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นาย ธีรวัฒน์ สหเสถียร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2550

CONTROLLED RELEASE OF AMOXICILLIN FROM ALGINATE-GELATIN-CHOTOSAN BEADS

Mr. Teerawat Sahasathian

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science Program in Petrochemistry and Polymer Science

Faculty of Science

Chulalongkorn University

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Thesis Title	CONTROLLED RELEASE OF AMOXICILLIN FROM ALGINATE-GELATIN-CHITOSAN BEADS
By	Mr. Teerawat Sahasathian
Field of Study	Petrochemistry and Polymer Science
Thesis Advisor	Associate Professor Nongnuj Muangsin, Ph.D.
Thesis Coadvisor	Nalena Praphairaksit, D.V.M., Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

..... Dean of the Faculty of Science
(Professor Piamsak Menasveta, Ph.D.)

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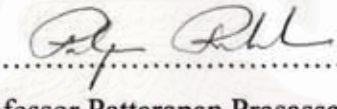

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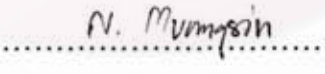

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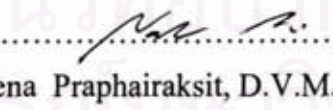

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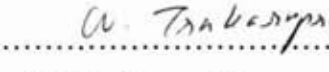

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งานวิจัยนี้มุ่งเน้นที่จะพัฒนาตำรับยาที่มีความสามารถในการหน่วงการปลดปล่อยยาให้ออกฤทธิ์เนิ่นในกระเพาะอาหาร โดยการใช้สูตรผสมของแอลจิเนตและเจลาตินเป็นเมทริกซ์สำหรับการเตรียมบีด และใช้ไคโตซานเป็นส่วนเคลือบบีดเพื่อช่วยทำให้บีดสามารถเกาะติดเยื่อเมือกในกระเพาะอาหารได้ดียิ่งขึ้น ทั้งนี้เพื่อเพิ่มประสิทธิภาพในการกำจัดเชื้อเฮลิโคแบคเตอร์ไพโลไร ซึ่งเป็นสาเหตุสำคัญที่ทำให้เกิดโรคแผลในกระเพาะอาหาร จากการศึกษาพบว่าการผสมเจลาตินลงในเมทริกซ์ของแอลจิเนตจะส่งผลต่อสมบัติต่าง ๆ ของบีดเพียงเล็กน้อยเท่านั้น ในขณะที่บีด แอลจิเนตที่เคลือบด้วยสารละลายไคโตซานความเข้มข้น 0.5 เปอร์เซ็นต์มวลโดยปริมาตร (ALG/0.5%CHI) ให้ผลการทดลองด้านความสามารถในการลอยตัวในสภาวะเลียนแบบของเหลวในกระเพาะอาหารและการเกาะติดเยื่อเมือกในกระเพาะอาหารที่ดี และเมื่อทำการศึกษาการปลดปล่อยยาในสภาวะจำลองพบว่า แอม็อกซิซิลลินจะถูกปลดปล่อยในสารละลายไฮโดรคลอริก พีเอช 1.2 ได้เร็วกว่าในสารละลายฟอสเฟตบัฟเฟอร์ พีเอช 7.4 อย่างไรก็ตาม จากผลการทดลองพบว่า ALG/0.5%CHI สามารถหน่วงการปลดปล่อยยาแอม็อกซิซิลลินให้ออกฤทธิ์เนิ่นในสภาวะเลียนแบบของเหลวในกระเพาะอาหารได้นานถึง 6 ชั่วโมง ซึ่งสูตรผสมที่เตรียมได้นี้นับว่าเป็นทางเลือกใหม่สำหรับใช้ในการรักษาโรคแผลในกระเพาะอาหารได้อย่างมีประสิทธิภาพ

สาขาวิชา.....ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์.....ลายมือชื่อนิสิต .....

ปีการศึกษา ..... 2550.....

ลายมือชื่ออาจารย์ที่ปรึกษา .....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

477 23285 23 : MAJOR PETROCHEMISTRY AND POLYMER SCIENCE
KEY WORD: CONTROLLED RELEASE / AMOXICILLIN / ALGINATE / CHITOSAN / BEADS

TEERAWAT SAHASATHIAN : CONTROLLED RELEASE OF AMOXICILLIN FROM ALGINATE-GELATIN-CHITOSAN BEADS. THESIS ADVISOR : ASSOC.PROF. NONGNUJ MUANGSIN, Ph.D., THESIS COADVISOR : NALENA PRAPHAIRAKSIT, D.V.M., Ph.D., 110 pp.

This work focused on the development of gastroretentive dosage forms of amoxicillin using alginate and gelatin as a matrix polymers and chitosan as a mucoadhesive polymer coating for the effective eradication of *Helicobacter Pylori*, a major causative agent of peptic ulcer. In all cases, an incorporation of gelatin in the alginate matrix presented only minor effect to the beads properties. The amoxicillin-loaded alginate beads coated with 0.5% (w/v) chitosan (ALG/0.5%CHI) showed the excellent floating ability as well as the *in vitro* mucoadhesive test. The beads were able to adhere strongly to the gastric mucosal layer. The *in vitro* release profiles revealed that amoxicillin released faster in pH 1.2 hydrochloric acid (HCl) than in pH 7.4 phosphate buffer. However, the result showed that ALG/0.5%CHI was able to sustain the release of amoxicillin for over 6 hours in the simulated gastric fluid (pH 1.2 HCl). In summary, it can be concluded that amoxicillin-loaded alginate beads coated with 0.5% (w/v) chitosan offer a novel alternative for the effective treatment of peptic ulcer.

Field of Study...Petrochemistry and Polymer Science...Student's Signature.....

Academic Year .....2007.....

Advisor's Signature.....N. Muangsin

Co-advisor's Signature.....

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ALG	Amoxicillin-loaded alginate beads
ALG-GEL	Amoxicillin-loaded alginate incorporated with gelatin beads
ALG/1%CHI	Amoxicillin-loaded alginate coated with 1% (w/v) chitosan beads
ALG-GEL/1%CHI	Amoxicillin-loaded alginate incorporated with gelatin and coated with 1% (w/v) chitosan beads
AMOX	Amoxicillin
°C	degree Celsius (centigrade)
cm^{-1}	Unit of wave number
DSC	Differential scanning calorimeter
EE	The encapsulation efficiency
FT-IR	Fourier transform infrared spectroscopy
HPLC	High Performance Liquid Chromatography
IEP	Isoelectric point
pH	The negative logarithm of the hydrogen ion concentration
ppm	Part per million
r^2	The correlation coefficient
S.D.	Standard deviation
SEM	Scanning electron microscope
S_w	The swelling ratio
w/v	Weight by volume
w/w	Weight by weight

CHAPTER I

INTRODUCTION

Amoxicillin (α-amino-hydroxybenzylpenicillin) is a moderate-spectrum β-Lactam antibiotics, which is effectively used to treat bacterial infections caused by susceptible microorganisms. It is usually the drug of choice within the class because of the better adsorption property, following oral administration, than other β-Lactam antibiotics. In addition, amoxicillin is widely used to treat *Helicobacter pylori* infection, the major causative agents of peptic ulcer [1].

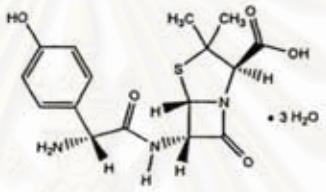


Figure 1.1 Structure of Amoxicillin trihydrate

Helicobacter pylori (*H. pylori*) is a bacterium that infects the mucus lining of the human stomach. Many peptic ulcers and some types of gastritis are caused by *H. pylori* infection, although most humans who are infected will never develop symptoms. This bacterium lives in the human stomach exclusively and is the only known organism that can thrive in that highly acidic environment. It is helix-shaped and can literally screw itself into the stomach lining to colonize [2].

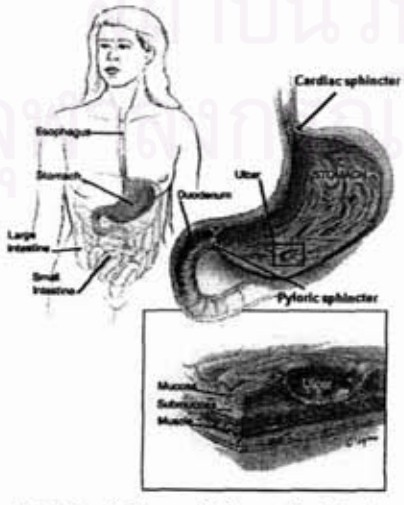


Figure 1.2 Position of *H. pylori* infection [3].

Figure 1.3 *Helicobacter pylori* [4].

However, some researchers have reported that the therapies using conventional amoxicillin can not completely eradicate *Helicobacter pylori* and suggest that the therapeutic effect needs more investigations [5,6].

In general, *Helicobacter pylori* exists in the gastric mucous layer or epithelial cell surfaces, thus the suitable amoxicillin concentration and residence time in the stomach should be considered for effective *Helicobacter pylori* eradication [7,8]. As the conventional amoxicillin has short residence time in the stomach and may be degraded in gastric acid resulting in lesser concentration in gastric blood [9,10], the extended residence time of amoxicillin was desirable to provide a more effective *Helicobacter pylori* eradication. Thus, some researchers had improved new amoxicillin formulations, such as floating tablets [11], mucoadhesive tablets [12], mucoadhesive microspheres [13-14], etc., which could retain in stomach for the extended period of time for more effective therapy.

Although mucoadhesive microspheres have recently gained considerable attention to obtain the extended period of time in stomach, the encapsulation efficiency was not satisfied [13,15]. Therefore, beads loaded with antibiotics may be an alternative formulation that would be useful for oral delivery to treat gastric diseases such as peptic ulcer [16-18] with significantly higher encapsulation efficiency. In addition, the bead formulations appear as the floating dosage form that could retain in stomach for the longer period of time and also provide the sustained release manner [19].

Polymers have recently become attractive materials in the pharmaceutical development. A number of natural polymers have increasingly been used for the bead preparation. Hydrophilic polysaccharides such as alginate (ALG) have gained a lot of attention owing to its excellent ability and simplicity to form the bead, simply by dropping an aqueous sodium alginate solution into a calcium chloride solution [20]. Nevertheless, this method presents a significant drug loss during the bead preparation especially in the case of soluble drugs [21]. To prevent this problem from occurring, some researchers have mixed alginate with other polymers such as ethycellulose [22], pectin [23], chitosan [24], konjac glucomannan [25], and gelatin [26-27]. In addition, the use of aldehydes as the cross-link of alginate has also been reported [27].

However, most literatures involving amoxicillin have only been focused on the use of synthetic polymers such as poly(vinyl pyrrolidone) [28], poly(acrylic acid) [28-30] due to their ability to adhere to the mucous layer, upon considering this as a main criteria, chitosan, a biodegradable natural polymer, has also exhibited excellent mucoadhesive property [31]. Therefore, it could be advantageous to study the controlled release of amoxicillin and mucoadhesive properties of beads formed by natural polymers such as alginate, gelatin and chitosan.

In this work, alginate and gelatin were used to prepare drug-loaded beads. Chitosan was chosen as a coating material to obtain the mucoadhesive properties and also increasing the encapsulation efficiency. In addition, freeze-dried technique was suggested for drying beads to obtain the floating dosage form.

Alginate is a natural anionic polysaccharides found mainly in brown seaweeds. It consisted of linear block copolymer of β-D-mannuronic acid and α-L-guluronic acid. Alginate has been known to form gel immediately when contact with calcium ions [27], from this property it is used in a wide variety of applications especially in food and pharmaceutics manufactures.

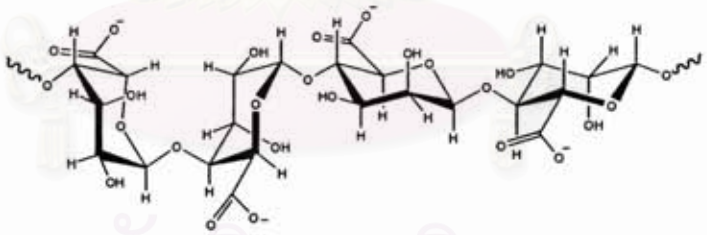


Figure 1.4 Structure of Alginate.

Gelatin is a heterogeneous water-soluble proteins derived through partial hydrolysis of the collagen from many different sources such as skin, bones, cartilage, ligaments. It can swell and absorb water 5-10 times its weight to form gel in aqueous solutions [32]. Gelatin is available in two types depending on the method of preparation. Type A gelatin (Porcine gelatin) is derived from an acid pretreatment process which has an isoelectric point between pH 7 and 9, type B gelatin (Bovine gelatin) is derived from a basic pretreatment process which has an isoelectric point

between pH 4.7 and 5.0. A structure of gelatin consists of both positive and negative charges. At pH values above or below the isoelectric point, gelatin will carry a net negative or positive charge, respectively [33]. Therefore, it is probable to use gelatin as the cross-linking agent of alginate, and also use to enhance the mucoadhesive properties.

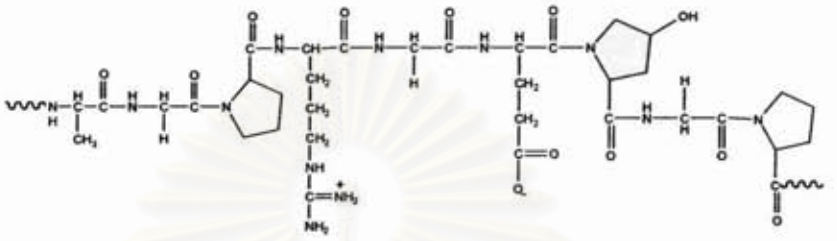


Figure 1.5 Structure of Gelatin.

Chitosan is a deacetylated derivative of chitin, found abundantly in marine crustaceans, insects and fungi. Properties such as biodegradability, good biocompatibility and non-toxicity make it suitable for using in biomedical and pharmaceutical formulations [34-35]. Chitosan was evaluated as a matrix for sustained release in various forms, e.g. microspheres [17-18], tablets [36] and beads [24,34]. Furthermore, the positively charged mucoadhesive chitosan could provide a strong electrostatic interaction with a negatively charged mucus surface [31]. Therefore, coating beads with chitosan may be used to obtain the mucoadhesive beads.

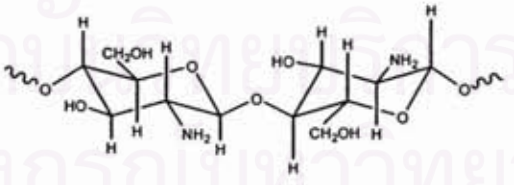


Figure 1.6 Structure of Chitosan.

1.1 The objectives of this research

The aims of this research were suggested by the amoxicillin-loaded beads based on sodium alginate which were further modified by incorporation of gelatin and coating with chitosan. The details of these objectives are described as follows:

1) To modify bead formulation based on sodium alginate and gelatin matrix coated with chitosan to obtain the effective controlled release and mucoadhesive properties.
2) To evaluate the effect of formulation variables such as bead preparation methods, chitosan concentrations used as coagulation fluid and the proportions of polymer matrix/drug.
3) To study floating and mucoadhesive properties, and release behavior of beads in gastric-intestine simulated condition.
4) To compare release behaviors of each formulation as well as amoxicillin commercial capsules.

1.2 The scope of research

The scope of this research was carried out by stepwise investigation as follows:

1) Review literatures survey for related research work.
2) Determine the preparation technique by preliminary study between two methods of preparations; suspension and solution method.
3) Prepare the beads by varying the proportions of amoxicillin, alginate, gelatin and chitosan.
4) Determine the encapsulation efficiency of beads.
5) Characterize and study the morphology of the beads using SEM, FT-IR, and a light microscope.
6) Study the buoyant properties of beads in simulated gastric fluid.
7) Study the swelling behavior of the beads in simulated gastric-intestine fluids under a microscope.
8) Study the mucoadhesive properties of beads by rinsing method with simulated gastric fluid.

9) Study the release behavior of the beads in simulated gastric-intestine fluids by using HPLC method.
10) Summarize the results.

CHAPTER II

BACKGROUND AND LITERATURE REVIEWS

2.1 Polymers in pharmaceutical field

The use of polymers in pharmaceutical research and development has increasingly gain interest for the past two decades, especially in order to control the release rate and target drug to a specific body site [37]. A conventional dosage form such as tablet, has the disadvantageous side effects of delivering an initially too high dose of active agent. Those drugs may be delivered to regions of the body where they may not be at their most effective, which then decays exponentially until the next administration.

Polymers have gained an importance in the pharmaceutical industry as both drug encapsulants and vehicles of drug carriage in order to either protect an active agent during its passage through the body until its release, or control its release. Carrier technology obtains the drug delivery system by coupling the drug to the carrier polymers in various dosage forms such as beads, microspheres, nanoparticles, liposomes. Those formulations could delay the release of drug and also generate a response in a specific area or organ of the body requiring treatment.

Gastric retention systems are also beneficial when the drug is effective locally in the stomach. However, the success of these novel drug delivery systems is limited due to their short resident time at the site of absorption especially in the upper of gastrointestinal tract due to variability in gastric emptying and gastrointestinal motility. Thus, it is known to those skilled in the art that an oral administration should be designed not only to control the rate at which it release the drug over the drug delivery time period but also to control on the location from which it is delivered (site-specific control). The site-specific control can be achieved by prolonging the period of retention of the system in the stomach. Therefore, to accomplish this purpose, the strategies for retarding drug transit through the GI tract were suggested.

2.2 Strategies for retarding drug transit through the gastrointestinal tract

The strategies for retarding drug transit through the gastrointestinal tract involved the pharmaceutical category might be considered into three strategies: super porous hydrogels, floating devices and mucoadhesive dosage forms. These strategies are achieved by a particular physical or physicochemical characteristic.

2.2.1 Superporous hydrogels

The gastric retention of superporous hydrogels is based on their fast swelling property. After oral administration, it swells quickly in the gastric medium to a large size and larger than the pyloric sphincter, so it will not expelled from the stomach. The size-related retention of a dosage form in the stomach has been studied with various systems to include systems such as swelling balloon hydrogels [38] or non-erodible or erodible tetrahedron shaped devices [39-40]. However, this approach has never passes beyond the experimental stage and clinical data are unavailable. In any case these gastric retention devices may not be safe. The hazard of lodging in esophagus or permanent retention in the stomach can cause bowel obstruction, intestinal adhesion and gastroplasy.

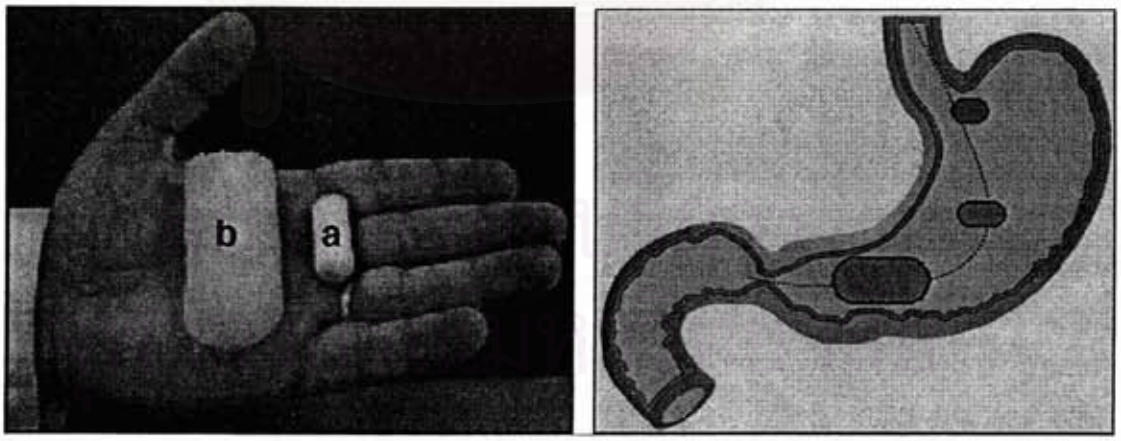


Figure 2.1 On the left, superporous hydrogel in its dry (a) and water-swollen (b) state. On the right, schematic illustration of the transit of superporous hydrogel [41].

2.2.2 Floating devices

The buoyant or floating dosage forms have a bulk density lower than that of gastric fluids and expected to remain floating on the stomach contents to prolong the gastric retention time. While the system is floating in the gastric medium, the drug is released slowly from the system at a desired rate. To achieve these floating dosage systems, many approaches were suggested; e.g. carbon dioxide gas-generating agents [42] and hollow systems prepared by solvent evaporation methods [43-44]. Among these systems, a hollow system is the most commonly prepared.

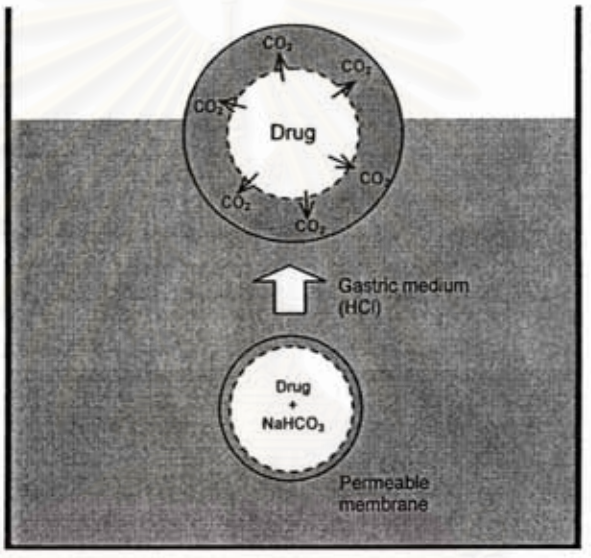


Figure 2.2 The mechanism of gas-generating system.

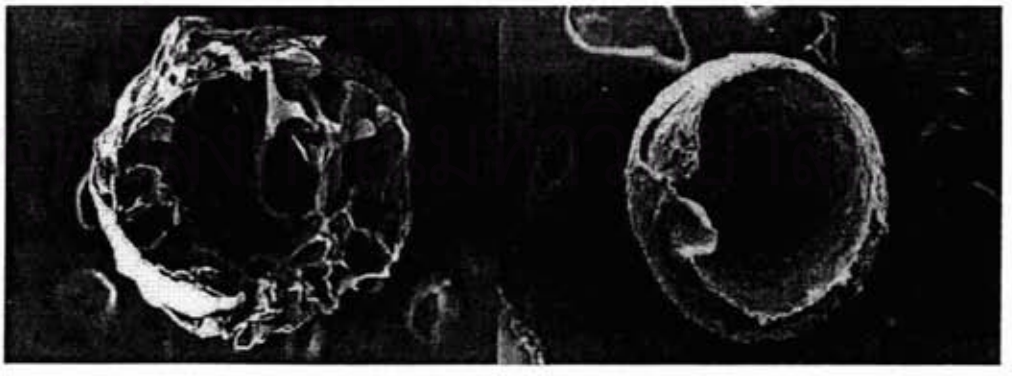


Figure 2.3 Cross-sectional morphologies of the hollow bead system [43].

2.2.3 Mucoadhesive dosage forms

"Bioadhesion" is a simple term of adhesive phenomena where at least one of the adherents is biological tissue. An adhesive bond may form with the epithelial cell layer, the continuous mucus layer or a combination of the two [45].

"Mucoadhesion" is a term used for a bioadhesive phenomenon where the biological substrate is a mucosal surface [46].

The adhesion between mucin and mucoadhesive polymers is explained by the various theories based on the molecular attractive and repulsive forces as listed in Table 2.1.

