บีดคอลลาเจน-แอลจิเนตชนิดใหม่ที่กักเก็บเคอร์คูมินอยด์ได้นาน

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NOVEL COLLAGEN-ALGINATE BEADS WITH LONG-LASTING CURCUMINOIDS ENCAPSULATION

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Petrochemistry and Polymer Science
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Thesis Title	NOVEL COLLAGEN-ALGINATE BEADS WITH LONG-LASTING CURCUMINOIDS ENCAPSULATION
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Field of Study	Petrochemistry and Polymer Science
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ปรียานุช ศศิวิลาสกร : บีดคอลลาเจน-แอลจิเนตชนิดใหม่ที่กักเก็บเคอร์คูมินอยด์ได้นาน (NOVEL COLLAGEN-ALGINATE BEADS WITH LONG-LASTING CURCUMINOIDS ENCAPSULATION) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร. พัฒทรา ธีรพิบูลย์เดช, 79 หน้า.

สามารถสร้างคอลลาเจน-แอลจิเนตบีดชนิดใหม่สำเร็จโดยการเกิดปฏิกิริยาทรานส์อะไคเลชันระหว่างหมู่เอสเทอร์ของโพรพิลีน ไกลคอล แอลจิเนตและหมู่อะมิโนของคอลลาเจน บีดที่เตรียมด้วยคอลลาเจน 10% มีรูปร่างที่กลมที่สุด ด้วยขนาดเส้นผ่านศูนย์กลางเท่ากับ 5.20 มิลลิเมตร ความแข็งแรงของบีดที่มีการเคลือบผิวชั้นนอกลดลงเมื่อความเข้มข้นของคอลลาเจนเพิ่มขึ้น พันธะเอไมด์ของเมมเบรนชั้นนอกที่เกิดขึ้นใหม่สามารถตรวจสอบจากผลของการวิเคราะห์ด้วย Light microscopy, FTIR และ DSC เคอร์คูมินอยด์ที่ถูกกักเก็บในบีดมีสองชนิดได้แก่ เคอร์คูมินอยด์อิสระและเคอร์คูมินอยด์ที่ถูกกักเก็บในอนุภาคเอทิลเซลลูโลส โดยเคอร์คูมินอยด์ที่ถูกกักเก็บในอนุภาคเอทิลเซลลูโลสนั้น มีการปลดปล่อยที่ช้ากว่าเคอร์คูมินอยด์อิสระในบัฟเฟอร์ pH 5.5 นอกจากนี้ยังพบว่าเมมเบรนชั้นนอกของคอลลาเจน-แอลจิเนตบีดสามารถลดอัตราเร็วในการปลดปล่อยของเคอร์คูมินอยด์

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต .....

ปีการศึกษา 2557 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

5572041423 : MAJOR PETROCHEMISTRY AND POLYMER SCIENCE
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PREEYANUCH SASIWILASSAKORN: NOVEL COLLAGEN-ALGINATE BEADS WITH LONG-LASTING CURCUMINOIDS ENCAPSULATION. ADVISOR: ASST. PROF. PATTARA THIRAPHIBUNDET, Ph.D., 79 pp.

New collagen-alginate coated beads (col-alg coated beads) were successfully prepared by using transacrylation reaction between ester group of propylene glycol alginate and amino group of collagen. The coated beads prepared by 10% collagen had resulted in the good sphere shape with diameter of 5.20 mm. The hardness of the coated beads were found to decrease when increase the collagen concentration. The new amide bond of the outer membrane was proved by the Light microscopy, FTIR and DSC results. Two types of curcuminoid were loaded in the beads which were free curcuminoid and Cur-loaded EC particles. Cur-loaded EC particles showed slower releasing than free curcuminoid in pH 5.5 buffer. Furthermore, the outer membrane of col-alg coated beads can retarded the release of curcuminoid.

Field of Study: Petrochemistry and Polymer Science
Student's Signature .....
Advisor's Signature .....
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LIST OF ABBREVIATIONS

%	percentage
°C	degree celsius
β	beta
α	alpha
Alg	alginate
Col	collagen
cm^{-1}	unit of wavenumber (IR)
cP	centipoise
Cur	curcuminoid
Da	dalton
DLS	dynamic light scattering
DSC	Differential scanning calorimetry
EC	ethyl cellulose
EE	entrapment efficiency
et al.	et alli, and other
ATR-FTIR	attenuated total reflectance-fourier transform infrared
g	gram/unit of strength
hr	hour
HPLC	High performance liquid chromatography

HSA	human serum albumin
i.e.	that is
in	inch
kV	kilovolt
LC	loading capacity
µL	microliter
M	molar
mg	milligram
min	minute
mL	milliliter
mm	millimeter
MWCO	molecular weight cut off
N	newton
nm	nanometer
PBS	phosphate buffer saline
PDI	polydispersity index
PGA	propylene glycol alginate
pH	negative logarithm of the activity of the hydrogen ion in an aqueous solution
rpm	revolution per minute
s	second

S.D.	Standard deviation
SEM	Scanning electron microscope
UV-vis	ultraviolet-visible
w/w	weight by weight

CHAPTER I

INTRODUCTION

1.1 Motivation of Research

Alginate is a naturally polysaccharides extracted from blown algae, Laminaria hyperborea, Laminaria digitata, Laminaria Japonica, Ascophyllum nodosum and Macrocystis pyrifera. Alginate is a linear copolymer containing blocks of (1,4)-linked β-D-mannuronate (M-blocks) and α-L-guluronate (G-blocks) residues [1]. According to its good solubility, non-toxicity, good compatibility and low cost, alginate is a widely used in many industries such as pharmaceutical, cosmetic and food industries. Alginate is able to form hydrogel beads by intermolecular cross-linking between divalent cations, such as Ca^{2+}, and carboxylate groups of guluronic acid unit [2]. Alginate beads have been extensively used for encapsulation many materials, such as drugs, living cells, fragrances and enzymes, and have been developed their properties for appropriate applications and products. Floating beads can prolong the release of active component in a gastric fluid [3, 4]. Mixing some polymers with alginate matrix can improve drug entrapment efficiency and also retard drug release [5]. Coating alginate beads with chitosan layer is found to protect active component from an aggressive stomach environment when administrate orally [6]. Interesting for our research is alginate beads in which their outer stable membrane is fabricated via transacrylation between ester groups of propylene glycol alginate (PGA) and amino

groups of human serum albumin (HSA) [7-9]. The stable membrane significantly improves the mechanical properties and be slow down the drug release. In this work, the outer membrane of alginate beads is constructed via the transacrylation between ester groups of PGA and amino groups of fish collagen. Fish collagen is now widely used as a biomaterial and pharmaceutical application because it has biocompatibility, high stability and a variety of bioactivities [10, 11]. We aimed that the outer membrane improves the stability of active agents and also protects the release of the active agents into a medium solution. The collagen-alginate coated beads contained active ingredients will be useful for many cosmetic products such as shampoo and shower cream.

1.2 The Objectives of Research

1. Fabricate new collagen-alginate coated beads by transacrylation reaction and loaded with free curcuminoid or curcuminoid-loaded ethyl cellulose particles.

2. Characterize physical and chemical properties of collagen-alginate coated beads.

3. Study releasing rate of curcuminoid from collagen-alginate coated beads.

CHAPTER II

THEORY AND LITERATURE REVIEWS

In this work, collagen-alginate coated beads were fabricated by external gelation technique and the outer membrane of beads was formed by transacrylation reaction between ester groups of propylene glycol alginate (PGA) and amino groups of collagen. Curcuminoid was chosen to be an active agent for studying the entrapment efficiency and controlled release from beads. These beads aimed to add in the cosmetic products such as shampoo and hand cream or lotion which can store the active ingredients inside the beads and release when rubs on the skin. This chapter presented the theory and literature reviews involved this work and research.

2.1 Alginate

Alginate is natural copolymer obtained from brown algae including to *Laminaria hyperborea, Laminaria digitata, Laminaria japonica, Ascophyllum nodosum,* and *Macrocystis pyrifera* [12]. It is linear polysaccharides consisted of 1⟶4 linked β-D mannuronic acid (M) and α-L guluronic acid (G) residues. The blocks are composed of sequences of M (M blocks) residues, sequences of G (G blocks) residues and alternating M and G (MG blocks) residues (Fig. 2.1) [13, 14]. Alginate extracted with different sources differs in M and G contents as well as the length of each block. It is widely used for many applications such as cosmetic,

pharmaceutical, food industry owing to its general biocompatibility, non-toxicity, low cost, biodegradability and gel formation ability. Alginate has been extensively used for biomedical application such as cell culture [15], protein delivery [16], wound dressing [17] and drug delivery [18].

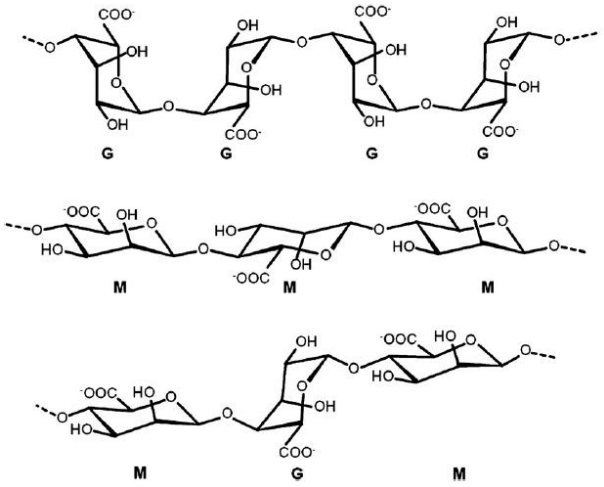


Figure 2.1 Chemical structures of G blocks, M blocks and MG blocks in alginate

The most common method to prepare hydrogel bead is adding the alginate solution with ionic cross-linking solutions such as divalent cations (i.e., Ca^{2+}). The divalent cations are ability to binding with guluronate blocks of the alginate chains. The structure of guluronate block allows to coordination with divalent cations. The one guluronate blocks of polymer formed junctions with the other guluronate blocks of adjoining polymer chain, the so call egg-box model (Fig. 2.2) [19, 20].

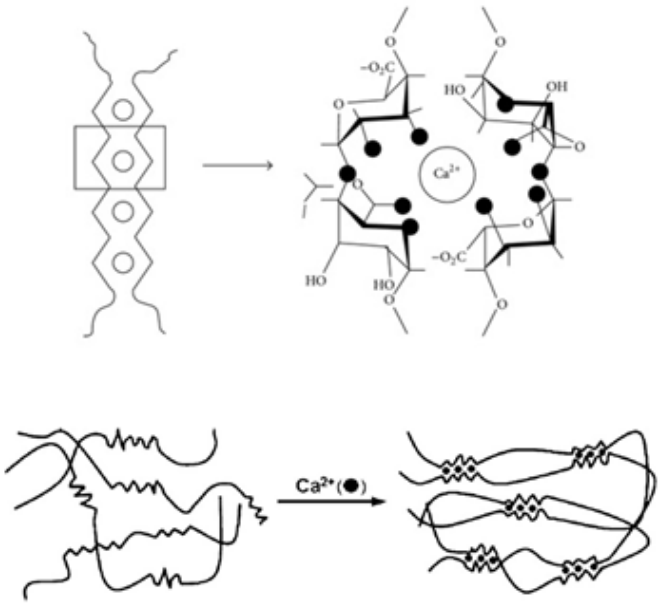


Figure 2.2 Binding to calcium ions by G-block in alginate (a) and Egg-box model structure of an alginate gel formation.

In spite of the fact that calcium alginate beads can be produced by simple method and mind process but this method has the majority of limitation. That is the drug loss during bead preparation, by leak through pore in the beads [21]. Recently, alginate has been modified for prolong entrapment of drug. Janyakul *et al*. [22] prepared composite beads consisting of xanthan gum, sodium alginate and diclofenac by using inotropic gelation method. Composite beads showed higher entrapment efficiency of diclofenac and longer lag time of diclofenac in pH 6.8 phosphate buffer were found. However, higher content of xanthan gum in beads increased the release rate of diclofenac. Cvitanovic *et al*. [23] fabricated alginate-base blend composed of carrageenan, pectin, chitosan from psyllium husk to entrap

caffeine. Alginate-psyllium blend beads exhibited the highest encapsulation efficiency while the alginate-chitosan coated beads showed the best releasing retardation of the bitter caffeine from alginate beads. Moreover, floating alginate beads have been developed to enhanced properties for special applications, such as prolonged gastric retention time in controlled release. Baljit et al. [24] fabricated gastroretentive floating sterculia-alginate beads by simultaneously ionotropic gelation. These floating beads have prolonged retention time, improved bioavailability and therapeutic efficacy of drug. Haroldo et al. [25] prepared floating alginate bead that made from alginate and cashew gum for capturing Lippia sidoides leaves oil (Fig 2.3). These beads were successful to resist the gastric fluid and prolonged gastric retention. These alginate-cashew gum beads presented improved entrapment efficiency, floating ability and controlled release of oil in beads.

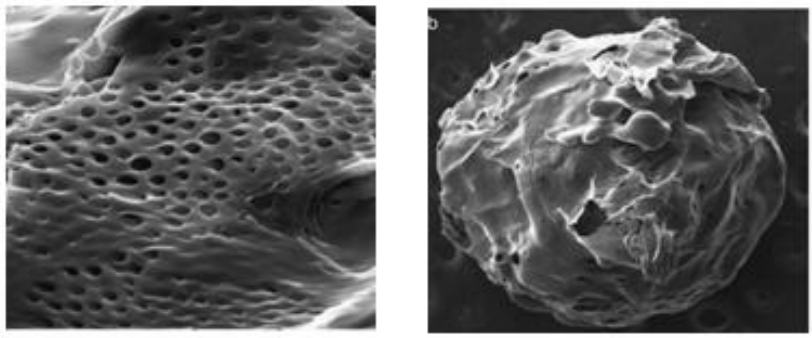


Figure 2.3 SEM image of alginate/cashew gum floating beads by Haroldo *et al*.

2.2 Human Serum Albumin-Alginate Coated Beads

The alginate beads are known to progressively degrade in aqueous media containing ionic compounds. This erosion can be prevented by coating the beads with a polycationic polymer such as poly-L-lysine, polyethylenimine or chitosan, which forms an ionic bonded membrane. However, this membrane had less prevention of the active core. It has been developed a stable membrane involving covalent bonds around alginate beads. Firstly, Levy *et al*. [7] fabricated the novel stable membrane around alginate beads by using tranacrylation reaction between propylene glycol alginate (PGA) and various proteins such as human serum albumin (HSA) (Fig 2.4).

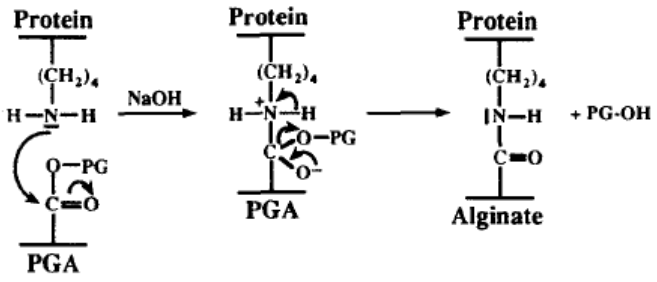


Figure 2.4 Reaction of propylene glycol alginate and protein

The beads with the PGA-HSA membrane were proved to display the good bioavailability. Edwards *et al*. [8] prepared HSA-alginate coated beads and studied the mechanical properties of beads as a function of the NaOH concentrations (Fig 2.5a). Increasing NaOH concentration revealed increasing the membrane thickness and led to more strength. Moreover, this bead had higher stability than PLL-alginate

coated beads. Furthermore, Hurteaux et al. [9] developed the method to prepare the small-sized HSA-alginate coated particles and encapsulate fluorescence peptide (Fig 2.5b). The mean diameter of bead was 50-60 μm and beads showed slower releasing the peptide than uncoated beads (Fig 2.5c).

