INVESTIGATION INTO THE PRODUCTION OF CALCIUM PECTINATE PARTICLES FOR ORAL DELIVERY TO THE COLON

By

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Abstract

The polysaccharide pectin can be cross linked with calcium ions forming calcium pectinate, a material that is ideal for drug release to the colon since it can be degraded by the colonic flora. Particles produced from calcium pectinate can allow an encapsulated drug to be released at a controlled rate through the polymers highly ordered and cross linked structure. To avoid drug loss before the colonic site is reached, particles can be coated in pH responsive polymer coats, facilitating colon targeting.

In this work, the production of calcium pectinate particles, loaded with 5 aminosalicylic acid (5-ASA) was explored. Their potential for use in controlled drug release to the colon was evaluated. In general, two production methods for the particles were explored; the 'syringe droplet' method and the 'water in oil' emulsion method, where the processing conditions that produced high quality particles were investigated. The characteristics of these particles were found to be dependent on the processing parameters selected. In the syringe droplet method, light mixing speeds and high pectin concentrations were essential and in the water in oil emulsion method, high emulsifier concentrations with fast mixing speeds were necessary in order to produce a satisfactory emulsion. Loading of 5-ASA into the particles was investigated by loading the drug in solution and suspension. Due to the poor solubility of 5-ASA in water, encapsulation of the drug in solution resulted in a 0% encapsulation efficiency. Therefore, the 5-ASA was re-encapsulated though a suspension where reasonable drug loadings, which were affected by the calcium chloride concentration, were evaluated.

However the loaded drug caused a deformity in the structure of the particles. Particles were also subject to *in vitro* release in conditions simulating the gastrointestinal tract, where the 5-ASA was rapidly released though all simulations. This rapid release was believed to be due to the incompatibility of the encapsulation of 5-ASA into calcium pectinate particles produced by the water in oil emulsion method. The particles were finally subject to preliminary coating studies with a pH responsive polymer by a solvent evaporation method where it was believed some degree of coating was achieved. In this method, it was believed that an effective polymer coating was produced by sufficient agitation and a satisfactory coating solution concentration.

Acknowledgements

I am thankful to my supervisor Dr Rachel Bridson, co supervisor Dr Richard Greenwood and fellow members of my research group Dr Ian Lee, Mr Matt Barea and Mr John Willett for their help and support throughout this project.

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Contents

Chapter 1

Introduction

1.0 Background and aims

Over the last two decades, attention has been focused on the colonic region as a site for the delivery of some drugs administrated by the oral route (Chen 1998). The oral route is attractive since it is liked by patients encouraging compliance. Production of oral dosage forms can sometimes be cheaper than parental forms since sterile environments may not be needed (Chen 1998). Compared to other regions of the gastrointestinal tract (GIT) the colon can be highly responsive to absorption enhancers, offers a less hostile environment for liable drugs, and may allow increased bioavailability (Holta 2000).

However the colon is the last part of the GIT which means obstacles such as change in pH and differing enzymes need to be considered where designing an oral dosage form to be targeted to the colon. The acidic pH of the stomach can give rise to some issues. Vandamme *et al* (2001) have discussed that drugs can be prematurely released and degraded in the stomach due to its strongly acidic environment. Also since most absorption and digestion occurs in the in small intestine, large volumes of a drug may be lost while travelling though this region (Linshu *et al* 2003). Many of these disadvantages can be overcome by the addition of a pH responsive polymer coat that protects a drug until it reaches the colon.

The success of colonic delivery systems is therefore dependent on the ability of the formulation to protect the incorporated drug from the conditions of the upper GIT. When designing a formulation several factors have to be taken into account;

- Firstly the drug and its formulation must remain intact from the moment it is taken through the mouth and during its journey though the GIT until it reaches the colon,

being protected from the harsh acidic environments of the stomach and degradation in the small intestine.

- Secondly the formulation must allow the drug to be released upon entry to the colon.
- Lastly drug release upon entry to the colon must be predictable and at an appropriate rate at the colon site.

To date, some research has shown that dosage forms composed of polysaccharides can be targeted to the colon since they are naturally degraded by the colonic flora (Vandammei *et al* 2001), where calcium pectinate has shown much promise (Rubinstein *et al* 1993). However, particles composed of calcium pectinate can be susceptible to premature release; therefore they are often coated with pH responsive polymers, protecting the dosage form en route to the colon (Siepmann *et al* 2008)

The overall objective of this project was to explore the production of particulate systems composed of calcium pectinate that could be used for the oral delivery of drugs to the colon. Particularly, emphasis was placed on exploring production methods for the particles then evaluating their drug loading and release properties. More specifically the aims were too;

- Explore the syringe droplet method for the production of the calcium pectinate particles, determining the effect of the key processing parameters on the particle characteristics.
- Explore an alternative production method based on emulsions and again, evaluating the effect of the key processing parameters on particle characteristics.
- Determine whether the particles can be successfully loaded with the model drug, 5-ASA, a drug used for inflammatory bowel disease.
- Evaluate the release characteristics of the particles in various *in vitro* release media simulating the stomach, small intestine and colon.

- Carry out a preliminary investigation on how to coat the particles in a pH responsive coat, which can protect the core en route to the colon.

1.1 Physiology of the human GIT

The GIT's (Figure 1.1) function is to digest food, absorb nutrients and to prevent the absorption of harmful substances (Chourasia 2003). The stomach is the first organ and contents can remain here for up to 2 hours (Scheline 1973). The small intestine is the longest section with a high surface area and is therefore where most of the absorbance occurs and contents can remain here for around 3 to 6 hours. The colon is the last organ and contents can remain here from 12 to 24 hours (Luishu *et al* 2003). These residence times can vary from person to person and are also affected by conditions such as diet and age.

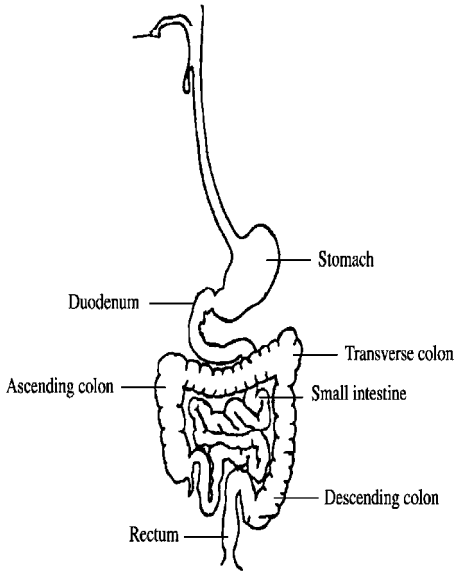

Figure 1.1 – The GIT. The main function is to digest food and protect against harmful substances. The tracts main organs are the stomach, small intestine and colon

1.1.1 pH variations in the GIT

Following the route of the GIT from stomach to colon, there is a general increase in the tracks pH. The stomach has a pH of 1.5 to 3.5 which varies depending on whether the stomach is in a fasted or fed condition (Luishu *et al* 2003). The small intestine has a pH of 5.5 to 6.8. The

colon is made up of the ascending, transverse and descending colon and the pH rises from 7 in the ascending colon and reaches 7.4 in the descending colon (Luishu *et al* 2003). These pH values can differ from person to person and are dependent on the age, diet and physiological condition of the site (Savage 1977).

1.1.2 Gastrointestinal micro flora

Vandamme *et al* (2001) have discussed that some delivery systems targeted to the colon can be triggered by the micro flora that are present in the colonic region. Yang (2000) has discussed that the micro flora in the colon survives by fermenting the various types of substrates that have been left undigested in the small intestine. The stomach contains only a small number of bacteria and the total number of bacteria increases along the gastrointestinal tract. Factors such as the short transit time of material through the stomach and small intestine and the low pH of the stomach are most likely responsible for the low bacterial content of these organs (Yang 2000). In contrast, the long transit time of the colon, together with the high content of nutrients, allows about 400 different bacterial species to thrive in the colon (Luishu 2003).

Studies by Drasar *et al* (1969) and Semde *et al* (2000) have seen that many polymers that are naturally degraded by these enzymes can be used for colonic drug delivery. Delivery systems produced from these polymers are activated upon entry to the colon. Most of the recent research by Wong *et al* (2002) and Surajit *et al* (2010) includes natural polysaccharides being applied to create biodegradable colon delivery systems. The ability of the flora to act as the triggering mechanism for a delivery system can vary from person to person. Factors that affect the flora are age, diet and the physiological condition of the site (Safran 1986 and Shantha 1995).

1.2 Controlled release systems for colonic delivery

1.2.1 Slow release matrices

A variety of dosage forms have proven to successfully deliver drugs to the colon at a controlled rate. Such dosage forms include those containing pro drugs, time dependent systems and slow release matrices (Singh 2001).

Slow release matrices have successfully delivered drugs to the colon under a controlled release rate by researchers including Wong *et al* (2002) and Maestrelli *et al* (2008). The method used for the encapsulation is dependent on the chemical characteristics of the drug. Wong *et al* (2002) encapsulated the water soluble sulphanilamide by the water in oil emulsion when Maestrelli *et al* (2008) entrapped the hydrophobic theophylline by the syringe droplet method. Many of these matrices are composed of polymers that are naturally degraded by the micro flora in the GIT where polysaccharides such as pectin and chitosan have been used. A variety of production methods have successfully produced particles composed of slow release matrices, where common methods are based on emulsions and spray drying (Vauthier *et al* 2008).

When at the colon site, the colonic fluids will penetrate into the matrices' and induce swelling. The incorporated drug diffuses out of the particle while the pectin is degraded by the colonic flora, though pores created by the penetrating colonic fluids. This rate can be controlled by processing the polymer in way that allows a controlled release rate. This is often preformed in many natural polymers by cross linking the polymer chains (with and without use of chemical cross linkers) from a random arrangement into an ordered arrangement, producing a denser structure allowing controlled release to be achieved (Chourasia *et al* 2003). A variety of synthetic and natural polymers can be used for slow release matrices. Ideally they should have low toxicities, give no inflammatory effects or

have any harmful by products. Also their mechanical features should be well suited for the indented application (Lakshmi *et al* 2007).

1.2.2 pH responsive polymers

Many matrice blends used for colonic delivery would instantly swell and release the incorporated drug in the upper GIT leaving virtually nothing by the time the system enters the colon. This problem can be overcome by coating the matrice with a polymer that dissolves when it enters a particular pH environment. These "smart" polymers that respond to the pH changes along the GIT can be used to trigger the drug release. Polymers are chosen by the pH at which they will begin to dissolve so they can target the core matrix to the right part of the body. Table 1.1 shows some common polymers used and their pH solubility thresholds (Siepmann *et al* 2007). Poly (methacrylates) known as Eudragits have been extensively used in colonic delivery. Eudragits with a pH solubility of pH 7 are ideal where Eudragit S100 has been extensively used.

Table 1.1 – pH solubility threshold of some common pH responsive polymers

Polymer	**pH Solubility**
Eudragit L 100	5.6
Eudragit S 100	7
Eudragit L 30D	5.5

Polymers with a threshold of pH 7 are ideal for colonic delivery since they will be resistant to the conditions of the upper gastrointestinal tract and will degrade upon entry to the colon allowing the full dose to be delivered at the colon site (Figure 1.2).

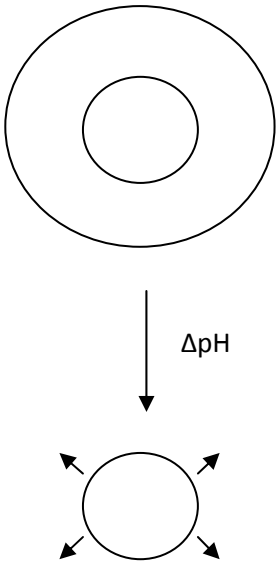

Figure 1.2 – Slow release matrice coated in a pH responsive polymer. The outer eudragit coating protects against the conditions of the lower GIT. Upon entry to the colon, the eudragit degrades, allowing the drug to diffuse though the inner matrice core.

1.3 Pectin

Pectin (Figure 1.3) is a polysaccharide that is obtained from the walls of some plants such as citrus fruit and apples. It is a fairly hydrophilic polymer that forms a gel in aqueous media (Luishu *et al* 2003) which has made it an idea polymer for drug delivery applications. Its various chemical characteristics are suited for a range of applications in colonic delivery.

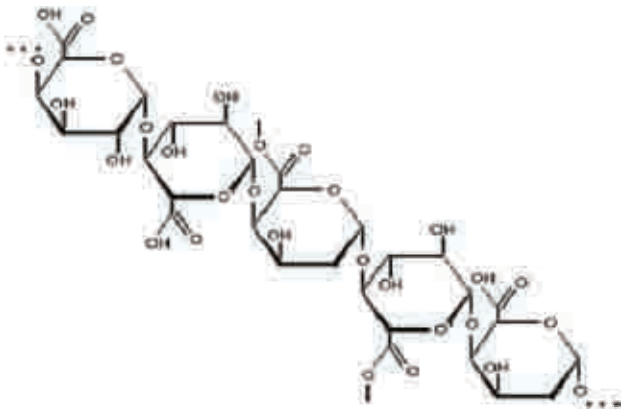

Figure 1.3 –Pectin

1.3.1 Degrees of esterfication and amidation

Pectin's characteristics are dependent on the number of carbonyl groups that are present in the molecule. Pectin is classed as having either a high degree of esterfication (DE) or a low DE expressed as a percentage. DEs below 50% esterfication are classed as low DE and those greater than 50% are high DE (Luishu *et al* 2003). Some of the carbonyl groups when in the presence of ammonia can be converted into amide groups producing amidated pectin. These types of pectin groups are known as having degrees of amidation (DA) expressed as a percentage. Both DE and DA determine the number of carbonyl groups in various pectin types, when low DE pectin and amidated pectin have more carbonyl groups than high DE pectin. This is an important characteristic to know since pectin with various DEs will be better suited for a given application in colonic delivery, where if the wrong type is used, it could have a significant impact on the performance of the delivery vehicle. For example high DE pectin is ideal to use in polymer coatings for a core drug since they are less water permeable and can be blended with other polymers (Sajeev *et al* 2009). Low DE pectin is ideal for slow release matrices since more carbonyl groups are present and can be cross linked with divalent ions to create the slow release matrices' mesh like structure (Wong *et al* 2002).

1.3.2 Pectin applications for colonic delivery

A range of particulate systems, produced from pectin, have shown great promise for colonic delivery. Slow release matrices have shown their potential in a variety of forms. Microspheres of high DE pectin were produced showing a sustained release of 5-Fluorouracil to the colon, where the pectin particles were coated with a Eudragit S100 coating, protecting the drug from premature release until the colon was reached (Paharia *et al* 2007). Also high DE pectin is often used as a coating to protect an inner drug core since it is less permeable making it ideal for controlled release coatings. Mcloed *et al* 1997 discussed that the pectin

coating could be enhanced, manipulating the coating's permeability using blends of pectin with other polymers such as ethyl cellulose and hydroxypropylmethyl cellulose for the coating application, where various release patterns could be achieved by altering the ratio of the polymer and pectin. Pectin systems have also been enhanced by cross linking the pectin chains with divalent ions, where calcium ions have been extensively used, to form calcium pectinate.

1.3.3 Calcium pectinate

Calcium pectinate is an attractive material for colonic delivery since its cross linked structure can encapsulate drug molecules allowing a sustained release to be achieved while still leaving the pectin liable to enzymatic degradation (Ashford *et al* 2004). This however is dependent on factors such as diet, age and can vary from person to person. Strong ionic bonds between the pectin's carbonyl groups and the Ca^{2+} ions are formed. This causes the pectin chains to form highly ordered mesh like structures though arrangements known as the "egg box" structure (Figure 1.4). This procedure is known as ionotropic gelation (Maestrelli *et al* 2008) where pectin and other polysaccharides have been frequently used. The strength of the calcium chloride used to cross link the pectin and the number of carbonyl groups in the pectin influences the permeability of the material and the release kinetics of an incorporated drug (Maestrelli *et al* 2008). The permeability of the pectin is highly reduced since the cross linked structure increases the pectin's density, due to the increase in order of the polymer chains. This in turn, reduces the rate which the colonic fluids penetrate into particle produced of calcium pectinate, allowing a controlled release of a drug to be achieved (Morris 1982 and Powell 1982).

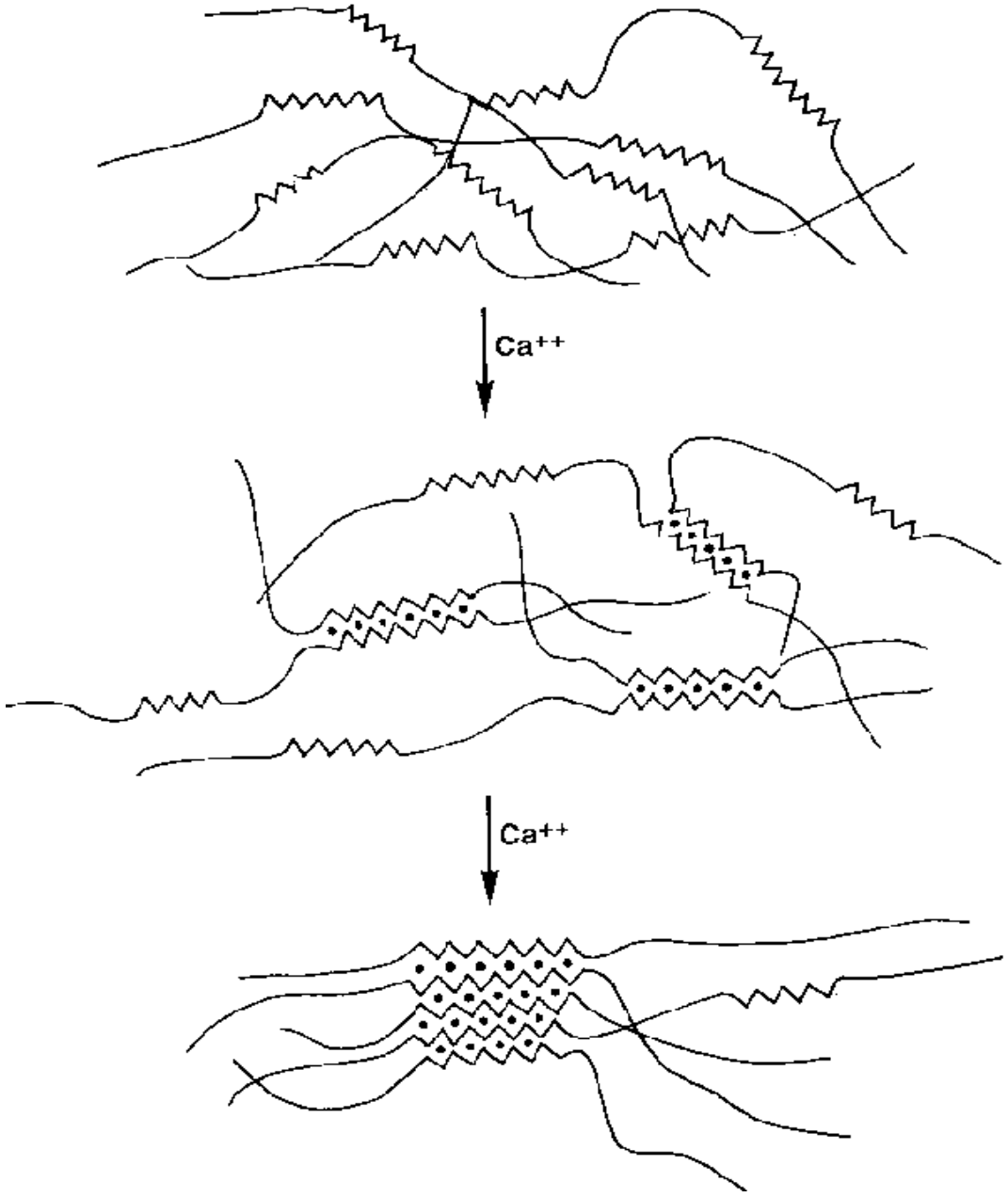

Figure 1.4 – Diagram of the egg box model. The random polymer chains in pectin which are crosslinked into more orderly packed structures forming an egg box like model.

Wong *et al* (2002) entrapped drugs of various physiochemical characteristics into low DE pectin microspheres cross linked with calcium ions producing calcium pectinate microspheres, capable of sustained release. These microspheres were coated with pH responsive polymers protecting the cores along the upper GIT, allowing the full dose to be release at a controlled rate. Maestrelli *et al* (2007), Surajit *et al* (2009) and Sriamornsak

(2008) also entrapped a range of drugs into larger calcium pectinate gel beads displaying a sustained release in systems simulating the colon. Production times for this method are as little as 20 minutes and hardening of the gel beads can be promoted by the addition of hardening agents such as polyethyleneimine (PEI) (Surajit *et al* 2006). Calcium pectinate has also shown its potential as a protective coating, where inner drug cores are coated in calcium pectinate by the compression coating method (Ashford *et al* 1993).

1.4 Emulsions

Emulsions are systems composed of a continuous phase, a dispersed phase and a stabiliser and are a common method for producing particles in drug delivery (Vauthier et al 2009). When two immiscible liquids are mixed, one phase (the dispersed phase) is distributed throughout the continuous phase in the form of small droplets which are thermodynamically unstable. A variety of types exist depending on the materials and the production process.

1.4.1 Types of emulsions

There are a variety of emulsions types. When an aqueous phase is dispersed into an oily continuous phase this is known as water in oil (w/o) emulsion. Likewise when an oily phase is dispersed into an aqueous continuous phase this is known as an oil in water emulsion (o/w). More complex emulsions know as multiple emulsions exist where oil droplets will contain smaller water droplets while being dispersed in a continuous aqueous phase, this is known as a water in oil in water (o/w/o) emulsion and likewise water in oil in water (w/o/w) emulsions exist.

1.4.2 Emulsion stabilisation

A variety of theories have been discussed to explain how stabilised emulsions are formed. One common theory is the surface tension theory (Billany 2002). When two immiscible liquids are mixed together one phase forms small droplets. The droplets have internal forces

that promote distortion of the droplets shape. Droplets can be attracted by van der Waals forces, when approaching they coalesce forming bigger droplets with a decrease in their internal force (Billany 2002). Addition of surfactants or wetting agents stabilise the emulsion system allowing the droplets to remain uniformly distributed in the continuous phase by adding an interfacial film which is absorbed into the surface of the droplets lowering their internal force preventing particle coalescence (Billany 2002).

Another theory is where the surfactant places itself around the droplets in a protective film at the interface of the oil and water. This film prevents particle coalescence where the tougher the film, the more stabilised emulsion is achieved (Billany 2002). Enough of a surfactant has to be introduced into the emulsion in order to keep the droplets uniformly dispersed throughout the continuous phase.

Chapter 2

Syringe droplet method

2.0 Introduction

The syringe droplet method was investigated for the production of calcium pectinate particles. The processing parameters of pectin concentration and mixer speed were investigated and their effects on particle shape were evaluated. The effects of calcium chloride concentration and cross linking time on particle swellability were also explored.

2.1 Materials

Pectin with a low degree of esterfication (<50%) was supplied as a gift sample by S Black Ltd, Herts, UK. Calcium chloride dihydrate was supplied by Fisher Scientific, Leicestershire, UK. Double distilled water was used to produce the pectin and calcium chloride solutions.

