the following time points after placement in the stability oven.

- Samples that had not been placed in the stability oven were analysed approximately one month after manufacture. (labelled as t = 0 months).
- Samples that had been in the stability oven for three months were analysed approximately one month after removal from the stability oven and stored in the laboratory. (labelled as t = 3 months)
- Samples that had been stored in the oven for 5.5 months were analysed shortly after removal from the stability oven. Some were analysed with and without their internal spines present at this time point.

A total of six CIDR inserts were analysed at each time point, of which three were made from the alternative supplier silicone (E08106) and three were from the Dow Corning Q7-4840 silicone (E08106). At t = 5.5 months four CIDR 330 inserts were tested with the spine removed, and two CIDR 330 inserts were tested with their spines intact as manufactured.

The results from the drug release rate tests are shown in Figure 6.3. Figure 6.3 shows no statistical difference between CIDR inserts made with different silicone feedstocks. It is of note however that the drug release rate stabilised at the three month time point. Although not statistically significant there is a trend that shows that the Dow Corning CIDR insert (batch E08105) has the higher drug release rate compared with the CIDR insert made with the alternative supplier silicone (batch E08106) at the t = 5.5 months. This is expected to be due to the natural variation in drug release rates as observed at previous time points and not due to differences in the progesterone release rate from the different silicones.

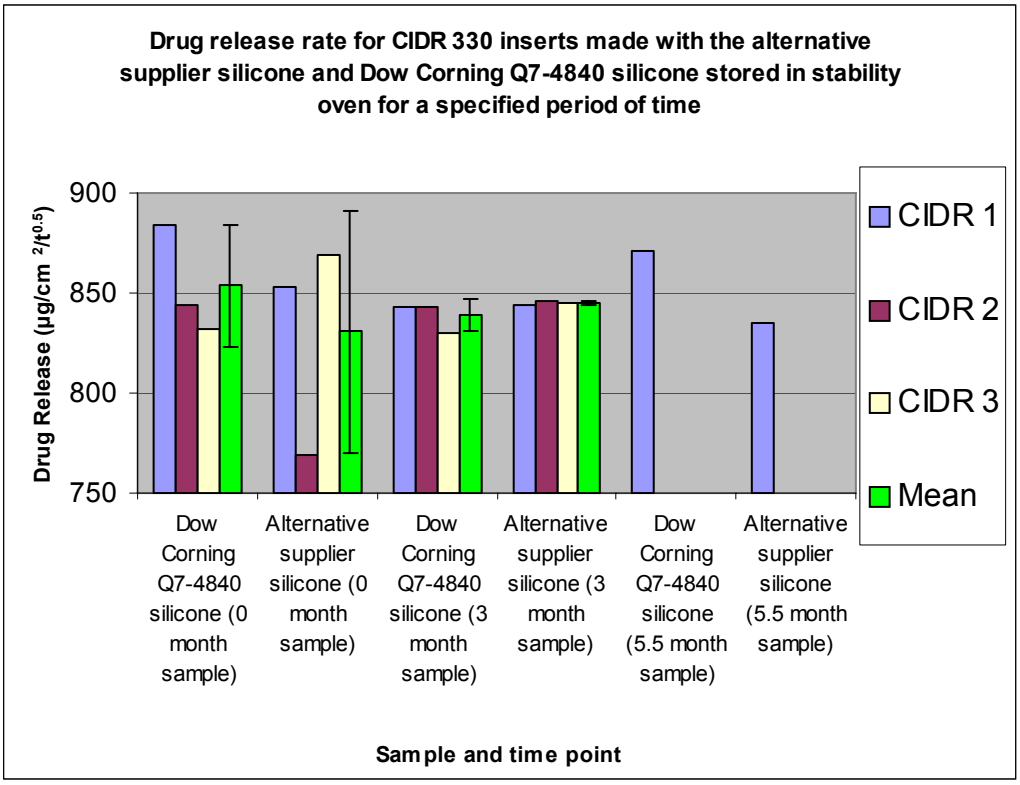


Figure 6.3 Drug release rate of CIDR 330 inserts made with Dow Corning Q7-4840 silicone (batch E08105) and alternative supplier silicone (batch E08106) over time in the stability oven. At t = 5.5 months only one sample was tested and hence no mean is recorded. Error bars are the 95 % confidence interval.

Reardon (Reardon, 2004a) found that CIDR inserts made from Dow Corning silicone inserts had a lower drug release rate compared to the alternative supplier silicone. Reardon hypothesised that different cure times could be causing differences in drug release rate, however Laird (Laird, 2004b) found that it was difficult to make conclusions of drug release rate with respect to cure times of the CIDR inserts. It is noted that diffusion is the rate-determining step in 'in vitro' drug release rate (Rathbone, et. al., 2000), and the results from this section indicate that there is no difference in diffusion between the different silicone feedstocks.

It is also known that there is a tentative relationship between drug release rate and increasing surface progesterone (Wong, 2003e) (NIICAR FL390, 2005), however these results show that there is no difference in the drug release rate between the two silicone feedstocks despite the observed differences in surface progesterone.

Spineless CIDR 330 inserts that had been made with different silicone feedstocks were analysed at t = 5.5 months by Hanson Dissolution apparatus to determine the drug release rate. CIDR inserts were tested without their spines as work by Laird (Laird, 2004f) on CIDR 1380 inserts found that there was a linear relationship between increasing drug release rate and the decreasing adherence of the skin to the spine. Removal of the spine increases the surface area of the silicone skin that is exposed to the release media, hence increasing the drug release rate. The results are shown in Figure 6.4. From Figure 6.4 there is a trend that inserts made with an alternative silicone supplier (batch E08106) have a greater drug release rate compared with the CIDR inserts made with the Dow Corning Q7-4840 silicone (batch E08105). However this is not statistically different (Student T tests indicate that there is an insignificant difference between the different feedstocks).

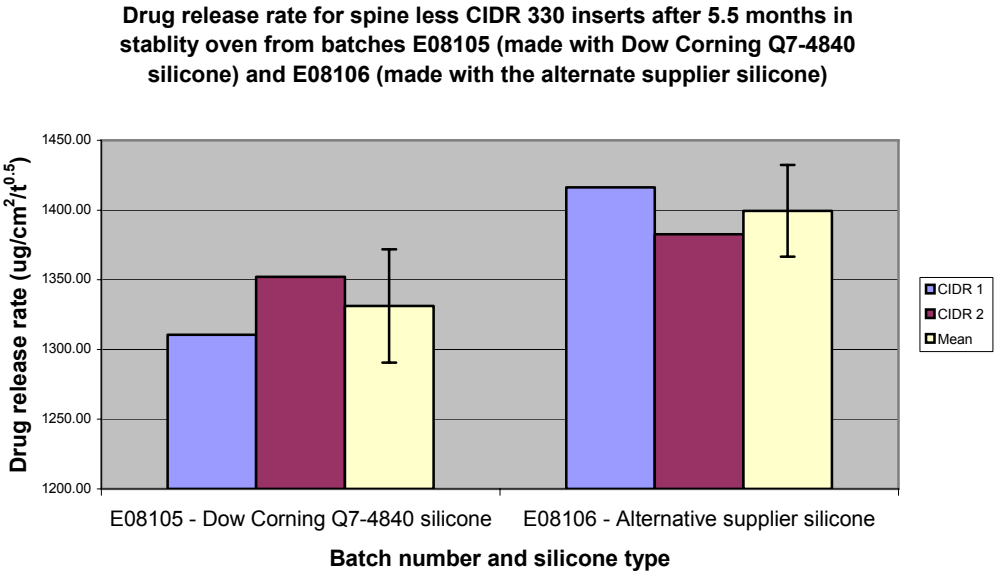


Figure 6.4 Drug release rate analysed by Hanson Dissolution from CIDR 330 inserts, which had the spine removed, tested at t = 5.5 months in stability oven. Error bars are the 95 % confidence interval. Drug release rate calculated from a surface area of 28 cm^2.

There is no statistically significant difference in drug release rate between CIDR inserts made with different silicone feedstocks as tested by Hanson Dissolution apparatus, regardless whether the CIDR insert was tested with or without the spine.

6.1.2.1 Surface progesterone on CIDR 330 inserts made with different silicones analysed by Hanson Dissolution drug release rate test after storage in the stability oven

The surface progesterone on CIDR inserts made with the different silicone feedstocks were analysed, using the mass of progesterone released after one hour in the Hanson Dissolution apparatus drug release rate test (As discussed in the Introduction). The results are shown in Figure 6.5. Student T tests for CIDR inserts made with the alternative supplier silicone (E08106) found the mass of progesterone released after one hour to be insignificant between the zero and three month time points. Student T tests for CIDR inserts made with the Dow Corning silicone (E08106) show a significant difference between the 0 and three month time points. There is a clear statistical difference in the surface progesterone, with CIDR inserts made with the alternative supplier silicone having less surface progesterone compared to CIDR inserts made with Dow Corning Q7-4840 silicone at both time points.

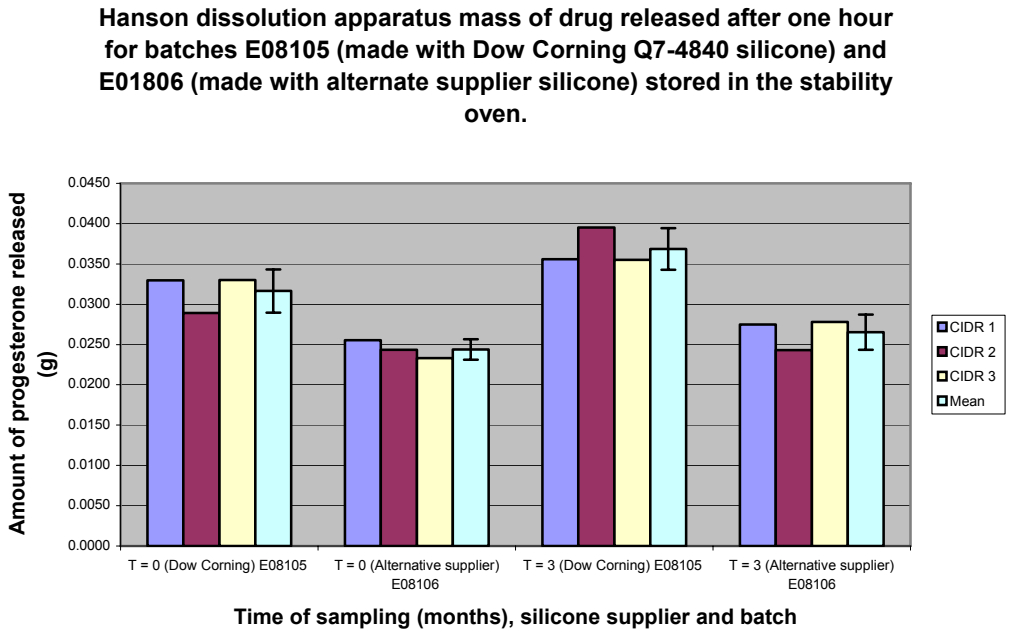


Figure 6.5 Amount of progesterone released after one hour in a Hanson Dissolution apparatus drug release rate test for CIDR 330 inserts made with Dow Corning Q7-4840 Silicone and alternative supplier silicone. The mean is the mean of CIDR insert 1 to CIDR insert 3. Error bars are the 95 % confidence interval.

In the Section 6.1.1 it was shown that after two months in the stability oven the surface progesterone on CIDR 330 inserts made with Dow Corning Q7-4840 silicone increased, compared with the alternative supplier silicone CIDR 330 inserts. The results from that experiment are in accordance with this result.

6.1.3 SEM on CIDR 330 inserts made with different silicone feedstocks after storage in the stability oven

In order to gain an understanding of the changes in surface morphology on CIDR inserts made with different silicone feedstocks, SEM was undertaken on samples that had been stored in the stability oven. The time points are listed in Table 6.1.

Table 6.1 SEM samples and date of scan.

Time in stability oven.	**Samples Scanned.**
1 month	Batch E08105 (Dow Corning Q7-4840 silicone), and batch E01806 (alternative supplier silicone), and one CIDR from batch E08016, which had been stored in the laboratory. See Figure 6.7 to 6.8.
1 month	Samples wiped with ethanol before scanning to remove progesterone from the surface of the sample. Samples from batch E08105 (Dow Corning Q7-4840 silicone), and batch E01806 (Alternative supplier silicone).
3 months	CIDR 330 inserts made with Dow Corning Q7-4840 silicone (batch E08015) and the alternative supplier silicone (batch E08106).

SEM samples were cut from the top of the wing of the CIDR insert. Along the top of the wing of the CIDR insert is a small ridge formed by flash. Flash is caused by silicone squeezing between the two sides of the closed tool, and would have received increased heat during cure because of the close contact with the two sides of the tool. Flash is minimized in normal manufacturing procedure. Figure 6.6

shows an example of flash that occurs if the shot volume is too large. Flash is usually minimal in normal production in order to increase productivity.

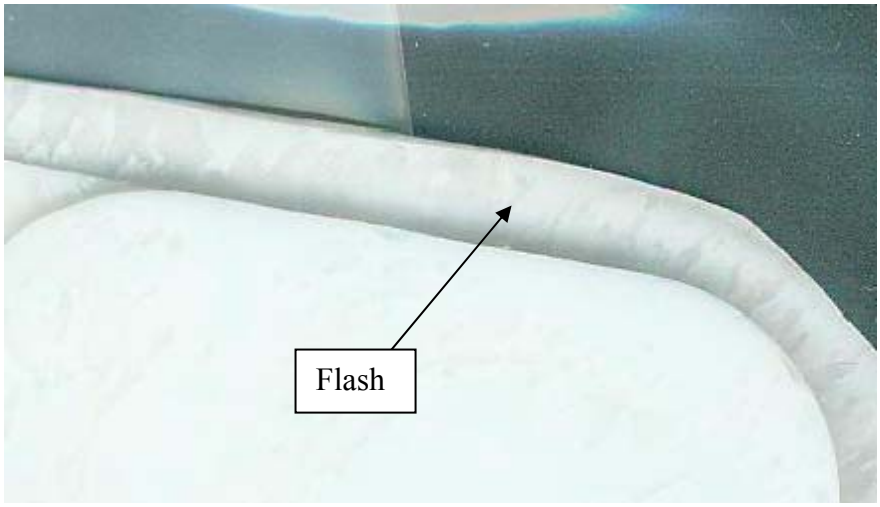


Figure 6.6 Example of flash on a hand moulded slab if the shot volume is too large. Image not to scale.

6.1.3.1 SEM observations on CIDR 330 inserts after one month in the stability oven

Analysis of samples at t = 1 month showed that the CIDR 330 insert made with alternative supplier silicone had fewer surface crystals (see Figure 6.7) compared to the CIDR 330 insert made with Dow Corning Q7-4840 silicone (see Figure 6.8)

Figures 6.7.1 and 6.7.2 show the crystals on the surface of CIDR 330 insert made with the alternative supplier silicone (batch E08106) that had been in the stability oven for one month. Figure 6.7.3 shows a close up on the flash of the CIDR insert showing crystals growing on the flash. Figure 6.7.4 is a higher magnification image of the clear region to the left of the flash in Figure 6.7.1, however close-ups of Figure 6.7.4 (Figure 6.7.5 and 6.7.6) show small crystals on the CIDR insert surface. In Figure 6.7.2 the crystals on the surface are rectangular and different from the other crystals shown in the 6.7. These crystals clearly cover the surface as shown if Figure 6.7.1. It is known that the alternative supplier silicone does undergo an initial blooming after manufacture (Reardon, 2004b), which would bring a progesterone layer to the surface of the CIDR insert. This progesterone

could then transform into the observed crystals observed in Figures 6.7.1 and 6.7.2.

Figure 6.7 SEM images of CIDR 330 insert made with the alternative supplier silicone (batch E08106) analysed at t = 1 month.

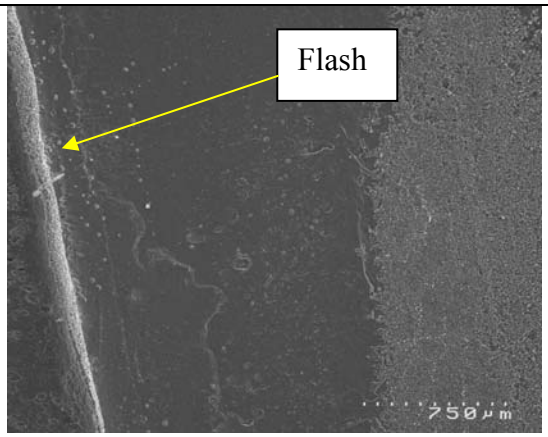
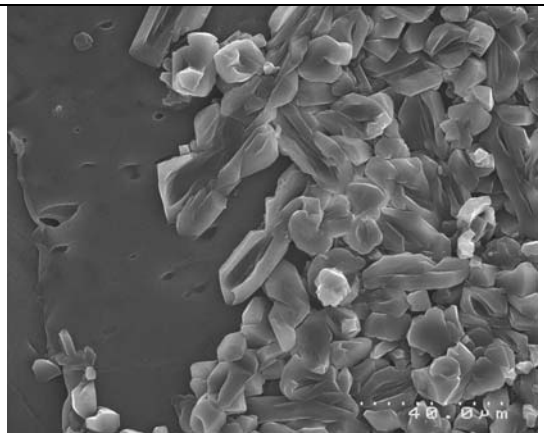
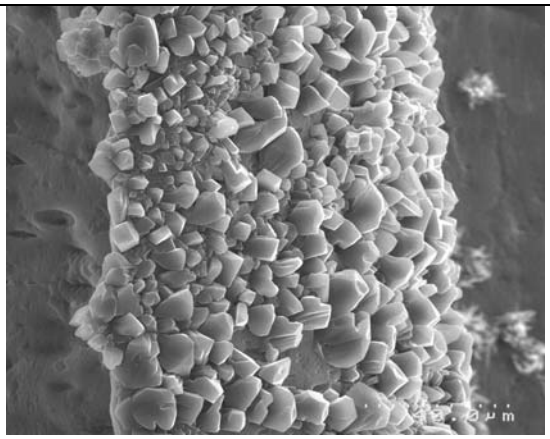
Figure 6.7.1 Low magnification image of CIDR 330 insert made with the alternative supplier silicone.	
Figure 6.7.2 High magnification image of CIDR 330 image made with the alternative supplier silicone. Image of the border between the crystalline region and the smooth region in Figure 6.7.1.	
Figure 6.7.3 High magnification image of the flash (see also Figure 6.7.1).	

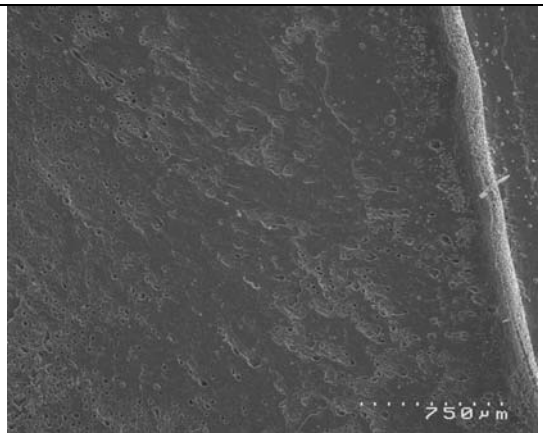
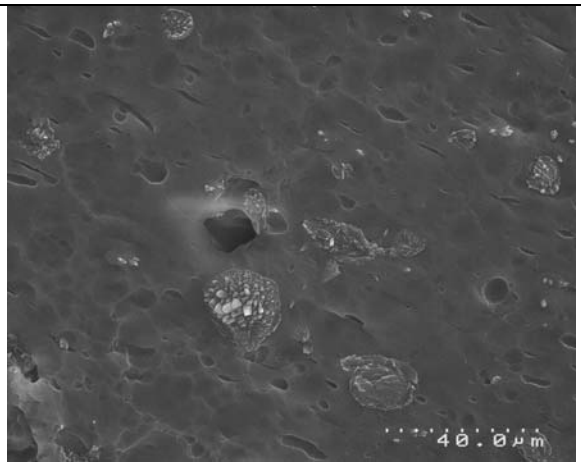
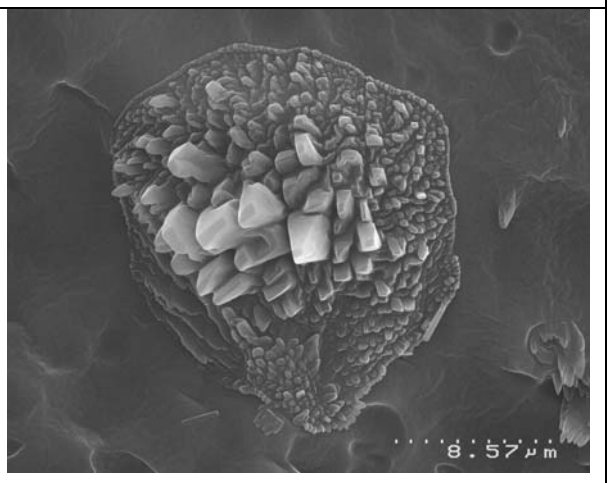
Figure 6.7.4 Low magnification image to the left of the flash shown in Figure 6.7.1.	
Figure 6.7.5 Higher magnification image of Figure 6.7.4. showing small crystal clusters on the insert surface.	
Figure 6.7.6 Close-up of a progesterone cluster from Figure 6.7.5.	

Figure 6.8.1 shows a low magnification SEM image of the Dow Corning Q7-4840 silicone CIDR 330 insert made with the Dow Corning Q7-4840 silicone (batch E08015). This image clearly shows that there is a difference in the surface morphology between CIDR 330 inserts made with the alternative supplier silicone and CIDR 330 inserts made with Dow Corning Q7-4840 silicone. There appear to be two types of crystals observed on the surface of the CIDR insert, as Figure 6.8.2 shows a block like crystals and Figure 6.8.4 that shows rectangular crystals.

These longer and more regular crystals are similar in morphology to the crystals on the CIDR 330 insert made with the alternative supplier silicone (batch E08106) as observed when comparing with Figures 6.7.2, and 6.7.3. Figure 6.8.5 shows a close up of the flash from the CIDR 330 insert made with Dow Corning Q7-4840 silicone (batch E08105).

Figure 6.8 SEM images of CIDR 330 insert made with Dow Corning silicone (batch E08105) analysed at t = 1 month.

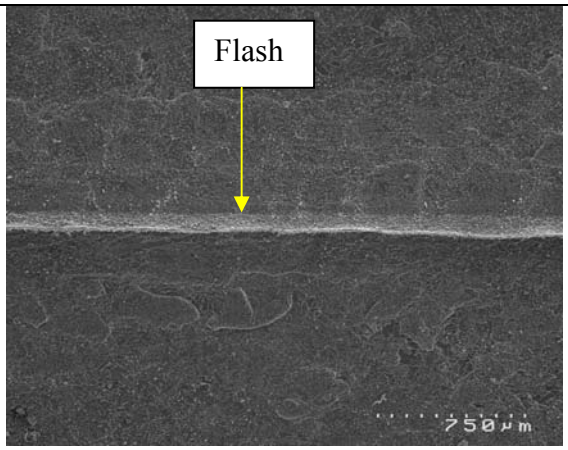
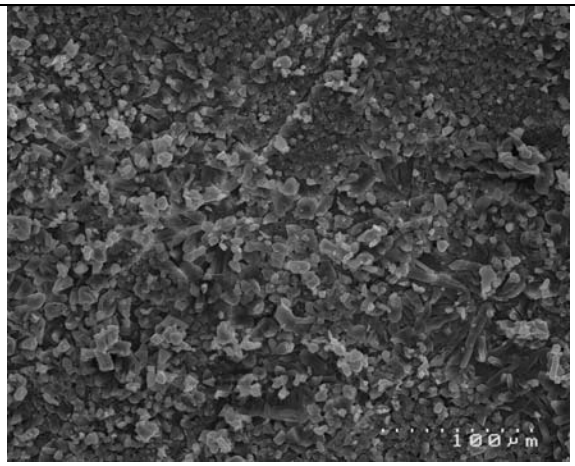
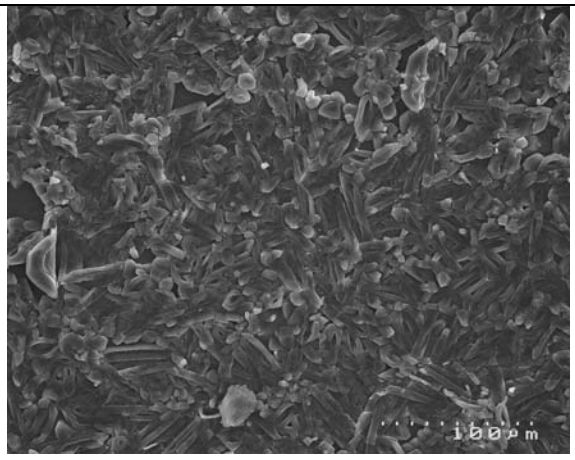
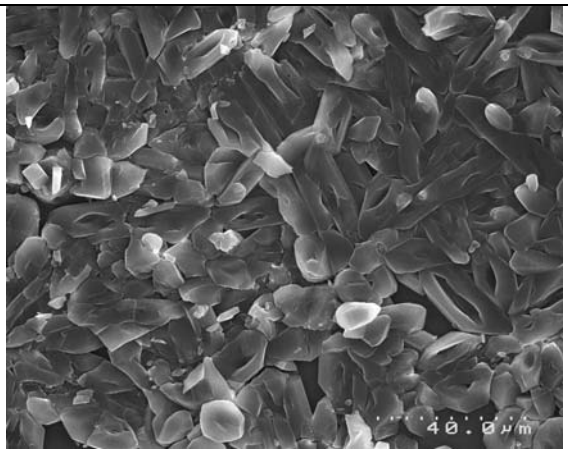
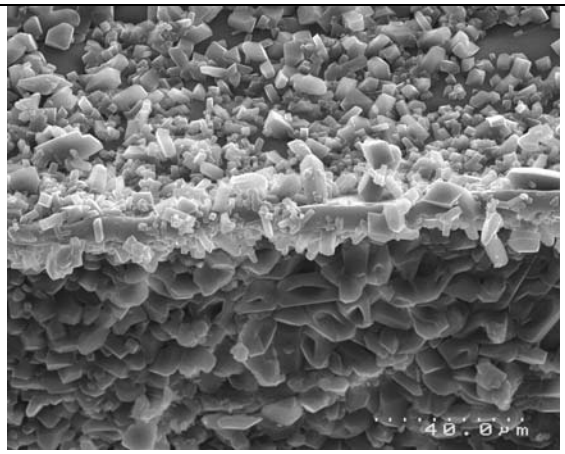
Figure 6.8.1 Low magnification image of the CIDR insert.	
Figure 6.8.2 Higher magnification image of crystals on the surface of the insert.	
Figure 6.8.3 Higher magnification image of crystals on the surface of the insert.	

Figure 6.8.4 Close-up of crystals on the insert surface.	
Figure 6.8.5 Close-up of crystals on the flash.	

The flash on both samples made from the different silicone feedstocks (Figures 6.7.3 and 6.8.5) shows that both are well covered in crystals. However the flash of the insert made with Dow Corning Q7-4840 silicone (batch E08105) is surrounded with crystals (Figure 6.8.5) whereas the flash on the CIDR insert made with the alternative supplier silicone (batch E08106) (Figure 6.7.3) is not. The increased heat and/or pressure has appeared to increase the blooming on the flash from the alternative supplier silicone with respect to the surrounding silicone, whereas there appears to be little difference in the blooming on the flash of the insert made with the Dow Corning silicone with respect to the surrounding areas of the CIDR insert. The crystals on the flash made with the alternative supplier silicone are different from the other crystal shapes observed on the same insert (Figure 6.7).

