Development and Evaluation of Sustained Release Alginate Beads Containing Tetrahydrocurcumin and Curcumin

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Thesis Title	Development and evaluation of sustained release alginate beads containing tetrahydrocurcumin and curcumin
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This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

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I hereby certify that this work has not already been accepted in substance for any degree, and is not being concurrently submitted in candidature for any degree.

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(Miss Sirirat Sriraksa)

Candidate

ชื่อวิทยานิพนธ์ การพัฒนาและประเมินอัลจิเนตบีดชนิดออกฤทธิ์เนิ่นที่บรรจุเตทตระไฮโดรเคอคูมินและเคอคูมิน

ผู้เขียน นางสาวศิริรัตน์ ศรีรักษา

สาขาวิชา เภสัชศาสตร์

ปีการศึกษา 2556

บทคัดย่อ

วัตถุประสงค์ของงานวิจัยคือ การพัฒนาแคลเซียมอัลจิเนตบีดชนิดออกฤทธิ์เนิ่นที่บรรจุสูตรตำรับเซลฟ์ไมโครอิมัลซิฟายด์ของสารประกอบละลายน้ำยากสองชนิด ได้แก่ เตทตระไฮโดรเคอคูมินและเคอคูมิน เตทตระไฮโดรเคอคูมินและเคอคูมินเป็นสารประกอบฟีนอลิคที่ไม่ชอบน้ำได้จากเหง้าของขมิ้นชัน ซึ่งมีฤทธิ์ทางด้านเภสัชวิทยาหลากหลาย ได้แก่ ฤทธิ์ต้านอนุมูลอิสระ ฤทธิ์ต้านการอักเสบ และฤทธิ์ต้านการเกิดมะเร็ง อย่างไรก็ตาม สารประกอบทั้งสองชนิดมีค่าการละลายน้ำต่ำ ซึ่งเป็นข้อจำกัดที่สำคัญจากการใช้ประโยชน์ในการรักษาของเตทตระไฮโดรเคอคูมินและเคอคูมิน

แคลเซียมอัลจิเนตบีดแบบลอยตัวที่บรรจุสูตรตำรับเซลฟ์ไมโครอิมัลซิฟายด์ของเตทตระไฮโดรเคอคูมินถูกเตรียมด้วยวิธีไอโอโนโทรปิคเจลเลชั่น และอาศัยเทคนิคการฟูของก๊าซ ความเข้มข้นของโซเดียมอัลจิเนต แคลเซียมคลอไรด์ สารก่อรูพรุนชนิดละลายน้ำ (คอลลีโคทไอาร์) ในสูตรตำรับบีด และวิธีการทำบีดให้แห้ง มีผลต่อความสามารถในการลอยตัวและอัตราการปลดปล่อยเตทตระไฮโดรเคอคูมิน สูตรตำรับที่เหมาะสม (E1) ประกอบด้วย สูตรตำรับเซลฟ์ไมโครอิมัลซิฟายด์ของเตทตระไฮโดรเคอคูมิน 10% โซเดียมอัลจิเนต 2% แคลเซียมคลอไรด์ 0.05 โมลาร์ คอลลีโคทไอาร์ 3% และทำให้แห้งด้วยเครื่องเตาอบลมร้อนที่อุณหภูมิ 50 องศาเซลเซียส เป็นเวลา 12 ชั่วโมง สูตรตำรับที่เหมาะสมสามารถควบคุมการปลดปล่อยของ

เตทตระไฮโดรเคอคูมิน ภายในเวลา 8 ชั่วโมง โดยมียาถูกปลดปล่อยประมาณ 80% และสามารถลอยตัวอย่างรวดเร็วภายในเวลาประมาณ 30 วินาทีและลอยอยู่ได้นานมากกว่า 8 ชั่วโมง ขนาดของไมโครอิมัลชั่นที่ปลดปล่อยจากบีดมีค่า 31.5±0.1 นาโนเมตร ซึ่งมีค่าใกล้เคียงกับสูตรตำรับเซลฟ์ไมโครอิมัลซิฟายด์ชนิดของเหลวของเตทตระไฮโดรเคอคูมิน (30.0±0.3 นาโนเมตร)

แคลเซียมอัลจิเนตบีดที่บรรจุสูตรตำรับเซลฟ์ไมโครอิมัลซิฟายด์ของเคอคูมินถูกเตรียมด้วยวิธีไอโอโนโทรปิคเจลเลชั่น และเคลือบด้วยไคโตซาน การเกิดสารประกอบเชิงซ้อนพอลิอิเล็กโทรไลต์ระหว่างอัลจิเนตและไคโตซาน และค่า pH ของสารละลายตัวกลางที่ใช้ศึกษามีผลต่อพฤติกรรมการปลดปล่อยเคอคูมินจากบีด การปลดปล่อยเคอคูมินจากสูตรตำรับต่างๆ ที่ pH 1.2 มีการปลดปล่อยยาที่จำกัด ในขณะที่ pH 6.8 สูตรตำรับ F2 ให้ค่าการปลดปล่อยยาสูงสุด สูตรตำรับที่เหมาะสม F2 ประกอบด้วย สูตรตำรับเซลฟ์ไมโครอิมัลซิฟายด์ชนิดของแข็งของเคอคูมิน 5% โซเดียมอัลจิเนต 2.5% แคลเซียมคลอไรด์ 0.1 โมลาร์ และเคลือบด้วยไคโตซาน 0.25% สามารถควบคุมการปลดปล่อยของเคอคูมินภายในเวลา 10 ชั่วโมง โดยมียาถูกปลดปล่อยประมาณ 70% ขนาดของไมโครอิมัลชั่นที่ปลดปล่อยจากบีดมีค่า 30.7±0.2 นาโนเมตร ซึ่งมีค่าใกล้เคียงกับสูตรตำรับเซลฟ์ไมโครอิมัลซิฟายด์ชนิดของเหลวของเคอคูมิน (29.2±0.7 นาโนเมตร)

โดยสรุป การพัฒนาสูตรตำรับแคลเซียมอัลจิเนตบีดชนิดออกฤทธิ์เนิ่นที่บรรจุสูตรตำรับเซลฟ์ไมโครอิมัลซิฟายด์เป็นรูปแบบยาเตรียมชนิดของแข็งที่มีศักยภาพในการเพิ่มอัตราการละลายและการดูดซึมของเตทตระไฮโดรเคอคูมินและเคอคูมินเมื่อให้โดยการรับประทานได้

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ABSTRACT

The aim of this study was to develop sustained release calcium alginate beads containing self-microemulsifying formulations of two poorly water soluble compounds, tetrahydrocurcumin (THC) and curcumin. THC and curcumin are hydrophobic polyphenol compounds derived from *Curcuma longa* L. Both compounds exhibit numerous pharmacological activities such as anti-oxidant, anti-inflammatory and anti-carcinogenic properties. However, they have low aqueous solubility, which is an important restriction on their therapeutic usefulness.

Floating calcium alginate beads containing self-microemulsifying formulation of THC were prepared by ionotropic gelation method and based on gas formation technique. The concentration of sodium alginate, calcium chloride and water soluble pore former (Polyvinylalcohol- polyethylene glycol copolymer; Kollicoat® IR) in bead formulations, and the drying method had different effects on the floating abilities and *in vitro* rate of THC release. The optimal formulation (E1) contained 10% w/v THC self-emulsifying mixture, 2% w/v sodium alginate, 0.05 M calcium chloride, 3% w/v Kollicoat® IR and dried by hot air oven at 50°C 12 h. It provided a sustained release of about 80% of THC over an 8 h period from floating calcium alginate beads with short floating lag time within 30 seconds and prolonged floating duration (>8 h). The droplet size of the microemulsion released from the beads was in the range of 31.5±0.1 nm, which was similar to that of the liquid THC self-microemulsifying formulations (30.0±0.3 nm).

Calcium alginate beads containing self-microemulsifying formulation of curcumin were prepared by ionotropic gelation method followed by coating with chitosan. The presence of the polyelectrolyte complex between alginate and chitosan and the pH of dissolution medium had a significant effect on drug release behaviors

from the beads. The *in vitro* release of curcumin from various formulations was limited at pH 1.2, whereas the release was fast for chitosan coated alginate beads (F2) at pH 6.8. The optimal formulation F2 contained 5% w/v solid curcumin-SMEDDS, 2.5% w/v sodium alginate, 0.1 M calcium chloride and coated with 0.25% w/v chitosan. It provided a sustained release of about 70% of curcumin from the beads over an 10 h period. The droplet size of the microemulsion released from the beads was in the range of 30.7±0.2 nm, which was similar to that of the liquid curcumin self-microemulsifying formulations (29.2±0.7 nm).

In conclusion, the developed sustained release calcium alginate beads containing self-microemulsifying formulations provided a useful solid dosage form with the potential to improve dissolution and oral absorption of THC and curcumin.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 General Introduction

Curcumin is a hydrophobic polyphenol compound derived from the powdered rhizome of *Curcuma longa* L. It has a wide biological and pharmacological profile. Tetrahydrocurcumin (THC), as one of the major metabolites of curcumin (Pan *et al.,* 1999), has been widely used in pharmaceutical and cosmetic preparations. THC and curcumin have identical Beta-diketone structures and phenolic group, but differ in that THC lacks the double bonds. THC was reported to exhibit similar physiological and pharmacological properties including anti-oxidative, anti-inflammatory and anti-carcinogenic activities as curcumin (Sugiyama *et al.,* 1996; Okada *et al.,* 2001; Johnson and Mukhtar, 2007; Sandur *et al.,* 2007; Menon and Sudheer, 2007; Hsu *et al.,* 2007; Murakami *et al.,* 2008; Jurenka, 2009). Although these compounds possesses multiple medicinal benefits, their clinical application are limited due to their low aqueous solubility (Kaminaga *et al.,* 2003), poor absorption and extensive metabolism (Pan *et al*., 1999; Anand *et al*., 2007) which results in low oral bioavailability (BA). In addition, a relative short gastric emptying time can result in an incomplete release of compounds from the dosage form at the site of absorption, and lead to a reduced efficacy of the administered dose.

To improve the bioavailability of curcumin and THC, different approaches have been investigated. These approaches include forming curcumin phospholipid complex, o/w nanoemulsions, nanoparticle encapsulation, self-emulsifying system (Maiti *et al.,* 2007; sheikh *et al.,* 2009; Setthacheewakul *et al.,*

2010). One of the most popular and commercially suitable approaches is to formulate self-emulsifying drug delivery systems (SEDDS). SEDDS are isotropic mixtures of oils, surfactants and co-surfactants which form fine oil-in-water (o/w) emulsions upon mild agitation in aqueous media such as gastrointestinal (GI) fluids (Constantinides, 1995). SEDDS are considered as an attractive approach because of the improvement in solubility, dissolution and oral absorption of poorly water soluble compounds. However, the normally prepared SEDDS in liquid form produces some disadvantages for example the cost of manufacturing process is high, low stability and portability. To overcome these problems, solid SEDDS have been introduced as alternative methods (Prajapati and Patel, 2007). Such systems require the solidification of liquid self-emulsifying (SE) ingredients into powders/ nanoparticles to create various solid dosage forms such as SE nanoparticle, SE tablets and SE pellets (Hu *et al.*, 2004; Wei *et al*., 2007; Setthacheewakul *et al.*, 2011; Sermkaew *et al.*, 2013). Alginate beads containing self-emulsifying formulation of curcumin or THC are a proposed novel oral delivery system in this study. Sodium alginate has several unique properties that have enabled it to be used as a matrix for the entrapment of a variety of drug, proteins and cells. Sodium alginate is naturally occurring polysaccharides obtained from marine brown algae and can form gel in the presence of divalent cations such as Ca^{2+}. The gelation is explained by the egg-box model in which divalent cation binds to two carboxyl groups on the adjacent alginate molecules (Bajpai *et al.*, 2004). Moreover, the alginate bead preparation utilizes simple technique and there is no use of any organic solvent.

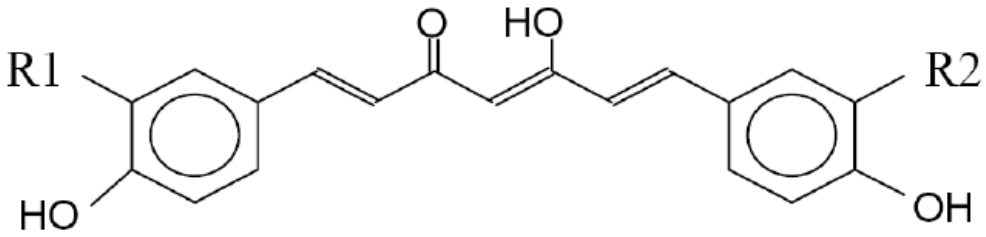
The objective of this study was to develop calcium alginate beads containing self-microemulsifying (SME) formulation of THC or curcumin to improve

the solubility, dissolution and provide sustained release of these active compounds. The optimized bead formulations were evaluated for their physical properties and *in vitro* drug release.

1.2 Curcumin and Tetrahydrocurcumin

1.2.1 Introduction

Turmeric has been used for many ailments, particularly as an anti-inflammatory agent, and curcumin has been identified as the active principle of turmeric (Aggarwal *et al.*, 2003). It is the yellow pigment presenting in rhizome of turmeric of *Curcuma longa* L. belonging to the Zingiberaceae family. The fractions of turmeric known as curcuminoids are mixture of three principal compounds: 77% diferuloylmethane (curcumin), 17% demethoxycurcumin, and 6% bisdemethoxycurcumin. (Anand *et al.*, 2007). The structures of these compounds are presented in Figure 1.1.



Curcumin I : R1 = R2 = OCH_3

Curcumin II: R1 = OCH_3, R2 = H

Curcumin III : R1 = R2 = H

Figure 1.1 Chemical structures of curcuminoids (Aggarwal *et al.*, 2007).

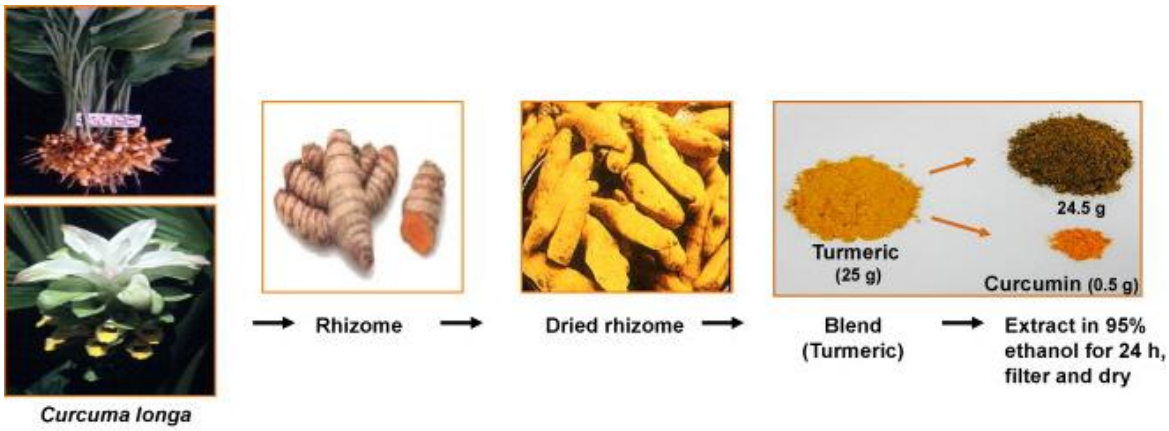


Figure 1.2 The extraction of curcumin (Goel *et al.*, 2008)

Curcumin is a hydrophobic polyphenol compound. It has a widely spectrum of the pharmacological activities such as exhibit anti-inflammatory (Rao *et al.*, 1982: Lukita *et al.*, 2002), anti-carcinogenic (Mahady *et al.*, 2002; Yoysungnoen *et al.*, 2008), anti-oxidant activities (Ruby *et al.*, 1995; Sugiyama, 1996), and low toxicity with promising clinical application (Hsu *et al.*, 2007). Curcumin, an orange-yellow crystalline powder, has a molecular weight of 368.38 Da and a melting point of 183ºC. Its molecular formula is $C_{21}H_{20}O_6$ ((1E, 6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-Dione). Chemically, curcumin is a bis-α,β-unsaturated β-diketone (commonly called diferuloylmethane). Curcumin is practically insoluble in water at acidic or neutral pH. The water solubility of curcumin was found to be 30 pmol/mL (Kaminaga *et al.*, 2003), and the maximum solubility of which in aqueous buffer (pH 5.0) was reported to be 11 ng/mL (Tonnesen *et al.*, 2002). Curcumin dissolves in ethanol, acetone, dimethylsulfoxide and glacial acetic acid.

Wang *et al.* (1997) studied the degradation kinetics of curcumin in buffer solutions at pH ranging from 3 to 10, 37°C. The result showed that decomposition of

curcumin was pH-dependent and occurred faster at neutral-basic pH conditions. When curcumin was incubated in 0.1 M phosphate buffer, pH 7.2 at 37°C, more than 90% decomposed within 30 min (Figure 1.3). Curcumin is unstable at neutral and basic pH values. It would be stable in the stomach and small intestine, since degradation of curcumin is extremely slow at pH between 1 and 6.

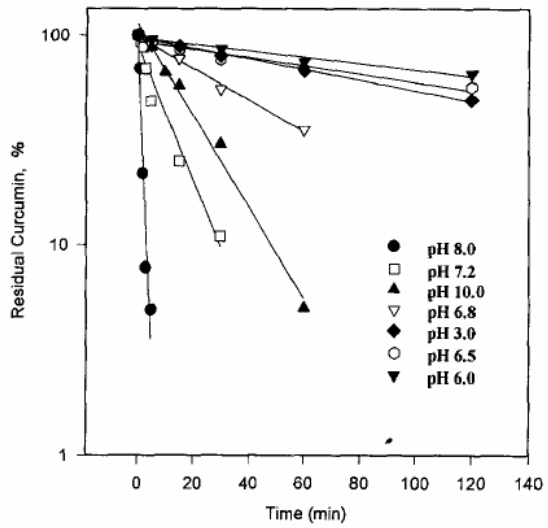
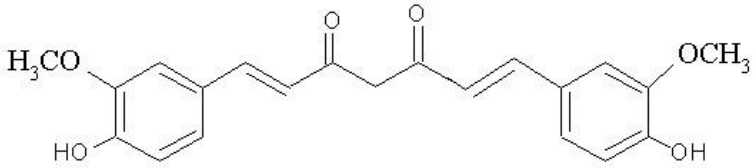


Figure 1.3 Apparent first-order plots for the degradation of curcumin at various pH values. The data are normalized to a value of 100 at zero time. Points represent the experimental data and the solid lines were drawn using linear least-squares regression analysis (Wang *et al.*, 1997)

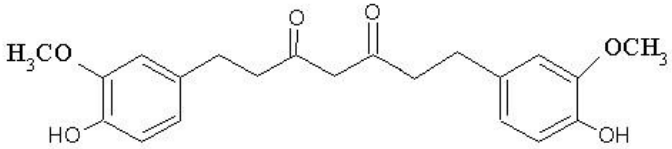
Tetrahydrocurcumin (THC) is one of the major metabolites of curcumin (Pan *et al.,* 1999). It was reported to exhibit the same physiological and pharmacological properties of curcumin, but this compound has more potent than in antioxidant activity in both *in vitro* and *in vivo* systems (Sugiyama *et al.,* 1996) and the mechanism could be implied by β-diketo moiety structure (Okada *et al.,* 2001). Furthermore, Pari and Murugan, (2006) reported that the anti-diabetic and anti-oxidant effects of THC are more efficacy than those of curcumin at the same dose (80

mg/kg body weight of rats) after oral administration. In addition, toxicity studies of THC published in peer-reviewed literatures are somewhat limited. There is a paper reporting that a dose of 3000 mg/kg of THC per day orally administered in mice did not show any toxicity (Yoysungnoen *et al.*, 2008).

Structurally, THC and curcumin have identical β-diketone structures and phenolic groups, but THC lacks the double bonds of curcumin (Sugiyama *et al.*, 1996; Okada *et al.*, 2001) (Figure 1.4).



Structure of curcumin



Structure of tetrahydrocurcumin

Figure 1.4 Chemical structures of curcumin and tetrahydrocurcumin (Murugan and Pari, 2007)

THC, in a white to off-white powder form, has a molecular weight of 372.41 Da and melting points between 85 and 100 ºC. The stability of THC at 37°C in phosphate buffers of various pH is presented in Figure 1.5. THC is very stable in 0.1 M phosphate buffers at pH ranges between of 3-10. THC was not decomposed after incubation in 0.1 M phosphate buffer for 8 h, regardless of pH (Pan *et al.*, 1999).

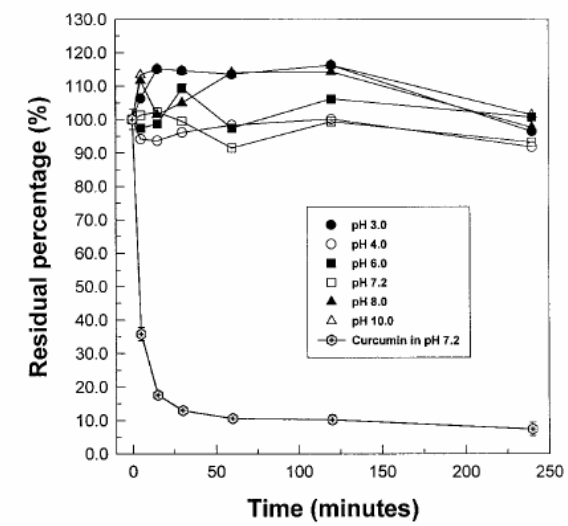


Figure 1.5 Effect of pH on the stability of THC (Pan *et al.*, 1999)

One of the reasons for the low bioavailability of curcumin and THC are that to be its curcumin is almost insoluble in water at acidic or neutral conditions, making it difficult to absorb (Maiti *et al.*, 2007).

1.2.2 Delivery systems for improved oral bioavailability of curcumin

To improve the bioavailability of curcumin numerous approaches have been investigated.

Recently, nanoparticles have been used as vehicles for drug delivery systems to improve bioavailability of curcumin. The curcumin is encapsulated in cross-linked polymeric particles with a hydrophobic core and a hydrophilic shell. The particles size of nanocurcumin less than 100 nm. The nanocurcumin was tested on pancreatic cancer cells and reported to be effective in inhibition of these cells and has similar *in vitro* activity as that of free curcumin in pancreatic cancer cells (Bisht *et al.*, 2007).

Solid lipid nanoparticles (SLNs) are usually consisting of natural or synthetic lipid or lipoid. The particles are in the solid at room temperature (Pardeike *et al.,* 2009). SLNs dosage forms provide the advantages such as, protect labile compounds from chemical degradation, provide sustained release, and target the effect to improve the efficiency of the drugs (Zhu *et al.,* 2009). Kakkar *et al.* (2011) prepared curcumin-SLNs using the microemulsification method. The mean particle sizes of curcumin-SLNs were 134.6 nm and the drug content of 92.33±1.63%. *In vivo* studies demonstrated significant improvement in bioavailability after administration of C-SLNs at all the doses other than curcumin suspension.

To overcome these problems, self-microemulsifying drug delivery systems (SMEDDS) have been introduced as the most popular and commercially methods. This system is basically consist of isotropic mixtures of oils, surfactants, co-solvents and drug compound which have the ability to form o/w microemulsion when it comes in contact with aqueous medium in GI tract after oral intake (Borhade *et al.,* 2008). The curcumin-SMEDDS formulation was composed of 57.5% surfactant, 30% cosurfactant and 12.5% oil. The in situ evaluation of this formulation showed that the absorption percentage of curcumin-loaded SMEDDS was 3.86 times higher than that of curcumin suspension (Cui *et al.,* 2009).

To improve the absorption of curcumin are numerous studies approaches of delivery systems. All of these studies have concluded that it is possible to develop formulations and methods which can improve the bioavailability and give higher plasma concentrations.

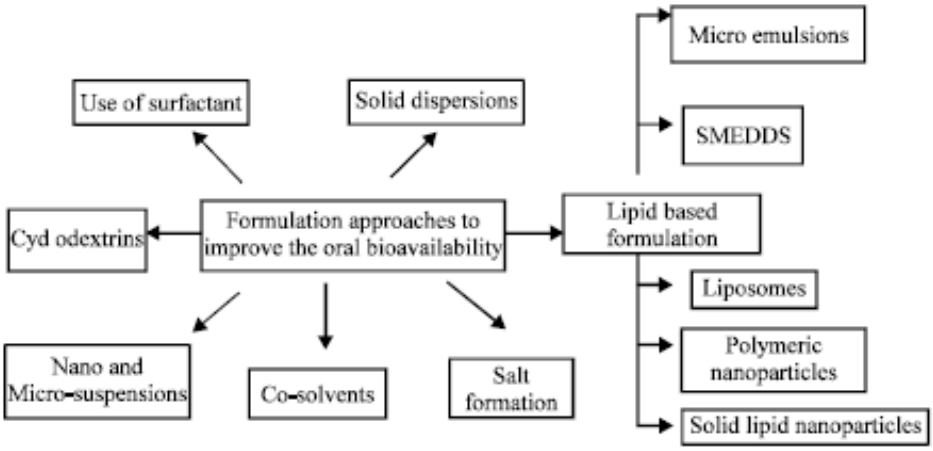


Figure 1.6 Some formulation approaches to improve the oral bioavailability of poorly water soluble drugs (Rajput *et al.,* 2012)

1.3 Solid SEDDS

1.3.1 Introduction

Approximately 35-40% of new drug candidates have poor water solubility. The oral delivery of such drugs is frequently associated with low bioavailability (Vijay *et al.,* 2011). To overcome these problems, different formulation methods have been developed to improve the oral bioavailability such as cyclodextrins, nanoparticles and solid dispersions (Gursoy *et al.,* 2004; Abdalla *et al.,* 2008). Recently, SEDDS have been focused in recent years as drug carriers to improve the oral bioavailability of insoluble lipophilic compounds.