2.2 Methods

2.2.1 Particle production

The syringe droplet method (Figure 2.1) investigated by Maestrelli *et al* (2007), Surajit *et al* (2009) and Sriamornsak (1998) was used to produce calcium pectinate particles.

Pectin solutions were produced by adding 1.5 g and 2.5 g of pectin in its powder form to a 100 mL beaker and made up to 50 mL with double distilled water, producing two separate solutions of 3% w/v and 5% w/v respectively. The mixtures were stirred at 500 rpm over night using a magnetic mixer (Fisher Scientific FB15001) and magnetic flea with a diameter of 2.5 cm to ensure complete dissolution of the pectin.

Calcium chloride solutions were produced by adding 20 g, 40 g and 60 g of calcium chloride dihydrate in its solid form into beakers and making up to 200 mL with double distilled water and mixed with a 3.5 cm mixing flea producing three separate solutions of 10% w/v, 20% w/v and 30% w/v.

10 mL of the pectin solution was added drop wise though a 10 mL syringe fitted with an 21G needle under magnetic mixing at speeds of 500, 1000 and 1500 rpm into the 250 mL beaker containing 200 mL of calcium chloride solution.

Particles were produced instantly by ionotropic gelation and left to mix for 15 minutes. The mixture was removed from the magnetic mixer and left to cross link. Cross linking times of 20, 45, 90 and 180 minutes were investigated.

The particles were then collected by suction filtration on 1.2 µm filter paper (Fisher Scientific) and washed with 200 mL of double distilled water.

Particles were then left to dry overnight in a petri dish stored in an air circulated oven at 37 ºC. Particles were dated, labelled and then stored in an air tight sample vial for further studies.

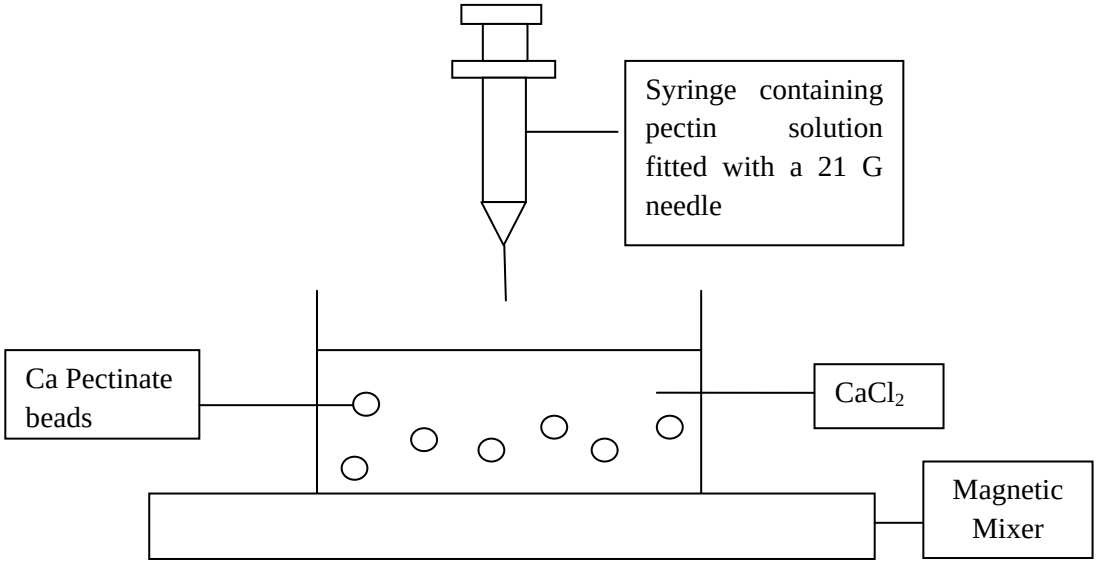

Figure 2.1 – Apparatus used for production of calcium pectinate particles produced by the syringe droplet method.

2.2.2 Particle characterisation

The dry particles' form and shape characteristics were characterised by scanning election microscopy (SEM) (JEOL 6060). Three beads were placed on a double adhesive disc stuck to a metal SEM stub. The beads were coated using a gold coater to protect the beads from the

election beam. Hydrated particles were analysed with a light microscope fitted with a digital camera (Olympus BH2). Three particles were placed on a viewing slide and several drops of water were added to hydrate the particles and they were analysed using a magnification of × 10.

2.2.3 Swellability

The particle swellability, expressed as a percentage was investigated as described by Dashora *et al* (2009) and Maestrelli *et al* (2007). 1 g of dry particles were left to swell in 50 mL of double distilled water for 3 minutes. The particles were collected, weighed (4 decimal places) and the swelling percentage was found by the following;

$$S = (W_1 - W_i) / W_i \times 100 \qquad (2.1)$$

Where S is the swell percentage, W_i is the initial weight of particles and W_1 the hydrated weight of the particles. The swelling percentage was found using independent triplicate batches and a mean value was determined.

2.3 Results and discussion

The SEM images of the particles produced with 3% w/v and the 5% w/v pectin solutions are shown in Figures 2.2a and 2.2b respectively and the light microscopy images of the particles are shown in Figures 2.3a and 2.3b. Each of the figures shows the effects of the various calcium chloride strengths and mixing speeds that were investigated.

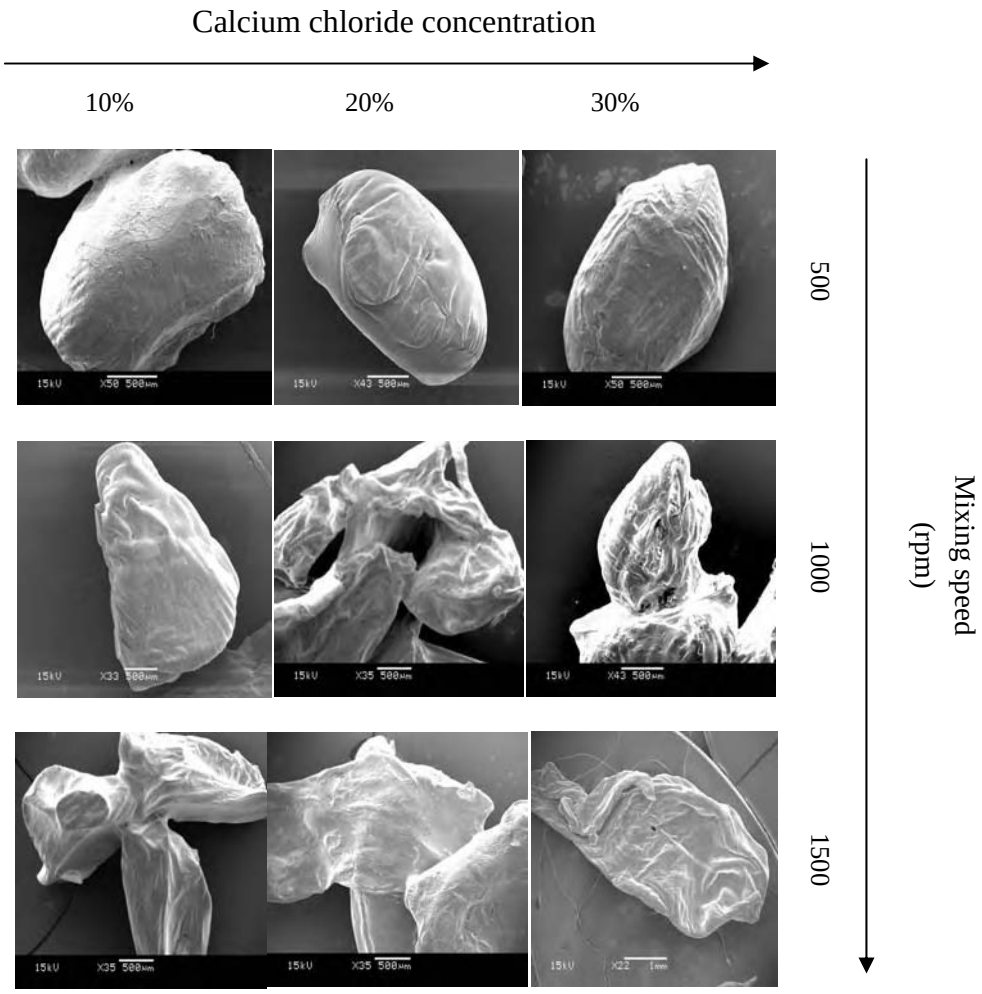

Figure 2.2a – SEM images of dry pectin particles produced from the 3% w/v pectin solution. The effects of the calcium chloride concentration (10% left, 20% middle and 30% right) and mixing speed (500 rpm top, 1000 rpm centre and 1500 rpm bottom) on the particle shape characteristics can be seen.

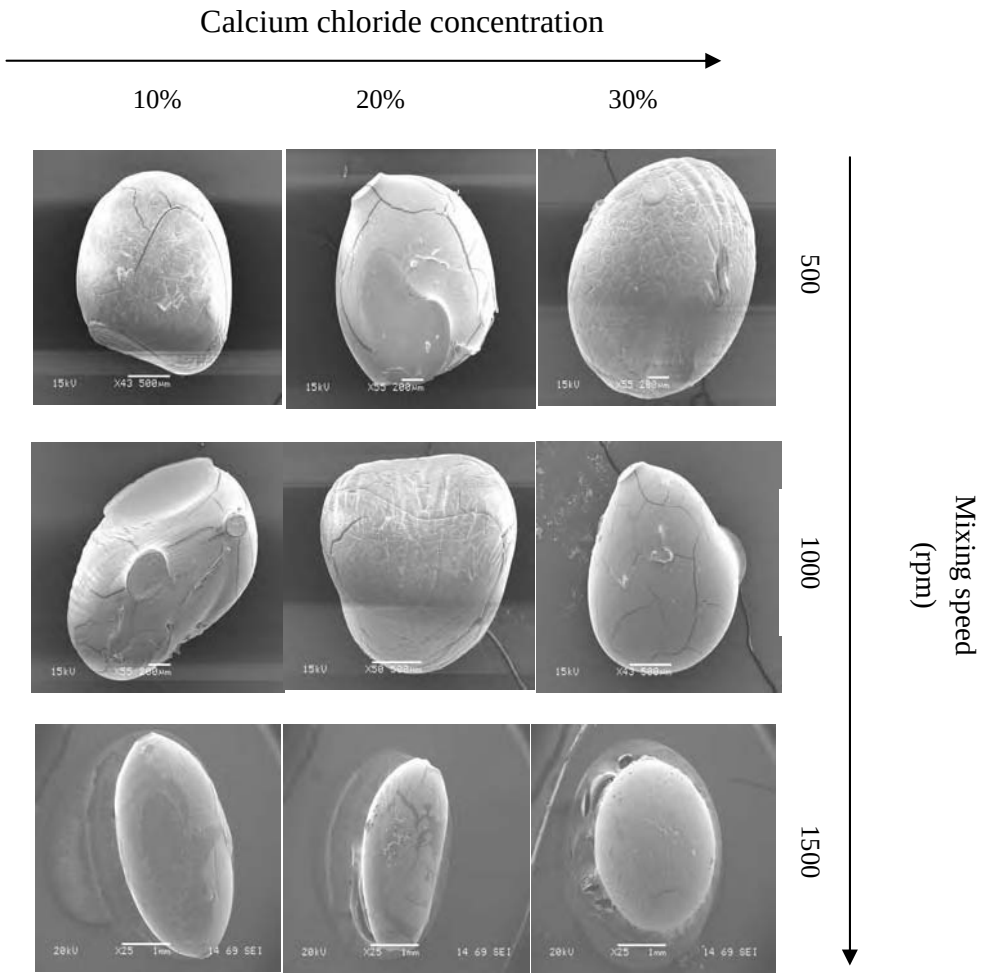

Figure 2.2b – SEM images of dry pectin particles produced from the 5% w/v pectin solution. The effects of the calcium chloride concentration (10% left, 20% middle and 30% right) and mixing speed (500 rpm top, 1000 rpm centre and 1500 rpm bottom) on particle shape characteristics can been seen.

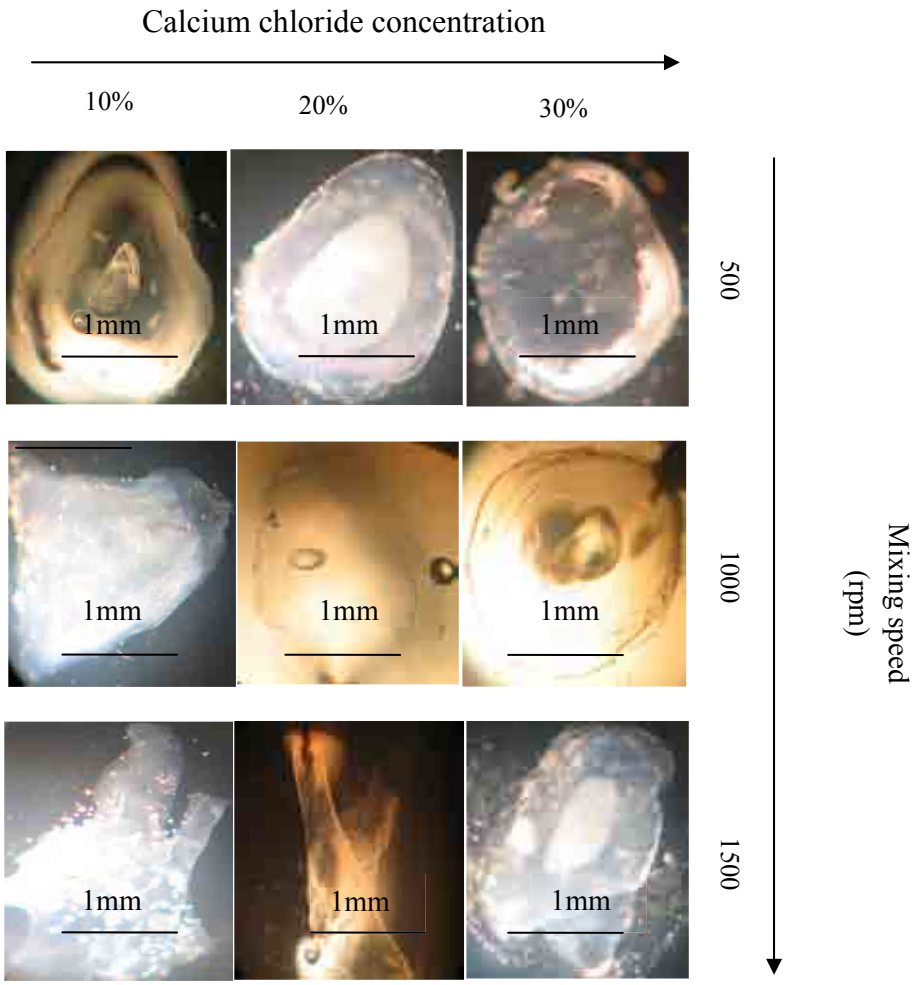

Figure 2.3a – Light microscopy images of hydrated pectin particles produced from the 3% w/v pectin solution. The effects of the calcium chloride concentration (10% left, 20% middle and 30% right) and mixing speed (500 rpm top, 1000 rpm centre and 1500 rpm bottom) on particle shape characteristics can be seen.

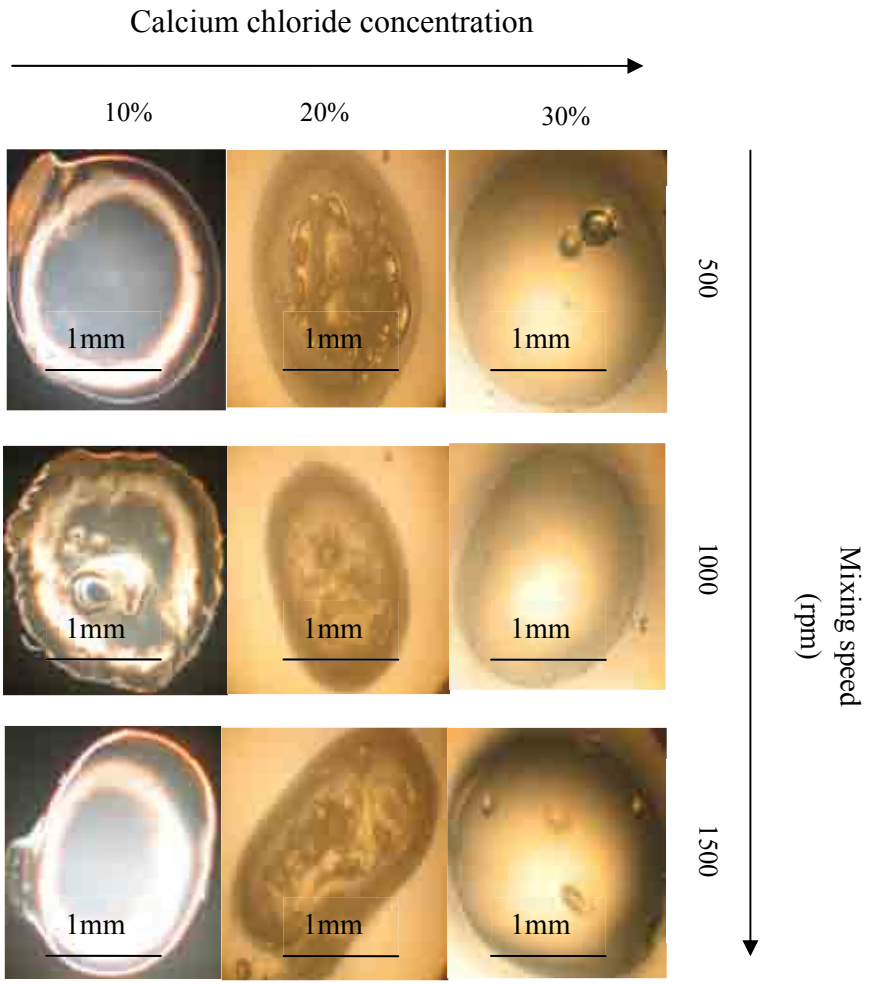

Figure 2.3b – Light microscopy images of hydrated pectin particles produced from the 5% w/v pectin solution. The effects of the calcium chloride concentration (10% left, 20% middle and 30% right) and mixing speed (500 rpm top, 1000 rpm centre and 1500 rpm bottom) on particle shape characteristics can be seen.

2.3.1 Effect of pectin concentration on particle formation

The concentration of the pectin solution had a distinct effect on the form of the particles that were produced. The particles that were produced with the 3% w/v pectin solution only gave spherical particles at stirring speeds of 500 rpm. Stirring speeds above 500 rpm gave irregularly shaped pectin products while the particles produced with the 5% w/v pectin solution gave particles with a more defined spherical geometry over the range of investigated parameters.

The 3% w/v pectin solutions had a lower viscosity and density. Any particles which begin to form can be deformed by the shear stresses created during the mixing process. Particles produced with the 5% w/v pectin solutions had a higher viscosity and density and were able to keep the spherical geometry when exposed to the stresses of the mixing process.

Maestrelli *et al* (2007), Surajit *et al* (2009) and Sriamornsak (1998) have discussed the effect of pectin concentration in the production of calcium pectinate particles however none of the authors have used a concentration less than 5% w/v. Concentrations in their studies varied from 5% to 8% w/v and the spherical particles of approximately 1.7mm in diameter were produced.

It is believed that no other author has previously used solutions of 3% w/v in the syringe droplet method, it was therefore hypothesised that the cause of the deformation in the 3% pectin particles was because the pectin's carbonyl groups and the calcium ions do not form as strong ionic bonds compared to the 5% w/v solutions. This leaves the pectin's polymer chains with lower degrees of order and therefore with lower densities making the particles vulnerable to deformation at high mixing speeds. Maestrelli *et al* (2007) also noticed that the type of pectin can affect particle shape characteristics, where various ranges of low DE pectin (20%, 30% and 40%) were investigated.

2.3.2 Effect of calcium chloride concentration on particle formation

The calcium chloride's function was to act as the cross linker between the pectin's carbonyl groups and the calcium ions forming the solid particles with the "egg box" structures. The increase in calcium chloride concentration produced particles with higher degrees of sphericity when particles were produced with the 5% w/v pectin solution. However the 3% pectin solutions gave distorted particles over the range of calcium chloride concentrations.

The effect of the calcium chloride on the particles form, which has been discussed by Maestrelli *et al* (2007), was attributed to the efficiency of the ionic bonds produced between the pectin carbonyl groups and the calcium ions producing particles with the "egg box" structures. The higher concentrations of calcium chloride were able to produce strong ionic bonds resulting in beads with high degrees of sphericity which decreased with the weaker calcium chloride concentrations.

Maestrelli *et al* (2007) investigated a range of calcium chloride concentrations from 2.5% to 30% and saw that solutions of 2.5% gave noodle like structures when solutions of 20% gave highly spherical beads of approximately 1.7mm in size.

2.3.3 Effect of calcium chloride concentration on swellability

The increase in calcium chloride concentration reduced the pectin's ability to swell as shown by the particle swellability percentage (Tables 2.1a and 2.1b).

By increasing the calcium chloride concentration, the degree of cross linking in the particles increases which reduces the amount of water that can penetrate into the particle. The higher degrees of cross linking produced particles with denser structures that reduce the permeability of the particle giving the decreasing trend in particle swellability.

It is ideal to have particles with high degrees of cross linking since this will prevent colonic fluids rapidly swelling the pectin particle which would lead to a rapid release rate of an incorporated drug.

The swellability percentage of the particle provides a numerical value that illustrates the swellability for particles produced with different calcium chloride concentrations.

Maestrelli *et al* (2007) have discussed the effects of calcium chloride concentration on particle swellability. The increase of calcium chloride concentration promotes the ionic bonding leading to denser particles where swelling percentages of 90%, 80% and 70% were found for calcium chloride concentrations of 10% 20% and 30% respectively.

Surajit *et al* (2006) have shown the effects on cross linking time and their effects on the permeability on the pectin microspheres. Longer periods of time lead to a slightly higher degree of cross linking, however the investigated cross linking times in this study had no extra effects on the swelling of the particles.

Table 2.1a – Swellability percentages of particles produced with 3% w/v pectin. The percentage decreases with increasing calcium chloride concentration since less water penetrates into the sphere. The cross link time had no effect on the percentage. Each value represents the mean from three independent studies ± standard deviation.

	Calcium Chloride Concentration		
Cross linking (mins)	10%	20%	30%
0	78 ±1.30	60 ±1.53	40 ±1.56
20	78 ±1.42	59 ±1.37	39 ±1.33
45	75 ±1.67	58 ±1.86	37 ±1.21
90	74 ±1.54	57 ±1.42	37±1.47
180	73 ±1.29	57 ±1.97	37 ±1.86

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Table 2.1b – Swellability percentages of particles produced with 5% w/v pectin. The percentage decreases with increasing calcium chloride concentration since less water penetrates into the sphere. The cross link time had no effect on the percentage. Each value represents the mean from three independent studies ± standard deviation.