SEM has been used to analyse mottled and non-mottled Sections of CIDR 330 inserts. SEM analysis (Bourke, 2004) on slabs made with alternative supplier silicone showed that after 14 days that there were a few small crystal structures

present, and bubbles in the silicone were unpopulated with crystals. This contrasted with samples made with Dow Corning Q7-4840 silicone, which had crystal deposits on the CIDR insert and also had air bubbles (inside the slab) filled with crystals (Bourke, 2004).

SEM images of the alternative supplier silicone CIDR 330 insert that was stored in the laboratory are shown in Figure 6.9. The crystal morphology on the CIDR insert is different from the other CIDR inserts made at this time point, with the crystals in Figure 6.9.2 being the dominant crystal formation. SEM images show that there is less crystallisation compared with the CIDR insert stored in the stability oven. The flash appears to have a reduced density of crystal coverage compared to the flashes from the other inserts analysed at this time point. The reduction in crystallisation could be a random phenomena or related to the stability oven, as no SEM scans were done at t = 0 months. None of the rectangular crystals (such as those shown in Figure 6.7.2) are observed on this inserts.

Figure 6.9 SEM images of CIDR 330 insert not stored in the stability oven one month after manufacture.

Figure 6.9.1 Low magnification image of CIDR insert showing the flash and regions of crystals.

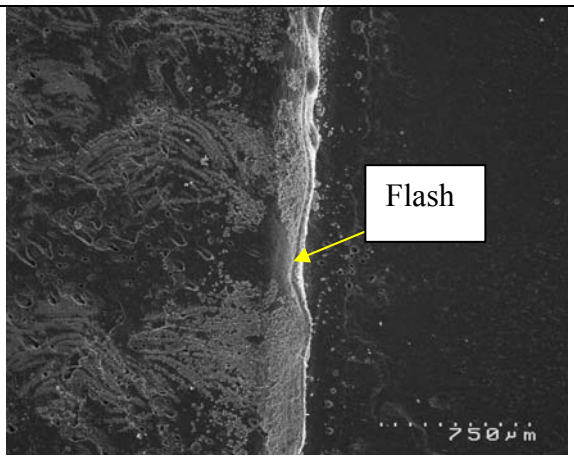
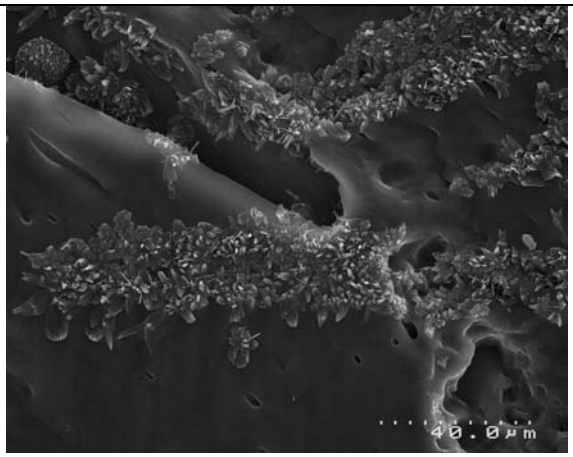
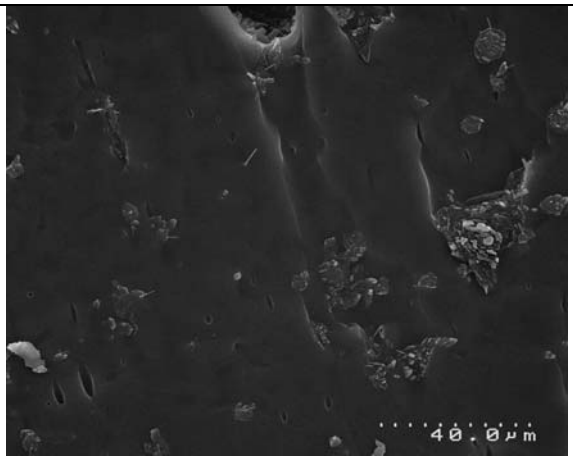
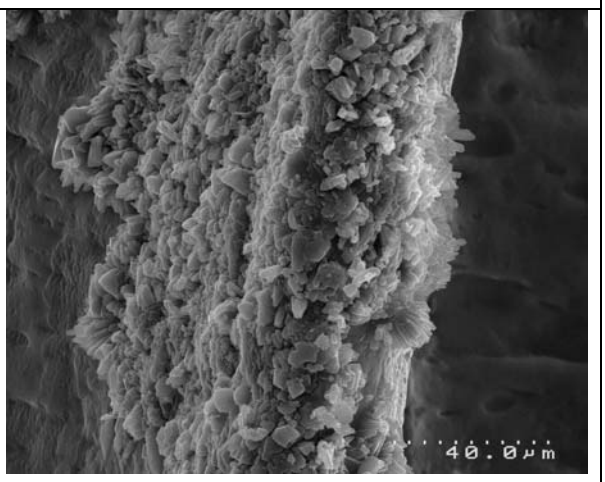


Figure 6.9.2 Close-up of crystals on the surface of the insert.	
Figure 6.9.3 Close-up of crystals on a clear area away from the flash on the surface of the insert.	
Figure 6.9.4 Close-up of the crystals on the flash.	

It is also useful to compare the crystals obtained as part of this SEM study with crystals of α and β polymorphs of progesterone obtained by Muramatsu et. al., (Muramatsu et. al., 1979). Comparing the crystals from Muramatsu et. al. (Figure 6.10) with the block like crystals observed in both the CIDR insert from batch E08105 (CIDR insert made with the Dow Corning Q7-4840 silicone, Figure 6.8.4) and the longer (more rectangular) crystals on the CIDR insert from batch E08106 (CIDR insert made with the alternative supplier silicone, Figure 6.7.2), there is no

similarity with either of the polymorphs pictured by Muramatsu et. al (see Figure 6.10). However the crystals observed in Figure 6.7.6 from insert made with the alternative supplier silicone, has crystals that are more like the α progesterone crystals due to their pointed tips. However without XRD or DSC data these observations are purely speculative.

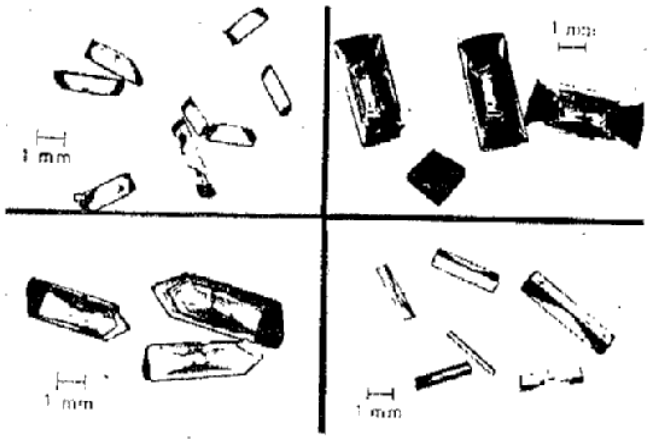


Figure 1—*Single crystals in α- (left) and β- (right) forms obtained by seeding–recrystallization from ethanolic mixtures (1:1 v/v) with water (top) and n-hexane (bottom).*

Figure 6.10 Crystals of α and β progesterone polymorphs from Muramatsu et. al. (Muramatsu, et. al., 1979)

6.1.3.2 SEM observations at t = 1 month in the stability oven and wiping with ethanol

In order to see if there is any difference in the surface morphology of a CIDR 330 insert made with Dow Corning Q7-4840 silicone and a CIDR 330 insert made with the alternative supplier silicone (t = 1 month), the surface of the samples were wiped with ethanol to remove surface progesterone then analysed by SEM.

Figure 6.11 shows SEM images of the CIDR insert made with the alternative supplier silicone (batch E08106). The surface of the device is clear with no visible surface progesterone. Holes are also observed in the silicone. Holes in the CIDR were also observed in the samples made with the alternative supplier silicone (batch E08106) stored for one month in the stability oven (Figures 6.7.5 and 6.9.2).

Figure 6.11 SEM images of CIDR 330 insert (batch E08106) made with alternative supplier silicone, stored in the stability oven and wiped down with ethanol. t = 1 month.

Figure 6.11.1 Low magnification image of CIDR insert showing the flash devoid of crystals.

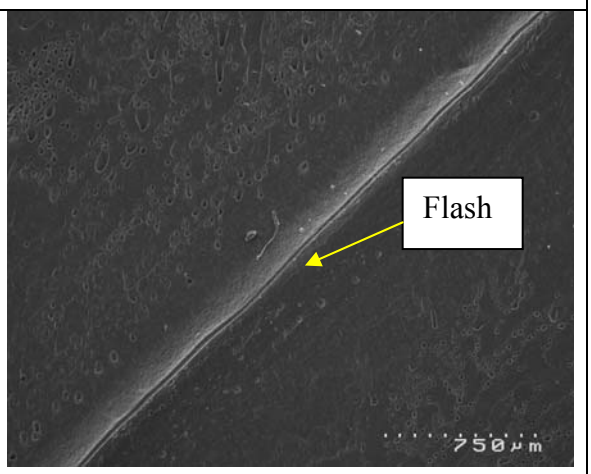


Figure 6.11.2 Close-up of the flash.

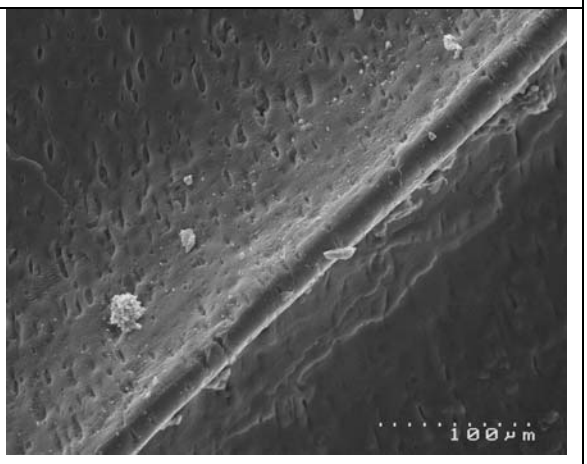
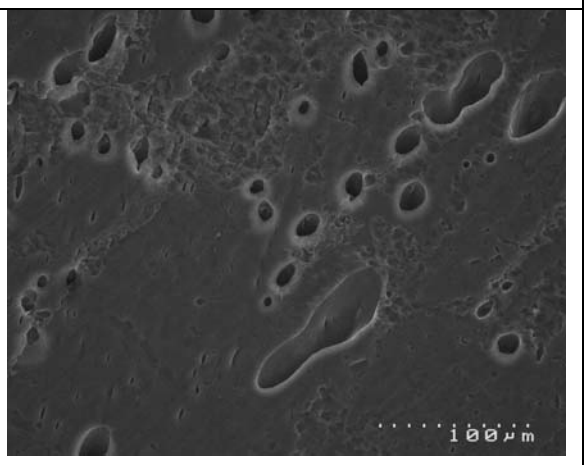


Figure 6.11.3 Close-up of holes in the sample surface.



Figures 6.12 shows SEM images of the CIDR made with Dow Corning Q7-4840 silicone. It is clear that there is some material remaining on the surface of the CIDR insert indicating that wiping the surface of the insert was not totally successful in removing all of the surface progesterone (Figure 6.12.2). The CIDR

330 insert made with the Dow Corning Q7-4840 silicone is observed to be featureless, with only small holes observed in the silicone.

Figure 6.12 SEM images of CIDR 330 insert (batch E08106) made with alternative supplier silicone, stored in the stability oven and wiped down with ethanol. t = 1 month.

Figure 6.12.1 Low magnification image of CIDR insert showing the flash devoid of most crystals.

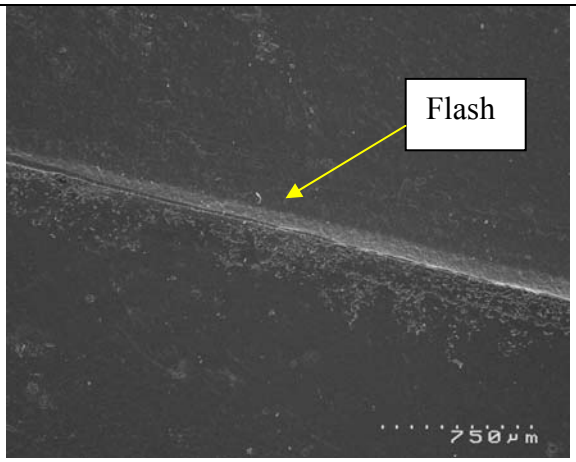


Figure 6.12.2 Higher magnification image of the flash showing that there is still some remaining progesterone not removed by ethanol wiping.

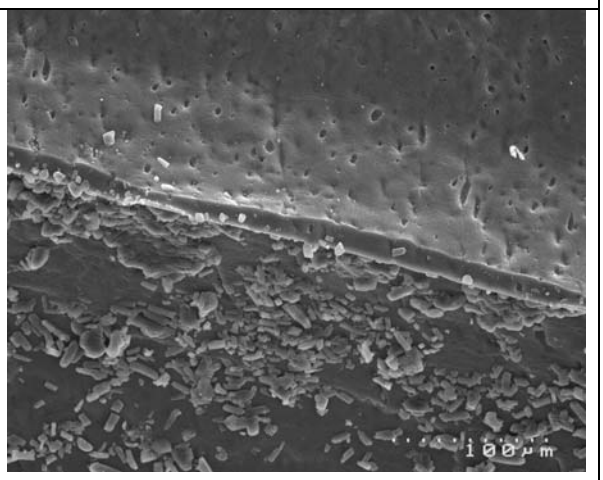
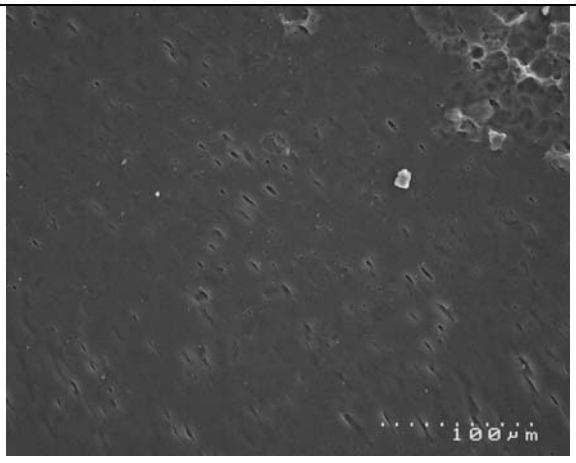


Figure 6.12.3 Higher magnification image of holes in the CIDR insert.



Comparing the two feedstocks, it is observed that the alternative supplier silicone has larger holes (Figure 6.11.3) compared to the CIDR insert made with Dow Corning Q7-4840 silicone. The hole sizes could be related to the feedstock type, however it is also possible that the holes are created by differences in mixing, or due to regional hole variation in the CIDR insert.

6.1.3.3 SEM observations at t = 3 months in the stability oven

SEM images of a CIDR 330 insert from batch E08106 made with the alternative supplier silicone is shown in Figure 6.13. These images show that there has been a change in the crystal type observed, compared with the one month stability samples that had block and rod shaped crystals, along with longer semi rectangular crystals (Figure 6.7.2). The rod like crystals observed in Figure 6.13.3 are similar to the β progesterone polymorph crystals from the work of Muramatsu et. al. (Muramatsu, et. al., 1979) shown in Figure 6.10. However it is also possible that what is observed is a crystal formation similar to the cluster observed on the alternative supplier silicone CIDR 330 insert after one month in the stability oven (see Figure 6.7.5) that had been disturbed by mechanical means

Figure 6.13 SEM images of CIDR 330 insert (batch E08106) made with alternative supplier silicone, t = 3 months.

Figure 6.13.1 Low magnification image of CIDR insert showing the flash devoid of most crystals. Tear in silicone created when detaching the sample from the CIDR insert.

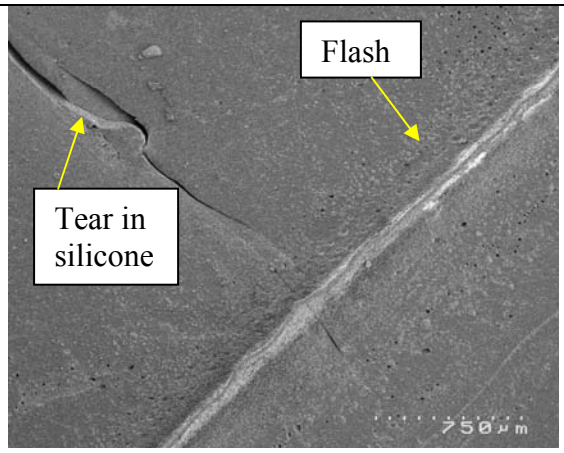


Figure 6.13.2 Higher magnification image of CIDR insert, taken above the flash in Figure 6.13.1.

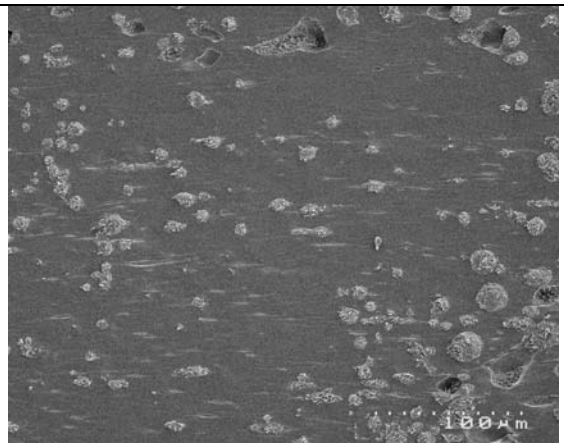


Figure 6.13.3 Close-up of CIDR insert showing a crystal formation.

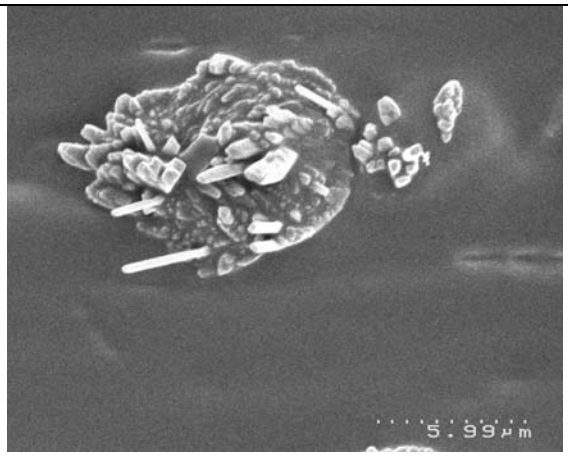


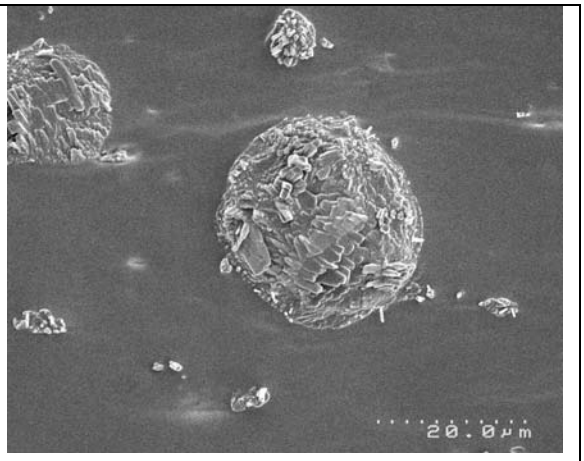
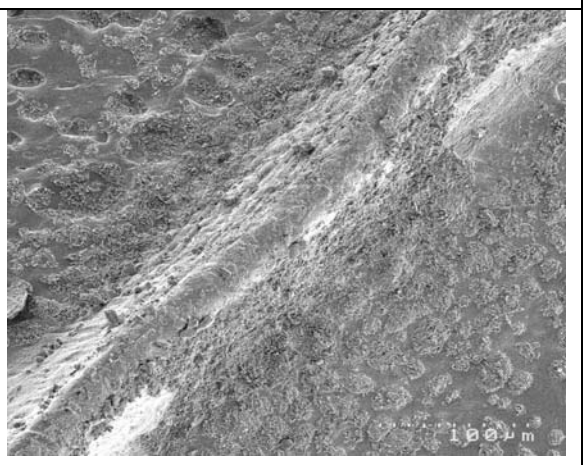
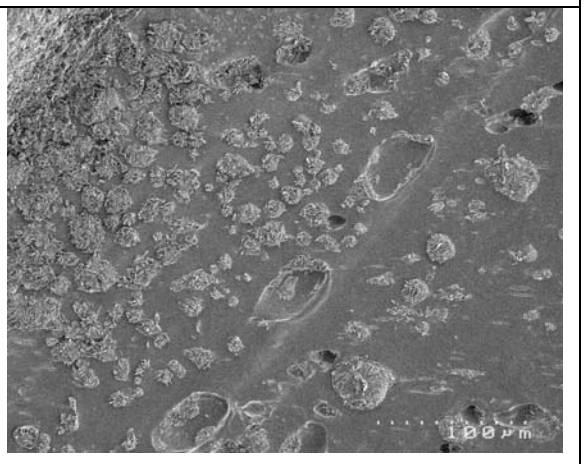
Figure 6.13.4 Crystals on the CIDR insert surface.	
Figure 6.13.5 Higher magnification image of the flash.	
Figure 6.13.6 SEM image taken just below the flash.	

Figure 6.14 shows SEM images of the CIDR 330 insert made with Dow Corning Q7-4840 silicone. These images also show a significant difference compared with the t = 1 month sample. The crystals observed on the surface are block like and cover most of the surface. It is noted that the crystals do not appear to cover the entire surface as observed in Figure 6.14.5. There are no crystals observed on the type observed in Figure 6.7.2

Figure 6.14 SEM images of CIDR inserts made with Dow Corning Q7-4840 silicone (batch E08105) t = 3 months.

Figure 6.14.4 Image of crystals on the insert surface on both sides of the slabs.

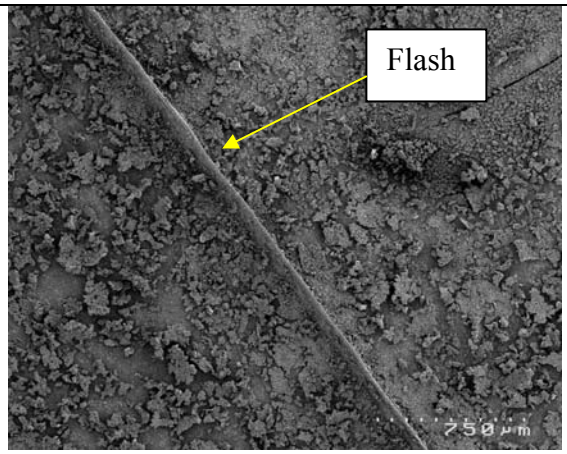


Figure 6.14.2 Close-up of the flash, showing crystals around and on the flash.

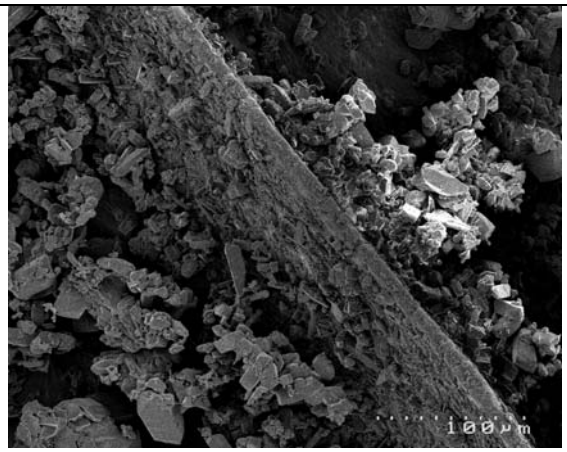


Figure 6.14.3 Close-up of crystals on the CIDR insert surface.

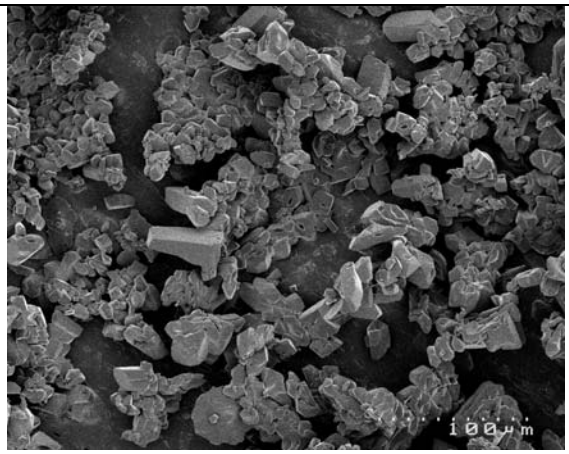
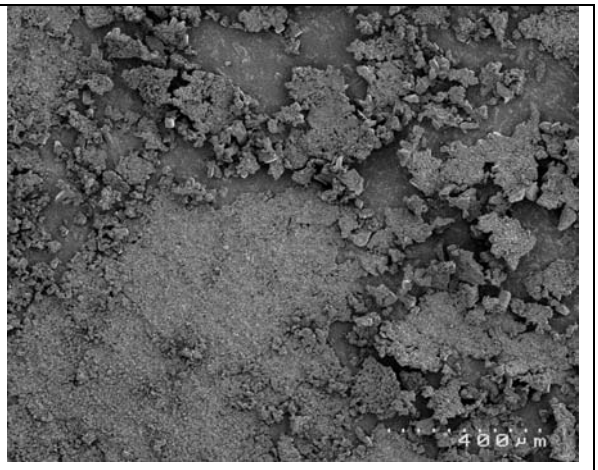
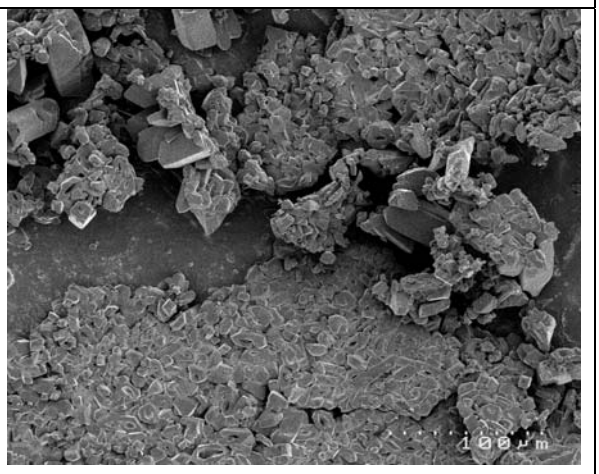


Figure 6.14.4 Image of crystals on the CIDR insert surface to the left of the flash in Figure 6.14.1.	
Figure 6.14.5 Image of crystals on the CIDR insert surface, image a close-up of Figure 6.14.5.	

Surface progesterone levels measured using the surface progesterone method on CIDR 330 inserts made with Dow Corning Q7-4840 silicone were found to have levelled out after two months in the stability oven (see Section 6.1.1). No surface progesterone tests were undertaken at t = 1 month on CIDR inserts made with either of the two silicone feedstocks. A DEC Manufacturing stability report (STAB001,1999-2004) notes migration of progesterone to the surface of the CIDR has been found to occur for two to three months after manufacture of the CIDR before stopping. Hence it is possible that the surface morphology changes that occurred between the t = 1 and t = 3 month samples, are from progesterone migrating to the surface. It would also be possible that the progesterone is undergoing changes in some manner over the two months between SEM observations. However Muramatsu et al. (Muramatsu, et. al., 1979) noted that both the α and β progesterone polymorphs are stable at room temperature for many months. Work in Chapter Four also found that there was no polymorphic transformation of either the α or β progesterone polymorphs in the stability oven.

Progesterone travelling to the surface may undergo polymorphic changes, and it is known that work by Rades & McFetridge (Rades & McFetridge, 2003) found that the progesterone scraped of a CIDR insert was the α progesterone polymorph.