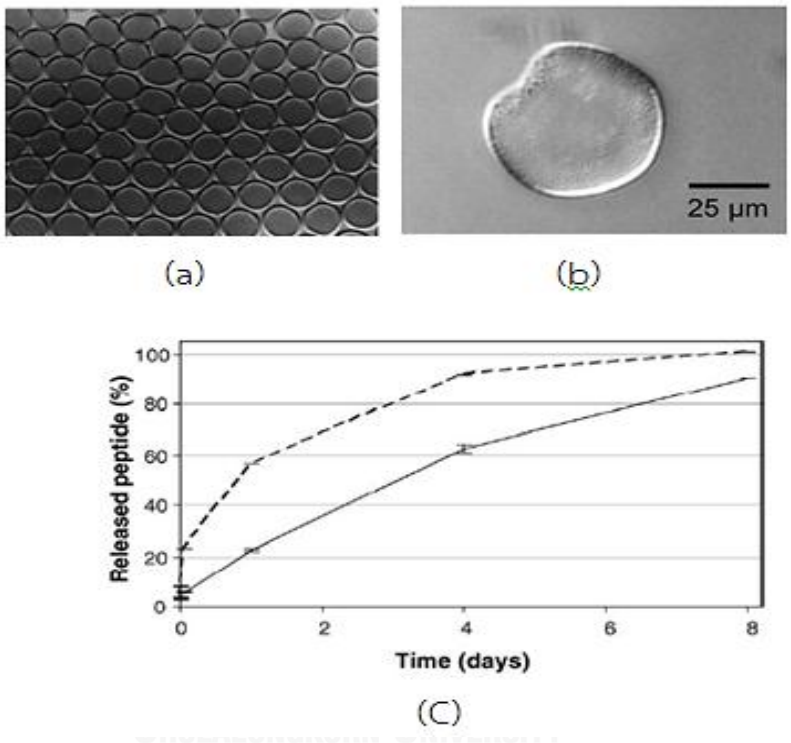


Figure 2.5 Macrophotographs of HSA-alginate beads by: (a) Edwards *et al*., (b) optical photomicrograph by Hurteaux *et al.*and (c) released profile of the fluorescence peptide from microcapsule as compared with controlled uncoated microspheres.

2.3 Curcuminoid

Curcuminoids are polyphenols isolated from the rhizomes of *Curcuma longa* Linn. in which composed of three active components i.e. curcumin, demethoxycurcumin and bisdemethoxtcurucmin (Figs 2.6a and b) [26]. The

appearance of curcuminoid is yellow powder. Curcuminoid is commonly used as colouring agent in food cosmetic and textile. Moreover, curcuminoid have been known to possess a variety of pharmaceutical activities such as anti-tumor, anti-oxidant, anti-inflammatory, diabetics, anti-cancer and hepatoprotective activities [27-29].

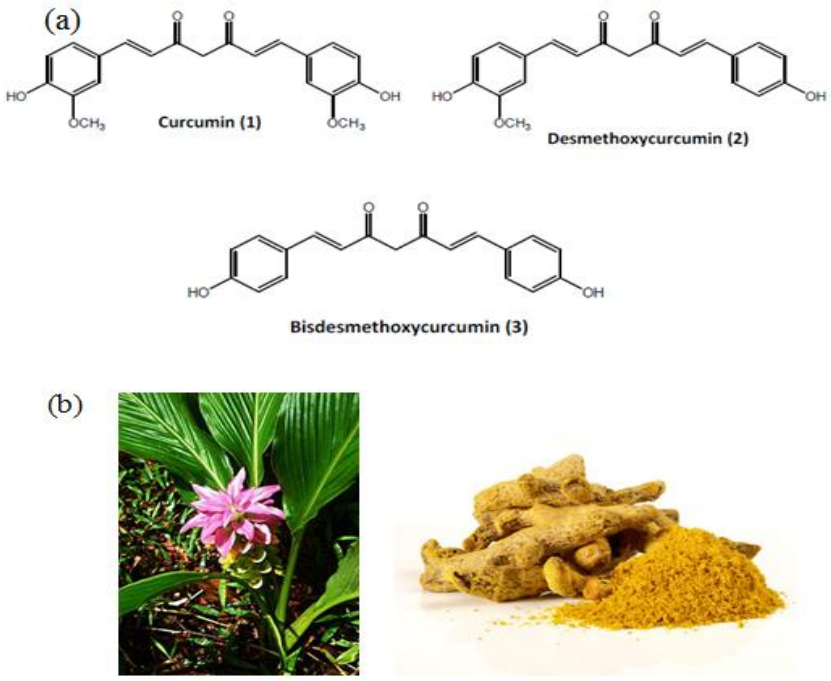


Figure 2.6 Structure of chemical constituents from *Curcuma longa* Linn (a), *Curcuminoid longa* rhizome (b) and *Curcuma longa* Linn. Plant.

However, curcuminoid is limited to use due to its poor aqueous solubility, poor bioavailability and high sensitivity to light. Therefore many researches have been reported to solve these problems by encapsulation. In the recently years, Nayak *et al.* [30] fabricated the nanostructure lipid carriers (NLC) (100-250 nm) by

using nanoemulsion technique and ultrasonic probe for delivery curcuminoid. Three emulsifiers, trimyristin, tristerin and glyceryl monostearate, were used to produce nanoemulsion. This lipid carrier can prolong the curcumionoid release and improve bioavailability. In 2011, Nattakitta *et al.* [31] prepared nanoencapsulation systems by using the biocompatible, safe and inexpensive polymers ethyl cellulose (EC) and methylcellulose (MC) (Fig 2.7). These nanospheres can improve bioavailability of curcumin when administered orally. Moreover, the ethyl cellulose nanospheres showed the highest mucoadhesive and controlled release. Aditya *et al.* [32] prepared curcuminoid-loaded liposomes from phosphatidylcholide by thin-film hydration technique to improve the therapeutic efficiency of curcuminoid for non-parasitic diseases. Liposomes improve the bioavailability of curcuminoid, since they can protect curcuminoid from the gastrointestinal fluid. Moreover, liposomes delivery system significantly increased efficient anti-malarial activity of curcuminoid.

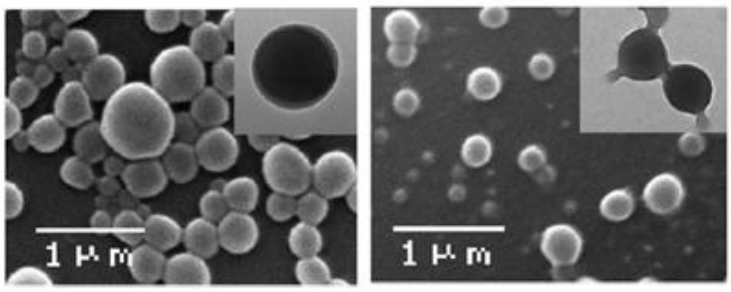


Figure 2.7 SEM and TEM images of ethyl cellulose (left) and ethyl cellulose-methyl cellulose (right)

2.4 Polymersomes

Polymersomes are self-assembled spherical vesicles based on amphiphilic block copolymer. They can be chemically functionalized for an active targeting mechanism and increase the pharmaco-dynamic profiles of drugs including enhance cellular uptake and *in vivo* stability. Polymersomes can be hollow, lamellar and spherical structures whose dimension and morphology can be controlled by chemical constitution, size of copolymer, preparation method and solution properties. The representation structures of copolymer used are demonstrated in Fig 2.8 [33]. Compare to liposomes, polymersomes offer the high membrane stability, chemical versatility of polymer assemblies, tunable permeability, higher circulation times, reduce water permeability, stable in biologically relevant temperature and exhibited remarkable mechanical stability (Fig 2.9) [34]. These are owing to the high molecular weight of polymer chain compared to lipids molecules. Importantly, copolymer molecular weight can be considerably larger membrane than those of natural lipid membrane. Moreover, polymersomes offered the unique opportunity combine both advantages of liposome and block copolymer assemblies. Thus they emerged as a very promising candidate for drug vesicles which can encapsulate both hydrophilic and hydrophobic molecules including anticancer drug, gene, proteins and diagnostic probes [35, 36].

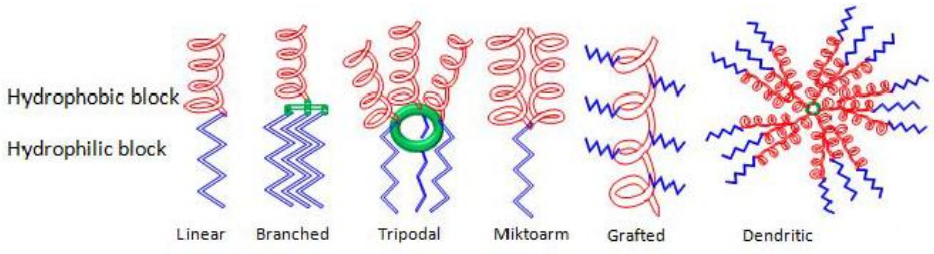


Figure 2.8 Systematic demonstration of various types of amphiphilic block copolymers which can self-assemble into polymersomes.

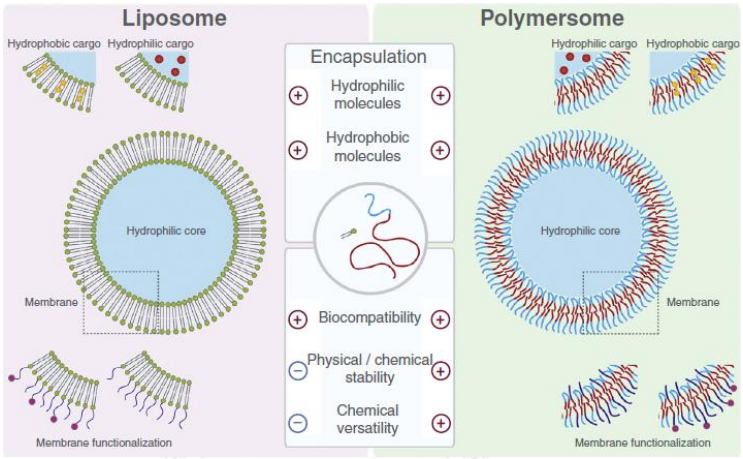


Figure 2.9 Structure and usability of liposomes (left) versus polymersomes (right).

Controlled Release [37, 38]

Controlled release system is designed to enable control of drug exposure, enhance administer drug which are encapsulated in polymer device to release at specific rate, time and condition. Controlled release system may also prevent stability, prolonged drug efficacy and effective delivering drug in the sustain manner. Controlled release system is used in many administration routes such as transdermal, oral and vaginal administration.

Advantage of Controlled Release

Controlled released system provides numerous benefits over conventional dosage form. Controlled release dose forms are able to control the rate of drug delivery, the target drug area of drug administration and maintain therapeutic levels of drug with narrow fluctuation. Efficacious, non-toxic therapy requires that drug concentration in plasma lies within the therapeutic range, which is bound below by the minimum effective concentration (MEC) and above by the minimum toxic concentration (MTC) that so-call plasma concentration profile of drug (Fig 2.10).

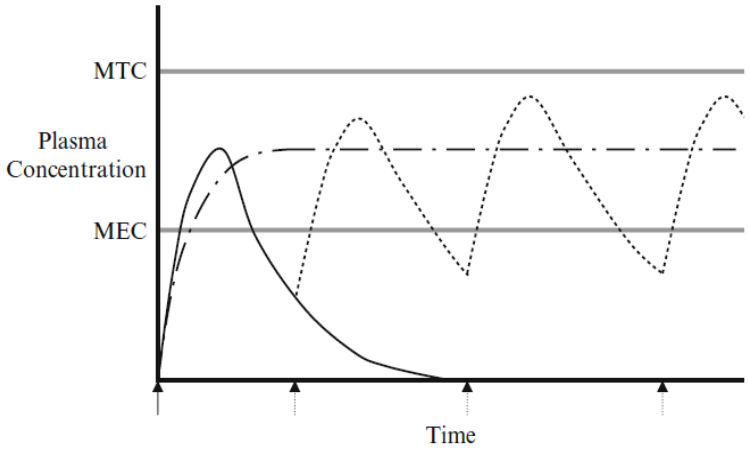


Figure 2.10 Plasma concentration profile [14]

Controlled Release Mechanisms

There are 3 mechanisms which drugs can be released from the system.

- *Diffusion Controlled Release*

Diffusion occurs when drug molecule have to diffuse through a polymer membrane or polymer matrix to the external environment (Fig 2.11). Diffusion can be split into reservoir and monolithic system, depends on a drug is surrounded by polymer membrane and/or distributed through the polymer matrix. There are basically 2 types of diffusion system including nonporous reservoir system and microporous reservoir system. In nonporous reservoir system, drug molecule can be diffuse from polymer membrane while in microporous reservoir system, drug is released by diffuse through micropores that are usually filled with water or oil.

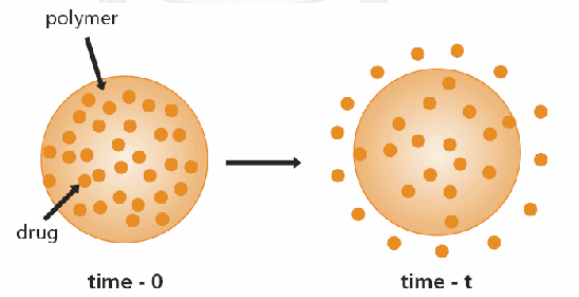


Figure 2.11 Presentation of diffusion controlled release

- *Swelling Controlled Release* [38]

Swelling refers to the uptake of water by polymer system with increase in the volumn. The drug can be diffuse through the swollen network of polymer into the external environment. The popularly materials used to make swell carriers are based on hydrogel. The swelling can be triggered by a chance in external parameters such as temperature, pH and ionic strength (Fig 2.12).

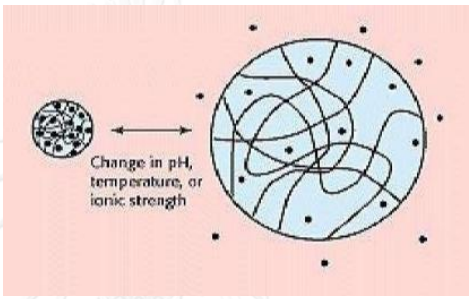


Figure 2.12 Presentation of swelling controlled release [38]

- *Eroison Controlled Release*

Drug can be released from polymer matrix owing to erosion of polymer (Fig 2.13). Erosion mechanism can be classified into 2 types as follows:

Bulk erosion: polymer degradation may occur through bulk hydrolysis.

Surface erosion: polymer degradation occurs only at the surface of polymer, resulting in the release rate that is proportional to the surface area of delivery system.