	Calcium Chloride Concentration		
Cross linking (mins)	10%	20%	30%
0	81 ±1.43	60 ±1.54	40 ±1.63
20	78 ±1.76	59 ±1.87	39 ±1.78
45	77 ±1.53	58 ±1.21	37 ±1.18
90	76 ±1.64	57 ±1.15	35 ±1.29
180	75 ±1.38	57 ±1.24	35 ±1.60

2.3.4 Effect of mixing speed

The increase in mixing speed gave a distortion in the particle's form due to the increased shearing produced in the mixing process. With the increasing velocity, the flow becomes turbulent where the fluid flows in irregular motions producing higher degrees of agitation which is believed to deform the particles (Twitchell 2002). Maestrelli *et al* (2007), Surajit *et al* (2009) and Sriamornsak (1998) all produced particles operating at "gentle" mixing speeds but do not state a specific speed or the type of mixer used. It is believed that no other author has discussed the effects on mixing speeds above 500 rpm on the formation of the calcium pectinate particles produced by the syringe droplet method.

Since the solid particle forms instantly though ionotropic gelation upon entry into the calcium chloride, the size of the particles is completely dependent on the diameter of the needle from which the pectin solution is being added drop wise into the calcium chloride solution. Various needle sizes were investigated to reduce the droplet size; however, the pectin solution was too viscous to squeeze though smaller needles. Pectin solutions less than 1% may be possible; however since pectin concentrations of 5% w/v are needed to obtain spherical particles, this low concentration would lead to deformed particles being produced.

2.4 Conclusion

Calcium pectinate particles were produced using the syringe droplet method. The effects of pectin concentration, calcium chloride concentration, cross linking time and mixing speed were investigated. High concentrations of pectin and calcium chloride were ideal to give spherical particles and low mixing speeds were needed during the production process to avoid the shear stress from deforming the pectin particles though turbulent flow. Spherical particles are ideal since they are more elegant. The Particles would also be likely to undergo a coating stage, where spherical particles would be easier to coat. Cross linking times had no effect on the swellability percentage however the swellability was strongly affected by the change in calcium chloride concentration.

In some respects this method is ideal for the production of particles for pharmaceutical use since only pectin, calcium chloride and double distilled water are required for their production and the method avoided the use of environmentally hazardous chemicals. Also the particles were produced instantly giving short production times which would be more economical for larger scales.

The disadvantages were that the method was limited to the production of relatively large particles where alteration of particle size was limited. Multi particulate systems on the micro/nano scale are ideal in colonic delivery since they would be uniformly distributed throughout the colon avoiding irregular absorption of a drug.

Chapter 3

Water in oil emulsion method

3.0 Introduction

A method for the production of the calcium pectinate particles using a water in oil emulsion was investigated for producing the particles in the micron size range. The processing parameters of surfactant concentration and mixing speed were investigated showing their effects on particle size. The effect of calcium chloride concentration on particle swellability was also explored.

3.1 Materials

Pectin of a low DE (< 50%) was obtained as a gift sample from S Black Ltd, Herts, UK. Isooctane and calcium chloride where supplied by Fisher Scientific, Leicestershire, UK. Span 85 and Tween 85 were supplied by Sigma Aldrich, UK. Double distilled water was used to produce all aqueous solutions.

3.2 Methods

3.2.1 Original particle production method from the literature

The following method was described by Wong *et al* (2002) and the first to be investigated. 1.5 g of pectin in a powder form was dissolved in a 100 mL beaker and made up to 50 mL with double distilled water and mixed over night on a magnetic mixer (Fisher Scientific FB15001) using a 2.5 cm flea at 500 rpm producing a 50 mL 3% w/v solution.

This 50 mL solution was added drop wise in five 10 mL portions though a syringe fitted with an 18G needle into a 250 mL beaker containing 75 mL of isooctane, which contained 1.75 g of Span 85, under continuous mixing at 1000 rpm for 10 minutes. The 18G needle was chosen since it had a small diameter but still allowed the pectin to be dispersed with ease.

After the tenth minute 5 mL of an aqueous solution containing 1.8 g of Tween 85 was added through another 18G needle and mixed for 5 minutes.

After the fifth minute 20 mL of calcium chloride solution 30% w/v was added through a third 18G needle to cross link the pectin particles and mixed for a further 10 minutes.

The cross linked particles were collected by suction filtration on 1.2 µm filter paper (Fisher Scientific) and washed with 200 mL of double distilled water. The microspheres were then dried in an air circulated oven in a petri dish at 37 ºC overnight and stored in air tight sample vials.

The method of Wong *et al* (2002) was unsuccessful and therefore further method development studies were carried out.

3.2.2 Exploring suitability of magnetic mixing on particle formation

The method by Wong *et al* (2002) was performed on a magnetic mixer at mixing speeds of 1000 rpm.

3.2.3 Exploring the effect of cross linking time on particle formation

Before the particles were filtered, they were left to cross link for periods of 20, 45, and 90 and 180 minutes as investigated by Maestrelli *et al* (2007) in the syringe droplet method. After this cross linking time the particles were collected and dried.

3.2.4 Exploring the suitability of overhead mixing on particle formation

The effect of mixer type was investigated by utilising an over head axial flow down pumping stirrer with an impeller diameter of 3.5 cm (Fisher Scientific IKARW 20). The quantities of chemicals from the method described by Wong e*t al* (2002) had to be doubled to give a sufficient volume to produce particles with this stirrer. The production times were also doubled.

3.2.5 Exploring the effect of emulsifier concentration on emulsion stability

The Span 85 was increased from 1.7 g to 10 g giving a concentration of 5% w/v.

3.2.6 Optimised method for particle production

Particles were produced using an over head mechanical stirrer (Figure 3.1) with some modifications to the method described by Wong *et al* (2002).

50 mL of the 3% w/v pectin solution was dispersed into a 400 mL beaker with 200 mL of isooctane containing 10 g of Span 85 and mixed for 10 minutes at 1000 rpm with an impeller with a 3.5 cm diameter. After this time 10 mL of an aqueous solution containing 2 g of Tween 85 was added and mixed for 3 minutes. Then 20 mL of calcium chloride solution was added to cross link the pectin and mixed for 5 minutes.

The emulsions were left for 1.5 hours to cross link after which they were collected by suction filtration, washed with 50 mL of double distilled water and dried in an air circulated oven at 37 ºC overnight in a petri dish.

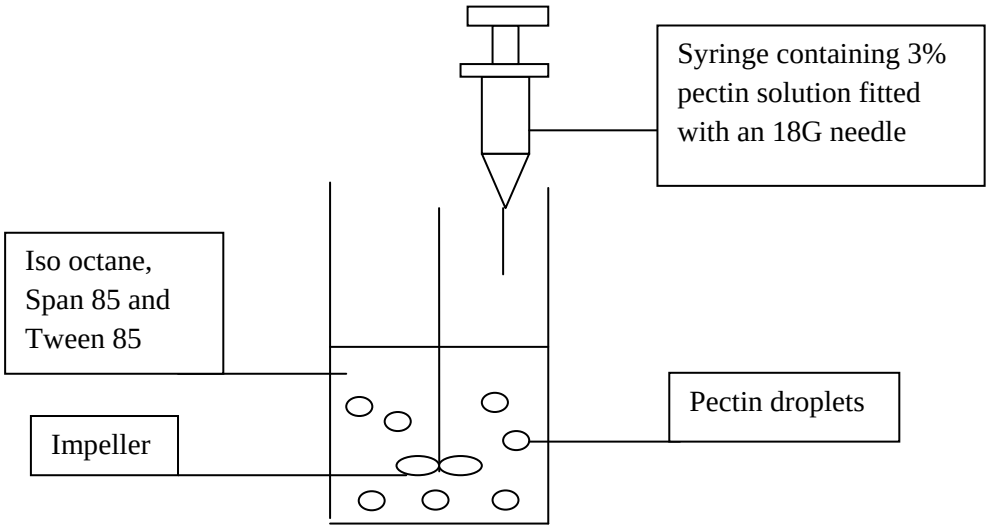

Figure 3.1 – Apparatus for the water in oil emulsion method for particle production. The emulsion system comprised isooctane as the continuous phase, Span 85 and Tween 85 as the emulsifier and pectin as the dispersed phase.

3.2.7 Particle characterisation

The dry particles' size and shape were characterised by scanning electron microscopy (SEM) (JEOL 6060). A small amount of dry particles were placed onto a double adhesive SEM disc stuck to a metal SEM stub. The particles were coated using a gold SEM coater to protect the particles from the electron beam.

Hydrated Particles were analysed with a light microscope fitted with a digital camera (Olympus BH2). A small mass of particles were placed onto a viewing slide then a few drops of water were added to hydrate the particles. The particles were analysed with × 200 magnification.

The average size and size distribution were analysed with laser diffraction particle sizing (Mastersizer 2000). The sizer sample chamber was cleaned with double distilled water and particles were added in their dry powdered form into the sample chamber under mixing at 2000 rpm. Five readings were recorded for each formulation to calculate the average particle diameter along with the size distribution curve.

The particles were also sized by suspending them in a solution of Tween 85 before sizing. 100 mg of microspheres were suspended into 5 mL of a 5% w/v Tween 85 solution and added into the sample chamber drop wise though a pipette under mixing at 2000 rpm. Five readings were run for each formulation. The average particle diameter along with the size distribution curve was obtained.

3.2.8 Swellability percentage

The swellability percentage, investigated by Surajit *et al* (2009), was used to investigate the swellability of the particles by finding the mass of water lost from the particles during drying. Before the particles were dried during production, 100 mg of the particles were weighed onto a petri dish and dried overnight. The weight of the dry particles was recorded and the swellability was found by;

$$S = (W_i - W_1) / W_i \qquad (3.1)$$

Where S is the swellability, W_i is the initial weight of particles before drying and W_1 the weight of the dried particles.

This method was chosen instead of the particle swelling method described in the syringe droplet method chapter since it would be difficult to ensure all full mass of spheres were recovered after swelling in water due to their small size.

3.3 Results and discussion

3.3.1 Exploring the suitability of magnetic mixing on particle formation

The initial method described by Wong *et al* (2002) was performed under magnetic mixing to produce the calcium pectin particles. The product was an indiscreet mass of calcium pectinate with no particulate products (Figure 3.1).

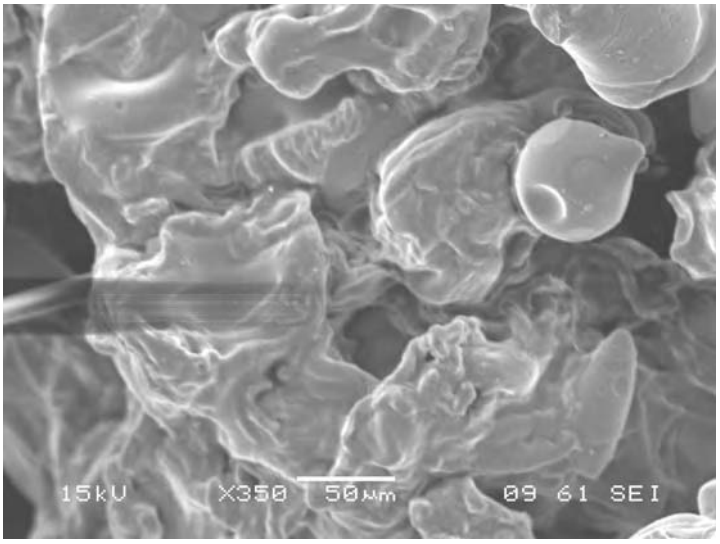

Figure 3.2 – SEM of the calcium pectinate product produced using the magnetic mixer. The product obtained was an indiscreet mass of polymer with no particulate products.

At this early stage the unsuccessful production attempt producing the indiscreet pectin product could have been for a number of reasons. Firstly the particles could not be receiving the sufficient amount of cross linking time in order to form the pectin droplets into solid particles. Also the magnetic mixer could not be giving the necessary shearing forces in order to produce spherical droplets. Lastly, the emulsion could have an insufficient level of

stabilisation in order to keep the droplets form coalescing. These findings were investigated and discussed further in the following sections.

3.3.2 Exploring the effect of cross linking time on particle formation

Before the separation process, the emulsions were left on light mixing to allow the particles to cross link further. Cross linking times of 20, 45, 90 and 180 minutes were investigated. Cross linking times below 90 minutes were unable to produce spherical particles (Figure 3.3a). However cross linking times of 90 and 180 minutes gave some spherical products (Figure 3.3b).

Once dispersed into the isooctane and mixed, the immiscible pectin forms small droplets throughout the isooctane. When the calcium chloride solution is added, the calcium ions cross link the pectin chains forming the pectin droplets into solid calcium pectinate particles.

It is hypothesised that the pectin droplets do not completely cross link in the 10 minute mixing period once the calcium chloride is added. When left for 90 minutes under gentle mixing, the additional cross linking time allows the pectin's carbonyl groups and calcium ions to form the strong ionic bonds forming the egg box structures. This yields the solid calcium pectinate products with some spherical geometries.

Although the products gained had some degree of sphericity, they were not a uniform population of individual spheres. This would not be ideal since they will have to undergo a coating process and pharmaceutical products should be uniform in nature.

Maestrelli *et al* (2007) have investigated the effects of cross linking time on calcium pectinate particles produced with the syringe droplet method. However it is believed that no other author has repeated the effect of this parameter in the context of an emulsion method since they claimed that their methods gave spherical products without the additional cross linking times.

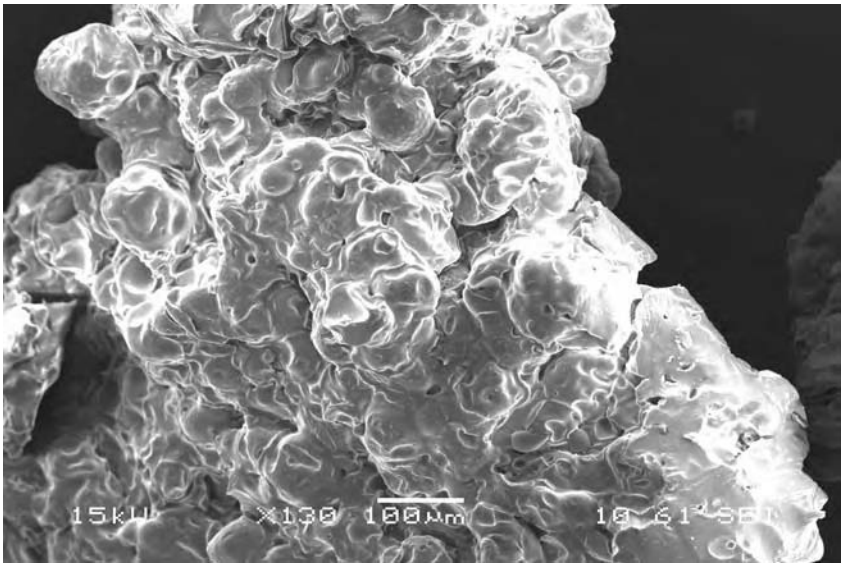

Figure 3.3a – Effect of cross linking time of the calcium pectinate. Cross linking times of 20 and 45 minutes were not sufficient to give spherical particles.

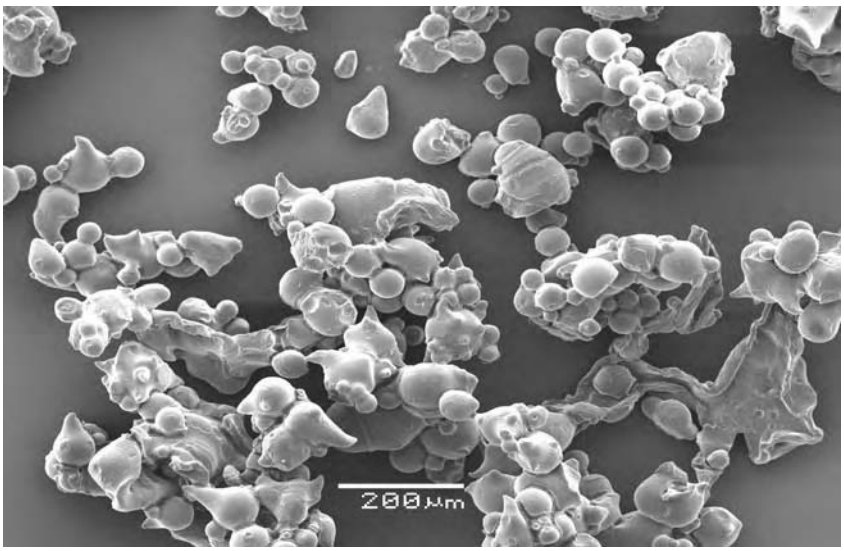

Figure 3.3b – Effect of cross linking time of the calcium pectinate. Cross linking times of 90 and 180 minutes were sufficient to give the structures required to form solid particles.

3.3.3 Exploring the suitability of overhead mixing on particle formation

An overhead mechanical stirrer was used to produce the particles giving better agitation and prevented the beaker from falling over at higher mixing speeds. In order to achieve a satisfactory emulsion volume to produce the particles the reagent volumes and mixing times were doubled. As the 100 mL of pectin was added, the emulsion became rather thick

compared to previous batches and the product obtained was a thick white paste with no spherical products (Figure 3.4).

Figure 3.4 – Photograph of the product that was obtained using 100 mL of pectin solution. The product was a creamy paste.

The pectin was reduced back to the initial 50 mL volume to produce the particles. Once these were collected, the product gained was a population of particles that were in an agglomerate form (Figure 3.5).

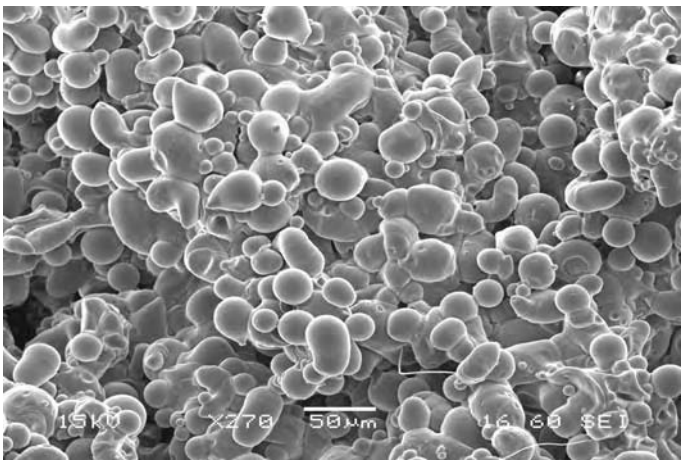

Figure 3.5 – SEM image of the dried particles. Spherical products were in an agglomerate form.

The particles produced with the overhead mixer saw that particulate products were being produced due to the increase in agitation. Magnetic mixing provided a crude method of production where it was believed that it did not provide the efficient level of shearing to

produce spherical particles. When greater levels of shear are needed, overhead mixers and homogenisers can be better suited to the mixing process (Twitchell 2002). The effect on mixer type is a concept that is not often discussed in the literature; however this concept will go in to further discussion in a later section.

3.3.4 Exploring the suitability of surfactant concentration on particle form

The particles produced were in an agglomerate form which was most likely due to inefficient level of stabilisation in the emulsion. All batches produced so far were done using 1% w/v of Span 85 as the stabiliser in the isooctane. This was increased to 5% w/v. With this emulsifier increase the products gained were a population of spheres with high degrees of sphericity (Figure 3.6). Span 85 was used since it is the ideal emulsifier for water in oil emulsions (hlb 1.8) (Wong *et al* 2002).

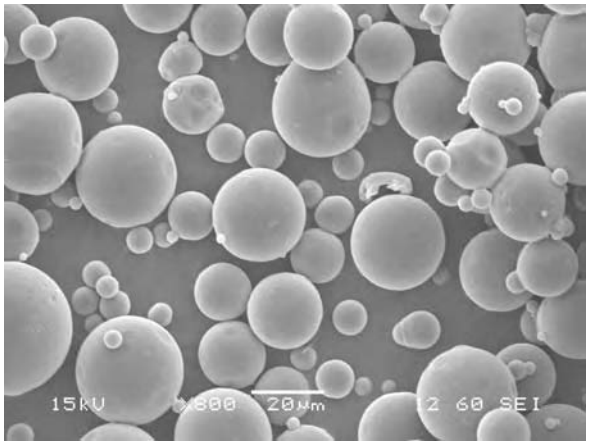

Figure 3.6 – SEM showing the effect of increasing the surfactant concentration to 5%. This gave the emulsions the required level of stabilisation in order to give individual spherical particles.

This increase in surfactant concentration provided the emulsions with the sufficient level of stabilisation in order to keep the droplets individually dispersed throughout the isooctane (to be discussed in the next section). It is believed that no other author has discussed the use of a surfactant concentration as high as 5% w/v when using water in oil emulsion to prepare calcium pectinate microspheres.

So far the studies allowed a standardised particle production method to be produced where the surfactant concentration and level of agitation had the biggest effects on the formation of the pectin particles. The next stage would be to undergo further investigation into the key processing parameters of surfactant concentration, mixing speed and calcium chloride concentration. The parameters investigated were;

- Surfactant concentration (Span 85) – 1%, 3% and 5% w/v
- Calcium chloride concentration – 10%, 20% and 30% w/v
- Mixing speed – 500, 1000 and 1500 rpm

Particles that were produced were analysed with SEM (Figure 3.7a - c) and light microscopy (Figure 3.8a - c).

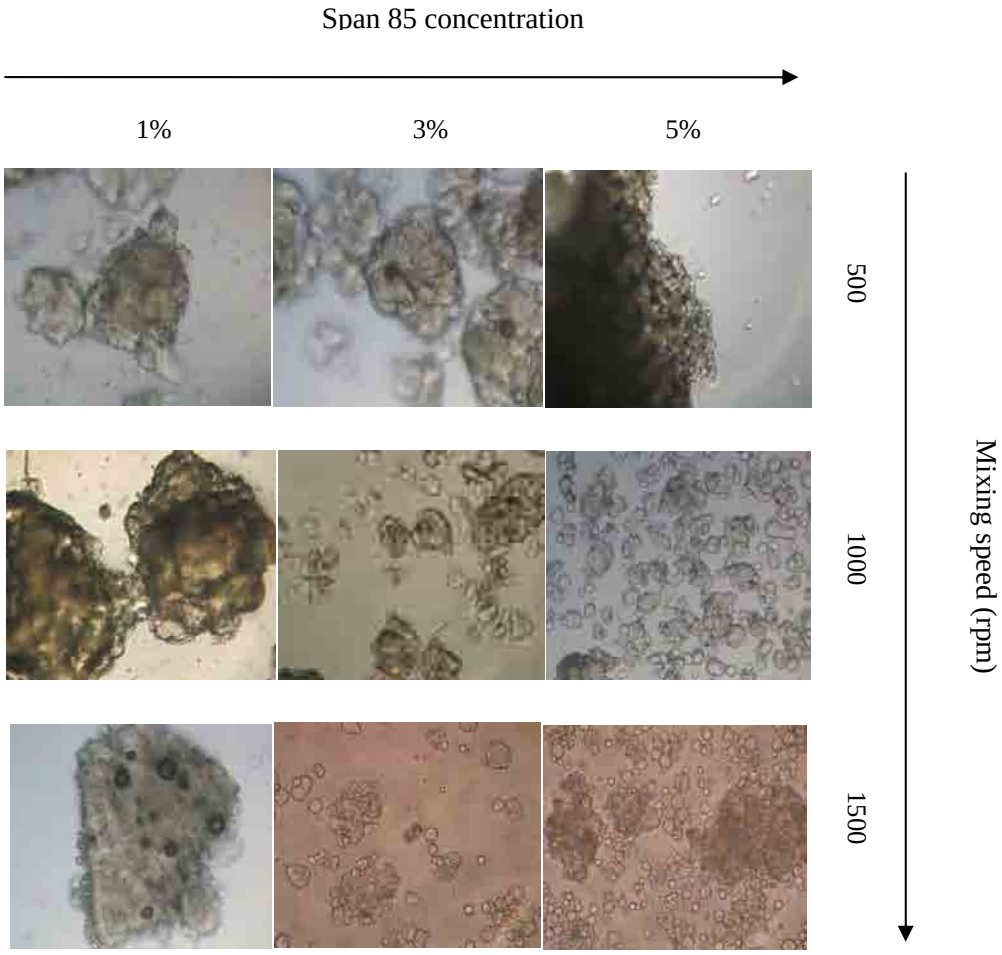

Figure 3.7 a – Light microscopy images of particles produced with 10% calcium chloride solution showing the effect of Span 85 concentrations of 1%, 3% and 5% (left, middle and right) and mixing speeds of 500, 1000 and 1500 rpm (top, centre and bottom) on the particles' size and form.