SEDDS are isotropic mixtures oils, surfactants, co-solvents/surfactants and drug compounds. Generally, SE formulations form a fine emulsion when contact with aqueous medium in conditions of gentle agitation (Figure 1.7). The resulting o/w emulsions are thermodynamically stable due to the relatively small volume of the

dispersed oil phase. A narrow range of droplet size distribution with small size is also. The emulsions droplet size of SEDDS was between 100 and 300 nm while SMEDDS form transparent microemulsions with a droplet size of less than 50 nm (Shah *et al.*, 1994). Fine oil droplets would pass rapidly from the stomach and promote wide distribution of the drug throughout the GI tract, thereby minimizing the irritation frequently encountered during extended contact between bulk drug substances and the gut wall. SEDDS have gained considerable interest after the commercial success of Sandimmune Neoral (Cyclosporine A), Fortovase (Saquinavir) and Norvir (Ritonavir). The advantages of SEDDS such as easily manufactured, improvement in oral bioavailability, quick onset of action, reduce drug dose, increased drug loading, no effect of lipid digestion process, increasing absorption by surfactant induced membrane fluidity, and then permeability changes (Hauss and Fogal, 1998; Patel and Chaulang, 2008)

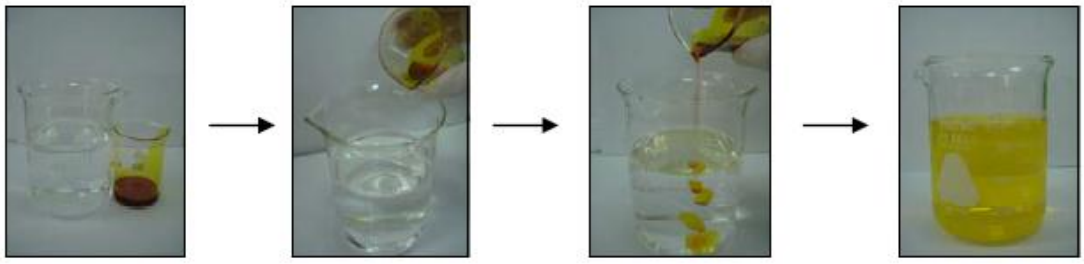


Figure 1.7 Photographic images of self-emulsifying systems introduced into distilled water (Setthacheewakul *et al.*, 2010)

However, the liquid SEDDS formulations exhibit some disadvantages as the incorporation of liquid into the soft or hard gelatin capsules can lead to drug leakage and precipitation as well as to problems with capsule aging (Wang *et al.*,

2010), the cost of the manufacturing process is high. More importantly, the high amount of surfactants present in these formulations can induce GI side-effects.

To overcome these problems of liquid SEDDS, solid SEDDS have been put forward as an alternative approaches. Such systems require the solidification of liquid SEDDS ingredients into powders/nanoparticles to create several solid dosage forms such as SE granules (Dixit and Nagarsenker, 2008; Beg *et al.,* 2013), SE nanoparticle (Yunxia *et al.,* 2005; Trickler *et al.,* 2008), SE tablets (Wei *et al.,* 2007; Sermkaew *et al.,* 2013) and SE pellets (Abdalla *et al.,* 2007; Setthacheewakul *et al.,* 2010; Setthacheewakul *et al.,* 2011) and so on. Solid SEDDS dosage forms provide the advantages such as low production cost, easily of process control, high stability and reproducibility, better patient compliance. Recently, there have been various studies that mainly attention on the preparation and characterization of solid SEDDS dosage form.

1.3.2 Solidification of liquid to solid SEDDS

1.3.2.1 Adsorption to solid carriers

The liquid SEDDS are adsorbed onto inert carriers to produce solid SEDDS by mixing in a blender. A formulation of liquid SMEDDS which is converted to solid SMEDDS using maltodextrin as a solid carrier was represented in Figure 1.8.

Figure 1.8 Liquid SMEDDS and solid SMEDDS before and after diluted with water (Ito *et al,* 2005).

1.3.2.2 Spray drying

The formulation is prepared by mixing lipids, surfactants, drug, solid carrier, and solubilization the mixture before spraying into a drying chamber through a nozzle. The volatile vehicles first evaporate leaving behind small solid particles. These particles can be further processed into capsules or compressed into tablets (Yi *et al,* 2008).

1.3.2.3 Dry Emulsion

Dry emulsions are powders in which emulsion spontaneously occurs *in vivo* or after exposed to an aqueous solution. The powder of dry emulsions are typically prepared from o/w emulsions containing a solid carrier (for example lactose, maltodextrin) in the aqueous phase by freeze-drying (Hansen *et al.,* 2004), spray drying (Toorisaka *et al.,* 2005; Jang *et al.,* 2006; Cui *et al.,* 2007), or rotary evaporation (Christensen *et al.*, 2001).

1.3.3 Dosage forms of solid SEDDS

1.3.3.1 Self-emulsifying nanoparticles

Nanoparticle methods have been used in the production of SE nanoparticles. Solvent injection is one of these methods in which the lipid, surfactant, and drugs compound were melted together, and then injected drop wise into a stirred non-solvent. The resulting SE nanoparticles were thereafter filtered out and dried. These method yielded nanoparticles of about 100 nm with a high drug loading efficiency of 74% (Attama and Mpamaugo, 2006).

1.3.3.2 Self-emulsifying pellets

SEDDS have been widely formulated as pellets. Pellets are prepared by extrusion-sphereonization technique. Setthacheewakul *et al.* (2010) successfully development and evaluation of a new SMEDDS in liquid and pellet formulations of curcumin that result in improved solubility and oral absorption. The study demonstrated that, a new SMEDDS in both forms are promising approaches for the formulation of lipophilic compounds with low oral bioavailability. Setthacheewakul *et al.* (2011) successfully developed novel self-emulsifying floating drug delivery systems (SEFDDS) in both forms of THC that result in improved solubility. The study demonstrated that, the optimum formulation of floating pellets containing SEDDS of THC had a floating efficiency of 93% at 6 h. It provided a controlled release of about 80% within 2 h and 80% within 8 h of liquid and pellets forms, respectively, of SEDDS-THC over an 8 h period were significantly higher than that of unformulated THC (only 30% within 8 h).

1.3.3.3 Self-emulsifying tablets

Novel SME floating tablets were developed by Sermkaew *et al.* (2012). The optimum formulation of floating tablets containing SME of THC with short floating lag time together with a prolonged buoyancy (>12 h) and provided a controlled released of about 72% of THC from tablets over an 12 h period.

1.3.3.4 Self-emulsifying microspheres/ beads

You *et al.* (2005) prepared solid SEDDS sustained-release microspheres using the quasi-emulsion-solvent-diffusion method for the spherical crystallization technique. The release of zedoary turmeric oil (ZTO) in the formulation could be controlled by the ratio of aerosil 200 to hydroxypropyl methylcellulose acetate succinate (HPMCAS-HG). The plasma concentration time profiles were achieved after oral intake of microspheres into rabbits, with a bioavailability of 135.6%, respect to the conventional liquid SEDDS. (Figure 1.9)

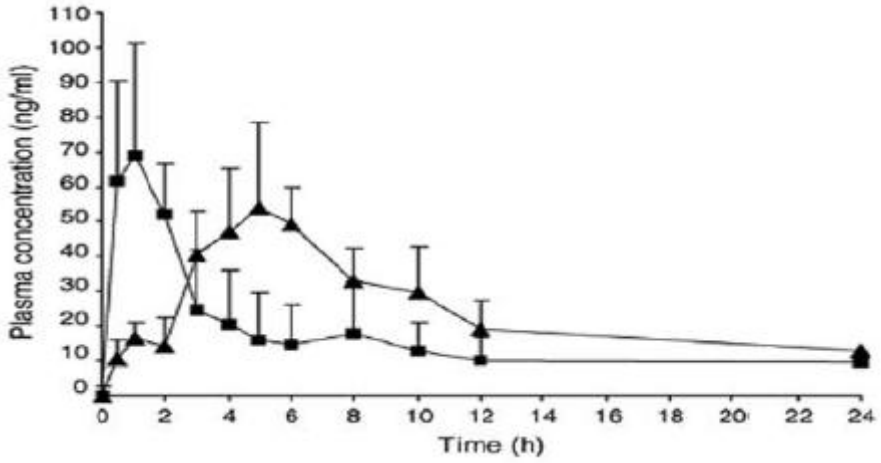


Figure 1.9 The mean plasma concentration–time profiles after oral intake (160 mg/kg ZTO dose) of the conventional SES and the SE microspheres. Each point represents the mean (±SD) (n = 6).

Patil and Paradkar (2006) discovered loading SEDDS into microporous polystyrene beads (PPB) using the solvent evaporation method. PPB with complex internal void structures was typically produced by copolymerising styrene and divinyl benzene. They are inert and stable over a wide pH range, temperature and humidity. Geometrical features, such as bead size and pore architecture of PPB, were found to govern the loading efficiency and *in vitro* drug release from the SEDDS-loaded PPB.

1.4 Polymers for drug delivery

1.4.1 Sodium alginate

1.4.1.1 Sources of sodium alginate

Sodium alginate is a polysaccharide mainly occurring as a skeletal component of all marine brown algae (*Phaeophyta*) (Painter, 1983). A polymeric material resembling alginates from brown algae is, however, produced as a capsular component in soil bacteria such as *Azotobacter vinelandii* (Gorin and Spencer, 1966) and several species of *Pseudomonas* (Govan *et al*., 1981; Linker and Jones, 1966). In particular, the alginate-secreting bacterium *Pseudomonas aeruginosa* in cystic fibrosis patients (Pedersen *et al.,* 1990) has gained much focus in recent years. All of these algae, sodium alginate is the primary polysaccharide present and it may comprise up to 40% of the dry weight. The sodium alginate forms mixed salts with numerous cations naturally found in seawater including magnesium, calcium, strontium, barium, and sodium, and the native species is usually found as an insoluble calcium cross-linked gel (Sutherland, 1991). Its main function is to contribute to the strength and

flexibility of the seaweed plant, and the composition of the alginate varies both within different species of algae and within different parts of the plant (Andresen, 1997). Industrially, sodium alginate has for many decades been used for its gelling, viscosifying and stabilizing properties and for its ability to retain water plant (Onsøyen, 1996). More recently, new biotechnological, biomedical and pharmaceutical applications have been added to the list, and some new sodium alginate based materials have also emerged from extensive research in the field plant (Skjåk-Bræk and Espevik, 1996; Draget *et al.,* 1997). Although present research holds many promises to the possibility of sodium alginate production by microbial fermentation and post-polymerization modification of the molecule, all commercially available sodium alginates are at present extracted from algae plant (Skjåk-Bræk and Espevik, 1996; Draget *et al.,* 2005).

1.4.1.2 Chemical structure

Sodium alginate is water-soluble linear unbranched polysaccharide consist of 1-4 linked α-L-guluronic and β-D-mannuronic acid residues. Sodium alginates are copolymers containing mannuronic acid (M) and guluronic acid (G). The uronic acid residues are distributed along the polymer chain in a pattern of blocks, where homopolymeric blocks of G residues (G-blocks), homopolymeric blocks of M residues (M-blocks) and blocks with alternating sequence of M and G units (MG-blocks) co-exist. In G-blocks are buckled and less flexible while in M-blocks have a shape referred to as an extended ribbon-like structure. If two G-block regions are aligned side by side, a diamond shaped hole results. This hole has dimensions that are

ideal for the cooperative binding of calcium ions (Haug *et al.*, 1967; Haug and Larsen, 1962). (Figure 1.10)

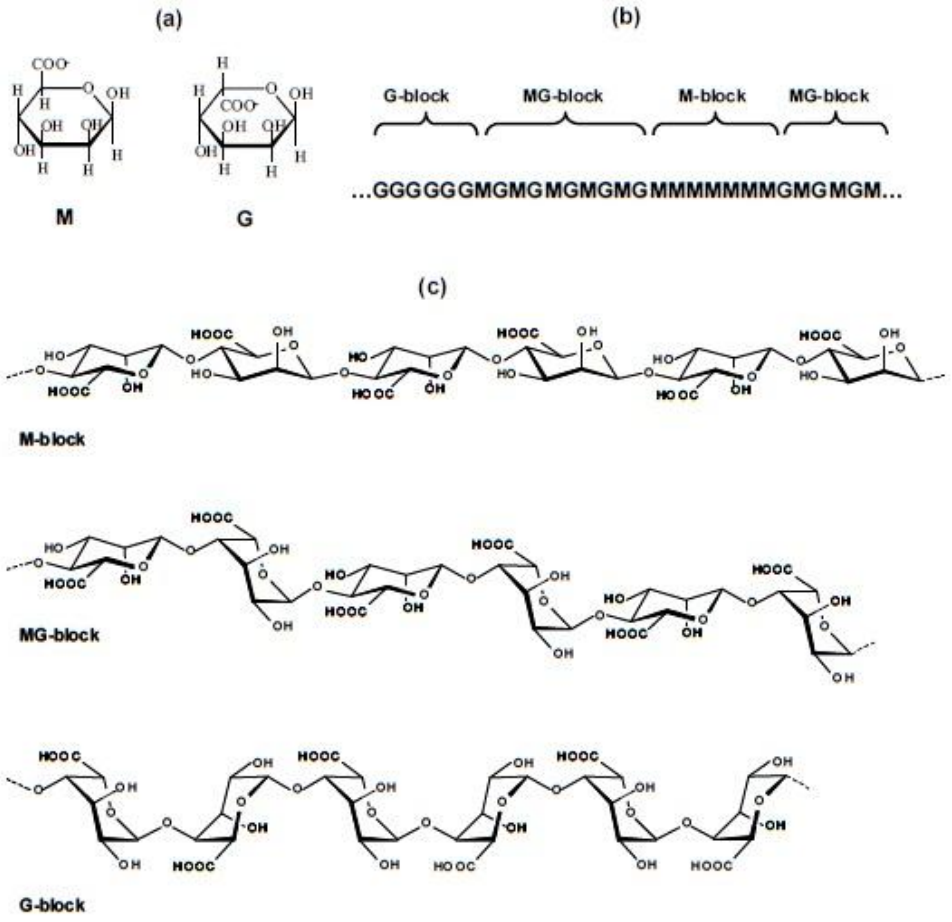


Figure 1.10 Sodium alginate chemical structures. (a) Illustration of the Haworth formulas of the two monomers. M=β-D-mannuronic acid and G=α-L-guluronic acid. (Smidsrod, 1974) (b) Block composition in alginates. (Haug *et al.*, 1996) (c) Ring 4C1 for M and 1C4 for G). (Smidsrod *et al.*, 1973: Stokke *et al.*, 1993: Dentini *et al.*, 2005)

1.4.1.3 Gel formation

In general, alginate beads can be prepared by ionotropic gelation method when the mixture of sodium alginate solution and the desired substance are extruded as droplets into the solution of divalent ion such as calcium, strontium, or barium, while monovalent cations and magnesium do not induce gelation while barium and strontium ions produce very strong alginate gels than calcium (Clark and Ross-Murphy, 1987). Other divalent cations including lead, copper, cadmium, nickel, zinc, and manganese will induce gelation, but their use is limit due to toxicity. In the gelation and crosslinking process, the polymer chains are cross-linked by the exchange of sodium ions from gluronic acids with divalent cations, and the stacking of these guluronic groups to form the egg-box structure as represented in Figure 1.11 (Rees and Welsh, 1981).

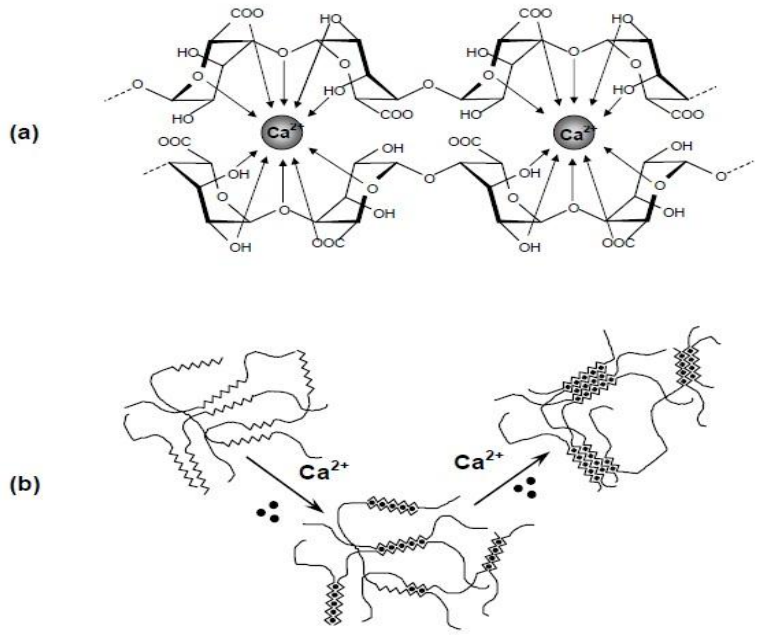


Figure 1.11 Binding of divalent ions to alginate the egg-box model. (a) Chelation of divalent cations. (b) Lateral association of chains. (Mørch, 2008).

1.4.1.4 Properties of alginate making it suitable for oral delivery

a) Biocompatibility

Sodium alginates are generally regarded as safe (GRAS) by the food and drug administration (FDA). The oral administration of sodium alginate has not been shown to stimulate immune response unlike the intravenously administered forms and it is reported that sodium alginate is non-toxic and biodegradable when given orally (Espevik *et al.,* 1993).

b) Bioadhesiveness

Sodium alginate has a very strong bioadhesive property, which again makes it a viable candidate for a potential delivery vehicle for drugs to mucosal tissues. With carboxyl end groups, alginate is classified as an anionic mucoadhesive polymer (Gombotz *et al.,* 1998). Research has shown that sodium alginate has the highest mucoadhesive strength when compared to other polymers such as polystyrene, chitosan, carboxy-methylcellulose and poly (lactic acid) (Chang *et al.,* 1985; Kwok *et al.,* 1989). Due to the adherence of sodium alginate particles to the mucosal tissues, transit time is delayed and the drug is localized to the absorptive surfaces. It improves the overall drug bioavailability and effectiveness with mucosal delivery.

c) pH sensitivity

Macromolecules release from alginate beads in low pH condition is also significantly reduced which could be advantageous in the development of an oral delivery system (Yotsuyanagi *et al.,* 1987; Kim and Lee, 1992; Sugawara *et al.,* 1994). Theoretically, alginate beads have the property of shrinking in low pH (gastric

environment) and drugs are not released (Chen *et al.*, 2004). In gastric condition, the hydrated sodium alginate is converted into a porous, insoluble so-called alginic acid while in the higher pH of the intestinal tract, the alginic acid skin is converted to a soluble viscous layer. This pH dependent behavior of sodium alginate can be exploited to customize release profiles. On the other hand, the rapid dissolution of sodium alginate matrices in the higher pH ranges may result in burst release of drugs. Therefore many modifications in the physicochemical properties are needed for the prolonged controlled release of drugs.

1.4.2 Chitosan

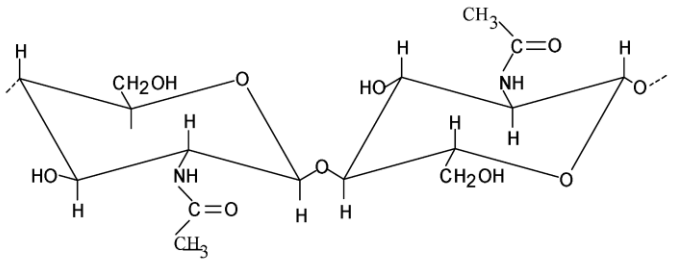
1.4.2.1 Sources of chitosan

Chitosan is a polycationic polysaccharide derived from chitin. Chitin is found in the outer skeletons of crustaceans, insects and some fungi. The most commonly obtained form of chitosan is the α-chitosan from crustacean chitin obtained from crab- and shrimp shell wastes (Roberts, 1992; Shepherd *et al.*, 1997).

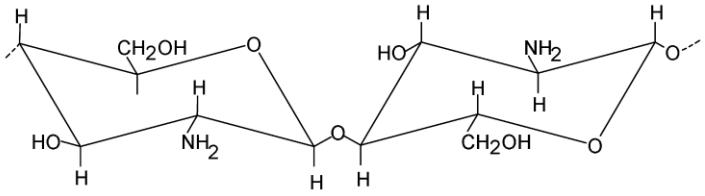
1.4.2.2 Chemical structure

Chitosan is obtained by the alkaline deacetylation of chitin. It is a linear polysaccharide composed a copolymer of β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) (Roberts, 1992; Domard and Cartier, 1992; Seiler, 1994) (Figure 1.12). The molecular structures of chitosan are very similar to cellulose, except that the acetylamino group replaces the hydroxyl group on the C-2 position. Thus chitosan is poly (N-acetyl-2-amino-2-

deoxy-D-glucopyranose), where the N-acetyl-2-amino-2-deoxy-D-glucopyranose (or Glu-NH) units are linked by (1-4)-β-glycosidic bonds (Kurita, 1986; Roberts, 1992).



(A)



(B)

Figure 1.12 Structures of chitin (A) and chitosan (B) (Hejazi and Amiji, 2003).

1.4.2.3 Properties of chitosan making it suitable for oral delivery

a) Biocompatibility and biodegradability

Chitosan has also been marketed throughout the world as a component in non-medical products, as a fat binder in cholesterol-lowering and slimming formulations (Shahidi et al., 1999). The study demonstrated that, chitosan entraps lipids in the intestine, because of its cationic nature (Kanauchi *et al.*, 1995). It has been found to be highly biocompatible. (Muzzarelli *et al.*, 1998). Also, chitosan is metabolized by certain human enzymes, especially lysozyme, and is considered biodegradable (Muzzarelli *et al.*, 1997).

b) Mucoadhesiveness

The mucoadhesive properties of chitosan have been demonstrated by its ability to adhere to porcine gastric mucosa *in vitro* (Gåserød *et al.,* 1998), and hence it could be useful for localized drug delivery. It has been suggested that residence time of formulations at the sites of action (e.g. within the GI tract) and suggested that chitosan may be valuable for drug delivery to specific regions of the GI tract like the stomach (Gåserød *et al.,* 1998; López *et al.,* 2000), small intestine (He *et al.,* 1998; Shimoda *et al.,* 2001), and buccal mucosa (López, 1998).

c) pH sensitiveness

Chitosan shows a pH-sensitive behavior as a weak poly-base due to the high amounts of amino groups in its chain. It is readily soluble at low pH while it is insoluble at higher pH. The mechanism of pH sensitive swelling involves the protonation of the free amino groups in chitosan chains at acidic conditions and shrinks at basic condition (Yao *et al.,* 1994).

1.4.2.4 Modifications of chitosan

The ability of chitosan has limited for controlled drug release. Chitosan is hydrophilic nature and dissolves easily at acidic condition. Several modifications of chitosan in the physicochemical properties have been studied to improve the oral drug delivery.

Alginate-chitosan complexes as a pH sensitive hydrogel can be of important use in oral delivery systems. The property of alginate has shrinking at lower pH and getting dissolved at higher pH, while chitosan dissolves easily at lower pH

and is insoluble at higher pH ranges. In view of these limitations encountered in pure alginate and chitosan bead systems, the concept of alginate-chitosan polyelectrolyte complexes gained acceptance (Murata *et al.,* 1993; Hugues *et al.,* 1996). Upon mixing, the carboxyl residues of alginate and the amino groups of chitosan ionically interacts to form the polyelectrolyte complex. Complexation of chitosan with alginate reduces the porosity of alginate beads and decreases the leakage of the encapsulated drugs (Sezer and Akbuga, 1999). The dissolves easily of chitosan at low pH is prevented by the alginate network since alginate is insoluble at low pH. The possible dissolves easily of alginate in higher pH is prevented by the chitosan which is stable in higher pH.

1.5 Methods for preparation of the beads

Generally, three methods have been utilized for the production of gels: namely the ionic gelation method, emulsion cross-linking, and spray drying. These methods have been employed for making gels and also microencapsulation /encapsulation of certain core materials, such as food ingredients, drugs and probiotics.