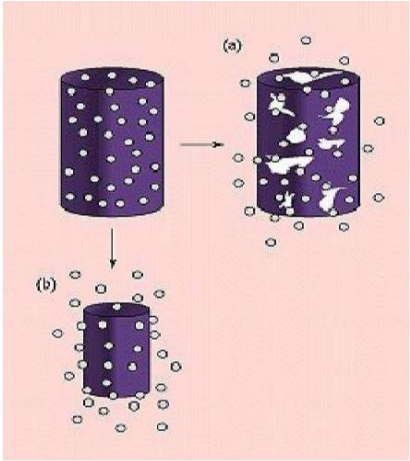


Figure 2.13 Presentation of erosion controlled release (a) bulk erosion (b) surface erosion [38]

CHAPTER III

EXPERIMENTALS

3.1 Chemicals and Materials

All chemicals and reagents were analytical grade. Sodium alginate from brown algae (viscosity of 2% solution at 25°C = 250 cP) was purchased from Sigma (USA). Propylene glycol alginate (Food grade PGA, 1.25% solutions at 25°C, 600-1200 cP) was gifted by FMC BioPolymer (Thailand). Fish collagen was purchased from the Specialty Natural Products (Thailand). Ethyl cellulose (viscosity 100 cP) was purchased from Sigma-Aldrich, St. Louis, USA.

Filtering centrifugal tube MWCO 100,000 (Amicon Ultra-15) was purchased from Millipore (Billerica, USA). Dialysis tubing cellulose membranes (MWCO 11011 Da) was purchased from Sigma-Aldrich, Heintein, Germany.

Ethanol, dichloromethane, hexane, acetone (analytical grade) were purchased from Merck, Darmstadt, Germany. Imidazole, 99% was purchased from Acros, Geel, Belgium.

3.2 Curcuminoid Extraction

Curcuminoid was isolated from *Curcuma longa* (Turmeric) rhizomes which were collected from Nongkhai province, Thailand in 2012. Turmeric rhizomes were cut into small pieces and dried in the open air for 3 days. Five kilograms of dried rhizomes was macerated in ethanol for 3 days. After filtration, a half of ethanol in the extract solution was removed by a rotary evaporator and then *n*-hexane (2 times to ethanol amount) was added in. Mildly stirring overnight, curcuminoid will then precipitated in the hexane layer. After filtration, 36.84 g of yellow curcuminoid was obtained. The purity of curcuminoid was analyzed by HPLC using an ACE® 5 C18-AR column (150 x 4.6 mm). The mobile phase was the mixture of 40% acetonitrile and 60% water containing 2% acetic acid with a flow rate of 1 mL/min. The 25 µL of sample was injected and UV adsorption was detected at 254 nm. Three separated peaks of curcumin, demethoxycurcumin and bisdemethoxycurcumin in curcuminoid showed the retention times at 16.9, 15.1, 13.5 mins, respectively. Thus, the purity of curcumin in curcuminoid was 85.7% while that of demethoxycurcumin and bisdemethoxycurcumin was 11.3% and 3.0%, respectively.

3.3 Curcuminoid-loaded Ethyl Cellulose Particles

3.3.1 Preparation

Curcuminoid-loaded ethyl cellulose particles (Cur-loaded EC particles) were prepared by solvent displacement dialysis method using the previous method [31]. Briefly, 150 mg of ethyl cellulose and 150 mg of curcuminoid were dissolved in 15 mL of ethanol. The solution was dialyzed using a dialysis tube (cellulose membrane, MWCO 11011, 76 mm flat width, 3.0 in., Sigma-Aldrich, USA) in the distilled water. Finally, the aqueous suspension of Cur-loaded EC particles was collected and characterized by SEM, DLS and UV-vis spectrophotometers.

3.3.2 Entrapment Efficiency, Size and SEM Characterization

Entrapment Efficiency Percentage and Loading Capacity Percentage

The Cur-loaded EC suspension was centrifugally filtered using centrifugal-filtering devices with MWCO of 100000 (Merck Millipore) at 6000 rpm for 45 minute. Sediment was extracted in 10 mL of ethanol solution. The solution was analyzed by UV–vis spectrophotometer at 421 nm. The percentages of encapsulation efficiency (%EE) and loading capacity (%LC) were calculated using equations (1) and (2), respectively:

$$\%EE = \frac{\text{Actual curcuminoid loading}}{\text{Total curcuminoid loading}} \times 100 \quad (1)$$

$$\%LC = \frac{\text{Weight of curcuminoid in particles}}{\text{Weight of particles}} \times 100 \quad (2)$$

Dynamic Light Scattering (DLS)

The mean hydrodynamic diameter, size distribution and zeta potential of Cur-loaded EC particles were measured in distilled water at 25°C on a Malvern 3000HSA Zetasizer (UK) based on the dynamic light scattering (DLS) techniques.

Scanning Electron Microscopy (SEM)

The morphology of particles was examined by scanning electron micrograph (SEM). The suspension of Cur-loaded EC particles was diluted in distilled water and ultra-sonicated for 2 minute. The sample was placed on the double-sided adhesive tape, air-dried and gold coated under vacuum and then examined under scanning electron microscope (Phillips, XL30CP).

3.4 Collagen-Alginate Coated Beads

3.4.1 Preparation

Alginate, propylene glycol alginate (PGA) and collagen aqueous solutions were prepared separately using 1% NaCl solution. These solutions were well mixed together for 30 min using a magnetic stirrer. Then, this solution was extruded through syringe tube connected with silicone needle into 2% $CaCl_2$ by a syringe pump (NE-300 new era system, USA). These gel beads were retained in the $CaCl_2$ solution for 30 min. The wet beads (col-alg beads) were filtered and washed with distilled water for

three times. The beads were then incubated in 0.02 M NaOH solution for 5 min to create membrane at the surface of the wet beads via the transacrylation reaction between ester groups of PGA and amino groups of collagen. The collagen-alginate coated beads (col-alg coated beads) were filtered and washed with distilled water for three times. The transacrylation was then quenched by incubating col-alg coated beads in a pH 7 imidazole buffer for 15 min. The wet col-alg coated beads were then evaluated the hardness study and light microscopy. These beads were dried in an oven at 70°C for 2 hr and used for ninhydrin test, FT-IR, DSC, and SEM analysis.

Collagen-alginate coated beads containing cur-loaded EC particles (or free curcuminoid) were prepared similarly as above method, excepted that the free curcuminoid or curcuminoid-loaded ethyl cellulose particles were added into the mixture solution before extrude through syringe tube.

Alginate beads were prepared by 1.5% w/w of alginate solution in 1% NaCl solution. The alginate solution was dropwised into 2% $CaCl_2$ solution and incubated for 30 min before filtration and washing. These gel beads were evaluated the hardness study by texture analyzer comparing to those col-alg and col-alg coated beads.

3.4.2 Physical, Chemical and Morphology Characterization

Fourier Transformed Infrared (FTIR) Spectroscopy

Infrared (IR) spectra of col-alg beads and col-alg coated beads were recorded with a Nicolet 6700 FT-IR (Thermo Electron Scientific, Madison, WI) spectrophotometer using the attenuated total reflection (ATR) method. Samples were scanned between 600 cm^{-1} and 4000 cm^{-1}.

Differential Scanning Calorimetry Analysis (DSC)

DSC Thermograms of alginate, PGA, collagen, col-alg beads and col-alg coated beads were recorded using differential scanning calorimeter (DSC204F1, Selb, Germany). Each sample (5-7 mg) was accurately weighted into aluminum pan. The measurement was performed between 25 and 300°C at heating rate of 10°C/min.

Ninhydrin Test

Ninhydrin test was used to confirm the formation of additional amide bonds on the bead surface. The 0.1% ninhydrin in ethanol solution was sprayed on the dried col-alg coated beads containing 10% collagen which were placed in a glass petri dish. The beads were heated on the hot plate at 65 ℃ for 5 min and then cool down to the room temperature. The darker purple color observed indicates the relative number of amide bonds on the surface.

Hardness Study

The hardness of fresh beads were determined by a texture analyzer (TA.XT2i, Germany) equipped with a force transducer and a texture expert software for data processing. The gel beads were placed under the probe and the mechanical measurement was performed at room temperature. The probe was placed at a height of 6 mm from the ground and moved down at the rate of 0.6 mm/s until a resistance force of 0.03 N was detected. The probe was then left motionless at this position for 60 s, before being retracted to the initial position. The result was obtained as a texture profile analysis graph. The hardness was determined from the maximum point of the force peak.

Light Microscopy

The surface morphology of the wet beads was observed and recorded by a light microscope (Olympus, Japan) equipped with a digital camera.

Scanning Electron Microscopy (SEM)

The surface and cross-section texture of dry bead was examined by scanning electron microscope (SEM) (JSM-6480LV, JEOL, Japan). The samples were mounted on the metal stub, using double sided adhesive tape, gold coated under vacuum and the images were taken at 20 kV.

3.5 Entrapment Efficiency Percentage and Loading Capacity Percentage

The dry beads containing Cur-loaded EC particles (or free curcuminoid) were added into 50 mL of 10% NaCl and stirred overnight. Beads swelled and then were burst. Then this solution was partitioned with CH_2Cl_2. Curcuminoid will move into the CH_2Cl_2 layer and CH_2Cl_2 was removed by the rotary evaporator. The dry curcuminoid was re-dissolved with ethanol and determined its amount by the UV spectrophotometer at 421 nm. The encapsulation efficiency (% EE) and loading capacity (% LC) were calculated using equations 1 and 2, respectively.

3.6 Curcuminoid Release Study

The 50 mg of dry beads was put into 100 mL of phosphate buffer saline (PBS, 0.2 M and pH 5.5) at room temperature and the mixture was gently stirred by a magnetic stirrer. At predetermined time (1, 2, 3, 4, 5, 6, 7, 24, 48, 96 and 144 hr.) intervals, one milliliter release medium was withdrawn from beaker and replaced with the same amount of fresh medium. The amount of curcuminoid release was analyzed by UV spectrophotometer at 430 nm. Percent curcuminoid released was calculated from the following equation (3):

$$\% \text{ release} = \frac{\text{Curcuminoid released}}{\text{Total curcuminoid used}} \text{x100} \qquad (3)$$

3.7 Statistical Analysis

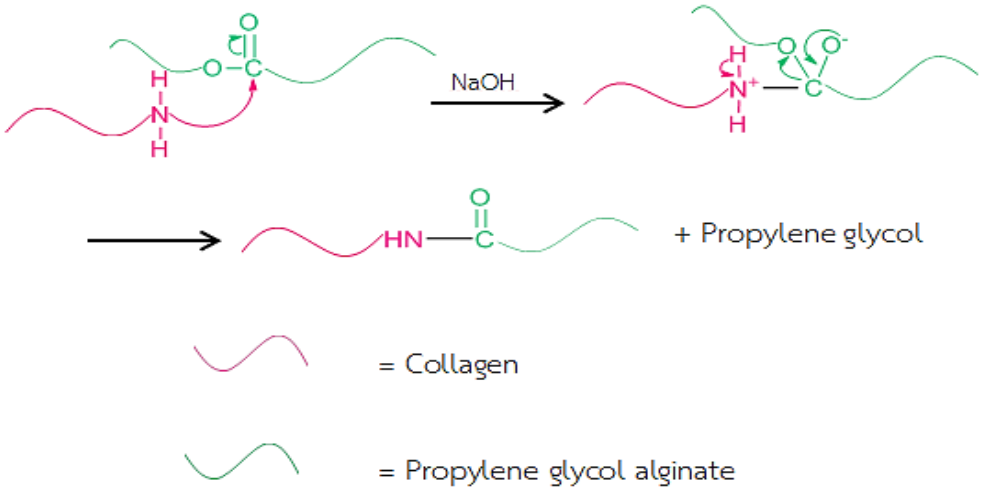
Quantitative data were reported as means ± standard deviations, where indicated. Statistical analysis was performed using a one-way Anova analysis, followed by the Turkeys HSD for multiple comparisons. A p-value <0.05 was considered statistically significant.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Formation and Characterization of Unloaded Collagen-Alginate Coated Beads

The outer membrane of the col-alg coated beads was fabricated *via* transacrylation reaction between ester group of propylene glycol alginate (PGA) and amino group of collagen. The mechanism of new amide bond from this reaction is shown in Scheme 4.1

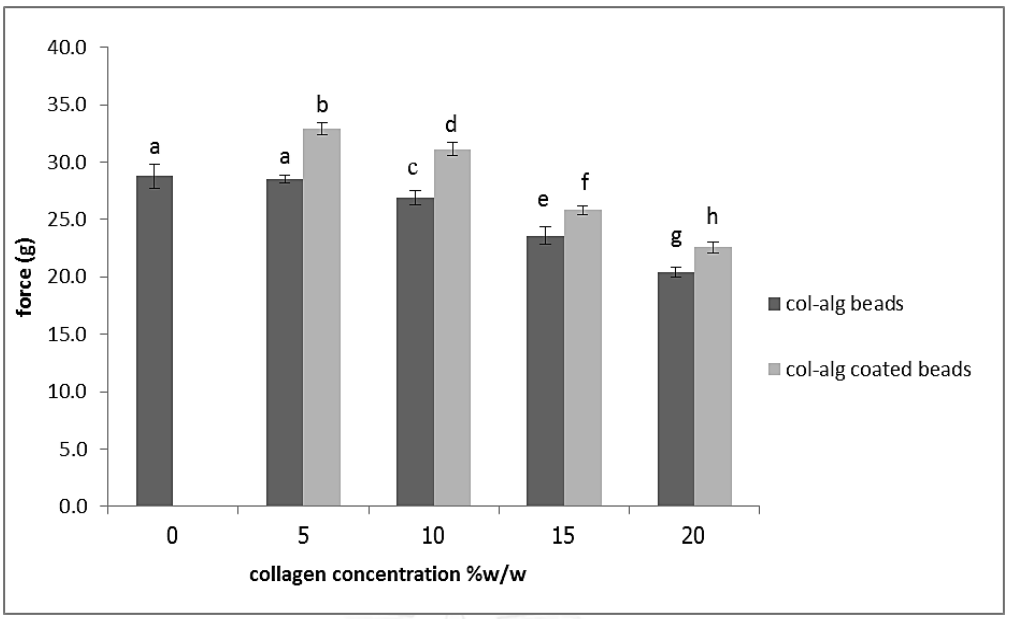


Scheme 4.1 The mechanism of amide bond formation between PGA and collagen

In order to prove the new membrane of alginate beads by collagen and PGA, the hardness was the first experiment to evaluate. Alginate (1.5%) and PGA (0.5%) mixed with various concentration of collagen was dropwised into 2% $CaCl_2$ solution.

Fresh beads were then incubated in 0.02 M NaOH to form the outer membrane. The obtained beads from this method are “col-alg coated bead” while fresh beads without incubated in NaOH is “col-alg bead”. We expected that the new amide bond around the bead surface should increase the hardness of bead. Thus, the col-alg coated bead was measured the hardness and compare with col-alg bead and alginate bead.

The hardness of beads is the maximum force during compression of beads and determined using texture analysis. The hardness result is shown in Fig. 4.1 and Table 4.1. The col-alg beads had hardness significantly lower than col-alg coated beads. The results are consistent with the formation of additional amide bonds around the bead surface. Moreover, the decrease of the bead hardness at high collagen content might be the result of the reduced number of the carboxylate ion of alginic acid required for the ionic-crosslinking with Ca^{2+}.



Note: a, b, c indicate significant different hardness ($p<0.05$)

Figure 4.1 The hardness of the col-alg beads and col-alg coated beads as a function of the amount of collagen. Data are shown as mean ± 1 S.D. and are derived from three repeats.

Table 4.1 The hardness of the col-alg beads and col-alg coated beads as a function of the amount of collagen.