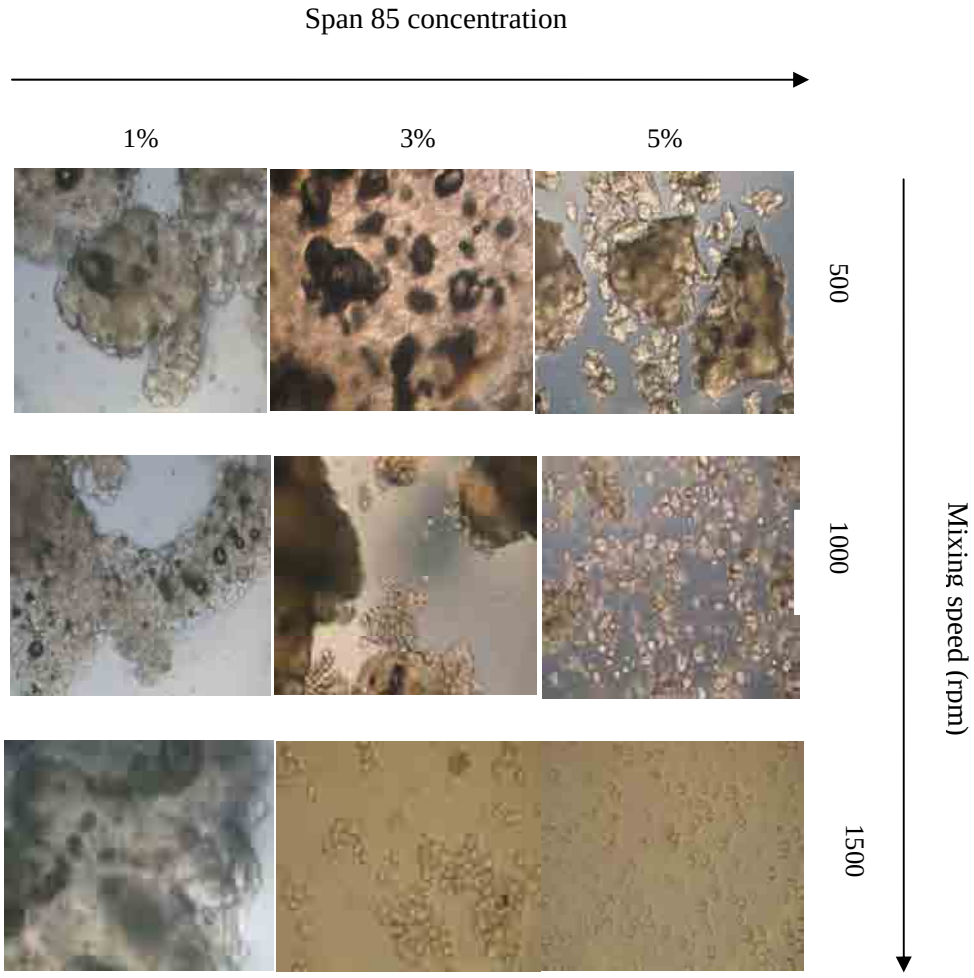

Figure 3.7 b – Light microscopy images of particles produced with 20% calcium chloride solution showing the effect of Span 85 concentrations of 1%, 3% and 5% (left, middle and right) and mixing speeds of 500, 1000 and 1500 rpm (top, centre and bottom) on the particles' size and form.

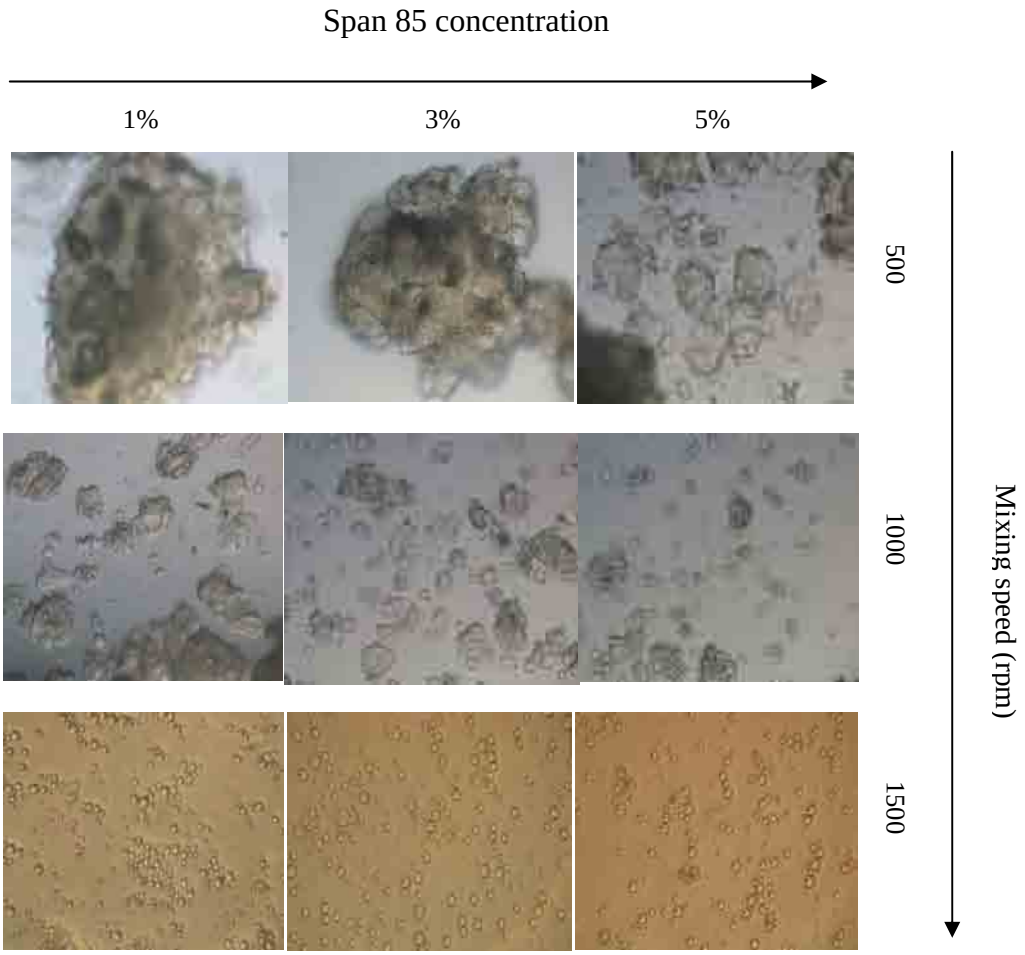

Figure 3.7 c – Light microscopy images of particles produced with 30% calcium chloride solution showing the effect of Span 85 concentrations of 1%, 3% and 5% (left, middle and right) and mixing speeds of 500, 1000 and 1500 rpm (top, centre and bottom) on the particles' size and form.

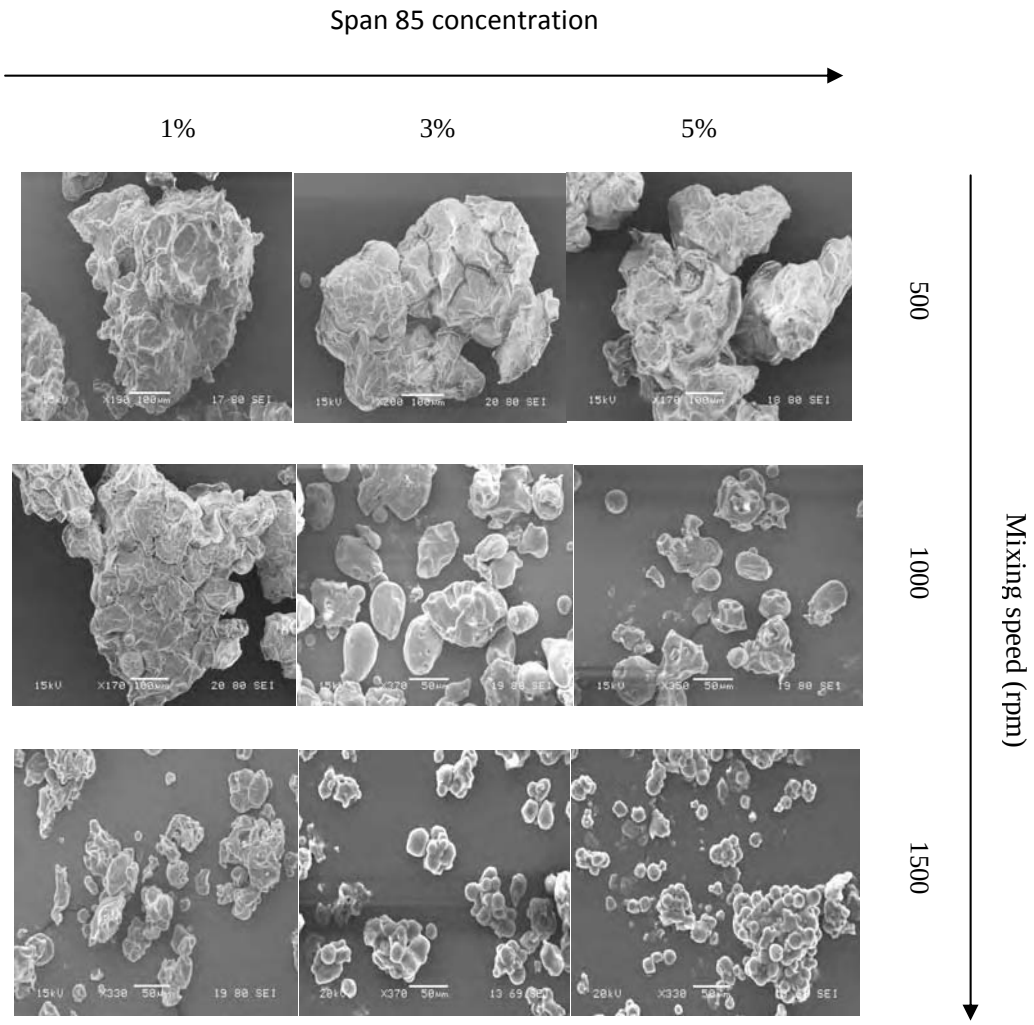

Figure 3.8 a SEM images of particles produced with 10% calcium chloride solution showing the effect of Span 85 concentrations of 1%, 3% and 5% (left, middle and right) and mixing speeds of 500, 1000 and 1500 rpm (top, centre and bottom) on the particles' size and form.

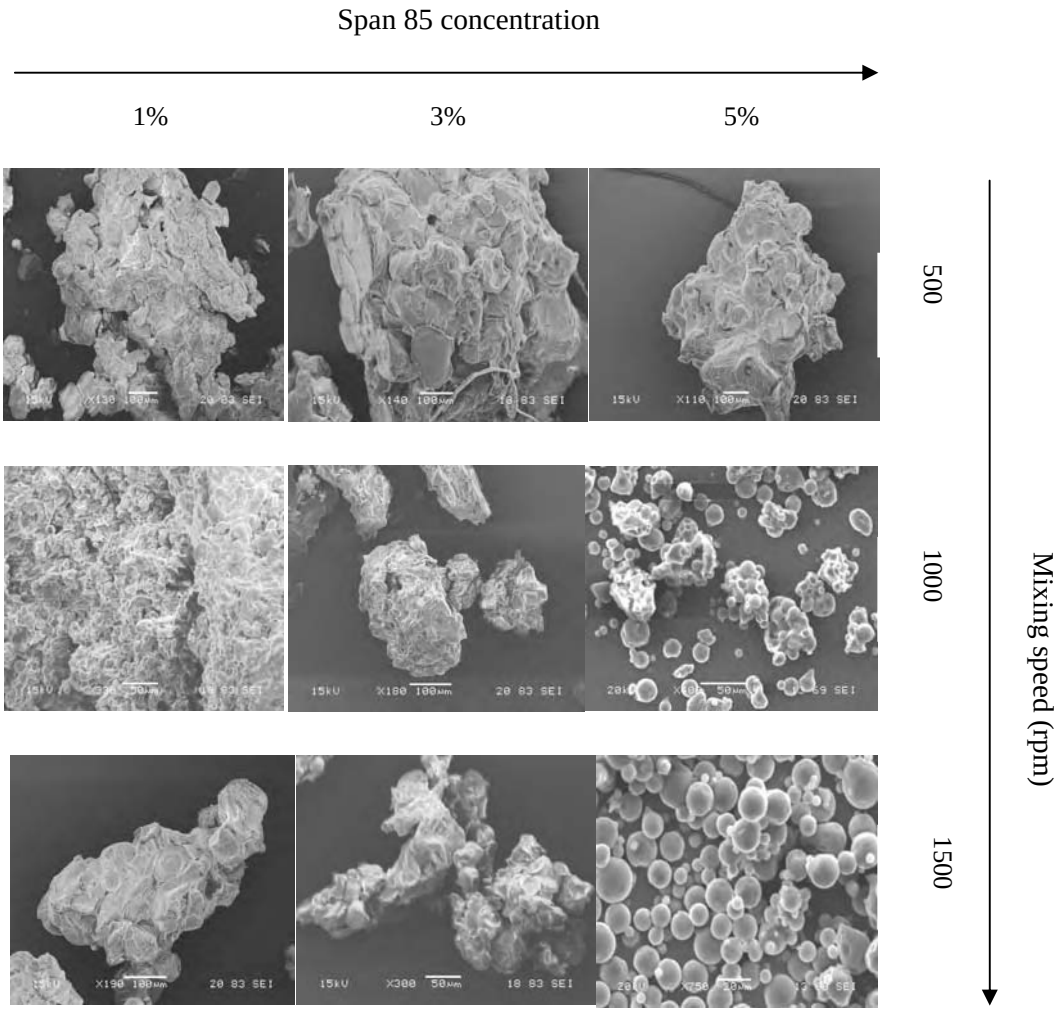

Figure 3.8 b SEM images of particles produced with 20% calcium chloride solution showing the effect of Span 85 concentrations of 1%, 3% and 5% (left, middle and right) and mixing speeds of 500, 1000 and 1500 rpm (top, centre and bottom) on the particles' size and form.

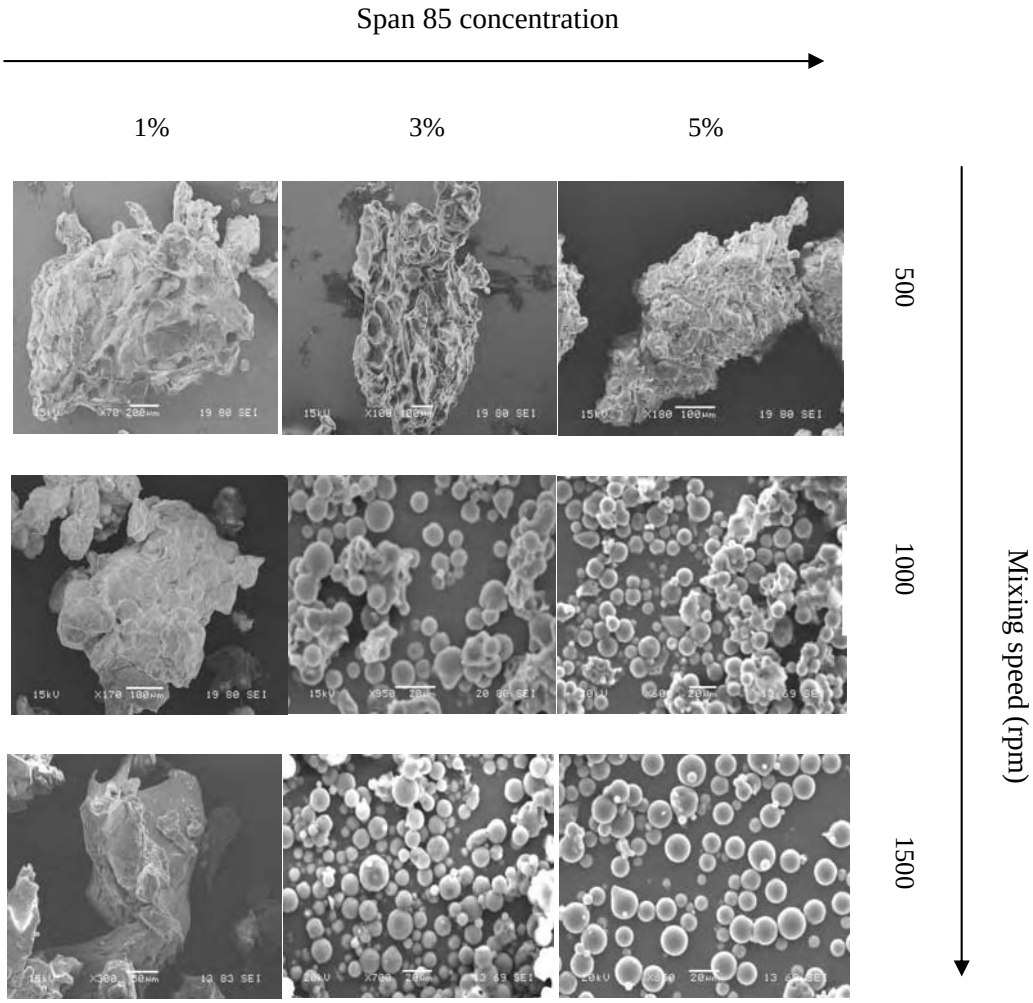

Figure 3.8 c SEM images of particles produced with 30% calcium chloride solution showing the effect of Span 85 concentrations of 1%, 3% and 5% (left, middle and right) and mixing speeds of 500, 1000 and 1500 rpm (top, centre and bottom) on the particles' size and form.

3.3.5 Effect of surfactant concentration on particle formation

The concentration of Span 85 used in the emulsions had a distinct effect on the morphology of the particles that were produced. Concentrations of 1% w/v gave indiscreet pectin forms with no particulate products. Concentrations of 3% w/v gave some spherical particles however most were distorted in shape. Concentrations of 5% w/v gave highly defined spherical particles where the populations were individually distributed. (Figures 3.7 and 3.8)

The Span 85 acted as the stabiliser between the pectin droplets and the isooctane enabling the droplets to stay uniformly dispersed throughout the emulsion. When two immiscible liquids are mixed the dispersed phase forms small droplets and the system becomes thermodynamically unstable (Billany 2002). The addition of the Span 85 adds a repulsive force between pectin droplets and the isooctane phase and avoids the particles from approaching each other and coalescing as described by the internal film theory. Without any form of stabilisation the droplets will be attracted by van der Waals forces forming into bigger droplets until both liquids return to their individual states.

Wong *et al* (2002), Paharia *et al* (2006) and Esposito *et al* (2001) have suggested using 0.5% - 1.75% w/v of Span 85 in the isooctane and have reported gaining spherical pectin microspheres in a size range of 50 to 110 microns in diameter. These concentrations when investigated were unable to stabilise the emulsions leading to a minimum concentration of 5% w/v being needed to sufficiently stabilise the emulsion. With these conditions microspheres in the size range of 20 microns were formed indicating the increase in surfactant concentration could possibly decrease the particle diameter. Maia *et al* (2006) have discussed that increasing surfactant concentration can give smaller diameter droplets since the droplets have a lower internal force and avoid coalescence.

It is believed that no other author has discussed the use of Span 85 concentrations above 1.75% when using a water in oil emulsion for the production of calcium pectinate microspheres. It is believed that the concentrations below 5% were unable to provide films that covered the entire surface of the droplets leading to the higher concentrations being used. It has been discussed by Maia *et al* (2006) that the type of pectin used effects the choice of surfactant concentration, however these concentrations were still in a range of 0.5 to 1.75% w/v.

3.3.6 Effect of mixing speed on particle size and formation

The mixing speed of the emulsions affected the size and formation of the microspheres produced. Mixing speeds of 500 rpm gave indiscreet pectin forms. Once this was increased to 1000 rpm some spherical particles were visible, however, the majority of the population was in an indiscreet form. Batches produced at 1500 rpm gave a population of highly defined spherical spheres (Figures 3.7 and 3.8).

The mixing process creates shearing which produces the spherical droplets of a desired size. Faster mixing speeds creates smaller droplets likewise with slower mixing speed creating larger droplets (Twitchell 2002). Depending on the materials being mixed, a specific level of shearing will be needed in order to create the spherical droplets, where in some cases a particular piece of mixing apparatus (overhead mixers/homogenisers for high shear) will need to be used (Twitchell 2002).

Paharia *et al* (2006) have discussed the effect that these mixing speeds have on the particle's size and from when using a similar over head stirrer. Increasing the speeds from 500, 1000 and 1500 rpm produced particles with diameters of 31, 27 and 25 microns respectively showing a decreasing particle diameter with increasing mixing speed. Although the size of the pectin product did decrease as shown in the sizing data (Table 3.1), mixing speeds of

1500 rpm was the only speed which was able to produce populations of spherical microspheres when Paharia *et al* (2006) claimed the mixing speeds of 500, 1000 and 1500 rpm all produced populations of spherical spheres.

It is believed that mixing speeds of 500 rpm did not provide the turbulent flow in order to produce spherical microspheres. One of the mechanisms of liquid mixing is by turbulent flow where an impeller in a mixing vessel forces molecules to move in a turbulent manner with random changes in the fluid's motion (Twitchell 2002). Turbulent flow is necessary in the mixing process since it creates the random motions in the fluid flow that gives the high degrees of agitation that laminar flow does not provide. The Reynolds numbers (Re) which is a number that identifies if a fluid flow is either laminar or turbulent was found for each mixing speed (Table 3.1).

Table 3.1 – Re numbers for the investigated mixing speeds

Mixing speed (rpm)	Re
500	14041
1000	28098
1500	42140

The Re numbers show that the mixing speeds of 500 rpm gave fluid flow patterns that were classed as transitional flow, which is a mixture of laminar and turbulent flow, but not fully turbulent. Turbulent flow (Re > 20000) was required in order to provide the necessary flow regime to produce the spherical droplets. It is believed that this could be the reason that no particulate forms were produced at the mixing speed of 500 rpm. It is believed that in the production of calcium pectinate microspheres by the water in oil emulsion method, no other author has discussed the concept of flow type on the formation of the particles since it is a rarely discussed topic in any pharmaceutical review.