Both types of CIDR 330 inserts made with the alternative supplier silicone and Dow Corning Q7-4840 had differences in the progesterone morphology on the surfaces between the one month and three month samples. Work by Reardon (Reardon, 2004b) found that the alternative supplier silicone does not undergo secondary blooming (although slowly undergoing an initial blooming), hence it is possible that the changes observed in the alternative supplier silicone between one and three months is due to crystallisation of progesterone on the surface, through low level migration or crystalline dislodgement.

6.1.4 Progesterone content differences between CIDR 330 inserts made with alternative supplier and Dow Corning Q7-4840 silicones stored in the stability oven

As part of the study into the differences between CIDR 330 inserts were made with different feedstock silicones and stored in the stability oven. The inserts were analysed for their progesterone content using the method described in Chapter Three. Initial results found that after three months in the stability oven, samples made with Dow Corning Q7-4840 silicone (batch E08105) had a decrease in the amount of total extractable progesterone. In order to verify this result more samples of CIDR 330 inserts were taken and analysed and the same result was found. The results are shown in Figure 6.15.

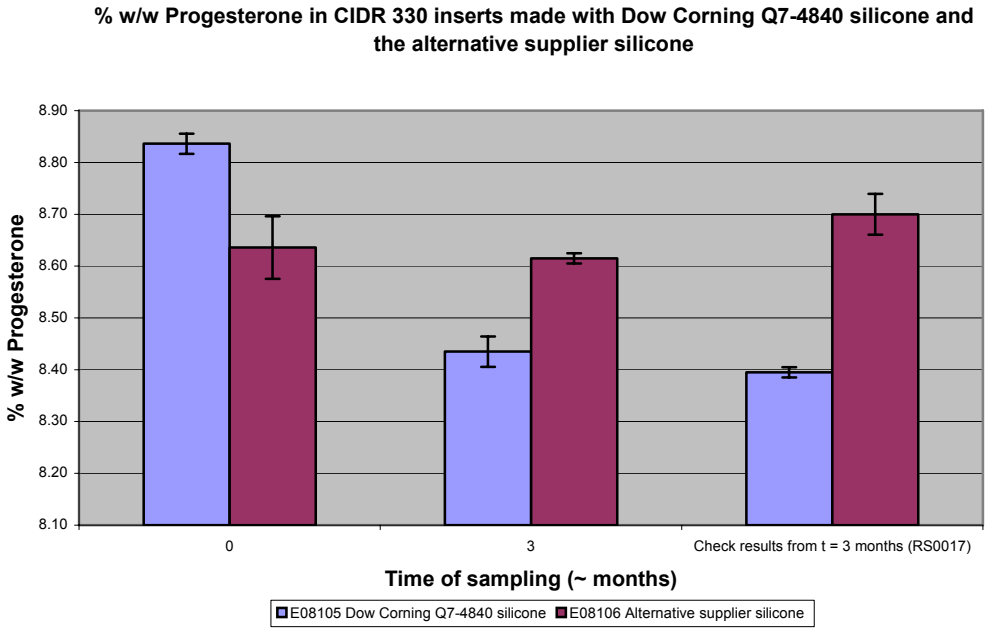


Figure 6.15 % w/w Progesterone in CIDR 330 Inserts made with Dow Corning Q7-4840 silicone (batch E08105) and an alternative supplier silicone (batch E08106). Error bars are the 95 % confidence interval. t = 0, results are from normal QC testing on the insert samples. At t = 0 n ≥ 8, at t = 3 n = 2.

Wong (Wong, 2003e) found that higher mottling can lead to lower progesterone content values. Wong reasoned that progesterone on the surface of a CIDR insert could be dislodged by handling and hence reduce total progesterone content values. Previous studies in this Chapter showed that there are higher surface progesterone levels on a CIDR 330 insert made with Dow Corning Q7-4840 silicone compared to a CIDR 330 insert made with the alternative supplier silicone.

6.1.5 Differences in % mottling of CIDR 330 inserts made with different silicone feedstocks

CIDR 330 made with different silicone feedstocks that had been placed in the stability oven for 15.5 months were analysed for % mottling. During the time in the stability oven the water container ran dry, but as discussed in Chapter Three this was of concern.

A total of 13 CIDR 330 inserts made with Dow Corning Q7-4840 silicone from batch E08105 were found to all exhibit very strong blooming. It was easy to dislodge progesterone from the device. It was found that six of these CIDR 330 inserts had some degree of mottling. The high level of progesterone observed on the surface of the CIDR inserts agrees with previous results in this Chapter. The lack of mottling for these inserts made with the Dow Corning Q7-4840 silicone (while possessing a high level of blooming) is interesting. Higher mottling brings about a higher mass of progesterone released on a CIDR 1380 insert as determined from a indicative Hanson Dissolution drug release test (sampling after one hour) (Wong, 2003e) (Wong, 2003j) (NICAR FL390). The CIDR 1380 insert has a thinner skin compared with the CIDR 330 insert, which may have an effect on drug migration. It is also known that the CIDR 330 insert has a reduced drug load of 9 % w/w compared to either the CIDR 1900 or 1380 inserts (10 % w/w), which could be a cause of this phenomena.

A total of 12 CIDR 330 inserts made with the alternative supplier silicone were analysed for % mottling. These CIDR inserts exhibited no mottling, or secondary blooming and in fact had the expected appearance. It was discovered that the black paper the CIDR inserts were placed on during analysis possessed some white stains after removal of the CIDR inserts. Previous SEM scans have clearly show that there is some surface progesterone on the CIDR inserts made with the alternative supplier silicone

The lack of mottling for the CIDR 330 inserts made with the alternative supplier silicone agrees with previous studies by Reardon (Reardon, 2004b), who found that CIDR 1380 inserts made with Dow Corning Q7-4840 silicone did not exhibit any mottling or blooming.

6.2 Variations in raw materials

6.2.1 Yield stress differences between different batches of liquid silicone

Historically the viscosity between different batches of silicone varies, with some batches being very thick and others described as “almost pourable”. In order to

investigate this phenomena the yield stress of various batches of liquid silicone (parts A and B of Dow Corning Q7-4840) were measured. Yield stress was measured using a constant rate yield test described in Chapter Three. The yield stress of a sample is the stress required to initiate flow.

Yield stress versus Hanson Dissolution drug release rate

Since the drug release rate can be related to secondary blooming (Wong, 2003e), the yield stress of a range of silicone batches were analysed for yield stress with respect to the drug release rate of CIDR 1380 inserts made with that silicone batch. Drug release rate data was taken from work by Dougal Laird, who collated a wide range of drug release rate results for CIDR 1380 inserts, from data from general laboratory testing. The mean yield stress from a range of measurements was plotted versus the average drug release rate. This resulted in a trend of increasing drug release rate versus increasing yield stress for both parts A and B of the liquid silicone (see Figures 6.16 and 6.17).

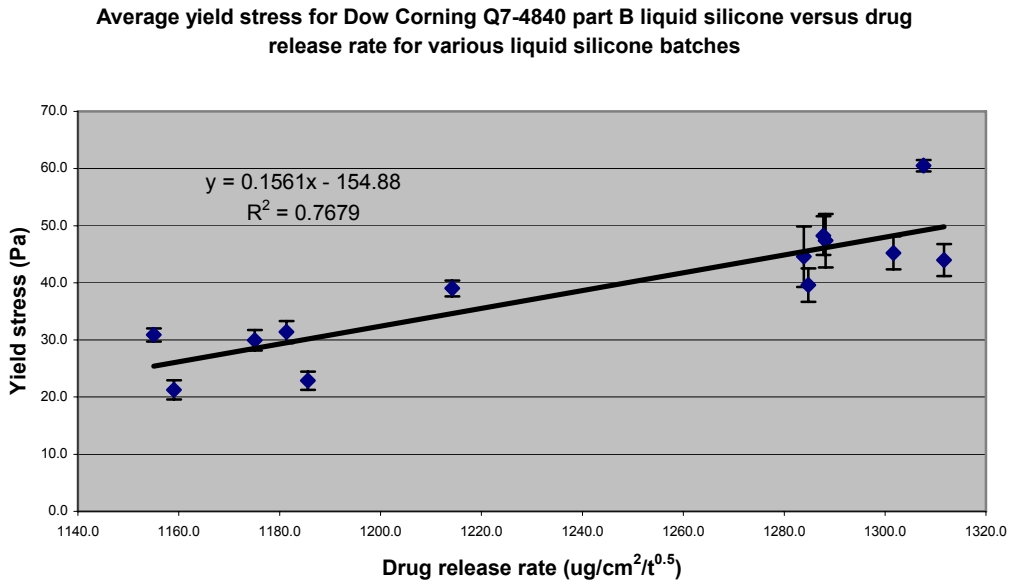


Figure 6.16 Average yield stress of part B liquid silicone versus average Hanson Dissolution drug release rate for a range of silicone batches. Error bars are the standard deviation.

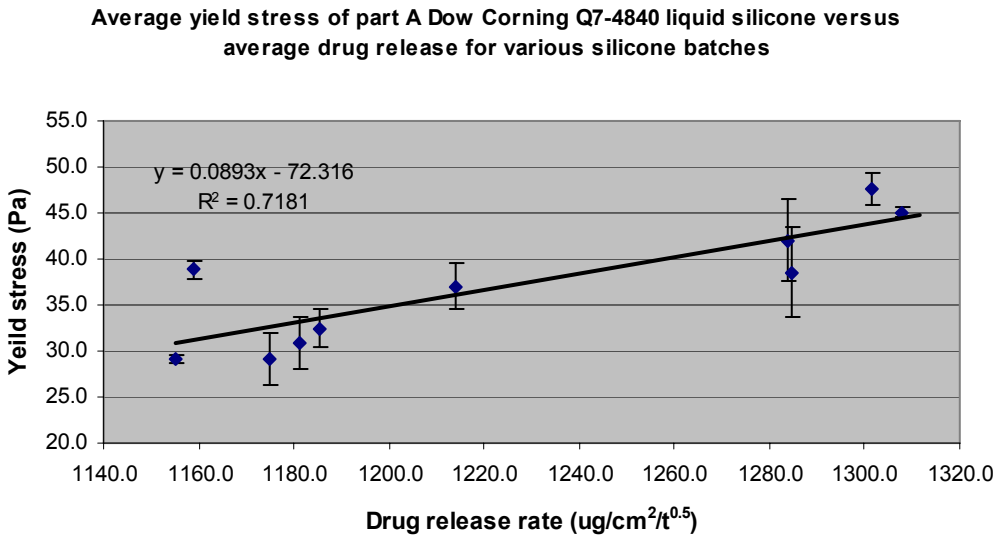


Figure 6.17 Average yield stress from many measurements of the sample of part A liquid silicone versus average Hanson Dissolution drug release rate for a range of silicone batches. Error bars are the standard deviation.

It is important to ensure that the data being observed in Figure 6.16 are not caused by the differences in sample age. Figure 6.18 clearly shows that there is no trend of manufacture date versus part B liquid silicone yield stress. Dow Corning state that their silicone is suitable for use for 18 months after manufacture (Dow Corning, 2005).

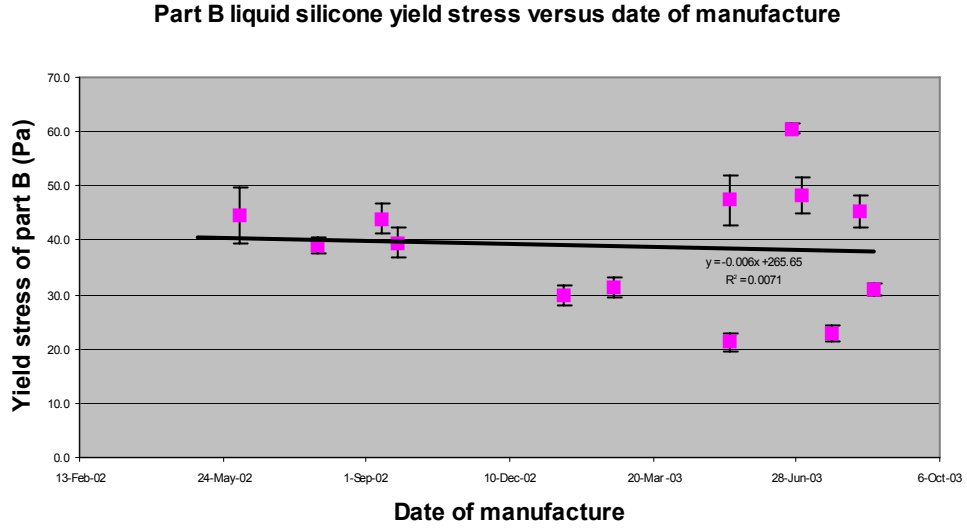


Figure 6.18 Average yield stress of liquid silicone part B versus date of manufacture for a range of silicone batches. Error bars are the standard deviation.

The slope of the trend line for part A average yield stress (Figure 6.17) is less than the slope of the trend line for part B average yield stress (Figure 6.16). Figure 6.19 shows that there is no trend between the yield stress of parts A and B from the same silicone batch. One part of Dow Corning Q7-4840 will contain the crosslinker, whereas the other part will contain the platinum catalyst (Lee, et. al., 1979). Figure 6.18 clearly shows that there is no relationship between age and yield stress, which indicates that any curing of the part B liquid silicone per se, would be minimal.

It is also known that there are differences in the compositions of parts A and B Dow Corning Q7-4840 liquid silicone, with the GCMS TIC (see Chapter 5) of part A having fewer peaks compared to the GCMS TIC of part B. Hence the observed differences in the composition between parts A and B of the Dow Corning Q7-4840 silicone, may be causing the differences in observed yield stress.

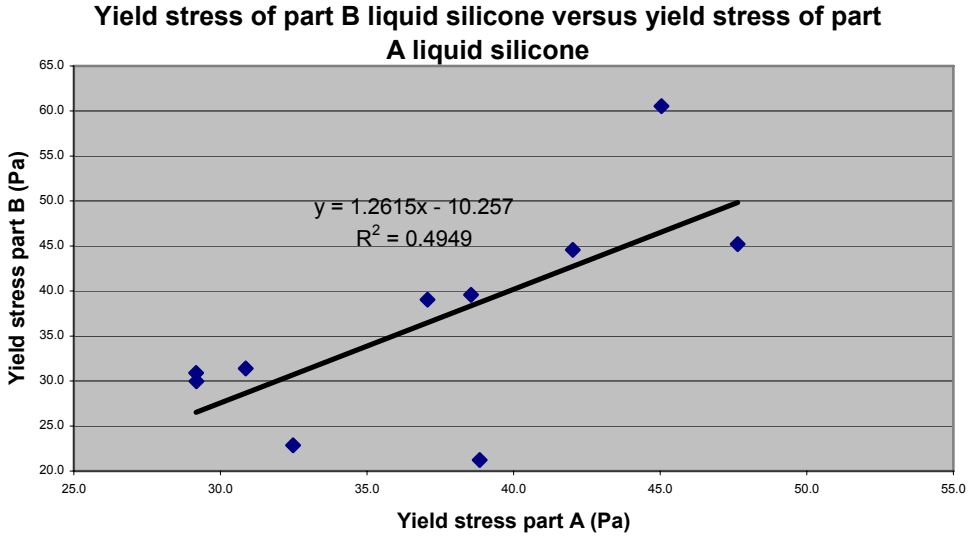


Figure 6.19 Average yield stress from many measurements of the same sample of liquid silicone of part B liquid silicone versus part A liquid silicone from the same silicone batch. Measurements conducted using Dow Corning Q7-4840 silicone.

As different batches of liquid silicone have different yield stresses this could have an effect on the mixing of progesterone, with batches of high yield stress being less amenable to progesterone mixing resulting in reduced mixing of the progesterone in the silicone.

The yield stress of a room temperature vulcanised silicone rubber is believed by Cochrane & Lin (Cochrane & Lin, 1985) "to be a measure of the number and strength of the interaggregate interactions between the particles forming the silica network in the sealant". Room temperature vulcanised silicone rubber consists of a silicone gum, reinforcing filler, and pigments (Rochow, 1987). These silicones have a different mechanism of cure to platinum cured silicones such as Dow Corning Q7-4840 because of the different temperatures of cure. Work by Cochrane and Lin (Cochrane & Lin, 1985) into the effects on the silica on yield stress in room temperature vulcanised silicone rubber found that there was an increases in yield stress as the fumed silica concentration increased. Cochrane and Lin (Cochrane & Lin, 1985) also found that replacing silanol groups on the silica with organosilicone groups that allow weak van der Waals interactions between the aggregates, results in a reduction of the yield stress. Increasing either the surface area, or % loading of fumed silica will also increase the yield stress (Cochrane & Lin, 1985). All of these factors could be causing a change in the drug release rate.

The slope of the drug release rate plot is defined as $[2AC_pD_p]^{½}$ (Rathbone, et. al., 2000) from the Higuchi square root of time model (see Chapter Two). D_p is the diffusion coefficient, and C_p is the solubility of drug in the polymer, and A is the initial amount of drug in the polymer (by unit volume). The drug exists in the polymer either dissolved in the matrix or as discrete particles but $A >> C_p$ (Rathbone, et. al., 2000). An effect on the drug release rate can be caused from changes in either A, D_p or C_p. A is constant in all samples analysed. Hence the variation in yield stress is having an effect on D_p or C_p. The diffusion co-efficient of progesterone through a silicone matrix decreases with increasing fumed silica loading (Mazin, et. al., 1992) and since yield stress of room temperature vulcanised silicone rubber is caused by the interaction of the silica network (Cochrane & Lin, 1985) it can be hypothesised that the silica is causing the changes observed in Figures 6.17 and 6.18. It would be necessary to know the mass of fumed silica used in these batches to confirm this hypothesis, or find a link between progesterone diffusion and other factors such as the fumed silica surface area.

It should also be noted that work by Burggraaf (Burggraaf, 2004) found that a significant cause of variation in drug release rate is adherence of the spine to the silicone skin, which results in an increased device surface area allowing increased contact with the release media, and Larid (Laird, 2004f) found that there was a linear trend of increasing drug release rate with increasing insert ballooning (adherence of the skin to the spine). Hence the effect of yield stress on the drug release rate may be through the adherence of the silicone skin to the insert spine. It should be stated that the 'in vivo' drug release will not be affected by ballooning (Burggraaf, 2006e).

Intra batch variations in the yield stress of part B

As mottling and secondary blooming varies between CIDR inserts from the same batch the yield stress was analysed between a number of different pails of Dow Corning Q7-4840 silicone from batch 0001883500 to determine if there was any intra batch variation.

Figure 6.20 clearly shows that there is no significant intra batch variations in yield stress within a silicone batch. As there is no significant variation in the viscosity of silicone versus height of sampling in the same pail of silicone (Fraser, 2006) it can be concluded that the yield stress of part B in the same silicone batch is constant and not causing secondary blooming and mottling. Work by Wong (Wong, 2002d) found that significant intra batch variations exist in the level of secondary blooming and mottling.

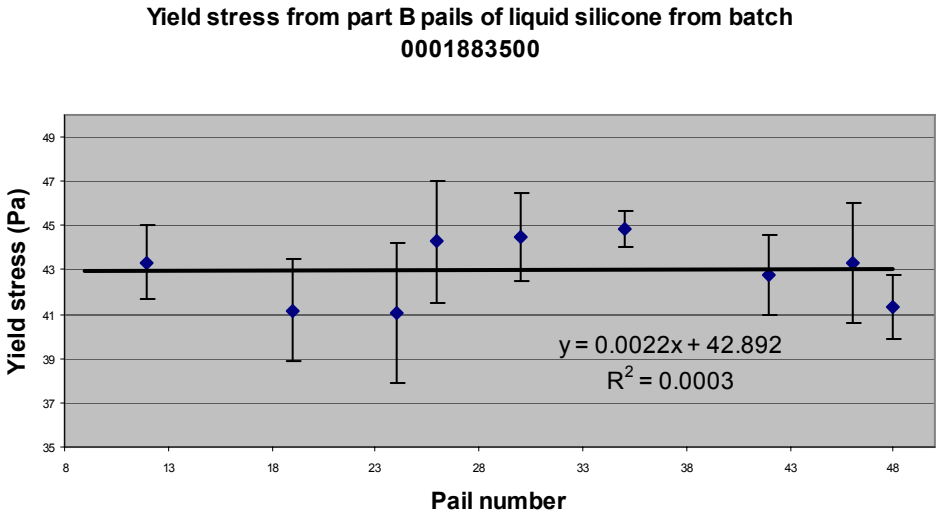


Figure 6.20 Yield stress from pails of part B liquid silicone from batch 0001883500. Error bars are the standard deviation.

6.2.2 Amount of crosslinker and the resulting effect on secondary blooming and mottling

A crosslinker is required as part of heat cured silicone. The crosslinker binds the silicone network together during the process of curing to form the silicone rubber. A range of studies have been undertaken to determine the effect of different concentrations of crosslinker on secondary blooming and mottling.

6.2.2.1 Amount of crosslinker in Dow Corning silicone

A study was undertaken to determine the effect on secondary blooming and mottling from different amounts of extra crosslinker. An increase in the amount of crosslinking would make the matrix more rigid. Slabs were made using mixed residue silicone from batch E09338 from manufacturing. Using the residue from batch E09338 slabs were made with the following % w/w amounts of extra crosslinker; 0 %, 0.26 %, 0.50 %, 0.80 % and 2.01 %. Slabs were made using the hand moulder and cured for a minimum of 30 seconds. The method of manufacture is described in detail in Chapter Three.

After manufacture, slabs were placed into a stability oven to promote secondary blooming and mottling over the length of the experiment (4 months). Samples were stored in the stability oven in ziplock bags, with each bag holding 3 x 100 mL containers serving as space fillers to promote airflow around the slabs. Holes were added to each bag to allow moisture to enter the bags.

6.2.2.1.1 Progesterone content on slabs made with extra crosslinker

It was found that the amount of progesterone extracted from the slabs decreased with the amount of extra crosslinker added. This is shown in Figure 6.21.

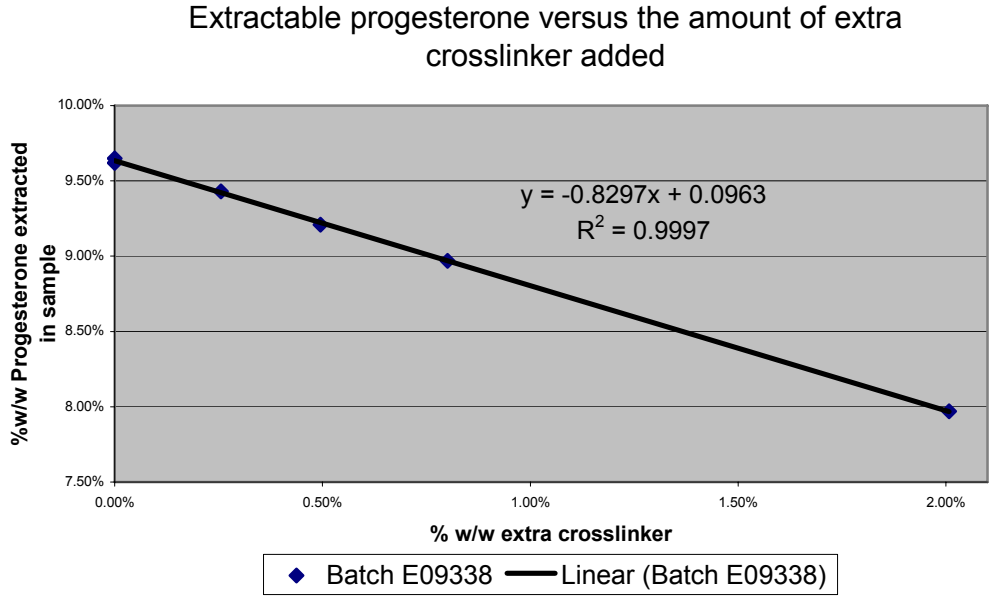


Figure 6.21 Percentage of progesterone extracted from samples made with extra crosslinker.

Hence it can be concluded that an increase in the amount of crosslinker increases the retention of progesterone in the matrix (from soxhlet extraction). Drug release away from a polymer (Rathbone, et. al., 2000) occurs in a number of steps that are described in Chapter Two. The rate-determining step in drug release has been assumed to be diffusion of the drug to the surface of the sample (Rathbone, et. al., 2000). Hence the most likely explanation of the decrease in progesterone extraction, is a decrease in diffusion caused by increased crosslinking closing up the matrix. Moreover it is unlikely that the observed decrease in drug content

would be caused by dislodging progesterone from the surface of the sample, as Figure 6.21 clearly shows a strong linear trend with a R^2 value of 0.9997. However drug release rate results (from Hanson Dissolution apparatus rather than soxhlet extraction) discussed in the next Section do not support the theory of crosslinking affecting diffusion. Also it is unknown if extra extraction would remove further progesterone from the slabs, which would inidcate a decrease in the diffusion rate rather than progesterone being locked into the matrix (hence being non-extractable).

6.2.2.1.2 Hanson Dissolution drug release rate analysis on slabs made with different levels of extra crosslinker

Some slabs also underwent drug release rate analysis. Figure 6.22 shows that there is no effect between drug release rate and the amount of extra crosslinker in the slabs, except that there appears to be a difference in drug release rate between slabs made with 0.50 % w/w and 0.26 % w/w extra crosslinker. There is also a difference in drug release rate between the slabs made with 0.80 % w/w and 2.01 % w/w extra crosslinker. This could be due to the slab used for the 2.01 % w/w sample being smaller than the other slabs.

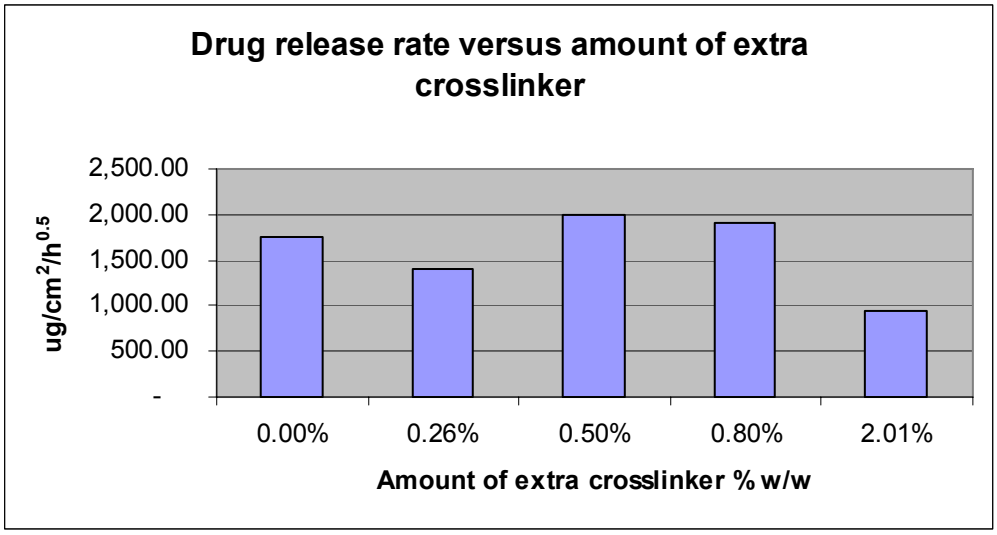


Figure 6.22 Hanson Dissolution release rate versus the amount of extra crosslinker added to slabs made with Dow Corning Q7-4840 silicone. Drug release rate calculated from a surface area of ~ 62 cm^2 for all slabs except the slab made with 2.01 % w/w extra crosslinker, which had a surface area of 30.87 cm^2.