%w/w of collagen in the solution	hardness (or force)(g)	
	col-alg beads	col-alg coated beads
0	28.8 ± 1.0^{a}	N/A
5	28.5 ± 0.3^{a}	32.9 ± 0.5^{b}
10	26.9 ± 0.6^{c}	31.1 ± 0.6^{d}
15	23.6 ± 0.8^{e}	25.8 ± 0.4^{f}
20	20.4 ± 0.4^{g}	22.6 ± 0.4^{h}

N/A = Not applicable

Note: a, b, c, d, e, f, g and h indicate significant different hardness ($p<0.05$)

Photographic images of col-alg coated beads were shown in Fig 4.2. The fresh beads had average diameters at 5.20 ± 0.02 mm while dry beads had average diameters at 1.47± 0.02 mm and bead shape was spherical. However, the beads were become less spherical shape when the collagen concentration increased. Based on the visual appearance of col-alg coated beads produced by the extrusion method, we selected beads with 10% collagen to further investigation by FTIR, DSC, ninhydrin test, light microscopy and SEM.

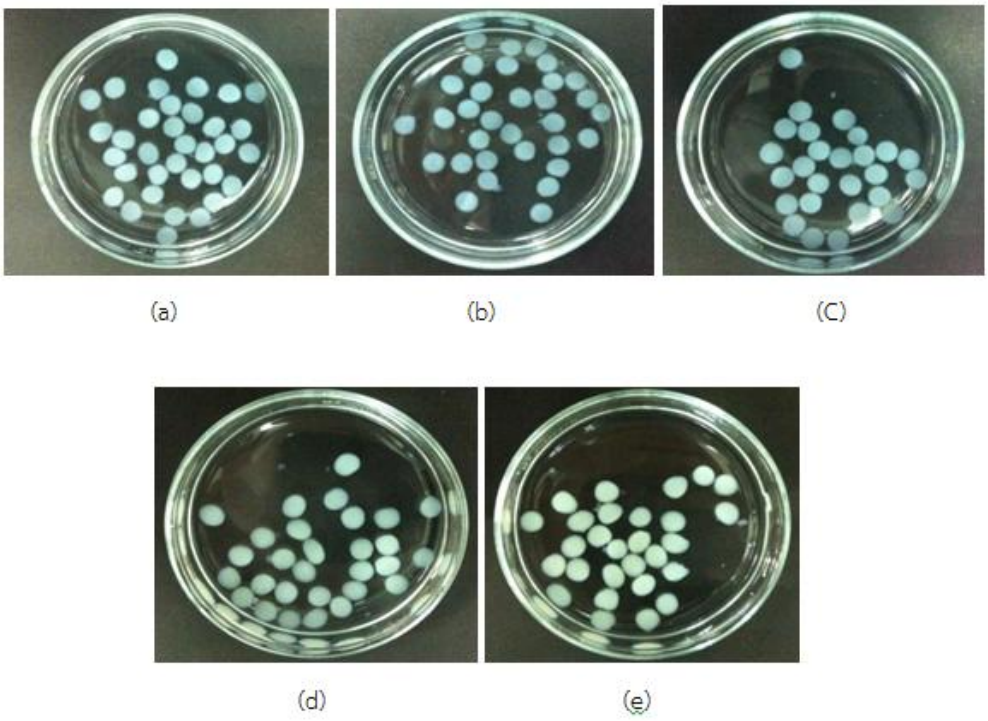


Figure 4.2 Photographic images of col-alg coated beads prepared with collagen concentration of (a) 0%, (b) 5%, (c) 10%, (d) 15%, (e) 20% w/w.

ATR-FTIR Spectroscopy

The FTIR spectra of col-alg beads (Fig 4.3a) showed the absorption band at 1735 and at 3264 cm^{-1} corresponds to stretching of C=O of ester group of the PGA and –NH of amino group of the collagen, respectively. In the case of col-alg coated beads (Fig 4.3b), the new amide bond between ester group of PGA and amino group of collagen showed the absorption band of –NH stretching at 3270 cm^{-1} while that of C=O stretching was shifted to low wavenumbers and overlapped with a broad absorption band at 1591 cm^{-1}. These result supported the assumption that the membrane via transacrylation reaction occurred around the surface of col-alg coated beads.

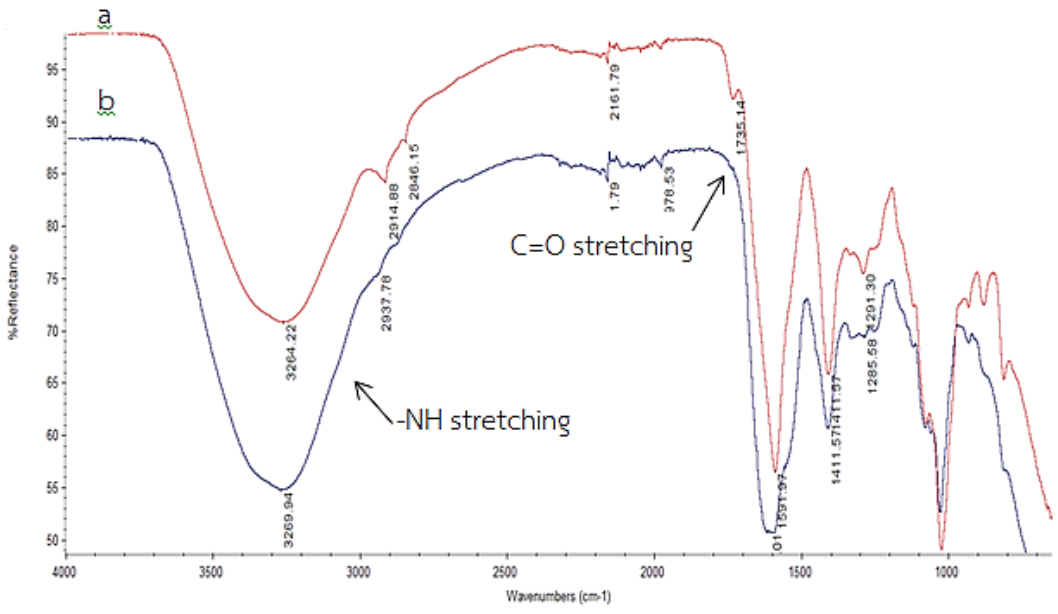


Figure 4.3 ATR-FTIR spectra of col-alg beads (a) and col-alg coated beads (b)

Differential Scanning Calorimetric (DSC)

Table 4.2 presented the thermograms for dehydration, decomposition and melting temperature of collagen, PGA, col-alg beads and col-alg coated beads. The results exhibited the different thermograms of col-alg beads and col-alg coated beads from the pure components. In the case of col-alg beads, the endothermic peaks were seen at 193.8 and 202.1°C (Fig 4.4c) might be due to the melting process of polymer in col-alg beads and produced hydrogen bond between polymers. While col-alg coated beads showed endothermic peaks at 176, 186 and 208.7°C (Fig 4.4d) attributed to the melting process of the polymer mixture in beads and formation of the respective amide unit of membrane. Peaks of col-alg beads appeared to be combinations of each components but it was different from col-alg coated beads probably because col-alg coated beads resulted in the new chemical bond.

Table 4.2 Thermogram data of collagen, PGA, col-alg beads and col-alg coated beads.

Sample	peak (°C)	attribution
Collagen	95.8	dehydration
	181.3	melting temperature
PGA	95.8	dehydration
	141.5	melting
	232.3	decomposition
Col-alg beads	193.8	melting temperature
	202.1	melting temperature
col-alg coated beads	176.9	melting temperature
	186	melting temperature
	208.7	melting temperature

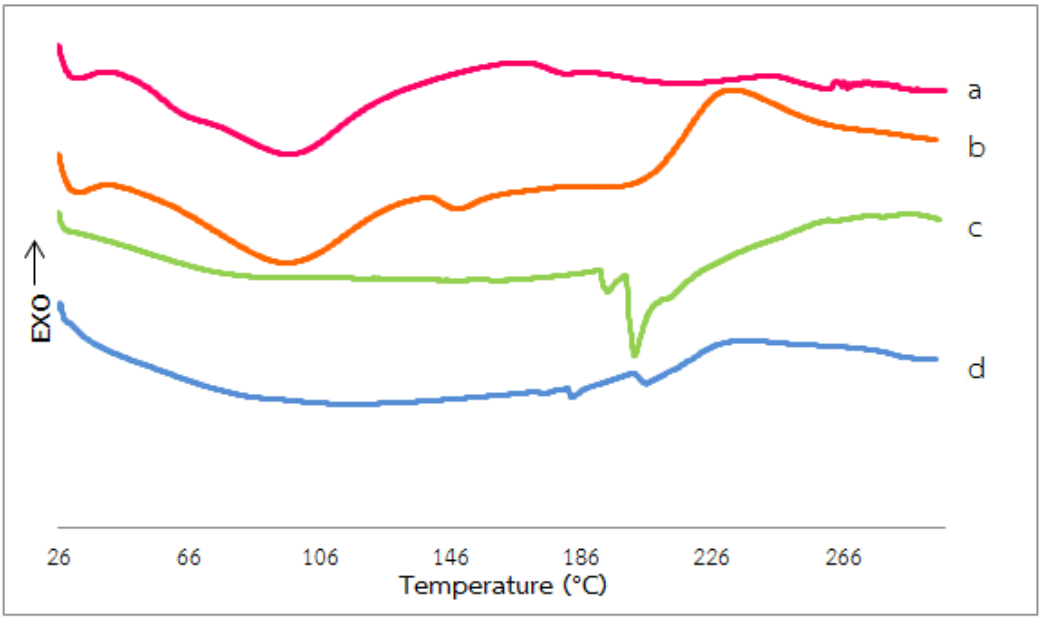


Figure 4.4 DSC thermograms of: collagen (a), propylene glycol alginate (b), col-alg beads (c) and col-alg coated beads (d)

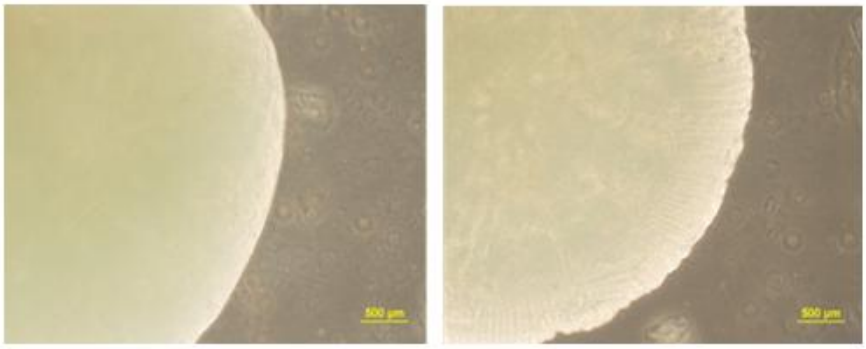
Ninhydrin Test

A colorimetric ninhydrin test was used for the confirmation of the additional amide bond formed on the bead surface. After spraying the ninhydrin solution on the dried beads followed heating at 65 °C, the color of the surface of the col-alg coated beads turned to dark purple while those of the col-alg beads did not (Fig. 4.5). The results clearly confirmed the additional amide bonds on the col-alg coated bead surface.

Figure 4.5 Photographic images of col-alg beads (a) and col-alg coated beads (b) by ninhydrin test

Morphology of Col-alg Beads and Col-alg Coated Beads

The fresh beads observed under a light microscope revealed that the surface of the col-alg bead was rather smooth (Fig. 4.6a) while that of the col-alg coated bead had some parallel stripe lines from the surface inward (Fig. 4.6b). The results again confirmed that the transacrylation occurred at the outer surface of col-alg coated beads.



(a) (b)

Figure 4.6 Optical microphotographs of col-alg beads (a) and col-alg coated beads (b)

The morphology of the dried col-alg coated beads and col-alg beads were evaluated by SEM. The outer surface of col-alg bead was rather lumpy (Fig 4.7a) while that of col-alg coated bead was rather smooth with regular stripe lines (Fig 4.7d). The cross-section images clearly showed that both types of beads were hollow with dense outer layer and some inner sheets (Fig 4.7b and 4.7e). The enlarged views showed that the outer layer of the col-alg coated beads had some anisotropic orientation (Fig 4.7f) while that of col-alg beads was rather homogeneous (Fig 4.7c).

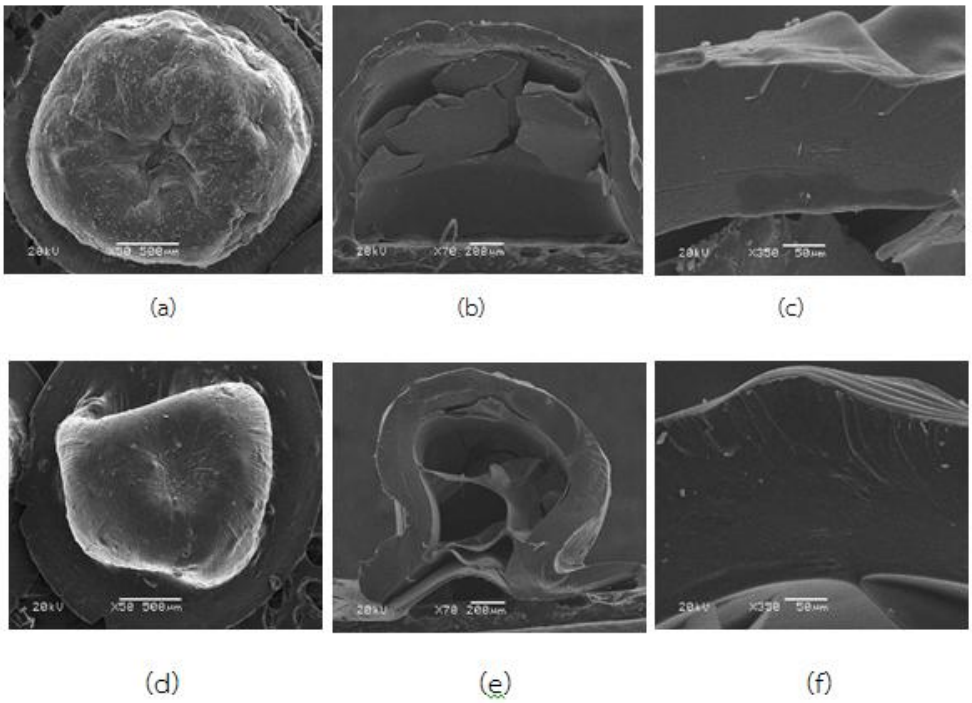


Figure 4.7 SEM micrographs of col-alg beads surface (a), cross section of col-alg beads (b-c), col-alg coated beads surface (d) and cross-section of col-alg coated beads (e-f)

4.2 Preparation of Curcuminoid-loaded Ethyl Cellulose Particles

Curcuminoid-loaded ethyl cellulose (Cur-loaded EC) particles were fabricated by solvent displacement method as described in the previous report [31]. In this study, the entrapment efficiency and loading capacity of curcuminoid in the particles were found to be 38.19 % ± 1.91 and 27.56 % ± 0.97, respectively. In our case, the result of % EE and % LC of Cur-loaded EC particle were lower than the previous research because in this research the direct method was used to analysis the amount of curcuminoid in particles by extracted curcuminoid in particles while indirect

method was used outer solvent to analyzed the amount of cucuminoid in particles. We decided to use direct method to calculate accurately the amount of curcuminoid due to it was directly extracted curcuminoid from particles to be correct amount of curcuminoid entrapment.