Table 2.1 Different theories explaining the mechanism of bioadhesion [45]

No.	Theory	Mechanism of bioadhesive	Comments
1	Electronic theory	Attractive electrostatic force between glycoprotein mucin network and the bioadhesive material	Electron transfer occurs between the two forming a double layer of electric charge at the interface
2	Adsorption theory	Surface forces resulting in chemical bonding	*Strong primary forces*: covalent bonds *Weak secondary forces*: ionic bonds, hydrogen bonds and van der Waal's forces
3	Wetting theory	Ability of bioadhesive polymers to spread and develop intimate contact with the mucus membranes	Spreading coefficients of polymers must be positive
4	Fracture theory	Analyses the maximum tensile stress developed during detachment of the bioadhesive drug delivery systems (BDDS) from the mucosal surfaces	Does not require physical entanglement of bioadhesive polymer chains and mucin strands, hence appropriate to study the bioadhesion of hard polymers, which lack flexible chains

Table 2.1 (continued) Different theories explaining the mechanism of bioadhesion

No.	Theory	Mechanism of bioadhesive	Comments
5	Diffusion theory	Physical entanglement of mucin strands and the flexible polymer chains	For maximum diffusion and best bioadhesive strength: solubility parameters (δ) of the bioadhesive polymer and the mucus glycoproteins must be similar
		Interpenetration of mucin strands into the porous structure of the polymer substrate	

"Mucoadhesive polymers" are natural or synthetic macromolecules which could attach to mucosal surfaces. The mucoadhesive polymers have received considerable attention in pharmaceutical field for more than 40 years. They are used in order to prolong the residence time and also improve the specific localization of drug delivery systems on various membranes [47].

Some of the polymeric structural characteristics necessary for mucoadhesive polymer can be summarized as follows: 1) Strong hydrogen bonding groups, e.g., carboxyl, hydroxyl, amino- and sulfate groups, 2) Strong anionic or cationic charges, 3) High molecular weight, 4) Chain flexibility, 5) Surface energy properties favoring spreading onto mucus and 6) Nontoxic, nonabsorbable and noninteracting with the drugs [48].

Typical polymers that have been used as mucoadhesive carriers include poly(acrylic acid), poly(methacrylic acid), chitosan, carboxymethylcellulose, cellulose ethers, sodium alginate, etc.

The most important requirement of a mucoadhesive is that it must be non-toxic with no undesirable physiological or pharmaceutical action, and should not be expensive. To obtain this purpose, mucoadhesive polymers in particular food grade are particularly attractive candidates [49].

2.2.3.1 Polyanionic polymer

Polymers with hydroxyl or carbonyl groups on their structure have been claimed as the most desirable candidates of mucoadhesive polymer. The synthetic polyacrylic acid derivatives known as Carbopol® have been gained to study in a wide range as mucoadhesive polymers. Carbopol exhibited good mucoadhesive properties has been largely attributed to the entanglement of the polymer chains with mucin as a results of swelling of the polymer in medium and hydrogen bonding possibly due to carboxyl groups being in their unionized state at low pH [50]. Other polyanionic polymers such as alginate, pectin, and carrageenan have yielded little or no mucoadhesive properties because both polymers and mucin are polyanionic.

2.2.3.2 Polycationic polymer

One of the important factors of mucoadhesion is electrostatic interaction. The positive charges of polymers become a significant influence to provide the molecular attraction forces to the anionic sites on the mucin. Chitosan obtained by deacetylation of chitin appears to be an ideal candidate as a mucoadhesive polycationic polymer which shows the excellent mucoadhesive properties because of its largely positive charges [51].

2.3 Hydrogel

Hydrogel is a three-dimension network of polymer chains that are water-insoluble and sometimes found as a colloidal gel in which water is the dispersion medium. Hydrogels are superabsorbent which could contain over 99% of water. Hydrogels possess also a degree of flexibility very similar to natural tissue, due to their significant water content.

2.3.1 Ionotropic and polyelectrolyte complex (PEC) hydrogel

Hydrogel formed with oppositely charged multivalent ion is known as an ionotropic hydrogel (Figure 2.4a) e.g. calcium alginate [19-21] and calcium pectinate gel [52]. In addition, when the oppositely charged hydrogels are mixed, they give a

product of such ion crosslinked systems which are known as complex coacervate or polyelectrolyte complex (PEC) hydrogel (Figure 2.4b). The mixtures of chitosan-alginate and chitosan-carrageenan are the samples of polyelectrolyte complexes [53].

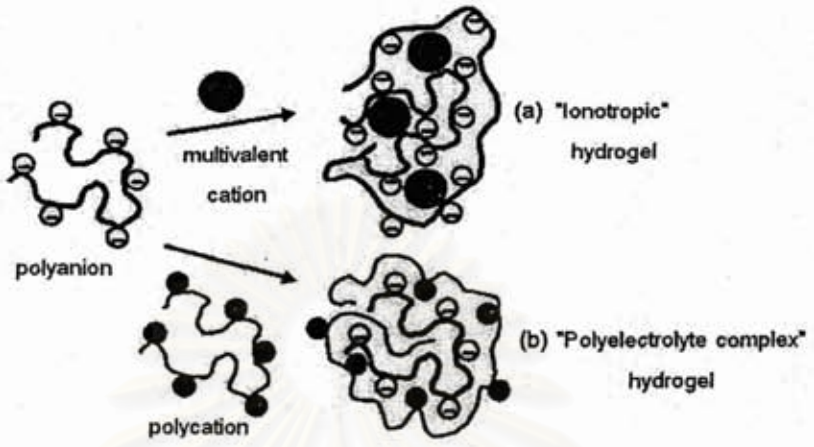


Figure 2.4 The ionotropic and polyelectrolyte complex (PEC) hydrogel [54].

2.4 Controlled drug release system

Controlled drug delivery occurs when a polymer, whether natural or synthetic, is judiciously combined with a drug or other active agent in such a way that the active agent is released from the material in a predesigned manner. The release of the active agent may be constant over a long period, it may be cyclic over a long period, or it may be triggered by the environment or other external events. In any case, the purpose behind controlling the drug delivery is to achieve more effective therapies while eliminating the potential for both under and overdosing (see Figure 2.5).

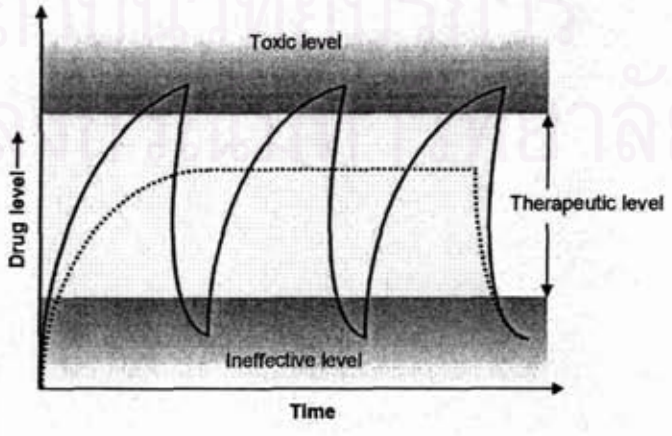


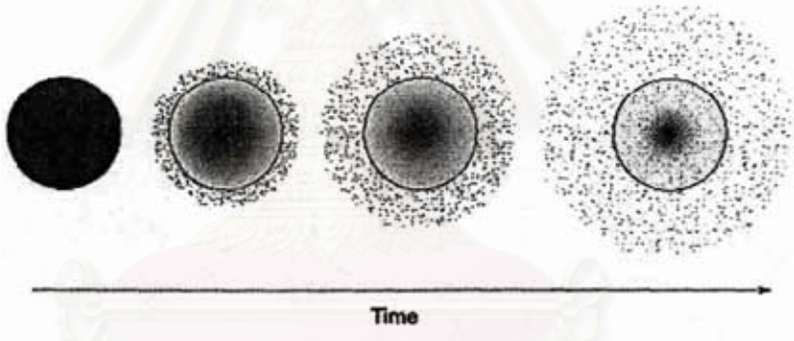
Figure 2.5 Drug level in the blood with (—) traditional drug dosing and (·····) controlled release dosing [55].

2.4.1 Controlled release mechanism [56]

There are three primary mechanisms by which active agents can be released from a delivery system: diffusion, swelling followed by diffusion, and degradation. Any or all of these mechanisms may occur in a given release system.

2.4.1.1 Diffusion-release systems

Diffusion occurs when a drug or other active agent passes through the polymer that forms the controlled-release device. The diffusion can occur on a macroscopic scale by passing through pores in the polymer matrix, or on a molecular level by passing between polymer chains. Examples of diffusion-release systems are shown in Figures 2.6.



Figures 2.6 Drug delivery from a typical matrix drug delivery system.

In Figure 2.6, a polymer and active agent have been mixed to form a homogeneous system, also referred to as a matrix system. Diffusion occurs when the drug passes from the polymer matrix into the external environment. As the release continues, its rate normally decreases with this type of system, since the active agent has a progressively longer distance to travel and therefore requires a longer diffusion time to release.

2.4.1.2 Swelling-release systems

The formation that follows the swelling-release systems is initial dry and when suspended in the body, it will absorb water or other body fluids, and swell. The swelling increases the aqueous solvent content within the formulation as well as the polymer mesh size, enabling the drug to diffuse through the swollen network into the external environment. Examples of these types of devices are shown in Figures 2.7a and 2.7b for reservoir and matrix systems, respectively. Most of the materials used in swelling-controlled release systems are based on hydrogels, which are polymers that will swell without dissolving when placed in water or other biological fluids.

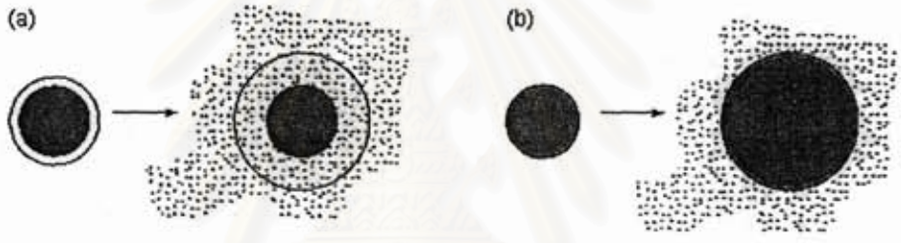


Figure 2.7 Drug delivery from (a) reservoir and (b) matrix swelling-controlled release systems.

One of the most remarkable and useful features of a polymer's swelling ability manifests itself when that swelling can be triggered by a change in the environment surrounding the delivery system. Depending upon the polymer, the environmental change can involve pH, temperature, or ionic strength, and the system can either shrink or swell upon a change in any of these environmental factors. A number of these environmentally sensitive or "intelligent" hydrogel materials are listed in Table 2.2.

For the formulation displaying the swelling-controlled release system, the drug release is performed only when the polymer swells. Because of the most useful pH-sensitive polymers swell at high pH values and collapse at low pH values, the triggered drug delivery occurs upon an increase in the pH of the environment.

This type of systems is an ideal for the oral delivery in which the drug is not released at low pH values in the stomach but rather at higher pH values in the upper small intestine.

Table 2.2 Environmentally sensitive polymers for drug delivery

Stimulus	Hydrogel	Mechanism
pH	Acidic or basic hydrogel	Change in pH → swelling → release of drug
Ionic strength	Ionic hydrogel	Change in ionic strength → change in concentration of ions inside gel → change in swelling → release of drug
Chemical species	Hydrogel containing electron-accepting groups	Electron-donating compounds → formation of charge/transfer complex → change in swelling → release of drug
Enzyme substrate	Hydrogel containing immobilized enzyme	Substrate present → enzymatic conversion → product change swelling of gel → release of drug
Magnetic	Magnetic particles dispersed in alginate microspheres	Applied magnetic field → change in pores in gel → change in swelling → release of drug
Thermal	Thermorespondsive hydrogel poly(N-isopropylacrylamide)	Change in temperature → change in polymer-polymer and water-polymer interactions → change in swelling → release of drug

Table 2.2 (continued) Environmentally sensitive polymers for drug delivery

Stimulus	Hydrogel	Mechanism
Electrical	Polyelectrolyte hydrogel	Applied electric field → membrane charging → electrophoresis of charged drug → change in swelling → release of drug
Ultrasound irradiation	Ethylene-vinyl alcohol hydrogel	Ultrasound irradiation → temperature increase → release of drug

2.4.1.3 Degradation-release systems

The release systems mentioned earlier are based on polymers that do not change their chemical structure beyond what occurs during swelling. However, a great deal of attention and research effort is being concentrated on biodegradable polymers. These materials degrade within the body as a result of natural biological processes, eliminating the need to remove a drug delivery system after release of the active agent has been completed.

Most biodegradable polymers are designed to degrade as a result of hydrolysis of the polymer chains into biologically acceptable and progressively smaller compounds. Degradation may take place through bulk hydrolysis, in which the polymer degrades in a fairly uniform manner throughout the matrix, as shown schematically in Figure 2.8a. For some degradable polymers, the degradation occurs only at the surface of the polymer, resulting in a release rate that is proportional to the surface area of the drug delivery system (see Figure 2.8b).

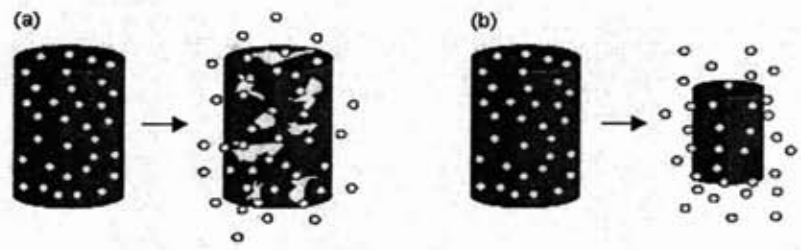


Figure 2.8 Drug delivery from (a) bulk-eroding and (b) surface-eroding biodegradable systems.

2.5 General characteristics of polymers used in controlled release dosage forms (CRDFs)

Polymers are generally used as the structural backbone for controlled drug release system. The characteristics of polymers selected in the preparation of the dosage form must comply with the following requirement [55].

(a) Biocompatibility: Harmful and toxic impurities must be eliminated from polymer before their inclusion in CRDFs. There must be minimal tissue response after injection or implantation into the body.

(b) Physical and mechanical properties: Properties of polymer should be the same as natural tissues or required for the dosage from design such as: elasticity, resistance to tensile, swelling, etc.

(c) Biodegradability: The polymer should be degraded by the body or in nature by enzymes at a well defined rate to non-toxic and rapidly excreted degradation product.

(d) Pharmacokinetic properties: Chemical degradation of the polymer matrix must be non-toxic, non-immunogenic and non-carcinogenic.

(e) Cost-effectiveness: The polymer should be low-cost or abundantly available, which could easily be adapted to the standard manufacture procedures.

2.6 Alginate

Alginate is the term used for the salts of alginic acid and its derivatives. Alginate is presented in the cell walls of brown algae as the calcium, magnesium and sodium salts of alginic acid. Alginate was originally thought to consist of a uniform polymer of mannuronic acid as shown in Figure 2.9. However later studies showed the presence of guluronic acid residues (Figure 2.10) and it is now understood that alginate is linear copolymer of β–D-Mannuronic acid and α–L-Guluronic acid (Figure 2.11).

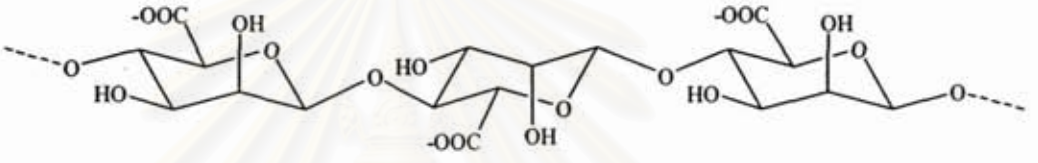


Figure 2.9 Polymannuronic acid.

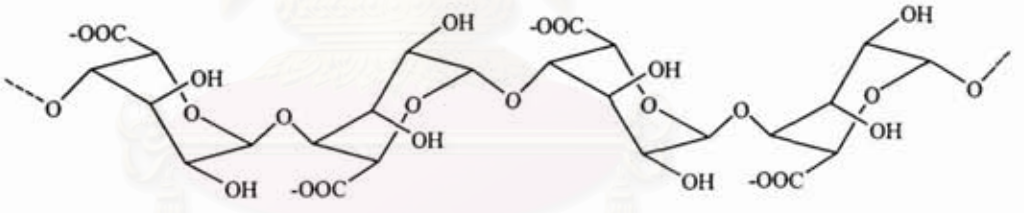


Figure 2.10 Polyguluronic acid.

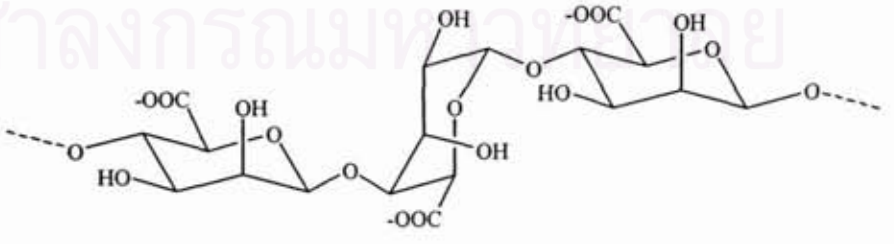


Figure 2.11 Copolymer of β–D-Mannuronic acid and α–L-Guluronic acid.

Depending on the weed source and growing conditions the ratio of mannuronic and guluronic acid can vary. It is also known that the block structure within the alginate can vary significantly. The polyguluronic acid blocks bind significantly more effectively with calcium ions than the polymannuronic acid blocks, thus the weed types with the higher guluronic acid levels normally show the stronger interaction with calcium and hence the strongest gel strength.

2.6.1 Alginate uses

The uses of alginates are based on three main properties. The first is their ability to increase the viscosity of aqueous solutions. The second property is to form gels when a calcium salt is added to a solution of sodium alginate in water. The gel is formed by chemical reaction with no heat required, the calcium displaces the sodium from the alginate, holds the long alginate molecules together and resulting in a gel. The third property of alginates is the ability to form films of sodium or calcium alginate and fibers of calcium alginates. Upon these properties, alginates are widely used in various applications such as textile printing, food and pharmaceutical uses [57].

1) Textile printing

In textile printing, alginates are used as thickeners for the paste containing the dyes. These pastes may be applied to the fabric by either screen or roller printing equipment. Alginates became important thickeners which do not react with the reactive dyes, whereas the usual thickeners, such as starch, react with the reactive dyes leads to lower color yields and sometimes by-products that difficult to wash out. Textile printing accounts for about 50 percent of the global alginate market.

2) Food

The thickening property of alginate is useful in food products such as sauces, syrups and toppings for ice-cream. In addition, alginate can be used as emulsifier in water-in-oil emulsions such as mayonnaise and salad dressings by preventing the separation into their original oil and water phase.

Alginates have some applications that are not related to either their viscosity or gel properties. They act as stabilizers in ice cream by reducing the formation of ice crystals during freezing, giving a smooth product. A variety of agents are used in the clarification of wine and removal of unwanted coloring, in some cases it has been found that the addition of sodium alginate can be effective.

The gelling properties of alginate were used in the production of artificial cherries since 1946. A flavored and colored solution of sodium alginate was allowed to drop into a solution of calcium salts. Moreover, calcium alginate films and coatings have been used to help to preserve frozen fish. If the fish is frozen in a calcium alginate jelly, the fish is protected from the air and rancidity from oxidation is very limited.

3) Pharmaceutical and medical uses

The fibers of calcium alginate are used in wound dressings. They have very good wound healing and haemostatic properties and can be absorbed by body fluids because the calcium in the fiber is exchanged for sodium from the body fluid to give a soluble sodium alginate. This also makes it easy to remove these dressings from the large open wounds or burns since they do not adhere to the wound. In addition, removal also can be assisted by rinsing the dressing with saline solution to ensure its conversion to soluble sodium alginate.

The good swelling properties of alginic acid powder led to its use as a tablet disintegrant for some specialized applications. Alginic acid has also been used in some dietary foods; it swells in the stomach and gives a full feeling if sufficient amount is taken so the person is dissuaded from further eating.

Alginate is widely used in the controlled release of drugs and other chemicals. In some applications, the active ingredient is placed in a calcium alginate bead and slowly released when the bead is exposed in the appropriate environment.

2.7 Gelatin

Gelatin (also gelatine) is a protein product derived through partial hydrolysis of the collagen extracted from connective tissue of animals such as porcine and bovine. It is a transparent brittle solid substance, colorless or slightly yellow, nearly tasteless and odorless.

Gelatin contains a large number of glycine (almost 1 in 3 residues, arranged in every third residue), proline and 4-hydroxyproline residues. A typical structure is -Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-Pro-.

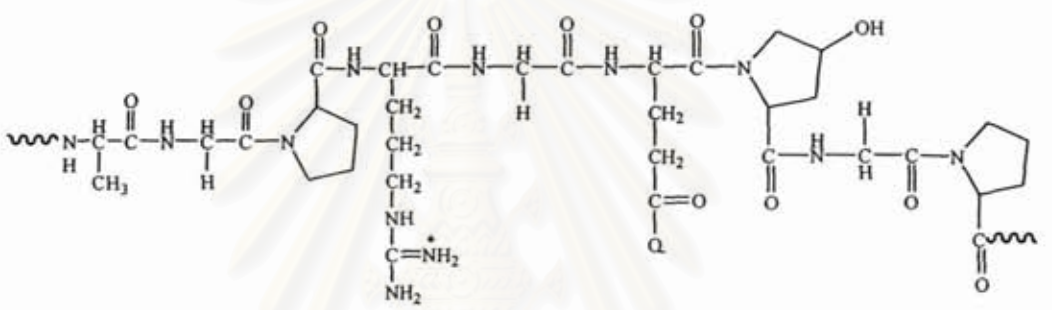


Figure 2.12 Gelatin.

There are two main types of gelatin. Type A gelatin (or porcine gelatin), with isoelectric point of 7 to 9, is derived from collagen with acid pretreatment. Type B gelatin (or bovine gelatin), with isoelectric point of 4.8 to 5.2, is the result of an alkaline pretreatment of the collagen.

2.7.1 Gelatin uses [58]

1) Food

Although gelatin is 98–99% protein by dry weight, it has less nutritional value than many other protein sources. Gelatin is unusually high in the non-essential amino acids glycine and proline.

Common examples of foods that contain gelatin are gelatin desserts or jelly, trifles, aspic, marshmallows. Gelatin may be used as a stabilizer, thickener, or texturizer in foods such as ice cream, jams, yogurt, cream cheese, margarine; it is used, as well, in fat-reduced foods to simulate the mouth feel of fat and to create volume without adding calories.

Gelatin is used for the clarification of juices, such as apple juice, and of vinegar. Isinglass, one of the oldest sources of gelatin from the swim bladders of fish, is still in use as a fining agent for wine and beer.

2) Pharmaceutical and medical uses

Gelatin is typically used as the shells of both hard and soft pharmaceutical capsules in order to make their contents easier to swallow. Various forms of gelatin are also used as common excipients in pharmaceutical formulations, including vaccines and a binder for tablets.

In some literatures, gelatin was suggested to crosslink with alginate for improving the release in the case of soluble drug [24].

3) Other uses

- Gelatin was used as animal glues.
- It is used to hold silver halide crystals in an emulsion in virtually all photographic films and photographic papers. Despite some efforts, no suitable substitutes with the stability and low cost of gelatin have been found.
- Used as a carrier, coating or separating agent for other substances, it, for example, makes beta-carotene water-soluble, thus imparting a yellow color to any soft drinks containing beta-carotene.
- Cosmetics may contain a non-gelling variant of gelatin under the name "hydrolyzed collagen".
- It is commonly used as a biological substrate to culture adherent cells.

2.8 Chitosan

Chitosan is a derivative of chitin which discovered by Rouget in 1859 and was formally named by Hoppe-Seyler in 1894 [59]. It is produced commercially by deacetylation of chitin, which present in outer structure in marine crustaceans such as crabs and shrimp.