The slope the drug release plot is defined as $[2AC_pD_p]^{½}$ (Rathbone, et. al., 2000) from the Higuchi square root of time model (see Chapter two). D_p is the diffusion coefficient, and C_p is the solubility of drug in the polymer, and A is the initial amount of drug in the polymer. The progesterone exists in the polymer in two forms, either dissolved in the matrix or as discrete particles but the mass of drug in the matrix is much greater than the solubility of drug in the matrix (Rathbone, et. al., 2000). An effect on drug release rate can hence be caused from changes to either A, D_p or C_p. In a previous Section it was thought that the decrease in extractable progesterone with extra crosslinker was caused by a decrease in D_p. The variable drug release rates shown in Figure 6.22 do not support such a theory as there is no trend of extra crosslinker versus drug release rate. However the method used in this Section is different, as drug release tests do not isolate extracted progesterone from the device, whereas content extractions isolate the extracted progesterone (using a soxhlet extractor). In a drug release rate test it is possible that progesterone can re-enter the matrix hence possibly affecting drug diffusion.

It is possible that the change in crosslinking density would have in some manner ‘locked’ progesterone into the matrix, preventing the progesterone from being extracted and hence decrease the value of A.

6.2.2.1.3 Surface progesterone analysis on slabs made with extra crosslinker

Samples made with different amounts of extra crosslinker, were analysed using the surface progesterone method. A sample consisted of a slab of silicone similar to the ones shown in Figure 6.26 with the flash removed prior to testing. Table 6.2 lists when particular samples were analysed. Figures 6.23 to 6.25 show the mass of progesterone released at the t = 0, 1 and 4 month time points.

Table 6.2 Sampling times for Slabs made with extra crosslinker.	
Time in oven	**Number and type of slabs tested.**
t = 0 months	Two slabs made using silicone from batch E09338 with 0.0 %, 0.26 %, 0.50 %, 0.80 %, and 2.01 % extra crosslinker respectively.
t = 1 months	Two slabs made using silicone from batch E09338 with 0.0 %, 0.26 %, and 0.50 %, w/w extra crosslinker respectively.
t = 4 months	At least two slabs made using silicone from batch E09338 with 0.0 %, 0.26 %, 0.50 %, 0.80 %, and 2.01 % w/w extra crosslinker respectively.

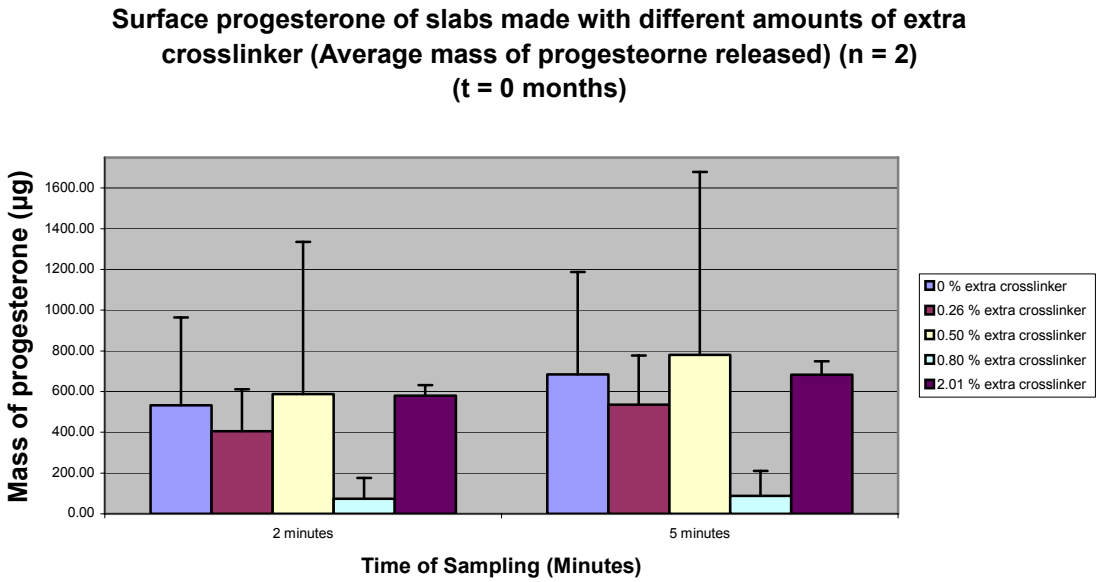


Figure 6.23 Surface progesterone of slabs made with extra crosslinker (Mass of progesterone released). t = 0 months, n = 2 . Error bar is the 95 % confidence interval (only half of the bar is shown for reasons of clarity). Slabs made with liquid silicone from batch E09338.

Figure 6.24 Surface progesterone (mass released) for samples made with extra crosslinker, t = 1 month, n = 2. Error bars are the 95 % confidence interval. Slabs made with liquid silicone from batch E09338.

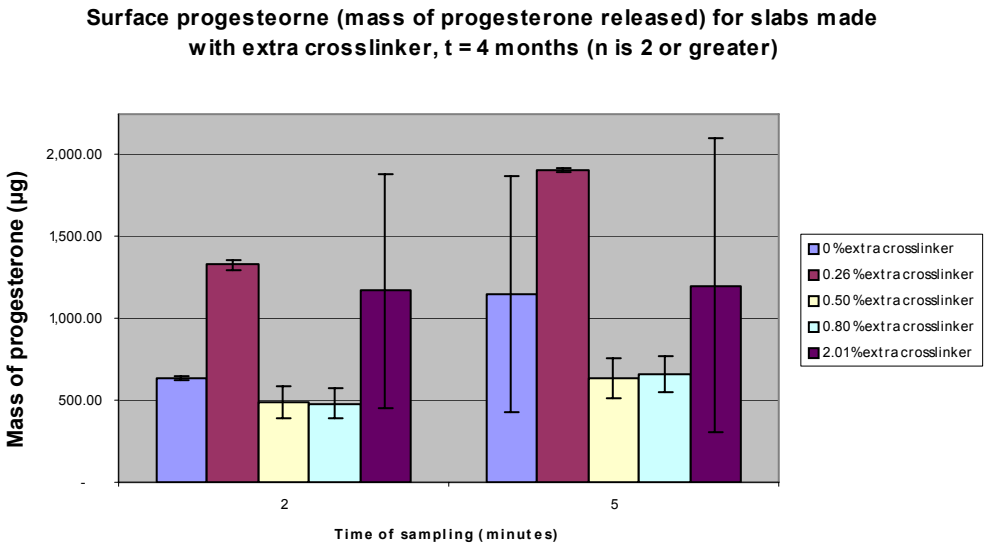


Figure 6.25 Surface progesterone of slabs (mass of progesterone released) t = 4 months. Error bars are the 95 % confidence interval. Slabs made with liquid silicone from batch E09338. n ≥3.

Figures 6.23 to 6.25 show no trend of reduced surface progesterone versus the amount of extra crosslinker. These Figures also show that there is a wide variation in the amount of surface progesterone observed. It is of note that there has been an increase in the level of surface progesterone on all samples analysed, as would be

expected after storage in the stability oven. It is possible that the wide variations in progesterone could have been caused by the progesterone dislodgment due to handling. The 100 mL containers used to promote air flow around the samples and pre-testing handling (such as sample weighing) could have caused variations in surface progesterone levels. It is also possible that the presence of the surface progesterone is caused by random variations.

6.2.2.1.4 Surface progesterone observations after four months in the stability oven

Samples analysed for surface progesterone at the four month time point were also visually analysed for surface progesterone before further testing. Table 6.3 shows the visual analysis results using a scale of high/medium/low/none.

Table 6.3 Surface Progesterone observations on slabs made with extra crosslinker, stored in the stability oven for four months. Made with silicone residue from batch E09338.

Amount of Extra Crosslinker (% w/w)	**Surface progesterone observations High/medium/low/none**		
	Sample 1	**Sample 2**	**Sample 3**
0.0 %	Medium	Between high and medium	-
0.26 %	High	High	-
0.50 %	Between high and medium	Sample is white	-
0.80 %	Low	Medium	-
2.01 %	Large translucent areas	High	High

Comparing Table 6.3 with Figure 6.25 it is clear that there is some degree of correlation between the level of observed progesterone on the slabs and the

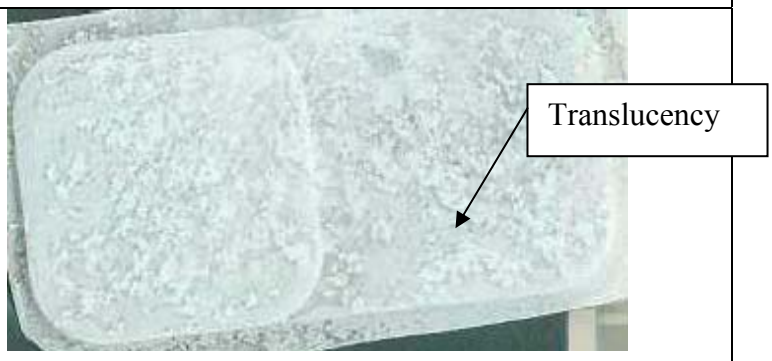
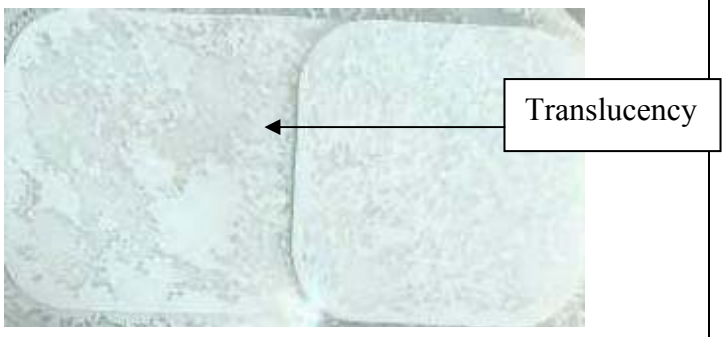
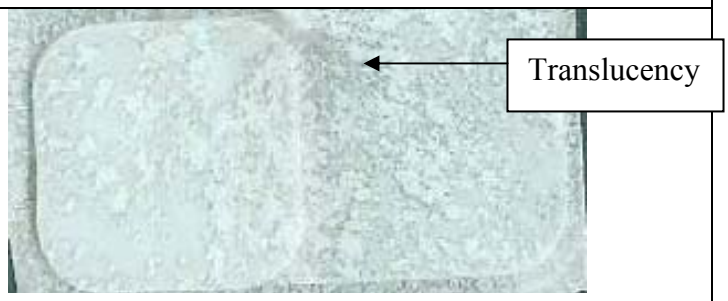
average surface progesterone. However there appears to be no correlation between the amount of extra crosslinker and the amount of surface progesterone on the slabs, as observed with previous results. Wong (Wong, 2002d) noted there was variation in the intra batch levels of surface progesterone and it is probable that this is being observed in this result.

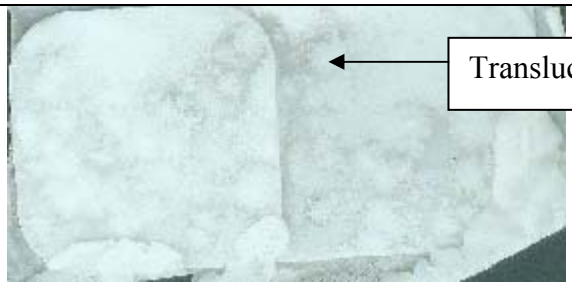
6.2.2.1.5 Visual observation of slabs stored in the stability oven for four months made with extra crosslinker

Scans were taken of slabs made with extra crosslinker (as outlined earlier) that were stored in the stability oven for four months (see Figure 6.27). This is to determine if there was an increase in mottling or other visual phenomena after four months in the stability oven. Figure 2.27.4 shows a slab made with liquid silicone residue from batch E09559 that had 0.51 % w/w extra crosslinker. This slab was also scanned to as a further point of reference.

Figure 6.26 Scans of slabs made with extra crosslinker after storage in stability oven for 4 months. Slabs made with liquid silicone residue from batch E09338 unless specified otherwise. Slabs shown are representative.

Amount of extra crosslinker added (% w/w)	**Image (not to scale)**
0.00 % w/w	 **Figure 6.26.1** Scan of slab that had no extra crosslinker added.

0.26 % w/w	 **Figure 6.26.2** Slab made with 0.26 % w/w extra crosslinker.
0.50 % w/w	 **Figure 6.26.3** Scan of slab made with 0.50% w/w crosslinker. Made with liquid silicone residue from batch E09338.  **Figure 6.26.4** Scan of slab made with 0.51 % w/w extra crosslinker. Made with liquid silicone residue from batch E09559.
0.80 % w/w	 **Figure 6.26.5** Scan of a slab made with 0.80 % crosslinker.

2.01 % w/w	 **Figure 6.26.6** Scan of slab made with 2.01 % w/w extra crosslinker.

From observations of the slabs in Figure 6.26 it is clear that slabs with more than 0.26 % w/w extra crosslinker exhibit translucency or mottling, while the slabs made with 0.26 % w/w or 0.0 % w/w crosslinker do not exhibit mottling.
In Figure 6.26 it is of note that there is a significant difference in the slab made with 2.01 % w/w extra crosslinker compared with the slab made with 0.80 % w/w extra crosslinker, with the white areas appearing ‘star like’ rather than the ‘island formations’ observed in the slab made with 0.80 % w/w extra crosslinker (Figure 6.27.5).

6.2.2.1.6 XRD on slabs made with extra crosslinker

Slabs made with extra crosslinker were analysed using XRD to determine if there was a difference in progesterone polymorphism on the surface of the slabs caused by the presence of the extra crosslinker. Samples were analysed at t = 0 and t = 4 months. Figure 6.27 shows the XRD diffractogram at t = 0 months while Figure 6.28 shows the XRD diffractogram at t = 4 months.

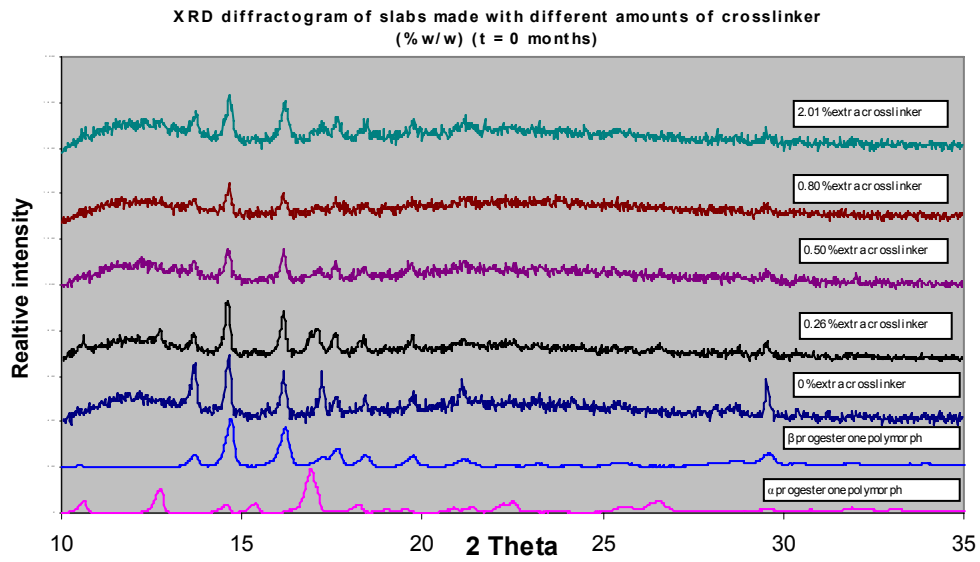


Figure 6.27 XRD Diffractogram of slabs made with extra crosslinker. t = 0 months.

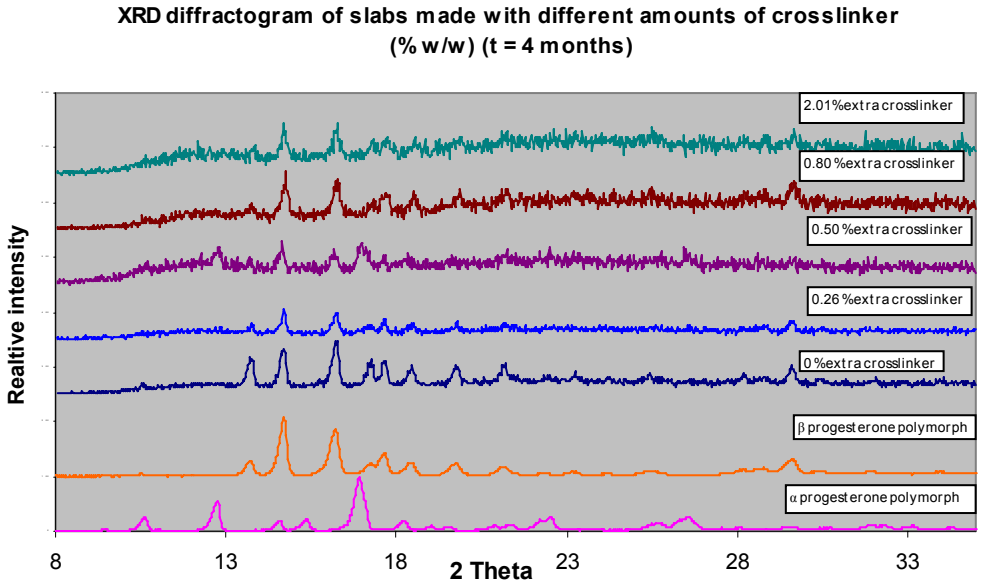


Figure 6.28 XRD diffractogram of slabs made with different amounts of crosslinker stored in the stability oven. t = 4 months.

From Figure 6.27 and 6.28 it is clear that the β progesterone polymorph is exhibited on the slabs at zero and four months, and there is no change in progesterone polymorphism after four months in the stability oven due to additional crosslinker. XRD diffractogram of mottled and non-mottled regions on CIDR inserts in Chapter Four showed that the mottled areas exhibited the

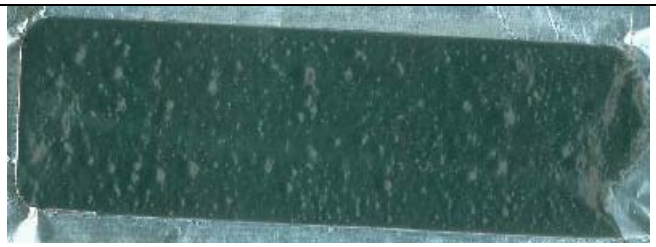
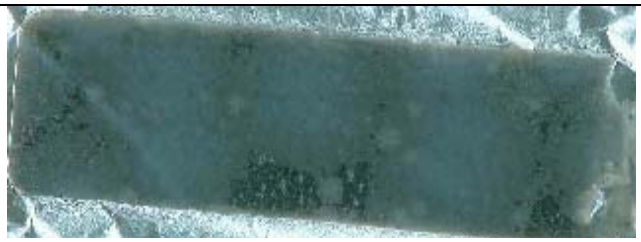
β progesterone polymorph, whereas the white non-mottled areas of CIDR inserts showed the α progesterone polymorph. However the effects on polymorphism of progesterone inside the sample remain unknown as samples were not analysed using a DSC, which is able to analyse below the surface of the sample. The results from Chapter Four found that both the α and β progesterone polymorphs are stable under humid conditions, agreeing with the result in this Section.

6.2.2.2 Effect on secondary blooming due to amount of crosslinker added to the alternative supplier silicone

It is useful to determine the effect of extra crosslinker on mottling and secondary blooming in the alternative silicone supplier silicone. In order to determine this the alternative supplier provided mixes of part B, with different amounts of extra crosslinker added, these being a mix with the normal level of crosslinker, a mix of part B silicone with twice the normal level of crosslinker and a mix of part B silicone with four times the normal level of crosslinker. Control slabs were made using Dow Corning Q7-4840 silicone (batch 0001854557). Progesterone from batch 15KDY was used in all slabs manufactured. Slabs were made with and without black dye to aid visualisation of surface progesterone. Slabs were cured for 60 seconds in a Ronson Benchtop cooker at some time during the cure period the oven was at 190 ^{o}C. Slabs were packed after cooling on the bench.

After manufacture slabs were placed into the stability oven in order to increase the rate of secondary blooming and mottling. During this period the water tray in the oven ran out, however as discussed in Chapter Three this is not of concern. Samples were scanned at t = 4 days, t = 1.5 months and t = 8 months after manufacture. Figure 6.29 to 6.31 shows the scans of these slabs.

Figure 6.29 Scans on slabs four days after manufacture, made with and without extra crosslinker, using alternative supplier silicone. Slab shown is representative.

Sample	**Scans four days after manufacture (slabs not to scale)**
Dow Corning Q7-4840 Control.	 **Figure 6.29.1** Scan of a slab at four days made with Dow Corning Q7-4840 silicone
Alternative supplier silicone control.	 **Figure 6.29.2** Scan of slab at four days made with the alternative supplier silicone. Slab contains normal level of crosslinker.
Alternative supplier silicone with twice the normal level of crosslinker.	 **Figure 6.29.3** Scan of slab at four days made with the alternative supplier silicone that contained twice the normal level of crosslinker.

Alternative supplier silicone with four times the normal level of crosslinker.	 **Figure 6.29.4** Scan of slab at four days made with the alternative supplier silicone that contained four times the normal level of crosslinker.

Figure 6.30 Scans on slabs 1.5 months after manufacture, made with and without extra crosslinker, using alternative supplier silicone. Slab shown is representative.

Sample	**Scans 1.5 months after manufacture (not to scale)**
Dow Corning Q7-4840 control.	 **Figure 6.30.1** Slab made with Dow Corning Q7-4840 silicone. Scanned 1.5 months after manufacture.
Alternative supplier silicone control.	 **Figure 6.30.2** Slab made with alternative supplier silicone. Normal level of crosslinker. Scanned 1.5 months after manufacture.

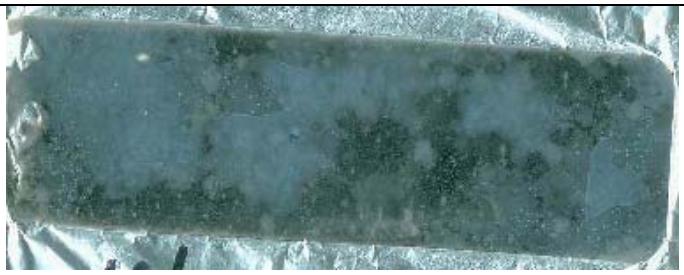
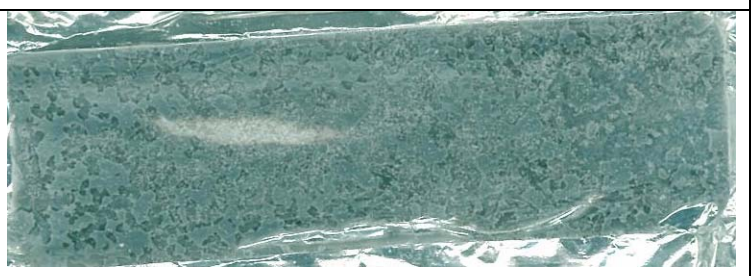
Alternative supplier silicone with twice the normal level of crosslinker.	 **Figure 6.30.3** Slab made with alternative supplier silicone containing twice the normal amount of crosslinker. Scanned 1.5 months after manufacture.
Alternative supplier silicone with four times the normal level of crosslinker.	 **Figure 6.30.4** Slab made with alternative supplier silicone containing four times the normal amount of crosslinker. Scanned 1.5 months after manufacture.

Figure 6.31 Scans on slabs eight months after manufacture, made with and without extra crosslinker, using alternative supplier silicone. Slab shown is representative.

Sample	**Observations eight months after manufacture**
Dow Corning Q7-4840 control.	 **Figure 6.31.1** Slab made with Dow Corning Q7-4840 silicone. Scanned eight months after manufacture. Shiny region on the sample is from plastic covering the slab during scanning. Slab observed to possess blooming.

Alternative supplier silicone control.	 **Figure 6.31.2** Slab made with alternative supplier silicone. Normal level of crosslinker. Scanned eight months after manufacture. No loose surface progesterone observed. Scraping makes the slab pale and gives off no noticeable powder.
Alternative supplier silicone with twice the normal level of crosslinker.	 **Figure 6.31.3** Slab made with alternative supplier silicone containing twice the normal amount of crosslinker. Scanned eight months after manufacture. No loose surface progesterone observed.
Alternative supplier silicone with four times the normal level of crosslinker.	 **Figure 6.31.4** Slab made with alternative supplier silicone containing four times the normal amount of crosslinker. Scanned eight months after manufacture. No loose surface progesterone, however scraping the white area with a scalpel does give a white powder.

Observations of the slabs made with the alternative supplier silicone containing extra crosslinker over the eight months (Figure 6.31.4) shows that there is formation of a surface layer on the slabs. This layer appears to be integrated into the surface of the sample rather than as a loose material. This is not observed in the slabs made without the extra crosslinker. The control Dow Corning Q7-4840 slabs clearly shows surface phenomena such as secondary blooming and ‘island

formation' (Figure 6.31.1). The slabs made without the extra alternative supplier silicone crosslinker clearly show no signs of surface phenomena, which is the desired outcome. It should be noted however that the SEM analysis of CIDR inserts from batch E08106 (made with the alternative supplier silicone) did exhibit limited surface crystallisation (see previous results in this Chapter).

The formation of a surface layer of progesterone on slabs made with the alternative supplier silicone (without extra crosslinker) has been known to be caused by objects touching a slab shortly after manufacture (Reardon, 2004b). Hence it is possible that the large grey areas observed on the slabs made with extra crosslinker is caused by post manufacture contact with surfaces such as packaging. This may have occurred if the packing was premature.
It is observed that the control slabs made with the alternative supplier silicone, (with normal levels of crosslinker) do not exhibit such phenomena, which would indicate that the grey areas are caused by the addition of crosslinker. Furthermore, close examination of these regions (shown in Figure 6.31.5) shows that there is crystallisation occurring at the edges of these regions showing that these regions may not have be created by post manufacture contact.

From Figure 6.31 it is clear that the surface material is tightly bound to the surface of the slab and is possibly a layer of tightly bound surface progesterone, however scraping of slabs in Chapter Seven also found that such a layer could be formed. Hence care should be taken interpreting this result as there are no SEM images results of this layer, and in Chapter Seven scraping of slabs resulted in the formation of a powder.

Further slabs were made using the same batch of alternative supplier silicone, using progesterone from Pfizer batch 24JAF. Slabs were made without any extra crosslinker and four times the normal level of crosslinker. Slabs were cured for 60 seconds between 144 and 204 ^{o}C. Slabs were stored in the stability oven, during this period the oven did run out of water, however as noted in Chapter Three this is not of concern. Observation of these slabs approximately six months after manufacture shows that the slabs made with extra crosslinker (Figure 6.32) have similar surface phenomena to the slabs made previously with extra

crosslinker (see Figure 6.31.5). Further the control slab made without the extra crosslinker (Figure 6.33) shows that the surface layer has not totally formed (not clear on Figure 6.33) which is in contrast to the slabs previously made in this Section. However this layer could be related to the initial blooming. The slab looks similar to slabs made by Reardon containing part A of the Dow Corning silicone and part B from the alternative supplier silicone shown Figure 6.35 in Section 6.2.2.3.

Figure 6.32 Scan of slab made with four times the normal amount of crosslinker. Slab made from alternative supplier silicone stored in the stability oven for six months. Slab shown is representative.

Figure 6.35 Scan of slab made with the normal amount of crosslinker. Slab made from alternative supplier silicone stored in the stability oven for six months. Slab shown is representative.

Slabs made without the black dye (at eight months) shows that the slabs with extra crosslinker added have similar surface phenomena to that observed in the blacked slabs, which enabled ease of writing on the slabs. The differences between the Dow Corning Q7-4840 silicone and the alternative supplier silicone observed in this Section agree with work by Reardon (Reardon, 2004b). Reardon (Reardon, 2004b) found that the alternative supplier silicone did not undergo secondary blooming.

The results obtained in this Section clearly show that there is a relationship between the addition of crosslinker and surface phenomena in the alternative supplier silicone. It has been found that the addition of crosslinker to the Dow Corning Q7-4840 silicone as noted earlier in this Chapter does increase mottling. The previous results also found no relationship between the level of surface progesterone and the level of extra crosslinker.