The SEM image of cur-loaded EC particles (Fig. 4.8) revealed a spherical in shape and non-aggregation. The average diameter of dried Cur-loaded EC particles estimated from SEM image was 346.15±0.1 nm. The hydrodynamic diameter and zeta potential of the hydrated (aqueous suspension) Cur-loaded EC particles were 353±2.82 nm (PDI = 0.1) and -27±0.66 mV, respectively (Fig 4.9). When comparing the average particles size from SEM and DLS, it showed that the particles gave different values. This might be attributed to the fact that SEM was measured using dry particles while DLS was measured using aqueous suspension particles. The negative zeta potential of particles indicated the presence of hydroxyl groups of ethyl cellulose on the surface of particles. The hydroxyl groups were dissociated negative charge on the surface of particles thus creating a negative zeta potential. Furthermore, the zeta potential value of stable particles in suspension should be positive more than +30 mV and negative less than -30 mV [2]. The zeta potential of the obtain particles was closer to -30 mV which indicates these particles were quite stable with minimal aggregation in water medium which was agreement with the SEM image.

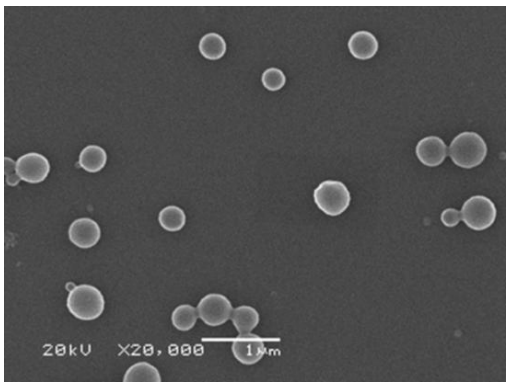


Figure 4.8 Scanning Electron Micrograph of curcuminoid-loaded ethyl cellulose particles

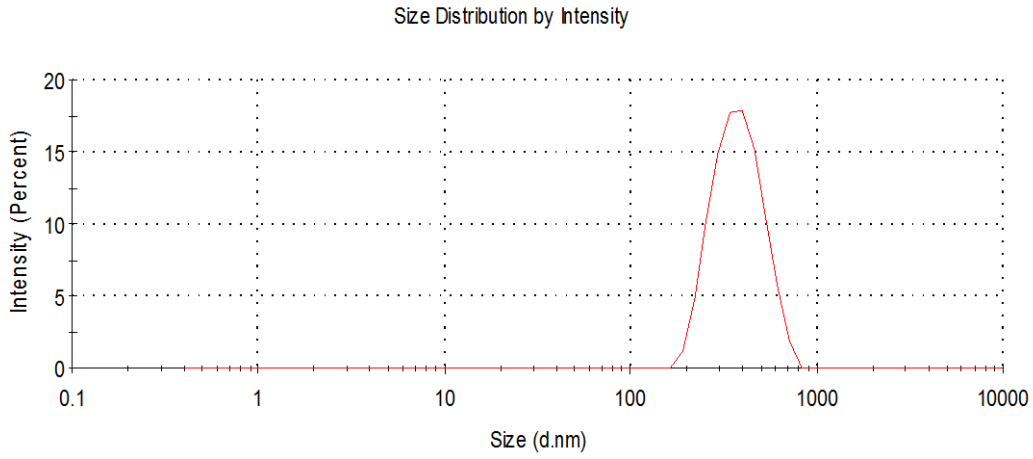


Figure 4.9 Zeta potential distribution graph of Cur-loaded EC particles

4.3 Preparation and Characterization of Curcuminoid-loaded Collagen-Alginate Coated Beads

Col-alg coated beads containing free curcuminoid (or cur-loaded EC particles) were prepared by adding the free curcuminoid (or Cur-loaded EC particles) into the mixture solution before extrude into 2% $CaCl_2$. The loading capacity and entrapment

efficiency of curcuminoid in different kinds of hydrogel beads showed in Table 4.3. The lower curcuminoid loading and entrapment efficiency of curcuminiod in beads might be curcuminoid could not be completely extracted from beads and be trapped by polymers in the water layer (Fig 4.10). The entrapment efficiency of free curcuminoid from col-alg beads was lower than that of col-alg coated beads. It was probably due to the instability of curcuminoid in a base solution.

Table 4.3 % of loading capacity and entrapment efficiency of curcuminoid in col-alg beads and col-alg coated beads.

sample	percentage of curcuminoid	
	%LC	%EE
free curcuminoid in col-alg beads	1.19 ± 0.19	65.2 ± 10.54
free curcuminoid in col-alg coated beads	1.12 ± 0.29	38.76 ± 10.48
Cur-loaded EC particles in col-alg beads	1.32 ± 0.28	64.9 ± 12.8
Cur-loaded EC particles in col-alg coated beads	1.59 ± 0.25	61.59 ± 6.82

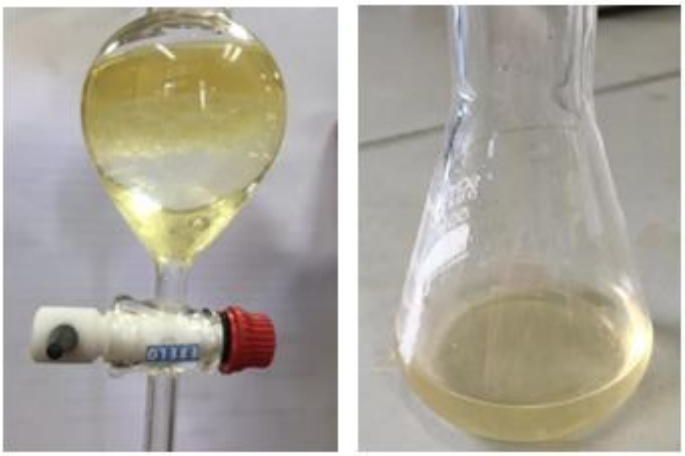


Figure 4.10 Representative images of curcuminoid entrapped by polymers in the water layer.

Morphology of Col-alg Coated Beads containing with Free Curcuminoid and Cur-loaded EC Particles.

The SEM images of the cross-section of free curcuminoid or Cur-loaded EC particles were shown in Fig 4.11. There are some curcuminoid or particles in the outer membrane of beads which indicated that free curcuminoid and Cur-loaded EC particles were entrapped in the polymer matrix. Moreover, we also prove the existence of curcuminoid by Confocal images (Fig. 4.12).

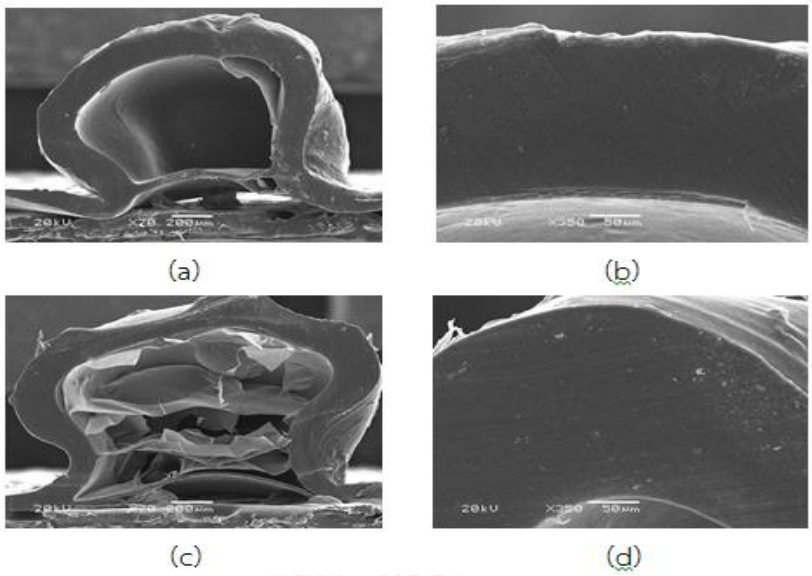


Figure 4.11 SEM images of the cross section of col-alg coated beads containing free curcuminoid (a-b) and Cur-loaded EC particles (c-d)

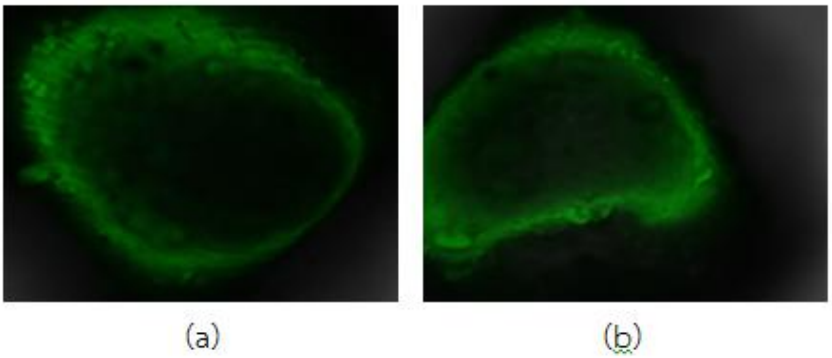


Figure 4.12 Confocal images of col-alg coated beads containing free curcuminoid (a) and Cur-loaded EC particles (b)

4.4 Curcuminoid Release Study

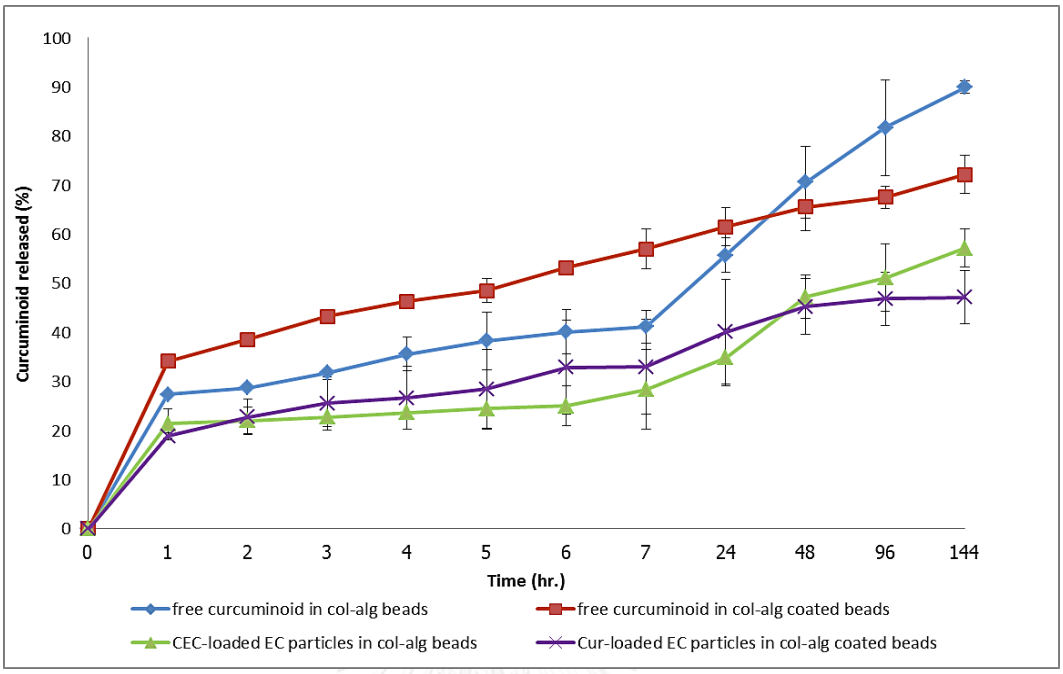


Figure 4.13 The release profiles of curcuminoid from col-alg beads and col-alg coated beads containing free curcuminoid and Cur-loaded EC particles at room temperature in PBS buffer pH 5.5

The release profiles of each kind of beads were shown in Fig 4.13. It is found that the untrapped curcuminoid adhered on the surface of the beads was fast dissociated into the release medium in the first hour. After that, the curcuminoid slowly release from beads depending on the swelling rate of the beads. Col-alg beads began to swell noticeably due to release medium was diffused into beads which led to an ion exchange between calcium ions in beads and monovalent ions from release medium. Therefore, the col-alg beads swelled and erosion. The release of free curcuminoid from uncoated and coated beads were about 90% and 74%,

respectively while the release of cur-loaded EC particles from uncoated and coated beads were 60% and 54%, respectively in PBS buffer within 6 days. The resulted revealed that free curcuminoid could diffuse to the medium faster than cur-loaded EC particles. This might be cause of the bigger molecule of Cur-loaded EC particles which could be trapped in the network of hydrogel alginate. Moreover, the coated beads enhanced the efficiency to trap the active component since the new amide bond was not destroyed by the ion-exchange and retarded the swelling rate.

CHAPTER V

CONCLUSION

In this research, collagen-alginate coated beads (col-alg coated beads) were successfully prepared using transacrylation reaction between ester group of propylene glycol alginate and amino group of collagen. The average diameters of fresh and dry collagen-alginate coated beads were 5.20±0.02 mm and 1.47±0.02 mm, respectively. The concentration of fish collagen affected the shape of hydrogel beads. If the concentration of collagen was more than 10%, the beads will have the sphere shape with tail. Thus, in this study, 10% collagen was chosen to prepare collagen-alginate beads for further study. The hardness, FTIR, DSC, ninhydrin, light microscope and SEM were employed to confirm the new amide bond at the outer membrane of beads. The new amide bond enhanced the strength of beads and was observed the new functional peaks in the FTIR analysis. The DSC thermograms showed different endothermic peaks from uncoated beads and ninhydrin solution turned to darker purples in the coated bead.

Two types of curcuminoid were loaded in the beads which were free curcuminoid and Cur-loaded EC particles. Cur-loaded EC particles showed slower releasing than free curcuminoid in pH5.5 buffer. It is found that the untrapped curcuminoid on the surface of the beads was released into the medium solution around 30% in the first hour. Then, the curcuminoid slowly release from beads owing

to the beads became swelling. Free curcuminoid was released out from the swell bead faster than Cur-loaded EC particles. This was likely due to the faster penetration of the smaller molecule of free curcuminoid. Moreover, the results indicated that the outer membrane of col-alg coated beads can retarded the release of curcuminoid.