1.5.1 The ionic gelation method

This method is a popular to produce capsules with hydrocolloids. It is easily manufactured without any sophisticate equipment, low cost, and has gentle formation conditions (King, 1995; Krasaekoopt *et al.,* 2003). This approach is based on the conjugation of oppositely charged macromolecules for preparing microparticles. Microparticles are produced by dissolving the sodium alginate in an

aqueous solution and then suspending the active ingredient in the sodium alginate solution. This mixer is extruded though a syringe needle in the form of droplets to free-fall into a hardening solution usually contains the solution of $CaCl_2$, when the divalent calcium ions crosslink with the polymer forming gel microspheres. Because of the complexation between oppositely charged species, alginate undergoes ionic gelation and precipitates to form spherical particles. (Figure 1.13)

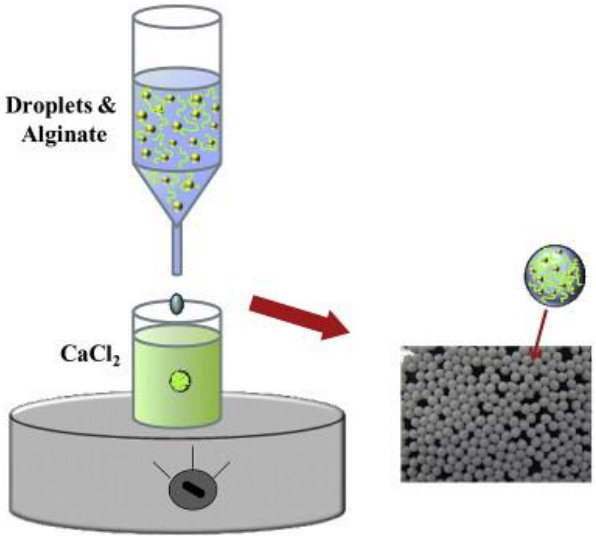


Figure 1.13 Schematic representation of the method used to form the filled calcium alginate beads. (Li *et al.*, 2011)

Sodium alginate can form gel by ionotropic gelation method with divalent calcium ions. When sodium alginate solution or mixture containing sodium alginate solution was dropped into $CaCl_2$ solution, spherical gel beads could be formed immediately in which intermolecular cross links were formed between the divalent calcium ions and the negatively charge carboxyl group of alginic acid (equation 1) (Choi *et al*., 2002). The gelation is explained by the egg-box model in which divalent metal ion binds to two carboxyl groups on adjacent alginate molecules (Figure 1.14).

$$Ca^{2+} + 2Na^{+}Alg \longrightarrow Ca^{2+}(Alg)^{2-} + 2Na^{+} \quad \text{Eq (1)}$$

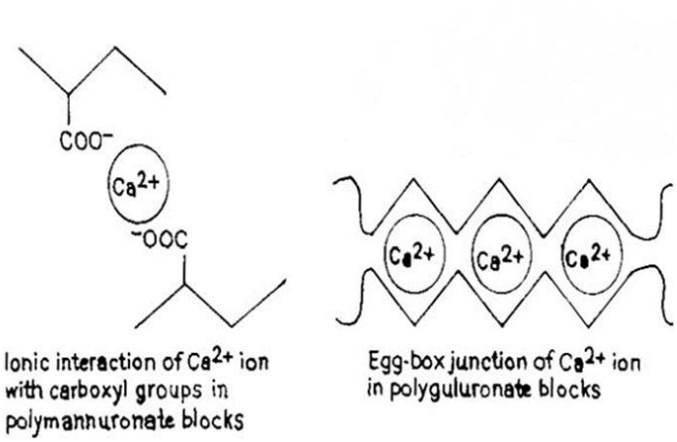


Figure 1.14 Bonding interactions between Ca^{2+} groups in the calcium alginate beads. (Bajpai and Sharma, 2004)

1.5.2 Emulsion cross-linking

The emulsion cross-linking creates a water-in-oil emulsion. A small volume of an alginate solution is added to a large volume of a vegetable oil. The mixture is homogenized, a solution containing a multivalent cation (normally Ca^{2+}) is added and the water-soluble alginate turns into insolubilized (cross-linked) tiny gel particles within the oil phase (Krasaekoopt *et al.*, 2003; Homayouni *et al.*, 2008).

The sizes of the final gel beads that are harvested later by filtration depend on the sizes of the internal phase particles of the emulsion. The size of the beads can be in a range from 25 µm to 2 mm. The bead size also is governed by the speed of agitation (Krasaekoopt *et al.*, 2003). In addition, adding emulsifiers in the water-in-oil emulsion can from a better emulsion because the emulsifiers are able to lower the surface tension of the emulsion. Thus the smaller spheres of gels are produced. For

example, Tween 80 at 0.2% is commonly applied as an emulsifier in this production (Sheu & Marshall, 1993)

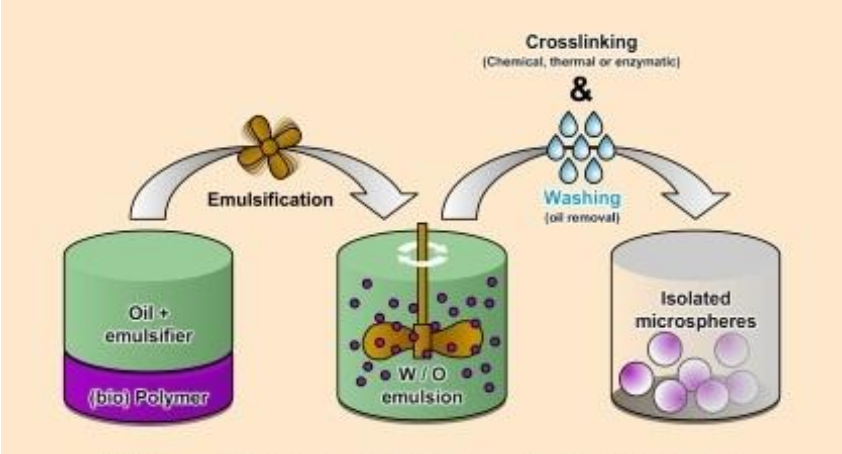


Figure 1.15 Processing scheme for microspheres-preparation by emulsion cross-linking (Yang *et al.*, 2001).

1.5.3 Spray-drying

Spray-drying is a common technique used in pharmaceuticals to produce powders, granules from the mixture of drug and excipient solutions as well as suspensions. This technique is based on drying of atomized droplets in a stream of hot air. The active ingredients dissolved or disperse in the sodium alginate solution and then, a suitable cross-linking agent is added. The mixture solutions are then atomized in a stream by hot air. Atomization leads to the formation of small droplets, from which solvent evaporates instantaneously leading to the formation of free flowing as depicted in Figure 1.16. The rate of spraying, the feed rate of polymer drug solution, nozzle size, and the drying temperature had effects on the size of microspheres.

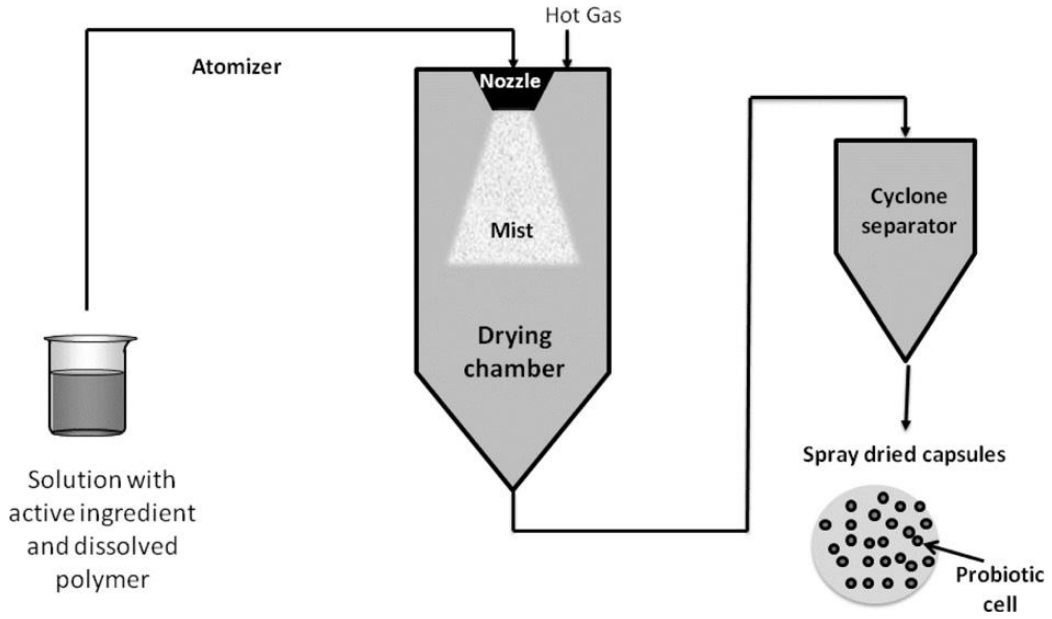


Figure 1.16 Schematic diagram of the encapsulation process of probiotics by spray drying (Burgain *et al.*, 2011)

1.6 Floating drug delivery systems (FDDS)

The FDDS are dosage forms which have a bulk density less than gastric fluids. While FDDS is remaining in the stomach, the continuous release of the drug at desired rate of the system is occurred. This FDDS provides local delivery to specific region such as stomach and proximal small intestine, controls of fluctuations in plasma drug concentration, and shows better bioavailability and therapeutic activity.

1.6.1 Classification of floating system

1.6.1.1 Effervescent systems

The buoyancy can also be achieved by generation of gas bubbles. These systems utilize matrices prepared by swellable polymers such as methylcellulose, chitosan and various effervescent compounds like carbonates or

bicarbonates, tartaric acid and citric acid (Harrigan, 1997). Carbon dioxide (CO_2) can be created *in situ* by incorporation effervescent components, which react with gastric acid in acid-either the natural, citric or tartaric acid in co-formulated.

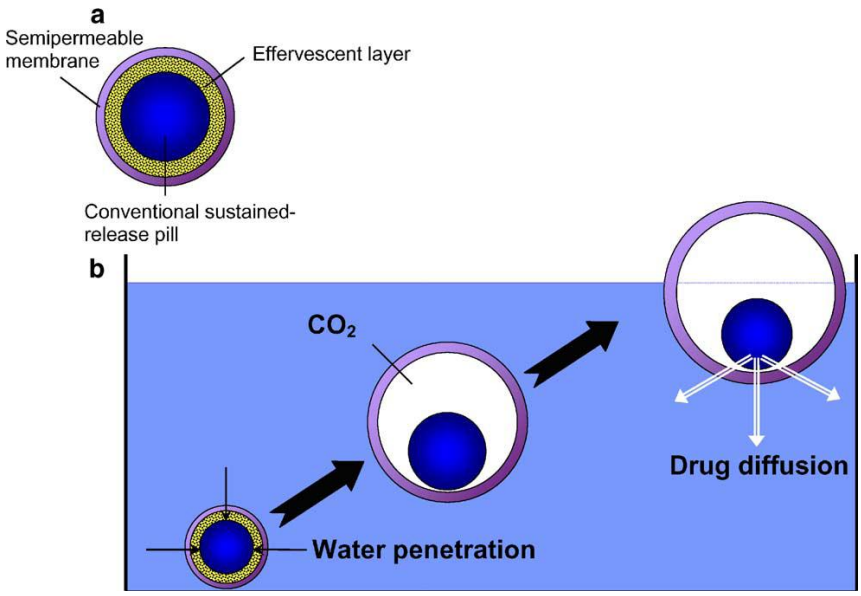


Figure 1.17 (a) Different layers i) Semi-permeable membrane, ii) Effervescent layer iii) Core pill layer (b) Mechanism of floatation via CO_2 generation. (Ichikawa, *et al*., 1996)

1.6.1.2 Non-effervescent systems

The non-effervescent FDDS based on mechanism of swelling of polymer or bioadhesion to mucosal layer in GI tract. These systems are normally prepared from gel-forming or swellable cellulose type hydrocolloids, polysaccharides and matrix forming polymers such as polycarbonate, polyacrylate. The formulation methods of such dosage forms involves the mixing of the drug and the gel-forming hydrocolloid, which swells in contact with gastric fluid after oral administration and

attains a bulk density of <1 within the outer gelatinous barrier (Ramesh *et al.*, 2009). The air trapped by the swollen polymer confers buoyancy to these dosage forms.

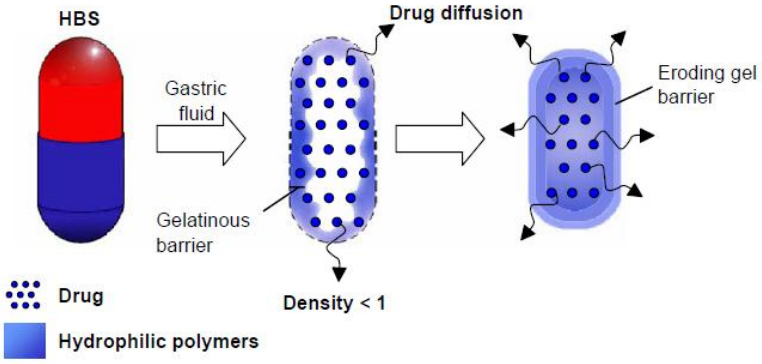


Figure 1.18 Hydrodynamically balanced system (HBS). The gelatinous polymer barrier formation results from hydrophilic polymer swelling. Drug is released by diffusion and erosion of the gel barrier. Based on Hwang et al. and Dubernet et al. Used with permissions.

1.6.2 Advantages and disadvantages of FDDS

Advantages (Nayak *et al.*, 2010)

1. Improvement of bioavailability
2. Reduction in plasma level fluctuations
3. Reduction in the variability in transit performance
4. Enhancement of therapeutic efficacy
5. Sustained drug delivery
6. Site specific drug delivery

Disadvantages (Mayavanshi and Gajjar, 2008)

1. The floating systems is requirement of a sufficiently high level of fluids in the stomach for the drug delivery. The dosage form can be administered with a glass full of water (200-250 ml) to provide the initial fluid for buoyancy.
2. Floating system is not feasible for those drugs that have solubility or stability problems in gastric fluids, e.g., phenytoin etc.
3. Drugs that are not stable at gastric pH are not suitable candidates to be formulated as gastric floating drug delivery system (GFDDS), e.g., erythromycin etc.
4. Drugs that irritate the mucosa are not suitable candidates and should be avoided to be formulated as GFDDS.

Table 1.1 Commercial floating formulations (Sarojini and Manavalan, 2012)

Brand Name	Drug (dose)	Company Country	Remarks
Madopar®	Levodopa and benserzide	Roche Products, USA	Floating, CR capsule
Cifran OD®	Ciprofloxacin (1g)	Ranbaxy, India	Gas generating floating form
Topalkan®	Aluminum magnesium antacid	Pierre Fabre Drug, France	Floating Liquid alginate
Valrelease®	Diazepam	HaffmanLaRoche, USA	Floating Capsule
Liquid Gaviscon®	Alginic acid and sodium bicarbonate	Glaxo Smith Kline, India	Effervescent floating liquid alginate preparation
Cytotec®	Misoprostol (100mcg/200mcg)	Pharmacia,USA	Bilayer floating capsule

CHAPTER 2

MATERIALS AND METHODS

2.1 FLOATING ALGINATE BEADS CONTAINING SELF-MICROEMULSIFYING FORMULATION FOR ORAL DELIVERY OF TETRAHYDROCURCUMIN

2.1.1 Materials

Tetrahydrocurcumin (THC) (white to off-white powder, 99.52% purity, lot no. C61260) was from Sabinsa Corporation (Piscataway, NJ, USA). Capryol 90™ (propylene glycol monocaprylate), Labrafac PG™ (propylene glycol caprylate/caprate), and Labrasol™ (caprylocaproyl macrogol-8 glycerides) were from Gattefossé (Saint-Priest, France). Cremophor EL™ (polyoxyethylene castor oil derivatives) was from BASF (Ludwigshafen, Germany). Sodium alginate (low viscosity 200 cP for 2.5% aqueous solution at 25 ºC) was from Sigma-Aldrich chemicals Ltd. (Germany). Kollicoat® IR (Polyvinylalcohol-polyethylene glycol) was from The Chemical Company (Bangkok, Thailand). Calcium carbonate ($CaCO_3$), calcium chloride ($CaCl_2$) and sodium chloride (NaCl) were from P.C. Drug Center Co., Ltd. (Bangkok, Thailand). Hard gelatin capsules (size 00) were from Capsugel (Bangkok, Thailand). Acetonitrile and methanol (HPLC grade) were obtained from RCI Labscan (Bangkok, Thailand). Glacial acetic acid and hydrochloric acid (analytical grade) were from RCI Labscan (Bangkok, Thailand).

2.1.2 HPLC analysis of THC

2.1.2.1 Chromatographic conditions

The analysis of THC was performed on chromatographic system of Agilent Technologies 1100 series HPLC including a quaternary pump, degasser, autosample, and photodiode array detector (HP 1100, Agilent, USA). A VertiSep^{TM} UPS C18 5-μm column (4.6 mm x 250 mm) and a guard VertiSep^{TM} UPS C18 5-μm column (4.6 mm x 10 mm) (Ligand Scientific, Bangkok, Thailand) were used for chromatographic separation. HPLC instrument was controlled by Agilent ChemStation software. The mobile phase consists of a mixture of 2% aqueous acetic acid and acetonitrile in the ratio of 30:70 v/v. The injection volume was 20 μL. The flow rate of mobile phase was 1 mL/min. The run time for each run was set for 8 min. Detection was performed by UV spectroscopy at a wave length of 282 nm.

2.1.2.2 Standard and sample preparations

Standard stock solution of THC was prepared in methanol at a concentration of 0.1 mg/mL. The stock solution of THC was diluted with methanol to give working standard solutions containing concentrations of 0.1 - 5 μg/mL. Twenty μL of each standard solutions were injected directly onto the HPLC column via the auto-injector three separate times (n = 3). The mean peak areas for each concentration were calculated, and standard calibration curve was constructed by plotting concentrations against peak areas. Samples were prepared in methanol to achieve a final concentration that ranged from 0.5 to 4 μg/mL. Twenty microliters of

the diluted samples were injected directly onto the HPLC column three separate times (n = 3).

2.1.2.3 Method development and validation

The validation parameters were carried out according to the ICH guidelines (2005) on the topic of Q2 (R1): validation of analytical procedure.

2.1.2.3.1 Specificity

Specificity of the method was determined through study of resolution factor of drug peak from the nearest resolving peak. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix.

2.1.2.3.2 Precision

The precision of the method was verified by repeatability (intra-day) and intermediate (inter-day) precision studies. Repeatability precision was determined by analyzing of the three different concentrations for three times in the same day. Intermediate precision of the method was assessed using above mentioned three concentrations analyzed on three different days. Each solution was injected in triplicate and the peak areas were used to calculate means and %RSD values. The acceptable %RSD should not exceed 2%.

%RSD = SD x 100 / mean

2.1.2.3.3 Accuracy

Accuracy of the developed method was tested in triplicate by fortifying a mixture of THC solutions with three different concentrations of the standard solutions and determining the recovery of added analyte. The percentage recovery should be in the range of 95-105%.

2.1.2.3.4 Linearity and range

Linearity of the method was studied by analysis of five concentrations of the standard solutions prepared in methanol in the range of 0.1-5 μg/mL (0.1, 0.5, 1, 2, and 5 μg/mL) using the HPLC method. The experiment was performed in triplicate. The peak areas versus concentration data were treated by least-squares linear regression analysis. The linearity test was carried out for 3 consecutive days. The minimum acceptable coefficient (r^2) to establish linearity was set at 0.999 a prior.

2.1.3 Preparation of liquid THC-SMEDDS

An optimum formulation of liquid THC-SMEDDS was prepared according to the procedure described by Setthacheewakul *et al.*, 2011. Cremophor EL™ (32.10% w/w), Labrasol™ (32.10% w/w), Capryol 90™ (13.65% w/w), Labrafac PG™ (13.65% w/w) and THC (8.51% w/w) were accurately weighed and mixed using a magnetic stirrer until a solution of THC-SMEDDS was obtained. The liquid formulation after mixing was left for 24 h at room temperature. The THC-SMEDDS were stored in air-tight glass containers and protected from light at room temperature until used.

2.1.4 Preparation of floating calcium alginate beads

2.1.4.1 Preparation of floating calcium alginate beads

The floating calcium alginate beads were formulated by ionotropic gelation method and designed based on gas formation technique. The beads were prepared by dispersing $CaCO_3$ (10% w/v) (gas forming agent) and THC powder (0.851% w/v) (control beads) into sodium alginate solution under stirring condition (at 1000 rpm, magnetic stirrer) for 60 min. The solution mixture (5 mL) was extruded through a 10 ml syringe (orifice diameter 0.076 in) into 40 mL of $CaCl_2$ solution containing acetic acid (10% v/v) maintained under gentle agitation (400 rpm) at room temperature. The solution containing suspend gel beads was stirred with a magnetic stir bar for 5 min to improve the mechanical strength of the gel beads and allowed to complete the reaction of gas producing. Since the carbonate salts are insoluble at neutral pH, the divalent ions were only released in the presence of acid, thereby preventing premature gelation. The fully formed gel beads were separated and washed with distilled water, and subsequently oven drying at 50 °C for 8 h.

2.1.4.2 Preparation of various formulation of the THC-SME floating calcium alginate beads

The various formulation of the THC-SME floating calcium alginate beads were prepared by dissolving THC-SME and Kollicoat® IR as water soluble pore-forming agent into mixture of sodium alginate and $CaCO_3$ (10% w/v) under stirring condition (at 1000 rpm, magnetic stirrer) for 60 min. The resulting homogenized mixture (5 mL) was extruded through a 10 ml syringe into 40 mL of

$CaCl_2$ solution containing acetic acid (10% v/v) maintained under gentle agitation (400 rpm) at room temperature. The formed gel beads were then treated in the same manner as the floating calcium alginate gel beads (see section 2.1.4.1). The resultant beads were separated, washed with distilled water and then dried by two different methods including oven drying at 50°C for 8 h, freeze drying. The different formulations of the beads were manufactured as shown in Table 2.1.

Table 2.1 Compositions of THC-SME floating calcium alginate beads

Formu-lation	THC-SME (%w/v)	Sodium alginate (%w/v)	$CaCl_2$ (M)[e]	$CaCO_3$ (%w/v)	Kollicoat® IR (%w/v)	Drying Method
Control[a]	-	2	0.05	10	-	Oven drying
A1	5	3	0.1	10	-	Oven drying
A2	10	3	0.1	10	-	Oven drying
A3	15	3	0.1	10	-	Oven drying
A4	20	3	0.1	10	-	Oven drying
B1	10	2	0.1	10	-	Oven drying
B2[b]	10	3	0.1	10	-	Oven drying
B3	10	4	0.1	10	-	Oven drying
C1	10	2	0.05	10	-	Oven drying
C2[c]	10	2	0.1	10	-	Oven drying
C3	10	2	3	10	-	Oven drying
C4	10	2	5	10	-	Oven drying
D1	10	2	0.05	10	1	Oven drying
D2	10	2	0.05	10	2	Oven drying
D3	10	2	0.05	10	3	Oven drying
E1[d]	10	2	0.05	10	3	Oven drying
E2	10	2	0.05	10	3	Freeze drying

[a]The control beads comprising of THC powder, [b]The compositions of formulation B2 is identical to that of formulation A2, [c]The compositions of formulation C2 is identical to that of formulation B1, [d]The compositions of formulation E1 is identical to that of formulation D3. [e] $CaCl_2$ solution containing 10% v/v acetic acid.

2.1.5 Physical characterization of THC-SME floating calcium alginate beads

2.1.5.1 Particle size analysis

THC-SME floating calcium alginate beads from each formulation were randomly sampled. The diameter of dried beads (n = 100) were measured by a Vernier caliper (Kanon, Japan), and the mean diameter was calculated for each formulation. (Ramya and Abdul, 2009)

2.1.5.2 Floating properties

The *in vitro* buoyancy studies were carried out using dissolution testing apparatus II; paddle method (Vankel® dissolution apparatus; VK 7000, USA). The floating ability of the beads from each formulation was determined by placing the beads in a dissolution apparatus containing 450 mL simulated gastric fluid (SGF, pH 1.2) without pepsin. The studies were performed at 100 rpm and 37.0 ± 0.5 °C. Floating lag time was defined as the time taken by all beads start to float at the surface of dissolution medium and floating duration was defined as the time period during which all beads constantly floated on the surface of the medium.

2.1.5.3 Morphology of the beads

Morphological examination of the surface and internal (cross-sectional) structure of the dried beads were carried out using a scanning electron microscope (Model LV-SEM 5800, JEOL, Japan) equipped with electron detector at an accelerating voltage of 10 kV. The samples were coated with gold in a vacuum

evaporator. The internal structure of the beads was examined by cutting them in half with a steel blade. SEM micrographs of the surfaces of THC-SME floating calcium alginate beads were photographed, and SEM analysis of the internal structure was also determined after splitting the samples.

2.1.5.4 Drug content and encapsulation efficiency

500 mg of dried beads were crushed into fine powder with a mortar and pestle, and then transferred to a 100 mL volumetric flask. The volume was made up to 100 ml with methanol and sonicated for 30 min. Then the mixture was filtered through a regular paper filter and a PVDF membrane filter 0.45 μm (Millipore®), respectively. The filtered solution was diluted with methanol. The amounts of THC extracted were analyzed by HPLC at wavelength of 282 nm. The drug content was calculated using a standard curve. The drug encapsulation efficiency was calculated using the following equation (Nimase and Vidyasagar, 2010).

% Encapsulation = (AQ/TQ)*100

Where:

AQ: Actual drug content

TQ: Theoretical drug content

2.1.5.5 *In vitro* drug release

The release profiles of the capsule filled with the THC-SME floating calcium alginate beads were studied using the USP30 rotating paddle apparatus. One capsule of each of different beads formulations was placed in 450 mL of simulated gastric fluid; SGF (pH 1.2) without pepsin at 37±0.5ºC and a rotating

speed of 100 rpm. Samples (5 mL) were withdrawn and replaced with 5 mL of the fresh medium after 5, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, and 480 min. One mL of the sample was made up to 10 mL with methanol in 10 mL volumetric flask. The samples were filtered using a 0.45 μm filter. The concentration of THC was assayed by HPLC analysis at the UV wavelength of 282 nm as described in section 2.1.2. A plot of the percent cumulative released of THC against time was constructed to illustrate the drug release profiles. Determinations were carried out in triplicate for each formulation, and the data was reported as a mean ±S.D.

2.1.6 Emulsion droplet size analysis

The droplet size and distribution of emulsions from SME were determined in triplicate at 25 ºC by photon correlation spectroscopy (Zeta potential analyzer, Model ZetaPALS, Brookhaven, USA) using dynamic light scattering. The liquid THC-SMEDDS and THC-SME floating calcium alginate beads were mixed with 200 mL of distilled water and subjected to mild agitation using a magnetic stirrer at a speed of 100 rpm at room temperature for 8 h, result in the formation of emulsions. The samples were diluted with distilled water that had been filtered through a 0.45 μm filter. The reconstituted emulsions were loaded into cuvette and measured after dilution to enable accurate measurement. The sample viscosity and the water refractive index were factored in particle size measurement using the instrument software.