In Chapter Five ESMS was undertaken on part B liquid silicone made by the alternative supplier that contained additional crosslinker. That work showed some increase in complexity of the spectra, and a limited similarity to the Dow Corning part B spectra. Furthermore since the compositions and mechanism of cure, between the two silicone suppliers are unknown, care should be taken in reaching any conclusion on the effects of the extra crosslinker on secondary blooming between the two silicone suppliers.

6.2.2.3 Slabs made with cross-mixed amounts of part B (Dow Corning and alternative supplier silicone) to find the minimum level of part B required to cause blooming

Reardon (Reardon, 2004b) had commented that part B of the alternative supplier silicone was responsible for retarding the secondary blooming. An experiment was undertaken to determine the minimum level of alternative supplier part B liquid silicone that would be required to prevent secondary blooming in cross-mixed slabs (slabs with different ratios of part B silicone from the two feedstocks). Slabs were made with various ratios of part B liquid silicone from the two silicone feedstocks. Slabs were made using Dow Corning Q7-4840 silicone from batch 0001854557 and progesterone from Pfizer batch 15KDY. All slabs were made using part A from the Dow Corning Q7-4840 silicone. The alternative supplier silicone used was from the same batch used in Section 6.2.2.2. Samples were cured for 40 seconds at ~ 190 ^{o}C in a Ronson Bench top oven. Slabs were left to cool before packing into non-sealed bags. After manufacture samples were placed into the stability oven. While in the stability oven the oven did run dry during for a time during the experiment, however as discussed in Chapter Three this should

have a minimal effect. Scans of the slabs at eight months are shown in Figures 6.34. All slabs exhibited strong blooming.

Figure 6.34 Scans of slabs after eight months in the stability oven that were made with differing ratios of part B liquid silicone from the alternative supplier and Dow Corning Q7-4840 silicone. Slab shown is representative.

Image (not to scale)

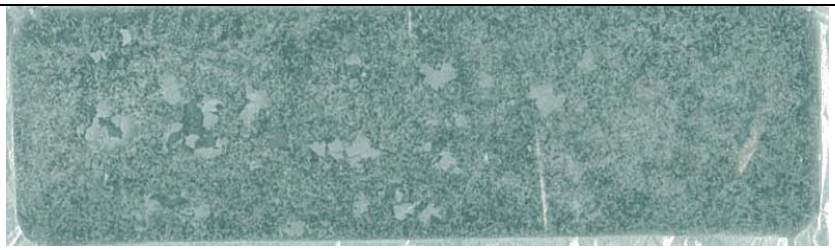


Figure 6.34.1 Scan of slab at eight months, made with Dow Corning Q7-4840 part B, no alternative supplier silicone part B added to this slab.
Flakes of progesterone observed on the surface.

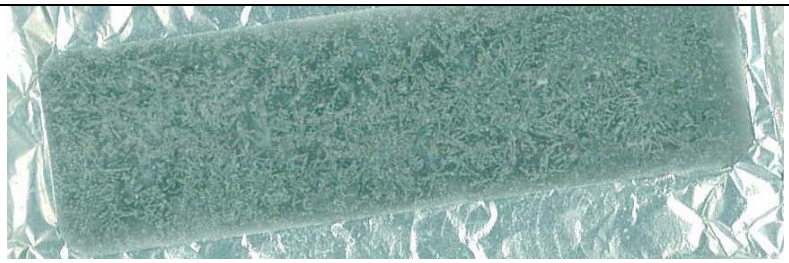


Figure 6.34.2 Scan of slab at eight months made with four parts Dow Corning Q7-4840 part B and one part alternative supplier silicone part B.
Crystals of progesterone observed on the surface.

Figure 6.34.3 Scan of slab at eight months, made with two parts of alternative suppler silicone part B and three parts of Dow Corning Q7-4840 part B.

Figure 6.34.4 Scan of slab at eight months, made with three parts of alternative suppler silicone part B and two parts of Dow Corning Q7-4840 part B. The streak to the right of the image is from the plastic covering the sample on the scanner.

Figure 6.34.5 Scan of slab at eight months, made with four parts of alternative suppler silicone part B and one part of Dow Corning Q7-4840 part B. The streak to the centre-right of the image is from the plastic covering the sample on the scanner.

Figure 6.34.6 Scan of slab at eight months, made with only the alternative suppler silicone part B. No Dow Corning silicone part B used in this slab.

The results shown in Figure 6.34 do not agree with Reardon's work (Reardon, 2004b), who observed that replacing part B of Dow Corning Q7-4840 silicone with part B alternative supplier silicone stopped secondary blooming. Figure 6.35 shows photos of the slabs made by Reardon 14 days after manufacture. No slab made in this Section had any resemblance to the slabs made by Reardon. Even the slab made with no part B from Dow Corning liquid silicone exhibited secondary blooming (Figure 6.34.6).

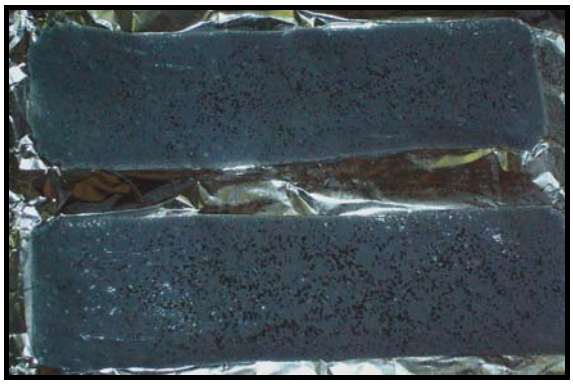


Figure 6.35 Slabs made with part A of Dow Corning Q7-4840 and part B of the alternative supplier silicone. From Reardon's work. (Reardon, 2004b).

Observation by the author of the cross-mixed slabs made by Reardon two years and six months after manufacture clearly show that there is secondary blooming on the control slabs made using Dow Corning Q7-4840 silicone (both parts A and B) and the slabs made using Dow Corning part B and alternative supplier silicone part A. Whereas the slabs made with the alternative supplier silicone part B and the Dow Corning part A exhibit the secondary blooming similar to that observed 14 days after manufacture (Figure 6.36) (Reardon, 2004b).

To investigate this deviation from Reardon's result (Reardon, 2004b), cross-mixed slabs were made mixing parts A and B from the different feedstock with the opposite feedstock (i.e. Dow Corning part A mixed with alternative supplier silicone part B and vice versa). Silicone from Dow Corning Q7-4840 batch 0001854557 was used. Pfizer progesterone from batch 24JAF was used. The alternative supplier silicone batch used was the same batch used in the crosslinking experiments (Section 6.2.2.2). Slabs were cured for 70 seconds between 168 to 188 ^{o}C. At the end of cure smoke was coming from the oven (Ronson Bench top oven).

Slabs were placed into the stability oven, which ran dry during the study however as noted in the Chapter One this was of little consequence. Figure 6.36 shows scans of the slabs taken six months after manufacture. Figure 6.36 shows that the slab made with the part B of the alternative supplier silicone has secondary blooming, as well as a large grey region that is similar to the top slab in Figure

6.36 (made with the part A alternative supplier silicone and part B from Dow Corning Q7-4840 silicone). Due to the low level of secondary blooming it is not possible to reach a conclusion.

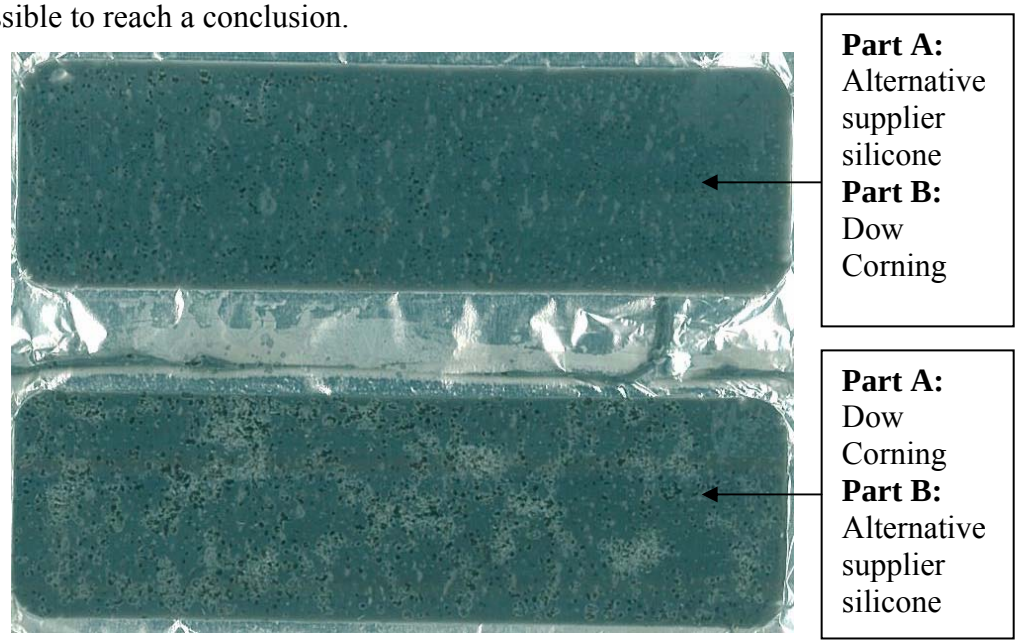


Figure 6.36 Scan of slabs cross-mixed with different silicone parts from different manufactures. Top slab made with part A of the alternative supplier silicone and part B of Dow Corning Q7-4840 silicone. Bottom slab made with part A of Dow Corning Q7-4840 and part B from the alternative supplier silicone, showing mild secondary blooming. Scan taken six months after manufacture. Slabs shown are representative.

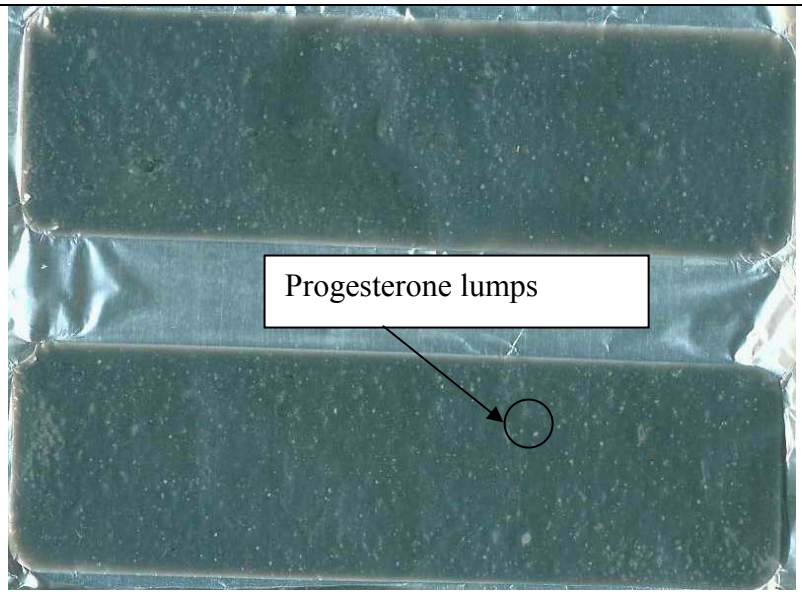
6.3 Effect on secondary blooming on slabs made with low bulk density progesterone

DEC Manufacturing uses progesterone with a bulk density between 3.5 to 4.33 mL/g with a preference for a bulk density of 4.17 to 4.33 mL/g (2.d.p) (Burggraaf, 2005b). To determine the effect of lower bulk density on secondary blooming and mottling, slabs were made with a low bulk density progesterone (batch 49MDR, bulk density of 2.9 mL/g (Pfizer, 2005)). Bulk density is the density of the compacted progesterone powder. Control slabs were made with Diosynth (batch L00024354) and Pfizer (batch 24JAF) progesterone, using silicone from Dow Corning Q7-4840 silicone (batch 0001854557). A black dye was added to slabs to aid visualisation of blooming.

The liquid silicone was mixed for more than four minutes and cured for two minutes at ~185 ^{o}C. The low bulk density progesterone (49MDR) did not properly mix into the silicone and remained as small white lumps (Figure 6.37.1) in the cured silicone. This is undesirable and hence this batch of progesterone was not used in the manufacture of CIDR inserts.

In order to investigate the effects on secondary blooming and mottling the slabs were placed into the stability oven one day after manufacture. The results from this work are shown in Figure 6.37. Figures 6.37.4 and 6.37.5 show that the control slabs have far more crystalline secondary blooming compared to the alternative suppler silicone. Figure 6.37.4 clearly shows that there is less secondary blooming on the slab made with the lower bulk density progesterone compared to the control slabs.

Figure 6.37 Scans of slabs made with low bulk density progesterone and control slabs. Slabs shown is representative.

Progesterone type and time in stability oven.	**Image (not to scale).**
Low bulk density progesterone t = 1 day after manufacture.	 **Figure 6.37.1** Scan of slabs made with progesterone from batch 49MDR (low bulk density) scanned after one day after manufacture

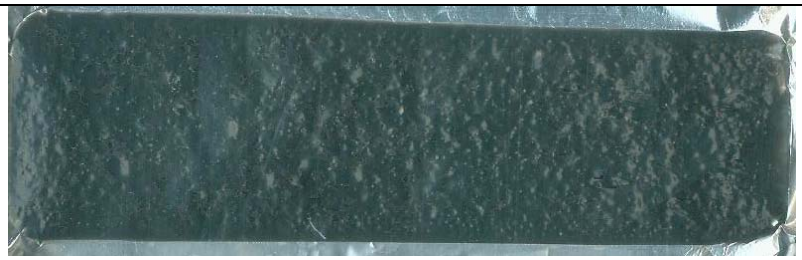
Control t = 1 day after manufacture.	 **Figure 6.37.2** Slab made with Diosynth progesterone, scan undertaken one day after manufacture.  **Figure 6.37.3** Slab made with Pfizer 24JAF progesterone, scan undertaken one day after manufacture. The streak to the right of the image is a reflection from the plastic covering the slab on the scanner.
Low bulk density progesterone t = 9 days after manufacture.	 **Figure 6.37.4** Scan of slabs made with 49MDR (low bulk density) progesterone after eight days in the stability oven.
Control t = 9 days after manufacture.	 **Figure 6.37.5** Scan of slab made with Diosynth Progesterone after eight days in the stability oven.

Figure 6.37.6 Scan of slab made with Pfizer 24 JAF progesterone after eight days in the stability oven.

The cause of this decline in secondary blooming would not be related to the unmixed lumps of progesterone in the silicone, as these are small (Figure 6.38.1). The amount of progesterone in these lumps should not have a major effect on the mass of progesterone available to undergo blooming. In batch 49MDR the progesterone particles would have packed close together. This may be through differences in the particle morphology as the batch progesterone particle size would have to pass Pfizer's micronized progesterone requirements.

While the particles will have melted during the curing process, it is possible that the particles formed upon cooling would have a smaller surface area. This may also occur though reduced mixing of the progesterone particles resulting in progesterone particles clumping together, hence reducing the surface area of the particle formed upon cooling. The particles would be denser and hence less affected by temperature changes during curing, and hence reduces blooming.

6.4 Purification of progesterone and effects on blooming

Progesterone undergoes re-crystallisation as part of the manufacturing process. A progesterone batch 74HBS has been identified as a poorly curing batch by manufacturing (Reardon, 2003c) and having high levels of impurities (Reardon, 2003c). A sample of progesterone from batch 74HBS was returned to Pfizer and underwent extra re-crystallisation. The aim of undertaking extra re-crystallisation was to reduce the impurity levels in the progesterone and determine if this is causing blooming. The additionally re-crystallised progesterone was then

ground up using a mortar and pestle. Slabs were made using the additionally re-crystallised progesterone.

The particle size of batch 74HBS was determined using a Malvern Mastersizer at the University of Waikato, finding that 84.33 % of particles were under 19.31 μm. This contrasts with the Pfizer Certificate of Analysis, which specified that 100% of particles were less than 20 μm. However this difference is minor. The particle size of the twice re-crystallized progesterone (after grinding) had 56.47 % of particles under 19.31 μm.

Slabs were made with Dow Corning Q7-4840 silicone (batch 0001854557) and were cured between 217 ^{o}C and ~ 260 ^{o}C for ~ 50 seconds. Slabs were left to cool on the bench for ~ 40 minutes before packing. Slabs were also made using a progesterone sample from the same batch (74HBS) that had not been additionally re-crystallised (as a control). Slabs were placed in the stability oven, and during storage the water container did run dry, however as discussed in Chapter Three this was of minor consequence. Two slabs were made with each of the progesterone types (two control slabs and two slabs with twice re-crystallized progesterone. Scans of the slabs are shown in Figure 6.38.

Figure 6.38 Slabs made with and without additionally re-crystallised progesterone from batch 74HBS.

Sample and date of image	**Image (not to scale)**
Control at t = 2 days.	 **Figure 6.38.1** Scan of slabs made with 74HBS progesterone that was not additionally re-crystallised. Sample scanned two days after manufacture.
74HBS additionally re-crystallised progesterone at t = 2 days.	 **Figure 6.38.2** Scan of slabs made with 74HBS progesterone that was additionally re-crystallised. Sample scanned two days after manufacture.

Control at t = 6 months.	 **Figure 6.38.3** Scan of slabs made with 74HBS progesterone that was not additionally re-crystallised. Sample scanned six months after manufacture.
74HBS additionally re-crystallised progesterone at t = 6 months.	 **Figure 6.38.4** Scan of slabs made with 74HBS progesterone that was additionally re-crystallised. Sample scanned six months after manufacture.

Observations of the slabs show that the slabs made with the additionally re-crystallised progesterone appear to have little difference between two and six months. This is contrast to the control slabs, which clearly show surface phenomena such as ‘island formations’ after storage in the stability oven. It is possible that further slabs made with 74HBS would exhibit blooming. Differences between the two feedstocks are apparent with the slabs made with the additionally

re-crystallised progesterone appearing ‘grainy’. Reardon (Reardon, 2003c) found that slabs made with non-micronized progesterone had a ‘grainy’ appearance. There is a difference in progesterone particle size between the two progesterone feedstocks used, which possibly causes the ‘grainy’ appearance. Due to the ‘grainy’ appearance of the slabs made with re-crystallised progesterone (Figure 6.38.4) that suggests that the progesterone particle size was too large, the known differences in particle size, and the low secondary blooming on the control slabs it is not possible to determine if there is a difference in secondary blooming due to progesterone re-crystallisation.

6.5 Swell tests of alternative supplier silicone and Dow Corning silicone

Swell testing is used to determine if there is a difference in the crosslinking in a silicone sample. Silicone that has more crosslinking should be more rigid and hence differences in the amount of ethanol adsorbed by the sample should be observed. An increase in crosslinking density should increase the drug release rate (Raul, 2004).

Blank slabs of silicone (containing no progesterone, n = 3) were made with Dow Corning Q7-4840 silicone (batch 0001854557) and the alternative supplier silicone. Slabs were weighed, and placed into ethanol for ~24 hours. They were subsequently weighed, and the % weight increase analysed. It was found (see Figure 6.39) that there was no statistical difference in % weight increases. It was also found that no ethanol had permeated to the air bubbles encapsulated in the alternative supplier silicone when the silicone was cut open. One Dow Corning sample had a small section of itself not immersed in the ethanol medium (but above the ethanol), however this slab had the highest percentage weight increase out of the Dow Corning samples analysed and hence the sample was not rejected.

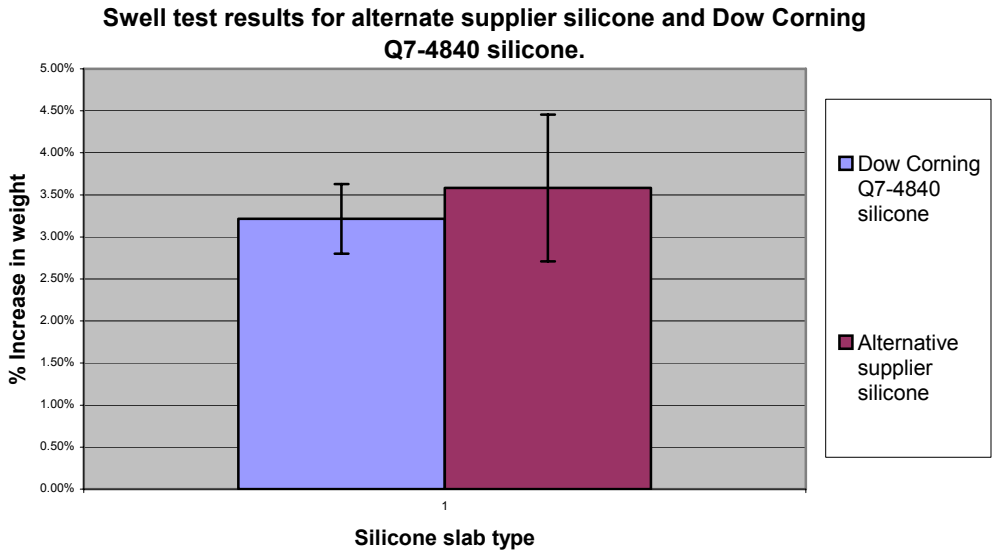


Figure 6.39 Swell test results for the alternative silicone supplier slabs and Dow Corning slabs. Error bars are the 95 % confidence interval.

Hence it appears there is no difference in crosslinking between the different feedstocks as determined by a swell test. Since only one batch of silicone from each supplier was used it is possible that differences in crosslinking may be detected with different batches of silicone. It is also possible that the analysis time was too short, as swell testing undertaken by Laird (Larid, 2004a) was done for a duration of three days. Swell tests by Golomb and Fisher (Golomb & Fisher, 1990) on Dow Corning Q7-4840 silicone containing 30 % w/w potassium dichromate in water, were undertaken for 120 days, and took ~ 70 days before the samples reached a constant weight. Further there was no ethanol permeation into the slabs, and no research was undertaken to determine the effect of swelling on slabs made with additional crosslinker, to determine if the observed results were real. Hence for these reasons no conclusion can be reached.

7.0 Manufacturing process alteration

The manufacture of CIDR inserts involves a large number of processes including annealing of CIDR insert spines, mixing of liquid silicone with progesterone, injection moulding of CIDR inserts, and packaging of CIDR inserts. Changes to these processes may reduce the effect of secondary blooming and mottling on CIDR inserts. For instance work by Rathbone and Ogle (Rathbone, & Ogle, 2000) found that curing at 120 ^{o}C prevented secondary blooming and mottling.

7.1 Packing in line of CIDR inserts

Packing in line is where CIDR inserts are packed shortly after removal from the tool. Currently CIDR inserts are not packed in line, however packing in line would result in increased manufacturing efficiency. The curing of the silicone results in the melting of the progesterone. As a result of packing in line the cooling profile of the CIDR insert after manufacture is changed. The polymorph of progesterone formed upon cooling depends on the cooling rate (Legendre, et. al., 2003). A slow cooling rate results in the formation of the α progesterone polymorph, while a fast rate of cooling results in the formation of the β progesterone polymorph (Legendre, et. al., 2003) (Duclos, et. al., 1991). Work by Reardon (Reardon, 2003a) found that slow cooling of CIDR inserts reduced the % mottling.

7.1.1 % Mottling versus cooling time

Mottling scores were undertaken on one year old CIDR 1380 inserts that were made with different bench cooling times (0 to 20 minutes) before packing (into plastic bags described in Chapter 3). The cooling time was the time the CIDR insert was left to cool on the manufacturing bench before the insert was packed. The CIDR inserts were made before the start of this research as part of a different study. At each time point there were six samples (which correspond to one rosette of CIDR 1380 inserts). The results from this experiment are outlined in Figure 7.1. From the results shown in Figure 7.1 it is clear that there is a linear relationship

between the cooling time of CIDR 1380 inserts and the average % mottling as shown by the R^2 value of 0.9413. This result indicates that packing in line reduces mottling on a CIDR insert. It is notable that packing in line does not totally prevent mottling. In Chapter Fopur it was found that mottled areas of the CIDR insert exhibit β progesterone polymorph, whereas the non-mottled regions (covered in powder) exhibited the α progesterone polymorph. However in Chapter four it was found that while slow cooling was able to change the progesterone polymorph (as detected by XRD), only once was a α progesterone polymorph formed through slow cooling (as detected by DSC).

It is possible that the packing of the CIDR insert after manufacture entraps gases released after cure. It is known that Dow Corning Q7-4840 silicone releases low molecular weight fluids and water vapour after cure (Dow Corning, 2005).

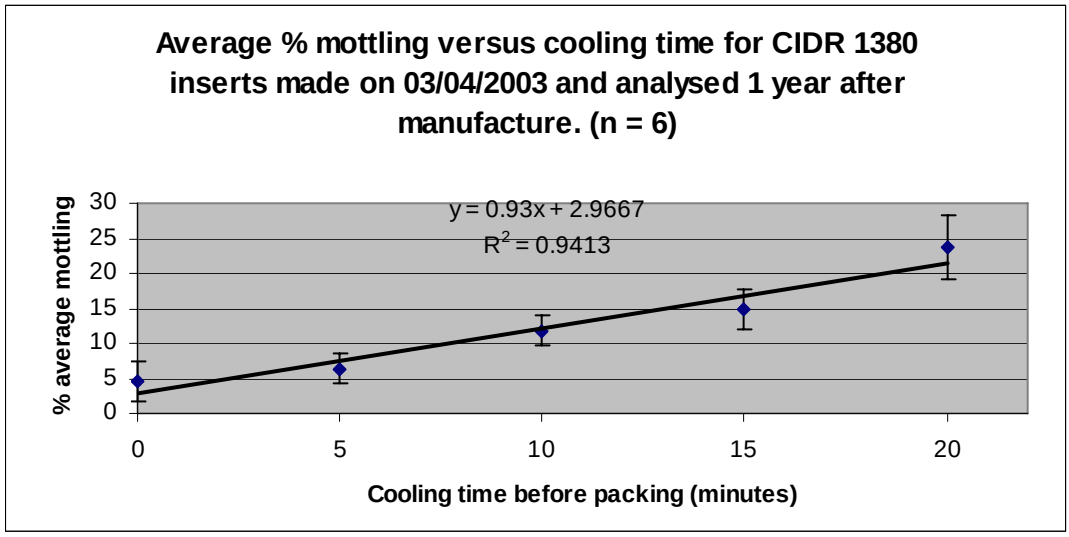


Figure 7.1 Average % mottling versus cooling time for CIDR 1380 inserts. Error bars are the 95 % confidence interval. n = 6.

To determine if there was any difference in the surface progesterone levels on CIDR inserts packed in line, some CIDR inserts were analysed using the surface progesterone method outlined in Chapter Three. Two CIDR inserts with cooling times of zero minutes, and two CIDR inserts with a cooling time of 20 minutes were analysed.

In Figure 7.2 it is clear that there is little statistical difference in surface progesterone levels between CIDR inserts that were packed in line after 20

minutes compared with the CIDR inserts that were packed in line immediately after manufacture, despite the trend of increasing mottling with cooling time (Figure 7.1). It is possible that surface progesterone was dislodged by handling, however it was noted that there was no observed surface progesterone on the samples that were packed at t = 0 and it was recorded that one sample packed at t = 20 minutes had some crystals on the surface. It is also possible that some of the progesterone may have migrated to the spine of the insert. It is known that there is a trend of increasing surface progesterone with increasing % mottling (Wong, 2003j) (NICAR FL320, 2005) (Wong, 2003e), but this is not observed in the experiments undertaken as part of this Section.