Chitosan is a liner polysaccharide composed of randomly distributed β-[1-4]-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). The degree of deacetylation (%DA) has a significant effect on the solubility and rheological properties of polymer. Generally, the %DA in commercial chitosans is in the range 60-100%.

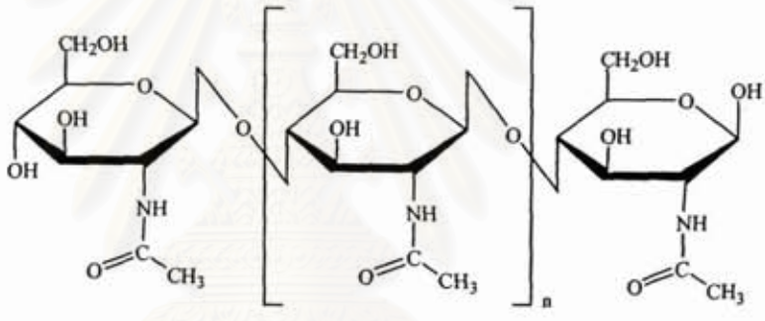


Figure 2.13 Chitin.

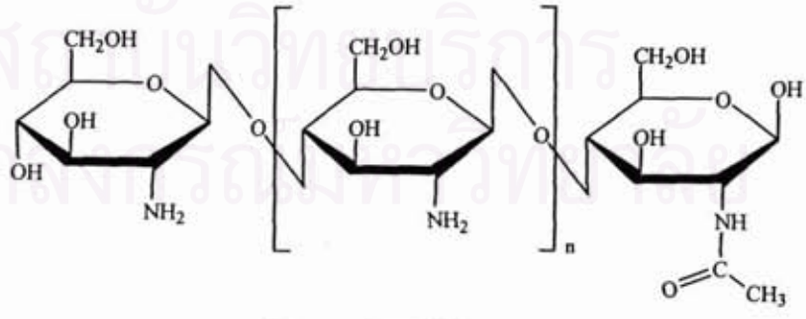


Figure 2.14 Chitosan.

Chitosan is soluble in dilute acidic solution and gives positively charged with a charge density depending on pH and %DA value.

2.8.1 Chitosan uses [60]

1) Cosmetic

Chitosan is a particularly effective hydrating agent which is able to supply water and avoid dehydration. Chitosan forms a protective tensor film on the skin's surface that can fix and allow the active principles to be placed in close contact with the skin. This is a new double advantage that makes chitosan of great interest in cosmetics. Therefore, chitosan are now widely used in skin creams, shampoos, etc.

2) Agriculture

Chitosan and its derivatives have plant protecting and antifungal properties. In very low concentration of chitosan, they can stimulate defensive mechanisms in plants against infections and parasite attacks. They can also be used as coatings of seeds which obtained to increase crop yield more than 20% in comparison with uncoated seeds.

3) Water treatment

Chitosan has been gaining interest for industry and nature conservation. They are remarkable as chelating agents and heavy metal traps. The Environmental Protection Agency (EPA) has already approved the use of chitosan in water at concentrations of up to 10 mg per litre. For sewage treatment, chitosan can be used at up to 5 ppm. It reduces the oxygen demand by 80 to 85% and reduces the phosphates level to less than 5 ppm.

4) Pharmaceutical and medical uses

Due to its biocompatibility with human body tissue, chitosan have demonstrated their effectiveness for all forms of dressings - artificial skin, corneal bandages and suture thread in surgery - as well as for implants or gum cicatrization in bone repair or dental surgery.

Lastly, chitosan is an excellent medium for carrying and slow release of medicinal active principles in plants, animals and man. The non-antigenic behavior of chitosan promises unlimited development in the health field.

2.9 Amoxicillin

Amoxicillin (α-amino-hydroxybenzylpenicillin) is an antibiotic that belongs to a class of antibiotics called penicillins. It is effective against many different bacteria by preventing them from forming a cell wall. Amoxicillin has been shown to be active against most strains of microorganisms including *H. pylori*, *H. influenzae*, *N. gonorrhoea*, *E. coli*, *Pneumococci*, *Streptococci*, and certain strains of *Staphylococci*.

2.9.1 Physicochemical properties [61]

Amoxicillin appears as a white, partially odorless, crystalline powder and is sparingly soluble in water.

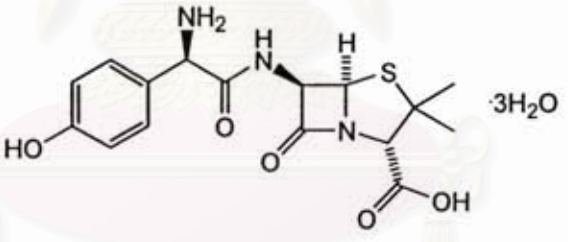


Figure 2.15 Chemical structure of amoxicillin trihydrate.

IUPAC name : 7-[2-amino-2-(4-hydroxyphenyl) -acetyl]amino-3,3-dimethyl-6-ox -2-thia-5-azabicyclo[3.2.0]heptane -4-carboxylic acid trihydrate

Empirical formula : $C_{16}H_{19}N_3O_5S.3H_2O$

Molecular weight : 419.46

CAS No. : 61336-70-7

2.9.2 Pharmacokinetics/Dynamic [62, 63]

Absorption : Rapid and nearly complete; food does not interfere.

Distribution : Diffuses into most body tissues and fluids; penetration in CNS is poor unless meninges are inflamed.

Metabolism : Partially hepatic (less than 30% biotransformed in liver)

Excretion : T½ is 61.3 minutes; approximately 60% excreted in the urine within 6 to 8 hours as unchanged drug.

2.9.3 Uses and indications [62, 63]

Treatment of ear, nose, throat, lower respiratory tract, gastric ulcer, skin and skin structure, and acute uncomplicated gonorrhea infections caused by susceptible strains of specific organisms.

2.9.4 Adverse reactions [62]

Central nervous system (CNS): Hyperactivity, anxiety, insomnia, confusion, convulsions, behavior changes, dizziness

Dermatologic : Acute exanthematous pustulosis, erythematous maculopapular rashes, erythema multiforme, Stevens-Johnson syndrome, exfoliative dermatitis, toxic epidermal necrolysis, hypersensitivity vasculitis, urticaria

Gastrointestinal: Nausea, vomiting, diarrhea, hemorrhagic colitis, pseudomembranous colitis, tooth discoloration (brown, yellow, or gray; rare)

Hematologic : Anemia, hemolytic anemia, thrombocytopenia, thrombocytopenia purpura, eosinophilia, leucopenia, agranulocytosis

Hepatic : Elevates AST (SGOT) and ALT (SGPT), cholestatic jaundice, hepaticcholestasis, acute cytolytic hepatitis

2.9.5 Overdosage/Toxicity [62]

Maximum dosing range of amoxicillin for adult is 2-3 grams/day. The overdosage of amoxicillin may cause symptoms similar to penicillin's, including neuromuscular hypersensitivity (agitation, hallucinations, asterixis, encephalopathy, confusion, and seizures) and electrolyte imbalance (with potassium or sodium salts), especially in renal failure. Hemodialysis may be helpful to aid in the removal of the drug from blood, otherwise most treatment is supportive or symptom-directed.

CHAPTER III

EXPERIMENT

3.1 Materials

The following materials were obtained from commercial suppliers.

3.1.1 Model drug

- Amoxicillin trihydrate (Chumchon Pharmaceutical, Thailand), using without further purification.

3.1.2 Polymers

- Alginic acid sodium salt from brown algae, Lot No. 1077205 (Fluka, UK)
- Gelatin from porcine skin, Lot. No. 424331/1, 180 g Bloom (FLuka, UK)
- Chitosan, food grade, Lot No. 496212, M.W. 50,000 – 300,000, Deacetylation 90% (Bonafides, Thailand)

3.1.3 Chemicals

- Acetic acid glacial 100%, AR grade (Scharlau, Spain)
- Acetonitrile, HPLC grade (Merck, Germany)
- Calcium chloride, AR grade (Merck, Germany)
- Methanol, HPLC grade (Merck, Germany)
- Hydrochloric acid fuming 37%, AR grade (Merck, Germany)
- Sodium hydroxide, AR grade (Merck, Germany)
- Sodium hydrogen phosphate, AR grade (Merck, Germany)
- Potassium chloride, AR grade (Merck, Germany)
- Potassium dihydrogen phosphate, AR grade (Merck, Germany)
- Potassium bromide, AR grade (Merck, Germany)

3.2 Instruments

The instruments used in this study are listed in Table 3.1.

Table 3.1 Instruments

Instrument	Manufacture	Model
Analytical balance	Mettler	AT 200
HPLC	ThermoFinnigan	P4000
UV-VIS Spectrophotometer	Milton Roy	Spectronic 601
	Cary 50	1.00
Fourier transform infrared spectrometer	Nicolet	Impact 410
Microscope	Olympus	CH-30
Scanning electron microscope	Jeol	JSM-5800 LV
Digital camera	Olympus	C-4040
	Canon	A 80
Horizontal shaking water-bath	Lab-line instruments	3575-1
pH-meter	Metrohm	744
Centrifuge	Sanyo	Centaur 2
Ultrasonic bath	Ney Ultrasonik	28 H
Thermogravimetric analyzer	NETZSCH	409 C/CD
Differential scanning calorimeter	NETZSCH	DSC 7
Freeze dryer	Labconco	Freeze 6
Micropipette (100-1000 µl)	Mettler Toledo	Volumate

3.3 Methods

3.3.1 Preparation of the amoxicillin-loaded alginate beads

The beads were prepared in various formulations for study of the effects on the properties of beads (e.g. encapsulation efficiency, floating properties, mucoadhesive ability, etc.). The procedures of the bead preparation were prepared as detailed by follows:

3.3.1.1 Preliminary study

In the preliminary study, the beads were prepared separately into two methods; suspension method and solution method.

For the suspension method, various types of beads were prepared and named after their components: alginate, gelatin and chitosan. The four formulations of beads were mainly studied; alginate beads (ALG), alginate mixed with gelatin beads (ALG-GEL), alginate coated with 1% (w/v) chitosan beads (ALG/1%CHI) and alginate-gelatin coated with 1% (w/v) chitosan beads (ALG-GEL/1%CHI).

ALG, amoxicillin trihydrate was directly suspended in 2% (w/v) sodium alginate at the alginate/amoxicillin ratio (w/w) of 1/1. The mixture was then extruded through 18G needle into stirred 2% (w/v) calcium chloride solution. The gel beads formed were left for 30 minutes in the solution prior to filtration and freeze drying.

ALG-GEL, gelatin was added to 2% (w/v) sodium alginate solution at the alginate/gelatin ratio (w/w) of 1/1. The mixture was left for 2 hours until the solution became clear. Amoxicillin trihydrate was then suspended in the solution with the alginate/gelatin/amoxicillin ratio (w/w) of 1/1/1. The mixtures were extruded through 18G needle into stirred 2% (w/v) calcium chloride solution. The gel beads formed were left for 30 minutes in the solution prior to filtration and freeze drying.

ALG/1%CHI, the mixture of alginate/amoxicillin was prepared as the same procedure of ALG beads, but different in the bead forming step. The coagulation fluid was produced by mixing in an equal volume of 2% (w/v) chitosan dissolved in 1% (v/v) acetic acid and 4% (w/v) calcium chloride solution. The solutions were mixed for 2 hours before use. The mixture of alginate/amoxicillin was then extruded through 18G needle into stirred coagulation fluid. The gel beads formed were left for 30 minutes in the coagulant prior to filtration and freeze drying.

ALG-GEL/1%CHI, the mixture of this formulation was prepared as the same procedure of ALG-GEL beads and the bead forming step was prepared as the same procedure of ALG/1%CHI beads.

For the solution method, each formulation was mainly prepared in the same way as the suspension method. However, amoxicillin trihydrate was dissolved in distilled water prior to mixing in the hydrogel.

3.3.1.2 Preparation of the amoxicillin-loaded alginate beads coated with various concentrations of chitosan

From the preliminary study, the beads prepared by the solution method and coated with chitosan solution showed the best encapsulation efficiency. Therefore, those formulations were chosen for the further study of the concentration of chitosan solution that might affect to the beads properties. The ALG/1%CHI and ALG-GEL/1%CHI beads were chosen to vary the concentration of chitosan for additional two concentrations; 0.5% (w/v) and 0.25% (w/v).

3.3.1.3 Preparation of the amoxicillin-loaded alginate beads with various drug contents

To summarize from all the studies, ALG/1%CHI beads showed the best results. Therefore, this formulation was then chosen for further study by varying the ratios (w/w) of drug contents. The beads were prepared as the same method as described previously with the exception of the amount of amoxicillin in various alginate/chitosan/amoxicillin ratios (w/w) of 1/1/1, 1/1/2, 1/1/3, 1/1/4 and 1/1/5.

3.3.2 Encapsulation efficiency

Accurately weighed quantities of 100 mg beads were ground to powder and then transferred into a 100 ml volumetric flask with distilled water to a volume of 90 ml. The suspension was sonicated in the ultrasonic bath for 60 minutes. Then, distilled water was added to adjust the volume to 100 ml. The suspension was filtered through 0.45 μm nylon membrane filter, 20 μl of filtrate was withdrawn and determined the encapsulation efficiency by HPLC. The conditions for HPLC assay were as follows: HPLC apparatus; ThermoFinnigan P4000 (pump), UV6000LP (UV detector); column: Pinnacle II C18 5 μm 200×4.6 mm; mobile phase: phosphate buffer (0.01 M, pH 6.0): acetonitrile (90:10); flow rate: 1 ml/min.

The encapsulation efficiency was calculated according to the following equation. All experiments were performed in triplicates.

$$EE(\%) = \frac{Accual\ drug\ content}{Theoritical\ drug\ content} \times 100\%$$

3.3.3 Morphological characterization of the beads

3.3.3.1 Scanning electron microscope (SEM)

The surfaces and inner part of the beads were observed via scanning electron microscope (JSM-5800 LV, JEOL, Japan). In the preparation of SEM examination, the samples were mounted on metal grids and coated by gold under vacuum before observation. The photographs were taken at different magnifications.

3.3.3.2 Fourier transform infrared spectroscopy (FT-IR)

The infrared spectra of all formulations were recorded with FT-IR (Impact 410, Nicolet). The dried sample was mixed with potassium bromide in agate mortar and pestle. The mixture was then transferred to a hydraulic pressing machine and pressed into a thin disc. The KBr disc was then measured within the wave numbers of 4000-400 cm^{-1}.

3.3.4 Floating properties

The floating properties were evaluated in a flask filled with 250 ml of simulated gastric fluid (SGF) without pepsin (0.1 N HCl pH 1.2). One hundred beads of each formulation were placed in the flask and then were shaken in a horizontal shaking water bath; shaking rate was 50 rounds per minute and incubated at 37±1°C. The percentage of floating samples was measured by visual observation at appropriate time intervals. All experiments were performed in triplicates.

3.3.5 Swelling properties

The swelling properties of the beads were determined at appropriate time intervals in two dissolution systems; SGF and SIF (simulated intestinal fluid of 0.1 M phosphate buffer pH 7.4).

The swelling behaviors were observed by measuring the change of diameters of the beads using a micrometer scale with standard light microscope. The percentage of swelling of the beads was calculated from the following formula [27].

$$S_w(\%) = \frac{D_t - D_0}{D_0}$$

Where D_t is the diameter of the beads at time *(t)* and D_o is the initial diameter of the dried beads.

3.3.6 In vitro evaluation of the mucoadhesiveness of the beads

The beads were tested for mucoadhesiveness according to the rinsing method designed by Ranga and Buri [28]. Briefly, the pig's stomachs were cut into pieces 10×15 cm and rinsed with 50 ml of physiological saline. One hundred beads of each formulation were scattered uniformly on the surface of the gastric mucosa. Then, the stomach with the beads was placed in a chamber maintained at 37°C and 93% relative humidity. After 20 minutes, the mucosa was taken out and fixed on a polyacrylic support at an angle of 45°. The stomach was then rinsed with 0.1N HCl pH 1.2 for 5

minutes at a rate of 300 ml/min. The beads remaining on the surface of gastric mucosa was counted, and the percentage of the remaining beads was calculated.

3.3.7 In vitro drug release

3.3.7.1 Amoxicillin release behavior in SGF

Accurately weighed quantities of 100 mg beads were suspended in 250 ml of 0.1 N HCl pH 1.2 in a conical flask. The flask was then placed in a shaken water bath at a speed of 50 strokes/minute with the temperature maintained at 37±1°C. At appropriate intervals, 3 ml of samples were collected and neutralized with 1 ml of 0.3 M NaOH to prevent the further degradation reaction. The samples were then filtered through a 0.45 µm nylon membrane filter and determined by a HPLC assay as the same conditions described in section 3.3.2. Drug release tests were performed in triplicate for each formulation.

3.3.7.1 Amoxicillin release behavior in SIF

Accurately weighed quantities of 100 mg beads were placed into conical flask with 250 ml of 0.1 M phosphate buffer saline (pH 7.4) and incubated at 37±1°C under shaking speed of 50 strokes/minute. At appropriate intervals, 3 ml of samples were collected. The samples were then filtered through a 0.45 µm nylon membrane filter and determined by a HPLC assay as the same conditions described in section 3.3.2. For each formulation, the samples were analyzed in triplicates.

3.3.8 Data analysis

All experimental data were analyzed statistically by using SPSS software for Windows, Version 13.0 (SPSS Inc. Chicago, Illinois). The Student's *t*-test was performed for two samples comparison. The analysis of variance (ANOVA) with the two-sides Dunnett's post-test analysis was suggested for multiple comparisons. A *P* value of ≤0.05 was considered to indicate statistical significance.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Preliminary study

In preliminary study, the beads were prepared into two different methods, suspension and solution method, to evaluate the effect of preparation method on the properties of encapsulation efficiency of beads.

The influence of the method of preparation on encapsulation efficiency (%EE) is given in Table 4.1 (P1-P8). The results showed that all formulations of beads prepared by the solution method exhibited higher encapsulation efficiencies comparing to the suspension method.

One factor of significant differences in the encapsulation efficiency between two preparation methods is mainly due to the homogeneity of amoxicillin and polymer matrix. In solution method, amoxicillin was dissolved in distilled water prior to mixing with the polymer matrix, showing more finely homogenous system and thus the beads having a higher %EE. The SEM micrographs could be used to confirm this phenomenon and the thorough results would be elaborated in the beads morphology.

Immersion time is another factor that may affect the percentage of encapsulation efficiency. In this study, to prevent the drug loss from diffusion to the surrounding medium, curing period in the coagulant of all formulations were limited to 30 minutes which is acceptable to complete the reaction between alginate and the calcium ions [64].

In this preliminary study, it could be summarized that the solution method is more preferable for the bead preparation because it gave the higher encapsulation efficiencies than the suspension method. Therefore, the solution method was chosen for the further study to evaluate the other variables that might effect on the properties of beads.

4.2 Encapsulation efficiency

Encapsulation efficiency is revealed as the percentage of total amount of amoxicillin in the dope that actually becomes entrapped in the beads.

Alginate beads in the absence of gelatin and chitosan (Table 4.1, P1 and P5) showed the low encapsulation efficiency (<80%). This may be due to insufficient cross-linking and large pore size permitting the amoxicillin to diffuse out during and after gelation.

The formulation incorporated with gelatin (Table 4.1, P2 and P8) showed insignificantly higher encapsulation efficiency (*t*-test, P>0.05). These results could be assumed that gelatin possibly acts as cross-linking agent. The isoelectric point of the gelatin used in this study had a value of 7, whereas at pH 6 of alginate solution it was slightly positively charged. Mixing gelatin in alginate solution resulted in the formation of a polyionic complex, thus, the drug was favorably entrapped into the network of alginate-gelatin.

High encapsulation efficiencies (~90%) were achieved for the beads coated with 1% (w/v) chitosan (Table 4.1, P7 and P8). This is probably due to the firmness of the alginate-chitosan complex during gelation caused by ionic interactions between carboxylate groups in the alginate and the protonated amine groups in the chitosan.

The variation in the concentrations of chitosan used as the bead coating (0.25-1.0% (w/v)) had a significant effect on the encapsulation efficiency (ANOVA, P<0.05). In the presence of higher concentration of chitosan, higher encapsulation efficiency was obtained. The results showed in Table 4.1 (Formulation P7, A, B and P8, C, D).

Moreover, the variation in drug contents (Table 4.1, Formulation B and E to H) had no significant effect on the encapsulation efficiency of beads (ANOVA, P>0.05). Furthermore, the high encapsulation efficiencies of all formulations were achieved (>86%). This is possibly due to a fairly homogeneous system of amoxicillin and polymer matrix being well maintained for all formulations.

Table 4.1 The compositions and encapsulation efficiencies of beads prepared by suspension and solution method

Formulation	Ratio of composition			Coagulation fluid (%w/v)		Encapsulation efficiency (%)	
	Alginate	Gelatin	Amoxicillin	Chitosan	$CaCl_2$	Suspension method	Solution method
P1	1	-	1	-	2.0	60.88±0.86	-
P2	1	1	1	-	2.0	74.59±2.74	-
P3	1	-	1	1.0	2.0	58.53±2.46	-
P4	1	1	1	1.0	2.0	73.62±2.75	-
P5	1	-	1	-	2.0	-	77.62±2.75
P6	1	1	1	-	2.0	-	80.57±0.93
P7	1	-	1	1.0	2.0	-	89.24±0.54
P8	1	1	1	1.0	2.0	-	92.86±1.50

Table 4.1 (continued) The compositions and encapsulation efficiencies of beads prepared by suspension and solution method

Formulation	Ratio of composition			Coagulation fluid (%w/v)		Encapsulation efficiency (%)	
	Alginate	Gelatin	Amoxicillin	Chitosan	$CaCl_2$	Suspension method	Solution method
A	1	-	1	0.25	2.0	-	79.28±1.24
B	1	-	1	0.5	2.0	-	86.12±0.99
C	1	1	1	0.25	2.0	-	81.30±0.60
D	1	1	1	0.5	2.0	-	88.54±2.69
E	1	-	2	0.5	2.0	-	87.34±1.62
F	1	-	3	0.5	2.0	-	89.96±0.87
G	1	-	4	0.5	2.0	-	88.58±0.28
H	1	-	5	0.5	2.0	-	90.39±0.90

4.3 Morphological characterization of the beads

4.3.1 Morphology of the beads

The size, shape and surface topography of the freeze-dried beads were observed by microscope and scanning electron microscopy (SEM).

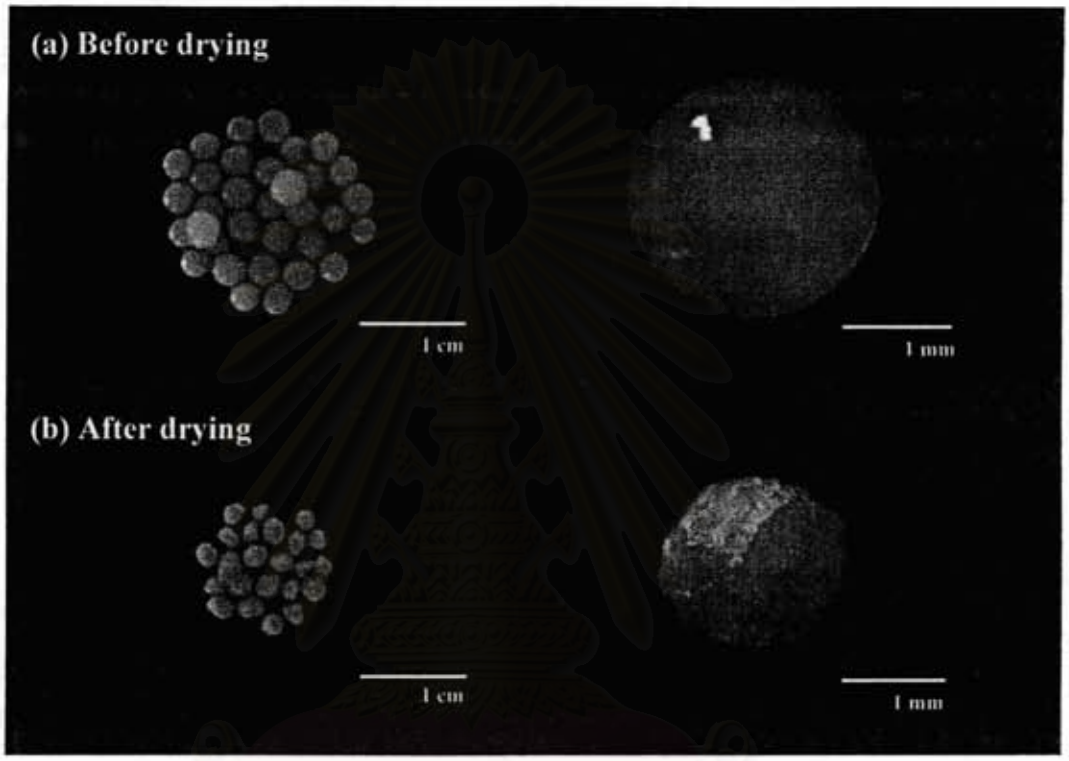


Figure 4.1 Macroscopic and microscopic aspect of amoxicillin-containing alginate beads: (a) before drying and (b) after drying.