2.1.7 Effect of formulation variables on THC release *in vitro*

In vitro release of THC from the optimum THC-SME floating calcium alginate beads formulation determined using the USP30 rotating paddle apparatus was compared with THC powder, THC floating calcium alginate beads, and liquid THC-SMEDDS. One capsule of each of the different formulations was placed in 450 mL of SGF (pH 1.2) without pepsin at 37±0.5ºC and a rotating speed of 100 rpm. Samples (5 mL) were withdrawn and replaced with 5 mL of the fresh medium after 5, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, and 480 min. One mL of the sample was made up to 10 mL with methanol in 10 mL volumetric flask. The samples were filtered using a 0.45 μm filter and analyzed using an HPLC assay as described in section 2.1.2. Three replicate release studies were performed for each formulation, and the data was reported as a mean ±S.D. A plot of the percent cumulative release of THC against time was constructed to illustrate the release profile.

2.1.8 Formulation stability studies

After determining the drug content, the optimized formulations were charged for the accelerated stability studies according to the ICH guidelines (2003) on the topic of Q1A (R2): stability testing of new drug substances and products. The optimum formulation (D3) was filled in hard gelatin capsules size 00 (500 mg/capsule) and stored in air-tight glass containers protected from light. Samples were maintained in a stability chamber (Patron AH-80, Taiwan) under intermediate conditions (Temperature 30±2ºC and humidity 65±5% for 6 months), and evaluated under accelerated storage conditions (Temperature 45±2ºC and humidity 75±5% for

6 months). The optimized formulation is evaluated for emulsion droplet size and drug content within the capsules.

2.2 ALGINATE BEADS CONTAINING SELF-MICROEMULSIFYING FORMULATION FOR ORAL DELIVERY OF CURCUMIN

2.2.1 Material

Curcumin (≥94% (curcuminoid content), ≥80% (curcumin)) was purchased from Sigma Aldrich (Buchs, Switzerland). Capryol 90™ (propylene glycol monocaprylate), Labrafac PG™ (propylene glycol caprylate/caprate) and Labrasol™ (caprylocaproyl macrogol-8 glycerides) were from Gattefossé (Saint-Priest, France). Cremophor EL™ (polyoxyethylene castor oil derivatives) was from BASF (Ludwigshafen, Germany). Sodium alginate (Low viscosity 200 cP for 2.5% aqueous solution at 25 ºC) was from Sigma-Aldrich chemicals Ltd. (Germany). Chitosan (85% degree of deacetylation, molecular weight 10^3 kD) and colloidal silicon dioxide (Aerosil 200) were obtained from Degussa-Hüls AG (Hanau, Germany). Calcium chloride ($CaCl_2$) and sodium chloride (NaCl) were from P.C. Drug Center Co., Ltd. (Bangkok, Thailand). Hard gelatin capsules (size 00) were from Capsugel (Bangkok, Thailand). Acetonitrile and methanol (HPLC grade) were obtained from RCI Labscan (Bangkok, Thailand). Glacial acetic acid and hydrochloric acid (analytical grade) were from RCI Labscan (Bangkok, Thailand).

2.2.2 HPLC analysis of curcumin

2.2.2.1 Chromatographic conditions

The HPLC method was performed on Agilent HPLC system (HP 1100, Agilent, USA) with a column VertiSep^{TM} UPS C18 5--µm column (4.6 mm x 250 mm(and a guard VertiSep^{TM} UPS C18 5-µm column (4.6 mm x 10 mm) (Ligand Scientific, Bangkok, Thailand) as a stationary phase, and a UV detector of curcumin at the wavelength of 425 nm. Data acquisition and analysis were performed by Agilent ChemStation software. The mobile phase was composed of 2% v/v acetic acid and acetonitrile in the ratio of 30:70. The flow rate and injection volume was 1 mL/min and 20 µL, respectively. Before each injection, the HPLC column was stabilized for 45 min with the initial mobile phase. The running time for each run was set for 10 min.

2.2.2.2 Standard and sample preparations

The stock solution (0.1 mg/mL) of standard curcumin was prepared by dissolving 10 mg of curcumin in 100 mL methanol. The working standard solutions containing 0.1 - 5 µg/mL concentrations of curcumin were freshly prepared by suitable dilution of stock solutions with methanol, and 20 µL of each standard solutions were injected directly onto the HPLC column via the auto-injector three separate times (n = 3). The mean peak areas for each concentration were calculated, and standard calibration curve was constructed by plotting concentrations against peak areas. The samples were prepared in methanol to achieve a final concentration of curcumin that ranged from 0.5 to 4 µg/mL within the standard curve range. The

resulting solution was then filtered and used for HPLC analysis. The injected volume was 20 μL. The analysis was performed in triplicate.

2.2.2.3 Method development and validation

The validation parameters were carried out according to the ICH guidelines (2005) on the topic of Q2 (R1): validation of analytical procedure.

2.2.2.3.1 Specificity

Specificity for an assay ensures that the signal measured comes from the substance of interest, and that there is no interference from excipient and/or degradation products and/or impurities. Determination of this can be carried out by assessing the peak identity and purity.

2.2.2.3.2 Precision

Precision of the method was verified as repeatability and intermediate precision studies. Repeatability studies were performed by analyses of three different concentrations of the standard solutions on the same day. Intermediate precision of the method was checked by repeating the studies on three different days. Each solution was injected in triplicate and the peak areas were used to calculate means and % RSD values.

2.2.2.3.3 Accuracy

Accuracy indicates the deviation between the mean value found and the true value. It is determined by applying the method to samples that is known

amounts of analyte. These should be analysed against standard and blank solutions to ensure that no interference exists. The accuracy is then calculated from the test results as a percentage of the analyte recovered by the assay.

2.2.2.3.4 Linearity and Range

Linearity of the method was studied by analysis of 5 concentrations of standard solutions prepared in methanol in the range of 0.1-5 μg/mL (0.1, 0.5, 1, 2, and 5 μg/mL) using the HPLC method. The experiment was performed in triplicate. The peak areas versus concentration data were treated by least-squares linear regression analysis. The minimum acceptable coefficient to establish linearity was set at 0.999 a prior.

2.2.3 Preparation of liquid curcumin-SMEDDS

An optimum liquid curcumin-SMEDDS was prepared according to the procedure described by Setthacheewakul *et al.*, 2010. Cremophor EL™ (33.52% w/w), Labrasol™ (33.52% w/w), Capryol 90™ (14.36% w/w), Labrafac PG™ (14.36% w/w) and curcumin (4.26% w/w) were accurately weighed and mixed using a magnetic stirrer until a solution of curcumin-SMEDDS was obtained. The liquid formulation after mixing was left for 24 h at room temperature. The curcumin-SMEDDS were stored in air-tight glass containers and protected from light at room temperature until used.

2.2.4 Preparation of curcumin-SME chitosan-alginate beads

2.2.4.1 Preparation of curcumin-SME alginate beads

The curcumin-SME alginate beads (F1) were prepared by the ionotropic gelation method. The powder of sodium alginate was dissolved in distilled water with gentle agitation and heating. The liquid curcumin-SMEDDS was adsorbed onto colloidal silicon dioxide as a solid adsorbent in the ratio of 100:60, by mixing in a mortar. Five percent weights by volume of curcumin-SME powder (containing 0.213 w/w of curcumin) were dispersed in sodium alginate solution by stirring (at 1000 rpm) with a magnetic stirrer for 60 min. Five milliliters of the homogeneous mixture were then extruded through a 10 ml syringe into 40 mL (0.1 M) $CaCl_2$ solution under gentle agitation (400 rpm) at room temperature. The resultant the beads were separated and washed with distilled water and oven drying at 50°C for 12 h.

2.2.4.2 Preparation of curcumin-SME chitosan coated alginate beads

The curcumin-SME chitosan coated alginate beads (F2, F3, and F4) were prepared as follows. The chitosan at various concentrations were used as a post-coagulation fluid. Chitosan (0.25% w/v, 0.5% w/v and 1.0% w/v for F2, F3 and F4, respectively) was dissolved in 3% (v/v) glacial acetic acid in distilled water at room temperature. The pH of post-coagulation fluid was adjusted to 5.0±0.1 by 0.1 N sodium hydroxide (NaOH). Five milliliters of homogeneous mixture (2.5% w/v sodium alginate solution and 5% w/v curcumin-SME powder) was then extruded through a 10 ml syringe into 40 mL (0.1 M) $CaCl_2$ solution with stirring at 400 rpm to obtain gel formation. The resultant the beads were separated, washed once with

distilled water, and then transferred into 200 mL of post-coagulation fluid solution with stirring for 10 min. The chitosan coated alginate beads were taken out, washed twice with distilled water and then oven drying at 50 °C for 12 h.

2.2.4.3 Preparation of curcumin-SME chitosan-alginate beads

The chitosan-alginate beads (M1, M2, M3, and M4) were prepared as follows. The homogenous mixture of 0.1 M $CaCl_2$ containing 0.25% w/v chitosan was used as a coagulation fluid. Chitosan was dissolved in 3% v/v of glacial acetic acid in distilled water at room temperature. The pH of the coagulation was adjusted to 4.5±0.1 by 0.1 N NaOH. Five milliliters of homogeneous mixture (2.5% w/v sodium alginate solution and 5% w/v curcumin-SME powder) was then extruded through a syringe (10 ml) into 40 mL of coagulation fluid under mechanical stirring at 200 rpm. The resultant the beads were collected, and washed once with distilled water, and followed by transfer into 200 mL of post-coagulation fluid solution (0.25% w/v, 0.5% w/v and 1.0% w/v of chitosan solution for M2, M3 and M4, respectively) with stirring for 10 min. The beads were selected, washed twice with distilled water, and then oven drying at 50°C for 12 h. In case of formulation M1, the beads were prepared as described above but not transferred to post-coagulation fluid. The compositions are listed in Table 2.2.

Table 2.2 Formulation design for the preparation of curcumin-SME alginate beads

Formulations	Drug-polymer mixture		Coagulation fluid		Post-coagulation fluid (% w/v chitosan)
	Sodium alginate (% w/v)	Curcumin-SMEDDS powder	$CaCl_2$ (M)	Chitosan (% w/v)	
F1	2.5	5	0.1	-	-
F2	2.5	5	0.1	-	0.25
F3	2.5	5	0.1	-	0.5
F4	2.5	5	0.1	-	1
M1	2.5	5	0.1	0.25	-
M2	2.5	5	0.1	0.25	0.25
M3	2.5	5	0.1	0.25	0.5
M4	2.5	5	0.1	0.25	1

2.2.5 Physical characterization of curcumin-SME alginate beads formulations

2.2.5.1 Emulsion droplet size analysis

The droplet sizes of the microemulsions from each of the formulations (F1-F4, M1-M4) were determined by photon correlation spectroscopy (Zeta potential analyzer, Model ZetaPALS, Brookhaven, USA). The liquid curcumin-SMEDDS and curcumin-SME beads were mixed with distilled water and subjected to mild agitation using a magnetic stirrer at a speed of 100 rpm at room temperature for 10 h, resulted in the formation of emulsions. The samples were diluted with distilled water that had been filtered through a 0.45 μm filter. Light scattering was monitored at a 90º angle and at a temperature of 25ºC. Three replicate analyses were carried out for each formulation, and the data presented as means ±S.D. (Setthacheewakul *et al*., 2010)

2.2.5.2 Particle size analysis

The particle size of the dried beads was measured with a Vernier caliper (Kanon, Japan) and calculated as the average value of the size of 100 beads. (Ramya and Abdul, 2009)

2.2.5.3 Morphology of the beads

The surface and cross-sectional morphologies of the dried beads were examined by a scanning electron microscope (Model LV-SEM 5800, JEOL, Japan) operating 10 kV. The beads were cut in half with a steel blade for determination of internal structure. The samples were coated under vacuum with gold in nitrogen atmosphere prior to observation.

2.2.5.4 Drug content and Encapsulation efficiency

The drug content and encapsulation efficiency of curcumin-SME alginate beads were determined using the same procedures described in section 2.1.5.4. All samples were analyzed by HPLC at wavelength of 425 nm as described in section 2.2.2.

2.2.5.5 Swelling property

The samples of beads of known weight (500 mg) were placed in SGF (pH 1.2) and in SIF (pH 6.8), respectively and allowed to swell at 37±0.5°C. At predetermined time intervals, the beads were removed and weighed. The weight of the swollen beads was determined (after blotting with filter paper to remove surface fluid) on an electronic balance, and thereafter returned to the SIF (pH 6.8) solution. The

swelling ratio of the beads was calculated from the formula (Rajendran and Basu, 2009).

Swelling ratio = (Wt-Wo)/Wo

Where:

Wo : Initial weight of beads

Wt : Final weight of beads

2.2.5.6 *In vitro* drug release

The release profiles of the capsule filled with the curcumin-SME beads were studied using the USP30 rotating paddle apparatus (Hanson Research Corporation, USA). One capsule of each of different the beads formulations was immerse in 450 mL of two dissolution media including SGF, pH 1.2 without pepsin for 2 h and simulated intestinal fluid; SIF, pH 6.8 for 8 h respectively, and stirred at 100 rpm at 37±0.5 ºC at certain time intervals, 5 mL of the sample was withdrawn and replaced with the same amount of the refresh dissolution medium. After the first 2 h in SGF, the sample was quickly filtered through a 100 mesh screen, blotted to remove the surface fluids, and charged into SIF for further test. The amount of curcumin released was assayed at the UV wavelength of 425 nm by HPLC analysis as described in section 2.2.2. A plot of the percent cumulative release of curcumin against time was constructed to illustrate the drug release profiles. Determination was carried out in triplicate times for each formulation and the data is reported as a mean ±S.D.

2.2.6 Effect of formulation variables on curcumin release *in vitro*

In vitro release of curcumin from the curcumin-SME beads compared to curcumin powder and liquid curcumin-SMEDDS were determined using the USP30 rotating paddle apparatus. One capsule of each of the different formulations was placed in 450 mL of SGF (pH 1.2) without pepsin at 37±0.5ºC and a rotating speed of 100 rpm for 2 h and then in SIF, pH 6.8 for 8 h. At certain time intervals, the samples (5 mL) were withdrawn and replaced with 5 mL of the fresh medium. Amount of released drug was analyzed using an HPLC assay as described in section 2.2.2. All measurements were carried out in triplicate and average values were plotted.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 FLOATING ALGINATE BEADS CONTAINING SELF-MICROEMULSIFYING FORMULATION FOR ORAL DELIVERY OF TETRAHYDROCURCUMIN

3.1.1 HPLC method for *in vitro* analysis of THC

A validated HPLC method developed by Setthacheewakul *et al.* (2011) was used to identify and determine THC in the active ingredient and its pharmaceutical formulations. The THC exhibited a well-defined chromatographic peak with a retention time of 6.9 min. Figure 3.1B and 3.1C show the chromatogram obtained by injection of a solution containing THC and THC-SMEDDS released from beads (5 μg/mL), respectively. The chromatogram shows the THC peak was clearly separated from the other peaks. This result confirmed the ability of the method to identify THC from the pharmaceutical excipients presented in the SMEDDS products.

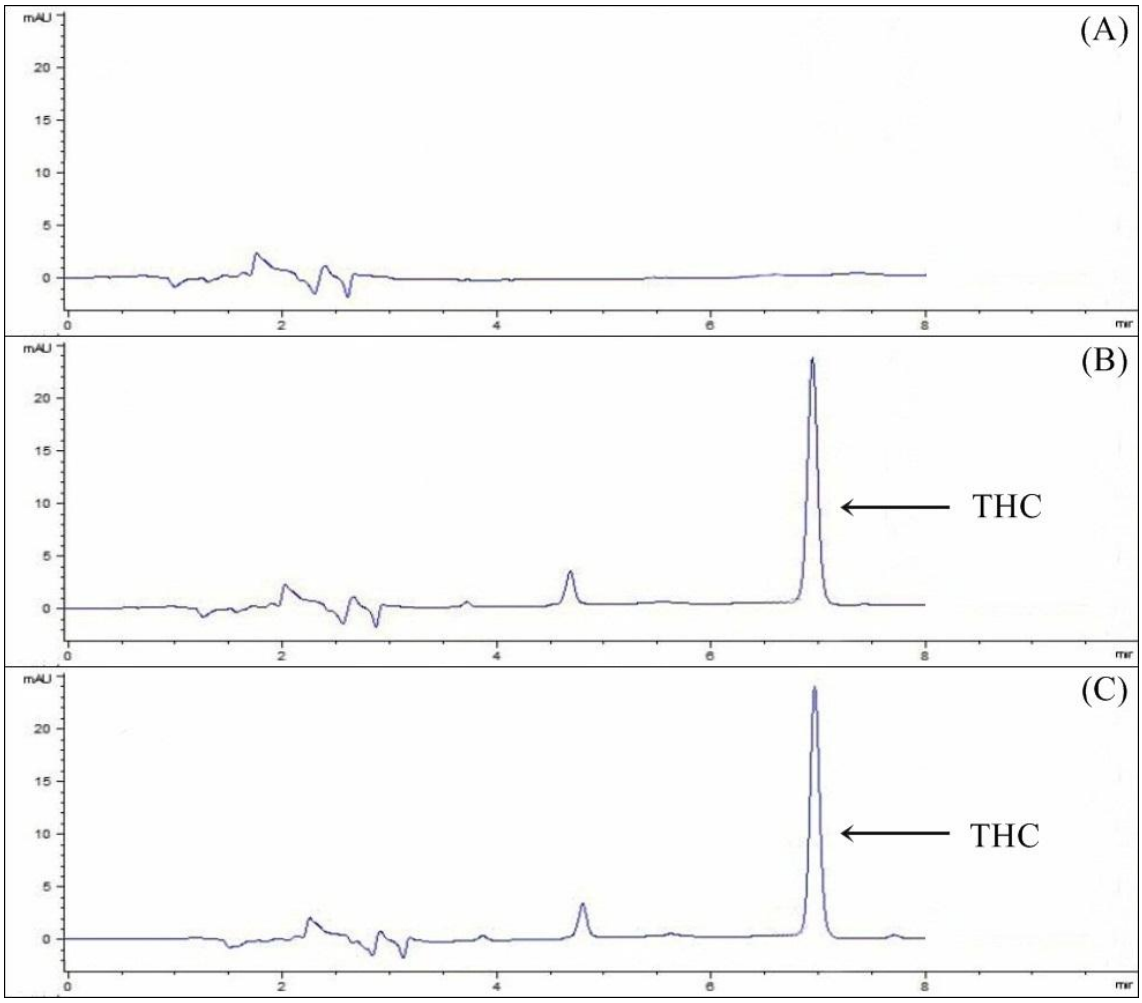


Figure 3.1 HPLC chromatogram of placebo-THC (a), THC (b) and THC-SMEDDS beads (c), using a C18 reverse phase column with a mixture of 2% aqueous acetic acid and acetonitrile (a linear gradient system), and a PDA detector (282 nm). Flow rate was 1 mL/min, injection volume was 20 µL

The calibration curve for THC was constructed by plotting concentrations *versus* corresponding mean peak areas (Figure 3.2). A good linearity was achieved with a correlation coefficient of 0.9999 over the concentration range of 0.1-5 µg/mL. The concentration of THC was calculated from the standard curve [peak area =

37.263 x conc. + 0.3317 (r^2 = 0.9999±0.0002)]. It was shown that an excellent correlation existed between the peak area and concentration of the analyte.

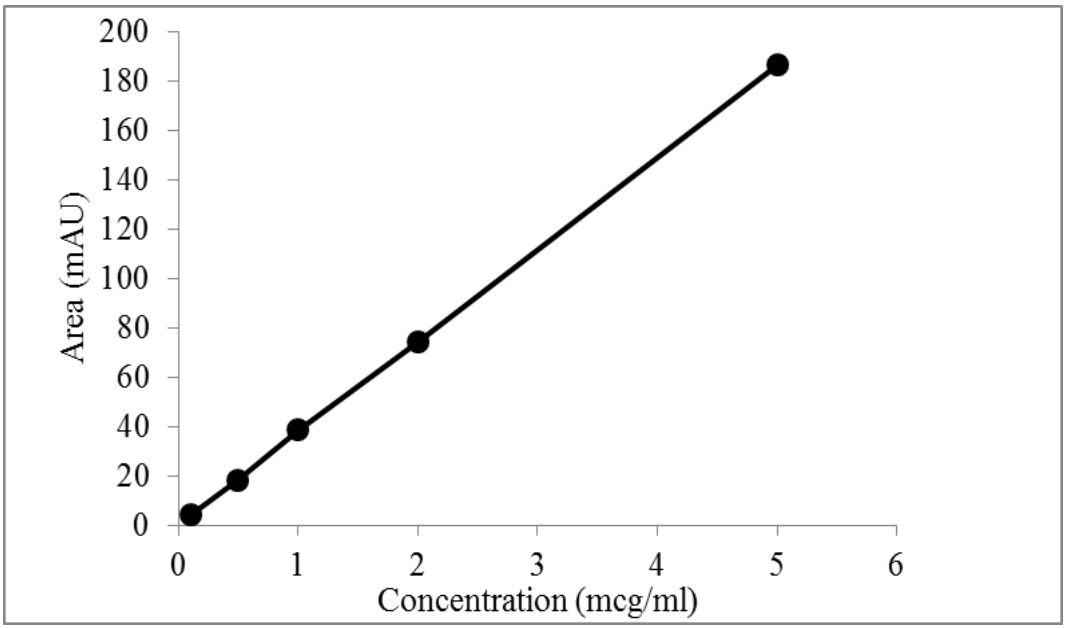


Figure 3.2 Calibration curve of THC

The precision of the method was determined by intra-day repeatability and intermediate precision studies. The reproducibility of the method was demonstrated by repeated injections of THC standards. The intra-day relative standard deviations (RSD) that ranged from 0.37% to 0.77%, whereas the inter-day relative standard deviations ranged from 0.71% to 1.72% respectively (Table 3.1)

Table 3.1 RSD (%) of intra-day repeatability and intermediate precision studies

Concentration µg/mL	RSD (%)	
	Intra-day repeatability	Intermediate precision
1.2	0.77	1.72
1.5	0.37	0.71
1.8	0.59	0.78

The accuracy of the method was verified with recovery values of 95.25-102.63% (Table 3.2).

Table 3.2 Recovery (%) of THC at various concentrations

Sample	Recovery (%) on each studied concentration		
	1.2 µg/mL	1.5 µg/mL	1.8 µg/mL
1	95.95	99.85	95.54
2	102.63	95.25	98.57
3	97.68	99.53	99.07
Average	98.75	98.21	97.73

The HPLC method validation results confirmed that determination of THC could be performed by the validated HPLC method described above with accepted accuracy and precision.

3.1.2 Preparation of floating calcium alginate beads

The $CaCO_3$ and THC-SME mixture were incorporated into the sodium alginate solution and the solution mixture was then extruded into a solution of $CaCl_2$ containing 10% (v/v) acetic acid resulted in porous-structured gel beads. During the formation of the calcium alginate beads, carbonate salts are reacted with acetic acid to produce carbon dioxide (equation 2) (Sriamornsak *et al.,* 2007). The evolving gas permeates through the calcium alginate structure leaving gas bubbles or pores (Figures 3.3), resulting in the highly porous and fragile beads. Most of the beads were spherical in shape, although some were found to be elongated or irregular.

$$CaCO_3 + 2CH_3COOH \longrightarrow (CH_3COO)_2Ca + H_2O + CO_2 \quad \text{.....Eq (2)}$$

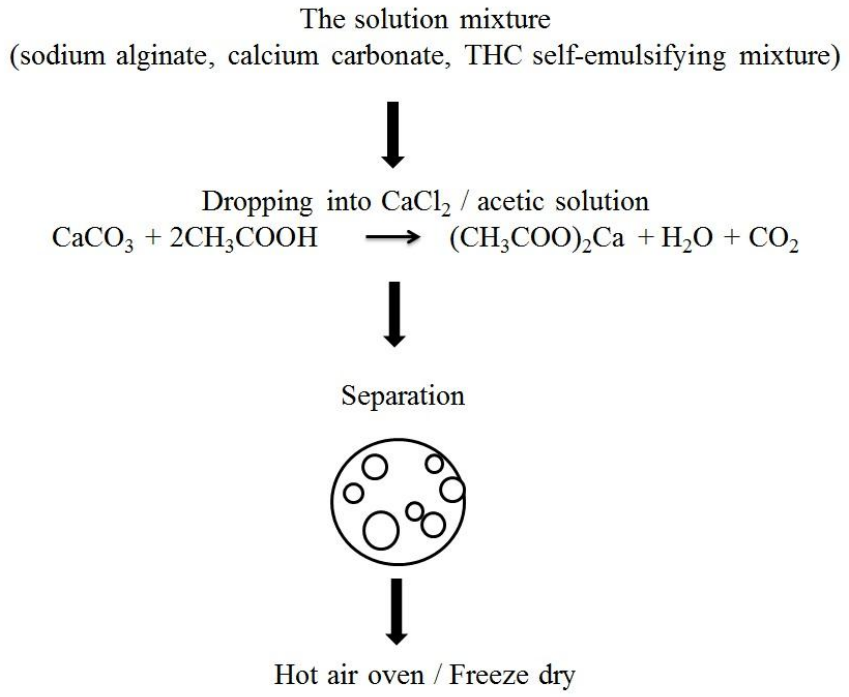


Figure 3.3 Schematic diagram of the preparation method for floating calcium alginate beads (adapt from Sriamornsak *et al.,* 2007)

3.1.3 Effect of formulation variables on physical properties of THC-SME floating calcium alginate beads

3.1.3.1 Effect of THC-SME mixture concentration

Drug content and Encapsulation efficiency

Drug content and encapsulation efficiency of bead formulations with different concentration of THC-SME mixture are given in Table 3.3. Increase in concentration of THC-SME mixture from 5 to 20 % w/w. (A1-A4) significantly decrease THC encapsulation efficiency. During preparation, the homogeneous mixture was not obtained when 15 or 20 % of THC-SME mixture was incorporated into sodium alginate solution. It might lead to the intra- and inter-batch variations in the drug content whereas A1 and A2 did not show this effect. Moreover, A2 (10% w/v THC-SME mixture) gave high drug content and percent encapsulation; therefore, this concentration of THC-SME mixture was fixed at 10 % w/v for further developed formulations.