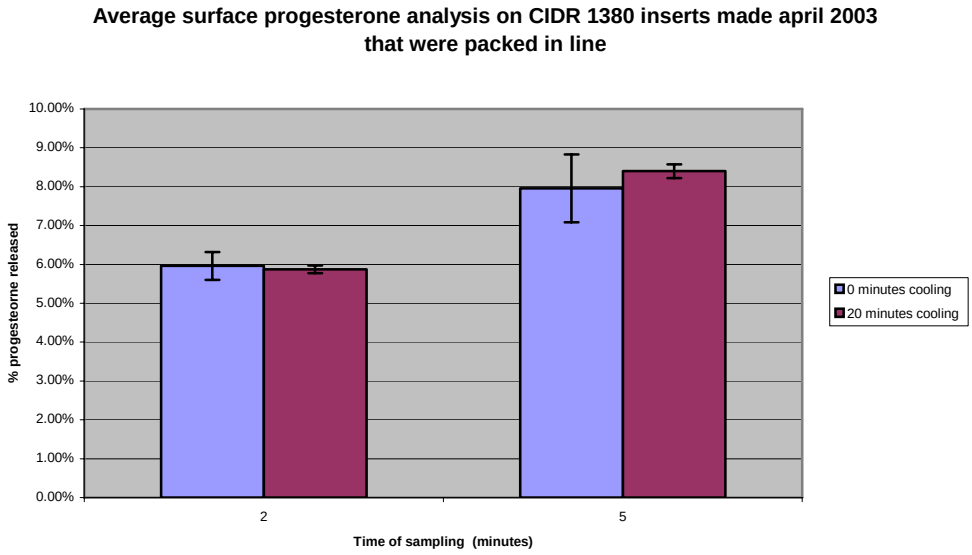


Figure 7.2 Surface progesterone analysis of CIDR 1380 inserts packed in line after different cooling times. Error bars are the 95 % confidence interval. n = 2. % Progesterone released as % of label claim (1380 mg).

7.1.2 The effect of packing in line of CIDR 1380 inserts into foil and plastic packaging

It has been shown in Section 7.1.1 that a longer cooling time before packing increases the % mottling on a CIDR 1380 insert. In order to gain an understanding of the effects of different packaging, and to verify the previous results. CIDR 1380 inserts were packed in line, and then placed into the stability oven. CIDR inserts were packaged into foil and plastic bags. It was thought that the foil bags

may have a heat conduction effect on the packed CIDR inserts, which would increase the CIDR insert cooling rate compared to plastic packaging.

A total of 36 CIDR 1380 inserts were manufactured from batch E09314. 12 CIDR inserts were packaged after bench cooling for 40 minutes (as a control), 12 CIDR inserts were packed in line into foil bags, and 12 CIDR inserts were packed in line into plastic bags. Each bag contained six CIDR inserts. At each time point at least two CIDR inserts from each packing condition were analysed to determine the surface progesterone levels. Time points of t = 0, 4 and 7 months were used. At the end of the study the Hanson Dissolution drug release rate analysis was undertaken on four CIDR inserts packed under each packing condition.

7.1.2.1 Surface progesterone results on CIDR 1380 inserts that were packed in line

The results from surface progesterone analysis on CIDR 1380 inserts packed in line are shown in Figure 7.3 and Figure 7.4. These Figures shows that there is an increase in the amount of progesterone on the surface of all inserts tested after four months. Furthermore there is little increase in the level of progesterone on the inserts after this period. There appears to be very little difference between amounts of surface progesterone on the inserts made under the different packing conditions. It is also of note that the 95 % confidence interval does vary quite remarkably, depending on the time point being analysed. This could be caused by dislodging of progesterone from the insert surface, hence giving the random variations.

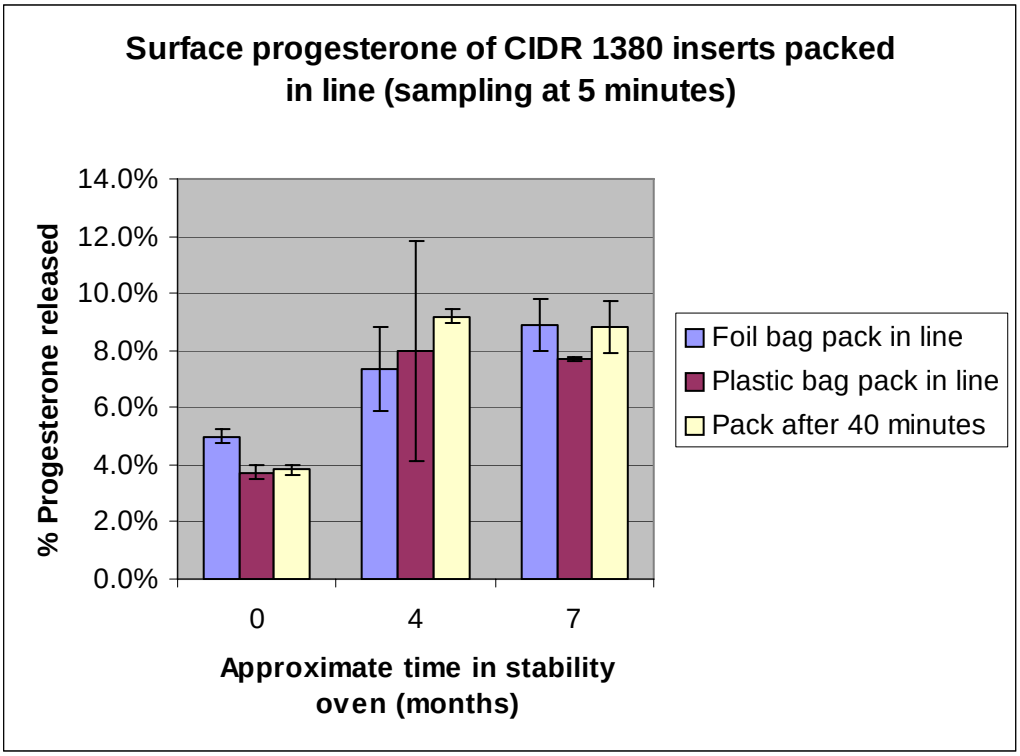


Figure 7.3 Surface progesterone at two minutes for CIDR 1380 inserts (batch E09314) packed in line. n ≥ 2. Error bars are the 95 % confidence interval. The % progesterone released is % of the label claim (1380 mg).

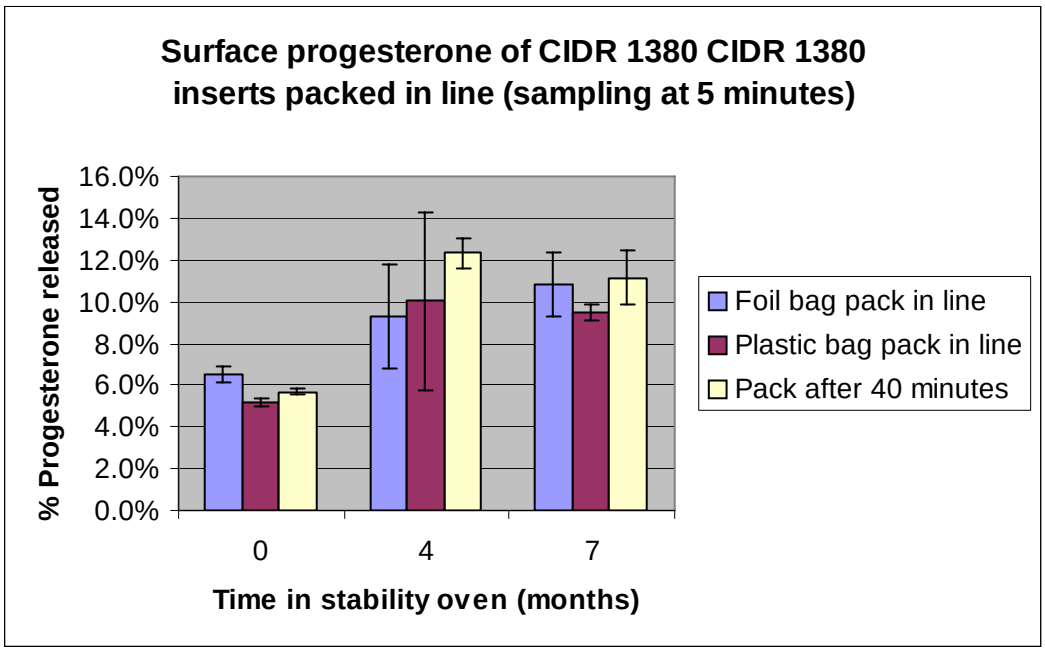


Figure 7.4 Surface progesterone at five minutes of CIDR inserts (batch E09314) packed in line sampling at five minutes. n ≥ 2. Error bars are the 95 % confidence interval. The % progesterone released is % of the label claim (1380 mg).

The lack of correlation between the surface progesterone amount and the packing environment agrees with the previous packing in line experiment surface progesterone results in Section 7.1.1. This is contrary to previous research, which indicated that there was a relationship between mottling and surface progesterone, with inserts that have higher levels of mottling also having higher levels of secondary blooming (Wong, 2003j) (NICAR FL390, 2005) (Wong, 2003e).

7.1.2.2 Hanson Dissolution drug release rate results on CIDR 1380 inserts that were packed in line

Drug release rates on CIDR 1380 inserts that were packed in line, were measured approximately nine months after manufacture. During this period the CIDR inserts were stored in the laboratory for two months. Samples were inspected on a number of occasions before testing, and this may have dislodged surface progesterone. The results are shown in Figure 7.5, that showing drug release rate and Figure 7.6 that shows the mass of progesterone released after one hour. Student T tests on the drug release results and the mass of progesterone released after one hour, show that there is no difference in either drug release rates or surface progesterone (% progesterone released after one hour) between the three different packing conditions. It is important that any means of reducing secondary blooming and mottling be as simple as possible to implement, without requiring amendment to the CIDR 1380 insert registration. In this experiment all CIDR 1380 inserts remained within drug release rate STP048 specifications of 998 to 1736 $\mu g/cm^2/\sqrt{t}$ (STP048, 2003).

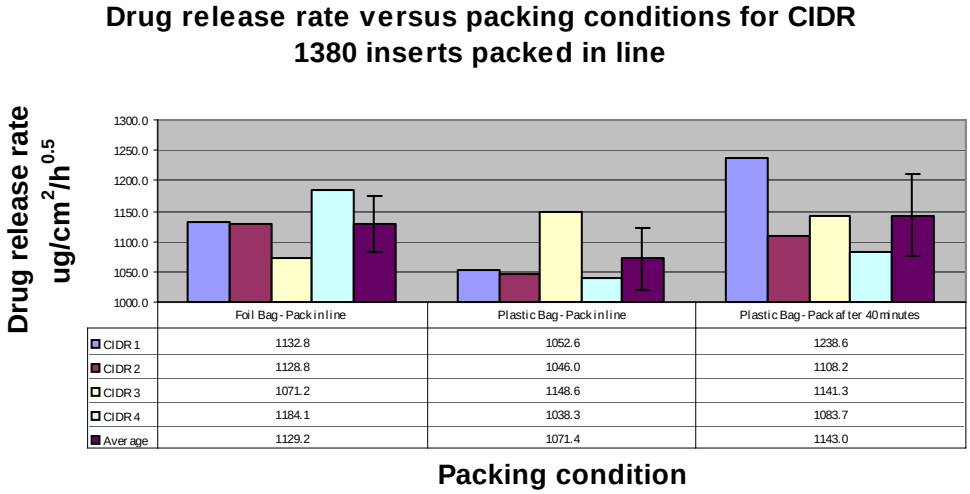


Figure 7.5. Drug release rate for CIDR 1380 inserts (batch E09314) packed in line and stored in stability oven. Units in the table below the Figure are $\mu g/cm^2/\sqrt{t}$. CIDR inserts from batch E09314. CIDR 1380 inserts had already undergone placement in a stability oven for seven months prior to transfer to the laboratory to await testing, which occurred two months later. Error bars for the average mass released is the 95 % confidence interval.

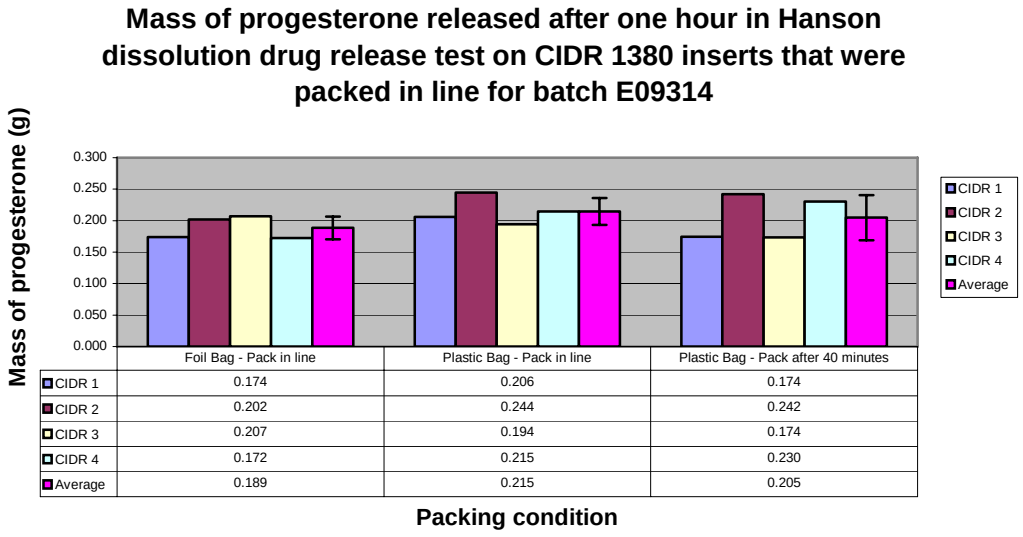


Figure 7.6 Amount of progesterone released after one hour of drug release rate test, for CIDR 1380 inserts (batch E09314) packed in line. CIDR 1380 inserts had already undergone placement in a stability oven for seven months prior to transfer to the laboratory to await testing, which occurred two months latter. Error bars for the average mass released is the 95 % confidence interval. Table contains mass of progesterone released in grams under each packing condition.

7.1.2.3 % Mottling versus packing condition

The average % mottling of CIDR 1380 inserts packed in line was analysed throughout the duration of the experiment (t = 0 to 8 months) the results are summarised in Figure 7.7. All CIDR inserts made for the packing in line study were analysed (36 CIDR inserts in total). Student T tests indicate that differences in % mottling are statistically insignificant between CIDR inserts packed in line in foil bags versus CIDR inserts packed in line into plastic bags. There is also an insignificant statistical difference in % mottling between CIDR inserts packed into plastic bags versus CIDR inserts packed after 40 minutes. However there is a significant statistical difference in % mottling between CIDR inserts packed into foil bags versus CIDR inserts packed after 40 minutes. From this analysis it is not possible to determine if there is a difference in the % mottling for CIDR inserts that are packed in line. It is interesting to note a significant difference between CIDR inserts packed into foil bags versus CIDR inserts packed after 40 minutes. The results suggest that the foil did not have a heat conduction effect (increasing the cooling rate) as previously hypothesised, however the cooling rate of CIDR inserts in foil bags is unknown. It is possible that the foil reflected heat back into the bag. Contrasting this result with the packing in line study in Section 7.1.1 (refer to Figure 7.1) there is a clear disagreement between the two different results.

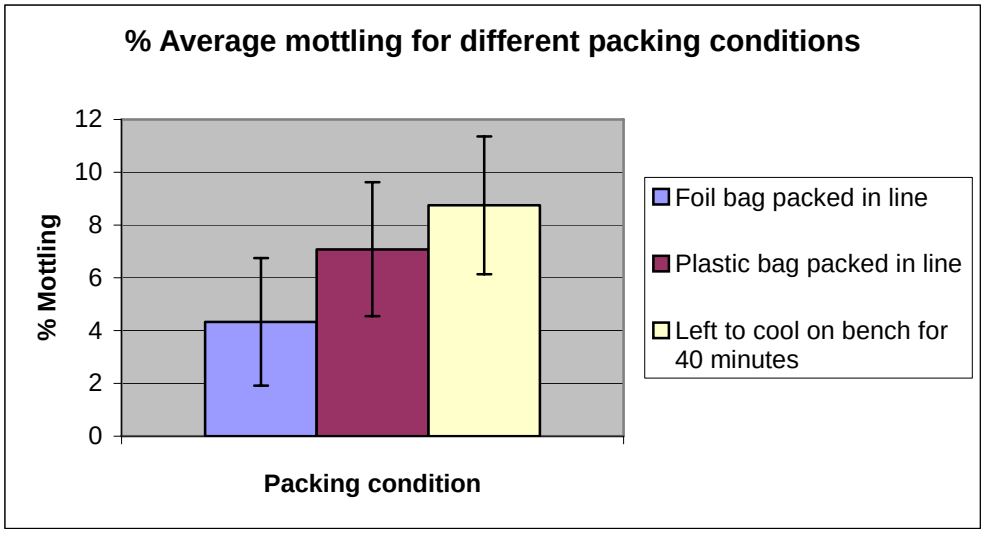


Figure 7.7 Average % mottling for different packing conditions for CIDR 1380 inserts (batch E09314). Error bars are the 95 % confidence interval. n = 12.

7.2 The effect of the static mixer on secondary blooming and mottling

The static mixer is used to mix the two parts of the silicone (pre-mixed with progesterone) as the silicone flows to the tool. In the normal manufacturing processes the static mixer is always used. The aim of this experiment was to determine if the presence of the static mixer reduced secondary blooming and mottling.

In order to investigate the effect of the static mixer on blooming and mottling a three month stability study was undertaken using CIDR 1900 inserts from batch E08572. CIDR inserts were cured for 40 seconds. Samples were analysed for surface progesterone, drug release rate, and % mottling at t = 0, and 3 months. At each time point two CIDR 1900 inserts made from each sample were analysed for surface progesterone.

7.2.1 Surface progesterone analysis on CIDR 1900 inserts made with and without the static mixer

Figures 7.8 and 7.9 show the surface progesterone analysis of CIDR 1900 inserts made with and without the static mixer (batch E08572) after storage in the stability oven. The surface progesterone levels increased over the three months in the stability oven, however Student T tests showed there was no statistical difference in the surface progesterone between the CIDR 1900 inserts made with or without the static mixer.

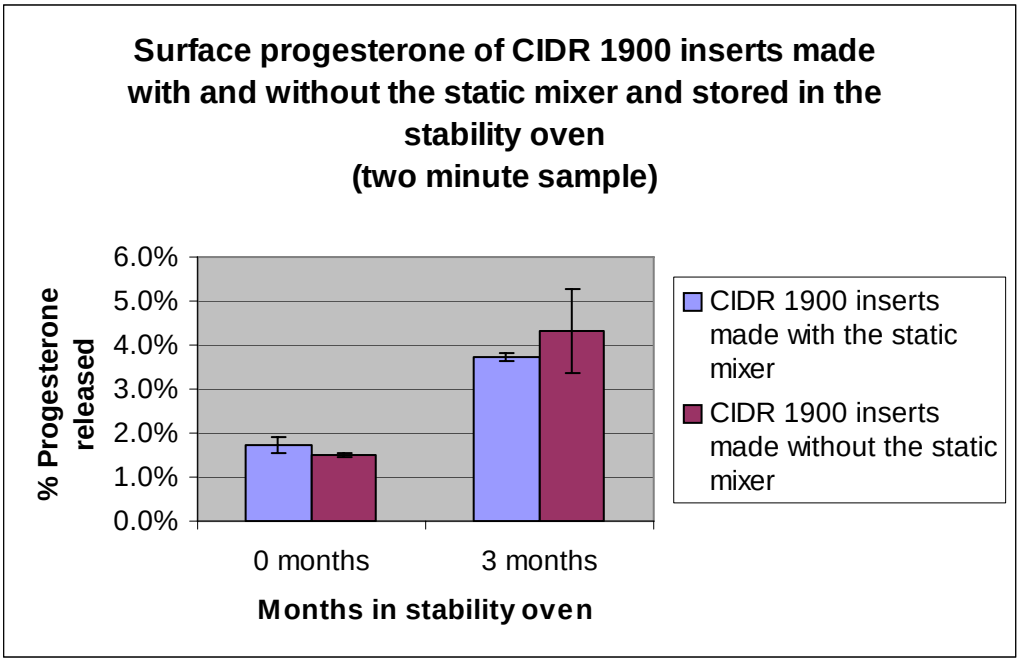


Figure 7.8 Surface progesterone at two minutes of CIDR 1900 inserts (batch E08572) made with and without the static mixer and stored in the stability oven. Error bars are the 95 % confidence interval. % Progesterone is % of label claim (1900 mg). n = 2.

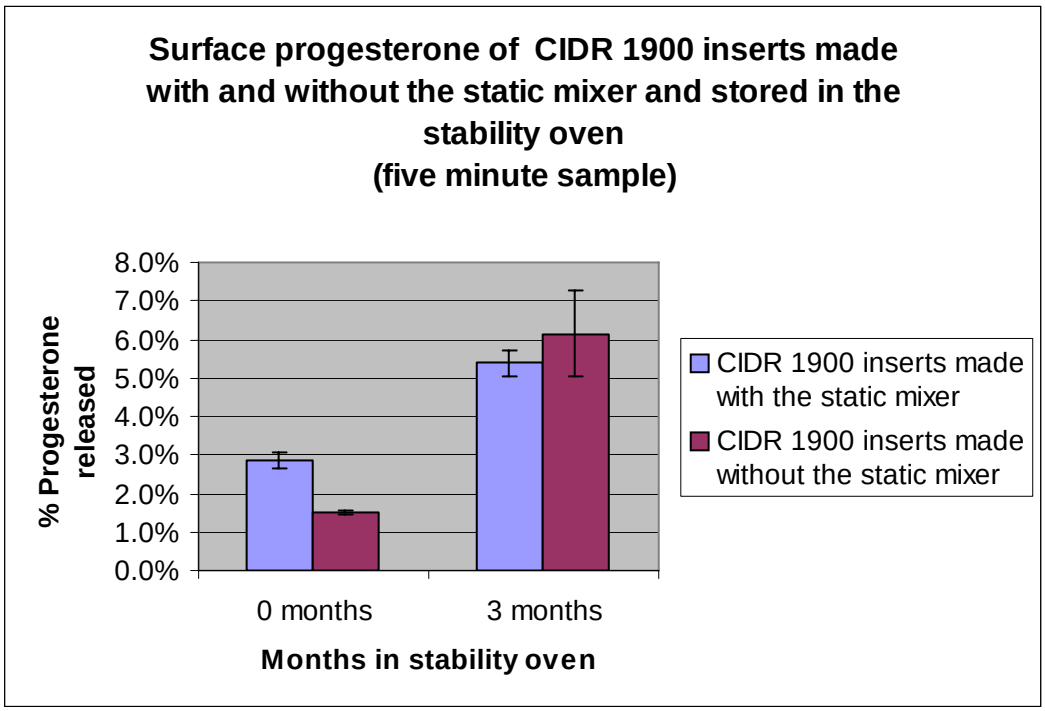


Figure 7.9 Surface progesterone analysis at five minutes on CIDR 1900 inserts (batch E08572) made with and without the static mixer and stored in the stability oven. % Progesterone released is % of label claim (1380 mg). Error is the 95 % confidence interval. n = 2.

7.2.2 % Mottling on CIDR 1900 inserts made with and without the static mixer

The % mottling on CIDR 1900 inserts made with and without the static mixer was analysed at t = 0. It was found (Figure 7.10) that there was a greater % mottling on CIDR inserts made without the static mixer compared to CIDR inserts made with the static mixer. Student T tests determined that the results were significantly different.

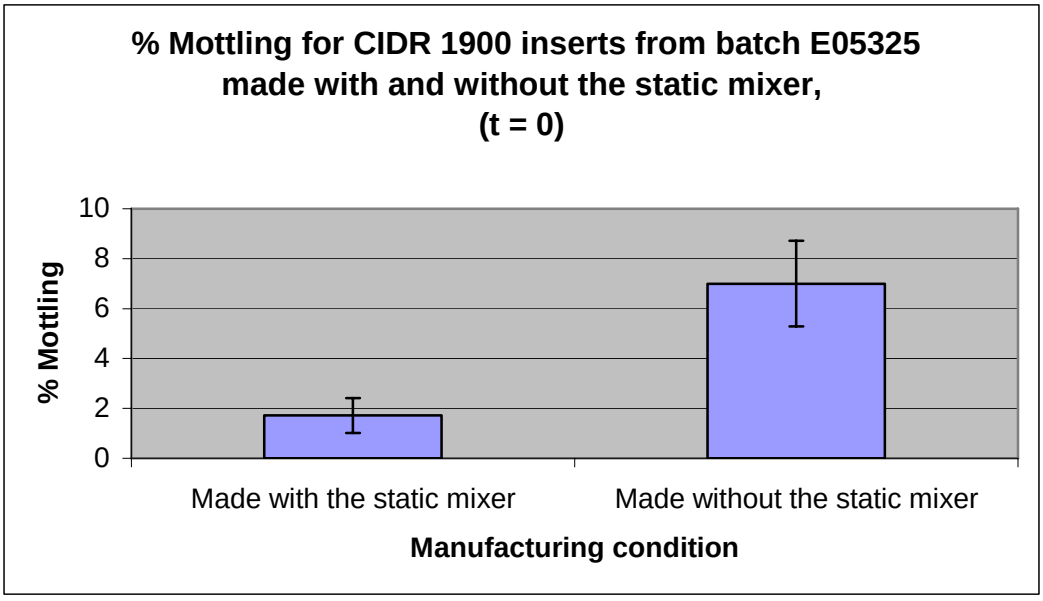


Figure 7.10 % Mottling for CIDR 1900 inserts from batch E05325 made with and without the static mixer. Error bars are the 95 % confidence interval. n = 7.

The lack of a static mixer reduces the mixing of the two parts of silicone before they enter the tool and are cured. This in turn would reduce the mixing of the crosslinker in with the platinum catalyst (Lee et. al, noted that keeping these two components apart was required to prevent immediate cure (Lee, et. al., 1979)) and hence would affect the processes occurring during silicone cure, such as crosslinking. This may make it easier for the progesterone to migrate around the CIDR insert and hence cause mottling. However it is noted that the surface progesterone levels on the CIDR inserts are not statistically different despite the manufacturing conditions. Experiments by others (Wong, 2003j) (NICAR FL390, 2005) (Wong, 2003e), shows a correlation between surface progesterone and % mottling, which was not observed in this experiment. These results however agree

with results from Aston (Aston, 2003), who found that decreasing mixing times increased mottling on slabs.

7.3 Slab spike studies (annealing residue and dust)

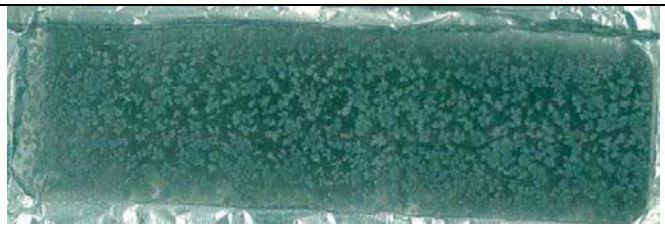
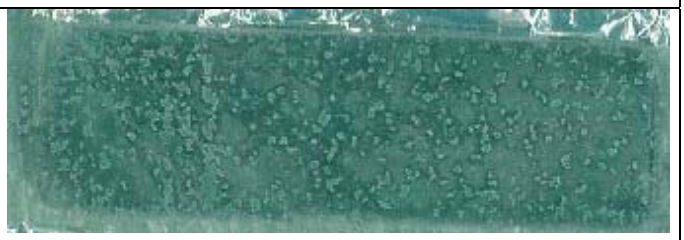
CIDR inserts are manufactured in controlled clean rooms to ensure that no adulteration of the product could occur through contamination. Manufacturing staffs are required to wear full body clean suits and to regularly clean manufacturing facilities. This Section investigates two possible contaminants that exist in the manufacturing environment, which may cause mottling and secondary blooming in CIDR inserts. These are dust and nylon residue from annealing. After annealing spines are placed into baskets, over drip trays. It is found that the drip trays collect a white material. XRD analysis (Wong, 2003i) determined that the material is nylon residue. Contaminants may affect mottling and secondary blooming through a range of possible mechanisms, from serving as a centre of crystallisation, or introducing contaminants, (which may affect the cure processes), or displacing space for progesterone in the matrix, or creating channels in the silicone matrix allowing the progesterone to flow through them.