Figure 4.1 showed the macroscopic aspect of amoxicillin-containing alginate beads (ALG) before and after drying. As expected, after freeze-drying process, the beads became smaller and partially shrunk as a result of the frozen water in the particle was sublimed directly from the solid phase to gas.

The SEM micrographs of the beads are exhibited in Figures 4.2-4.6. The effect of the compositions on the morphology of beads was summarized in Table 4.2. The results showed that the particle size of beads was distributed with a diameter ranging from 2 to 3 millimeters depending on their compositions (Table 4.2). The size of beads without gelatin was smaller when adding with gelatin. Likewise, the beads incorporated with a higher content of amoxicillin showed an increase in particle size. These size increments are mainly affected the viscosity of a mixture of the hydrogel. The higher viscosity of a mixture of hydrogels after adding gelatin or increasing drug content cause an increase in the beads size.

In addition, the inclusion of gelatin in the matrix created beads with a spherical shape, smooth surface and larger size (Figures 4.2 and 4.3). On dehydration during the drying process, the conformational changes of gelatin are taken place to be irreversible that may be utilized to preserve the structure of the formation [65].

In preliminary study (Figure 4.2, P1-P8), the shape of all formulations except P4 and P8 appeared as spherical. The loss of spherical shape of P4 and P8 is due to so high viscosity of a chitosan. It was observed that, with 1% (w/v) of chitosan, the viscosity of coagulation fluid became so high that the formation of drops was strongly impaired. Thus, the dropping process had to be improved by acceleration a stirring rate of coagulant, and this centrifugal force resulted in the oval shape.

Furthermore, the SEM micrographs shown in Figure 4.3 could be used to confirm the homogenicity of amoxicillin and polymer matrix that is the one factor effecting on the encapsulation efficiency in two different preparation methods. The crystals of amoxicillin could be seen apparently on the surface of beads prepared from suspension method, whereas the beads prepared from solution method appeared the smoother surfaces and the crystals of amoxicillin were not occurred.

Figures 4.4 and 4.5 demonstrated the SEM micrographs of the cross-section view of the beads. At the inner of the beads, a parallel alignment of honeycomb-like open cavities and many hollow zones with various pore diameters were identically formed in all formulations. These similar structures are probably due to the effect of gelling process. The gelling formation rapidly occurs on the surface of

bead on contact with the calcium ions, and the gelling is gently completed over the migration of calcium ions from the surface through the inner core. In addition, for the beads with higher amoxicillin content, the cavities inside the beads were denser and tightly packed. This was due to the higher proportion of amoxicillin comparing to the polymer mass, thus the beads being permeated with amoxicillin.

Furthermore, the crystals of amoxicillin could be apparently seen inside the beads for the formulations prepared from suspension method (Figure 4.5, formulation P1 to P4) and the formulations with increase of the drug content (Figure 4.5, formulation E to H). This might be indicated that the mixture of the polymer matrix and drug become to be not finely homogeneous mixtures, and some portions of suspended amoxicillin probably distribute over the surface of beads. In the other formulations, the amoxicillin appeared as amorphous, which indicated that those formulations exhibited a homogenous system.

Figure 4.6 gives the SEM micrographs of the rim of the beads coating with various concentration of chitosan used as coagulation. The beads with and without gelatin showed a similar skin morphology, whereas the chitosan-coated beads presented a thicker and denser skin. In the presence of a higher concentration of chitosan, a denser skin was obtained. This might be due to a membrane being formed properly with the greater number of alginate-chitosan linkages. The thickness of the skin of beads was found to be thicker with the increase of concentration of chitosan which was also suggested in the earlier reports [66-67].

Table 4.2 Effect of compositions on morphology of the beads

Formulation	Abbreviations	Shape	Bead size ± S.D. (mm)
P1	ALG-sus	Spherical	2.25 ± 0.10
P2	ALG-GEL-sus	Spherical	2.73 ± 0.16
P3	ALG/1%CHI-sus	Spherical	2.37 ± 0.14
P4	ALG-GEL/1%CHI-sus	oval	$x^{\dagger}$ = 2.34 ± 0.15
			$y^{\dagger}$ = 3.93 ± 0. 23
P5	ALG	Spherical	2.29 ± 0.15
P6	ALG-GEL	Spherical	2.80 ± 0.17
P7	ALG/1%CHI	Spherical	2.35 ± 0.13
P8	ALG-GEL/1%CHI	oval	$x^{\dagger}$ = 2.33 ± 0.15
			$y^{\dagger}$ = 3.95 ± 0.28
A	ALG/0.25%CHI	Spherical	2.45 ± 0.16
B	ALG/0.5%CHI	Spherical	2.49 ± 0.17
C	ALG-GEL/0.25%CHI	Spherical	2.83 ± 0.17
D	ALG-GEL/0.5%CHI	Spherical	2.70 ± 0.21
E	ALG/0.5%CHI (AMOX2X)	Spherical	2.84 ± 0.25
F	ALG/0.5%CHI (AMOX3X)	Spherical	2.86 ± 0.29
G	ALG/0.5%CHI (AMOX4X)	Spherical	2.93 ± 0.45
H	ALG/0.5%CHI (AMOX5X)	Spherical	2.96 ± 0.56

† Beads were determined on two axes lying orthogonally to one another.

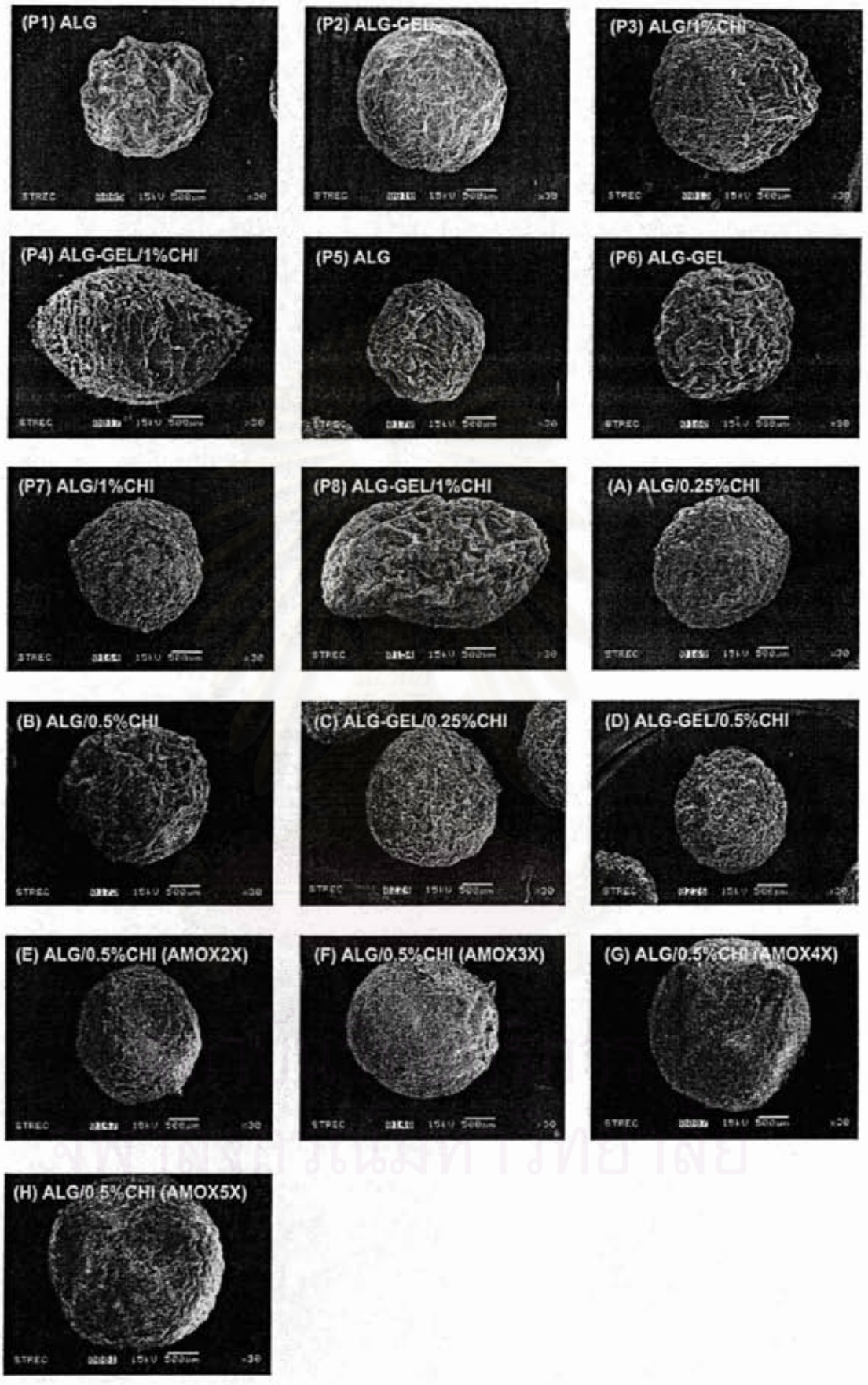


Figure 4.2 Scanning electron micrographs of the beads (30X).

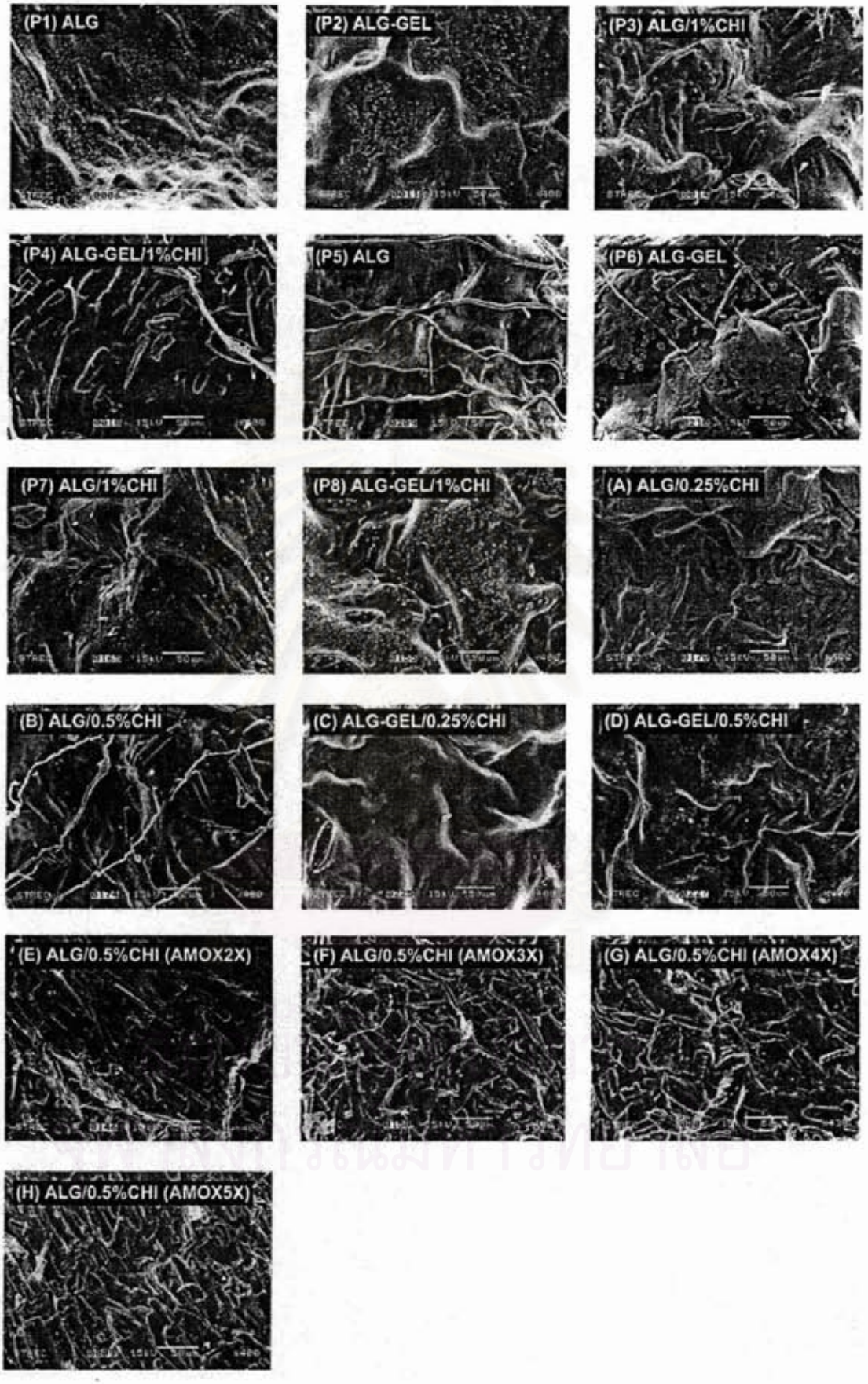


Figure 4.3 Scanning electron micrographs of the surface of beads (400X).

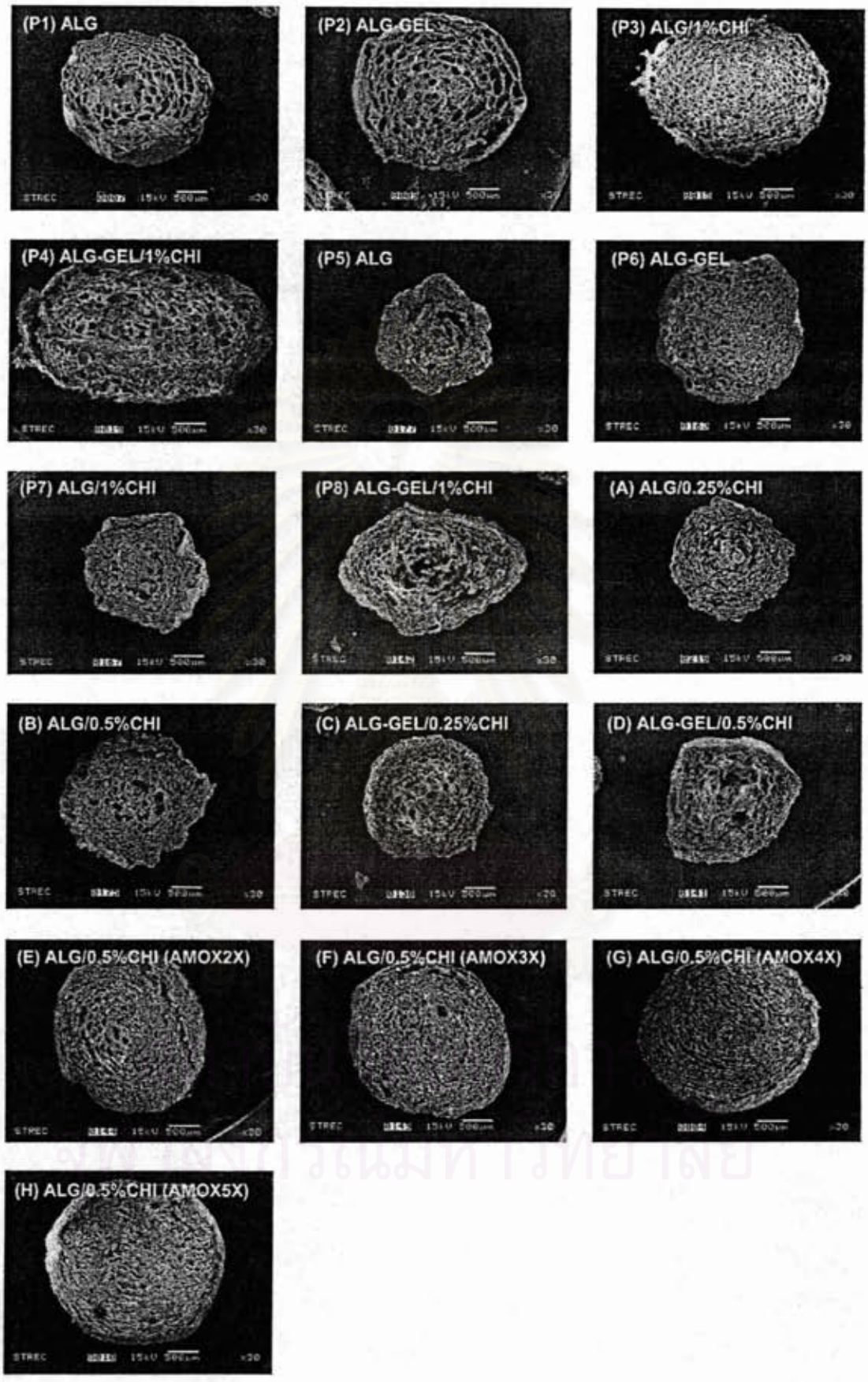


Figure 4.4 Scanning electron micrographs of the x-section of beads (30X).

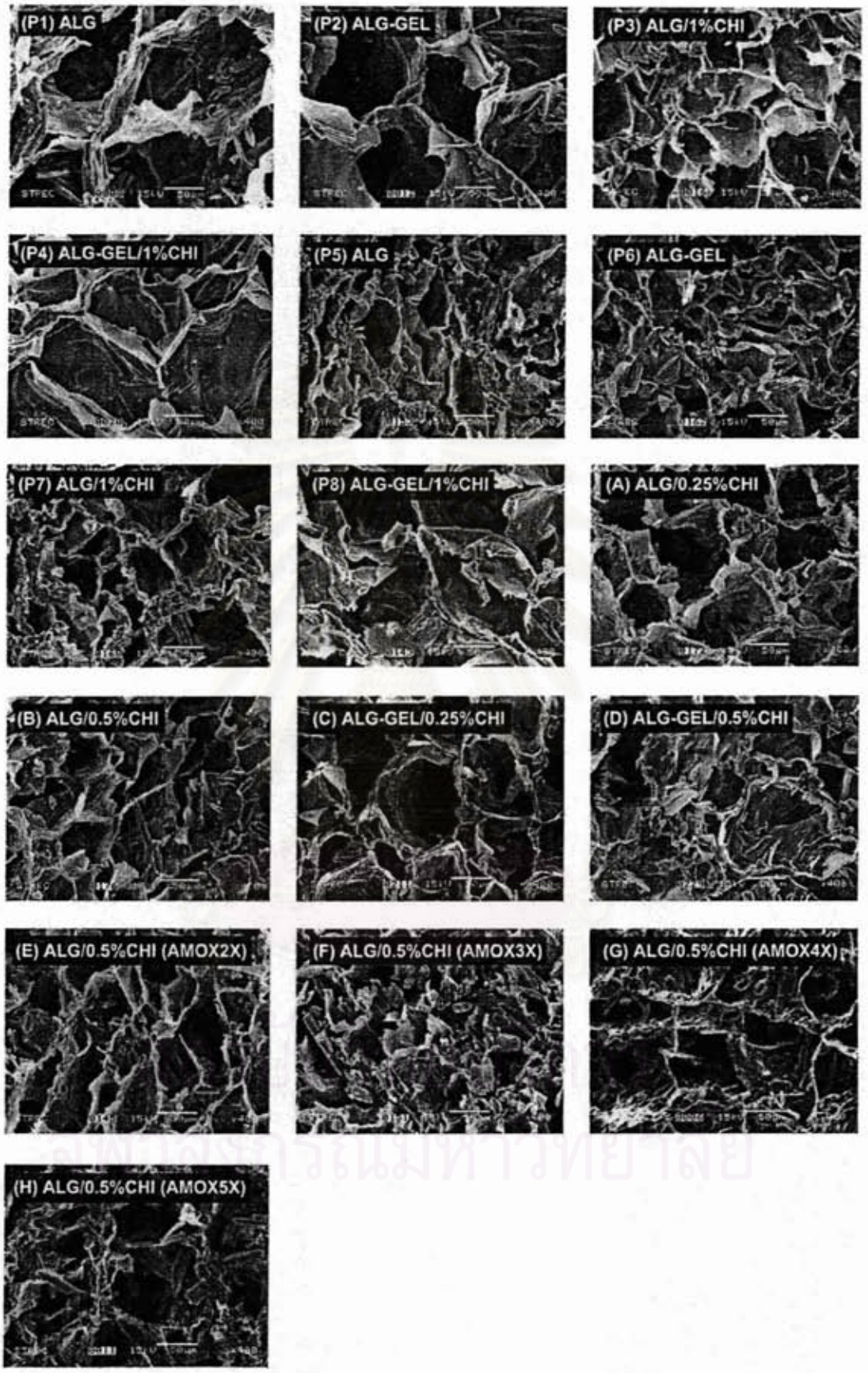


Figure 4.5 Scanning electron micrographs of the x-section of beads (400X).

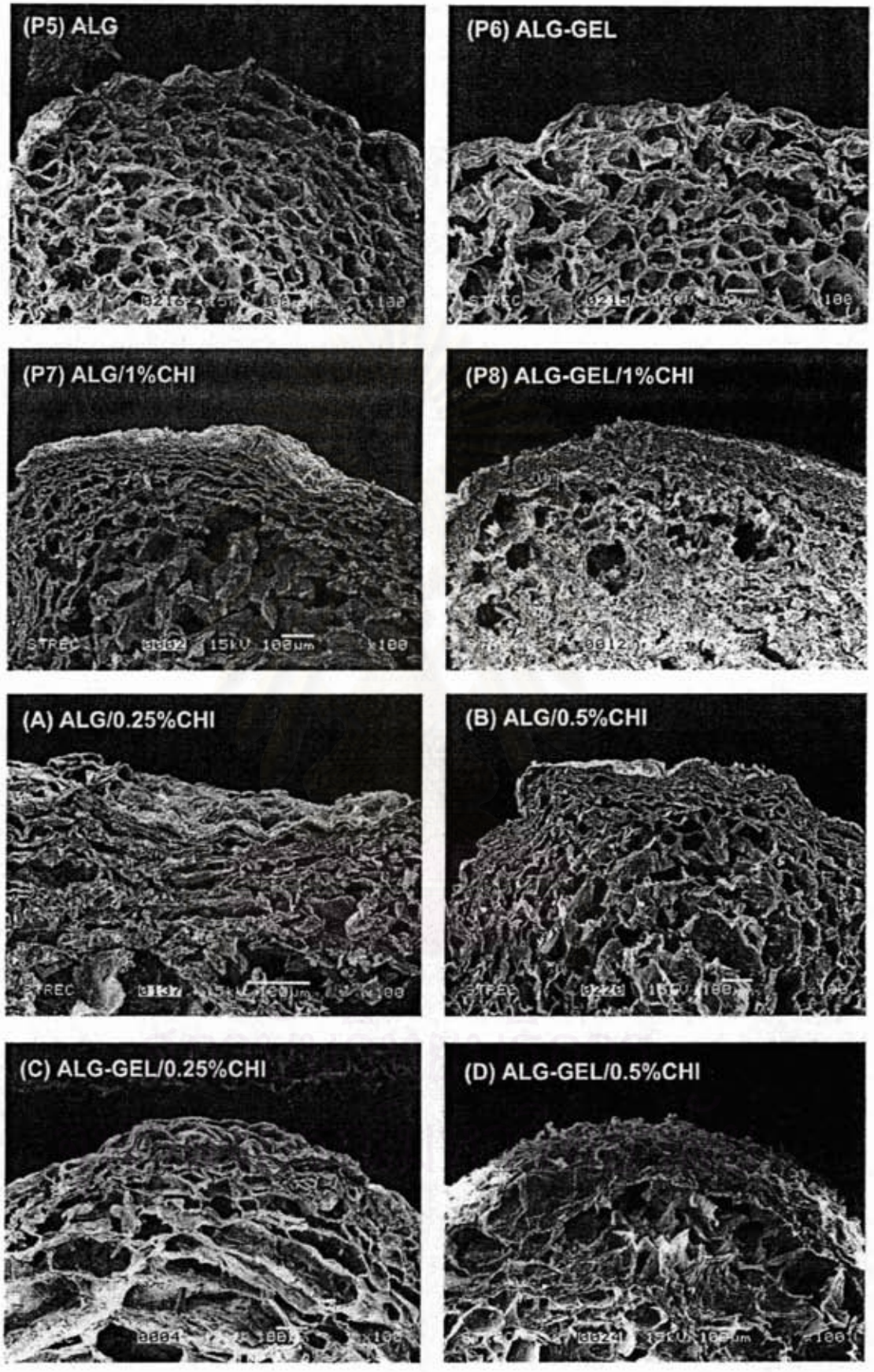


Figure 4.6 Scanning electron micrographs of the rim of beads (100X).