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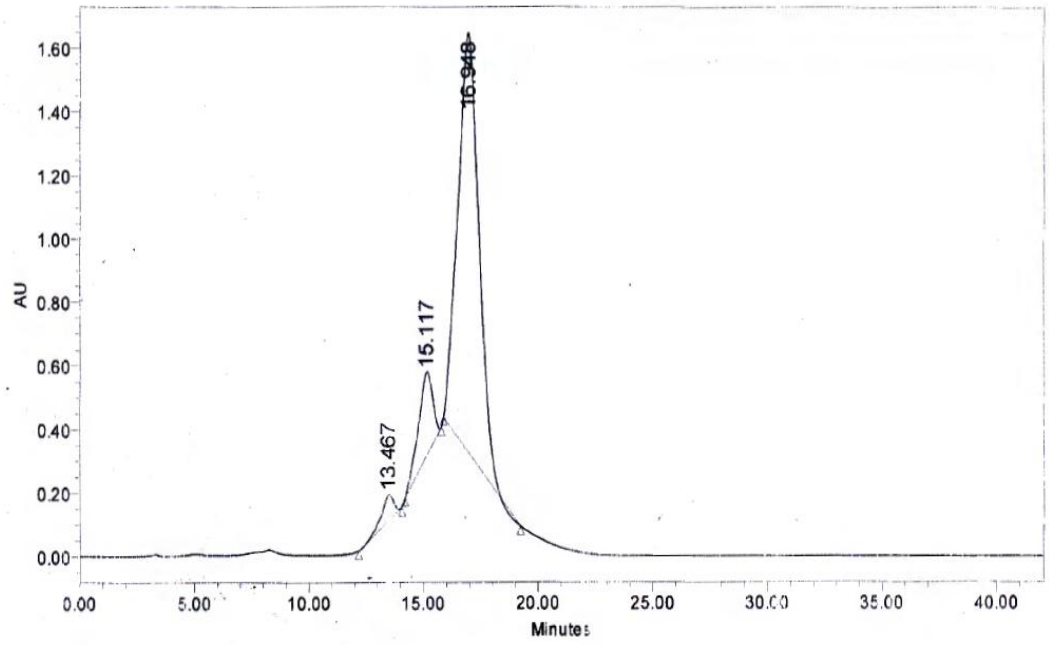
APPENDIX

Appendix A

HPLC

HPLC Chromatogram of Curcuminoid



	RT	Area	% Area	Height
1	13.467	2981079	3.00	81083
2	15.117	11282151	11.34	264062
3	16.948	85206863	85.66	1321232

Figure A1 Chromatogram of remained curcuminoid compound in the solution

Appendix B

Hardness of Col-alg Beads and Col-alg Coated Beads

Table B1 Hardness of col-alg beads and col-alg coated beads with 5%collagen

Test ID	force(g)		
		collagen 5%	
	alg 1.5%	col-alg beads	col-alg coated beads
1	29.682	28.515	33.401
2	29.755	28.807	33.766
3	29.39	28.734	32.891
4	27.13	28.588	33.328
5	28.515	28.734	32.745
6	27.859	28.88	32.235
7	27.932	28.442	33.328
8	28.005	27.859	32.599
9	30.193	28.296	32.453
10	29.172	28.442	32.162
Mean	**28.8**	**28.5**	**32.9**
S.D.	**1.0**	**0.3**	**0.5**

Table B2 Hardness of col-alg beads and col-alg coated beads with 10 and 15 %collagen

Test ID	force(g)			
	collagen 10%		collagen 15%	
	col-alg beads	col-alg coated beads	col-alg beads	col-alg coated beads
1	26.181	30.995	24.972	26.181
2	26.036	31.651	24.067	25.817
3	27.13	30.557	24.942	26.619
4	27.713	31.141	22.9	25.671
5	27.859	30.776	23.118	25.963
6	26.473	31.87	23.41	25.525
7	26.473	32.016	23.264	25.379
8	27.348	31.141	22.827	25.963
9	26.838	30.484	23.483	25.379
10	26.911	30.63	23.046	25.817
Mean	**26.9**	**31.1**	**23.6**	**25.8**
S.D.	**0.6**	**0.6**	**0.8**	**0.4**

Table B3 Hardness of col-alg beads and col-alg coated beads with 20%collagen

Test ID	force (g)	
	collagen 20%	
	col-alg beads	col-alg coated beads
1	20.42	22.9
2	20.201	22.243
3	20.493	22.483
4	21.295	22.316
5	20.712	22.462
6	20.42	22.535
7	19.982	22.17
8	20.785	23.046
9	19.982	22.025
10	20.055	23.483
Mean	**20.4**	**22.6**
S.D.	**0.4**	**0.4**

Appendix C

FTIR Spectrophotometr of Col-alg Beads and Col-alg Coated Beads

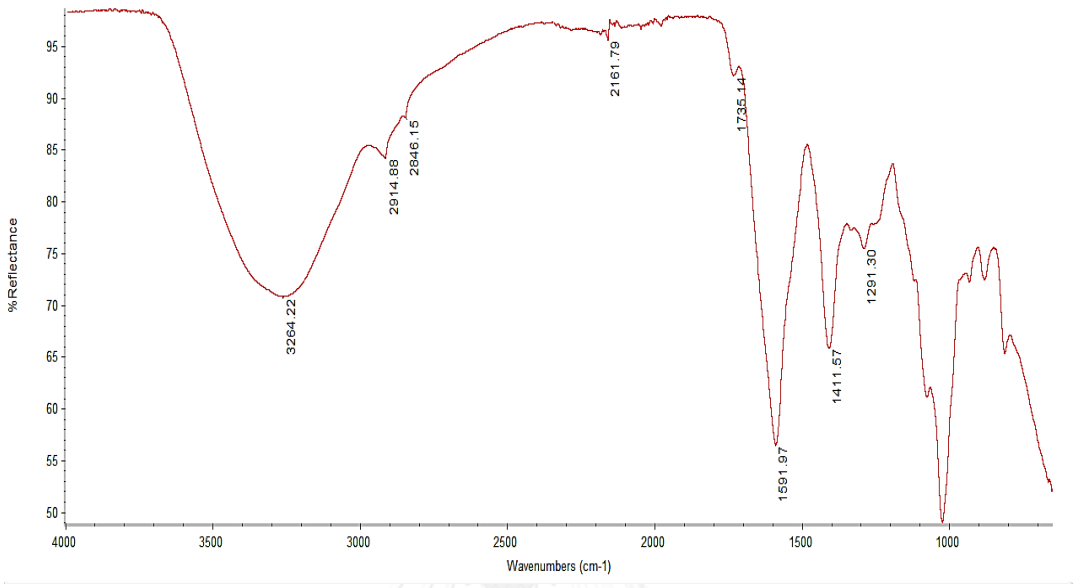


Figure C1 FTIR spectra of col-alg beads

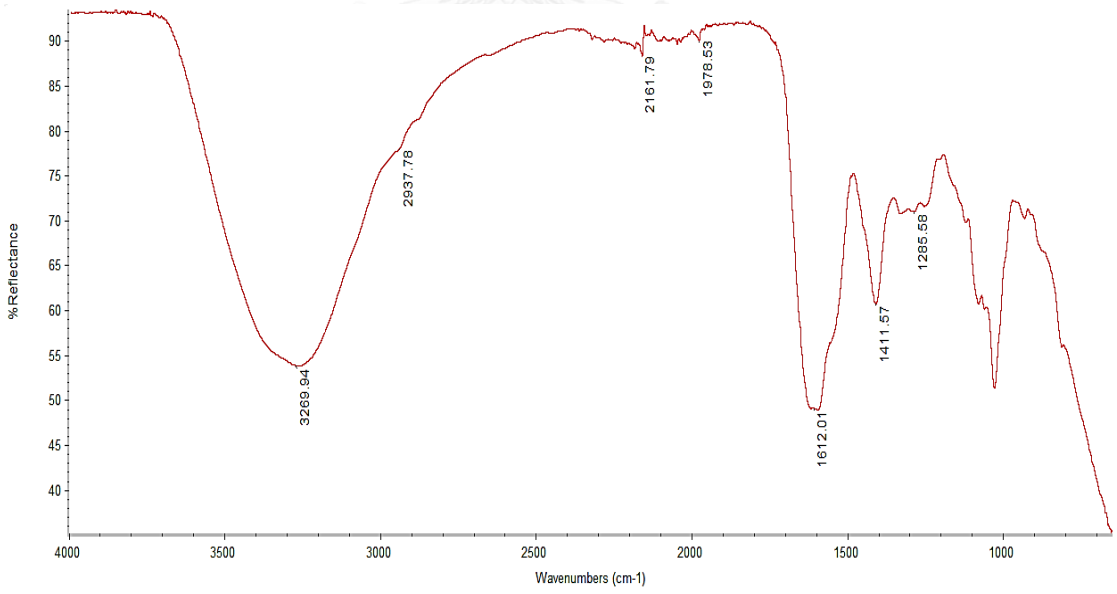


Figure C2 FTIR spectra of col-alg coated beads

Appendix D

Differential Scanning Calorimetric (DSC) of Collagen, Propylene Glycol Alginate (PGA), Col-alg Beads and Col-alg Coated Beads

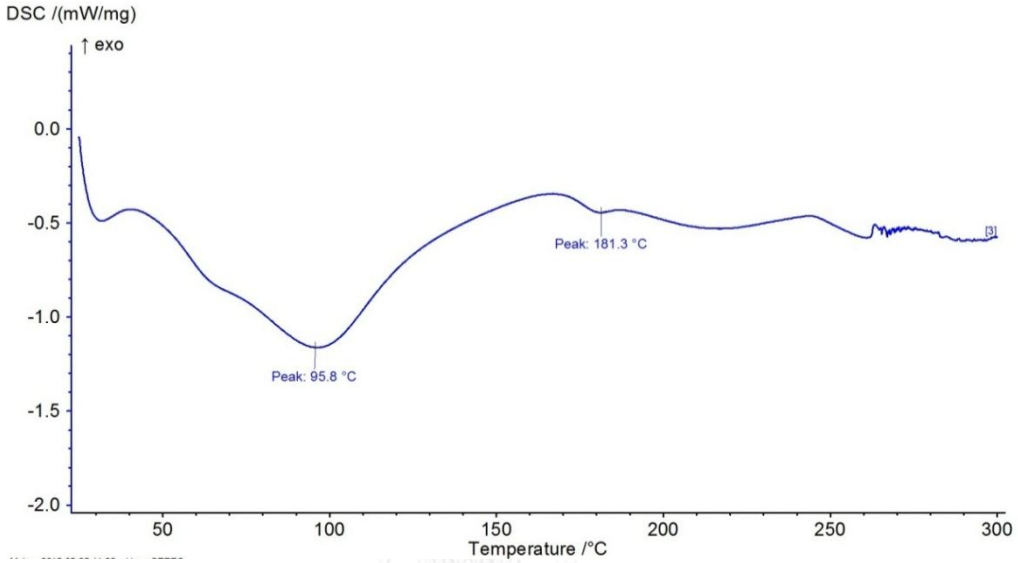


Figure D1 DSC thermogram of collagen

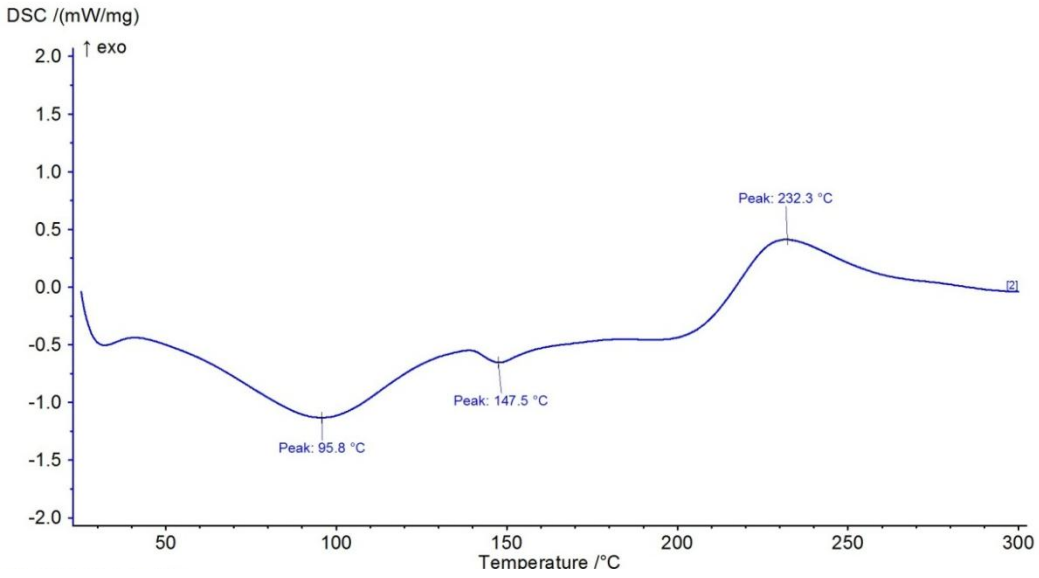


Figure D2 DSC thermogram of propylene glycol alginate (PGA)

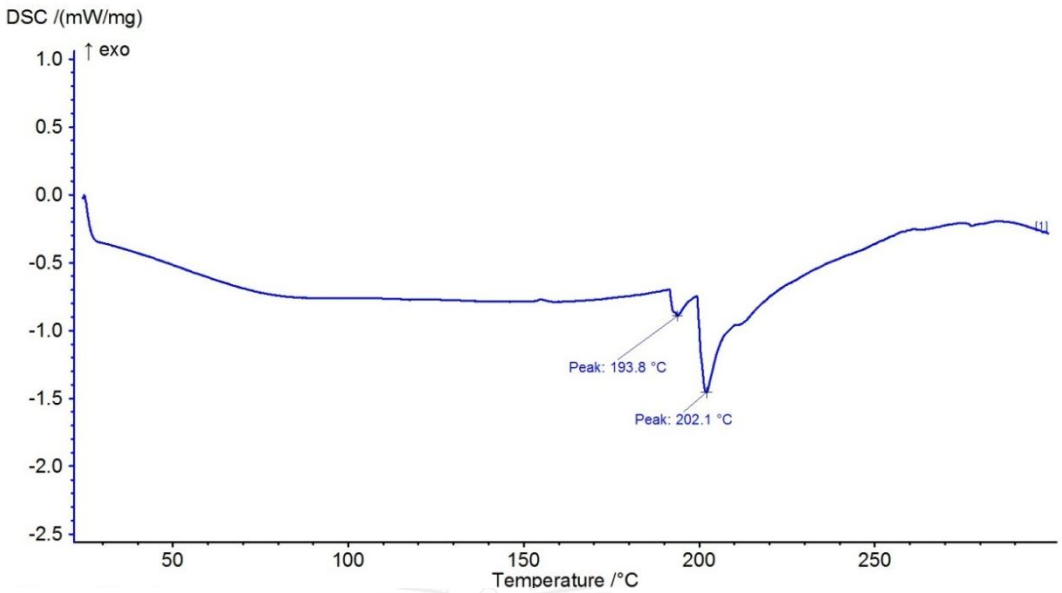


Figure D3 DSC thermogram of collagen-alginate beads

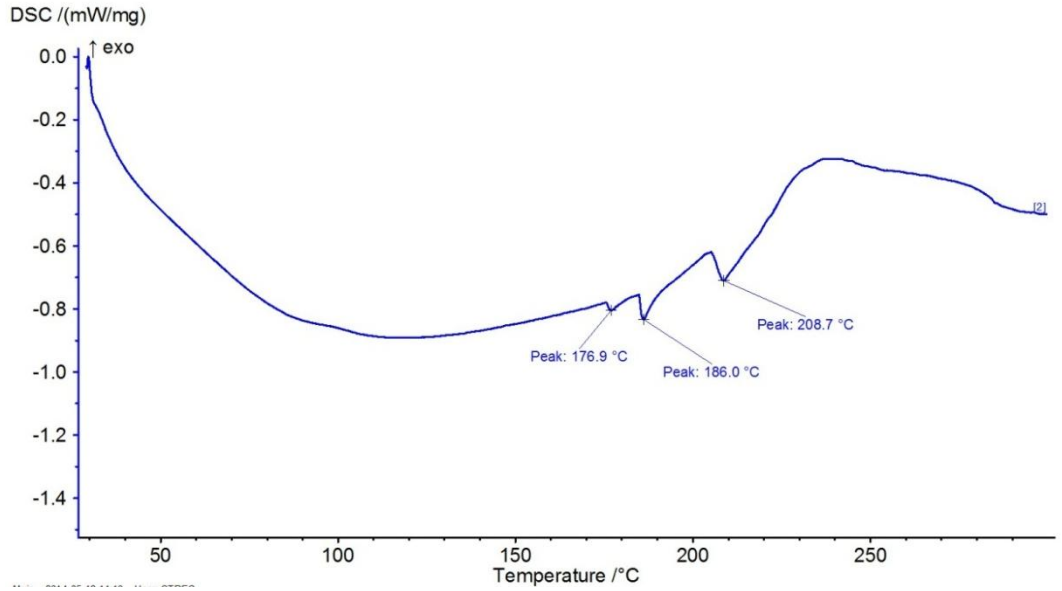


Figure D4 DSC thermogram of collagen-alginate coated beads

Appendix E

Dynamic Light Scattering (DLS) of Curcuminoid-loaded Ethyl Cellulose Particles

Table E1 The dynamic light scattering data of Cur-loaded EC particles in distilled water.