7.3.1 Slabs spiked with annealing powder

Nylon annealing powder was scraped off the annealing drip trays and dried and ground up to a powder (annealing powder). Slabs were made with mixed silicone residue collected from manufacturing (batch E09338) along with a small amount of the annealing powder and black dye. Control slabs were made without annealing powder. Slabs were cured for two minutes along with control slabs (no annealing powder) at ~185 ^{o}C in a Contherm oven. Slabs were stored in the stability oven after manufacture. During this period the stability oven water tray did become empty, however (as discussed in Chapter Three) this is not an impediment to the experiment. Figure 7.11 shows scans of slabs 13.5 months after manufacture. Figure 7.11 clearly shows that there are differences between the slabs made with and without the annealing powder spike. Figures 7.11.1 and 7.11.2 clearly show that are larger ‘island formations’ on the slab made without the annealing powder compared to the slab made with the annealing powder. The

undersides of the slab made with annealing powder (Figure 7.11.4) show the 'island formations' have conglomerated giving 'continental formations', however slabs made without the annealing powder (Figure 7.11.3), have smaller 'island formations', which are more dispersed and not conglomerated as observed in Figure 7.11.1 and 7.11.3.

Figure 7.11 Scan of slabs made with/without annealing powder 13.5 months after manufacture. Slabs shown are representative.

Slab	**Images (not to scale)**
Slab made without annealing powder.	 **Figure 7.11.1** Scan of slab made without annealing powder.
Slab made with annealing powder.	 **Figure 7.11.2** Scan of slab made with annealing powder.
Underside of slab made without annealing powder.	 **Figure 7.11.3** Underside of slab made without annealing powder.
Underside of slab made with annealing powder.	 **Figure 7.11.4** Underside of slab made with annealing powder.

Observations of the slabs made with/without the annealing powder spike for translucency (mottling) clearly show that there is translucency on both sets of slabs. Both sets of slabs also had very low levels of blooming. Hence it appears that the annealing powder has no effect on blooming, but has an effect on the 'formations' observed on the slabs. Both the 'island formations' and the 'continental masses' are not comprised of surface progesterone. Scraping both sets of samples with a scalpel resulted in a white powder forming on the surface, and on the undersides of the slabs; the scraped areas also became paler. It is possible that the white material is progesterone that has been brought up to the surface by the scrapping. In comparison scraping the surface of slabs made with the alternative supplier silicone found that (made in Chapter Six) one slab not produce surface progesterone (although going pale), another slab (Figure 6.34) did exhibit this phenomena. The areas around the islands appear to be translucent hence slabs made with the spike appear to have less mottling. Wong (Wong, 2003i) found that slabs made with lumps of nylon spine, did not have crystallisation specifically around the spine particles.

7.3.2 Slabs made in contact with annealing residue

After annealing spines are left to drip dry in large bins. It is thought that during this process water would dry on the spines leaving the nylon residue. This would result in some areas of the spines having higher concentrations of annealing powder compared with others such as ledges on the spine where liquid could collect. To investigate this possibility further, slabs were made using mixed silicone residue from batch E09338. In this experiment annealing residue was mixed with ethanol and painted onto the tin foil and allowed to dry. Liquid silicone was then applied over the areas with the annealing powder and slabs were cured for two minutes at ~185 ^{o}C. Some slabs were made using a black dye. These slabs were stored in the stability oven after manufacture. During this period the stability oven water tray did become empty, however (as discussed in Chapter Three) this is not an impediment to the experiment.

The slabs made with and without the annealing material contact, were found to possess translucency 13.5 months after manufacture. Figure 7.12 shows scans of slabs 13.5 month after manufacture. These show that there is secondary blooming on both sets of slabs, however there is more secondary blooming on the slabs made without the annealing powder than slabs made in contact with the annealing powder. It is also found that the undersides of the slabs made in contact with the annealing residue were similar to the slabs made with the annealing powder spike, as the control slabs had 'island formations', whereas the spiked slabs had a 'continental formation'.

Figure 7.12 Scans of slabs 13.5 months after manufacture made in contact / not in contact with annealing powder. Slab shown are representative.

Slab	**Scan (not to scale)**
Made without contact to annealing powder.	 **Figure 7.12.1** Slab made without annealing powder contact to (control).
Made with contact with annealing powder.	 **Figure 7.12.2** Slab made in contact with annealing powder.

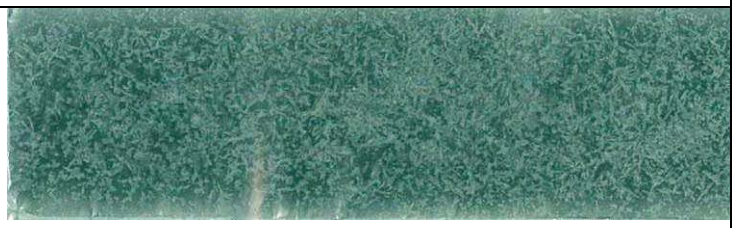
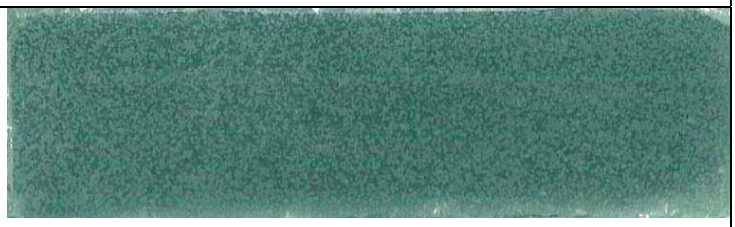
The slabs made in this Section did not possess significant differences in blooming, but did possess differences in surface phenomena such as 'island formations' and differences in the underside of the slabs. This may provide information on the causes of blooming, however work by Wong (Wong, 2003i) found that contact with the nylon spine did not cause increases in blooming. Hence it can be concluded that the accumulation of annealing powder in a specific location does not cause mottling and blooming, however it may affect how the mottling as differences were observed in island phenomena.

7.3.3 Slabs made with a dust spike

Dust is removed through regular cleaning of the manufacturing facilities. As part of cGMP requirements, manufacturing areas cannot be swept down, but must instead be vacuumed to prevent dust spreading. A sample of manufacturing dust was collected from a vacuum cleaner. Slabs were made with a dust spike using mixed liquid silicone residue from batch E09338, and a black dye. Control slabs were made without a dust spike. Slabs were cured for two minutes between ~150 to ~185 ^{o}C. These slabs were stored in the stability oven after manufacture. During this period the water tray did become empty, however (as discussed in Chapter Three) this is not an impediment to the experiment.

Figure 7.13 shows scans of slabs made with and without the dust spike, 13.5 months after manufacture. There is a clear difference between the slabs, with the slabs made without the dust spike showing strong secondary blooming while the slabs made with the dust spike are not. The scans show that there is a difference on the underside of the slabs in terms of 'island formations', as the slabs made with the dust spike have a grey 'conjoined island formation' in contrast to the control slabs, which have the 'island formations'. This result is less intense compared, to the slabs made with and without the annealing powder spike.

Figure 7.13 Scans of slabs made with and without a dust spike. Scans 13.5 months after manufacture. Slabs shown are representative.

Slabs	**Image (not to scale)**
Slab made without a dust spike.	 **Figure 7.13.1** Scan of slab made without a dust spike.
Slab made with a dust spike.	 **Figure 7.13.2** Scan of slab made with a dust spike.
Underside of slab made without the dust spike.	 **Figure 7.13.3** Scan of slab made without a dust spike.
Underside of slab made with a dust spike.	 **Figure 7.13.4** Scan of slab made with a dust spike.

7.4 The effect of cure time on mottling

7.4.1 The effect of cure time on % mottling on a range of CIDR inserts from the same batch

It is important to know the effect of cure time on solid CIDR inserts as this allows determination of the effect of cure time duration on % mottling with respect to the

state of the silicone matrix. This information is also useful in studying the effects of packing in line with different cure times. CIDR 1380 inserts made before the start of this research for another study were analysed for % mottling one year after manufacture. The CIDR insert cure times varied from 24 to 50 seconds. All CIDR inserts were made with the same raw materials. Unlike the previous packing in line study in Section 7.1 all CIDR inserts were packed in line after manufacture. The results are shown in Figure 7.14.

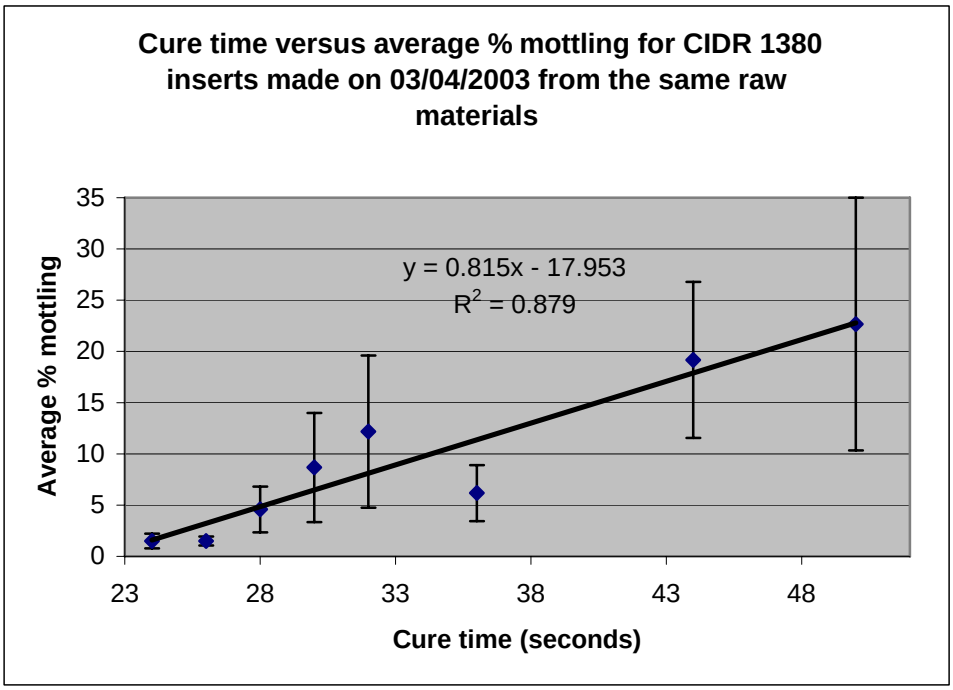


Figure 7.14 Cure time versus average % mottling for CIDR 1380 inserts with different cure times and packed in line after manufacture. Error bars are the 95 % confidence interval. n = 6.

Inspection of Figure 7.14 finds a tentative linear trend of increasing average % mottling with increasing cure times. However the 95 % confidence interval increases as the cure time increases, indicating a wider range of % mottling for the CIDR inserts made with longer cure times. These results indicate that a longer cure time on cured CIDR inserts increases the % mottling. Possible causes include the heat increasing randomisation of mottling in the CIDR insert. The longer period of cure may also drive off post cure compounds, principally water, and low molecular weight silicone fluids (Dow Corning, 2005).

Previous packing in line results from Section 7.1.1 show that the longer the cooling time the more the % mottling increases, as the CIDR inserts have a longer period to cool before packing (which slows the cooling rate). In this study the CIDR inserts were packed immediately after manufacture and hence the cooling rate is slowed, however the longer cure time does increase the time for the CIDR insert to adsorb heat. But other results in this Chapter have shown that there was effect on packing in line and % mottling, in contrast to the previously mentioned results.

Possible causes of the % mottling increasing with cure time cannot be linked with progesterone degradation products, as work by Laird (Laird, 2004e) found that there was no definite correlation of cure time versus progesterone degradation.

7.4.2 The effect of cure time on % mottling on a range of CIDR 1380 insert batches

The reserve samples from seven CIDR 1380 inserts batches with different cure times were analysed for their percentage mottling. The cure times of the various CIDR batches were calculated by Laird (Laird, 2004c). These batches were:

- E05334 – cure time 38 seconds
- E06301 – cure time 38 seconds
- E05332 – cure time 38 seconds
- E05325 – cure time 32 seconds
- E05323 – cure time 24 seconds
- E05317 – cure time 22 seconds
- E05320 – cure time 22 seconds

The CIDR inserts were all made in either May or June 2004 and were analysed on the 30th of June 2005. It should be noted that all CIDR insert reserve samples are stored in a temperature monitored warehouse. All reserve samples were analysed (n = 8 to 10). Figure 7.15 shows the mean mottling for each batch that was analysed. Figure 7.16 plots the mean % mottling versus cure time and Figure 7.17 shows the plot of % mottling versus cure time for all CIDR inserts analysed.

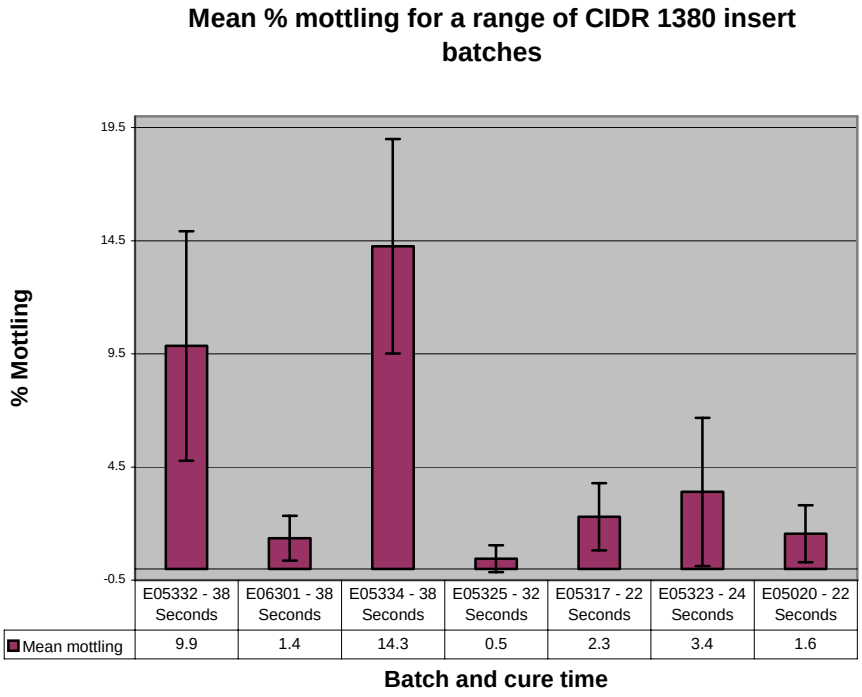


Figure 7.15 Mean mottling for a range of CIDR 1380 insert batches made with different cure times. Error bars are the 95 % confidence interval.

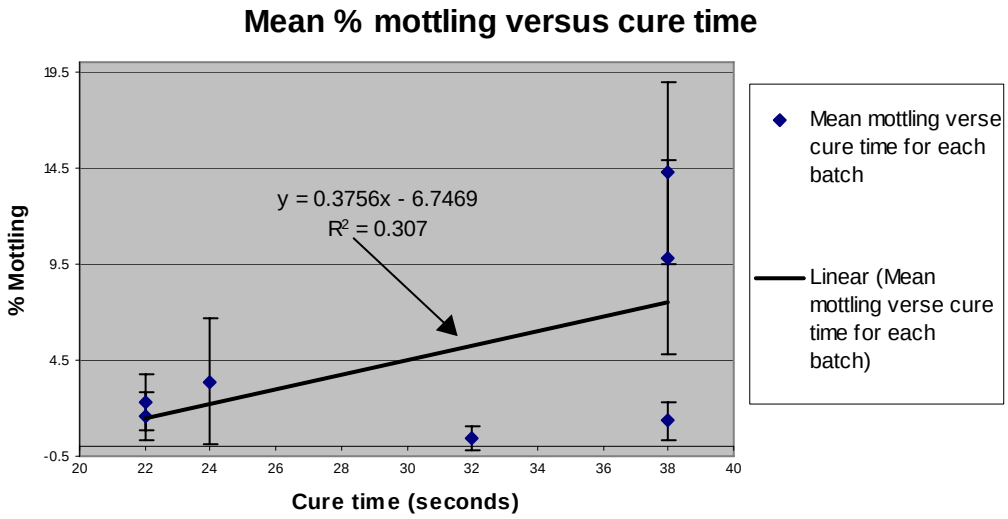


Figure 7.16 Average percentage mottling versus cure time for seven batches of CIDR 1380 inserts. Error bars are the 95 % confidence interval.

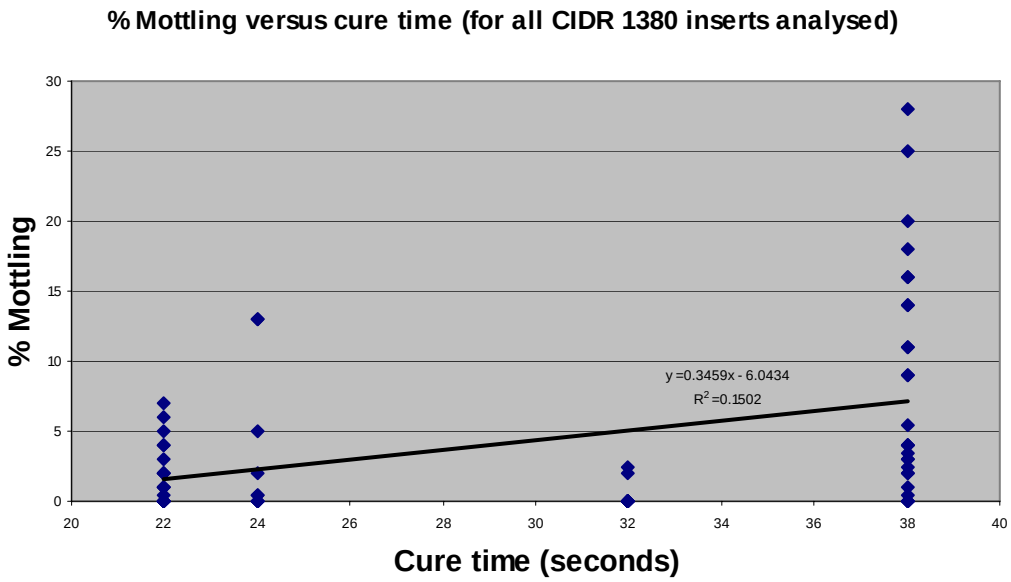


Figure 7.17 % Mottling versus batch cure time for all CIDR 1380 inserts analysed.

In Figures 7.15 to 7.17 it is clear that there is a tentative trend of increasing cure time resulting in increasing % mottling. Some of batches do appear to have low % mottling despite possessing a high cure time such as E06301 (38 seconds) and E05325 (32 seconds). Figure 7.18 shows the % of CIDR inserts analysed from the same batch that possessed mottling versus the cure time for that batch. Figure 7.18 shows no trend between the % of CIDR inserts from the same batch exhibiting mottling versus the cure time for the inserts from that batch.

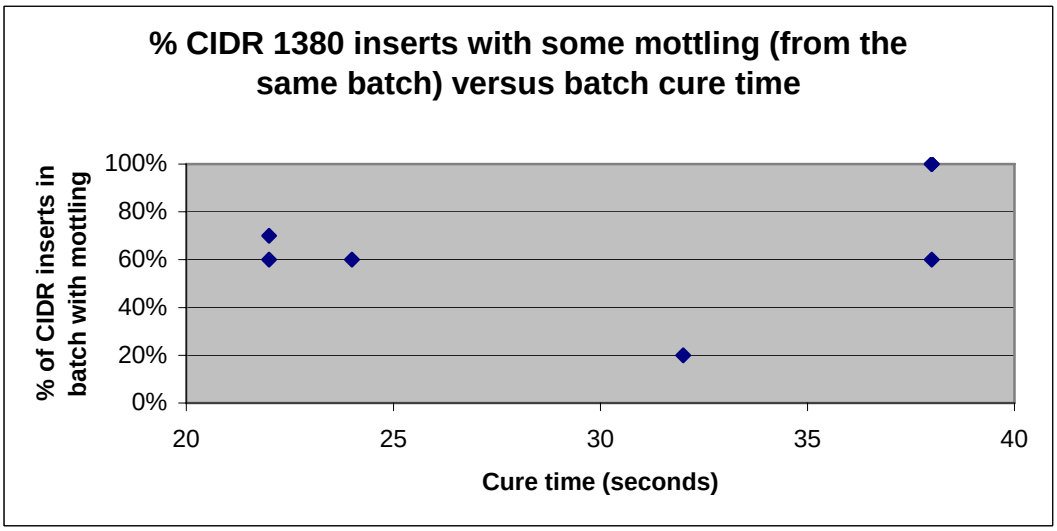


Figure 7.18 Percentage of CIDR 1380 inserts from each batch that exhibited non zero mottling

In previous work in this Chapter (Section 7.4) it was found that increasing the cure time on a solid CIDR 1380 insert that was packed in line increased the % mottling (Figure 7.14). CIDR inserts are cured for the minimum possible time to increase productivity, hence for the study in Section 7.4 the longer cure time simply means that the CIDR inserts have been cured, and then held at the cure temperature for longer. In this Section it is found that CIDR inserts cured for longer periods had increased mottling. In this case the CIDR inserts would have been in a semi cured state for a longer period compared to the CIDR inserts used in Section 7.4 In both cases increasing the cure time increased % mottling, hence it can be concluded that the state of the silicone (as the CIDR insert cures it will progress from liquid, to partially cured, to cured) does not have an effect on the % mottling of a CIDR 1380 insert, instead the length of the cure time is more critical. However as the relationship between % mottling and cure time is still tentative, further results are required to prove or disprove this hypothesis.

8.0 Conclusions and recommendations

8.1 Conclusions

The aim of this thesis was to determine the root cause of secondary blooming and mottling in the CIDR insert. This research determined a number of ways of reducing secondary blooming and mottling in the CIDR insert, however no definitive conclusion was reached into the root the cause of secondary blooming and mottling.

CIDR inserts were made with an alternative supplier silicone feedstock and the currently used silicone feedstock Dow Corning Q7-4840. Studies were undertaken to compare the differences between the two feedstocks. CIDR inserts made with the alternative supplier silicone did not usually exhibit any mottling or secondary blooming, agreeing with previous results by other researchers. Leachate was extracted into ethanol from CIDR inserts made with both types of silicone feedstocks and analysed by ESMS and GCMS. It was found that CIDR inserts made from the alternative supplier silicone had a greater ratio of cyclic silicone ions to straight chain silicone ions compared with CIDR inserts made with the Dow Corning Q7-4840 silicone. It was not established if this fact predisposed CIDR inserts made from the Dow Corning Q7-4840 silicone feedstock to undergo secondary blooming and mottling processes.

A study was undertaken using ESMS to determine the differences in leachate extracted from the liquid silicone feedstocks from Dow Corning Q7-4840 silicone and the alternative supplier silicone. It was found by ESMS that part B liquid silicone leachate from the alternative supplier silicone feedstock had a higher ratio of cyclic silicone ion peaks to the straight chain silicone ion peaks compared with the part Dow Corning Q7-4840 liquid silicone.

GCMS analysis of liquid silicone dissolved in dichloromethane found that the part B liquid silicone has a more complex matrix compared to part A Dow Corning Q7-4840 silicone (and compared to either part of the alternative supplier silicone).

ESMS analysis of the leachates from both parts of Dow Corning Q7-4840 silicone and the alternative supplier silicone feedstocks were in agreement with this result. The actual relationship to secondary blooming and mottling from this revelation remains unknown. In contrast to previous results, it was found that part B of Dow Corning Q7-4840 silicone did not cause secondary blooming.

Slabs were made with extra crosslinker to determine if there was any influence on secondary blooming and mottling it was found that addition of extra crosslinker to Dow Corning Q7-4840 silicone feedstocks increased mottling in the resultant slabs, but did not increase the overall observed extent of measured surface progesterone.

CIDR inserts were made with and without the static mixer (used to mix the two parts of liquid silicone before curing). Removal of the static mixer increased average % mottling, but did not increase surface progesterone levels on the CIDR inserts. Part B contains the crosslinker and it appears that mixing of the crosslinker may have an effect on mottling. Slabs made with the additional crosslinker were scanned by XRD and it was discovered that there was no effect on progesterone polymorphism with increasing addition of crosslinker as the β progesterone polymorph was exhibited in all samples.

XRD diffractograms of slabs made with Dow Corning Q7-4840 silicone found that the β progesterone polymorph was formed after manufacture. The polymorph observed on the slab remained stable for five months outside of the stability oven. Slabs made with the alternative supplier silicone exhibited the β progesterone polymorph (in a seven day study).

Mottled and non-mottled regions of CIDR inserts were scanned by XRD. The β progesterone polymorph was observed in the mottled areas, which disappeared after wiping with ethanol. Non-mottled regions showed the α progesterone polymorph, which did not persist after wiping with ethanol.

It has been found that pausing cooling of slabs at ~135 ^{o}C for ten minutes reduces secondary blooming and mottling in contrast to pausing at other temperatures

between (and just below) the melting points of the α and β progesterone polymorphs. However it was also observed that pausing cooling at ~125 ^{o}C reduced secondary blooming but not mottling. Work to repeat this experiment on pure progesterone using DSC and XRD only produced the β progesterone polymorph. Only with very slow cooling rates was it possible to produce the α progesterone polymorph. Therefore one can conclude that slow cooling under these conditions does not usually affect the polymorphism of progesterone per se.

Packing in line is where CIDR inserts are immediately packaged after cure rather than being allowed to cool on the bench. CIDR inserts were hence packed in line after manufacture. One study found that there was a decrease in % mottling from packing in line, whereas another study found no difference between the packing conditions. However there was no discernible difference in measured surface progesterone levels found between either packing method in each study.

Tentative trends were found between the cure time and % mottling for both solid CIDR inserts (CIDR inserts that had been cured and left in the closed tool for longer periods of time) and CIDR inserts that simply required a longer cure time to become solid. It was proposed that any increase in mottling through increasing cure times is definitely not caused by the physical state of the liquid silicone in the tool during cure (which ranged from liquid, to partly cured, and fully cured).

The yield stress of a range of liquid silicone batches (Dow Corning Q7-4840 silicone only) were analysed and compared to the average drug release rate form CIDR inserts made from these batches. A trend of increasing yield stress (of both parts A and B of liquid silicone) versus increasing ‘in vitro’ drug release rate was found. It is thought that this result is from the presence of the fumed silica in the silicone matrix (added to give reinforcement to the cured silicone), however the exact relationship between the yield stress and the drug release rate remains undetermined.

A number of results from this research (such as the packing in line experiment and the effect of extra crosslinker experiment) found that there was no correlation between measured surface progesterone and mottling, despite trends found by

other researchers to the contrary. However it is known that CIDR inserts with a high levels of measured progesterone tend to have a high degree of mottling, whereas CIDR inserts with a low level of measured progesterone tend to have a low degree of mottling. Hence it is likely that there is a non-linear relationship between secondary blooming and mottling. This result may be due to random progesterone dislodgement from the surface of the CIDR inserts durring handling.

8.2 The cause of secondary blooming and mottling

A prerequisite to secondary blooming and mottling occurring is the melting of progesterone. After melting the progesterone cools and initially forms an amorphous material in the silicone matrix. The mottled areas of a CIDR insert have the β progesterone polymorph at the surface, whereas the non-mottled areas have the α progesterone polymorph as a powder on the surface of the device. The progesterone powder on the surface of the device does not change its polymorphic form, however the matrix may cause the polymorphic phase biases that are often observed. Mottled regions are non-homogenous, and have reduced progesterone loading compared to the non-mottled regions on the CIDR insert indicating that they are the possible source of the progesterone in the secondary blooming. The movement of the progesterone to the surface of the insert possibly occurs via a medium (such as water (from cure or humidity), silicone fluids or trapped air), however the exact nature and properties of the medium is still at this point unknown.