The particle sizes and size distributions were analysed with laser diffraction particle sizing. Only particles made at 1000 and 1500 rpm were analysed since the formulations produced at 500 rpm contained no discreet particulate forms. The size distributions are shown in the Figures 3.9 a - f below and particle size statistics are given in Table 3.2

Table 3.2 – Average particulate size statistics for particles sized in the dry powder form. Each value is the mean for 5 measurements ± standard deviation.

	10% Calcium chloride			20% Calcium Chloride			30% Calcium Chloride		
Particulate size statistic	D10 (µm)	D50 (µm)	D90 (µm)	D10 (µm)	D50 (µm)	D90 (µm)	D10 (µm)	D50 (µm)	D90 (µm)
Sizes at 1000 rpm	66 ±4.4	405 ±32	668 ±57	66 ±4.3	418 ±45	924 ±72	46 ±5.9	273 ±22	911 ±78
Sizes at 1500 rpm	26 ±1.8	212 ±21	655 ±48	30 ±1.2	108 ±13	308 ±36	9 ±0.7	46 ±10	115 ±16

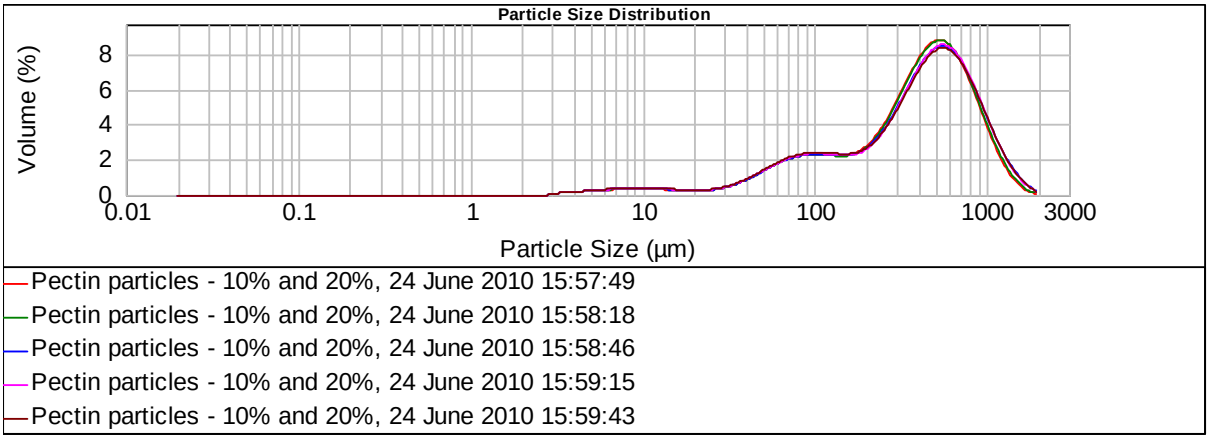

Figure 3.9a - Size distribution curve for the particles produced at the mixing speed of 1000 rpm with 10% calcium chloride.

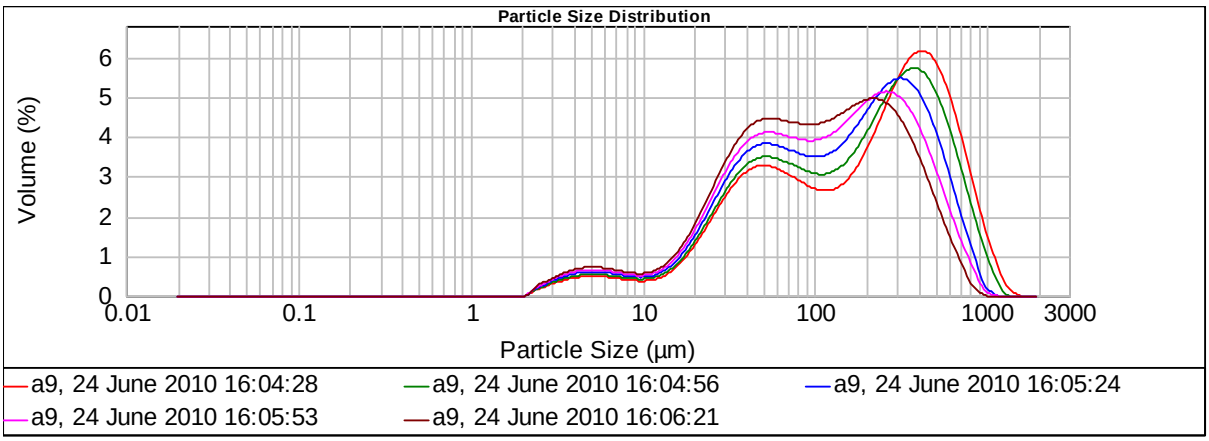

Figure 3.9b - Size distribution curve for the particles produced at the mixing speed of 1500 rpm with 10% calcium chloride.

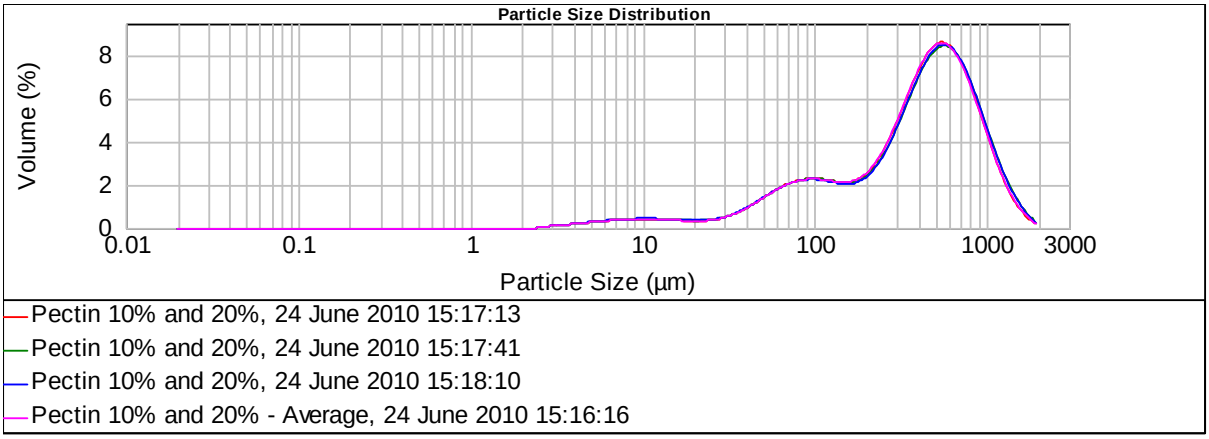

Figure 3.9c - Size distribution curve for the particles produced at the mixing speed of 1000 rpm with 20% calcium chloride.

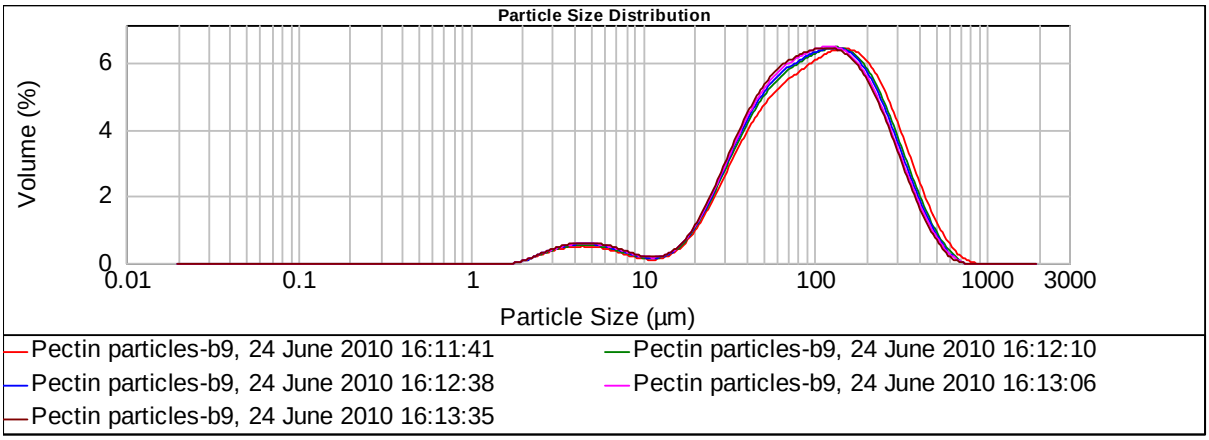

Figure 3.9d - Size distribution curve for the particles produced at the mixing speed of 1500 rpm with 20% calcium chloride.

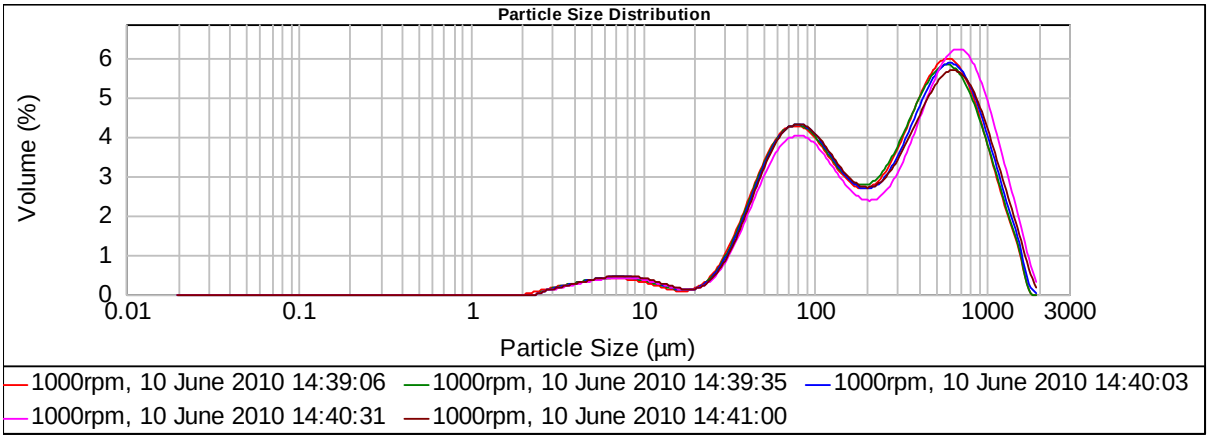

Figure 3.9 e - Size distribution curve for the particles produced at the mixing speed of 1000 rpm with 30% calcium chloride.

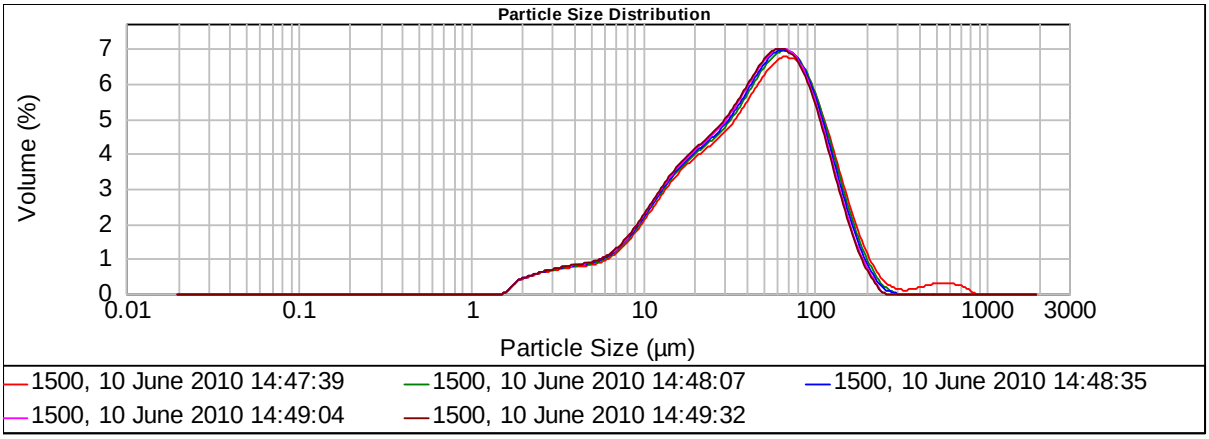

Figure 3.9f - Size distribution curve for the particles produced at the mixing speed of 1500 rpm with 30% calcium chloride.

In general the particle size distributions obtained from laser diffraction particle sizing were broad with multiple peaks. From the SEM images, the sizes of the particles are approximately 20 microns in diameter which do not match up to the results obtained from the laser sizing. The particles were added in a dried powder form and while mixing in the sample chamber, it was observed that the particles were liable to stick together forming agglomerates. It is believed that it was these agglomerates that were sized rather than the individual particles.

A 5% w/v solution of Tween 85 was used to suspend the particles before analysing in the sizer to prevent the agglomerates from forming. The Tween 85 was chosen since it was used in the production of the particles and Span 85 isn't very soluble in water. The particles' size distributions are shown in Figures 3.10 a – f and the size statistics in Table 3.3.

Table 3.3 – Average particulate size statistics for particles sized while suspended in Tween 85. Each value is the mean for 5 measurements ± standard deviation.

	10% Calcium chloride			20% Calcium Chloride			30% Calcium Chloride		
Average size range	D10 (µm)	D50 (µm)	D90 (µm)	D10 (µm)	D50 (µm)	D90 (µm)	D10 (µm)	D50 (µm)	D90 (µm)
Sizes at 1000 rpm	71 ±13	456 ±34	982 ±73	38 ±1.9	270 ±13	1028 ±97	46 ±1.9	35 ±1.5	911 ±74
Sizes at 1500 rpm	46 ±17	273 ±21	911 ±87	14 ±1.6	32 ±2	71 ±54	11 ±0.4	25 ±0.9	82 ±51

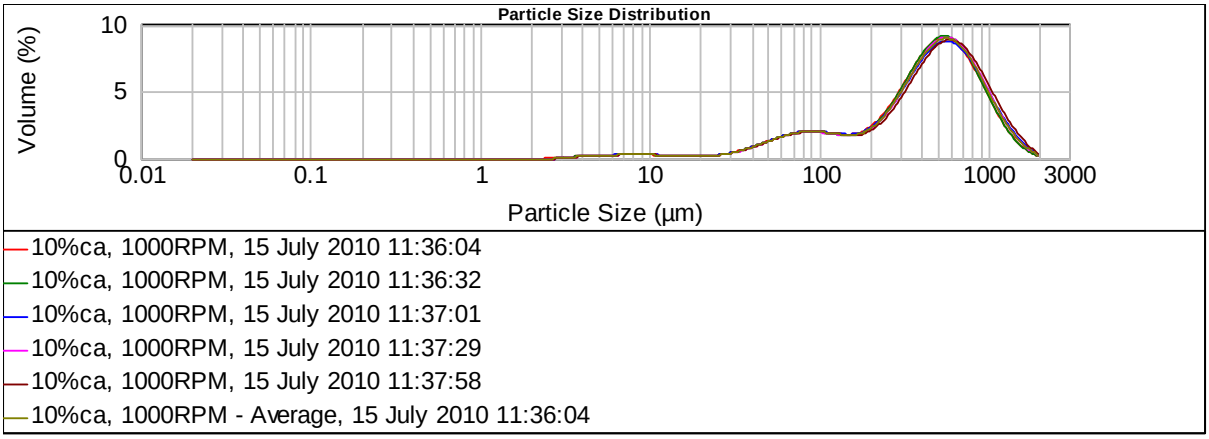

Figure 3.10a - Size distribution curve for the particles produced at the mixing speed of 1000 rpm with 10% calcium chloride.

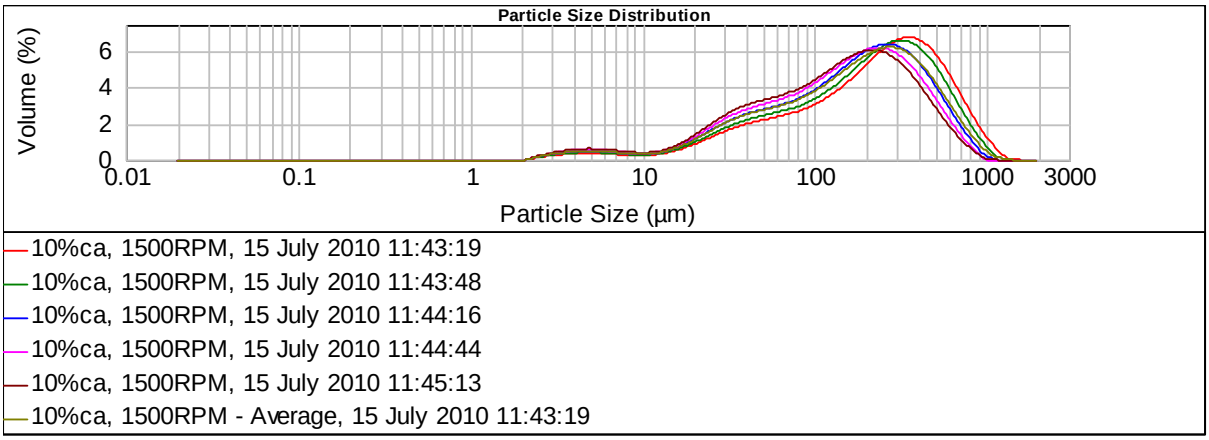

Figure 3.10b - Size distribution curve for the particles produced at the mixing speed of 1500 rpm with 10% calcium chloride.

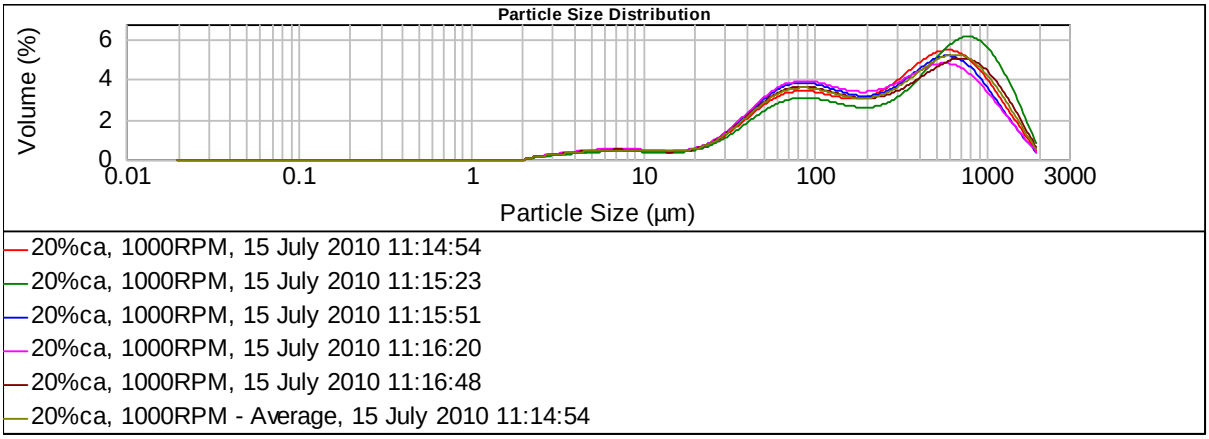

Figure 3.10c - Size distribution curve for the particles produced at the mixing speed of 1000 rpm with 20% calcium chloride.

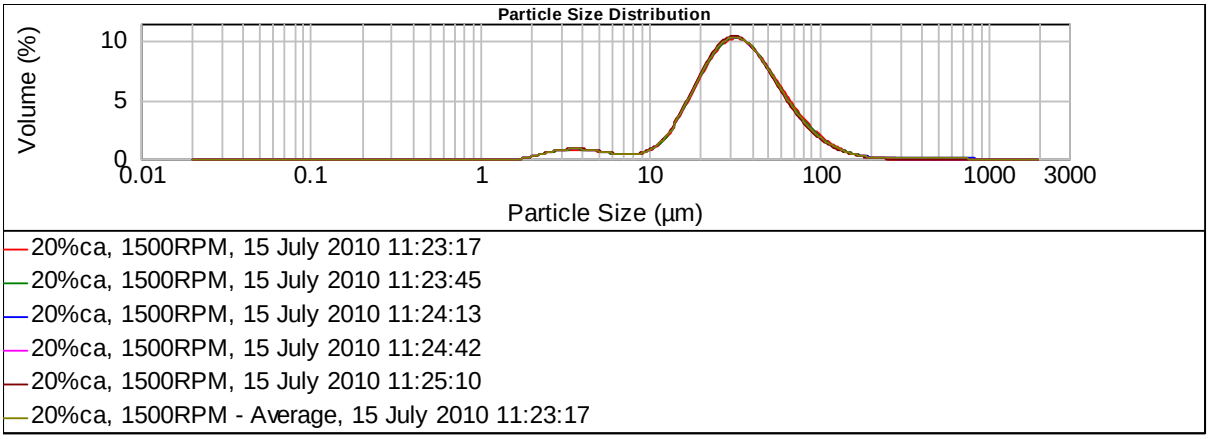

Figure 3.10d - Size distribution curve for the particles produced at the mixing speed of 1500 rpm with 20% calcium chloride.

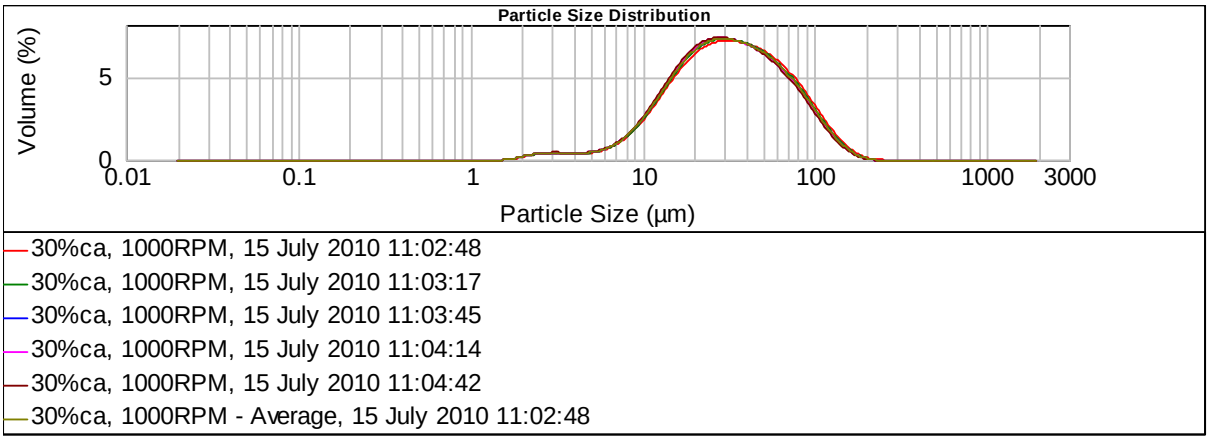

Figure 3.10e - Size distribution curve for the particles produced at the mixing speed of 1000 rpm with 30% calcium chloride.