Table 3.3 Drug content and encapsulation efficiency of bead formulations with different concentration of THC-SME mixture**.** Data represents the mean ±SD (n=3).

Physical properties	Formulations			
	A1 (5%w/v)	A2 (10%w/v)	A3 (15%w/v)	A4 (20%w/v)
Drug content (mg±SD/ 500 mg dried beads)	10.92±0.11	17.00±0.16	17.14±0.15	11.75±0.05
Encapsulation efficiency (±SD)	92.39±0.92	90.86±0.58	75.19±0.67	45.56±0.19

3.1.3.2 Effect of sodium alginate concentration

Drug content and Encapsulation efficiency

Drug content and encapsulation efficiency of bead formulations with different alginate concentration are given in Table 3.4. Increasing the concentration of sodium alginate (2, 3 and 4 %w/v) resulted in a high viscosity solution. The relatively high viscosity of the extruded sodium alginate solutions might have hindered the interaction between $CaCl_2$ and sodium alginate leading to a decrease in the drug loading in the beads (17.62, 16.74 and 15.91%, respectively), while there is little effect on encapsulation efficiency (91.08, 90.48 and 89.76%, respectively).

Table 3.4 Physical properties of bead formulations with different alginate concentration. Data represents the mean ±SD (n=3).

Physical properties	Formulations		
	B1 (2% w/v)	B2* (3% w/v)	B3 (4% w/v)
Drug content (mg±SD/ 500 mg dried beads)	17.62±0.06	16.74±0.03	15.91±0.09
Encapsulation efficiency (±SD)	91.08±0.30	90.48±0.15	89.76±0.49
Bead size (mm±SD)	1.60±0.09	1.77±0.13	2.10±0.15
Floating lag time (min±SD)	15±1.54	7±0.98	0.5±0.34
Floating duration (h)	>8	>8	>8

*The compositions of formulation B2 is identical to that of formulation A2 (Table 3.3)

Particle size analysis

Most the beads were found nearly spherical in appearance. There were differences in size between the samples with different percentage of sodium alginate (Table 3.4 and Figure 3.4). An increase in concentration of sodium alginate could lead to a greater viscosity of the solution, and hence a large drop needed to be dripped out of the needle. As a result, larger beads were formed. In addition, tailing of beads were observed in formulation B3 (4% sodium alginate concentration) due to the increase in viscosity of the pre-gelation liquid. This is in agreement to the previous findings of Sankalia *et al.,* 2005. They showed that the particle sizes were found to be directly proportional to sodium alginate concentration. Increased viscosity at a higher concentration of sodium alginate resulted in larger particles.

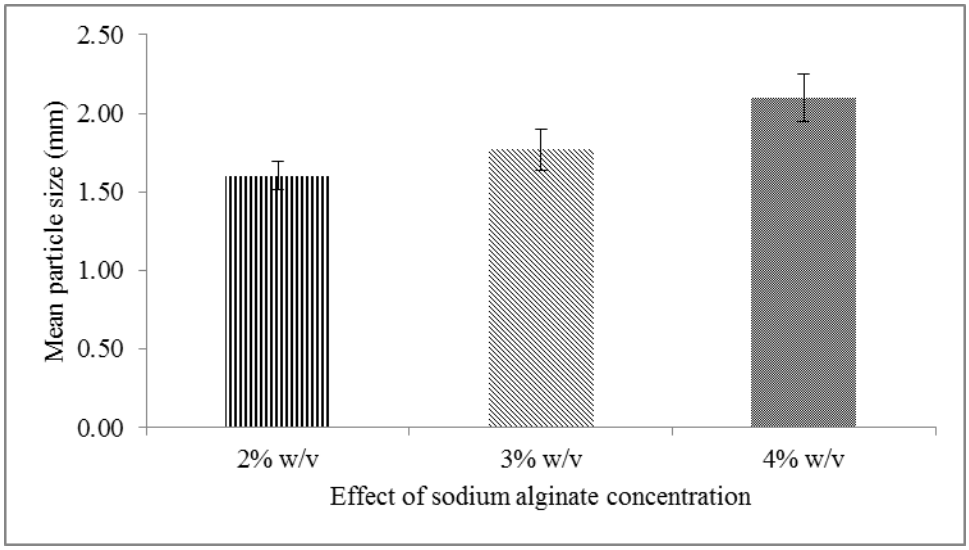


Figure 3.4 Effect of sodium alginate concentration on particle size of the beads. The means and standard deviation of triplicate data are plotted.

Floating properties

The *in vitro* buoyancy study of prepared beads was evaluated using dissolution apparatus II; paddle method containing 450 mL SGF (pH 1.2) without pepsin at rotational speed of 100 rpm and 37.0±0.5 ºC. The results are shown in Table 3.4 and Figure 3.5. The effect of alginate concentration on floating lag time of the beads was observed. As sodium alginate increased from 2 to 4% w/v, the floating lag time decreased due to the effect of increasing the viscosity of the emulsion mixture. When the viscosity increases, the amount of $CaCO_3$ that inserted into the layer of alginate was reduced. Most of $CaCO_3$ was perched around the beads. When the beads was immersed in SGF pH 1.2, it settled down and the carbonate salts are reacted with acid to produce CO_2, resulting in fast floating, while there is no effect on floating duration. All the bead formulations floated longer than 8 h.

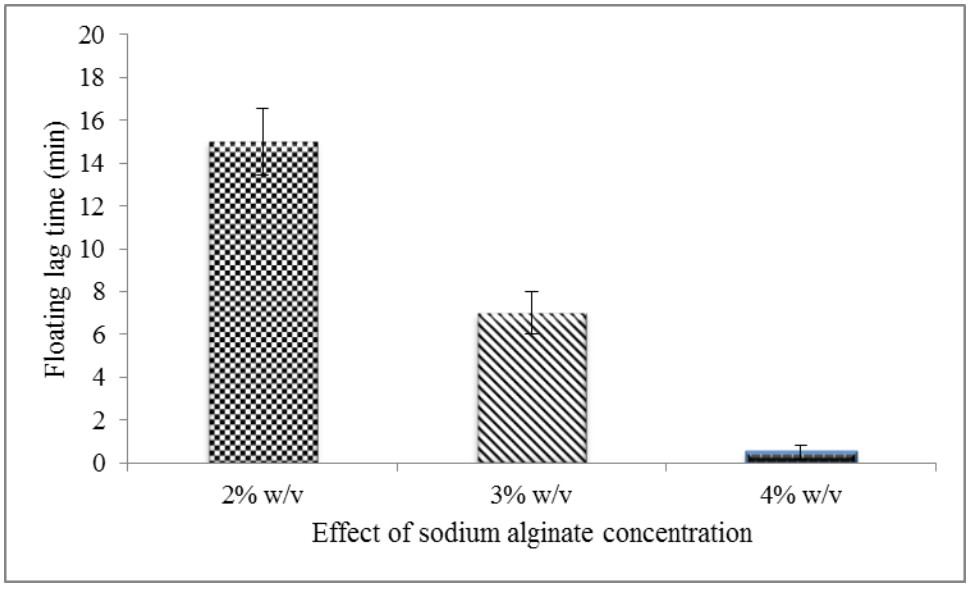


Figure 3.5 Effect of sodium alginate concentration on floating lag time of the beads. The means and standard deviation of triplicate data are plotted.

***In vitro* drug release**

The *in vitro* drug release behavior of THC from the beads was investigated in SGF; pH 1.2 without pepsin. The plot of cumulative % drug released vs. time for all formulations are depicted in Figure 3.6. It was found that the drug release was prolonged, and the release rates were proportional to an increase of sodium alginate concentration. The results showed that the rate and extent of THC decreased as the sodium alginate within the beads increased. The amount of THC release at 8 h were 58.51±1.31, 51.65±0.71, 43.10±1.43 for 2, 3, 4% w/v of sodium alginate, respectively. This decrease can at least partly be attributed to the formation of a denser gel network within the beads with increasing alginate content, which would slow down the movement of gastric medium into the beads.

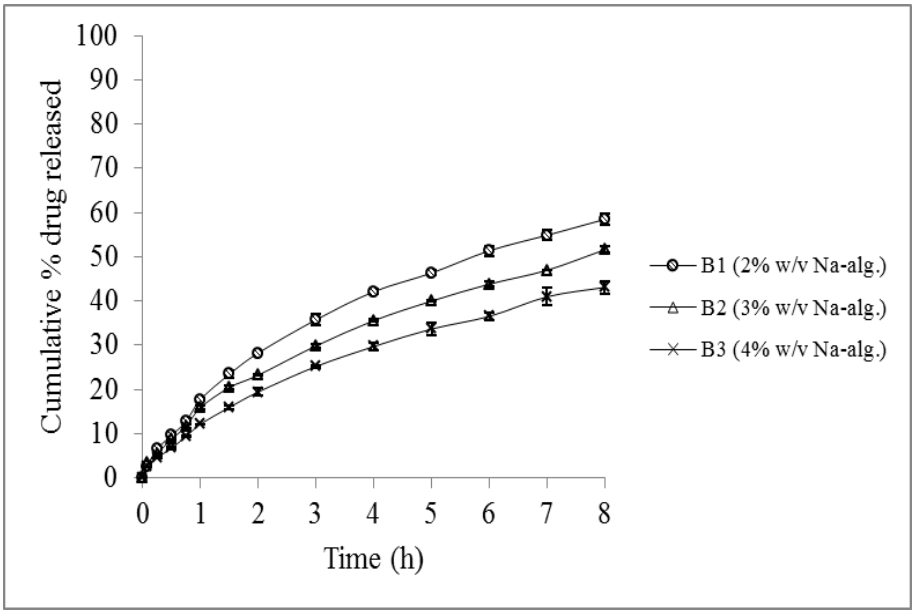


Figure 3.6 Effects of sodium alginate (B1-B3) on THC release from THC-SME floating calcium alginate beads in SGF (pH 1.2) without pepsin. The means and standard deviation of triplicate data are plotted.

Formulation B1 with 2% w/v sodium alginate had higher drug content, percent encapsulation and release rate when compared to other formulations; hence, this sodium alginate concentration was selected for further experiment.

3.1.3.3 Effect of $CaCl_2$ concentration

Drug content and Encapsulation efficiency

The drug content and encapsulation efficiency of the beads formulations with different $CaCl_2$ concentration were found to vary between 13.14±0.07% to 17.52±0.19% and 67.93±0.37% to 90.59±0.96%, respectively (Table 3.5). It was observed that increasing concentration of $CaCl_2$ from 0.1, 3, and 5 M (C2-C4) resulted in a decrease in both the drug content and encapsulation efficiency in the beads. It is possible that a greater interaction between calcium ions and the alginate gel network was formed, resulting in the diffusion of THC out of the matrix during gelation.

Table 3.5 Physical properties of bead formulations with different $CaCl_2$ concentration. Data represents the mean ±SD (n=3).

Physical properties	Formulations			
	C1(0.05M)	C2*(0.1M)	C3 (3M)	C4 (5M)
Drug content (mg±SD/ 500 mg dried beads)	17.09±0.17	17.52±0.19	14.47±0.26	13.14±0.07
Encapsulation efficiency (±SD)	88.37±0.90	90.59±0.96	74.79±1.35	67.93±0.37
Bead size (mm±SD)	1.77±0.07	1.60±0.09	1.35±0.09	1.18±0.13
Floating lag time (min±SD)	4±1.21	15±1.54	27±1.37	45±1.61
Floating duration (h)	>8	>8	>8	>8

*The compositions of formulation C2 is identical to that of formulation B1 (Table 3.4)

Particle size analysis

The formed beads were almost spherical. The mean particle size of the beads formulations was between 1.18±0.13 and 1.77±0.07 mm. It was observed that an increase in the concentration of $CaCl_2$ (0.05 to 5 M) led to a decrease in the size of the beads (Table 3.5 and Figure 3.7). This is in agreement to the findings of Smrdell *et al*., 2008; Sankalia *et al.*, 2005; Sriamornsak and Nunthanid, 1999; Kim and Lee, 1992. They observed that the high $CaCl_2$ concentration caused shrinkage of the beads and resulted in smaller particle size because of a high degree of crosslinking.

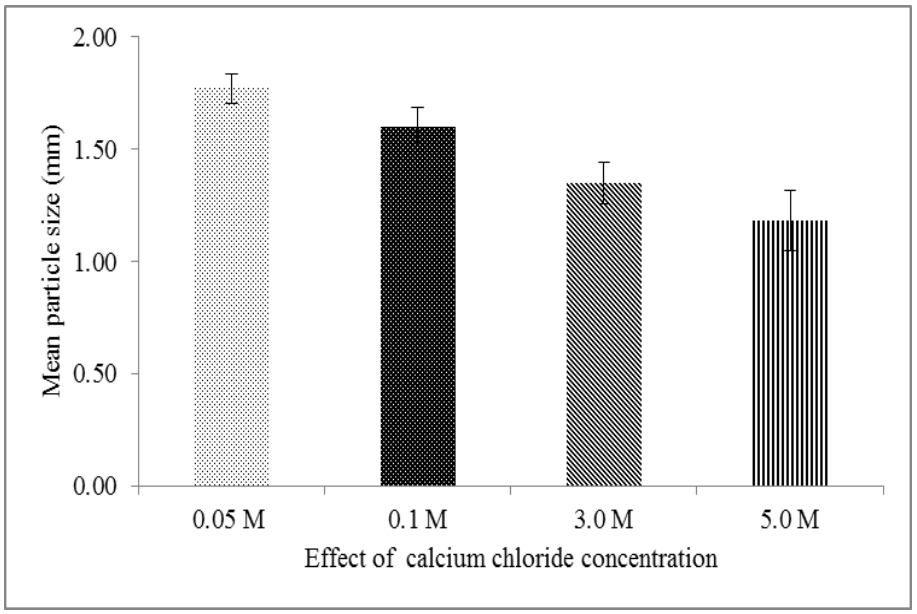


Figure 3.7 Effect of $CaCl_2$ concentration on particle size of THC-SME floating calcium alginate beads. The means and standard deviation of triplicate data are plotted.

Floating properties

The floating ability of the prepared beads was evaluated along with drug release studies. The results are shown in Table 3.5 and Figure 3.8. The effect of $CaCl_2$ concentration on floating lag time of the beads was studied. As $CaCl_2$ increased from 0.05, 0.1, 3.0 and 5.0 M, floating lag time increased (4±1.21, 15±1.54, 27±1.37 and 45±1.61 min, respectively) due to the higher concentration of $CaCl_2$ yielded denser structure; hence, gastric medium hardly diffused into the beads resulting in an increase of the floating lag time, while there is no effect on floating duration. All formulations floated longer than 8 h.

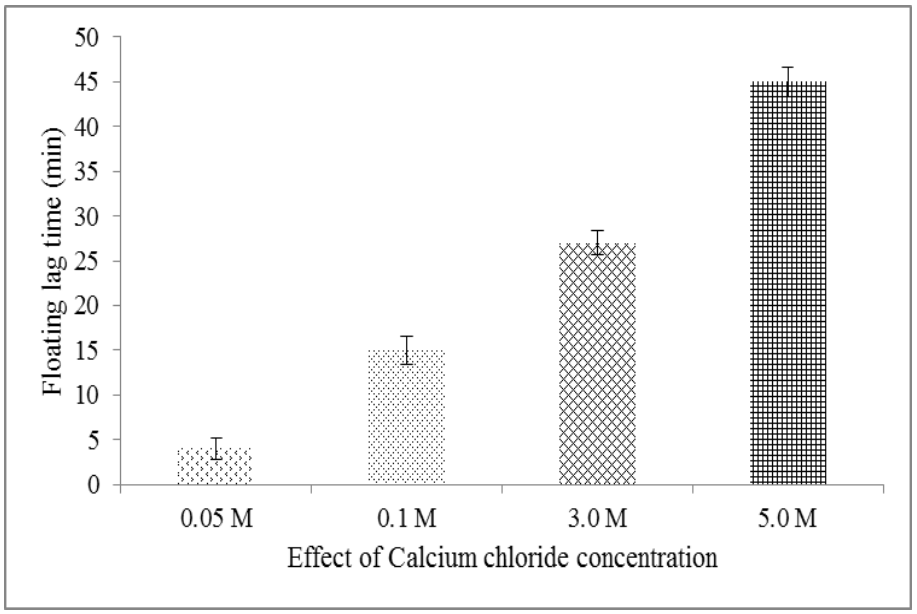


Figure 3.8 Effect of $CaCl_2$ concentration on floating lag time of THC-SME floating calcium alginate beads. The means and Standard deviation of triplicate data are plotted.

In vitro Drug release

Figure 3.9 shows the release profile of THC from the THC-SME floating calcium alginate beads. The beads were prepared using the same sodium alginate concentration, but different amounts of $CaCl_2$ concentration in the hardening solution. The rate and extent of THC release decreased as the calcium concentration used to harden the beads increased. For example, the percentages of THC released at 8 h were 61.50±0.46, 58.51±1.31, 45.73±1.04, and 35.27±0.32% for 0.05, 0.1, 3.0 and 5.0 M $CaCl_2$, respectively. These results may be explained by considering the structure of the beads. Low $CaCl_2$ concentration leads probably to a loose gel. As a consequence, the drug can be easily released from the beads, as the steric entanglements do not constitute a strong barrier. Further addition of $CaCl_2$ increases gives a more structured gel and the drug is more retained inside the beads due to steric reason, since the existence of physical entanglements of cross-linked polymer and calcium ion of lower dimensions controlling the drug diffusion flow within the beads. In contrast, at high concentration of $CaCl_2$, the strong and rigid gel is formed around the matrix and this strong gel reduce penetration rate of the dissolution medium into the matrix and prolong the release rate. Thus, in the sequential method, as the concentration of crosslinking agent increased, drug release decreased (El-Zatahry *et al.,* 2006). It was also evident from the literature (Rajinikanth *et al.,* 2003) that diffusion of drug from alginate matrix decreased as the concentration of $CaCl_2$ solution increased, probably due to greater cross-linking with sodium alginate.

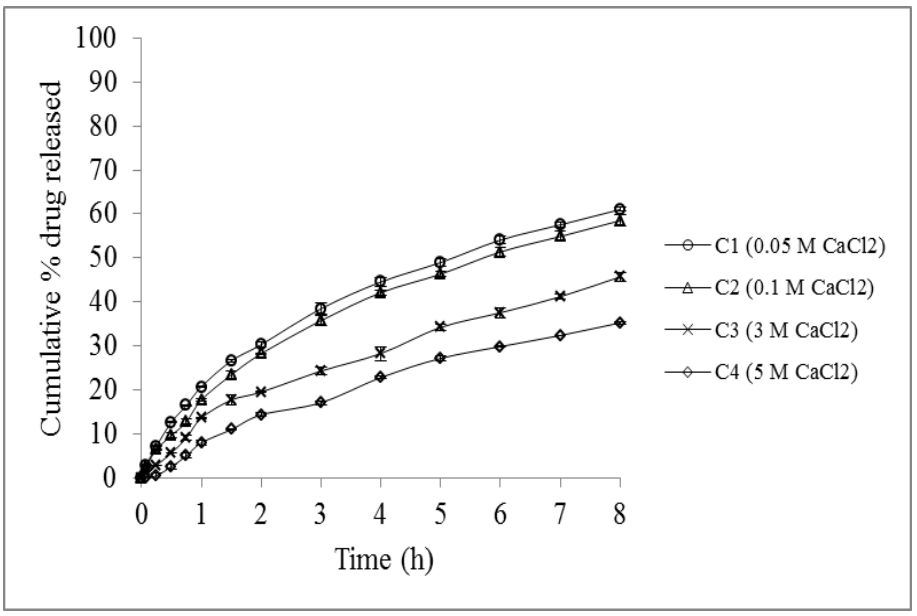


Figure 3.9 Effects of $CaCl_2$ (C1-C4) on THC release from THC-SME floating calcium alginate beads in SGF (pH 1.2) without pepsin. The means and standard deviation of triplicate data are plotted.

Thus, the formulation C1 with 0.05 M $CaCl_2$ was chosen for further experiment.

3.1.3.4 Effect of Kollicoat® IR concentration

Drug content and Encapsulation efficiency

The drug content of the bead formulations with different concentration of Kollicoat® IR was in the range of 14.38±0.26% and 16.56±0.50%. The entrapment efficiency was found in the range of 84.51±1.55% and 89.50±2.72% (Table 3.6). It was observed that an increase in the concentration of Kollicoat® IR from 1 to 3% w/v resulted in a little effect in both the drug content and encapsulation efficiency. Increasing the concentration of Kollicoat® IR (1, 2 and 3 % w/v) resulted in a high

viscosity of emulsion mixture. The relatively high viscosity of the emulsion mixture leading to a decrease in the drug loading in the beads.

Table 3.6 Physical properties of the bead formulations with different concentration of Kollicoat® IR. Data represents the mean ±SD (n=3).

Physical properties	Formulations		
	D1 (1% w/v)	D2 (2% w/v)	D3 (3% w/v)
Drug content (mg±SD/ 500 mg dried beads)	16.56±0.50	15.51±0.41	14.38±0.26
Encapsulation efficiency (±SD)	89.50±2.72	87.49±2.30	84.51±1.55
Bead size (mm±SD)	1.80±0.16	1.87±0.14	2.10±0.12
Floating lag time (s)	<30	<30	<30
Floating duration (h)	>8	>8	>8

Particle size analysis

The mean particle sizes of the beads formulations were obtained in the range between 1.80±0.16 and 2.10±0.12 mm (Table 3.6 and Figure 3.10). By increasing the concentration of Kollicoat® IR, the mean particle size of the beads increased.

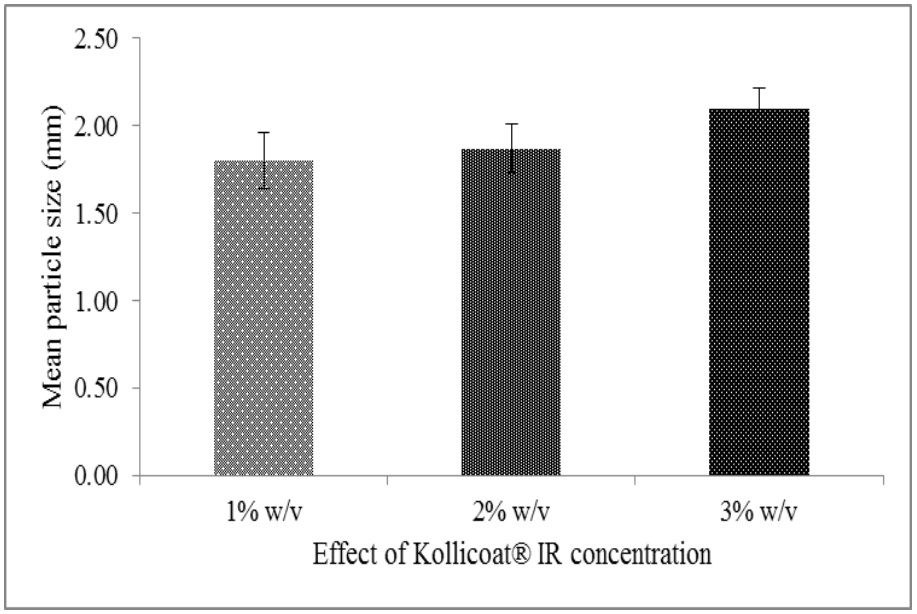


Figure 3.10 Effect of Kollicoat® IR concentration on particle size of THC-SME floating calcium alginate beads. The means and standard deviation of triplicate data are plotted.

Floating properties

The floating ability of the prepared beads was evaluated in SGF pH 1.2 and it was found that all formulation floated immediately or with a very short lag time of about <30 s. In addition, similarly floating duration time of all formulations which floated longer than 8 h was observed in Table 3.6. The results indicated that all bead formulations had good floating property.

***In vitro* drug release**

From the results of *in vitro* release studies, it revealed that the floating calcium alginate beads (D1, D2, and D3) gave a controlled release of THC during 8 h experiment. Among the formulations, formulation D3 provided an optimal modified release with a maximum % cumulative release within 8 h. The release rates of THC increased as the concentration of Kollicoat® IR increased due to its hydrophilic nature and thus readily soluble in water. It could create the pores in alginate matrix, this resulted in increased drug release (Figure 3.11).

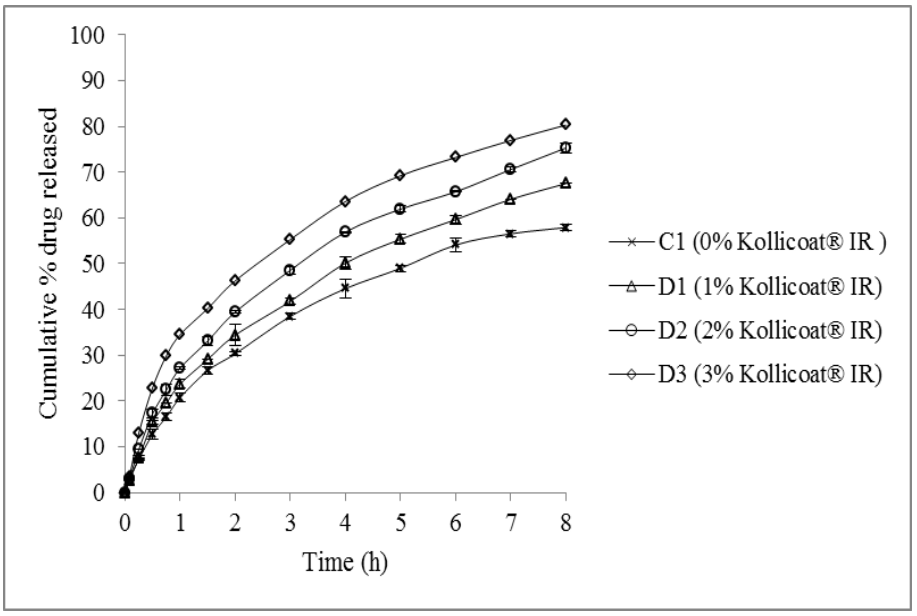


Figure 3.11 Effects of Kollicoat® IR (D1-D3) on THC release from THC-SME floating calcium alginate beads in SGF (pH 1.2) without pepsin. The means and standard deviation of triplicate data are plotted.