4.3.2 Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy was used to determine the chemical interaction of the samples as displayed in Figures 4.7 to 4.10.

Figure 4.7 showed the IR spectra of each composition of the ALG beads. The IR spectrum of pure alginate (Figure 4.7a) showed the characteristic absorption bands at 1632 cm^{-1}, 1421 cm^{-1} and 1028 cm^{-1} attributed to the asymmetric stretching vibration of -COO, the symmetric stretching vibration of -COO and the stretching of C-O-C, respectively [25]. The IR spectrum of amoxicillin-loaded alginate beads (Figure 4.7c) showed a coupling peak at 1607 cm^{-1} which was the combination peaks from both polymer and drug, while the major peaks of them were not shifted from the original positions and there were no new adsorption peaks of drug-loaded alginate beads. Therefore, it could indicate that there were no chemical interactions between the polymer and the drug used.

Figure 4.8 showed the IR spectra of each composition of the ALG-GEL beads. The characteristic absorption bands of gelatin (Figure 4.8b) at 1660 cm^{-1}, 1538 cm^{-1} and 1235 cm^{-1} were attributed to amide I (C=O stretching vibration), amide II and amide III (mainly N-H bending vibration and C-N stretching vibration), respectively [68]. Finally, the wide absorption band around 3430 cm^{-1} was due to the stretching vibration of O-H bonded to N-H [69]. From the IR spectrum of ALG-GEL (blank), it could be obviously seen that the characteristic absorption bands at 1660 cm^{-1} and 1538 cm^{-1} of gelatin shifted to the wave number at 1638 cm^{-1} and 1552 cm^{-1}. At the same time, the absorption band around 3430 cm^{-1}, concerned to the stretching vibration of N-H group bonded to O-H group, shifted to a lower wave number at 3420 cm^{-1}, suggesting an increase in the hydrogen bonding [69]. All those changes show a strong evidence of the intermolecular interactions and good molecular compatibility between alginate and gelatin. In Figure 4.8e, the IR spectrum of amoxicillin-loaded alginate-gelatin beads (ALG-GEL) showed a combination of characteristic absorption bands of alginate, gelatin and amoxicillin, while the principle peak of amoxicillin at 1772 cm^{-1} was not shifted, and the new absorption bands of drug-loaded beads were not presented. Thus, it can permit to conclude that there were no obvious chemical reactions between the drug and the polymer matrix.

Figure 4.9 illustrated the IR spectra of each composition of ALG/1%CHI beads. The IR spectrum of chitosan (Figure 4.9b) presented the peaks at 893 cm^{-1} and 1149 cm^{-1} assigned to the saccharine structure and a strong amino characteristic peak at around 1591 cm^{-1}. The peak at 1651 cm^{-1} was attributed to the amide of N-acylated chitosan [70]. The IR spectrum of the alginate coated with chitosan without loaded drug (Figure 4.9c) showed the band at 1635 cm^{-1} which was attributed to the formation of NH_3^+, it was indicative of the complexation between the amino groups of chitosan and the carboxylic groups of alginate. This polyelectrolytic complexation occurred when the beads were formed at the pH 5 of the coagulant (2%chitosan: 4%$CaCl_2$ = 1:1). As described earlier, alginate is composed of guluronic and manuronic acids which present a pK_a of 3.5 and 4, respectively. Thus, at the pH 5 of coagulation fluids, the degree of ionization of alginate at the surface changes and increases while the degree of ionization of chitosan (pK_a=6.3) does not change appreciably. During bead formation, the COO- of alginate induces a great number of ionic interactions between alginate and chitosan [71]. The skin of the beads thus formed denser as shown in the SEM morphology (see Figure 4.6). When incorporation the beads with amoxicillin, the principle peaks of both polymers and drug were also presented at the same positions and there were no new absorption bands of drug-loaded beads. Therefore, it may indicate that the chemical interaction between polymers/drug was unlikely to occur.

Figure 4.10 presented the IR spectra of each composition of the ALG-GEL/1%CHI beads. The IR spectrum of blank ALG-GEL/1%CHI (Figure 4.10d) displayed a combination of the characteristic absorption bands of ALG-GEL (blank) and ALG/1%CHI (blank). However, the peaks were observed to be much more weakened. This phenomenon is may be due to the multi-interaction among ALG, GEL and CHI. Moreover, in the presence of amoxicillin, the major peaks of drug-loaded beads (Figure 4.10f) showed unchanged position at 1772 cm^{-1} of amoxicillin and 891 cm^{-1} of chitosan. At the same time, the new peaks were not observed. Thus, it could indicate that there were no chemical interactions between polymers and drug.

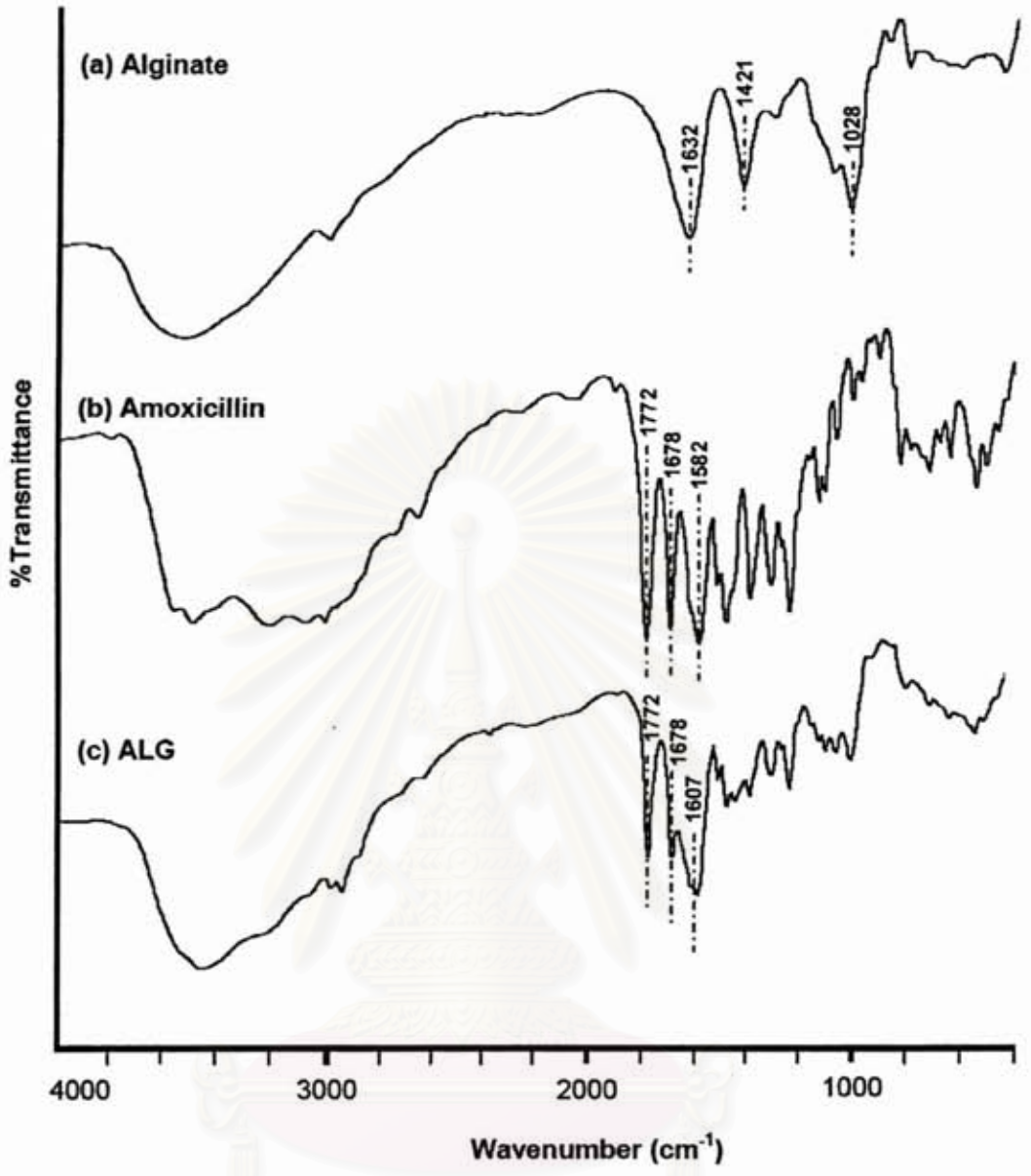


Figure 4.7 IR spectra of (a) alginate, (b) amoxicillin powder and (c) amoxicillin-loaded alginate beads (ALG).

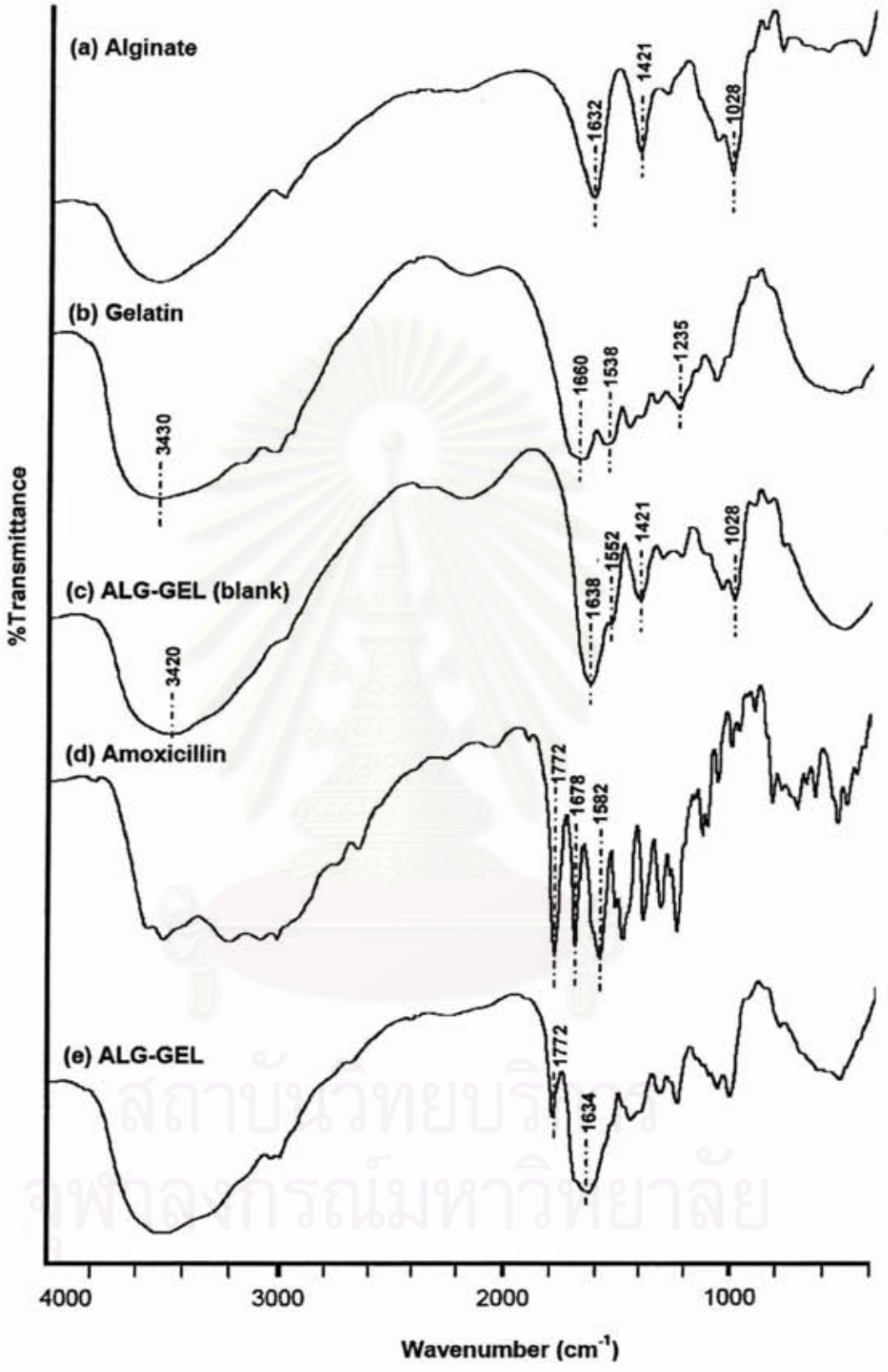


Figure 4.8 IR spectra of (a) alginate, (b) gelatin, (c) alginate-gelatin beads without amoxicillin, (d) amoxicillin powder and (e) amoxicillin-loaded alginate-gelatin beads (ALG-GEL).

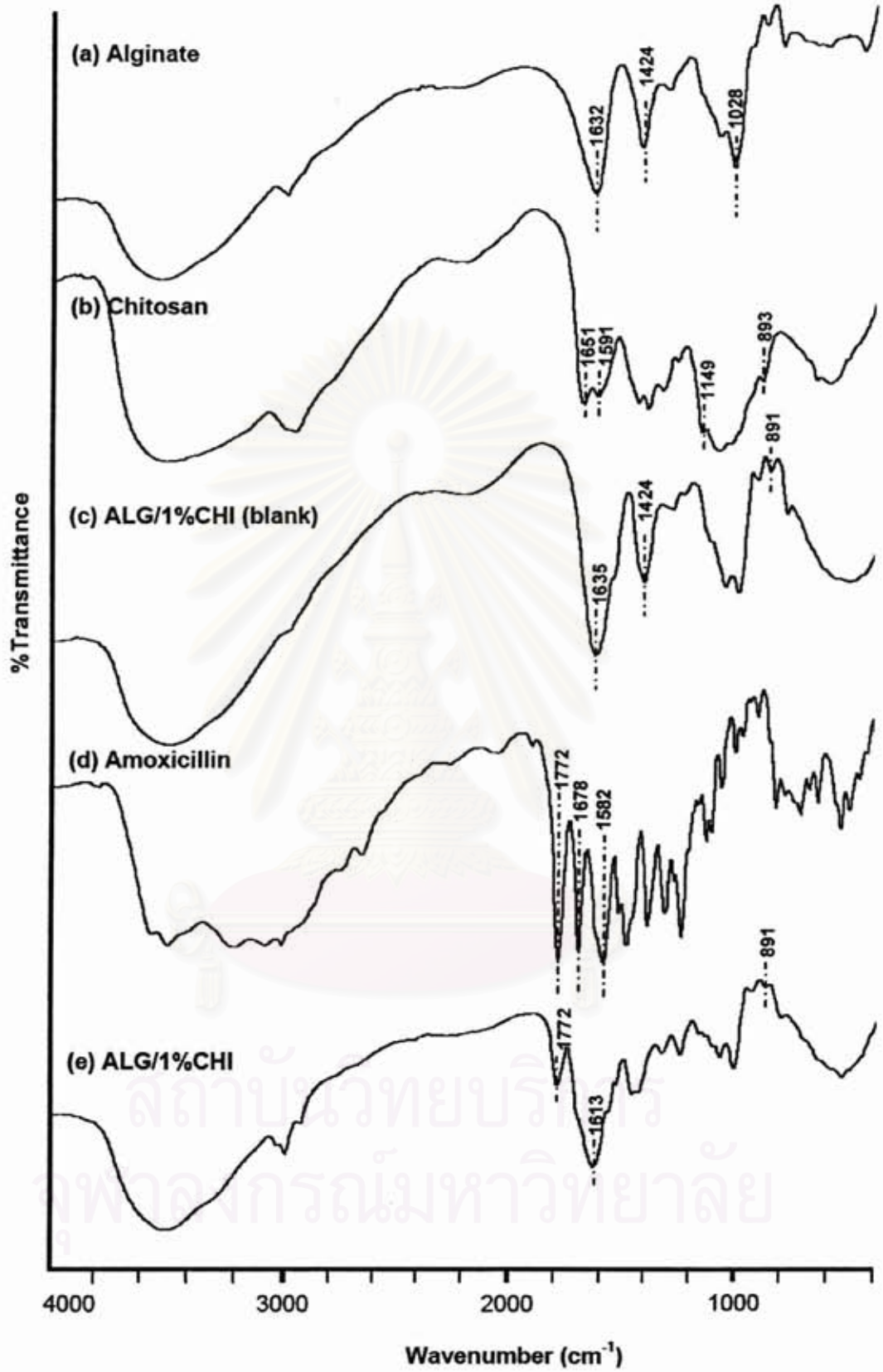


Figure 4.9 IR spectra of (a) alginate, (b) chitosan, (c) alginate beads coated with 1% (w/v) chitosan without amoxicillin, (d) amoxicillin powder and (e) amoxicillin-loaded alginate beads coated with 1% (w/v) chitosan (ALG/1%CHI).

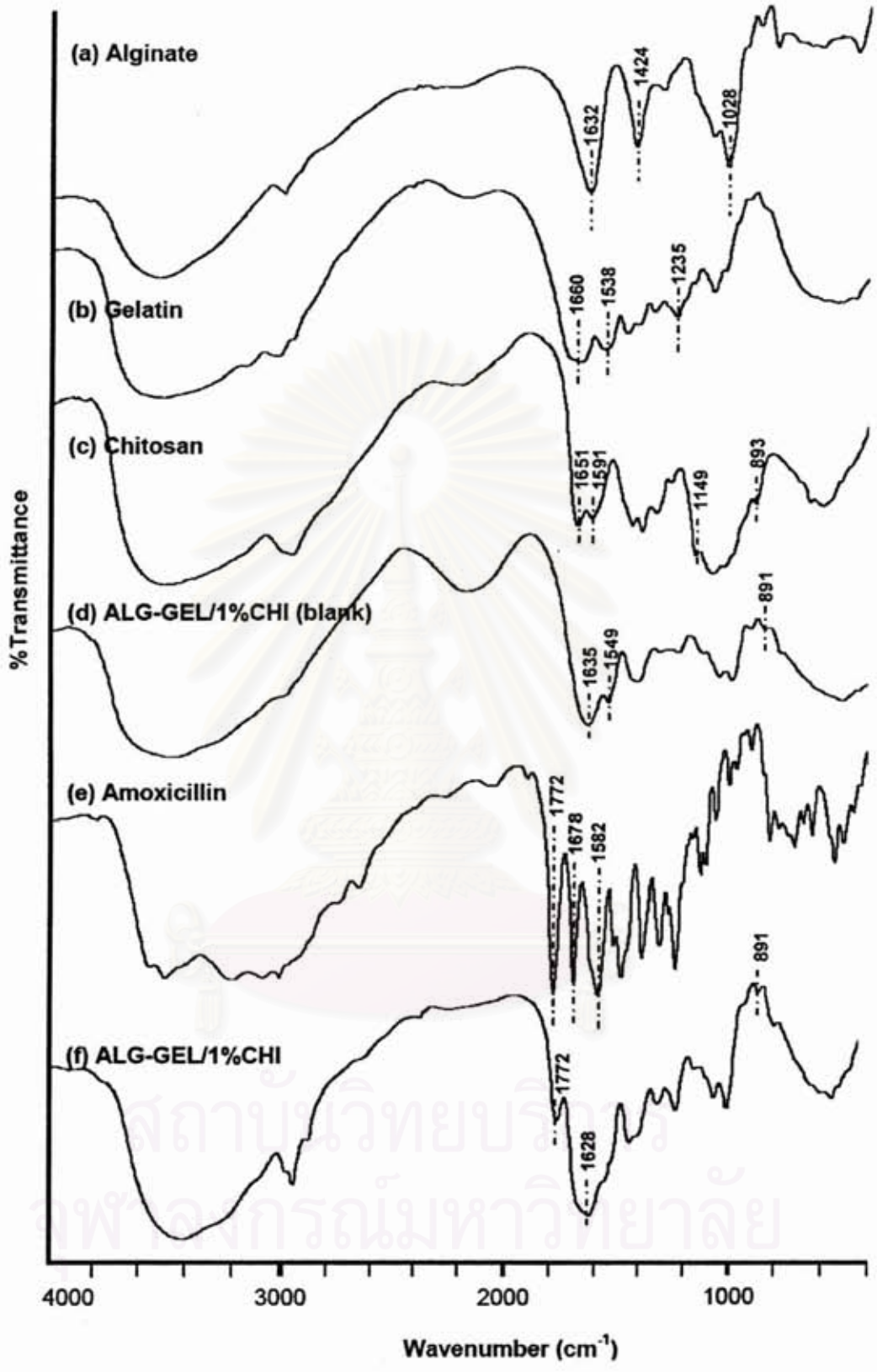


Figure 4.10 IR spectra of (a) alginate, (b) gelatin, (c) chitosan, (d) alginate-gelatin beads coated with 1% (w/v) chitosan without amoxicillin, (e) amoxicillin powder and (f) amoxicillin-loaded alginate-gelatin beads coated with 1% (w/v) chitosan (ALG-GEL/1%CHI).

4.4 The floating properties of the beads

The expectations to obtain a floating dosage form is to extend the gastric retention time, providing the sustained release manner and also giving support to the ability of a device to adhere to the mucosa of the stomach (see Figure 4.11). Therefore, the floating ability was considered as one strategy to achieve the site-specific therapy, especially in the stomach transit for treatment of peptic ulcer.

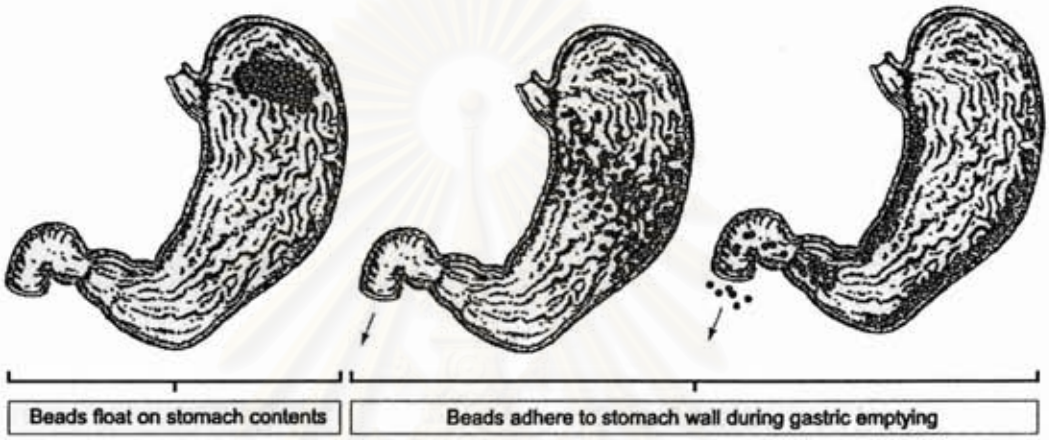


Figure 4.11 Proposed mechanism for retention of mucoadhesive beads in the human stomach [41].