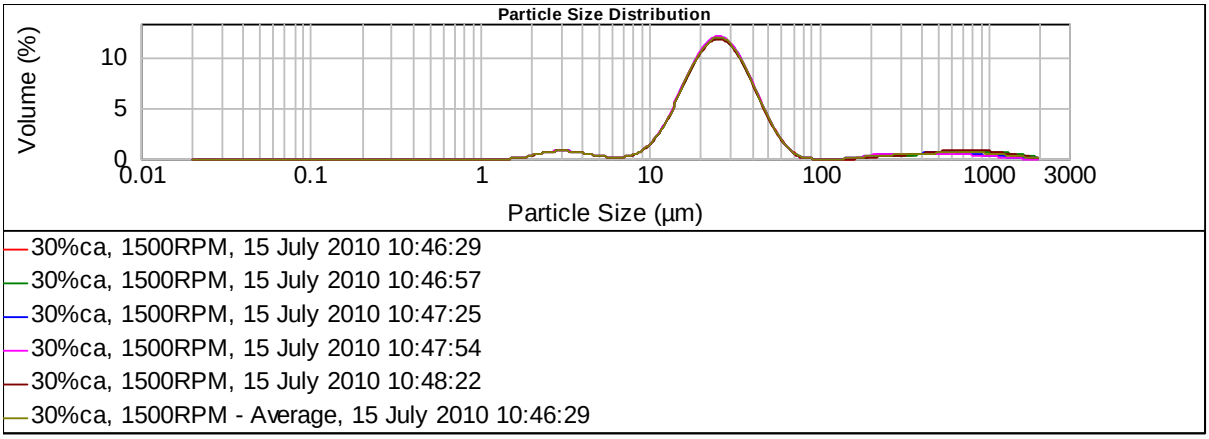

Figure 3.9f - Size distribution curve for the particles produced at the mixing speed of 1500 rpm with 30% calcium chloride.

The size distributions now illustrated some narrower distributions with average particle diameters that matched the sizes shown in the SEM images. Particles produced with 10% calcium chloride solutions gave sizes (D50) of 456 and 273 microns at mixing speeds of 1000 and 1500 rpm respectively. Batches made with 20% calcium chloride had average size (D50) of 270 and 32 microns at mixing speeds of 1000 and 1500 rpm respectively. Batches produced with 30% calcium chloride gave two clear distributions showing an average (D50) of 35 and 25 microns at 1000 and 1500 rpm respectively.

The particles produced with 30% calcium chloride solution with mixing speeds of 1500 rpm gave particles that were in a fine powder form which from the SEM images comprises individual spheres that are individually distributed. Particles produced at the other mixing speeds and calcium chloride concentrations were in an agglomerate form which is believed to have produced the broad distribution data with some multiple peaks and sizing data that didn't match that of the SEM images. In order to produce an accurate sizing data it was seen that the particles had to individually distribute to avoid agglomerates being sized. The process conditions of 10% and 20% calcium chloride and mixing speeds of 1000 rpm gave some agglomerate forms which were believed to still being sized giving sizing data that differed from the given SEM images.

3.3.7 Effect of calcium chloride on particle swellability

The increase in the calcium chloride concentration decreased the permeability of the pectin as shown by the swelling percentage (Table 3.3).

With the increase in calcium chloride concentration the pectin's density will also increase giving spheres with a lower permeability for the same reasons discussed in the previous chapter. The particles swellability percentage was found by measuring the mass of water that was lost during drying. Since the increase in calcium chloride concentration reduces the

permeability of the spheres then the spheres produced with higher calcium chloride concentrations will have a smaller mass of water being dried from the particle.

Table 3.4 – Swelling percentage for the range of calcium chloride concentrations. The increase in calcium chloride reduces the amount of water that the sphere can hold giving a reduced swelling percentage. Each value is a mean number from three independent studies ± standard deviation.

Calcium chloride concentration	Swellability percentage
10%	86% ±2.9
20%	80% ±3.4
30%	73% ±2.6

Maestrelli *et al* (2006) have investigated swelling percentages of 90%, 85% and 79% for calcium chloride concentrations of 10%, 20% and 30% respectively. This trend was found in this investigation. Maestrelli *et al* (2006) found the percentages by hydrating a known weight of dried spheres, blotting with filter paper then re weighing. This method was not used since it was hard to re weigh all the hydrated particles due to their small size therefore a method by finding the water loss was used. Bourgeois *et al* (2006) has seen that the swelling percentage is not only dependent on the efficiency of the ionic bonding between pectin and calcium ions but also on the type of pectin used.

3.4 Conclusion

This chapter looked into the production of the calcium pectinate microspheres using a water in oil emulsion method. Development of a method in the literature was required before a final standardised method was achieved. Initially a magnetic mixer was used to produce the spheres however production was switched to an overhead mechanical mixer providing better

agitation. Also it was seen that particles, once produced, had to be left to undergo additional cross linking in order to give defined spherical particles.

From the range of process parameters that were investigated, it was seen that the success of the production of defined spherical particles was dependent on achieving a satisfactory stabilised emulsion with span 85 concentrations of 5% (w/v), that were higher than that stated in the literature. Formation of individually distributed spherical particles also required a sufficient level of shearing at 1500 rpm to produce the pectin droplets which again the literature had not discussed. Also a calcium chloride concentration of 30% (w/v) was needed to give sufficient cross linking to produce the particles in a fine powdered form with a reduced permeability.

Chapter 4

Drug loading and encapsulation of 5-ASA

4.0 Introduction

This chapter describes the addition of the drug 5-amino salicylic acid (5-ASA) into the calcium pectinate microparticles. This drug is used for the treatment of inflammatory bowel disease. The optimised water in oil emulsion method for the production of the calcium pectinate particles described in the previous chapter was carried forward for the drug loading studies. These studies were carried out with 5-ASA solutions at concentrations of 0.02 % w/v and also with 5-ASA suspensions of 1 %, 2 % and 3 % w/v. The effects of the calcium chloride concentration on the drug loading of the particles were also investigated.

4.1 Materials

Pectin of a low DE (<50%) was supplied as a gift sample by S Black Ltd, Herts, UK. 5-ASA, Span 85, Tween 85 and pectinase were supplied by Sigma Aldrich, UK. Isooctane, calcium chloride dihydrate, phosphate buffered saline (PBS) were supplied by Fisher Scientific, Leicestershire, UK. Double distilled water was used to produce all aqueous solutions.

4.2 Methods

4.2.1 Drug loaded particles with 5-ASA solutions

The pectin and 5-ASA mixture was produced by dissolving 10 mg of 5-ASA in 20 mL of double distilled water. In a separate beaker 1.5 g of pectin was dissolved in 30 mL of double distilled water and mixed for 12 hours. The 20 mL 5-ASA solution was added under mixing to the 30 mL pectin solution. The final volume of the pectin/5-ASA mixture was 50 mL at concentrations of 3% and 0.02% w/v respectively. Particles were then produced using the optimised water in oil emulsion method. Briefly the surfactant concentration was 5% w/v

and mixing speed was 1500 rpm along with the various calcium chloride concentrations of 10%, 20% and 30% w/v.

4.2.2 Drug loaded particles with 5-ASA suspensions

1.5 g of pectin was weighed into a 100 mL beaker with 0.5 g, 1 g and 1.5 g of 5-ASA and made up to 50 mL with double distilled water forming the suspensions with a pectin concentration of 3% w/v and 5-ASA concentrations of 1%, 2% and 3% w/v. The suspensions were then used to produce the particles with the optimised water in oil emulsion method.

4.2.3 Particle characterisation

The particles' shape characteristics were analysed by the same methods discussed in the previous chapter by scanning electron microscopy (SEM) (JEOL 6060) and light microscopy (Olympus BH2).

4.2.4 Drug loading and encapsulation efficiency

The drug loaded particles were analysed for their drug content by investigation into the drug loading (DL) and encapsulation efficiency (EE). 100 mg of particles were mixed on a magnetic mixer in 200 mL of PBS containing 3% w/v pectinase for 12 hours. Three 1 mL samples were removed with a micro pipette and placed into Eppendorf tubes which were spun down at 2500 rpm for 5 minutes in a centrifuge. The supernatant was removed and analysed for 5-ASA content by UV spectroscopy (Jasco V-530) at a wavelength of 330 nm (Mladenovska *et al* 2007).

The drug concentration was determined by comparison to a calibration curve (Figure 4.1) which was prepared by serial dilution of a stock solution of 5-ASA with concentrations ranging from 0.1 mg/mL to 0.00625 mg/mL. The stock solution was prepared by dissolving

10 mg of 5-ASA in 100 mL of PBS (pH 7.4) producing a solution with a concentration of 0.1 mg/mL.

The drug loading in a unit of microspheres is defined as;

$$DL = (D/P) \times 100 \qquad (4.1)$$

Where DL is the percentage drug loading, D is the mass of drug (mg) released from a unit weight of particles and P is the mass of micro particles (mg) used in the drug loading study.

The encapsulation efficiency was also determined by the ratio of the drug loading found in each formulation to the initial drug loading of pectin and 5-ASA used for production. EE is defined as;

$$EE = (D_1/D_i) \times 100 \qquad (4.2)$$

Where EE is the encapsulation efficiency, D_i is the initial DL of pectin and 5-ASA used to prepare the formulation and D_1 is the DL obtained from the produced formulation.

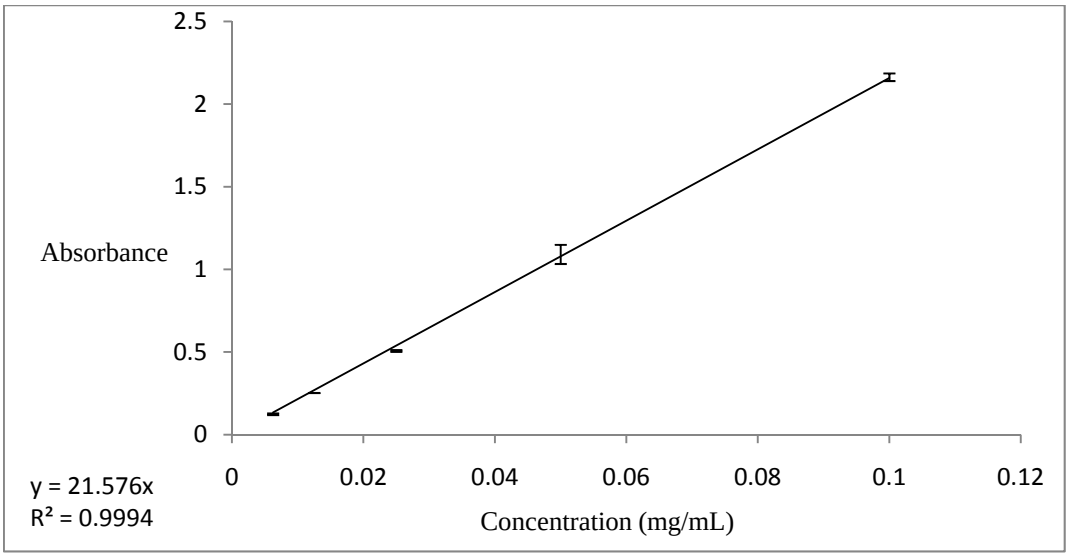

Figure 4.1 – Calibration Curve of 5-ASA in PBS (pH 7.2). Three 1 mL samples were assayed by UV spectroscopy where a mean value was determined. Each point is the mean value of three independent studies ± standard deviation.

4.3 Results and discussion

4.3.1 Particles prepared with 5-ASA in solution

The ability to load drug in solution into pectin particles was investigated along with the effects of calcium chloride concentration on particle morphology, DL and EE.

These formulations, as expected gave particles where the sphericity of the particle increased the increasing calcium chloride concentration (Figures 4.2 and 4.3).

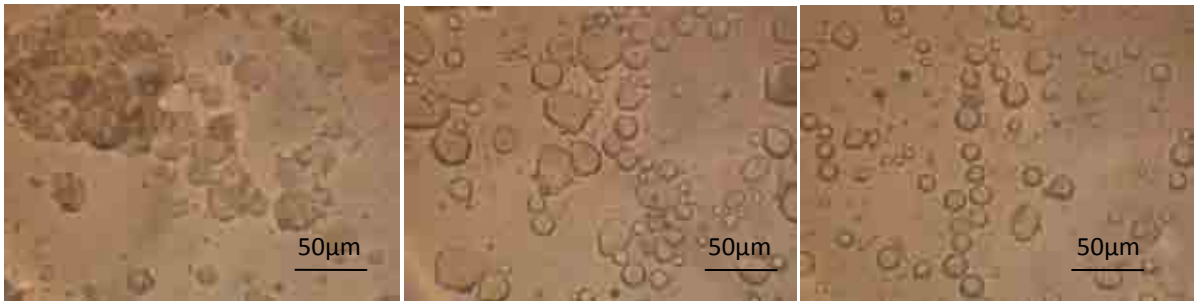

Figure 4.2 – Light microscope images of the 5-ASA drug loaded calcium pectinate particles produced with cross linking solutions of 10% (left), 20% (middle) and 30% calcium chloride (right).

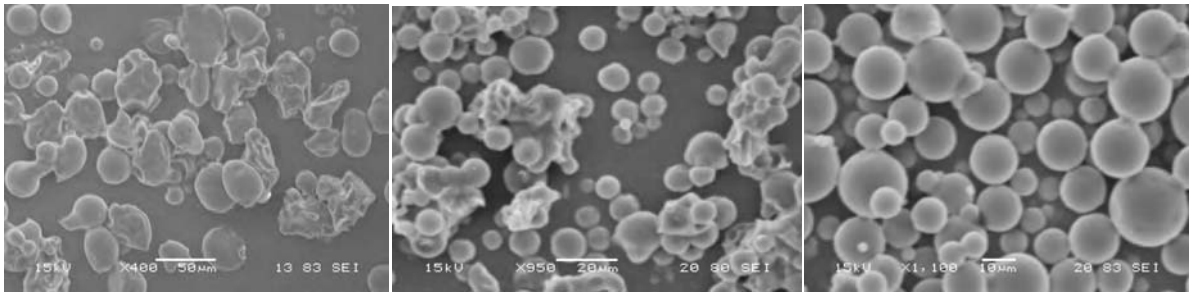

Figure 4.3 – SEM images of the 5-ASA drug loaded calcium pectinate particles produced with cross linking solutions of 10% (left), 20% (middle) and 30% (right).

Although the particles were produced, they were unable to encapsulate the drug (Table 4.1).

Table 4.1 – DL of the particles produced with 5-ASA loaded in solution. The particles were unable to encapsulate the drug.

	10% $CaCl_2$	20% $CaCl_2$	30% $CaCl_2$
DL	0 %	0 %	0 %

Although the quoted solubility of 5-ASA in water at 25 ºC is 1.7mg/mL (Mladenovska *et al* 2007) it was found that only 10 mg could be readily dissolved into each 50 mL batch for this study. In this emulsion system, a proportion of the drug can be lost to the continuous phase (Wong *et al* 2007). Since such a small proportion of 5-ASA is used, the drug could also be lost to the isooctane phases during the emulsification process (Esposito *et al 2001*).

A drugs physicochemical property has a high impact on the DL and EE. Drugs that are highly hydrophilic are known to have much higher DL and EE than drugs that are hydrophobic when being encapsulated by water in oil emulsions (Esposito *et al* 2001). Esposito *et al* (2001) saw that tetracycline, and metronidazol entrapped in pectin particles displayed two distinct EE. Tetracycline, a drug with greater hydrophilic character gave an EE of 11.5% compared to metronidazol, having a greater hydrophobic characteristics, gave an EE of 0.75%.

To increase the concentration of 5-ASA used to produce the particles by the water in oil emulsion, a higher volume of water will have to be included in the pectin/drug mixture which could lead to phase inversion of the emulsion, changing the mechanism of the emulsion system. For this reason and given the relatively low solubility of 5-ASA, it is believed that to successfully load 5-ASA solutions into pectin particles, it would be necessary to load particles produced with another production method.

Iruìn *et al* (2005) used 5-ASA concentrations of 0.5% w/v to produce particles by the syringe droplet method preparing calcium alginate particles as opposed to calcium pectinate particles.

These particles however were produced using the drug in suspensions. Mladenovska *et al* (2007) has successfully encapsulated 5-ASA with concentrations of 0.5% w/v into calcium alginate particles produced with a spray drying method. It is believed that no author has discussed the encapsulation of 5-ASA into calcium pectinate particles produced with the water in oil emulsion method, possibly since the method is not able to produce efficient DL since only a small proportion of the drug can be used and is liable to be lost to the isooctane.

4.3.2 Particles produced with 5-ASA in suspension

In order to improve drug loading, particles were prepared using the 5-ASA in suspension at concentrations of 1 %, 2 % and 3 % w/v. The resultant particles were irregular in shape with needle like formations (Figure 4.4) indicating that the pectin was developing around the entrapped 5-ASA's crystal like structure (Figure 4.5). The structure of 5-ASA in its dry powder form is shown in Figure 4.6.

Iruìn *et al* (2005) used the syringe droplet method to encapsulated 5-ASA at concentrations of 0.5% w/v into calcium alginate where the drug was loaded though a suspension. Their particles had diameters of approximately 1.7 mm which is believed to have made them big enough to encapsulate the drug without giving the irregular structures that the drug loaded though a suspension gave the micron sized particles.

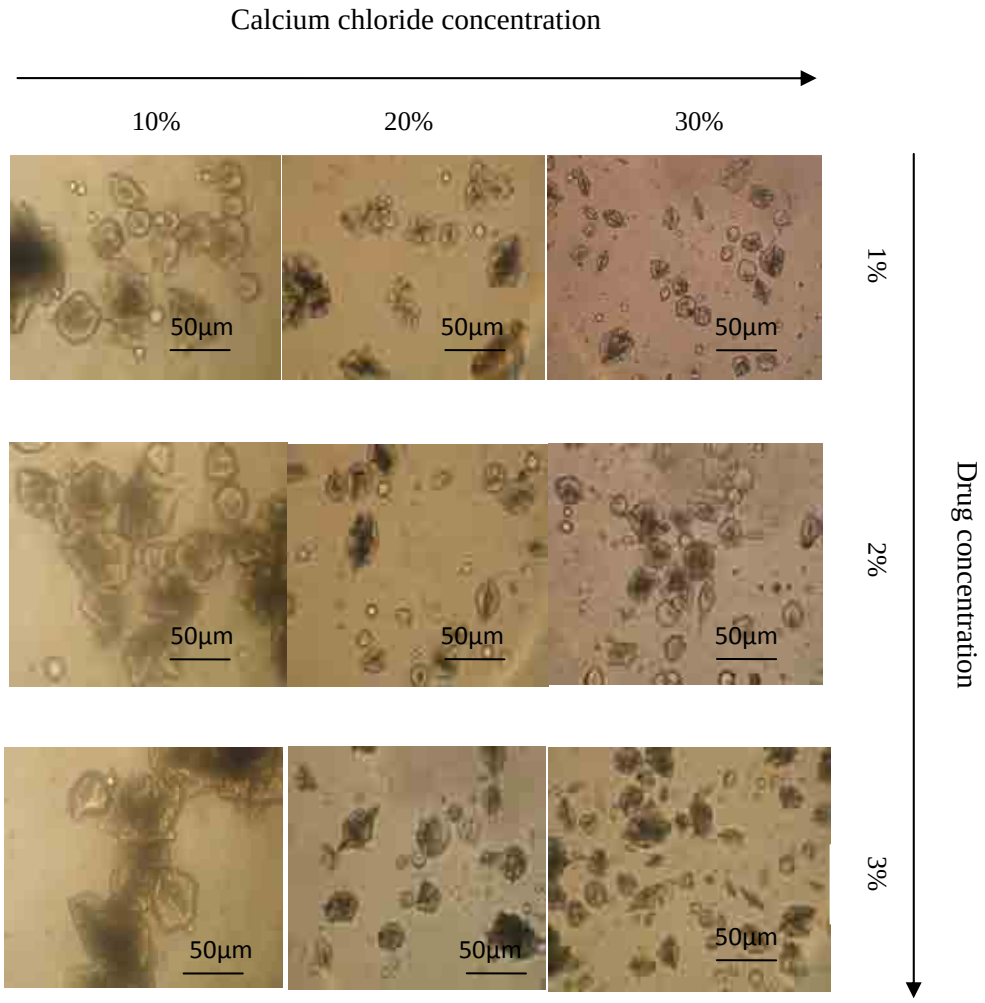

Figure 4.4 – Light microscopy images of the drug loaded particles produced with the drug in a suspension. The effects of calcium chloride concentration (10% left, 20% middle and 30% right) and drug concentration (1% top, 2% centre and 3% bottom) on the particle form can been seen.

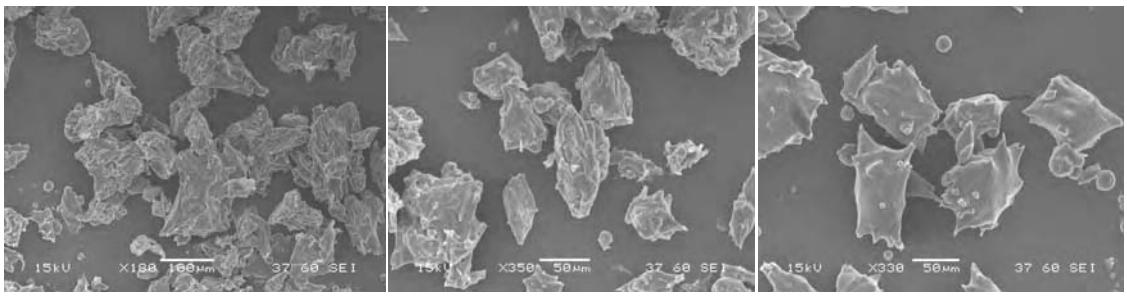

Figure 4.5 – SEM images of the drug loaded particles produced with the drug in a suspension using a drug a concentrations of 1% w/v. The effects of the calcium chloride concentrations (10% left, 20% middle, and 30% right) can be seen.

Figure 4.6 –5-ASA in its dry powder form. It is in a needle like structure.

4.3.3 Drug loading of 5-ASA loaded in suspension

The DL of the 100 mg of particles that were analysed for 5-ASA content is shown in Table 4.2.

Table 4.2 – DL of the different formulations produced. Each value is the loading of thee independent studies ± standard deviation.

	10% $CaCl_2$	20% $CaCl_2$	30% $CaCl_2$
1% 5-ASA	20% ±2.4	19% ±2.9	17% ±1.8
2% 5-ASA	36% ±2.7	33% ±2.2	30% ±2.5
3% 5-ASA	42% ±1.9	39% ±3.2	36% ±2.8

With the increasing drug concentration of 1%, 2% and 3% w/v the DL increased. With the increasing calcium chloride concentration of 10%, 20% and 30% w/v the DL decreased.

When the number of ionic bonds between the pectin and calcium ions is increased by increasing the concentration of calcium chloride, more orderly structures are formed which increases the pectin's density. This causes the drug to be squeezed out during the production process resulting in a lower DL with the increasing calcium chloride concentration (Wakerly *et al* 1997). Surajit *et al* (2009) investigated the effects of calcium chloride concentrations from 0.5% to 5% w/v in a syringe droplet method which saw a decrease in the DL from 25% to 18% with the increasing calcium chloride concentration.

The size of the particle is also known to affect DL. Particles with larger diameters have a larger volume for a drug to occupy resulting in higher DL. With the increasing calcium chloride concentration from 20% to 30%, the particle size decreases from 35 to 25 microns in diameter (as shown in the particle sizing data in the previous chapter) which could also contribute to the decreasing DL with increasing calcium chloride concentration. Yang *et al* (2000) saw that with increasing calcium chloride concentrations of 1%, 2% and 5% w/v the size of the particle decreased from 185 to 184 to 174 microns in diameter with DL of 25, 21 and 18 for the respective particle diameters.