Formulation D3 prepared with 2% w/v sodium alginate, 10% w/v THC-SME, 3% w/v Kollicoat® IR and in 0.05 M $CaCl_2$ solution was selected as an optimal formulation based on drug release studies. This formulation has shown to release

80.32±0.46% of THC in 8 h (Figure 3.11). The formulation D3 (3% Kollicoat® IR) was therefore be used for further evaluation.

3.1.3.5 Effect of Drying Method

Drug content and Encapsulation efficiency

The effect of drying method on the drug content and encapsulation efficiency of the two formulations prepared are shown in Table 3.7. There was no significant difference of drug loading and encapsulation efficiency between formulation E1 (oven drying) and E2 (freeze drying).

Table 3.7 Physical properties of the beads formulations prepared with different drying method. Data represents the mean ±SD (n=3).

Physical properties	Formulations	
	E1* (Oven drying)	E2 (Freeze drying)
Drug content (mg±SD/ 500 mg dried beads)	14.38±0.26	14.11±0.19
Encapsulation efficiency (±SD)	84.51±1.55	82.89±1.11
Bead size (mm±SD)	2.10±0.12	3.42±0.13
Floating lag time (s)	<30	<30
Floating Duration (h)	>8	>8

*The compositions of formulation E1 is identical to that of formulation D3 (Table 3.6)

Particle size analysis

As shown in Table 3.7 and Figure 3.12, the particle size of THC-SME floating calcium alginate beads was dependent on drying methods. Drying method could influence the mean particle size of the beads (Smrdel *et al*., 2008; Gal and Nussinovitch, 2007; George and Abraham, 2007; Fundueanu *et al*., 1999). The particle sizes were 2.10±0.12 mm for oven drying and 3.42±0.13 mm for freeze drying beads. The average beads size of the oven drying was smaller than that of the freeze drying beads due to the shrinking during the drying process at higher temperatures. However, the freeze drying almost prevented shrinking of the beads. Size of freeze dried beads remained almost the same as before drying while those of oven drying beads became small and dense.

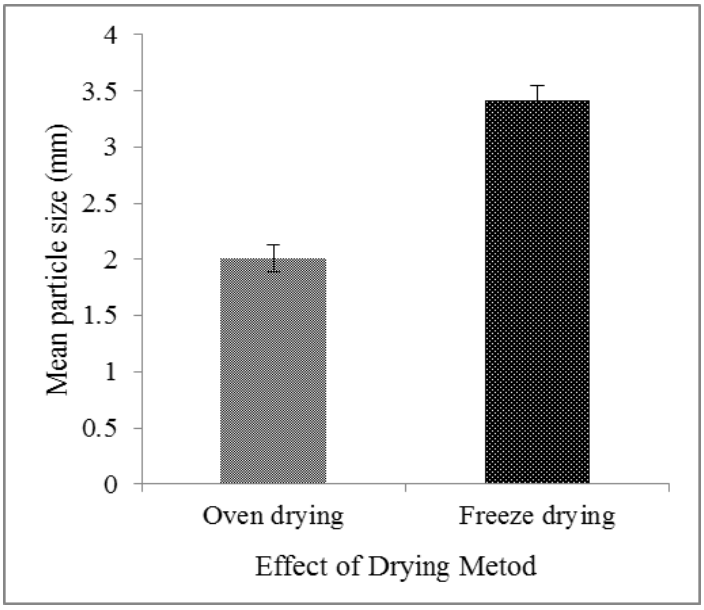


Figure 3.12 Effect of drying method on particle size of THC-SME floating calcium alginate beads. The means and standard deviation of triplicate data are plotted.

Morphological characterization

This study demonstrated that the drying method had great influence on shape and morphology of the beads including surface as well as internal structure characteristics of the beads (Figure 3.13 and 3.14). The freeze drying beads had larger size than oven drying beads and they remained almost of the same size as before drying. Their external structures were rather rough (Figure 3.14a,c) and their internal structure was very porous (Figure 3.14b,d) making them brittle to the touch. These characteristics of freeze drying beads are due to the fast sublimation of frozen water from alginate matrix resulting in formation of pores in areas of former ice crystals without having time to shrink. In contrast, the oven drying beads substantially shrank and became smaller during drying (Figure 3.15). They were more spherical probably due to slower water removal during oven drying resulting in slower and more uniform shrinkage of the beads. Similar findings regarding the size of freeze drying and oven drying beads were reported in the literature (Smrdel *et al*., 2008; Gal and Nussinovitch, 2007; George and Abraham, 2007; Fundueanu *et al*., 1999). The surface morphology of hot-air dried beads (Figure 3.13a,c) was smoother than that of freeze-dried (Figure 3.14a,c). From the internal structure of hot-air dried beads (Figsure 3.13b, d and 3.14b, d, respectively) a dense interior can be seen. The drying method influenced the beads dense, which increased with an increase in drying temperature. The freeze-dried beads demonstrated smaller apparent density because they are more porous than the hot-air dried beads. This is in accordance with the results of George *et al.* 2007 and Gal *et al.* 2007.

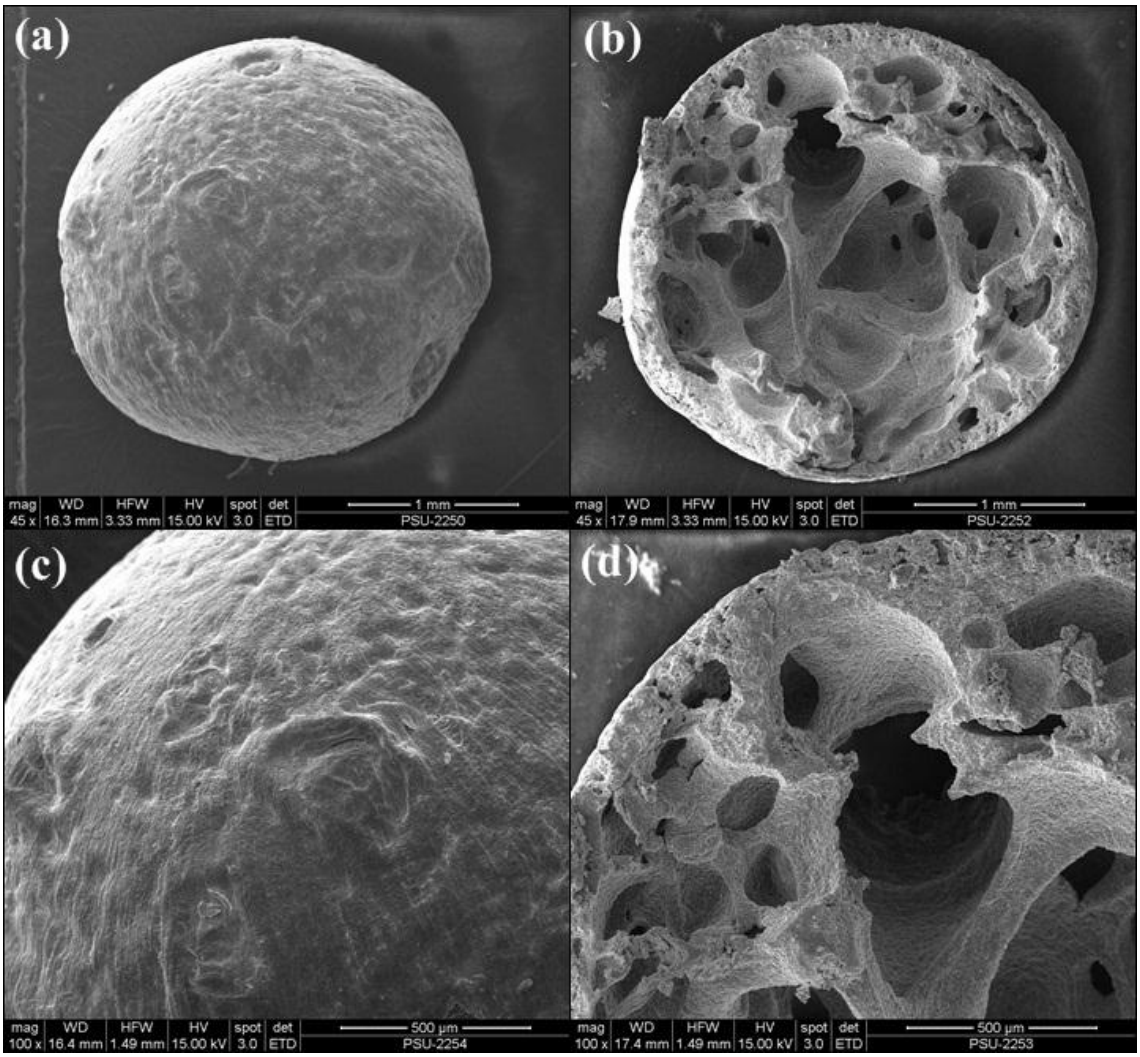


Figure 3.13 Scanning electron micrographs of (a and c) external and (b and d) internal structures of the THC-SME floating calcium alginate beads on drying by oven drying. Magnifications and scale bars are shown on the individual photographs.

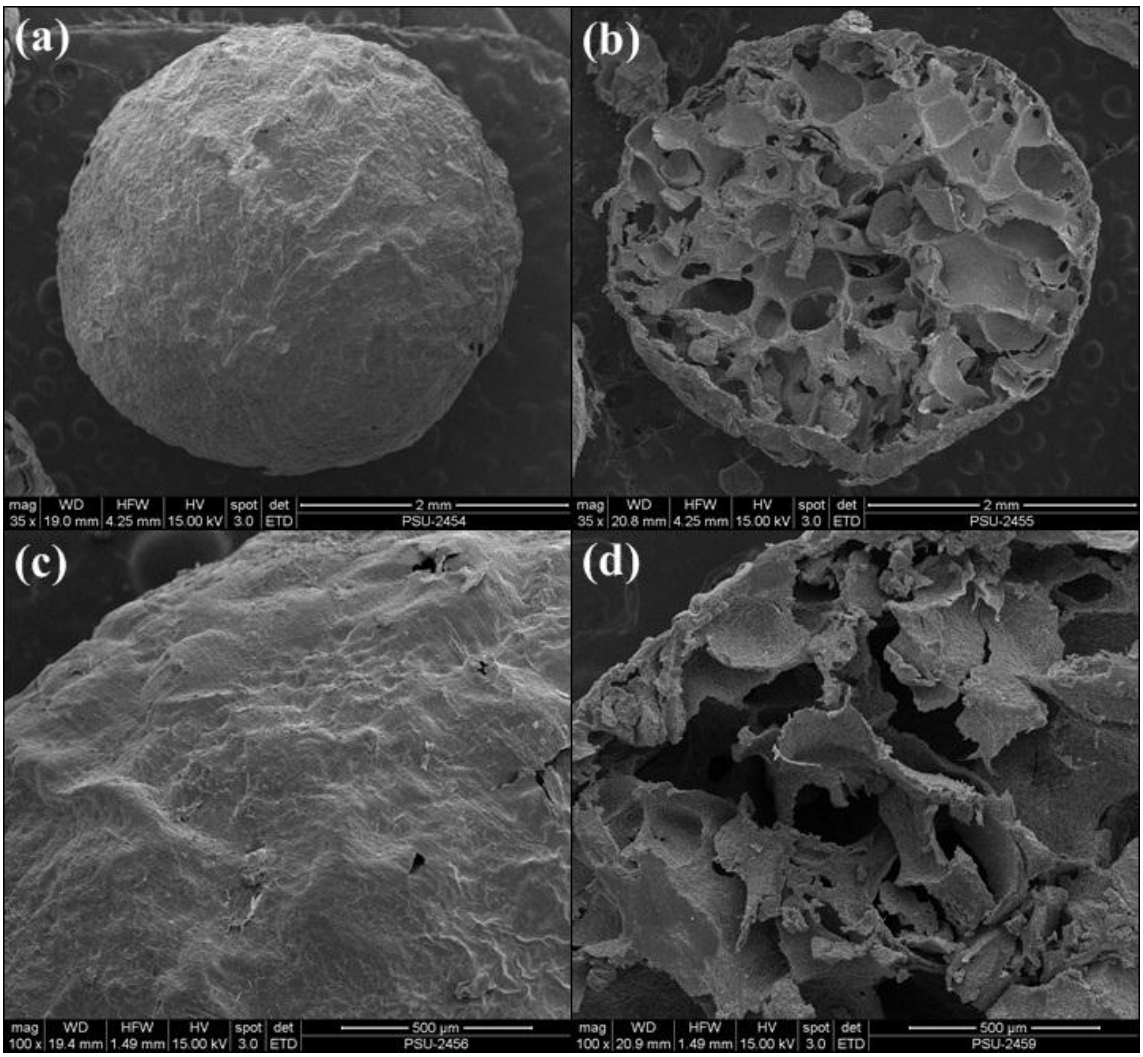


Figure 3.14 Scanning electron micrographs of (a and c) external and (b and d) internal structures of the THC-SME floating calcium alginate beads on drying by freeze drying. Magnifications and scale bars are shown on the individual photographs.

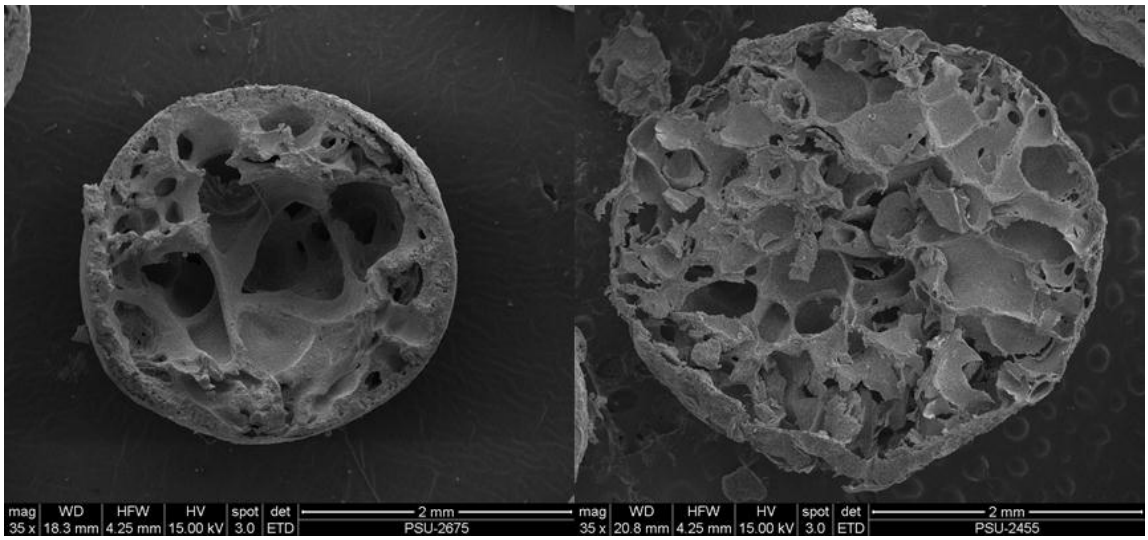


Figure 3.15 Scanning electron micrographs of cross-section of the THC-SME floating calcium alginate beads on drying by oven drying (left) and freeze drying (right). Magnifications and scale bars are shown on the individual photographs.

Floating properties

Floating studies were performed in SGF pH 1.2 without pepsin. The results showed that all formulations floated immediately or with a very short lag time of about <30 s on immersion in test media. Moreover, all formulations floated longer than 8 h (Table 3.7).

***In vitro* drug release**

The effect of drying method on the release of THC from THC-SME floating calcium alginate beads is shown in Figure 3.16. The faster drug release was observed with the freeze drying beads. This behavior has also been reported in the literatures (Sriamornsak, 1999; Sriamornsak, 1998). The freeze drying beads showed very high initial burst release fractions with rapid release, while the hot air beads demonstrated a lower burst and slower drug release. The faster drug release is probably explained by the higher porosity of the freeze drying product. However, for all formulations, the drug release was complete within 8 h.

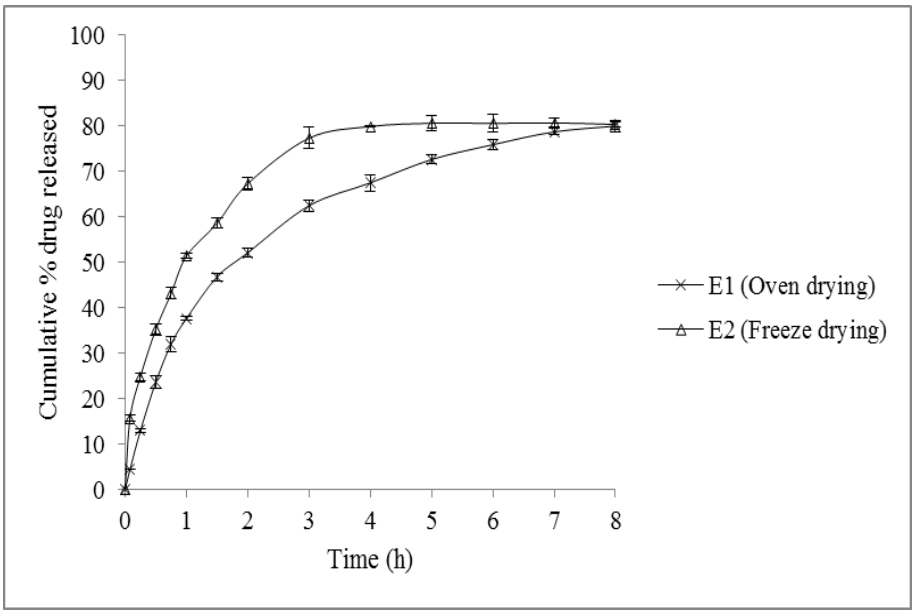


Figure 3.16 Effects of drying method on THC release from THC-SME floating calcium alginate beads. The means and standard deviation of triplicate data are plotted

3.1.4 The optimum formulation

The optimized THC-SME floating calcium alginate beads formulations (E1) contained 10% w/v THC-SME mixture, 2% w/v sodium alginate, 0.05 M $CaCl_2$, 3% w/v Kollicoat® IR and dried by oven drying at 50 °C 12 h. It provided a controlled release of 80% of the THC over an 8 h period. The droplet size of the emulsion from the optimum formulation E1 was 31.5±1.0 nm, which was similar to that of the liquid THC-SMEDDS (30.0±0.3 nm). Furthermore, incorporating liquid SMEDDS in a solid dosage form such as the beads did not have an effect on the emulsion droplet size. The optimum formulation of THC-SME floating calcium alginate beads prepared by ionotropic gelation method is shown in Figure 3.17.

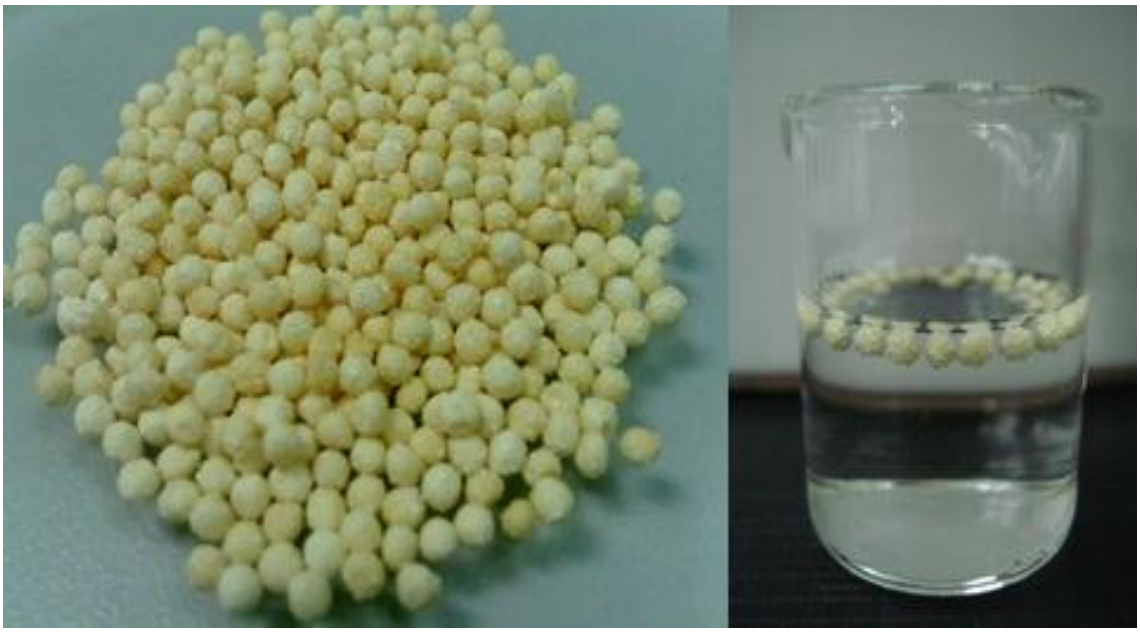


Figure 3.17 The THC-SME floating calcium alginate beads are almost spherical (left) and still float in SGF (pH1.2) after 8 h (right).

3.1.5 *In vitro* release of THC from the optimum formulation (E1) compared to the release from THC powder, THC floating calcium alginate beads and liquid THC-SMEDDS

Based on the data from various earlier experiments, formulation E1 was judged to be the ideal formulation. A comparative release study of THC powder, THC floating calcium alginate beads, liquid THC-SMEDDS and THC-SME floating calcium alginate beads (E1) was carried out to ascertain the superiority of E1 over the THC powder and THC-SMEDDS formulation. THC powder, as expected for a compound with low aqueous solubility, dissolved only slowly and only 30% of the drug was release at 8 h. THC floating calcium alginate beads dissolved only slowly and only 10% of the drug was release at 8 h. This may be a result of the controlled release barrier of alginate beads. Liquid THC-SMEDDS, as expected, release the THC almost instantly, since it rapidly forms an o/w microemulsion. Therefore, only the new developed THC-SME floating calcium alginate beads affords a controlled release of THC over an 8 h period (Figure 3.18) and the release was nearly complete (80%).

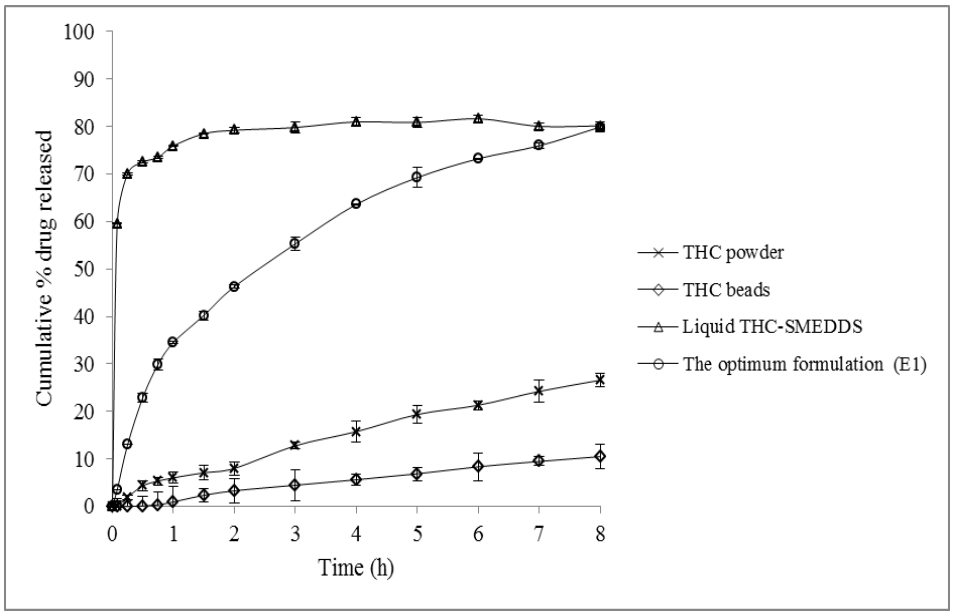


Figure 3.18 Release profile of THC from the optimum formulation (E1) compared with the release from THC powder, THC floating calcium alginate beads and liquid THC-SMEDDS in SGF. The mean±SD of triplicate data are plotted.

3.1.6 Formulation stability studies

Table 3.8 Stability data of capsule filled with the optimum formulation (E1). Data reported are mean±SD (n=3)

(A) 30±2ºC, 65±5% RH	
Sampling Time	Drug content (mg±SD/500 mg dried beads)
0 month	14.95±1.14
3 month	14.72±1.02
6 month	14.65±1.06
(B) 45±2ºC, 75±5% RH	
0 month	14.95±1.14
3 month	14.62±0.98
6 month	14.48±1.45

The stability data is summarized in Table 3.8. The optimum formulation E1 was found to be stable under intermediate storage condition (6 month) with content of THC in beads forms remaining in the range of 14.65±1.06 to 14.95±1.14 mg (500 mg dried beads). For under accelerate storage condition, the THC content from THC-SME floating calcium alginate beads (E1) was 14.48±1.45 to 14.95±1.14 (500 mg of beads) at 6 month. The droplet size of the emulsion from the optimum formulation D3 was 30.5±1.4 nm and 31.0±0.5 for intermediate storage condition and accelerate storage condition, respectively, which was similar to that of the liquid SEDDS (30.0±0.3 nm). There were no significant changes in the drug content, and the emulsion droplet size up to 6 months under both intermediate storage condition

(30±2ºC, 65±5% RH) and accelerate storage condition (45±2ºC, 75±5% RH; Table 3.8)

3.2 ALGINATE BEADS CONTAINING SELF-MICROEMULSIFYING FORMULATION FOR ORAL DELIVERY OF CURCUMIN

3.2.1 HPLC method for *in vitro* analysis of curcumin

A validated HPLC method developed by Setthacheewakul *et al.* (2010) was used to identify and determine of curcumin in the active ingredient and its pharmaceutical formulations. The curcumin exhibited a well-defined chromatographic peak with a retention time of curcumin approximately 8 min. Figure 3.19B and 3.19C shows the chromatogram obtained by injection of a solution containing curcumin and curcumin-SMEDDS released from beads (5 μg/mL), respectively. The chromatogram shows the curcumin peak was clearly separated from the other peaks. This result confirmed the ability of the method to identify curcumin from the pharmaceutical excipients presented in the SMEDDS products.