It is known that a number of factors affect the degree of secondary blooming and mottling on the CIDR insert. Increasing the amount of crosslinker has been showing to increase mottling, and appears to cause the formation of a closely bound layer of progesterone on slabs made with the alternative supplier silicone. The degree of mixing of the two parts of liquid silicone is observed to increase the amount of mottling on a CIDR insert. Increasing the extent of heating of the CIDR insert appears to increase the amount of mottling. Pausing the cooling of slabs at 135 ^{o}C for ten minutes also appears to decrease secondary blooming and mottling.

It was firmly established in this study that the alternative supplier silicone displays significantly lower to no secondary blooming and mottling in CIDR inserts relative to those made using the Dow Corning Q7-4840 silicone. Decreasing the bulk density of progesterone mixed into the silicone results in a decrease in secondary blooming on one hand, but on the other hand results in the formation of small unmixed progesterone lumps in the cured silicone matrix.

The research has given further valuable insights into the root cause of secondary blooming and mottling. From an industrial point of view some useful methods were found that can be used to minimise secondary blooming and mottling in CIDR inserts without to much change to currently used manufacturing methods and protocols.

8.3 Recommendations for future work

- The alternative supplier silicone leachates have been found to have a higher ratio of cyclic silicones ions to straight chain silicones ions compared to the Dow Corning Q7-4840 silicone, however the actual effect on this with respect to secondary blooming and mottling remains unknown. Hence further work should investigate:
    - Secondary blooming and mottling on slabs made with extra cyclic silicones.
    - Determining progesterone solubility in low molecular weight cyclic and straight chain silicone fluids (and combinations of both), as solubility of progesterone may either inhibit or assist in the secondary blooming and mottling in the matrix.
    - Analysing the absolute concentration of cyclic silicones versus straight chain silicones in the leachates from the two silicones feedstocks.
- Further analysis of the effect of mixing the two parts of liquid silicone with the static mixer. One liquid silicone part contains the platinum catalyst and the other contains the crosslinker. If there is incomplete mixing this may affect mottling and blooming.

  - Mixing a dye into one part of the silicone and then observing the mixing effects of the two parts by the static mixer, then repeating the experiment without the static mixer. If there were a difference in the colour variation at the end of the process then this would support the proposed hypothesis.
- It was found that part B of the Dow Corning Q7-4840 silicone was not responsible for causing secondary blooming, in contrast to work done by other researchers. Hence further work should be done to investigate this discrepancy.
- The fumed silica may affect diffusion of the progesterone through the silicone hence a study into the differences between the fumed silica used in the alternative supplier silicone compared to the fumed silica from the Dow Corning Q7-4840 silicone could be undertaken to determine if this is a factor.
- The alternative supplier and Dow Corning Q7-4840 silicones may give off different gases or relative concentrations of gases during cure, comparing the relative concentrations and compositions of cure gas given off by the two silicone feedstocks may lead to further insights into the cause of secondary blooming and mottling, along with more information on silicone composition.
- The results from this thesis gave contradictory results on the effect of packing in line on mottling, further work could to be undertaken to clarify these results.
- The studies into slow cooling of progesterone done in this research only used progesterone, undertaking these experiments inside a liquid silicone matrix comprising both parts A and B would give results that were more realistic to the real world conditions.
- The ions detected in the ESMS may be from a range of compounds or a few compounds, analysis of the leachates by LCMS would give further information in the differences in the number of components between the two silicone feedstocks and the two parts of the liquid silicone.
- Comparing the effect of molecular polarity with respect to secondary blooming and mottling. This can be undertaken by manufacturing slabs

containing steroids that have different polarities from progesterone, such as testosterone and cholesterol.

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Appendix A - Method discussion

Appendix A discuses issues related to techniques used in this thesis such as the surface progesterone measurement technique, and the % mottling analysis.

A.1 Development of a surface progesterone analysis method

The surface progesterone method in this thesis involves measuring the amount of progesterone released after two and five minutes into a volume of ethanol in a sonicator. The type of containers used depended on the test being undertaken. CIDR 1900 inserts and CIDR 1380 inserts were tested in 1.2 L Clickclack containers, and slabs and CIDR 330 inserts were tested in Techno-plas containers. In order to prevent the Techno-plas containers from floating in the sonicator during the tests, lead sinkers were added to the bottom of the container. The mass of progesterone released at each sample point is reported as % of label claim. The surface progesterone technique developed as part of this thesis is designed to be a simple and more quantitative method of measuring surface progesterone from blooming.

Analysis of the amount of surface progesterone (by measurement of the mass of progesterone released after one hour in a Hanson Dissolution drug release test) is commonly used at DEC Manufacturing. Unfortunately these machines are often in use for general laboratory testing. Additionally the Hanson Dissolution technique also measures not only the amount of progesterone on the surface of the device, but also the mass of progesterone released from the silicone matrix after one hour. But can differentiate between CIDR inserts with high levels of surface progesterone and low levels of surface progesterone.

Wong's (Wong, 2003c) method involved monitoring the release of progesterone from a CIDR insert after 20 minutes in a water/ethanol release medium. Whilst this method could distinguish between a fresh CIDR inserts and an old CIDR inserts, only 4 inserts were reported to have been analysed by this technique. Furthermore the sampling time involved (20 minutes) meant that the test took a

lot longer to run than was regarded as practical. Additionally Wong's test required the preparation of a ethanol water release media, which is not practical for a simple test.

Ogle's method (Ogle, 1999) involved rinsing the CIDR insert in ethanol. However this method does run the risk of not having the whole CIDR insert come in contact with the ethanol. It important to note that the methods discussed later in this Section show that it took approximately two minutes before the rate of progesterone dissolving in the ethanol begins to decline, which indicates that not all the progesterone on the surface has entered the solution until this time. However time required to dissolve and mix progesterone into ethanol is unknown. The method also faces the risk of having analyte lost from un-collected splashes. However Ogle's method does reduce amount of progesterone extracted from the matrix due to the ethanol having reduced contact time with the CIDR insert surface. Further more this method does not require equipment such as containers that require cleaning and does not require as much ethanol as Wong's method or the method discussed in this thesis.

A.1.1 Determination of optimal sampling times in the CIDR insert surface progesterone test

The optimum time point chosen should be able to distinguish progesterone released from the surface, but at the same time minimise the progesterone that would be continually released from the silicone matrix. Furthermore an efficient test should take as little time as possible to complete.

To determine the optimal sampling time (to ensure a short test time) a range of CIDR 1380 inserts with different degrees of mottling (0 % to 50 % mottling) were tested by UV analysis, with sample removal occurring at 20 second intervals for 5 minutes, to gain a plot of the release of the progesterone into the ethanol versus time. A total of ten CIDR inserts were used to generate the results in Figure A.1.

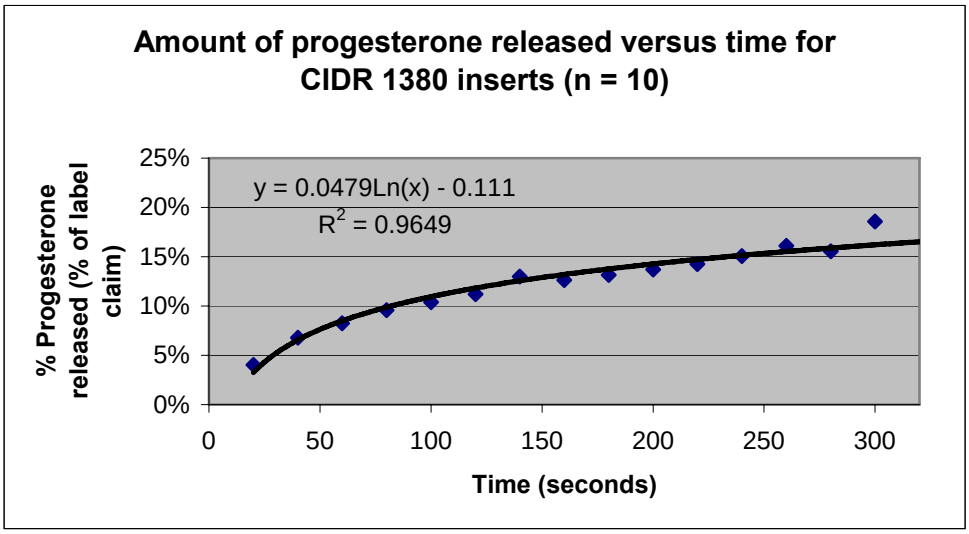


Figure A.1 Average percentage release from a range of CIDR 1380 inserts with different % mottling (between 0 % to 50 % mottling). The variation in points is due to the lack of data from particular samples at different time points resulting in spikes.

As can be observed in Figure A.1 there are two parts to the plot. Firstly there is an initial rapid release of progesterone from the sample. After ~1 minute the rate of progesterone release declines and becomes constant (or linear). Once the release has reached the linear region it is assumed that most of the surface progesterone has been released and progesterone released after two minutes would be released from the silicone matrix Hence two sample time points were determined, two minutes and five minutes. At two minutes the bulk of the progesterone release is from the surface of the CIDR insert (not from the silicone matrix). Some CIDR inserts may not have released all of the surface progesterone after two minutes as is observed in Figure A.2, which shows the release of progesterone on a CIDR 1380 inserts with 38 % mottling. This contrasts with Figure A.1.

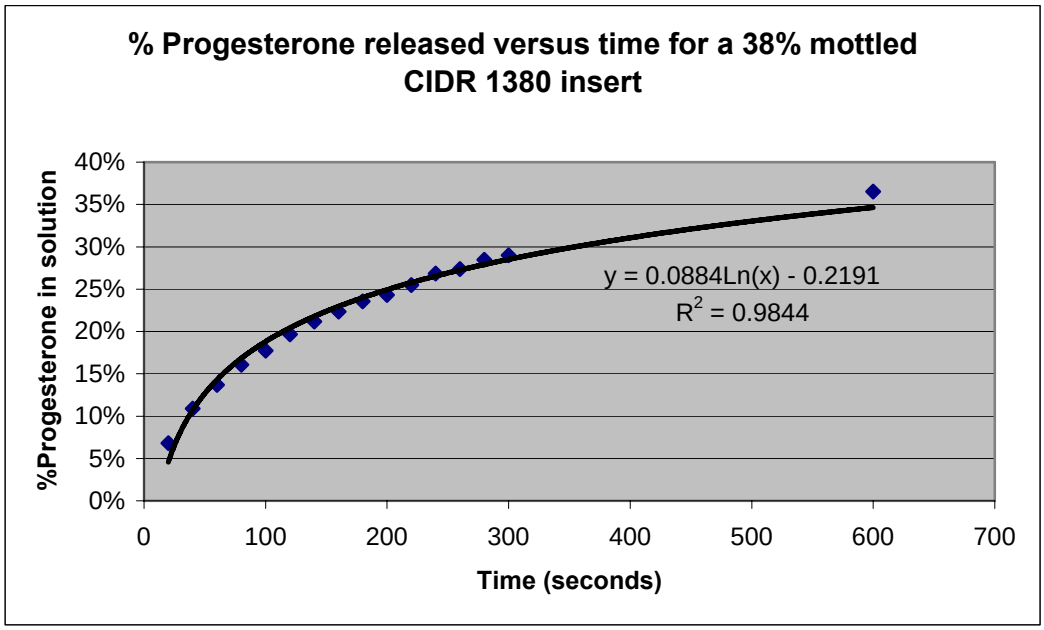


Figure A.2 Release of progesterone from a 38% mottled CIDR insert.

The five minute time point is selected in order that there will be both a back-up point and a means of verifying the result from the two minute point. If the two minute timepoint is adulterated for some reason, such as a delay in sampling or contamination (from unclean equipment) then some data will be recoverable from the five minute timepoint. Additionally if the five minute timepoint is lower than the two minute time point, then it is clear that the test results for the sample under analysis are unreliable. Samples that have high levels of surface progesterone such as the CIDR insert used in Figure A.2, will have released all surface progesterone after five minutes.

Method development tests were undertaken on CIDR 1380 inserts. As this technique was also used on slabs, CIDR 330 inserts and CIDR 1900 inserts it is important to note that it is assumed that these samples will release most of the progesterone after 2 minutes.

A.1.2 Sources of Error for the Surface Progesterone Method

In order for the technique discussed here to be reliable, consideration into sources of error from factors such as temperature, contamination and technique precision must be considered.

Contamination

It is possible that the plastic containers used may leach material in some form during the test. This is not of concern if the material has no UV absorbance at λ = 239 nm (the λ used to monitor the progesterone concentration in pure ethanol). A number of tests were run to determine the degree of leaching into the containers at λ_{max} = 239 nm.

A number of blank experiments were undertaken on Clickclack containers alongside regular CIDR 1380 insert testing and no leaching of concern was detected from these experiments. For example an undiluted blank sample collected after ten minutes and stored in the refrigerator and analysed on the UV, on the 21/01/05, found that the range in absorbance with three measurements was –0.0031 to –0.0035 AU. It was found that the Clickclack containers did not leach at λ = 239 nm during the corse of the experiment. Further any leaching that does occur will be minimized by dilution as was observed in a blank undertaken on the 28/01/04.

In order to investigate the leaching from Techno-plas containers some blank tests were undertaken using different volumes of ethanol. These tests ware outlined in Table A.1. No sample underwent any dilution before UV analysis. From Table A.1 it is clear that there is leaching from the Techno-plas containers. As observed in Table A.1 there is a clear difference between containers that have been cleaned with ethanol and those that were not. However even with such contamination present dilution of the collected sample should ensure that such contamination is minimized. It also should be noted that with the short length of the tests, leaching should be further reduced unless the contaminants at 239 nm are found only on the surface of the containers, which would mean that no leaching was occurring.

Table A.1 Blank absorbance ranges from samples in100 mL Techno-plas containers**.**

	Total time ethanol was left in the container	**100 mL ethanol (n = 1)**	**35 mL ethanol (n = 1)**	**35 mL container placed upside down (n = 1)**
Containers used as received	3 hours	0.0070 to 0.0062	0.0152 to 0.00158	0.0173 to 0.0178
Containers cleaned twice with ethanol	1 hour 20 minutes	-0.0034 to -0.0037	-0.0028 to -0.0033	-0.0021 (for both scans)

In order to assess the effect of lead sinkers on absorption at 239 nm in Techno-plas containers, glass bottles were rinsed several times in ethanol and then placed in a soak bath of Decon Nutracon for at least 15 hours. After rinsing with water, distilled water and ethanol, an approximate volume of 110mL of ethanol was added along with some lead sinkers and left for three nights. Then UV spectrophotometry was undertaken comparing the ethanol in the bottles with the blank. For both samples the mean absorbance at 239 nm was 0.0477 AU. While this absorbance is outside the machine tolerance, with further dilutions this value should be reduced enough to ensure that error is minimised.

Temperature

The rate of dissolution is influenced by temperature since drug release from the silicone matrix is occurring throughout the duration of the surface progesterone test. Operation of a sonicator for a long period of time does increase the water temperature. In order to determine if fluctuations in testing temperature has an effect on the mass of progesterone released, the temperature was recorded at the end of a number of extractions (final temperature) on similar samples (slabs made with different amounts of crosslinker and but from the same raw materials) and Figure A.3 was generated. From the low R^2 value (0.0763) it can be concluded

that there is little or no relationship between the temperature and the mass of progesterone released over this temperature range.

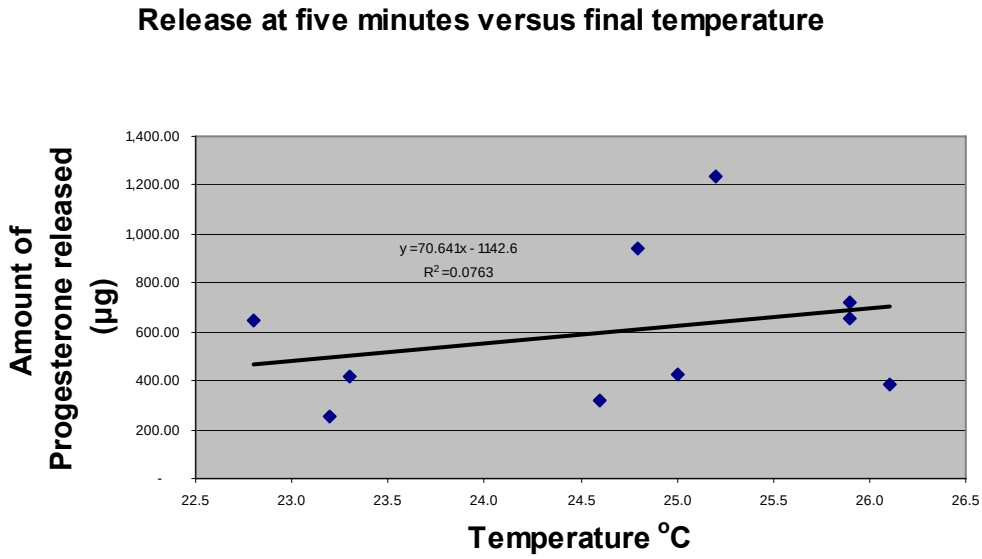


Figure A.3 Mass of progesterone released versus temperature at the end of test.

A.1.3 Precision of surface progesterone technique

In order to investigate the precision of the surface progesterone method, a total of eight control CIDR 1380 inserts were tested on two different days (four CIDR inserts on the 28 th of January 2005 and four CIDR inserts on the 3 rd of February 2005). These CIDR inserts were from the same batch (E09301) and had the control reference of CR116, and had % mottling of 1 % or less, and were stored in a freezer until testing started. Devices on the first day were given at least one hour to warm to room temperature whereas the devices on the second day were left overnight to warm to room temperature. Each day a different batch of ethanol was used to dilute samples and made standards. Samples were scanned on the same UV spectrophotometer (Beckman DU-600).

The percentage mass of progesterone released for both days is shown in Figure A.4. The % mass of progesterone released for each CIDR insert tested are shown in Table A.2. From Figure A.4 it is clear that undertaking of the surface progesterone on different days and using similar batches of ethanol produces similar results.

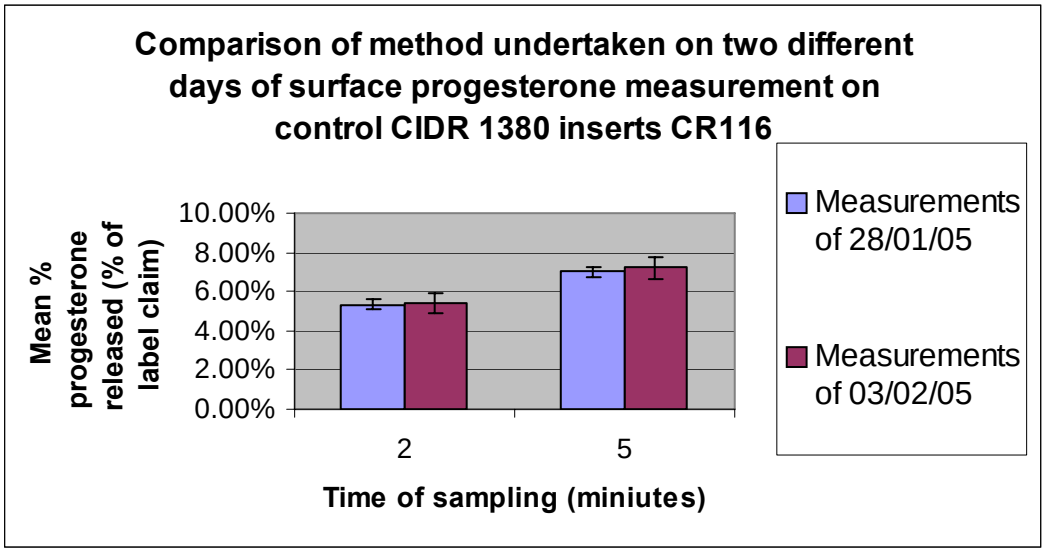


Figure A.4 Surface progesterone on CIDR 1380 inserts from batch E09301 Tested on two different days. n = 4, error is the standard deviation. % Progesterone release is % of label claim (1380 mg).

Student T tests were done on the raw data to determine if there was a difference between the samples tested on the first day and on the second day. It was found there is no significant variation between the two days for the % progesterone released for the two minute sample and the five minute sample

Table A.2 Amount of progesterone released from control CIDR 1380 inserts done on different days. % Progesterone release is % of label claim (1380 mg).					
	CIDR insert number 1	**CIDR insert number 2**	**CIDR insert number 3**	**CIDR insert number 4**	**Mean and standard deviation**
Two minute sample 28/01/05	5.35 %	5.63 %	5.35 %	4.97 %	Mean: 5.33 % Std. Dev.: 0.27 %
Two minute sample 03/02/05	4.79 %	6.03 %	5.40 %	5.48 %	Mean: 5.43 % Std. Dev.: 0.51 %
Two minute sample 28/01/05	6.99 %	7.30 %	7.03 %	6.68 %	Mean: 7.00 % Std. Dev.: 0.25 %
Five minute sample 03/02/05	6.51 %	7.78 %	7.16 %	7.34 %	Mean: 7.20 % Std. Dev.: 0.52 %

A.2 % Mottling error between different operators

This method of analysis is very subjective and hence the % mottling varies depending on the person undertaking the mottling score. For instance Figure A.5 from a study into the % mottling versus on the conditions of packing clearly shows that there is a clear difference in mean mottling scores determined by two different people.

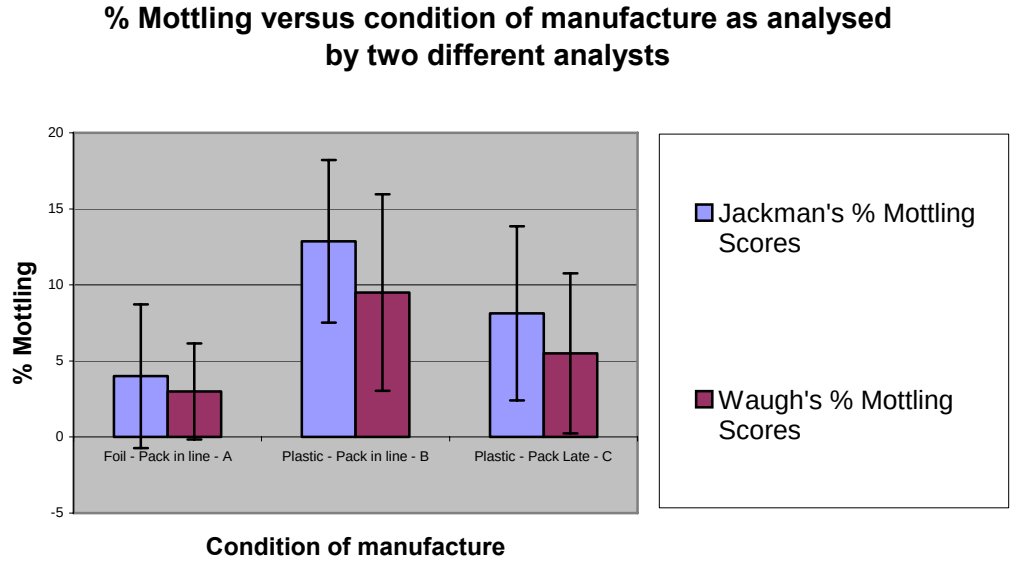


Figure A.5 % Mottling for CIDR inserts undertaken by two different people to demonstrate the differences between two analysts. CIDR insert packed in line. Analysis done in conjunction with Mark Jackman. n = 4.

These results can be compared with results from Wong and Aston, (Wong & Aston, 2003) who noted that the variation between two analysts is ~10 %. This variation is different from samples analysed by Waugh and Jackman in Figure A.5. This would be caused by the low level of mottling in these CIDR inserts whereas Wong's work had CIDR inserts that had over 40 % mottling.

Results gained by different analysts must be interpreted with care. Since the technique is subjective it is also possible that the same analyst could give different mottling scores to the same CIDR insert on different days. Wong's work (Wong, 2003g) found that one analyst could give a lower average % mottling at one time compared to a second analyst, and then at a latter time give a higher % mottling compared to the second analyst analysing the same CIDR inserts.

Appendix B - Brookfield R/S Soft Solids Tester

The following Figures B.1 and B.2 are from the Brookfield 2004 catalogue and from Brookfield's website on the R/S Soft Solids Tester.

Figure B.1 R/S Soft Solid tester as described by the Brookfield 2004 Catalogue (Brookfield, 2004).

Figure B.2 Extracted from a webpage from www.brookfieldenginering.com on the R/S Soft solids tester. (Brookfield, 2005). Figure continues over two pages.

R/S Soft Solids Tester (SST)

IDEAL for testing difficult-to-measure materials such as Slurries, Gels and Suspensions...

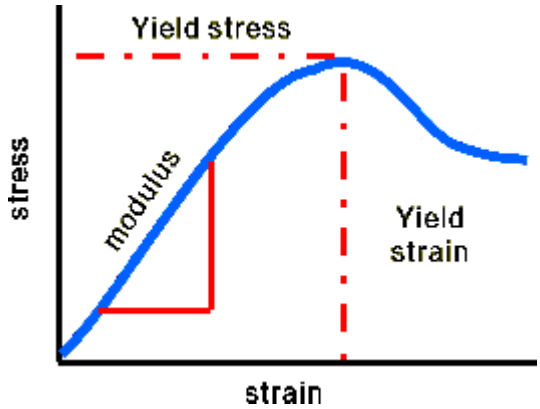
The Soft Solids Tester is IDEAL for testing difficult-to-measure materials such as Slurries, Gels and Suspensions and it provides Yield, Viscosity and Creep/Recovery data.

The flow properties of these materials are not easily measured using traditional coaxial cylinder or cone & plate equipment. Vane spindle geometry provides a solution. The vane spindle is lowered into the test sample with minimal disruption. The test is easily run in the sample's original container.

There are two significant Test Methods easily accomplished by the Soft Solids Tester:

1. Constant Rate Yield Test

- For Products Like: Stiff pastes, slurries, set gels, waxes
- Properties Measured:Yield Stress, Yield Strain, Modulus
- Test Description:This is a Constant Shear Rate Test. The vane is rotated at a constant low rotational rate and the Stress (torque) is measured against Time, Rotational Angle or Strain.
- Example Method for Mayonnaise:Constant Rotation at 0.2 rpm, 60 data points are taken in 60 seconds



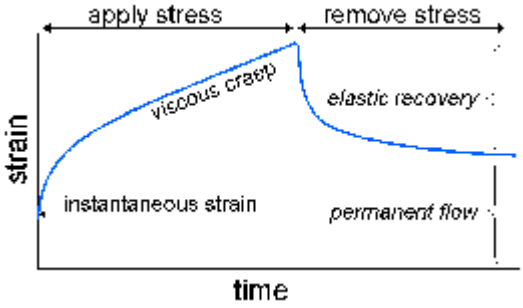
Typical Results Plot

2. The Creep/Recovery Test

- For Products Like:Gels, lumpy products like custard, gravy, sauces and jams

- Properties Measured: Instantaneous Strain, Viscous Creep, Elastic Recovery, Permanent Flow
- Test Description: This is a Constant Stress Test. The Stress (torque) is applied to the sample for a fixed time period, usually from one to five minutes. The degree of sample movement is recorded against time as Angular Displacement, Strain or Compliance. The Stress is removed and the Elastic Recovery and Permanent Flow is determined.
- Example Method for Fruit Preserves: Constant Stress of 1,250 Pa is applied over 120 seconds and 60 data points are taken. The stress is then removed and 60 data points are taken for another 120 seconds.

Typical Results Plot



Consult with Brookfield to determine if the Soft Solids Tester with Vane Spindle Geometry is a suitable solution to your testing needs.