4.3.4 Encapsulation efficiency

The encapsulation efficiency (Table 4.3) was obtained using equation 4.2.

Table 4.3 – EE of the different formulations produced. Each is a mean value of three independent studies ± standard deviation.

	10% $CaCl_2$	20% $CaCl_2$	30 % $CaCl_2$
1% 5 ASA	80 % ±2.8	76 % ±2.1	68 % ±2.0
2% 5 ASA	90 % ±2.3	83 % ±2.8	75 % ±2.6
3% 5 ASA	84 % ±1.7	78 % ±2.5	72 % ±1.8

With the increasing drug concentration from 1% to 2%, the encapsulation efficiency increased which then decreased when the drug concentration was increased to 3%. With the increasing calcium chloride concentrations, the encapsulation efficiency decreased.

The initial increase in EE following a decrease with the increasing 5-ASA concentration is most likely due to the increasing drug: polymer ratio reaching a limit were the 5-ASA loaded into the pectin reaches a maximum (between 5-ASA concentrations of 2% and 3% w/v). Sriamornsak (1998) loaded bovine serum albumin (BSA) into pectin beads produced by the syringe droplet method. 5 g, 10 g and 20 g of BSA were loaded giving EE of 60%, 24% and

23% where the increasing drug: polymer ratio reached a limit at BSA loadings of 10 g resulting in the decreased EE.

As with the drug loading, the encapsulation efficiency decreased with increasing calcium chloride concentrations, since the increase in particle density squeezed the drug out during the emulsification process.

Most colonic delivery reviews are based on the syringe droplet method as opposed to the water in oil emulsion method. Maestrelli *et al* (2007) used the syringe droplet method to produce calcium pectinate beads with EE as high as 99 % and DL of 32 %. It is believed that no comparison between the two methods has been discussed in the literature however the higher EE compared to the water in oil emulsion method is believed to be due to the short production time and instant hardening of the pectin particles.

4.4 Conclusion

The drug loaded particles were produced with the optimised water in oil emulsion method by loading the 5-ASA while in solution and also in suspension. The 5-ASA when loaded in a solution failed to produce any drug loaded particles. This was possibly since the solubility of the 5-ASA only allowed 10 mg of the drug to be loaded into each batch which was believed to be lost to the isooctane during the emulsification process. The particles produced with the drug loading in a suspension gave particles with fairly reasonable drug loadings and encapsulations efficiencies; however, these gave the particles irregular structures in line with the 5-ASA's needle like structure. If 5-ASA were to be used as a drug model in this study, it is believed that the syringe droplet method would be more efficient since the drug needs to be in suspension in order to produce particles with an effective DL and EE.

Chapter 5

In vitro release of 5-ASA from calcium pectinate microspheres

5.0 Introduction

The drug loaded particles produced with 2% w/v 5-ASA suspensions were subject to *in vitro* drug release studies in conditions simulating various regions of the GIT. Hydrochloric acid (HCl), Hanks buffer and phosphate buffered saline (PBS) were used to simulate the stomach, small intestine and colon respectively. The effect of the calcium chloride concentration on the release rate of the drug was investigated.

5.1 Materials

Concentrated HCl, Hanks buffer and PBS tablets were supplied by Fisher Scientific, Leicestershire, UK. Double distilled water was used to prepare all aqueous solutions.

5.2 Methods

300 mL of each drug release media used for the study were prepared as follows. HCl (0.1M) (pH 1.2) was prepared by diluting 8.33 mL of the concentrated HCl and making up to 1 L with double distilled water. The Hanks buffer (pH 6.4) was ready to use from Fisher Scientific. The PBS (pH 7.2) was prepared by dissolving 3 PBS tablets in 300 mL of double distilled water.

100 mg of drug loaded particles along with 300 mL of drug release media were weighed and measured out and placed into a 500 mL conical flask plugged with a foam plug. The conical flasks were placed into a shaking incubator and mixed at 100 rpm at 37 ºC.

The duration of the drug release study was 2 hours for the stomach, 6 hours for the small intestine and 20 hours for the colon. Three 1 mL samples were removed using a micro pipette every 30 minutes for 2 hours, then on an hourly basis. The volumes removed were replaced

with fresh media. The samples were placed into Eppendorf tubes and spun down in a centrifuge at 2500 rpm for 5 minutes. 0.75 mL of the supernatant was removed and analysed for 5-ASA content with UV spectroscopy at a wavelength of 330 nm (Mladenovska *et al* 2007). All release studies were preformed in triplicate.

The drug concentration was obtained by comparison to a calibration curve (Figures 5.1 – 5.3) of 5-ASA in each drug release media which was produced by the methods discussed in the previous chapter. The cumulative drug release was plotted against time on a separate graph for each simulated organ.

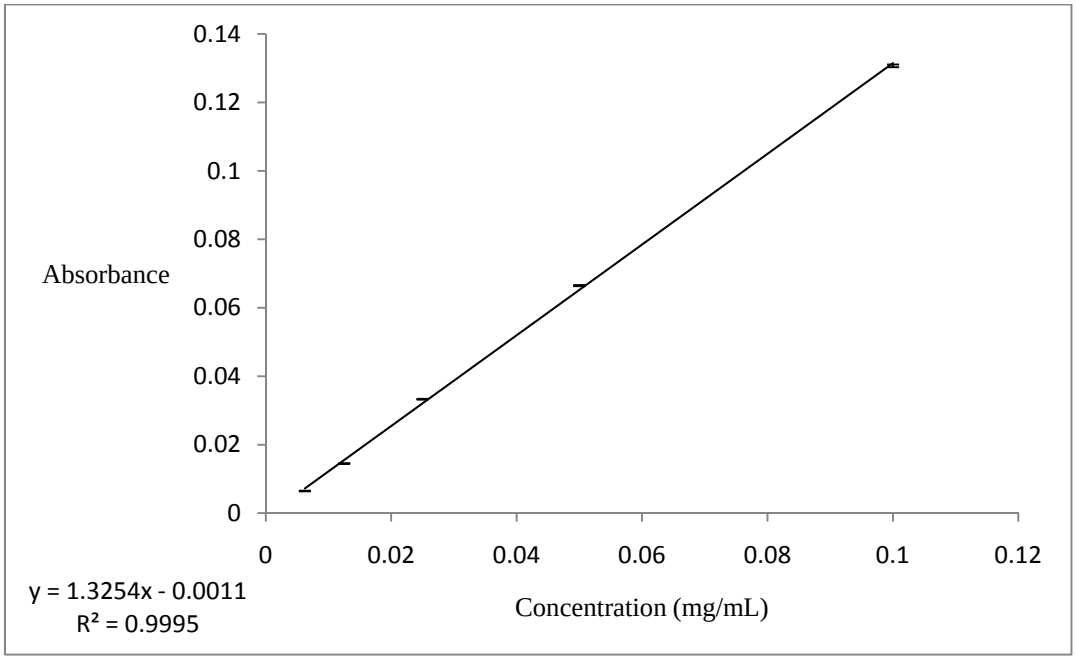

Figure 5.1 – Calibration curve of 5-ASA in Hydrochloric acid (pH 1.2). Three 1 mL samples were assayed by UV spectroscopy where a mean value was determined. Each point represents three independent studies ± Standard deviation.

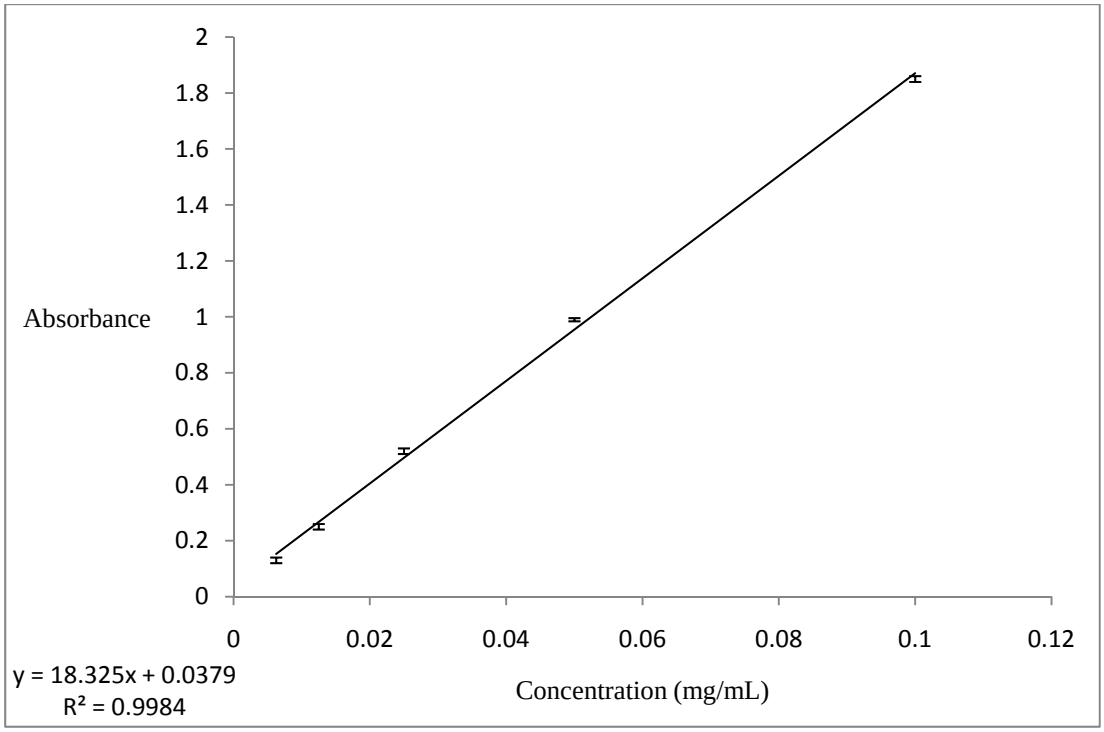

Figure 5.2 – Calibration curve of 5-ASA in Hanks Buffer (pH 6.4). Three 1 mL samples were assayed by UV spectroscopy where a mean value was determined. Each point represents three independent studies ± Standard deviation.

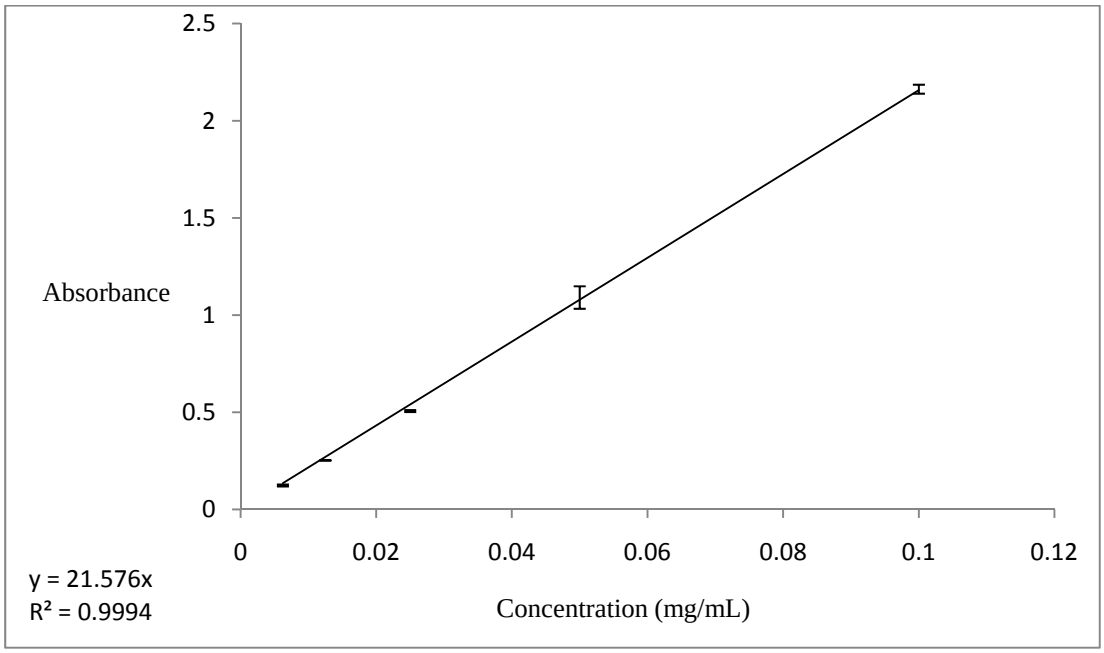

Figure 5.3 – Calibration curve of 5-ASA in PBS (pH 7.2). Three 1 mL samples were assayed by UV spectroscopy where a mean value was determined. Each point represents three independent studies ± Standard deviation.

5.3 Results and discussion

5.3.1 Drug release in 0.1M Hydrochloric acid simulating the conditions of the stomach

The cumulative drug release profile for particles produced with the various calcium chloride concentrations in conditions simulating the stomach is shown in Figure 5.4 along with the percentage release in Figure 5.5.

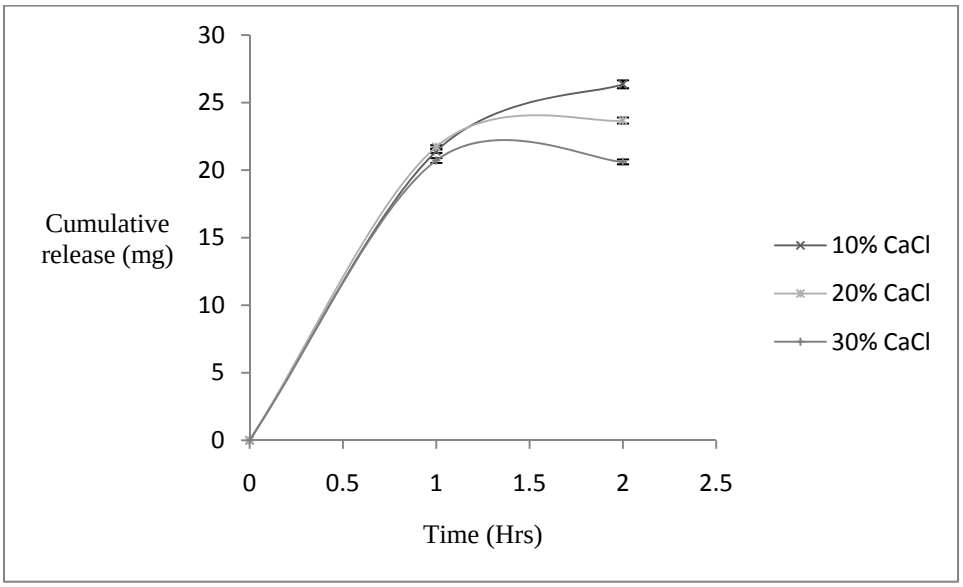

Figure 5.4 –Cumulative drug release profile from 100 mg of calcium pectinate microspheres in 300 mL 0.1M Hydrochloric acid (pH 1.2). The effects of the various calcium chloride concentrations of 10%, 20% and 30% on the release profile are shown. Three 1 mL samples were removed and assayed by UV spectroscopy where a mean value was determined, each point represents three independent studies ± standard deviation.

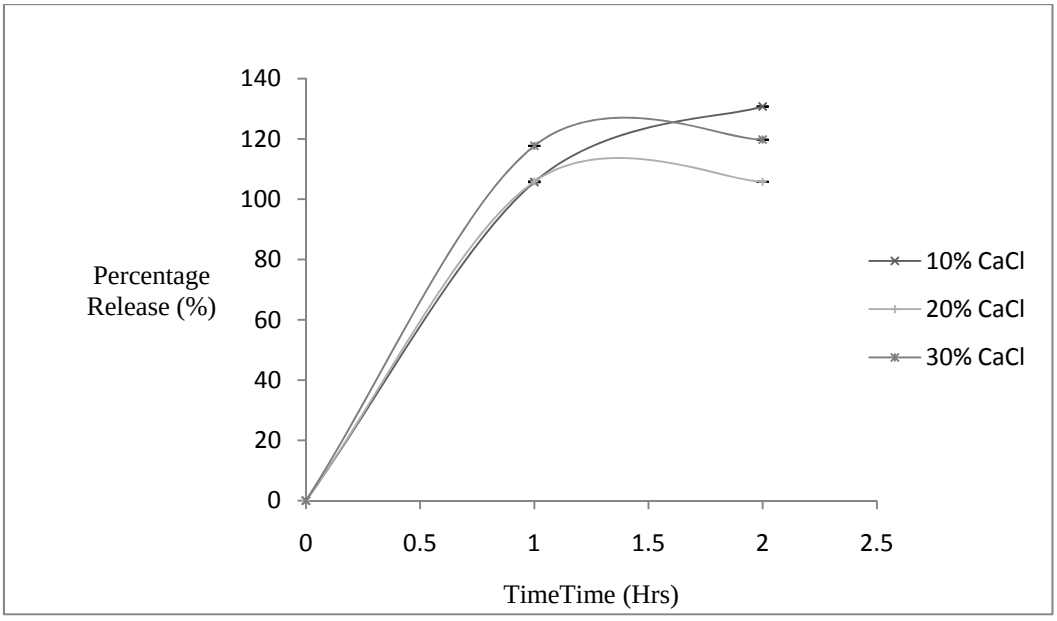

Figure 5.5 – Percentage drug release from 100 mg of calcium pectinate microspheres in 300 mL 0.1M hydrochloric acid (pH 1.2). The effects of the various calcium chloride concentrations of 10%, 20% and 30% on the release profile are shown. Three 1 mL samples were removed and assayed by UV spectroscopy where a mean value was determined, each point represents three independent studies ± standard deviation.

From the release profile the 5-ASA was rapidly released in the first hour. In acidic conditions calcium pectinate avoids degradation but will swell (Luishu *et al* 2003), however in reality the particles would normally be coated, protecting the pectin/drug matrice from the stomach conditions. Interestingly the results from the UV spectroscopy readings indicated that the cumulative release in the HCl was greater than was theoretically possible when compared to the DL studies in the previous chapter. For the calcium chloride concentrations of 10%, 20% and 30%, 5-ASA masses of 20 mg, 19 mg and 17 mg should have been released for the respective concentrations where in this study 5-ASA masses of up to 27 mg were being detected by the UV spectroscopy.

30 minutes into the release study, the particles were recovered and analysed with light microscopy (Figure 5.6). The image shows the pectin particles still intact without the 5-ASA crystals where it is believed that the crystals dropped out into the HCl media as the particles

swell. It is also believed that some of the 5-ASA remains in a solid form in the HCl leading to the higher than expected absorbance reading.

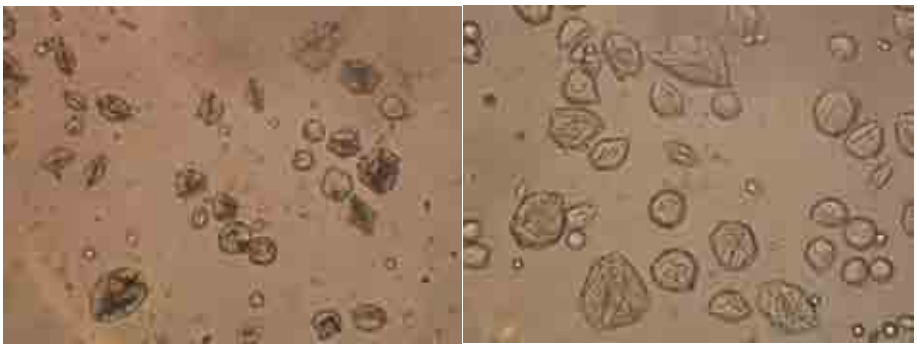

Figure 5.6 – Light microscope images of the drug loaded particles before the drug release study (left) and recovered after the 2 hour release study in HCl (right). It is believed that the particles swell and the 5-ASA drops out in a solid form into the release media rather than diffusing out.

Although the study was designed to ensure sink conditions at pH 7.4, the solubility of 5-ASA (an acid drug) in release media at pH 1.2 will be considerably lower than that at pH 7.4 (1.7mg/mL; Mladenovska *et al* 2007), which is believed to have kept some of the drug once it dropped out of the particles in its solid form. If this study was to be repeated it would be important to take into account the solubility of the drug at different pH levels.

Previously authors have failed to give a detailed review of drug release in conditions simulating the stomach since the particles will remain protected (e.g. by Eudragit coatings) in the regions of the lower GIT and their discussions focus on release in colonic conditions.

5.3.2 Drug release in Hanks Buffer simulating the conditions of the small intestine

The release profile for particles produced with the various calcium chloride concentrations in conditions simulating the small intestine is shown in Figure 5.7 along with the percentage release in Figure 5.8.

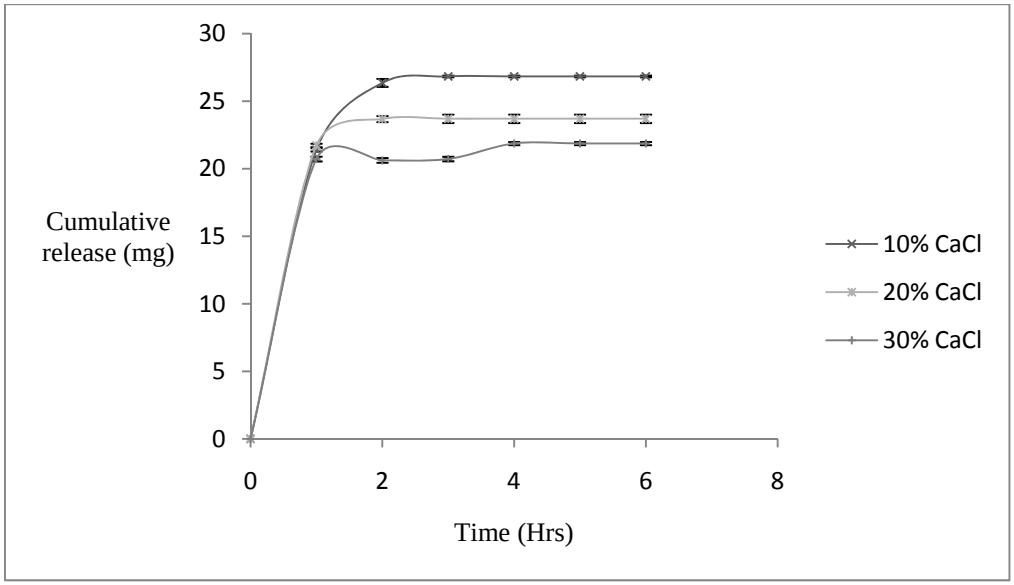

Figure 5.7 – Cumulative drug release profile from 100 mg of calcium pectinate microspheres in 300 mL Hanks buffer (pH 6.4). The effects of the various calcium chloride concentrations of 10%, 20% and 30% on the release profile are shown. Three 1 mL samples were removed and assayed by UV spectroscopy where a mean value was determined, each point represents three independent studies ± standard deviation.