The floating ability of the beads was evaluated in simulated gastric fluid (SGF) as shown in Table 4.3. The results showed that the alginate beads coated with 0.5% (w/v) of chitosan (Formulation B) demonstrated the excellent floating ability with 100%, whereas the alginate beads incorporated with gelatin showed poorer floating ability (Formulation P6, P8, C and D).

Coating the beads with chitosan was directly related to the floating ability. Alginate beads coated with chitosan gave the high floating ability (>95%). In this study, chitosan with the concentration of 0.5% (w/v) showed the best results and varying the drug content (Formulation E to H) did not affect the floating ability. Meanwhile, the chitosan concentration of 0.25% (w/v) (Formulation A) might not be sufficient to protect the air bubbles inside the beads from the surrounding medium, which caused the beads to be sunk. When treating the beads in 1% (w/v) chitosan (Formulation P7), the floating ability exhibited the result similarly to 0.25% (w/v)

chitosan. This is not due to an insufficient coating layer, but too high concentration of coagulation fluid gave the beads with a bigger size and thicker skin. The thicker skin of chitosan may cause the beads with a greater solution uptake and results in a higher bulk density than the external medium. When a bulk density of the beads becomes higher than the surrounding medium, the beads then sank.

Inclusion of gelatin in the alginate beads (Formulation P6, P8, C and D) showed the poorer floating ability. It is due to a high water-uptake property of gelatin which affects directly to the weight of beads. As the higher uptake of solution, a bulk density inside the beads became higher than gastric fluid, and thus the beads then sank.

Table 4.3 Floating ability of beads in simulated gastric fluid (n=6)

Formulation*	Abbreviations	Floating ability (%) ± S.D
P5	ALG	53.33 ± 1.15
P6	ALG-GEL	37.00 ± 1.00
P7	ALG/1%CHI	95.67 ± 4.51
P8	ALG-GEL/1%CHI	61.67 ± 0.58
A	ALG/0.25%CHI	96.00 ± 3.61
B	ALG/0.5%CHI	100.00 ± 0.00
C	ALG-GEL/0.25%CHI	68.67 ± 3.06
D	ALG-GEL/0.5%CHI	62.33 ± 1.53
E	ALG/0.5%CHI (AMOX2X)	100.00 ± 0.00
F	ALG/0.5%CHI (AMOX3X)	100.00 ± 0.00
G	ALG/0.5%CHI (AMOX4X)	100.00 ± 0.00
H	ALG/0.5%CHI (AMOX5X)	100.00 ± 0.00

* Formulation P1 to P4 prepared from the suspension method were not determined.

4.5 The swelling behaviors of beads

The swelling behavior of beads indicates the speed and the easiness of a liquid to penetrate into the polymer matrix as an important step for drug release.

The beads formation of sodium alginate was occurred by ionotropic gelation between Ca^{2+} ions in coagulant and $-COO^-$ groups in alginate. The polyguluronate units formed the coordinate structure with Ca^{2+}, so called an "egg-box" junction [72]. The junctions between the chains formed in this way were stable towards dissociation [73], while the polymannuronate units showed the normal polyelectrolyte characteristics of cations binding. The two interactions as illustrated in Figure 4.12 thus resulted in formation of spherical beads.

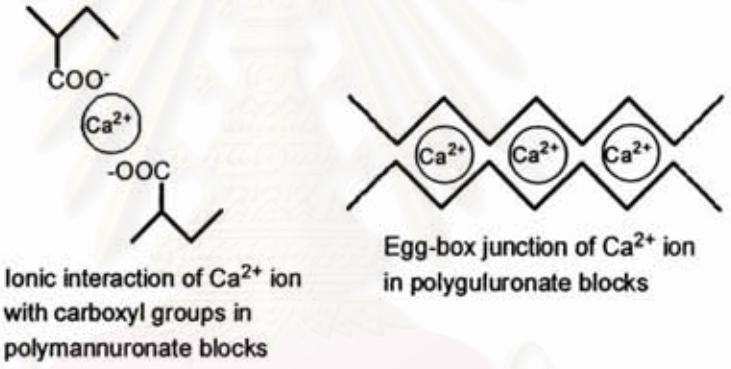


Figure 4.12 Bonding interactions between Ca^{2+} ions and $-COO^-$ groups in the calcium alginate beads [74].

4.5.1 The swelling behaviors in SGF

Figure 4.13 illustrated the samples of swelling behaviors in SGF (pH 1.2 HCl) of various beads formulation that are ALG, ALG-GEL, ALG/1%CHI and ALG-GEL/1%CHI.

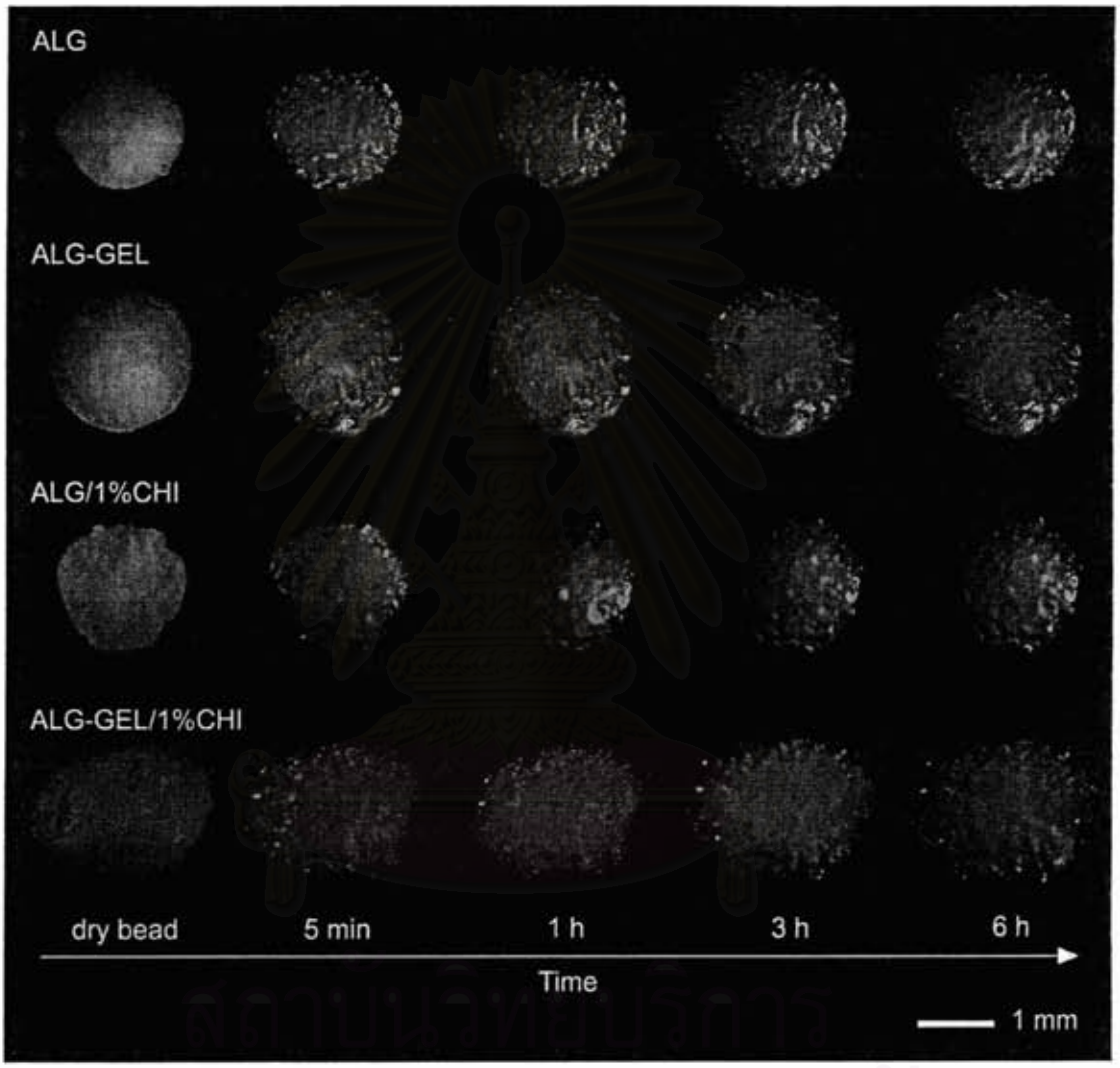


Figure 4.13 The swelling behaviors of beads in SGF.

Figures 4.14-4.16 showed the swelling behaviors of different formation of alginate beads in SGF. Under acidic condition, alginate beads (ALG) without gelatin or chitosan exhibited the lowest swelling degree (<10%). It is due to the complete deletion of calcium from alginate matrices, which are converted to the insoluble alginic acid [27].

When the beads incorporated with gelatin (Figure 4.14, ALG-GEL and ALG-GEL/1%CHI), the swelling was slightly increased. This was the cause of chemical properties of gelatin. Gelatin could behave as an acid or a base because of it consists of both positive charges from amino groups and negative charges from carboxylic groups. The stable structure of gelatin is at its isoelectric point (IEP). In this study, gelatin had a net zero charge at the pH around 7. In the case of the pH swings away from the IEP towards a more acidic condition, such as in this SGF, the amino groups would change to positive charge. As the charges on the coils change to a net positive charge, the coils will repel each other and uncoil slightly. This causes the gelatin to increase in solubility and swell significantly.

In addition, the bead coated with chitosan (Figure 4.14, ALG/1%CHI and ALG-GEL/1%CHI) showed the same trend of results as gelatin. Chitosan is well known that it simply swells in most of acidic medium. At low pH, protonation of the amino groups of chitosan take place. This causes the repulsion among polymer chains which allows more water into the gel network [75].

Figure 4.15 showed the swelling behaviors of ALG and ALG-GEL beads coated with various concentration of chitosan. As expected, lower concentrations of chitosan gave lower swelling properties. The different concentrations of chitosan directly affect to the swelling properties as above described.

Furthermore, the study in the effect of drug content on the swelling properties exhibited that the formulations with higher drug contents showed lower swelling properties (Figure 4.16). When the ratio of drug/polymer was increased to more than 1/1, the swelling behaviors of the beads became lower and showed similar swelling degrees at around 5%. It might indicate that the swelling behaviors of those formulations (Formulation E to H) were simply due to the effect of chitosan.

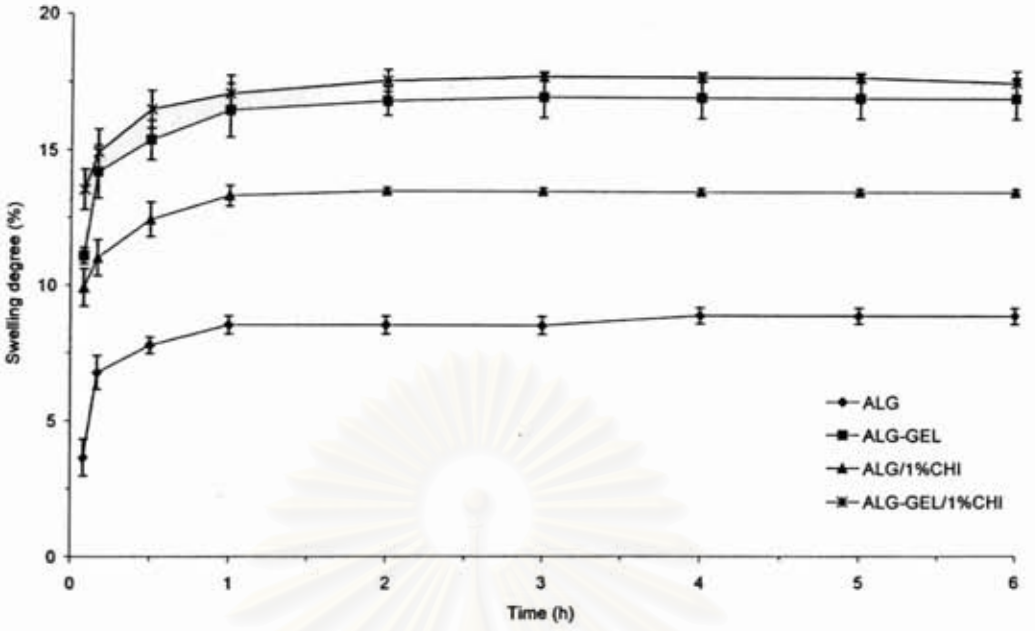


Figure 4.14 Swelling behaviors of different formulations of alginate beads in SGF (pH1.2 HCl).

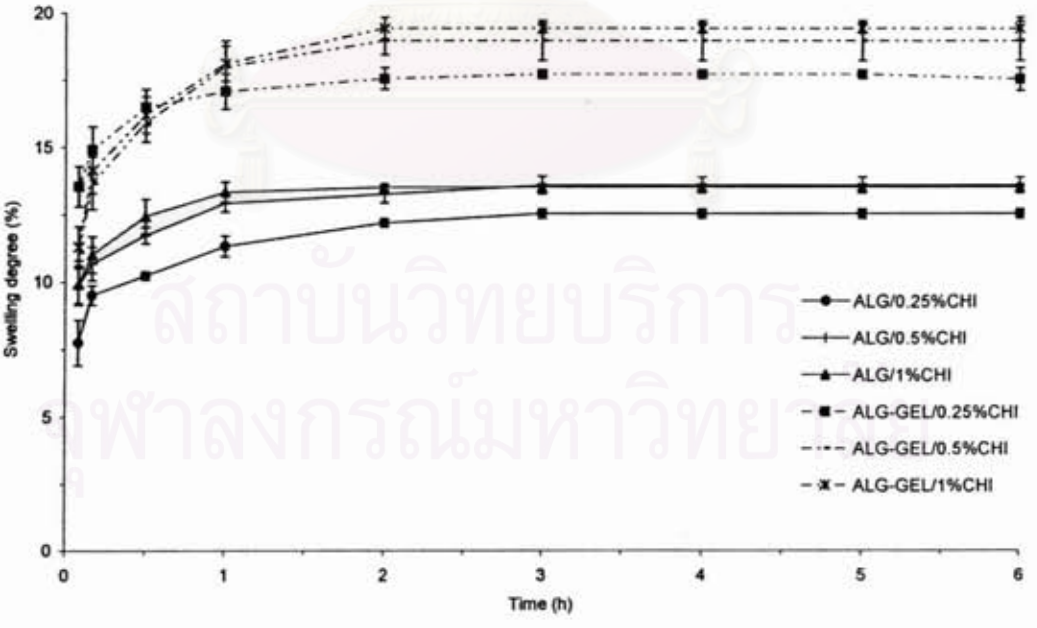


Figure 4.15 Swelling behaviors of ALG and ALG-GEL beads coated with various concentration of chitosan in SGF (pH1.2 HCl).

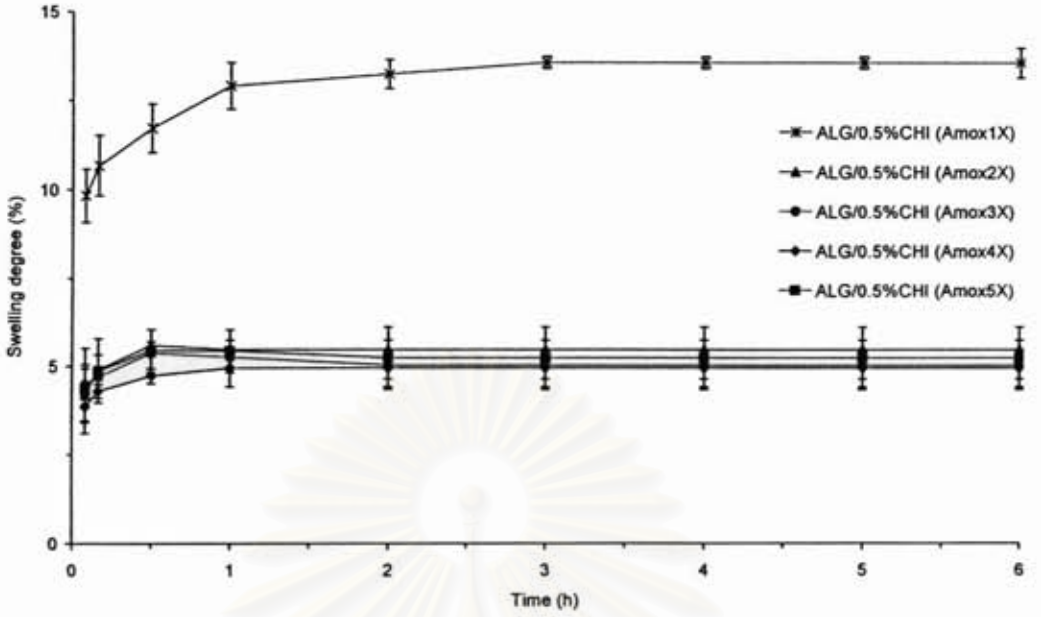


Figure 4.16 Swelling behavior of ALG/0.5%CHI with various drug contents in SGF (pH1.2 HCl).

4.5.2 The swelling behaviors in SIF

Figure 4.17-4.19 showed the swelling behaviors of different alginate beads in SIF (pH 7.4 phosphate buffer saline). When the beads were immersed in phosphate buffer saline pH 7.4, the ALG beads began immediately to swell. In this basic medium, the Na^+ ions appearing in the external solution undergo ion-exchange process with Ca^{2+} ions which are binding with COO^- groups mainly in the polymannuronate sequences of calcium alginate (see Figure 4.12). This phenomenon results in the electrostatic repulsion among negatively charged COO^- groups which causes the chain relaxation and enhances the swelling properties. In other words, it can be said that in the initial stage of the swelling process, the Ca^{2+} ions present in polymannuronate units are exchanged with Na^+ ions, thus causing the beads to swell along with uptake of water. This argument is further supported by the observation of some turbidity appears in the system due to formation of calcium phosphate. In the later stage of swelling process, the Ca^{2+} ions which are binding with $-COO^-$ groups of the polyguluronate units and thus form the tight egg-box structure also start to exchange with Na^+ ions of the buffer medium. This consideration is possibly due to polyguluronate sequences have a strong cooperative binding of Ca^{2+} ions and serve as a stable crosslinking structure within the gel. Finally, the disintegration of the alginate beads may occur when Ca^{2+} ions in the egg-box structure diffuse out into the medium [74].

Figure 4.17 showed the swelling behaviors in SIF of different formation based on alginate. ALG beads showed the highest swelling degree, meanwhile the other formulations which are incorporated with gelatin and/or coated with chitosan showed poorer swelling behaviors.

In SIF (phosphate buffer pH7.4), ALG beads showed the highest swelling degree. It is due to the fundamental properties of calcium alginate which can be swelled under basic medium. The mechanism of swelling of calcium alginate was described in earlier. Whereas, when inclusion the alginate beads with gelatin (ALG-GEL and ALG-GEL/1%CHI), the swelling degree of the beads became poorer. It is probably due to the gelatin does not swell or slightly swell at this basic condition (pH7.4). The background of the swelling behaviors of gelatin was described in

previous section. Briefly, The IEP of gelatin used in this study is around pH 7, therefore, the gelatin coils was not or partially changed to negative charges as the result of ionization of carboxylic groups in gelatin. This causes the less repulsion between the coils.

The beads coated with chitosan (ALG/1%CHI and ALG-GEL/1%CHI) showed the same results as the beads incorporated with gelatin. Because of a poorly swellable behavior of chitosan in basic medium, chitosan layer could protect the bead from outer fluids as well as envelop the whole bead with a fair firmness. Therefore, the swelling degree of the beads coated with chitosan was become poorer than uncoated alginate beads.

Figure 4.18 showed the swelling behaviors of ALG and ALG-GEL beads coated with various concentration of chitosan. The beads coated with 0.25% (w/v) of chitosan exhibited a higher swelling degree, meanwhile, those of the beads coated with 0.5% and 1% (w/v) of chitosan showed insignificantly different swelling behaviors (*t*-test, $P>0.05$). It is possibly due to coating the bead with chitosan at the concentration of 0.25% (w/v) is not sufficient to form a fairly firm network in order to envelop the beads. Whereas, when increase the concentration of chitosan to 0.5% and 1% (w/v), a moderately firm network was obtained and resulted the swelling of the beads become poorer.

The effect of the proportion of drug content in the swelling behaviors was studied. The results are shown in Figure 4.19. When increase the ratio of drug/polymer, the swelling degree was then lower. It could simply explain that when the polymer mass (alginate) become lower, the swelling degree is also lower. It is because of the composition that directly influence the swelling of the beads in SIF is supposed to alginate.

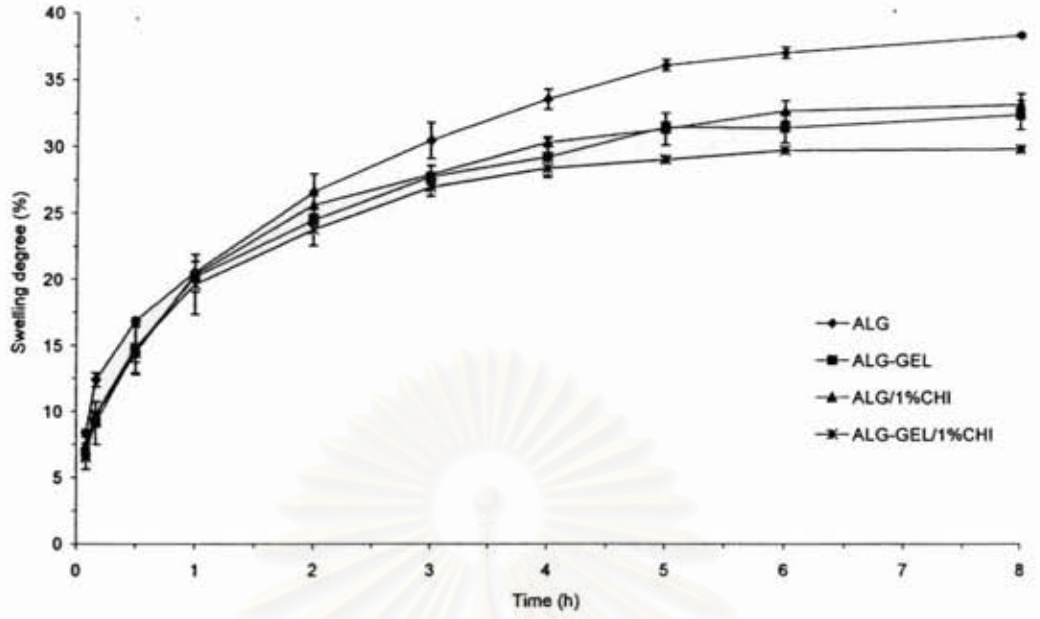


Figure 4.17 Swelling behaviors of different formation of alginate beads in SIF (pH7.4 phosphate buffer).