Sample ID	Hydrodynamic Diameter (nm)	Polydispersity Index	Zeta Potential (mV)
Cur-loaded EC 1	356	0.098	-27.60
Cur-loaded EC 2	350	0.103	-26.30
Cur-loaded EC 3	354	0.112	-27.1
mean	353.37	0.10	-27
S.D.	2.8	0.0	0.7

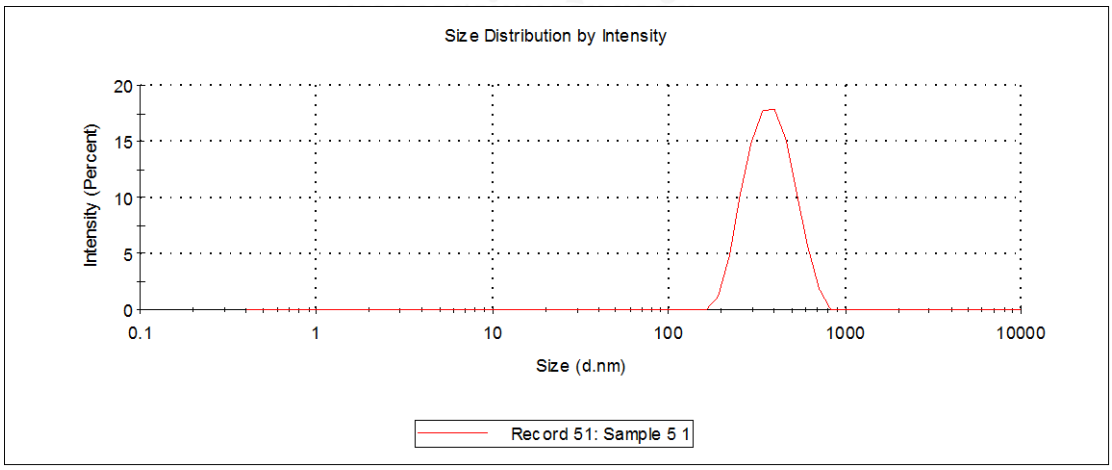


Figure E1 The size distribution graph of Cur-loaded EC particles 1

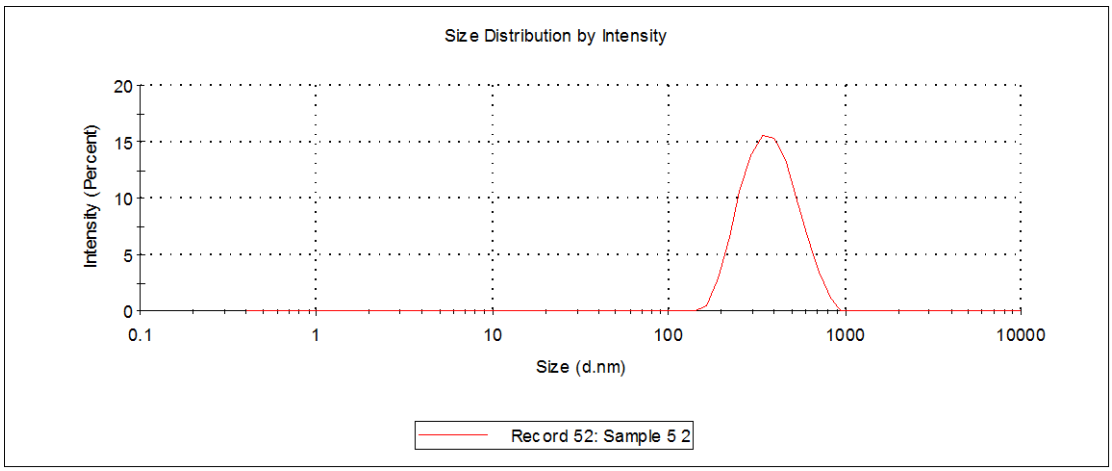


Figure E2 The size distribution graph of Cur-loaded EC particles 2

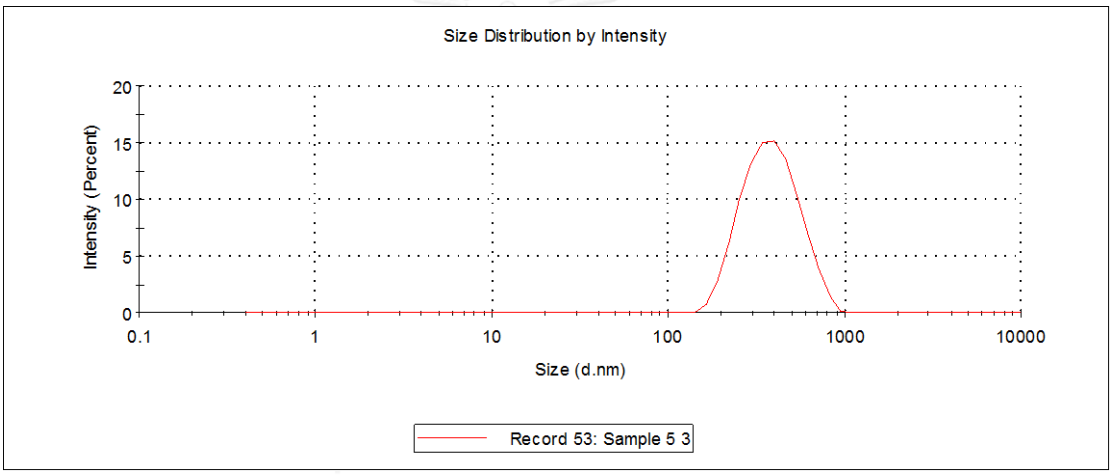


Figure E3 The size distribution graph of Cur-loaded EC particles 3

Appendix F

Calculation of the Entrapment Curcuminoid in Ethyl Cellulose Particles

1st times of Cur-loaded Ethyl Cellulose particles

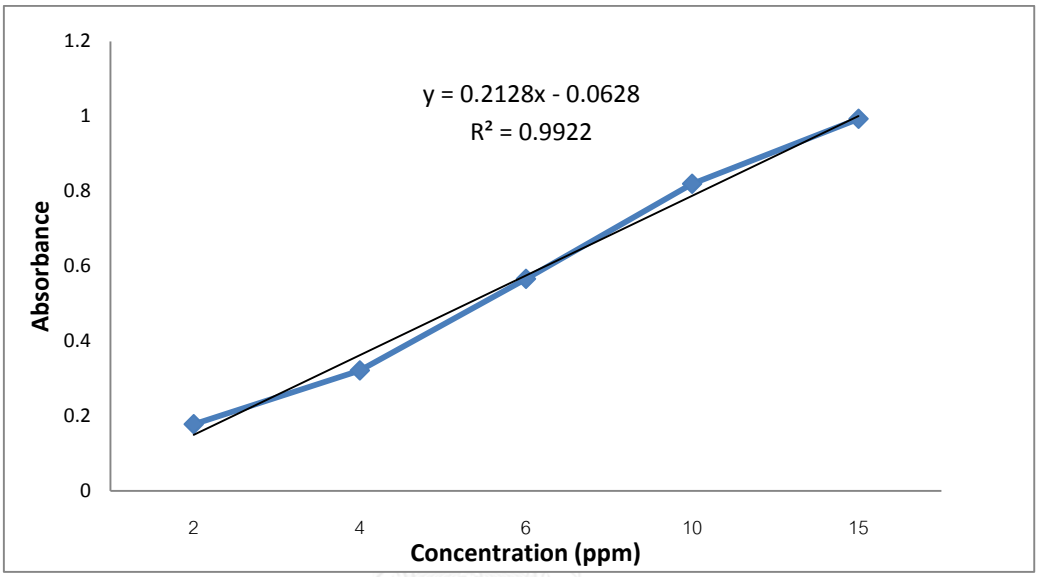


Figure F1 Calibration curve of curcuminoid at 421 nm

By the plotting a graph between absorbance and concentration of curcuminoid solution, a linear relationship was obtained and used for calculation of concentration of curcuminoid

From the equation of calibration curve;

$$Y = 0.2128X - 0.0628,\ R^2=0.9922 \qquad (1)$$

The amount of curcuminoid loaded in the nanoparticles was calculated by equation (1);

$$0.40857 = 0.2128X - 0.0628$$

$$X= 2.1832 \text{ ppm} = 2.1832 \text{ mg/L}$$

In the dilute solution of 10 mL had curcuminoid of (10x2.1832)/1000 = 0.0218 mg

It shown 200 μl from 10 ml of dilute solution had curcuminoid of 0.0218 mg

In the dilute solution of 10 mL had curcuminoid of (10x0.0218)/0.2 = 1.0916 mg

That mean in total volumn 1 mL had curcuminoid of 1.0916 mg

Weight of employed curcuminoid and ethyl cellulose (EC) were 3.026 and 3.03 mg

$$\%EE = \frac{\text{Actual curcuminoid loading}}{\text{Total curcuminoid loading}} x100$$

= (1.0916/3.026)x100

= 36.07 %

$$\%LC = \frac{\text{Weight of curcuminoid in particles}}{\text{Weight of particles}} x100$$

= [1.0916/(1.0916+3.03)]x100

= 26.48 %

2nd times of Cur-loaded Ethyl Cellulose particles

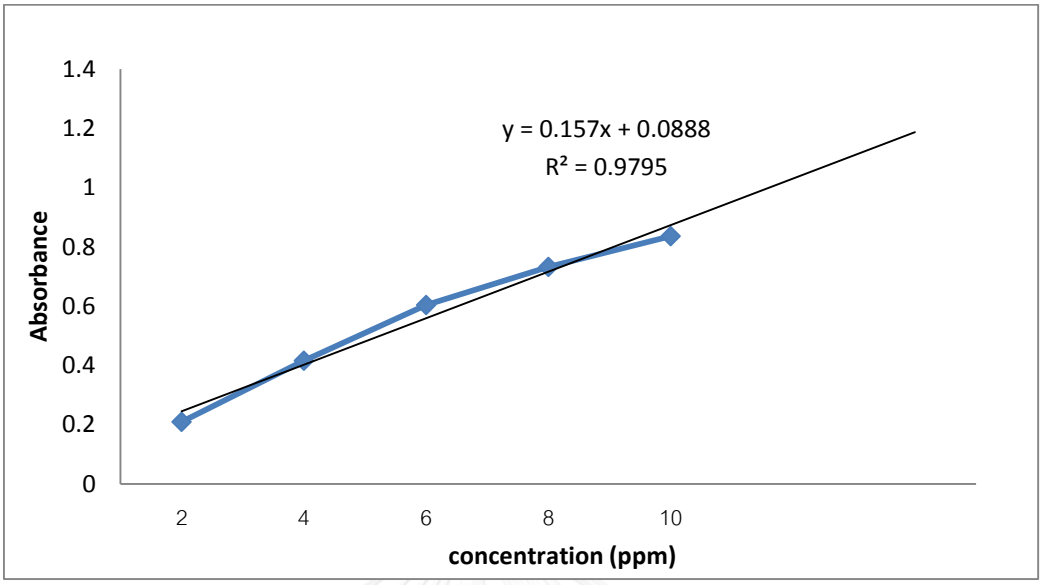


Figure F2 Calibration curve of curcuminoid at 421 nm

By the plotting a graph between absorbance and concentration of curcuminoid solution, a linear relationship was obtained and used for calculation of concentration of curcuminoid

From the equation of calibration curve;

$$Y = 0.157X + 0.0888,\ R^2=0.9795 \qquad (2)$$

The amount of curcuminoid loaded in the nanoparticles was calculated by equation (2);

$$0.75005 = 0.157X + 0.0888$$

$$X= 4.2118 \text{ ppm} = 4.2118 \text{ mg/L}$$

In the dilute solution of 5 mL had curcuminoid of (5x4.2118)/1000 = 0.0211 mg

It shown 200 μl from 10 ml of dilute solution had curcuminoid of 0.0211 mg

In the dilute solution of 10 mL had curcuminoid of (10x0.0211)/0.2 = 1.055 mg

That mean in total volumn 1 mL had curcuminoid of 1.055 mg

Weight of employed curcuminoid and ethyl cellulose (EC) were 2.79 and 2.80 mg

$$\%EE = \frac{\text{Actual curcuminoid loading}}{\text{Total curcuminoid loading}} x100$$

$$= (1.055/2.79)x100$$

$$= 37.81\ \%$$

$$\%LC = \frac{\text{Weight of curcuminoid in particles}}{\text{Weight of particles}} x100$$

$$= [1.055/(1.055+2.80)]x100$$

$$= 27.37\ \%$$

3rd times of Cur-loaded Ethyl Cellulose particles.

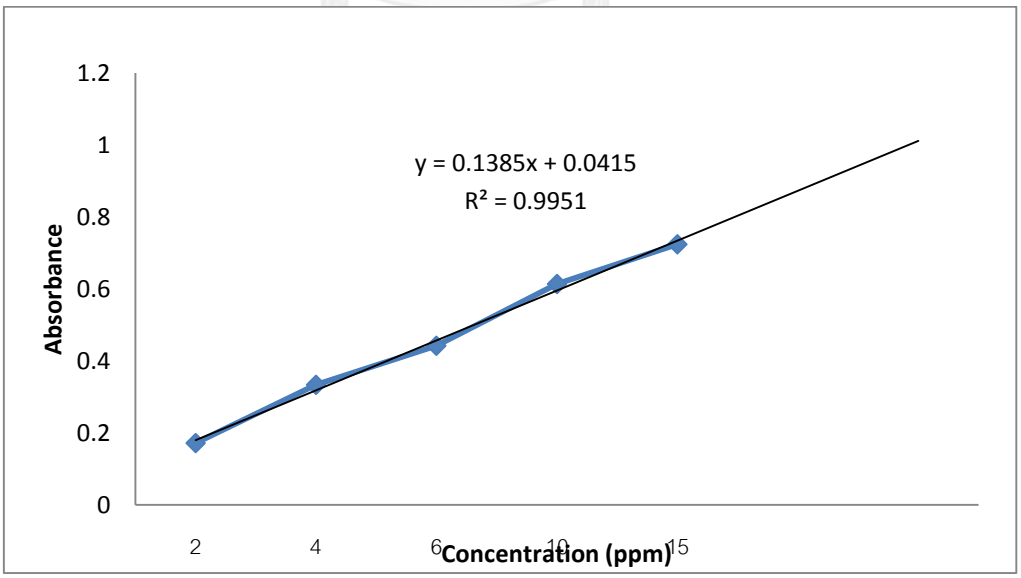


Figure F3 Calibration curve of curcuminoid at 421 nm

By the plotting a graph between absorbance and concentration of curcuminoid solution, a linear relationship was obtained and used for calculation of concentration of curcuminoid

From the equation of calibration curve;

$$Y = 0.1385X + 0.0415, R^2=0.9951 \quad (3)$$

The amount of curcuminoid loaded in the nanoparticles was calculated by equation (3);

$$0.65863 = 0.1385X + 0.0415$$

$$X= 4.4558 \text{ ppm} = 4.4558 \text{ mg/L}$$

In the dilute solution of 1 mL had curcuminoid of (1x4.2118)/1000 = 0.0044558 mg

It shown 50 μl from 10 ml of dilute solution had curcuminoid of 0.0044558 mg

In the dilute solution of 10 mL had curcuminoid of (10x0.0044558)/0.05 = 0.8912 mg

That mean in total volumn 1 mL had curcuminoid of 0.8912 mg

Weight of employed curcuminoid and ethyl cellulose (EC) were 2.19 and 2.2 mg

$$\%EE = \frac{\text{Actual curcuminoid loading}}{\text{Total curcuminoid loading}} x100$$

$$= (0.8912/2.19)x100$$

$$= 40.69 \%$$

$$\%LC = \frac{\text{Weight of curcuminoid in particles}}{\text{Weight of particles}} x100$$