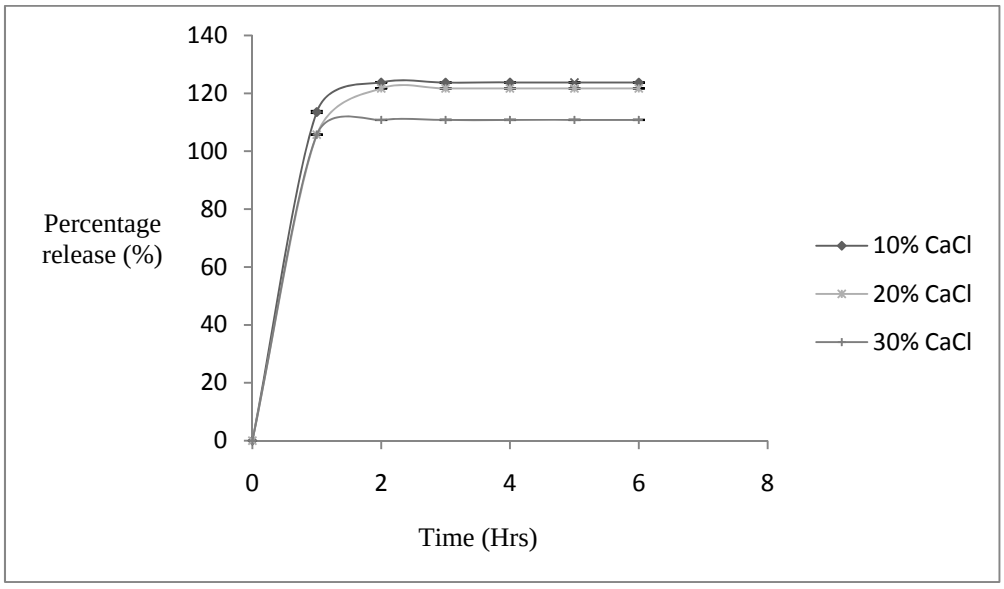

Figure 5.8 – Percentage drug release from 100 mg of calcium pectinate microspheres in 300 mL Hanks buffer (pH 6.4). The effects of the various calcium chloride concentrations of 10%, 20% and 30% on the release profile are shown. Three 1 mL samples were removed and assayed by UV spectroscopy where a mean value was determined, each point represents three independent studies ± standard deviation.

As with the HCl the drug release in the Hanks buffer gave higher cumulative release than expected, which is believed to be due to the same reasons in conditions simulating the stomach with undissolved solid 5-ASA crystals giving higher absorbance readings.

Like with the stomach conditions, reviews fail to give detailed discussions on the conditions of the small intestine again since the inner matrix will often be protected with a Eudragit coating. It is known in some pectin delivery systems coated in a Eudragit can release about 4 % of the encapsulated drug before reaching the colon (Paharia *et al* 2007). This is believed to be due to the increasing pH that can begin to degrade the coating and the release of a small percentage of the drug that is on the surface of the particles.

5.3.3 Drug release in PBS simulating the colon

The release profile for particles produced with the various calcium chloride concentrations in conditions simulating the colon is shown in Figure 5.9 along with the percentage release in Figure 5.10.

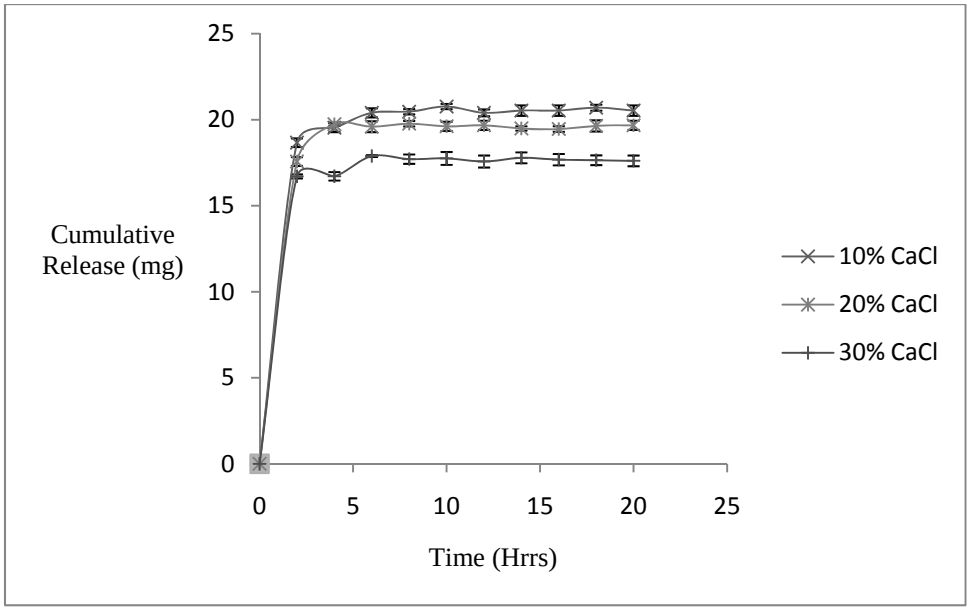

Figure 5.9 – Cumulative drug release profile from 100 mg calcium pectinate microspheres in 300 mL PBS (pH 7.2). The effects of the various calcium chloride concentrations of 10%, 20% and 30% on the release rate are shown. Three 1 mL samples were removed and assayed by UV spectroscopy where a mean value was determined, each point represents three independent studies ± standard deviation.

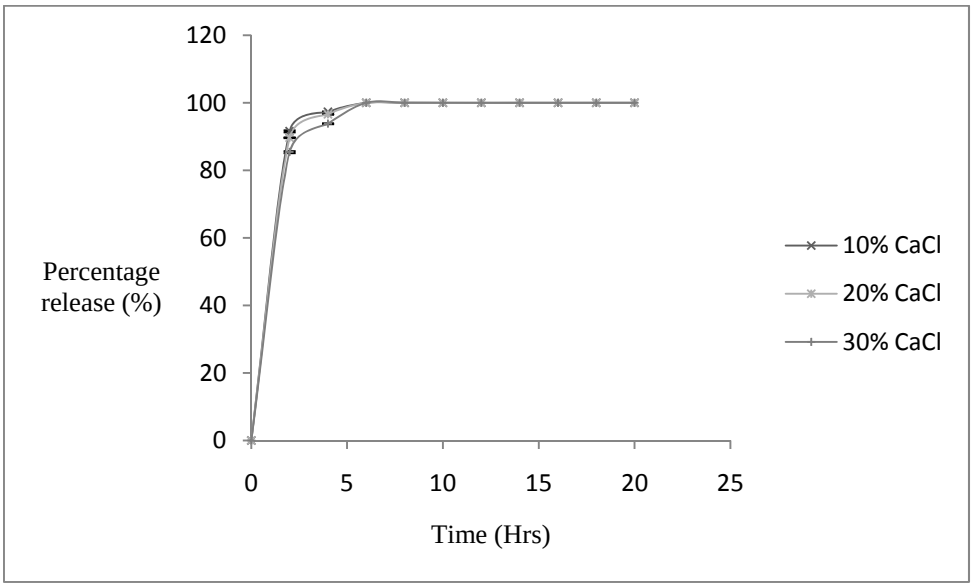

Figure 5.10 – Percentage drug release from 100 mg calcium pectinate microspheres in 300 mL PBS (pH 7.2). The effects of the various calcium chloride concentrations of 10%, 20% and 30% on the release profile are shown. Three 1 mL samples were removed and assayed by UV spectroscopy where a mean value was determined, each point represents three independent studies ± standard deviation.

From the release profile the drug was rapidly released from each batch of microspheres produced with the various calcium chloride strengths, but no change in release rate with the varying calcium chloride concentrations could be seen. The study was performed under sink conditions and the mass of drug released into the colonic media matched the theoretical result found in the DL of the particles. With the increasing calcium chloride concentration the total mass of drug released from the particles decreased. This was as expected since the DL decreases with increasing calcium chloride as discussed in the previous chapter.

The particles contained the solid 5-ASA crystals. The particles' shape adapted to that of the 5-ASA needle like crystals. It was believed that this left the crystal non-uniformly distributed in the particles where the 5-ASA crystal poked out with thin layers of pectin surrounding them (circled in Figure 5.11). It is believed that when the particles swell in the colonic media, as stated in the other release media sections, the drug rapidly drops out due to this non-uniform distribution.

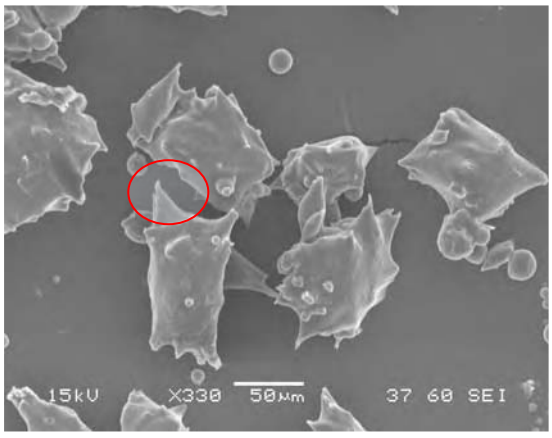

Figure 5.11 – Drug loaded particles where the particle shape adapted to the solid 5-ASA needle like structure.

It is hypothesised that there was not enough space for the solid 5-ASA crystals to occupy in the micron sized particles. As stated in the previous chapter it is believed that the pectin particles loaded with 5-ASA on the micron scale have only been achieved by a spray drying

method (Mladenovska *et al* 2007), and more commonly by particles produced on the millimetre scale by the syringe droplet method (Iruıìn *et al* 2005).

If the drug loaded particles had been produced with the syringe droplet method, it is believed that the bigger particles would have had a larger volume for the 5-ASA crystals to occupy allowing the drug to be properly encapsulated, and released at a controlled rate. The drug would be released by the colonic fluids penetrating into the particle inducing swelling; the drug would diffuse out at a slow rate since it is in a solid form though pores that are created by the colonic fluids. Particles produced with higher calcium chloride concentrations will swell at a slower rate resulting in a slower release rate.

5.4 Conclusion

The drug loaded particles produced with 2% w/v 5-ASA suspensions were put though *in vitro* studies in conditions simulating the stomach, small intestine and colon. From the results, the 5-ASA was rapidly released. Due to this rapid release a clear comparison on the effects of various calcium chloride concentrations on drug release could not be made. It was hypothesised that particles produced on the micron scale by the emulsion method had insufficient volumes to allow the 5-ASA to be properly encapsulated and released, resulting in solid 5-ASA crystals rapidly precipitating out into the release media. It is believed that a delivery vehicle composed of 5-ASA entrapped in calcium pectinate particles produced on the micron scale and showing a controlled release throughout the colon has not been discussed in the literature since most production methods are based on the syringe droplet method. For this reason it is believed that the syringe droplet method would have been better suited for encapsulation of the 5-ASA suspensions.

Chapter 6

Preliminary investigation into a polymer coating method

6.0 Introduction

The aim of this study was to attempt to coat the pectin particles. The polymer chosen for the coating was Eudragit S100 since its pH solubility threshold is pH 7. This would help to ensure that the pectin/5-ASA matrix would remain protected until the colon site is reached. The conditions that were briefly investigated were Eudragit S100 concentrations of 1%, 5% and 10% w/v and magnetic mixing speeds of 500, 1000 and 1500 rpm.

6.1 Materials

Liquid Paraffin and Span 85 were supplied by Sigma Aldrich, UK. Eudragit S100, acetone and hexane were supplied by Fisher Scientific, Leicestershire, UK.

6.2 Methods

The pectin microspheres were coated using a solvent evaporation technique (Lorenzo *et al* 1998). In this technique, a coating solution is prepared by dissolving Eudragit S100 in acetone. The particles are suspended in the coating solution which is then mixed in liquid paraffin. During the mixing process, the coating solution coats the particles which forms a solid Eudragit coating as the acetone evaporates.

Coating solutions were prepared by dissolving 0.05 g, 0.25 g and 0.5 g of Eudragit in 5 mL of acetone on a magnetic mixer at 40 ºC forming coating solutions of 1%, 5% and 10% w/v. 100 mg of microspheres were dispersed in the coating solution and the dispersion was added to an 80 mL beaker with 75 mL of liquid paraffin containing 1.7% Span 85. The mixture was mixed with a 2.5 cm mixing flea at speeds of 500, 1000 and 1500 rpm on a magnetic mixer for 3 hours to allow the complete solvent evaporation giving a core to coat ratio that was

dependent of the concentration of the coating solution used. The coated particles were collected by suction filtration and washed with three 20 mL portions of hexane and then left to dry in an air circulated oven overnight. Particles were characterised with SEM as described in the water in oil emulsion chapter.

6.3 Results and discussions

It was hypothesised that the coating solution concentration and mixing speed would affect the quality of the coating given to the particles (Figure 6.1). The uncoated particles are shown in Figure 6.2.

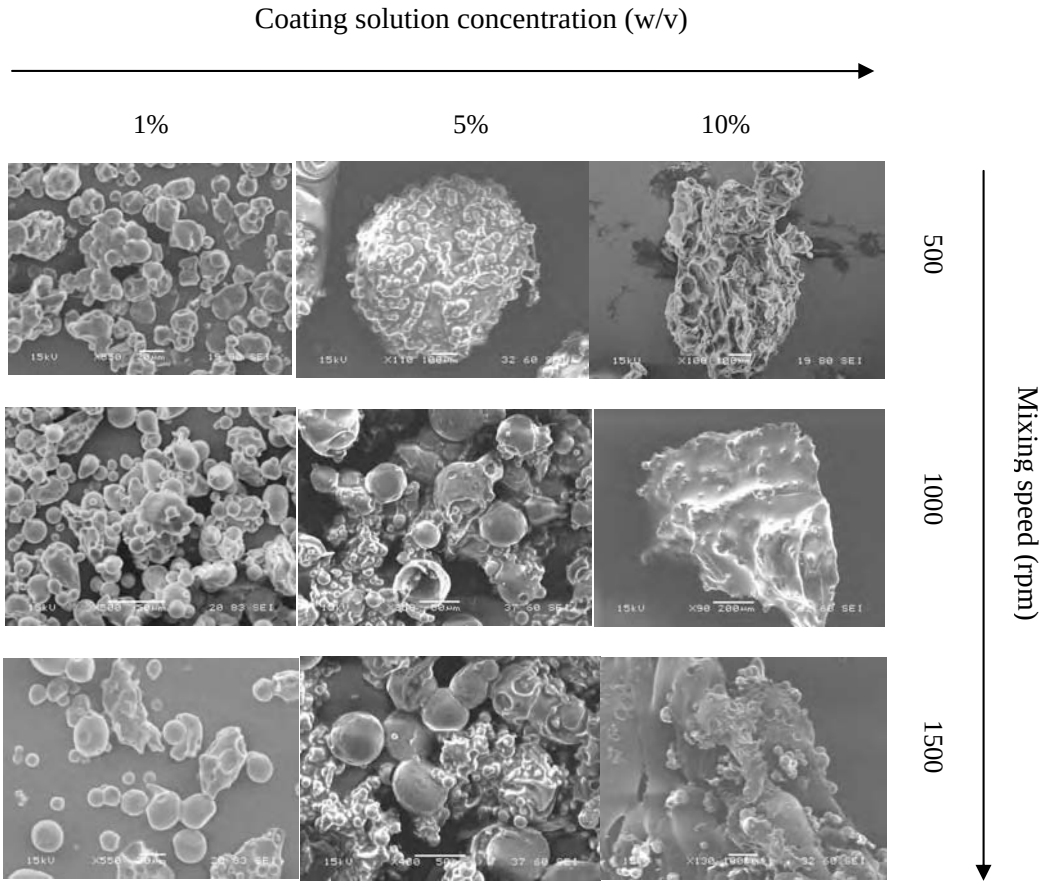

Figure 6.1 – SEM images of the attempt to coat the particles. Effects of coating solution concentration (1% left, 5% center, 10% right) and mixing speed (500 rpm top, 1000 rpm middle, 1500 rpm bottom) can be seen.

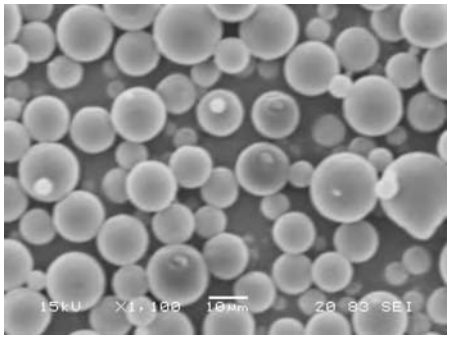

Figure 6.2 – SEM of the uncoated particles

6.3.1 Effect of mixing speed on Eudragit S100 coating

Mixing speeds of 500, 1000 and 1500 rpm were investigated in an attempt to coat the particles. Mixing speeds of 500 rpm produced what appeared to be a "ball" of Eudragit. It is believed that the particles were not been sufficiently mixed throughout the liquid paraffin since it was observed that the majority of the population of particles trailed along the base of the beaker during the mixing process. As this mixing speed was increased to 1000 and again to 1500 rpm, the particles were being mixed sufficiently throughout the liquid paraffin where it was believed that the particles gained some degree of coating judging by the size increase from approximately 10 microns in the uncoated particles to approximately 50 microns in the coated particles (Figure 6.3).

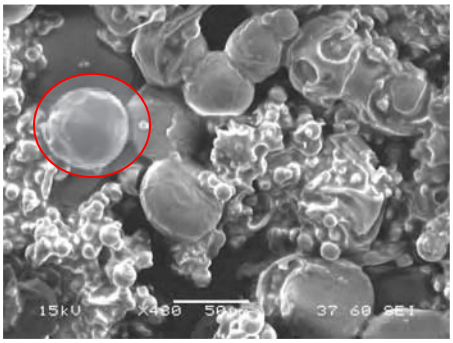

Figure 6.3 – SEM of what is believed to be coated pectin particles.

Paharia *et al* (2007) and Lorenzo *et al* (1998) have successfully coated pectin particles by solvent dehydration method at mixing speeds of 1000 rpm on a propeller mixer producing coated particles with spherical geometries approximately 100 microns in diameter; however it is believed other mixing speeds have not been discussed in the literature.

Paharia *et al* (2007) and Lorenzo *et al* (1998) both used a propeller mixer to coat their particles and judging from the better agitation the over head mixer gave opposed to the magnetic mixer during the production of the pectin particles discussed in chapter 3, it is believed that the higher agitation levels would allow the solvent dehydration to occur at a better rate yielding individually coated particles.

6.3.2 Effect of coating solution concentration

The coating solution concentration affects the core to coat ratio that is give to the particles. 10% w/v was originally used as investigated Paharia *et al* (2007) and Lorenzo *et al* (1998) however this led to large Eudragit masses being produced where the particles were believed to be stuck inside. Had the particles been produced with overhead mixing, it may have been possible that this concentration would have given a better coating quality since both authors using propeller mixers for their studies.

The coating solution was reduced to 5% w/v. Towards the end of the mixing process, small white spherical beads could be seen though the liquid paraffin which were believed to be spheres with some coating. Due to the decrease in the coating solution concentration, it is believed that this allowed the Eudragit to solidify at a faster rate during the solvent evaporation process yielding particles with some degree of coating as shown in the SEM images (Figure 6.3). Also the size of the particles produced in this study with 5% w/v solutions were smaller (50 microns) than particles that produced by Paharia *et al* (2007) with

10% w/v solutions (100 microns) showing that the reduction in coating solution concentration could be affecting the core: coat ratio.

6.4 Conclusion

In this chapter, initial coating studies were carried out in an attempt to coat the particles. From the results it was seen that the quality of coating given to the particles could be affected by the processing conditions. From the products that were produced, it was believed in order to produce high quality coats, a sufficient level of agitation may be required to allow the successful solvent dehydration during the coating procedure. Also it is believed that choice of polymer solution concentration could possibly affect the core: coat ratio where the higher concentrations would give thicker coats. Further investigation into coating is suggested as part of future work.

Chapter 7

Final conclusions and future work

7.0 Final conclusions

This project looked into the development of calcium pectinate particles for colonic delivery via the oral route. Two particle production methods were investigated, the syringe droplet method and the water in oil emulsion method. In the syringe droplet method, the effects of calcium chloride concentration, pectin concentration and magnetic mixing speed were investigated. From the investigated parameters it was seen that mixing speeds of 500 rpm and pectin concentrations of 5% w/v were needed to produce spherical particles and the increase in calcium chloride concentration decreased the pectin's permeability. The parameters investigated in the water in oil emulsion method were surfactant concentration, magnetic and overhead mixing speed and calcium chloride concentration. From the results it was found that surfactant concentrations of 5% w/v and overhead mixing speeds of 1500 rpm were needed in order to produce particles with high orders of sphericity and the increase in calcium chloride concentration reduced the particles ability to swell. Also the higher degree of mixing produced with the overhead stirrer also helped in producing spherical particles.

5-ASA was loaded into the particles produced with the water in oil emulsion method. Due to the drug's solubility, the drug was not successfully encapsulated in solution; therefore loading through suspension was investigated. Various suspension concentrations were investigated along with the effects of calcium chloride concentration on drug loading. From the results it was seen that drug loading decreased with increasing calcium chloride concentrations however the loaded drug caused the particles to form irregular structures.

The particles loaded with 5-ASA suspension were subject to *in vitro* studies simulating the stomach, small intestine and colon. The effects of calcium chloride concentration on drug

release rate were investigated. The release profiles indicated that the drugs were rapidly released in all simulations. This was believed to be due to the incompatibility between micron sized particles and large 5-ASA crystals, causing the crystals to rapidly drop out into the release media during the swelling process. It is believed that the bigger particles produced with the syringe droplet method may have been better suited for the encapsulation of 5-ASA and controlled release.

Some initial coating studies for the particles were attempted looking into processing conditions of mixing speed and coating solution concentration. It was observed that the addition of an effective coating could be down to using sufficient agitation to allow the effective solvent evaporation, to produce the solid coats. Also, the increase in coating solution concentration could affect the core: coat ratio.

7.1 Future work

A number of studies could be preformed to tie up the final conclusions. Firstly, the 5-ASA could be re-entrapped (both in solution and suspension) into the calcium pectinate particles produced by the syringe droplet method. Also various drugs with different physiochemical characteristics could be entrapped using the water in oil emulsion method to see whether or not the 5-ASA is just not compatible with the micron sized calcium pectinate microparticles produced using the emulsion method. Once entrapped, these new particles would then undergo the *in vitro* testing.

The particles then could undergo further coating studies in which the use of the overhead mixer at various speeds (500 – 2000 rpm) would be investigated, to see if better agitation would give an improved solvent dehydration during the coating process. Various coating solution concentrations (1% - 10% w/v) and possibly various surfactant concentrations could also be explored.

Siepmann *et al* (2007) have discussed the use of various polymer blends that can be used to coat particles that have changed the release rate and mechanisms of the delivery vehicle. Using polymer blends can be an alternative to adding plasticizers and can overcome sticky and brittle coats from being formed. Ideally two polymers with different physicochemical characteristics in the right blend can dramatically alter the delivery system. For example, blends of Eudragit S100 and Eudragit L100 have been blended in various ratios showing different release patterns. The use of polymer blends can also prevent the coat from ruptuing by using polymers with more flexability where Eudragit NE, a flexible pH repsonsive polymer, can prevent brittle coatings from rupturing. Some polymers are also known to prevent degradation in long periods of storage.

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