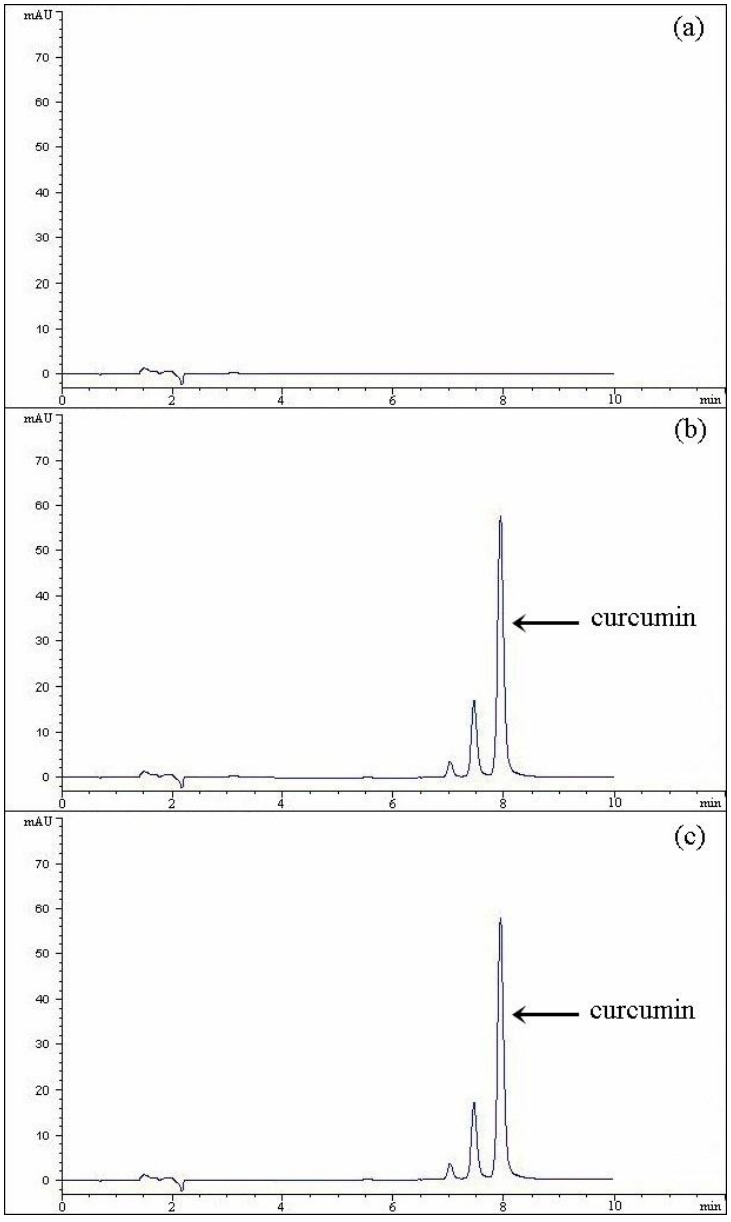


Figure 3.19 HPLC chromatogram of placebo-curcumin (a), curcumin (b) and curcumin-SMEDDS released from beads (c), using a C18 reverse phase column with a mixture of 2% aqueous acetic acid and acetonitrile (a linear gradient system), and a PDA detector (425 nm). Flow rate was 1 mL/min, injection volume was 20 µL

The calibration curve for curcumin was constructed by plotting concentrations *versus* corresponding mean peak areas (Figure 3.20). A good linearity was achieved with a correlation coefficient of 0.9999 over the concentration range of 0.1-5 µg/mL. The concentration of curcumin was calculated from the standard curve [peak area = 140.53 x conc. + 4.1508 (r^2 = 0.9999 ± 0.0002)]. It was shown that an excellent correlation existed between the peak area and concentration of the analyte.

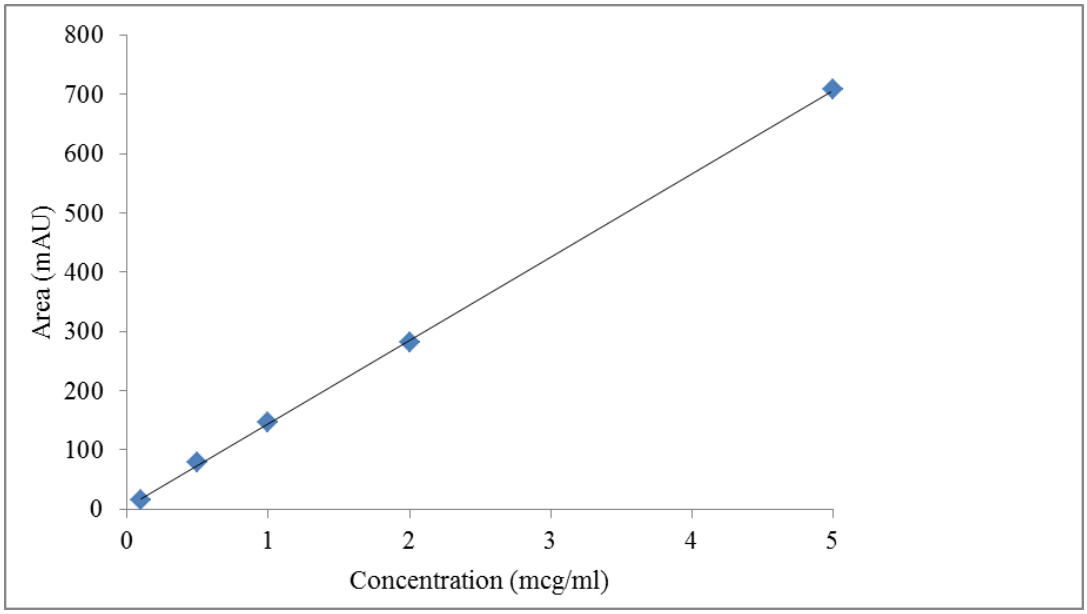


Figure 3.20 Calibration curve of curcumin

The precision of the method was determined by intra-day repeatability and intermediate precision studies. The reproducibility of the method was demonstrated by repeated injections of THC standards. The intra-day relative standard deviations (RSD) ranged from 0.68% to 1.61%, whereas the inter-day relative standard deviations ranged from 0.82% to 1.33% respectively (Table 3.9).

Table 3.9 RSD (%) of intra-day repeatability and intermediate precision studies

Concentration µg/mL	RSD (%)	
	Intra-day repeatability	Intermediate precision
0.4	1.61	0.82
0.5	0.68	1.01
0.6	1.09	1.33

The accuracy of the method was verified with recovery values of 98.99-100.98% (Table 3.10).

Table 3.10 Recovery (%) of curcumin at various concentrations

Sample	Recovery (%) on each studied concentration		
	0.4 µg/mL	0.5 µg/mL	0.6 µg/mL
1	102.10	100.70	101.59
2	100.14	97.11	100.37
3	100.69	99.15	99.77
Average	100.98	98.99	100.58

The HPLC method validation results showed that determination of curcumin could be performed by the validated HPLC method described above with accepted accuracy and precision.

3.2.2 Effect of formulation variables on physical properties of curcumin-SME alginate beads

In the present work, Formulation F1 (Table 3.11) was prepared by dropping an aqueous solution of sodium alginate containing curcumin-SME powder into $CaCl_2$ solutions and gelled spheres were formed instantaneously by ionotropic gelation. The preparation of chitosan coated alginate beads (Table 3.11, F2-F4) were prepared as described in section 2.2.4.2, which produced the beads and then transferred into post-coagulation fluid solution (0.25, 0.5 and 1% w/v of chitosan for F2, F3 and F4, respectively). The chitosan-alginates beads (Table 3.11, M2-M4) were obtained by dropping the homogeneous mixture (sodium alginate and curcumin-SME powder) into coagulation fluid ($CaCl_2$ solutions containing chitosan). Calcium ions and chitosan molecules had to diffuse into sodium alginate droplets to form the beads. The resultant the beads were followed by transfer into post-coagulation fluid solution (0.25, 0.5 and 1% w/v of chitosan for M2, M3 and M4, respectively). The beads after additional treatment with chitosan were intact and compact. In case of formulation M1, it was not transferred into post-coagulation fluid solution of chitosan.

Effect of formulation variables on emulsion droplet size analysis

The solid curcumin-SME of different formulations (F1–F4, M1-M4) preserved the self-emulsification performance of the liquid curcumin-SMEDDS, and the powder used for the beads had no remarkable effect on the droplet size of the reconstituted microemulsions. The curcumin-SME beads of eight different formulations (F1–F4, M1-M4) produced microemulsions with a droplet size in the range of 26.4 to 33.1 nm with a narrow distribution size, which was similar to the

droplet size of the emulsion from the liquid curcumin-SMEDDS formulation (29.18±0.7 nm).

Table 3.11 Composition of curcumin-SME alginate beads, curcumin-SME chitosan coated alginate beads and curcumin-SME chitosan-alginate beads. Data represents the mean ±SD (n=3)

Formulations	Physical properties			
	Drug content (mg±SD)	Encapsulation efficiency (±SD)	Beads size (mm±SD)	Time duration of beads in medium (h)
F1	5.78±.0.15	75.36±0.12	1.56±0.11	< 3
F2 (0.25%)	4.78±0.08	62.32±0.46	1.60±0.43	10
F3 (0.5%)	4.91±0.17	64.02±0.27	1.65±0.12	10
F4 (1%)	5.29±0.23	68.97±0.10	1.71±0.39	10
M1	6.15±0.19	80.18±0.34	1.59±0.07	< 5
M2 (0.25%)	5.57±0.25	72.62±0.51	1.64±0.34	10
M3 (0.5%)	5.72±0.09	74.58±0.40	1.75±0.03	10
M4 (1%)	6.02±0.61	78.49±0.39	2.10±0.12	10

Drug content and Encapsulation efficiency

Drug content and encapsulation efficiency of the different the beads formulations are given in Table 3.11. The concentrations of chitosan for coating of alginate beads had effect on the drug content and entrapment efficiency of curcumin in the beads prepared (F2-F4). As the chitosan concentration increased, the drug content and encapsulation efficiency was increase. This may be due to more sufficient cross-linking of alginate with chitosan during and after gelation caused by increased ionic interactions between the carboxylate groups in the alginate and the protonated amine groups in the chitosan. As a result, less drugs is lost during gelation (Anal and Stevens, 2005). However, drug content and entrapment efficiency were very high (75.36±0.56%, Table 3.11) in the absence of chitosan (F1). The drug content and entrapment efficiency for the chitosan-alginate gel beads (M1-M4) showed the same trends as in the chitosan coated alginate gel beads (Table 3.11).

Particle size analysis

The shape of the chitosan coated alginate beads (F1-F4) was spherical. The particle size of the beads was found to increase with the increase in percent concentration of chitosan (Sezer and Akbˆuga, 1995; Lim *et al*., 1997; Anal *et al*., 2003; Anal and Stevens, 2005). Mean particle size of formulations F1-F4 was between 1.56±1.15 and 1.71±0.93 mm (Table 3.11). The chitosan-alginate beads (M1-M4) showed an increase in particle size, probably due to extra coating with chitosan. Mean particle size of formulations M1-M4 was between 1.59±0.32 and 2.10±1.31 mm (Table 3.11).

Morphological characteristic

The SEM photos of typical surface (a,c) and cross-section (b) morphology of chitosan coated alginate beads and chitosan-alginate beads are presented in Figure 3.21; F1-F4 and Figure 3.22; M1-M4, respectively.

As shown in Figure 3.21 (F1-F4), it appeared that the beads coated with chitosan (F2-F4) have greatly altered the surface texture of the beads when compared with the uncoated beads (F1). The surface of F2 was found to be rougher than F3 and F4. Figure 3.22 presents the SEM of chitosan-alginate beads (M1-M4). We also could see that the surface structure of M1 was found to be rougher than M2, M3 and M4. It was clear that the surface of the coated beads with chitosan became smoother with increasing the concentration of chitosan, indicating that chitosan was coated on the surface of alginate beads.

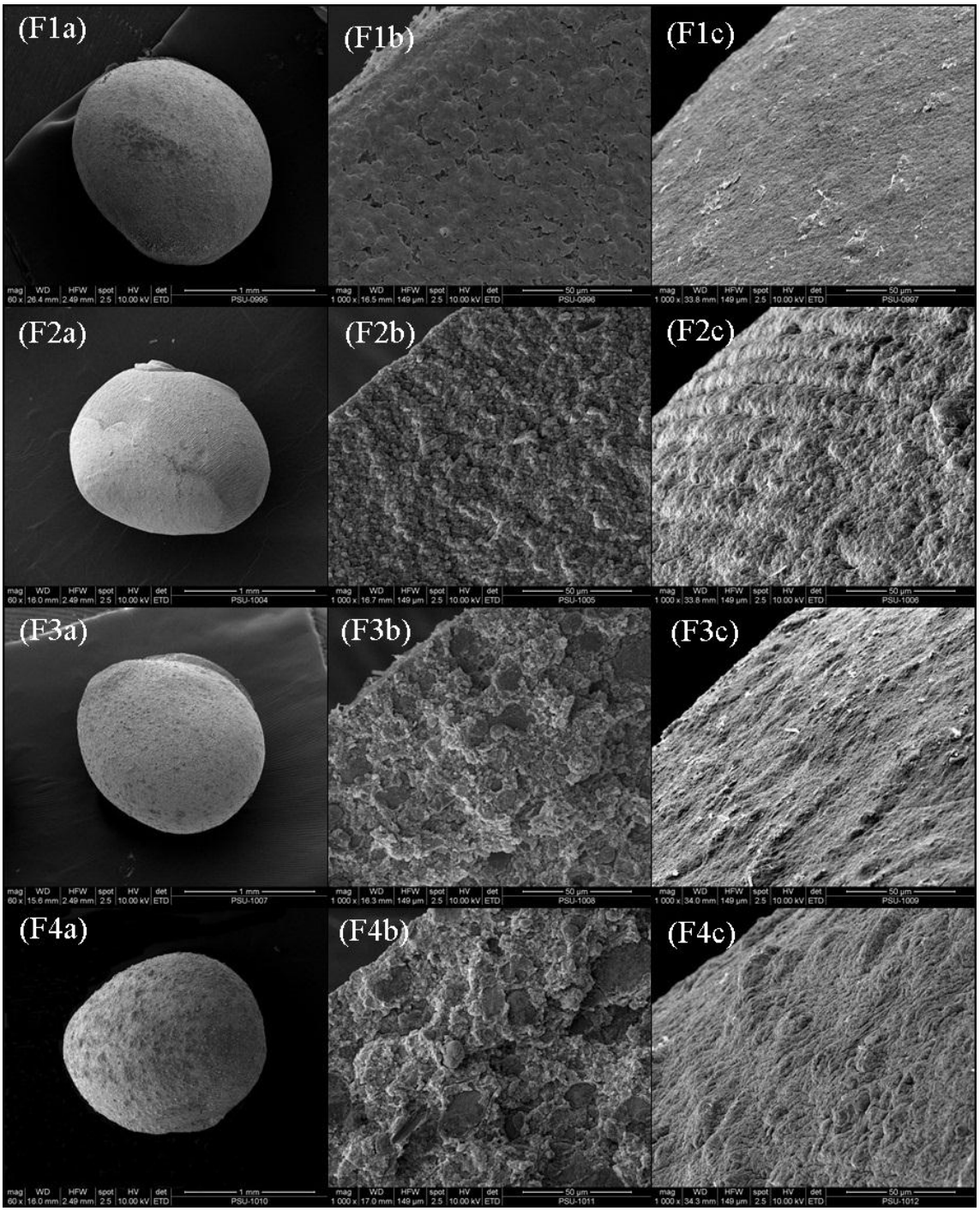


Figure 3.21 Scanning electron micrographs of typical surface (a and c) and cross (b) section morphology of the chitosan coated alginate beads. Magnifications and scale bars are shown on the individual photographs.

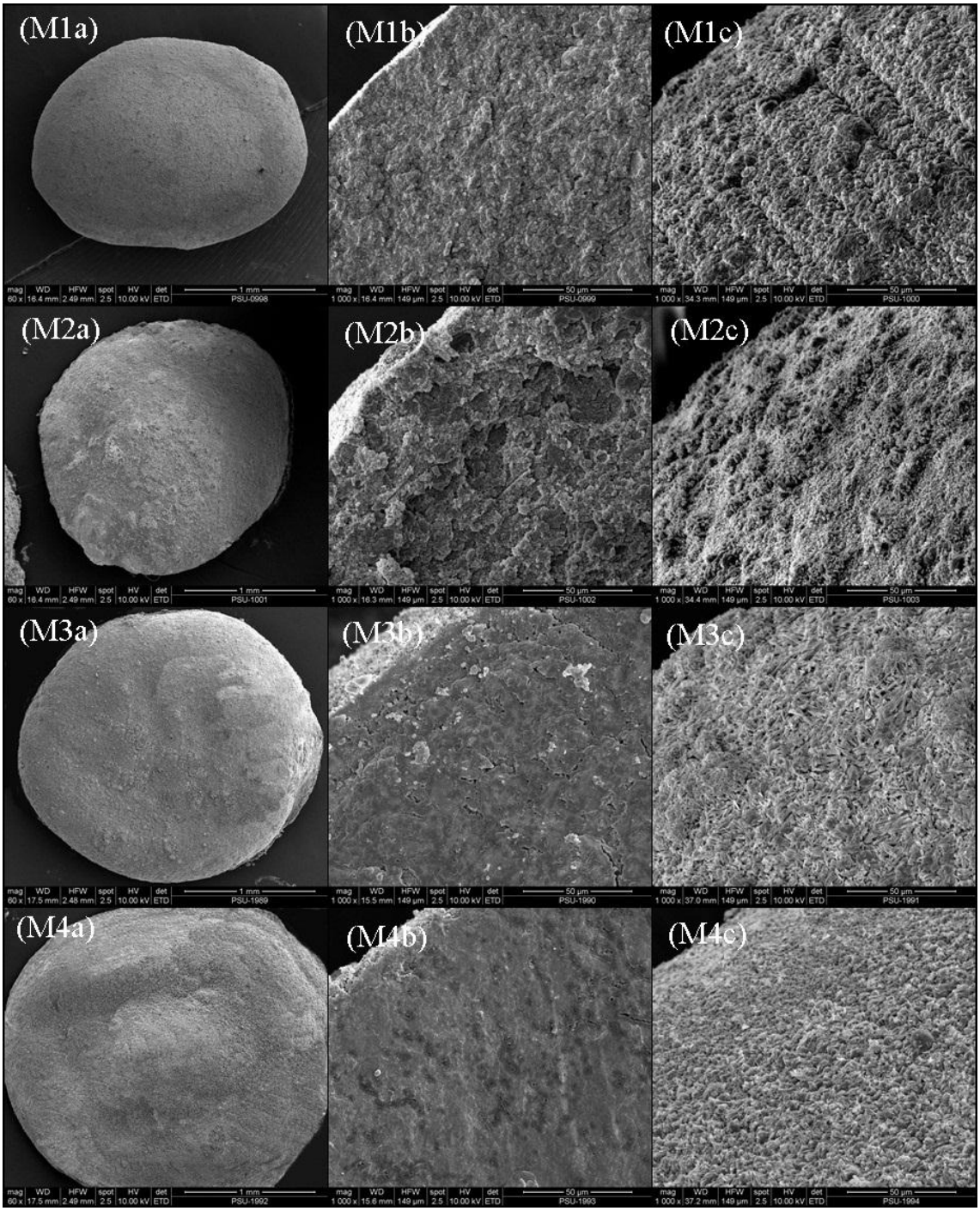


Figure 3.22 Scanning electron micrographs of typical surface (a and c) and cross (b) section morphology of the chitosan-alginate beads. Magnifications and scale bars are shown on the individual photographs.

Swelling property

The swelling properties of curcumin-SME loaded alginate beads (F1), chitosan coated alginate beads (F2-F4), chitosan-alginate beads (M1) and chitosan-alginate beads (M2-M4) were studied by measuring the water uptake in SGF (pH 1.2) for 2 h prior to test in (pH 6.8) for the next 10 h.

The results are shown in Figure 3.23. It was observed that when different formulations of the beads were placed into SGF (pH 1.2), the beads slightly swelled, which are similar for all formulations. The beads prepared without chitosan (formulation F1) was unstable in SGIF. Complete disintegration was observed within 3 h. In contrast, formulations F2-F4 gave the stronger beads with delayed disintegration and remained intact about 10 h in the SGIF. In a similar way, formulation M1 was completely disintegrated within 5 h. The chitosan-alginate beads (M2-M4) were more stable and remained intact about 10 h in the SGIF. It was observed that when the alginate beads coated with chitosan resulting in a decrease in the ion exchange between medium and alginate. As a result, less swelling behavior of the beads was detected.

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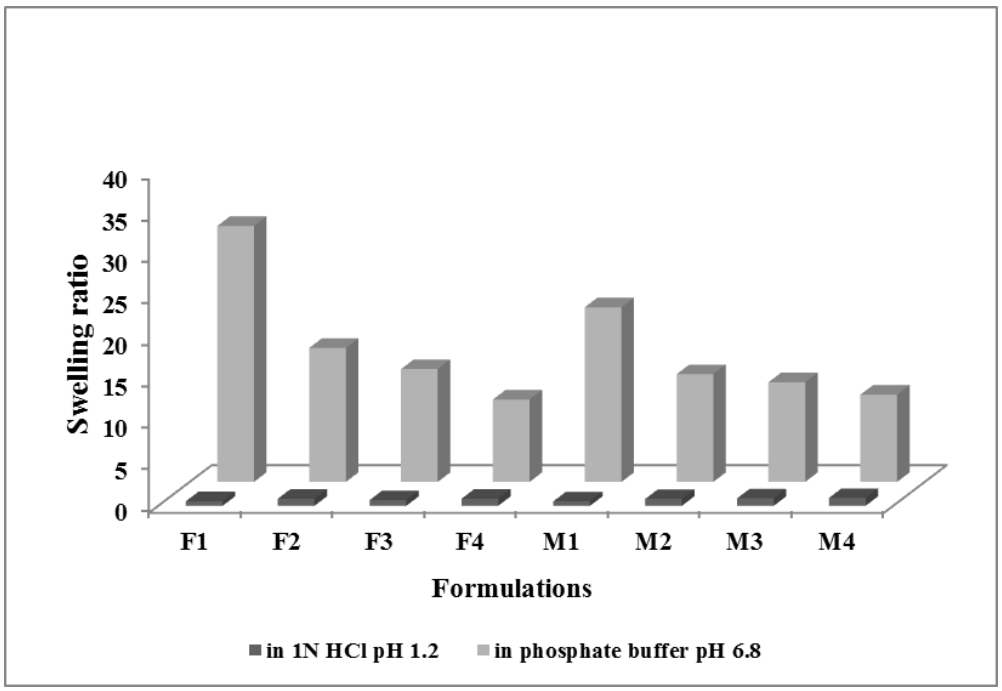

Figure 3.23 Effect of chitosan on swelling ratio of curcumin-SME beads. The means and standard deviation of triplicate data are plotted.

***In vitro* drug release in SGIF**

The release behavior of curcumin from different formulations of beads was investigated in SGF and SIF. The beads were incubated in SGF; pH 1.2 during the initial 2 h (approximately the residence time in the stomach) and then transfered into SIF; pH 6.8 (to simulate the intestinal conditions) for the next 8 h.

The results showed that the release rate of curcumin from different beads formulations in SIF was much higher than that in SGF (Figure 3.24 and 3.25). It was observed from the swelling study that the beads had swollen in SIF pH 6.8 more than in SGF pH 1.2. It can be said that the release will depend on diffusion of the curcumin through the insoluble matrix of alginate polymer in SGF pH 1.2 due to the inability of the dissolution medium to penetrate the beads. However, the dissolution medium

readily penetrated into the beads at pH 6.8, easily leaching out the curcumin incorporated in them.

The curcumin release, in SIF from the beads could be retarded by adding chitosan. This is similar to that of chitosan-reinforce calcium alginate beads reported by Murata *et al*. (2008). The increase concentration of chitosan showed a slower curcumin release. The ranged order for the rate of curcumin release from the beads in SGIF was as follows: F1>F2>F3>F4 for 0, 0.25, 0.5 and 1% w/v of concentration of chitosan coated alginate beads (Figure 3.24) and M1>M2>M3>M4 for 0, 0.25, 0.5 and 1% w/v of concentration of chitosan (post-coagulation fluid) (Figure 3.25). This may be also due to the electrostatic interaction between carboxyl groups of the alginate and the amino group of chitosan produced a compact surface layer that reduced diffusion of fluid into the gel beads (Rajendran and Basu, 2009). Curcumin release at pH 6.8 was accompanied by rapid swelling and disintegration of the alginate beads.