= [0.8912/(0.8912+2.2)]x100

= 28.83 %

So

%EE of curcuminoid in paticles = (36.07+37.81+40.69)/3

= 38.19±1.91%

%LC of curcuminoid in particles = (26.48+27.37+28.83)/3

= 27.56±0.97

Appendix G

Calculation of the Remain Curcuminoid in Col-alg Beads

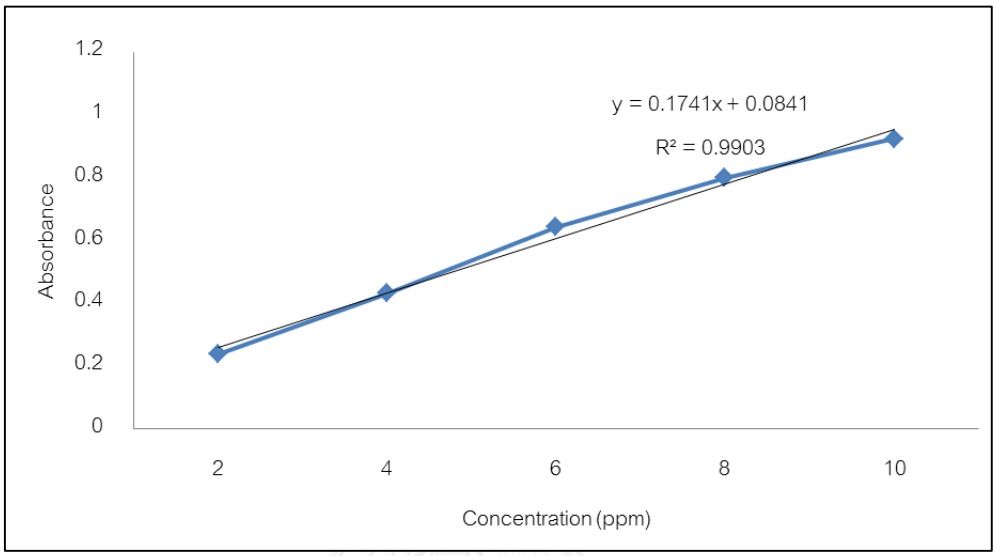


Figure G1 Calibration curve of curcuminoid at 421 nm

By the plotting a graph between absorbance and concentration of curcuminoid solution, a linear relationship was obtained and used for calculation of concentration of curcuminoid

From the equation of calibration curve;

$$Y = 0.1741X + 0.0841,\ R^2=0.9903 \qquad (4)$$

The amount of curcuminoid loaded in the nanoparticles was calculated by equation (4);

$$0.76178 = 0.1741X + 0.0841$$

$$X= 3.892 \text{ ppm} = 3.892 \text{ mg/L}$$

In the dilute solution of 1 mL had curcuminoid of (1x3.892)/1000 = 0.003892 mg

It shown 70 μl from 10 ml of dilute solution had curcuminoid of 0.003892 mg

In the dilute solution of 10 mL had curcuminoid of (10x0.003892)/0.07 = 0.556 mg

$$\%LC = \frac{\text{Weight of curcuminoid in particles}}{\text{Weight of particles}} x100$$

= (0.556/52.8)x100

= 1.05%

$$\%EE = \frac{\text{Actual curcuminoid loading}}{\text{Total curcuminoid loading}} x100$$

= (5.97/10.5)x100

= 56.86 %

Table G1 Loading Capacity of curcuminoid in col-alg beads

Sample Name	Loading Capacity				
	1	2	3	Mean	S.D.
Free curcuminoid in col-alg beads	1.05	1.12	1.4	1.19	0.2
Free curcuminoid in col-alg coated beads	0.97	0.94	1.45	1.12	0.3
Cur-loaded EC particles in col-alg beads	1.01	1.38	1.57	1.32	0.3
Cur-loaded EC particles in col-alg coated beads	1.68	1.31	1.79	1.59	0.3

Table G2 Entrapment Efficiency of curcuminoid in col-alg beads

Sample Name	Entrapment Efficiency				
	1	2	3	Mean	S.D.
Free curcuminoid in col-alg beads	56.86	61.7	77.05	65.2	10.5
Free curcuminoid in col-alg coated beads	32.67	32.76	38.76	38.76	10.5
Cur-loaded EC particles in col-alg beads	55.9	79.55	59.24	64.9	12.8
Cur-loaded EC particles in col-alg coated beads	63.24	54.1	67.43	61.59	6.8

Appendix H

Controlled Release of Curcuminoid in Col-alg Beads and Col-alg Coated Beads

Calibration curve of Curcuminoid in PBS buffer pH 5.5

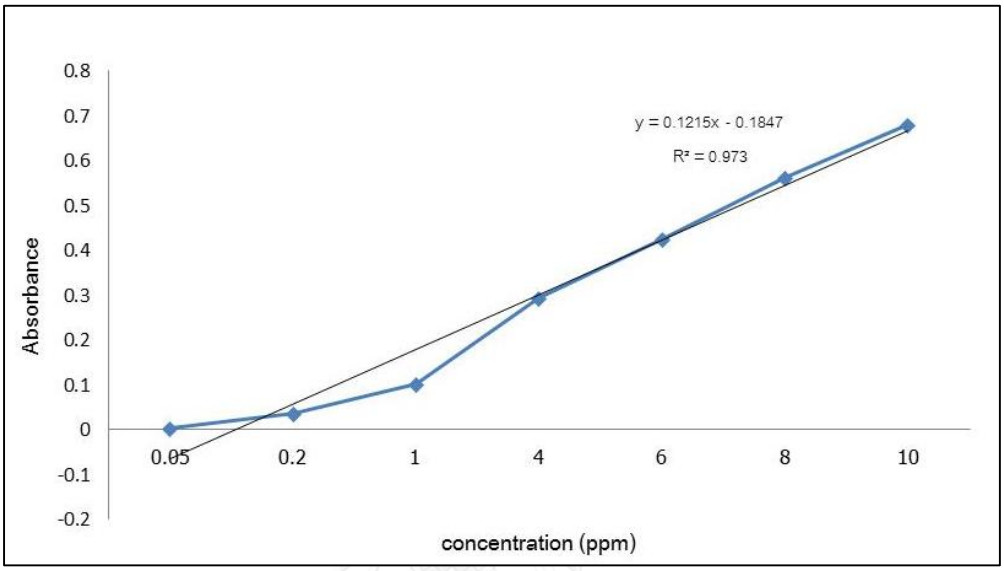


Figure H1 Calibration curve for release study of curcuminoid at 430 nm

-Free curcuminoid in col-alg coated beads which in the PBS medium 1 hr

From equation Y=0.1215X – 0.1847

0.034778=0.1215X – 0.1847

X = 1.806 ppm = 1.806 mg/L

Weight of curcuminoid in 1 mL of sampling = (1.806x1)/1000

= 0.001806 mg

Weight of curcuminoid in 100 mL of release medium = 0.001806x100

= 0.1806 mg

Weight of curcuminoid in col-alg coated beads 53.6 mg = 0.518 mg

Cumulative release of curcuminoid 1 h = (0.1806x100)/0.518

= 34.86%

-Free curcuminoid in beads which in the PBS medium 2 hr

From equation Y=0.1215X – 0.1847

0.063068=0.1215X – 0.1847

X = 2.039 ppm = 2.039 mg/L

Weight of curcuminoid in 1 mL of sampling = (2.039x1)/1000

= 0.002039 mg

Weight of curcuminoid in 100 mL of release medium = 0.002039x100

= 0.2039 mg

Weight of curcuminoid in col-alg coated beads 53.6 mg = 0.518 mg

Cumulative release of curcuminoid 2 h = (0.2039x100)/0.518

= 39.36%

-Free curcuminoid in beads which in the PBS medium 3 hr

From equation Y=0.1215X – 0.1847

0.089467=0.1215X – 0.1847

X = 2.257 ppm = 2.257 mg/L

Weight of curcuminoid in 1 mL of sampling = (2.257x1)/1000

= 0.002257 mg

Weight of curcuminoid in 100 mL of release medium = 0.002257x100

= 0.2257 mg

Weight of curcuminoid in col-alg coated beads 53.6 mg = 0.518 mg

Cumulative release of curcuminoid 3 hr = (0.2257x100)/0.418

= 43.57%

-Free curcuminoid in beads which in the PBS medium 4 hr

From equation Y=0.1215X – 0.1847

0.113529=0.1215X – 0.1847

X = 2.455 ppm = 2.455 mg/L

Weight of curcuminoid in 1 mL of sampling = (2.455x1)/1000

= 0.002455 mg

Weight of curcuminoid in 100 mL of release medium = 0.002455x100

= 0.2455 mg

Weight of curcuminoid in col-alg coated beads 53.6 mg = 0.518 mg

Cumulative release of curcuminoid 4 hr = (0.2455x100)/0.518

= 47.39%

-Free curcuminoid in beads which in the PBS medium 5 hr

From equation Y=0.1215X – 0.1847

0.131199=0.1215X – 0.1847

X = 2.6 ppm = 2.6 mg/L

Weight of curcuminoid in 1 mL of sampling = (2.6x1)/1000

= 0.0026 mg

Weight of curcuminoid in 100 mL of release medium = 0.0026x100

= 0.26 mg

Weight of curcuminoid in col-alg coated beads 53.6 mg = 0.518 mg

Cumulative release of curcuminoid 5 hr = (0.26x100)/0.518

= 50.19%

-Free curcuminoid in beads which in the PBS medium 6 hr

From equation Y=0.1215X – 0.1847

0.155968=0.1215X – 0.1847

X = 2.804 ppm = 2.804 mg/L

Weight of curcuminoid in 1 mL of sampling = (2.804)/1000

= 0.002804 mg

Weight of curcuminoid in 100 mL of release medium = 0.002804x100

= 0.2804 mg

Weight of curcuminoid in col-alg coated beads 53.6 mg = 0.518 mg

Cumulative release of curcuminoid 6 hr = (0.2804x100)/0.518

= 54.13 %

-Free curcuminoid in beads which in the PBS medium 7 hr

From equation Y=0.1215X – 0.1847

0.192249=0.1215X – 0.1847

X = 3.102 ppm = 3.102 mg/L

Weight of curcuminoid in 1 mL of sampling = (3.102)/1000

= 0.003102 mg

Weight of curcuminoid in 100 mL of release medium = 0.003102x100

= 0.3102 mg

Weight of curcuminoid in col-alg coated beads 53.6 mg = 0.518 mg

Cumulative release of curcuminoid 7 hr = (0.3102x100)/0.518

= 59.88%

-Free curcuminoid in beads which in the PBS medium 24 hr

From equation Y=0.1215X – 0.1847

0.219671=0.1215X – 0.1847

X = 3.328 ppm = 3.328 mg/L

Weight of curcuminoid in 1 mL of sampling = (3.328x1)/1000

= 0.003328 mg

Weight of curcuminoid in 100 mL of release medium = 0.003328x100

= 0.3328 mg

Weight of curcuminoid in col-alg coated beads 53.6 mg = 0.518 mg

Cumulative release of curcuminoid 24 hr = (0.3328x100)/0.518

= 64.25%

-Free curcuminoid in beads which in the PBS medium 48 hr

From equation Y=0.1215X – 0.1847

0.250033=0.1215X – 0.1847

X = 3.578 ppm = 3.578 mg/L

Weight of curcuminoid in 1 mL of sampling = (3.578x1)/1000

= 0.003578 mg

Weight of curcuminoid in 100 mL of release medium = 0.003578x100

= 0.3578 mg

Weight of curcuminoid in col-alg coated beads 53.6 mg = 0.518 mg

Cumulative release of curcuminoid 48 hr = (0.3578x100)/0.518

= 69.07 %

-Free curcuminoid in beads which in the PBS medium 96 hr

From equation Y=0.1215X – 0.1847

0.250231=0.1215X – 0.1847

X = 3.58 ppm = 3.58 mg/L

Weight of curcuminoid in 1 mL of sampling = (3.58x1)/1000

= 0.00358 mg

Weight of curcuminoid in 100 mL of release medium = 0.00358x100

= 0.358 mg

Weight of curcuminoid in col-alg coated beads 53.6 mg = 0.518 mg

Cumulative release of curcuminoid 96 hr = (0.358x100)/0.518

= 69.11 %

-Free curcuminoid in beads which in the PBS medium 144 hr

From equation Y=0.1215X – 0.1847

0.287202=0.1215X – 0.1847

X = 3.884 ppm = 3.884 mg/L

Weight of curcuminoid in 1 mL of sampling = (3.884)/1000

= 0.003884 mg

Weight of curcuminoid in 100 mL of release medium = 0.003884x100

= 0.3884 mg

Weight of curcuminoid in col-alg coated beads 53.6 mg = 0.518 mg

Cumulative release of curcuminoid 144 hr = (0.3884x100)/0.518

= 74.98%

Table H1 Release of free curcuminoid in col-alg beads

Time (hr)	% curcuminoid released			
	1	2	Mean	S.D.
0	0	0	0	0
1	27.75	27.09	27.42	0.5
2	28.99	28.51	28.75	0.3
3	31.24	32.32	31.78	0.8
4	38	33.16	35.58	3.4
5	42.46	34.18	38.32	5.9
6	43.26	36.9	40.08	4.5
7	43.53	38.85	41.19	3.3
24	53.21	58.29	55.75	3.6
48	65.41	75.75	70.58	7.3
96	74.79	88.58	81.69	9.8
144	89.08	90.83	89.96	1.2

Table H2 Release of free curcuminoid in col-alg coated beads

Time (hr)	% curcuminoid released			
	1	2	Mean	S.D.
0	0	0	0	0
1	34.86	33.42	34.14	1
2	39.36	37.7	38.53	1.2
3	43.57	42.92	43.25	0.5
4	47.39	45.33	46.36	1.5
5	50.19	46.74	48.47	2.4
6	54.13	52.2	53.17	1.4
7	59.88	54.09	56.99	4.1
24	64.25	58.76	61.51	3.9
48	69.07	62.12	65.6	4.9
96	69.11	65.98	67.55	2.2
144	74.98	69.4	72.19	3.9

Table H3 Release of Cur-loaded EC particles in col-alg beads

Time (hr)	% curcuminoid released			
	1	2	Mean	S.D.
0	0	0	0	0
1	23.5	19.3	21.4	3
2	23.98	20.16	22.07	2.7
3	24.77	20.84	22.81	2.8
4	25.97	21.33	23.65	3.3
5	27.45	21.47	24.46	4.2
6	27.91	22.15	25.03	4.1
7	34.19	22.62	28.41	8.2
24	39.04	30.77	34.91	5.8
48	44.13	50.39	47.26	4.4
96	46.27	56.02	51.15	6.9
144	54.46	59.96	57.21	3.9

Table H4 Release of Cur-loaded EC particles in col-alg coated beads

Time (hr)	% curcuminoid released			
	1	2	Mean	S.D.
0	0	0	0	0
1	19.44	18.39	18.92	0.7
2	25.35	20.27	22.81	3.6
3	28.96	22.17	25.57	4.8
4	31.18	22.18	26.68	6.4
5	34.11	22.78	28.45	8
6	39.7	26.13	32.92	9.6
7	39.81	26.19	33	9.6
24	47.68	32.56	40.12	10.7
48	49.34	41.21	45.28	5.7
96	50.7	43.07	46.89	5.4
144	51.04	43.34	47.19	5.4

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