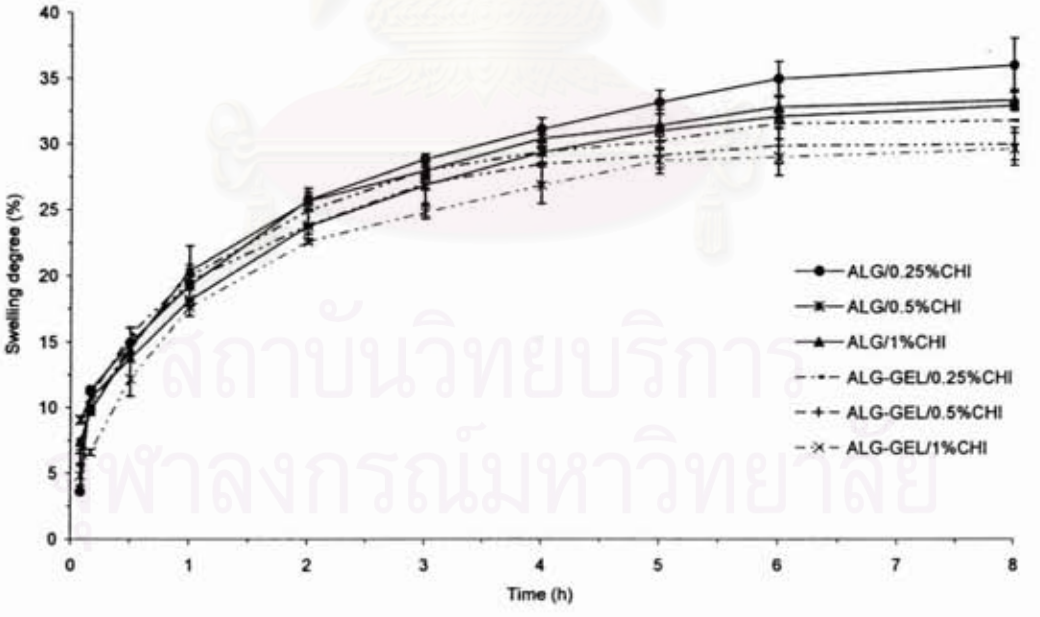


Figure 4.18 Swelling behaviors of different formation of alginate beads coated with various concentration of chitosan (pH7.4 phosphate buffer).

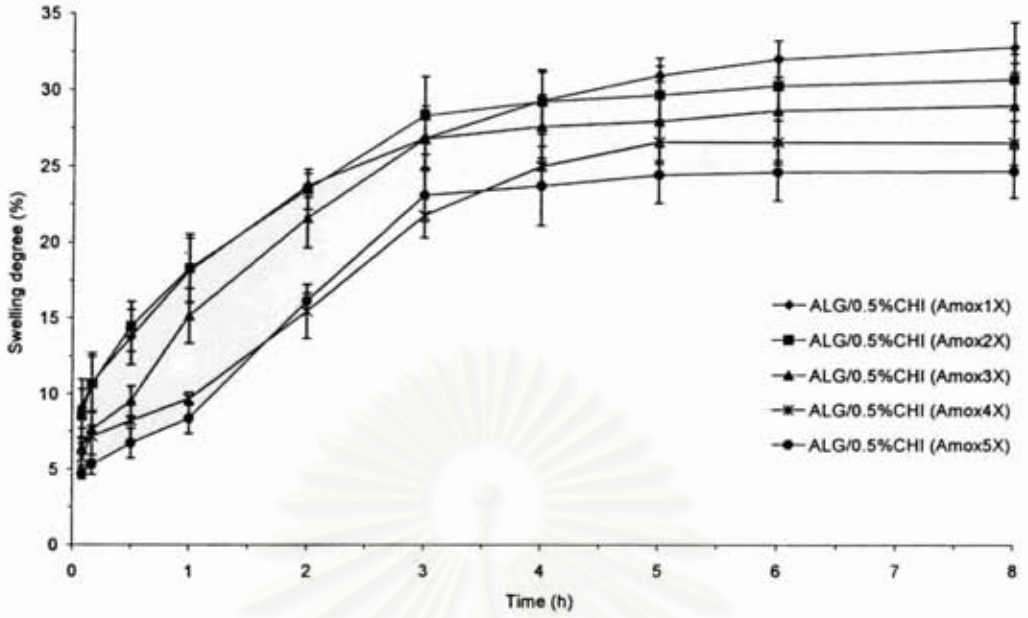


Figure 4.19 Swelling behaviors of ALG/0.5%CHI with various drug contents (pH7.4 phosphate buffer).

4.6 *In vitro* evaluation of mucoadhesiveness

Mucoadhesive controlled release devices can improve the effectiveness of drugs by allowing targeting and localization of drugs at a specific site. In this study, gastroretentive dosage forms (GRDFs) is studied especially to complete the eradication of *H. pylori*, which is the cause of peptic ulcer.

The *in vitro* mucoadhesiveness test (Table 4.4) showed that the percentage of the beads coated with 1% (w/v) chitosan (Formulation P7 and P8) remaining on the gastric mucosa (95.50% ± 1.29% and 96.67% ± 1.53%, respectively) was higher than that of the uncoated beads referred as Formulation P5 and P6 (75.33% ± 2.08% and 79.40% ± 3.85%, respectively). This indicated that the alginate beads coated with chitosan adhere to the gastric mucus more strongly than those without chitosan. This result corresponds to earlier work by Ping He et al. [31], who proposed that chitosan has excellent mucoadhesive properties which is mainly due to a strong electrostatic attraction between positively charged chitosan and negatively charged mucus glycoprotein.

In addition, swelling behavior is another factor that might be effect to the mucoadhesive properties. As described in the theories of mechanism of bioadhesion in Chapter II, the swelling behavior is directly related to the wetting theory, a higher swelling degree gives a higher ability of polymers to spread and develop intimate contact with mucus membrane. This phenomenon conformed to the results of swelling properties in SGF. The beads coated with chitosan, which showed a higher swelling degree, exhibited a greater mucoadhesiveness.

The further study is to evaluate the effect of the concentration of chitosan on the mucoadhesive properties. The results showed that the remaining percentage of those alginate beads coated with 0.5% (w/v) chitosan (Formulation B and D) was not different significantly from the beads coated with 1% (w/v) chitosan (*t*-test, P>0.05). However, when coating the beads with 0.25% (w/v) chitosan (Formulation A and C), the percentage of the beads remaining on the mucosa was lower than those of beads coated with 1 and 0.5 % (w/v) chitosan.

Moreover, it was observed that when the drug content increased (Formulation B, E to H), the remaining percentage of the mucoadhesive beads were slightly lower. It was probably due to the higher weight of the beads along with the amount of the drug added, which possibly overcame the mucoadhesive force and resulted in a reduction of mucoadhesive ability.

Table 4.4 Percentage of the beads remaining on gastric mucosa of pig (n=3)

Formulation*	Abbreviations	Percentage of beads remaining on gastric mucosa (%) ± S.D
P5	ALG	75.33 ± 2.08
P6	ALG-GEL	79.40 ± 3.85
P7	ALG/1%CHI	95.50 ± 1.29
P8	ALG-GEL/1%CHI	96.67 ± 1.53
A	ALG/0.25%CHI	89.80 ± 3.96
B	ALG/0.5%CHI	95.00 ± 2.00
C	ALG-GEL/0.25%CHI	90.67 ± 1.53
D	ALG-GEL/0.5%CHI	96.00 ± 2.65
E	ALG/0.5%CHI (AMOX2X)	94.67 ± 1.15
F	ALG/0.5%CHI (AMOX3X)	93.33 ± 0.58
G	ALG/0.5%CHI (AMOX4X)	93.67 ± 2.92
H	ALG/0.5%CHI (AMOX5X)	91.00 ± 2.00

* Formulation P1 to P4 prepared from the suspension method were not determined.

4.7 *In vitro* drug release

4.7.1 Amoxicillin release behavior in SGF

In the previous studies, the medium of pH 1.2 HCl has been used to evaluate functions of the dosage forms in the stomach. However, the stability of amoxicillin was reported to be rather unstable in an acidic solution with pH below 2 [76-77]. Nägele and Moritz [78] proposed the degradation pathway of amoxicillin as shown in Figure 4.20.

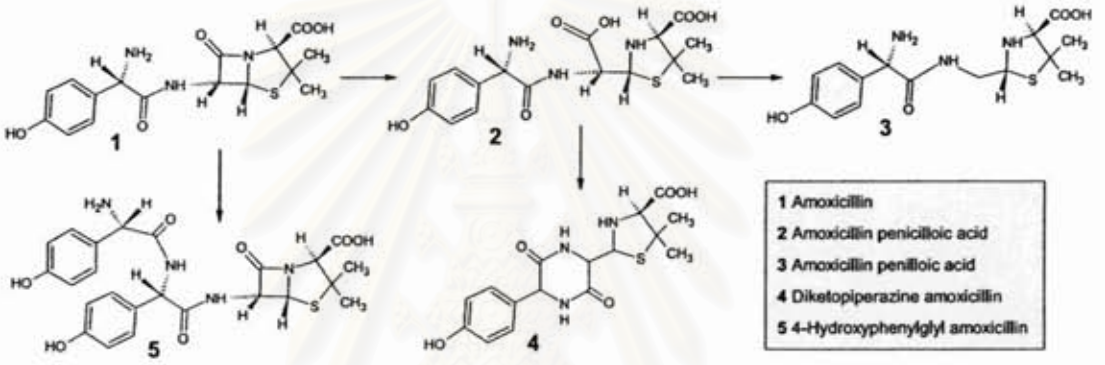


Figure 4.20 Degradation pathway of amoxicillin [78]

Therefore, to evaluate the release behavior of amoxicillin-loaded dosage form, the degradation factor of pure amoxicillin was concerned as an important factor to correct the release profiles.

From the experiment, the typical degradation of pure amoxicillin in pH 1.2 HCl medium was demonstrated in Figure 4.21. The degradation behavior was fitted to the exponential decay equation:

$$C_t = C_0 e^{-k_d t} \quad (1)$$

where C_t is the amount of amoxicillin remaining in the solution at time t, C_0 is the initial amount of amoxicillin, k_d is the degradation rate constant.

From equation (1), it can be shown as the first-order kinetics by the equation:

$$lnC_t = lnC_0 - k_d t \quad (2)$$

The plot between *ln* C_t against t was a straight line (Figure 4.22). A value of the degradation rate constant (k_d) can be calculated from the slope of the straight line plot which was -0.0988.

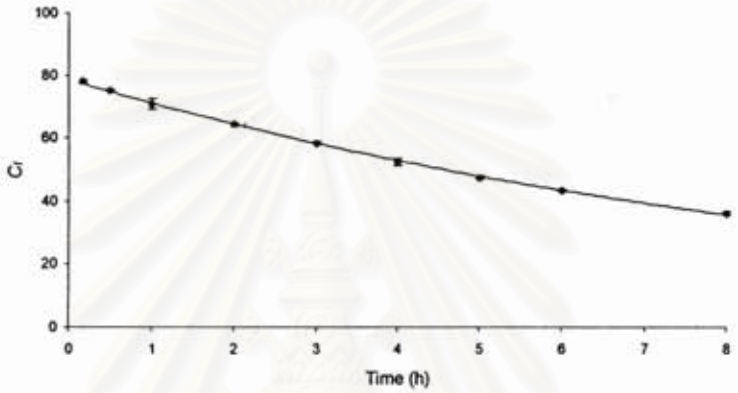


Figure 4.21 The degradation of amoxicillin (pure) in pH 1.2 HCl medium

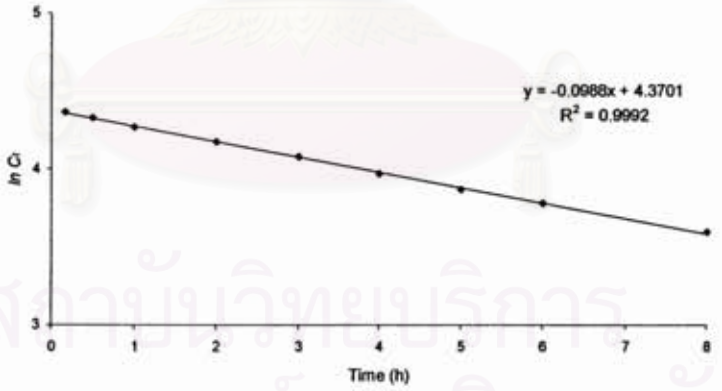


Figure 4.22 The relationship between *ln* C_t and t

The measured amount of amoxicillin released at a certain time can be determined by the following equation:

$$D_t = A_t - (C_0 - C_t) \quad (3)$$

where D_t is the measured amount of amoxicillin released at time t, A_t is the actual amount of amoxicillin released without degradation at time t, (C_0-C_t) is the amount of amoxicillin degraded at time t.

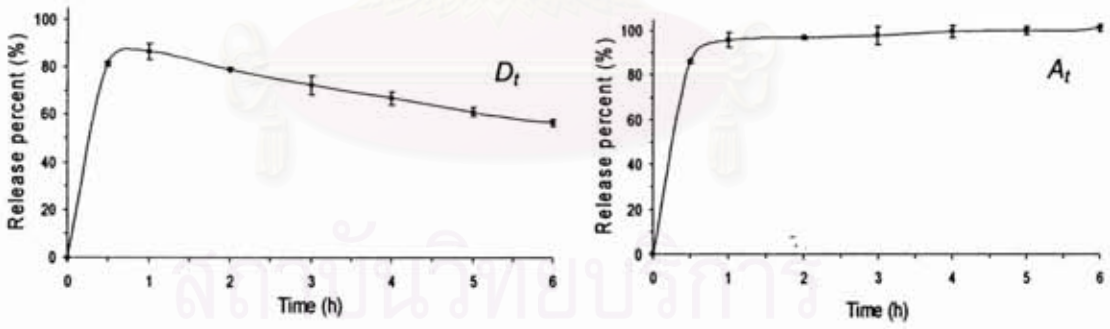
Substitution of C_t in Equation (3) with its equivalents in Equation (1) gives:

$$D_t = A_t - C_0(1-e^{-k_d t}) \quad (4)$$

where C_0 is the initial amount of amoxicillin prior to degradation, k_d is the degradation rate constant.

From the experiment, we know D_t, C_0 and meanwhile k_d can be obtained from the plots of degradation of amoxicillin. Therefore, we can calculate A_t by rearrangement of Equation (4) as:

$$A_t = D_t + C_0(1-e^{-k_d t}) \quad (5)$$



(a) (b)

Figure 4.23 The release profiles of amoxicillin from the formulation (a) before and (b) after correction by the degradation factor.

Figure 4.23 showed the plot of release profile of amoxicillin prior to and after correction by the degradation factor. The plot in term of D_t (Figure 4.23a) illustrated the release of drug decreased in time, but it was the inaccurate profile as it had not been corrected with the degradation factor. Therefore, to study the actual release

behaviors of amoxicillin, it was necessary to correct the plot with the degradation factor and reveal in term of A_t (Figure 4.23b). The following dissolution profiles of amoxicillin from the beads in SGF would be demonstrated as the actual profiles. The results were shown in Figures 4.24-4.29.

4.7.1.1 Effect of the bead compositions

Figure 4.24 showed amoxicillin release profile in SGF (pH 1.2 HCl). The alginate beads (ALG) showed the same dissolution results to the commercial capsules, amoxicillin was released rapidly and 100% completed dissolutions were attained within 1 hour. In the same way, the drug release from the beads incorporated with gelatin (ALG-GEL) reached to 96% within 1 hour. Those two formulations were unable to extend drug retention, and at the same time, it was observed that the beads remained intact in size and shape throughout the dissolution test. This finding corresponded to the observations by Østberg [75] and Almeida [27] who reported a general inability of calcium alginate matrices to retard drug release in pH 1.2 HCl, as well as the calcium ions form alginate matrices are deleted and converted in an insoluble alginic acid. In addition, the burst effect at the initial release was seen, presumably due to the rapid release from the surface. However, the initial burst effect was considerably reduced by coating the beads with chitosan.

After adding chitosan in the coagulation fluids (ALG/1%CHI, ALG-GEL/1%CHI), the release of entrapped amoxicillin was significantly reduced by 70% in 1 hour. It is because the chitosan layer could moderately prevent the drug to leak out. Simultaneously, it was observed that those beads coated with chitosan could maintain the release of drug over 6 hours (see Figure 4.25). In other words, it could say that the sustained release of amoxicillin was achieved when the beads were cured in chitosan.

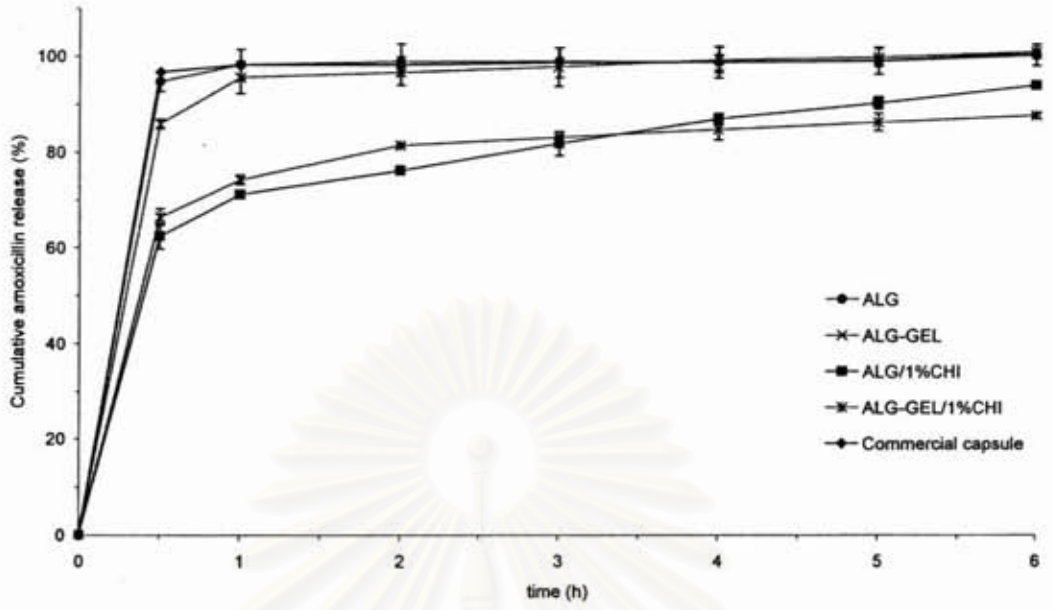


Figure 4.24 The release profiles of amoxicillin from the beads with various compositions and commercial capsules in SGF (pH 1.2 HCl).

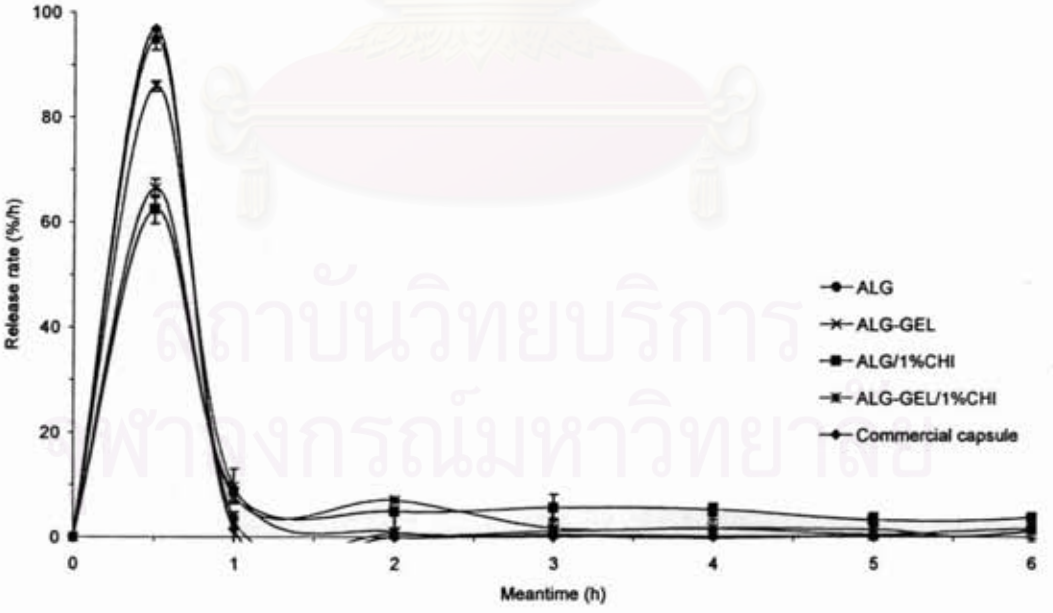


Figure 4.25 The release rate profiles of amoxicillin from the beads with various compositions and commercial capsules in SGF (pH 1.2 HCl).

4.7.1.2 Effect of the concentration of chitosan

From the previous study, it was found that the beads coated with chitosan showed the best results to control the release of amoxicillin. Thus, the concentrations of chitosan were studied further as the considerable effect to retard the drug release.

Figure 4.26 gives the influence of the concentration of chitosan on the amoxicillin release in SGF. The beads coated with 0.25% (w/v) chitosan (ALG/0.25%CHI, ALG-GEL/0.25%CHI) showed an inability to delay the drug release. The release of amoxicillin of those formulations exhibited nearly 90% within the first 2 hours and after that there was no further release (see Figure 4.27). When increase the concentration of chitosan to 0.5% (w/v) (ALG/0.5%CHI, ALG-GEL/0.5%CHI), the beads showed the release profiles similar to that of those beads coated with 1% (w/v) chitosan (ALG/1%CHI, ALG-GEL/1%CHI). However, the drug release of ALG/0.5%CHI and ALG/1%CHI exhibited the best results to extend a longer period of time. It was observed that those formulations of ALG/0.5%CHI and ALG/1%CHI could obviously sustain the release of drug for over 6 hours.

To summarize from all the studies (i.e. encapsulation efficiency, floating properties and mucoadhesive properties), ALG/0.5%CHI and ALG/1%CHI were considered as the optimal formulations. However, comparing between the formulations of ALG/0.5%CHI and ALG/1%CHI, treating the beads with 0.5% (w/v) chitosan was more convenient to prepare than those of the beads curing with 1% (w/v) chitosan. The shape of the beads could confirm this agreement (see Figure 4.2). The stirring acceleration was required in the dropping process of the beads using 1%CHI as coagulation fluid, while curing the beads with 0.5%CHI had not to be stirred. Therefore, ALG/0.5%CHI was chosen as the most suitable formulation for the further study.

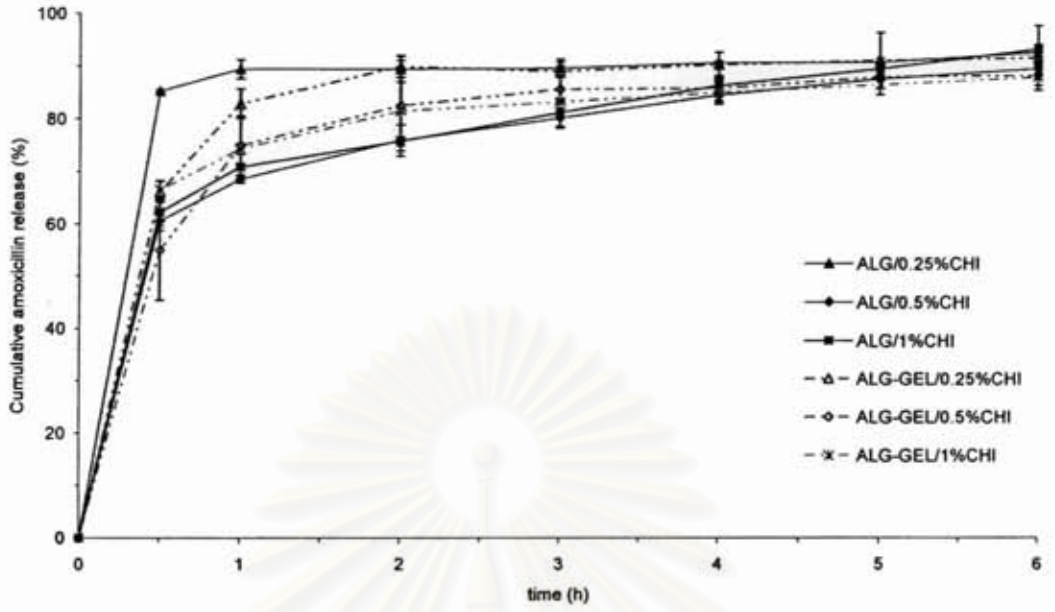


Figure 4.26 The release profiles of amoxicillin from the beads coated with various concentration of chitosan in SGF (pH 1.2 HCl).