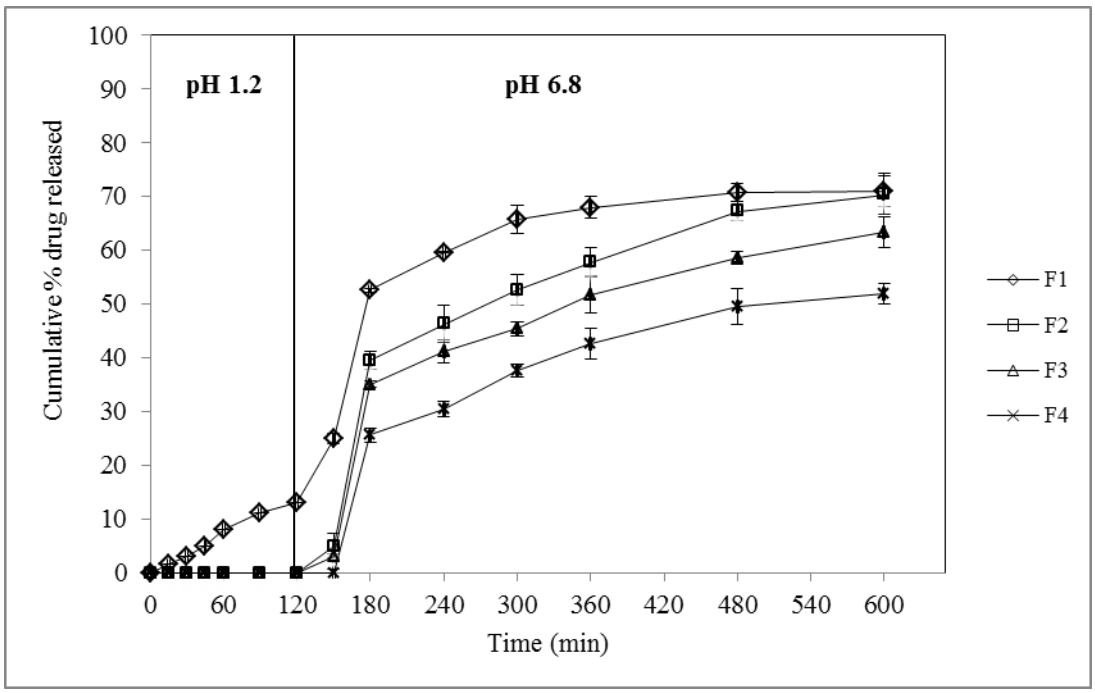

Figure 3.24 Effects of concentration chitosan coated alginate beads (0, 0.25, 0.5 and 1% w/v of chitosan for F1, F2, F3 and F4, respectively) on curcumin release from curcumin-SME chitosan coated alginate beads in SGIF. The means and standard deviation of triplicate data are plotted.

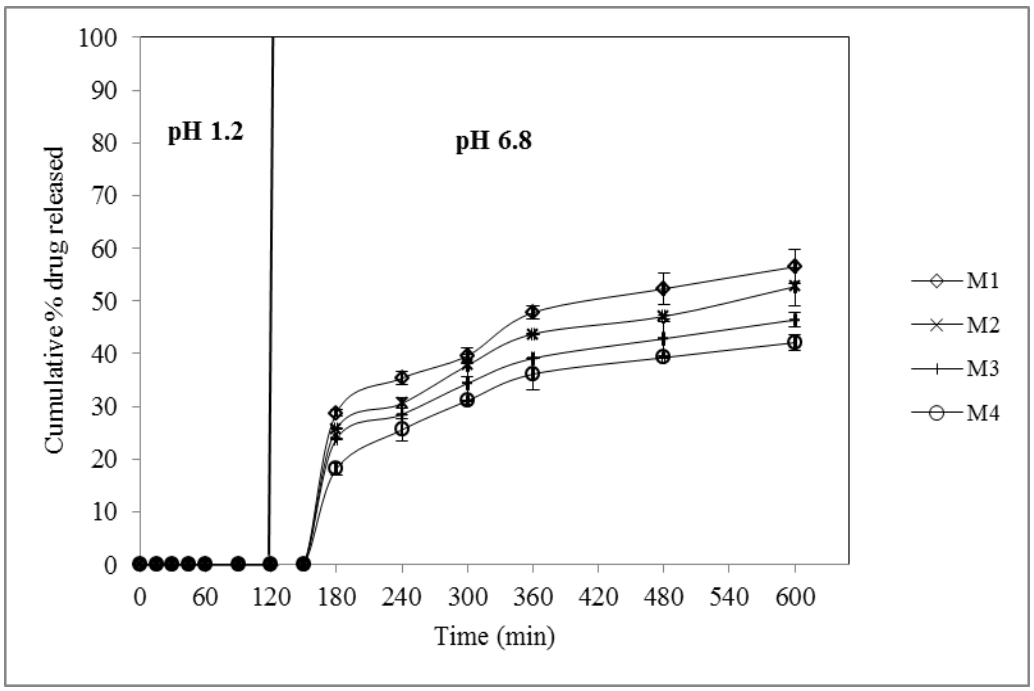

Figure 3.25 Effects of concentration chitosan in post-coagulation fluid (0, 0.25, 0.5 and 1% w/v of chitosan for M1, M2, M3 and M4, respectively) on curcumin release from curcumin-SME chitosan-alginate beads in SGIF. The means and Standard deviation of triplicate data are plotted.

3.2.3 The effect of formulation variables on curcumin release *in vitro*

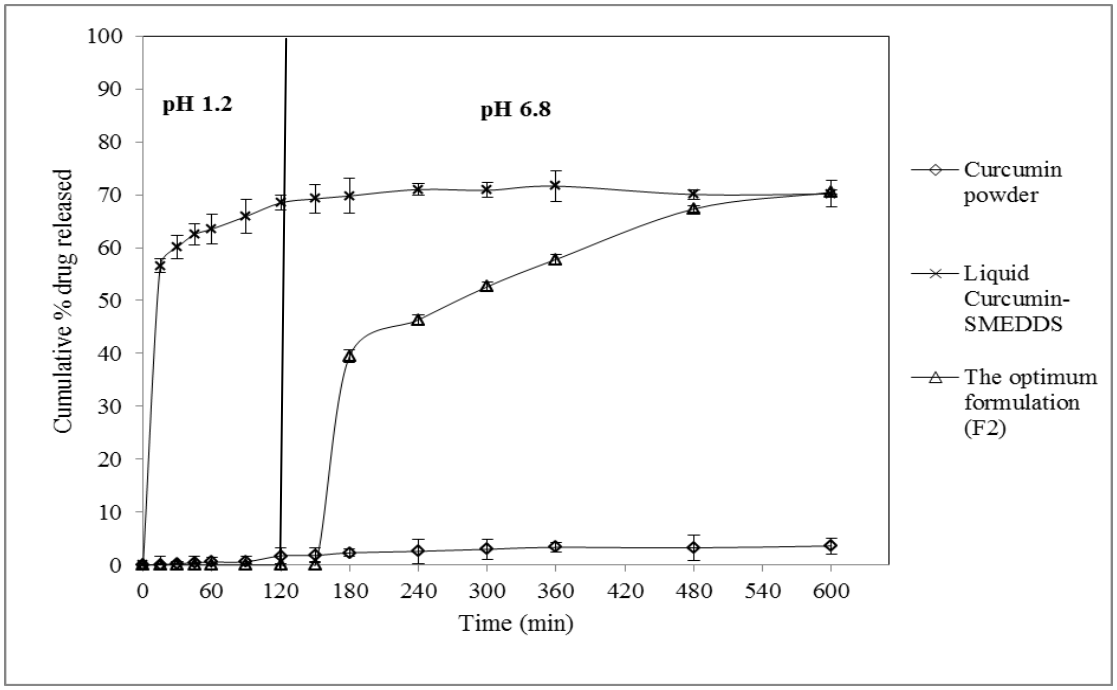

Figure 3.26 Release profile of curcumin release from the chitosan coated alginate beads (F2) and chitosan-alginate beads (M2) compared with curcumin powder and liquid curcumin-SMEDDS in SGIF. The mean±SD of triplicate data are plotted.

A comparative release study of curcumin powder, liquid curcumin-SMEDDS and solid curcumin-SME (F2 and M2) was carried out to ascertain the superiority of F2 over all formulation. Curcumin powder, as expected for a compound with low aqueous solubility, dissolved only slowly and only 3% of the drug was in solution at 10 h. In contrast, the liquid curcumin-SMEDDS, released the curcumin almost instantly, since it rapidly formed an o/w microemulsion when contacted with the dissolution medium. The optimum formulation of chitosan coated alginate beads (F2) provided a sustained release of 70 % of curcumin over an 10 h period. (Figure 3.26)

CHAPTER 4

CONCLUSION

Curcumin and THC are well-known for their pharmacological properties including anti-oxidant, anti-inflammatory, and anti-cancer. Nevertheless, both compounds are limited in their clinical applicability by the low bioavailability during oral administration. In the present study, two types of sustained release alginate beads containing self-microemulsifying formulation of curcumin or THC were developed to enhance its solubility and oral absorption.

This study has demonstrated that THC-SME formulation could be successfully encapsulated into a floating drug delivery system composed of calcium cross-linked alginate. The THC-SME floating alginate beads could be prepared using ionotropic gelation method and based on gas formation technique. This system could float and prolong the drug residence time in the stomach and provided the control drug release from the beads that resulted in improved solubility and dissolution of THC. The optimal formulation (E1) provided a controlled release of about 80 % of THC release from floating alginate beads over an 8 h period with short floating lag time within 30 seconds and prolonged floating duration (>8 h). The droplet size of the THC released from the beads was in the microemulsion range (31.5±0.1 nm).

The curcumin-SME formulation could also be encapsulated in sustained release alginate beads. The liquid curcumin-SME formulation was adsorbed onto solid adsorbent (colloidal silicon dioxide) and dispersed in alginate solution. The curcumin-SME alginate beads could be prepared using ionotropic gelation method

followed by coating with chitosan. Dissolution experiments indicated that the beads containing curcumin SME formulation and coated with chitosan enhanced the drug release of curcumin and that the chitosan coating considerably controlled the drug release. The optimal formulation F2 provided a controlled release of about 70% of curcumin release from the beads over a 10 h period. The droplet size of the emulsion released from the beads was in the range of 30.7±0.2 nm.

It is concluded that both sustained release THC-SME floating alginate beads and curcumin-SME alginate beads coated with chitosan can be suitable approaches to improve the oral bioavailability of THC and curcumin. In addition, the developed systems may reduce daily oral dose, and lead to the reduction of dosing frequency. In the future, further investigations to evaluate the *in vivo* bioavailability of these systems are necessary.

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APPENDIX

In vitro drug release

Table 1 Effects of sodium alginate (B1-B3) on THC release from THC-SME floating calcium alginate beads in SGF (pH 1.2) without pepsin.

Time (min)	Formulations								
	B1 (2% w/v)			B2 (3% w/v)			B3 (4% w/v)		
	%	Mean	±SD	%	Mean	±SD	%	Mean	±SD
5-1	3.25	3.27	0.16	2.95	2.94	0.07	2.58	2.57	0.21
5-2	3.45			3.01			2.78		
5-3	3.10			2.85			2.36		
15-1	7.20	7.28	0.08	5.59	5.45	0.12	4.49	4.42	0.27
15-2	7.38			5.43			4.64		
15-3	7.25			5.34			4.12		
30-1	12.40	12.67	0.32	8.99	8.53	0.44	6.94	6.84	0.23
30-2	13.07			8.59			6.59		
30-3	12.54			8.01			7.01		
45-1	16.09	16.53	0.40	12.06	11.63	0.36	9.53	9.46	0.17
45-2	16.98			11.28			9.27		
45-3	16.52			11.56			9.58		
60-1	20.48	20.65	0.30	16.23	15.95	0.27	12.17	12.36	0.34
60-2	21.03			15.63			12.16		
60-3	20.44			16.00			12.76		
90-1	25.09	25.80	0.55	20.69	20.62	0.34	16.32	15.95	0.37
90-2	26.19			20.21			15.58		
90-3	26.12			20.96			15.94		
120-1	28.42	28.23	0.19	23.31	23.17	0.15	18.80	19.33	0.68
120-2	28.27			23.21			20.10		
120-3	28.00			22.98			19.10		
180-1	36.62	35.42	0.93	30.22	29.93	0.33	25.06	25.12	0.21
180-2	34.96			29.51			25.36		
180-3	34.69			30.05			24.96		
240-1	42.50	42.08	0.35	35.80	35.42	0.31	30.32	29.62	0.62
240-2	41.74			35.35			29.14		
240-3	42.00			35.12			29.39		
300-1	48.76	48.34	0.37	40.14	39.91	0.19	34.64	33.44	1.04
300-2	47.93			39.86			32.73		
300-3	48.32			39.73			32.95		
360-1	53.20	52.80	0.96	43.47	43.88	0.34	37.07	36.50	0.54
360-2	51.58			44.21			36.01		
360-3	53.61			43.95			36.43		
420-1	57.74	56.91	0.76	47.06	46.99	0.08	39.54	41.32	1.56
420-2	56.04			46.89			42.40		
420-3	56.95			47.02			42.03		
480-1	59.44	58.71	0.60	51.15	50.64	0.45	45.11	44.03	1.02
480-2	58.58			50.14			43.09		
480-3	58.12			50.63			43.89		

Table 2 Effects of $CaCl_2$ (C1-C4) on THC release from THC-SME floating calcium alginate beads in SGF (pH 1.2) without pepsin.

Time (min)	Formulations											
	C1(0.05 M)			*C2 (0.1 M)			C3 (3 M)			C4 (5 M)		
	%	Mean	±SD	%	Mean	±SD	%	Mean	±SD	%	Mean	±SD
5-1	2.98	2.85	0.12	3.25	3.27	0.16	1.76	1.76	0.07	0.11	0.09	0.04
5-2	2.76			3.45			1.69			0.05		
5-3	2.80			3.10			1.83			0.12		
15-1	7.29	7.27	0.13	7.20	7.28	0.08	2.98	2.86	0.22	0.55	0.62	0.23
15-2	7.14			7.38			2.61			0.87		
15-3	7.39			7.25			3.00			0.43		
30-1	12.73	12.77	0.23	12.40	12.67	0.32	5.74	5.46	0.25	2.61	2.68	0.09
30-2	12.56			13.07			5.36			2.79		
30-3	13.02			12.54			5.27			2.65		
45-1	16.53	16.62	0.34	16.09	16.53	0.40	9.13	9.26	0.19	5.08	5.22	0.20
45-2	16.34			16.98			9.48			5.13		
45-3	17.00			16.52			9.16			5.44		
60-1	20.75	20.47	0.27	20.48	20.65	0.30	13.65	13.40	0.33	8.03	8.18	0.15
60-2	20.21			21.03			13.03			8.32		
60-3	20.45			20.44			13.52			8.18		
90-1	26.64	26.70	0.26	25.09	25.80	0.55	17.73	17.72	0.27	11.03	11.34	0.27
90-2	26.99			26.19			17.98			11.46		
90-3	26.48			26.12			17.45			11.53		
120-1	30.34	30.30	0.16	28.42	28.23	0.19	19.56	19.37	0.18	14.38	14.40	0.12
120-2	30.43			28.27			19.21			14.29		
120-3	30.12			28.00			19.34			14.53		
180-1	38.49	38.52	0.19	36.62	35.42	0.93	24.32	24.48	0.22	17.14	17.22	0.10
180-2	38.72			34.96			24.73			17.19		
180-3	38.34			34.69			24.39			17.34		
240-1	44.57	44.46	0.43	42.50	42.08	0.35	28.31	28.42	0.35	22.88	22.90	0.14
240-2	43.99			41.74			28.13			23.05		
240-3	44.82			42.00			28.81			22.78		
300-1	48.99	48.84	0.27	48.76	48.34	0.37	34.24	34.51	0.23	27.21	27.38	0.16
300-2	48.53			47.93			34.67			27.41		
300-3	49.01			48.32			34.61			27.53		
360-1	54.10	54.11	0.34	53.20	52.80	0.96	37.59	37.81	0.21	29.89	29.87	0.15
360-2	53.78			51.58			38.01			29.72		
360-3	54.46			53.61			37.83			30.01		
420-1	57.55	57.18	0.38	57.74	56.91	0.76	41.22	41.74	0.65	32.42	32.41	0.18
420-2	57.21			56.04			42.47			32.23		
420-3	56.79			56.95			41.53			32.59		
480-1	61.04	60.60	0.84	59.44	58.71	0.60	45.73	45.41	0.29	35.27	35.35	0.28
480-2	61.14			58.58			45.18			35.12		
480-3	59.63			58.12			45.32			35.67		

*The compositions of formulation C2 is identical to that of formulation B1 (Table 1)

Table 3 Effects of Kollicoat® IR (D1-D3) on THC release from THC-SME floating calcium alginate beads in SGF (pH 1.2) without pepsin.

Time (min)	Formulations								
	D1 (1% w/v)			D2 (2% w/v)			D3 (3% w/v)		
	%	Mean	±SD	%	Mean	±SD	%	Mean	±SD
5-1	2.79	2.72	0.07	3.01	2.97	0.05	3.59	3.53	0.05
5-2	2.65			3.00			3.52		
5-3	2.72			2.91			3.49		
15-1	7.51	7.44	0.10	8.66	9.03	0.44	13.03	13.30	0.28
15-2	7.32			9.52			13.30		
15-3	7.48			8.92			13.59		
30-1	15.17	15.73	0.71	17.45	17.45	0.44	22.31	22.88	0.66
30-2	15.48			17.02			23.60		
30-3	16.53			17.89			22.73		
45-1	20.01	20.03	0.49	22.36	22.22	0.36	30.71	29.87	0.80
45-2	19.56			22.49			29.11		
45-3	20.53			21.82			29.81		
60-1	23.44	23.43	0.40	27.94	26.97	0.84	34.65	34.44	0.20
60-2	23.02			26.47			34.41		
60-3	23.82			26.49			34.26		
90-1	30.61	29.82	0.70	33.02	33.33	0.40	40.89	40.14	0.68
90-2	29.60			33.20			39.57		
90-3	29.26			33.78			39.97		
120-1	34.64	34.31	0.36	40.03	39.82	0.21	46.07	46.37	0.26
120-2	34.36			39.83			46.51		
120-3	33.93			39.61			46.53		
180-1	41.30	41.74	0.52	49.12	48.64	0.54	55.86	55.20	0.60
180-2	42.32			48.05			54.71		
180-3	41.59			48.75			55.01		
240-1	50.55	50.96	0.64	57.81	57.00	0.71	63.70	63.51	0.21
240-2	51.70			56.51			63.54		
240-3	50.63			56.69			63.28		
300-1	54.84	55.12	0.69	61.23	61.75	0.77	69.76	69.01	0.66
300-2	55.90			62.64			68.74		
300-3	54.62			61.38			68.53		
360-1	60.64	59.81	0.77	66.38	65.61	0.68	73.32	73.43	0.37
360-2	59.65			65.07			73.13		
360-3	59.13			65.39			73.84		
420-1	64.67	63.86	0.70	69.19	69.12	0.07	75.54	75.76	0.55
420-2	63.52			69.04			76.38		
420-3	63.39			69.13			75.36		
480-1	68.02	67.51	0.50	75.31	75.28	0.04	79.15	80.03	0.79
480-2	67.02			75.29			80.67		
480-3	67.48			75.23			80.28		

Table 4 Effects of drying method (E1 and E2) on THC release from THC-SME floating calcium alginate beads in SGF (pH 1.2) without pepsin.

Time (min)	Formulations					
	*E1 (Oven drying)			E2 (Freeze drying)		
	%	Mean	±SD	%	Mean	±SD
5-1	3.59	3.53	0.05	15.61	15.70	0.11
5-2	3.52			15.82		
5-3	3.49			15.67		
15-1	13.03	13.30	0.28	24.49	24.88	0.44
15-2	13.30			25.36		
15-3	13.59			24.78		
30-1	22.31	22.88	0.66	30.27	31.28	1.02
30-2	23.60			32.31		
30-3	22.73			31.25		
45-1	30.71	29.87	0.80	40.14	41.45	1.14
45-2	29.11			42.26		
45-3	29.81			41.96		
60-1	34.65	34.44	0.20	51.13	52.15	0.88
60-2	34.41			52.71		
60-3	34.26			52.61		
90-1	40.89	40.14	0.68	59.31	59.91	0.54
90-2	39.57			60.02		
90-3	39.97			60.38		
120-1	46.07	46.37	0.26	65.43	65.87	0.42
120-2	46.51			66.26		
120-3	46.53			65.92		
180-1	55.86	55.20	0.60	77.92	77.52	0.61
180-2	54.71			76.82		
180-3	55.01			77.83		
240-1	63.70	63.51	0.21	80.44	80.58	0.27
240-2	63.54			80.89		
240-3	63.28			80.41		
300-1	69.76	69.01	0.66	80.79	80.14	0.90
300-2	68.74			79.11		
300-3	68.53			80.52		
360-1	73.32	73.43	0.37	81.47	80.32	1.00
360-2	73.13			79.79		
360-3	73.84			79.69		
420-1	75.54	75.76	0.55	80.80	80.67	0.15
420-2	76.38			80.69		
420-3	75.36			80.51		
480-1	79.15	80.03	0.79	79.02	80.11	0.96
480-2	80.67			80.79		
480-3	80.28			80.53		

*The compositions of formulation E1 is identical to that of formulation D3 (Table 3)

Table 5 Effects of concentration chitosan coated alginate beads on curcumin release from curcumin-SME chitosan coated alginate beads in SGIF.

Time (min)	Formulations											
	F1			F2 (0.25% w/v)			F3 (0.5% w/v)			F4 (1% w/v)		
	%	Mean	±SD	%	Mean	±SD	%	Mean	±SD	%	Mean	±SD
15-1	1.46	1.26	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15-2	1.23			0.00			0.00			0.00		
15-3	1.09			0.00			0.00			0.00		
30-1	3.16	2.97	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30-2	2.99			0.00			0.00			0.00		
30-3	2.76			0.00			0.00			0.00		
45-1	5.39	4.92	0.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
45-2	4.87			0.00			0.00			0.00		
45-3	4.51			0.00			0.00			0.00		
60-1	7.56	7.69	0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
60-2	7.49			0.00			0.00			0.00		
60-3	8.02			0.00			0.00			0.00		
90-1	11.21	11.25	0.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
90-2	11.62			0.00			0.00			0.00		
90-3	10.93			0.00			0.00			0.00		
120-1	13.64	12.84	0.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
120-2	12.14			0.00			0.00			0.00		
120-3	12.74			0.00			0.00			0.00		
150-1	25.61	24.84	0.76	5.23	5.01	0.20	3.19	2.90	0.29	0.00	0.00	0.00
150-2	24.83			4.92			2.61			0.00		
150-3	24.09			4.87			2.89			0.00		
180-1	52.50	52.33	0.48	39.50	39.04	0.42	35.05	33.70	1.17	25.57	24.88	0.82
180-2	52.71			38.94			33.08			23.98		
180-3	51.79			38.69			32.97			25.09		
240-1	59.55	59.94	0.59	45.35	46.27	0.87	41.16	40.13	0.89	30.44	29.80	0.77
240-2	60.62			46.39			39.61			28.95		
240-3	59.65			47.08			39.63			30.01		
300-1	65.68	65.28	0.92	49.62	50.98	1.36	45.36	44.28	1.00	37.64	36.87	0.81
300-2	64.23			52.34			44.08			36.96		
300-3	65.93			50.98			43.39			36.02		
360-1	67.95	67.68	0.55	57.79	57.96	0.44	51.70	50.67	0.99	42.58	41.73	1.37
360-2	67.05			58.45			50.59			40.15		
360-3	68.04			57.63			49.73			42.45		
480-1	70.77	70.03	0.68	67.32	66.64	0.63	58.55	58.65	0.67	49.54	48.90	1.19
480-2	69.89			66.08			58.04			47.52		
480-3	69.43			66.53			59.37			49.63		
600-1	71.04	70.82	0.21	70.49	70.71	0.85	63.29	63.07	0.95	51.91	51.45	1.02
600-2	70.63			71.65			63.89			52.15		
600-3	70.79			69.99			62.03			50.28		

Table 6 Effects of concentration chitosan in post-coagulation fluid on curcumin release from curcumin-SME chitosan-alginate beads in SGIF.

Time (min)	Formulations											
	M1			M2 (0.25% w/v)			M3 (0.5% w/v)			M4 (1% w/v)		
	%	Mean	±SD	%	Mean	±SD	%	Mean	±SD	%	Mean	±SD
15-1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15-2	0.00			0.00			0.00			0.00		
15-3	0.00			0.00			0.00			0.00		
30-1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30-2	0.00			0.00			0.00			0.00		
30-3	0.00			0.00			0.00			0.00		
45-1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
45-2	0.00			0.00			0.00			0.00		
45-3	0.00			0.00			0.00			0.00		
60-1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
60-2	0.00			0.00			0.00			0.00		
60-3	0.00			0.00			0.00			0.00		
90-1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
90-2	0.00			0.00			0.00			0.00		
90-3	0.00			0.00			0.00			0.00		
120-1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
120-2	0.00			0.00			0.00			0.00		
120-3	0.00			0.00			0.00			0.00		
150-1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
150-2	0.00			0.00			0.00			0.00		
150-3	0.00			0.00			0.00			0.00		
180-1	28.77	27.86	0.82	25.72	25.00	0.96	23.85	22.78	0.95	18.18	17.86	0.35
180-2	27.63			23.91			22.49			17.92		
180-3	27.17			25.38			22.01			17.49		
240-1	35.35	34.66	0.94	30.56	29.59	1.21	28.47	27.93	0.80	25.57	25.05	0.53
240-2	35.04			28.23			28.31			25.06		
240-3	33.59			29.98			27.01			24.51		
300-1	39.62	38.76	1.47	37.78	37.09	0.67	34.35	33.29	0.95	31.09	30.70	0.35
300-2	37.06			37.07			32.53			30.59		
300-3	39.61			36.43			32.99			30.41		
360-1	47.79	47.17	0.64	43.72	42.66	1.24	39.10	38.38	0.87	36.11	35.84	1.12
360-2	46.51			41.29			37.41			36.79		
360-3	47.21			42.98			38.63			34.61		
480-1	52.32	51.66	0.84	47.06	46.15	0.88	42.85	41.68	1.22	39.27	38.42	0.85
480-2	51.94			45.31			41.78			38.41		
480-3	50.71			46.09			40.42			37.57		
600-1	56.49	55.95	1.38	51.71	51.61	1.28	46.42	46.04	0.41	42.10	42.55	0.88
600-2	56.98			50.29			46.08			43.56		
600-3	54.38			52.84			45.61			41.99		