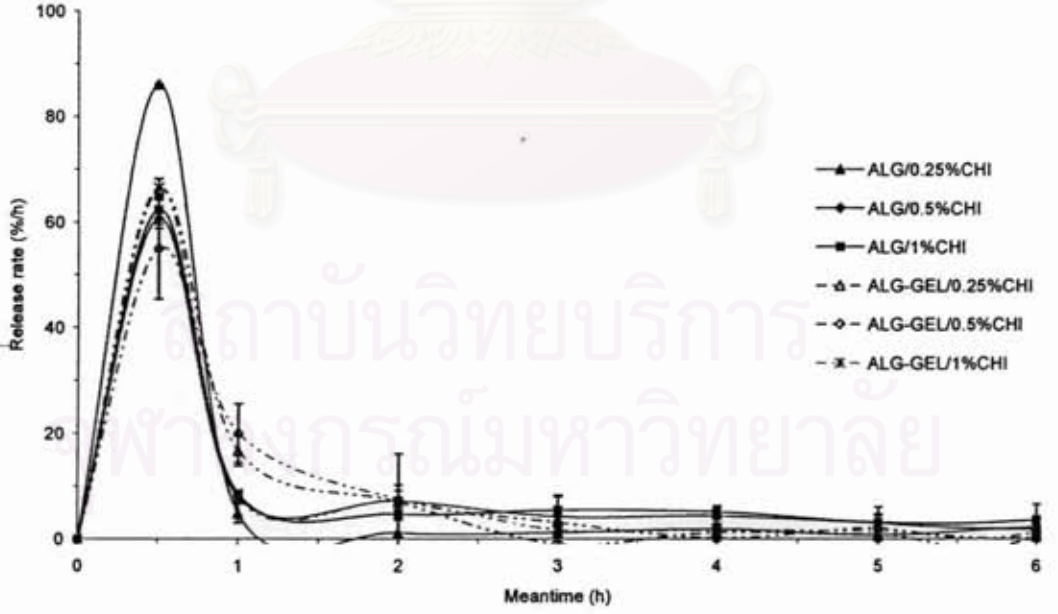


Figure 4.27 The release rate profiles of amoxicillin from the beads coated with various concentration of chitosan in SGF (pH 1.2 HCl).

4.7.1.3 Effect of the drug content

From the previous study, ALG/0.5%CHI was suggested as the most suitable formulation to obtain the sustained release in SGF for over 6 hours.

In this study, ALG/0.5%CHI was varied in the drug content (polymer/drug ratios from 1/1 to 1/5). Figure 4.28 showed the release profiles of amoxicillin from ALG/0.5%CHI beads with various drug contents. Probably because of the inability of alginate to retard the drug release as described previously, the release profiles of those formulations were almost identical. All formulations showed the sustained release in SGF for over 6 hours (see Figure 4.29).

In an acid medium, the beads based on alginate as a particular polymer matrix were unable to control the release of drug as mentioned the earlier results (see Figure 4.24). However, developing the beads by coating with chitosan could significantly improve the release of drug. The chitosan layers were considered as an important parameter to retard the release of drug. Thus, the release behaviors of the beads with varying in drug content, while the other constituents were unchanged, were not significantly different. In other words, it could indicate that the drug content did not affect the release behaviors.

However, it should be noted that the variation of drug content in this study was limited just to the maximum drug/polymer ratio of 5/1 (or 10% (w/v)). It was due to the high viscosity of the mixtures, which was difficult to be extruded through the needle. The standard deviation (S.D.) of the size could be confirmed this incident. The external pressure was required to drop the high viscosity mixtures into the coagulation fluids. This caused the beads varying in sizes, which could be represented by higher S.D. (see Table 4.2).

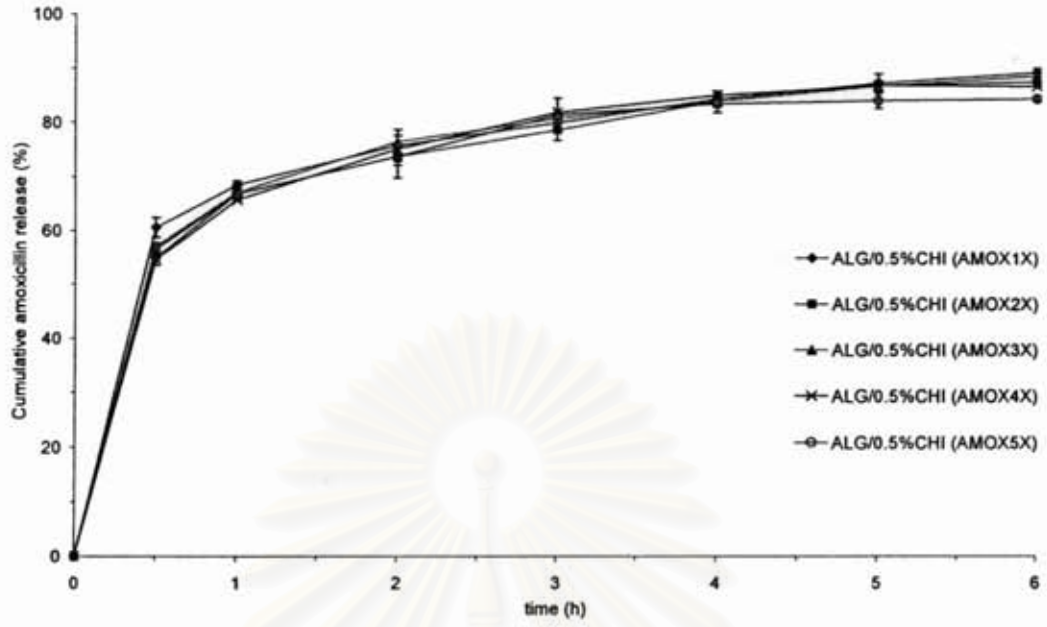


Figure 4.28 The release profiles of amoxicillin from ALG/0.5%CHI beads with various drug contents in SGF (pH 1.2 HCl).

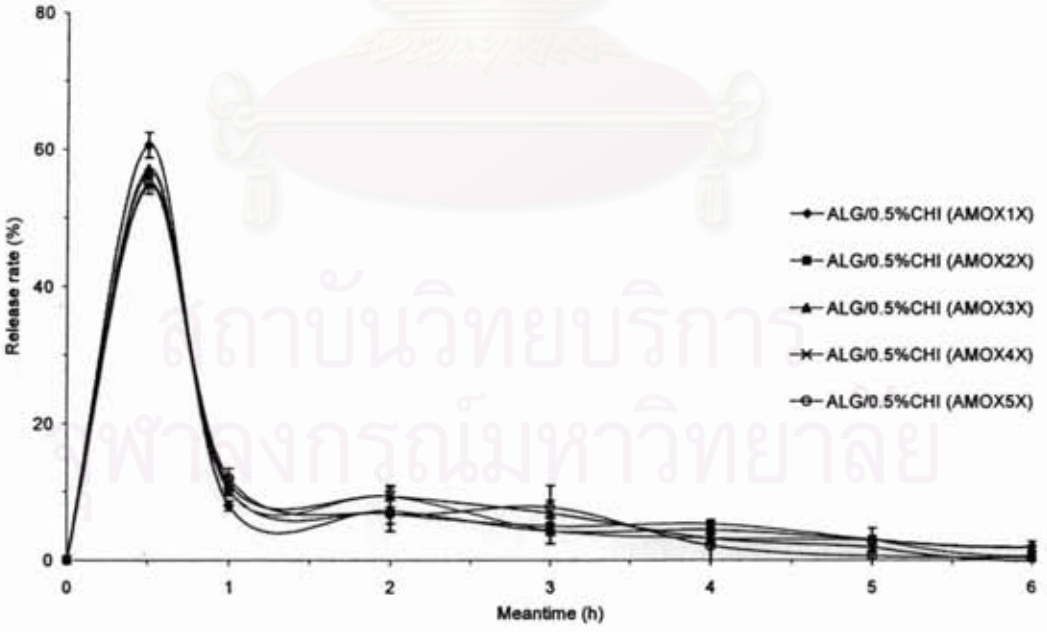


Figure 4.29 The release rate profiles of amoxicillin from ALG/0.5%CHI beads with various drug contents in SGF (pH 1.2 HCl).

4.7.2 Amoxicillin release behavior in SIF

It has been reported earlier that amoxicillin was not observed to degrade under neutral or basic conditions [77]. Therefore, the degradation factor was not concerned to achieve the actual release profiles of amoxicillin in pH 7.4 phosphate buffer medium.

4.7.2.1 *Effect of bead compositions*

Figure 4.30 illustrated the effect of the bead compositions on the release profiles in SIF (pH 7.4 phosphate buffer). The amoxicillin release of the commercial capsules was significantly different from those of amoxicillin-loaded beads. At the interval of 1 hour, 90% of amoxicillin from the commercial capsules were released, while the release from ALG, ALG-GEL, ALG/1%CHI and ALG-GEL/1%CHI were 60%, 54%, 56% and 48%, respectively. Furthermore, it was observed that when the release time was 2 hours, all formulations could maintain the release rate up to 20%/h, while the commercial capsules exhibited for less than 5%/h (see Figure 4.31).

Under a neutral medium (pH 7.4 phosphate buffer), the delayed release pattern was obtained for all formulations. The results showed that there was no significant difference among those formulations. This could be attributed to the release rate of amoxicillin from the beads based on alginate was directly depended on the swelling behavior of alginate in phosphate buffer. As described earlier, the disruption of calcium-alginate occurs faster in phosphate buffer due to the chelating action of phosphate ions. At this neutral medium, the affinity of phosphate for calcium is higher than that of alginate as well as the solubility of calcium-phosphate complex is high. The repulsion among negatively charged COO^- groups in alginate causes the swelling properties. In addition, the beads incorporated with gelatin or coated with chitosan gave no significant effect on the release behaviors. In this neutral medium, the gelatin did not swell or slightly swell, as well as the chitosan, it lost of its positive charges to form the ionic interaction with alginate, but the fair firmness of its network might be possibly still occurred. Because of those reasons, the swelling of the alginate beads became slightly poorer and thus the drug release was slightly retarded.

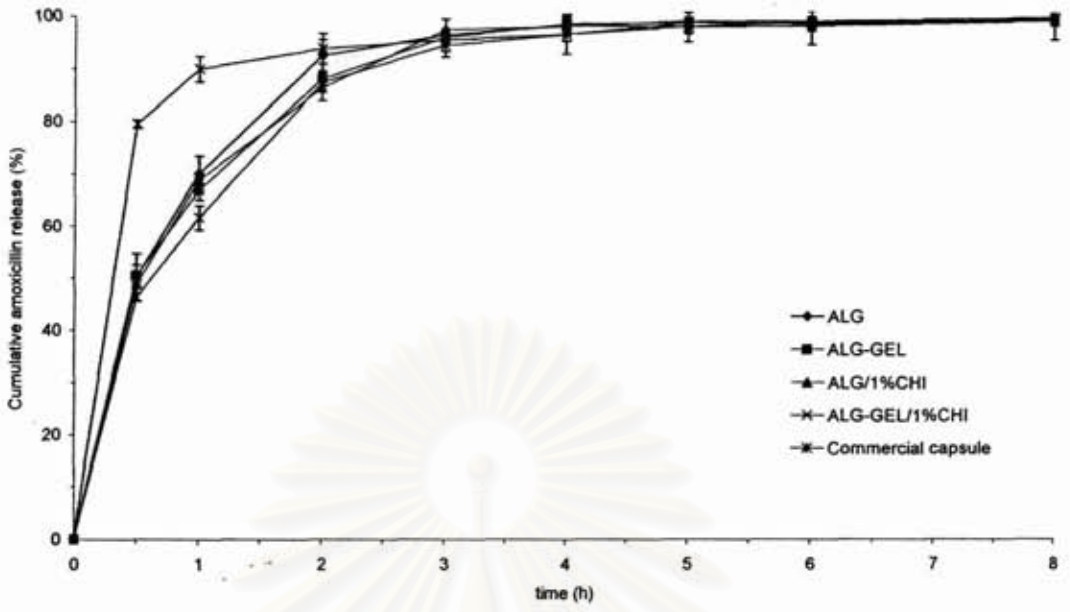


Figure 4.30 The release profiles of amoxicillin from the beads with various compositions and commercial capsules in SIF (pH 7.4 phosphate buffer).

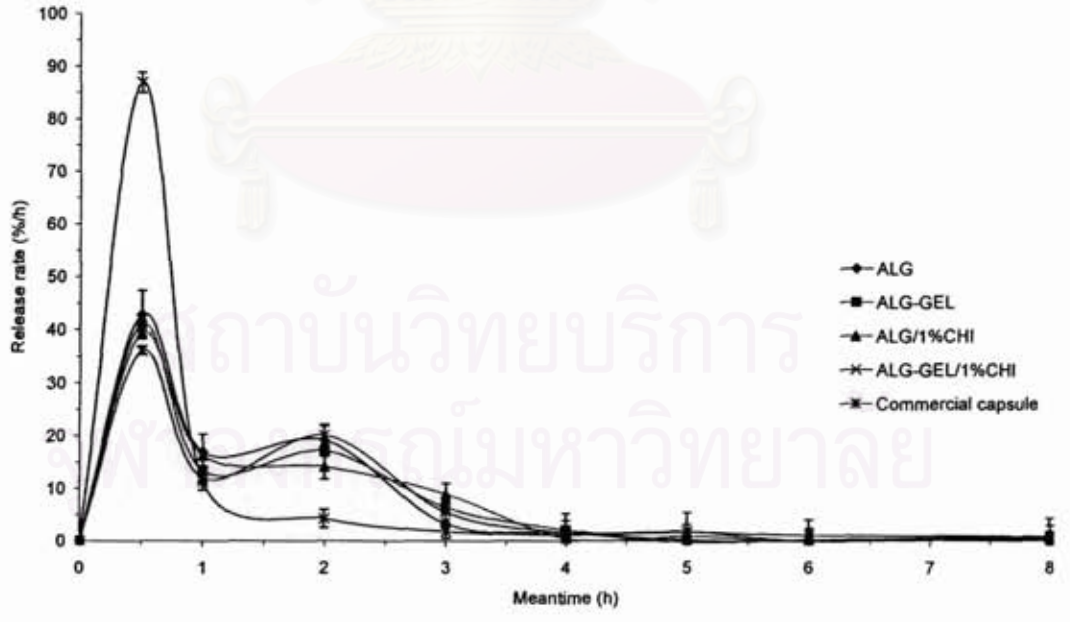


Figure 4.31 The release rate profiles of amoxicillin from the beads with various compositions and commercial capsules in SIF (pH 7.4 phosphate buffer).

4.7.2.2 Effect of the concentration of chitosan

Figure 4.32 showed the effect of the concentration of chitosan on the release behaviors. When comparing at the same concentration of chitosan, it was observed that the beads incorporated with gelatin and coated with chitosan could retard the initial stage of the drug release better than those of the beads without gelatin. At the first hour, the amoxicillin release of ALG/1%CHI, ALG/0.5%CHI and ALG/0.25%CHI was about 69%, 79% and 81%, respectively, while ALG-GEL/1%CHI, ALG-GEL/0.5%CHI and ALG-GEL/0.25%CHI showed the release about 62%, 76% and 74%, respectively. In this neutral medium, chitosan was not considered as an important effect to the drug release behaviors.

As mentioned in the swelling behaviors, the swelling of the beads incorporated with gelatin showed a slightly lower than those of the beads without gelatin. Therefore, the drug release from the beads incorporated with gelatin was slightly lower than those of the beads without gelatin. Moreover, the beads coated with chitosan did not affect the drug release behaviors. Chitosan will lose its positive charge when suspended in the neutral pH of SIF. As that result, the complex will be dissociated and thus the beads lose the ability to retard the release of drug. The swelling properties of the beads were considered as the important factor to control the release of amoxicillin. The rapid swelling behaviors of the beads in SIF may possibly accelerate the initial stage of the drug release, and the complete dissolution may take only few hours. In this dissolution study, the complete release was obtained within 3-4 hours for all formulations (see Figure 4.33).

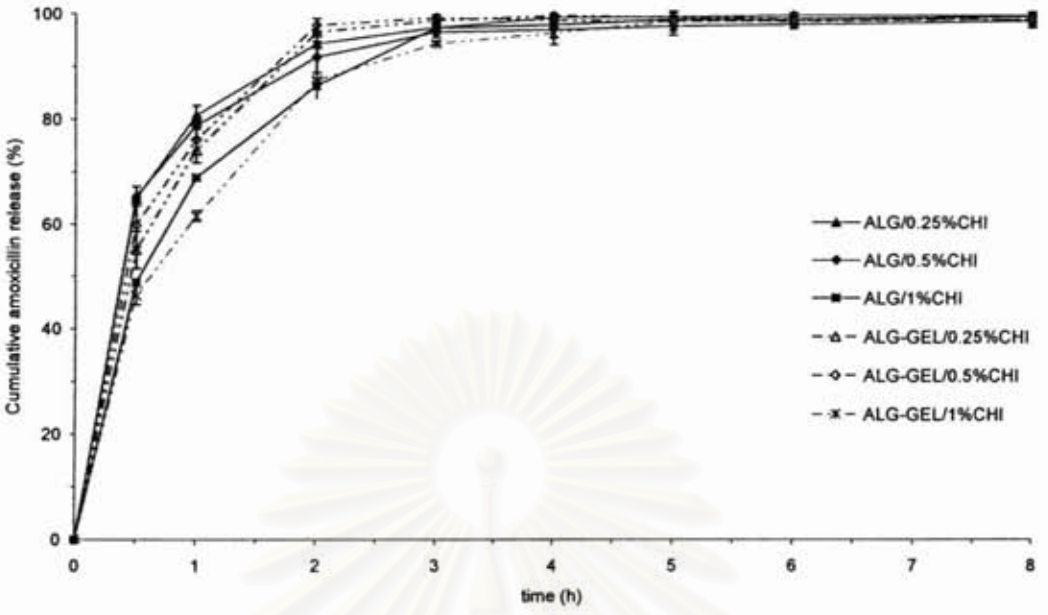


Figure 4.32 The release profiles of amoxicillin from the beads coated with various concentration of chitosan in SIF (pH 7.4 phosphate buffer).

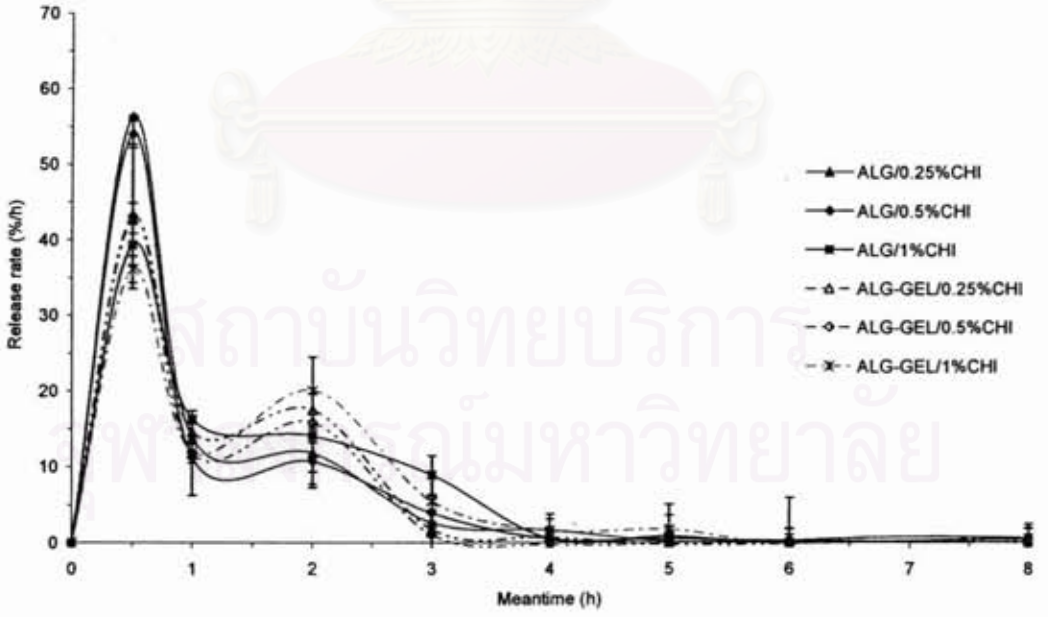


Figure 4.33 The release rate profiles of amoxicillin from the beads coated with various concentration of chitosan in SIF (pH 7.4 phosphate buffer).

4.7.2.3 Effect of the drug content

The release behaviors of ALG/0.5%CHI with various drug contents are shown in Figure 4.34. The release patterns of all formulations were not significantly different. It is possibly due to all formulations comprising with the same polymer matrix. All formulations showed the complete release within 3-4 hours, except for ALG/0.5%CHI (AMOX5X) which could sustain the release of drug up to 4-5 hours (see Figure 4.35). This might be because the drug release of that formulation, ALG/0.5%CHI (AMOX5X), was not only followed by the swelling mechanism but the diffusion mechanism was also considered. The beads with the excess amoxicillin content caused the cavities inside the beads packed and denser. Therefore, the diffusion of the amoxicillin remaining inside the core became more difficult to pass through from the polymer matrix to the external medium, and the drugs required a longer diffuse time to release.

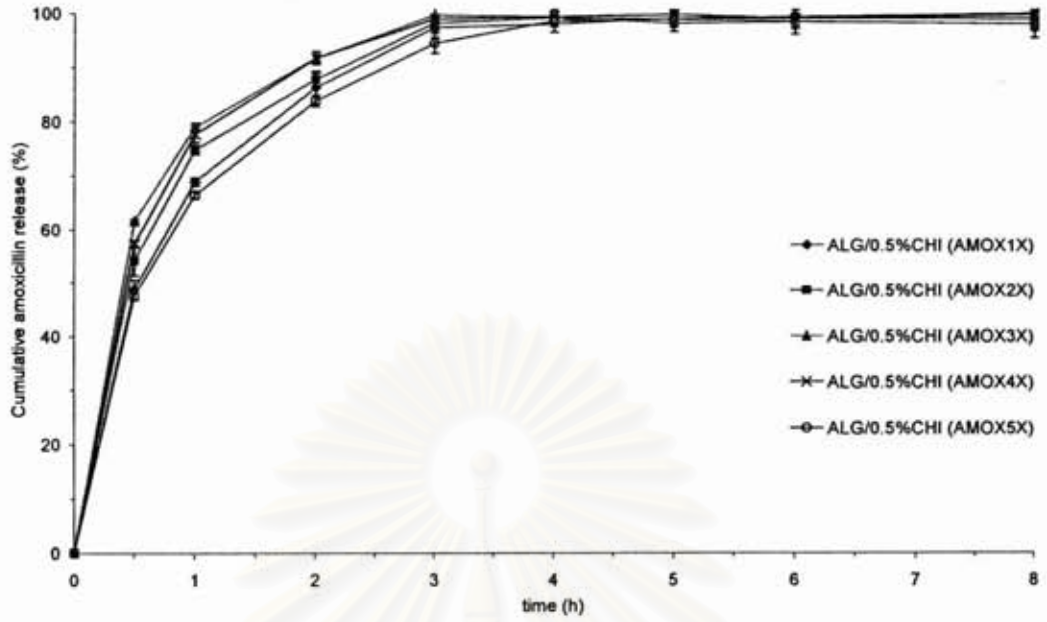


Figure 4.34 The release profiles of amoxicillin from ALG/0.5%CHI beads with various drug contents in SIF (pH 7.4 phosphate buffer).

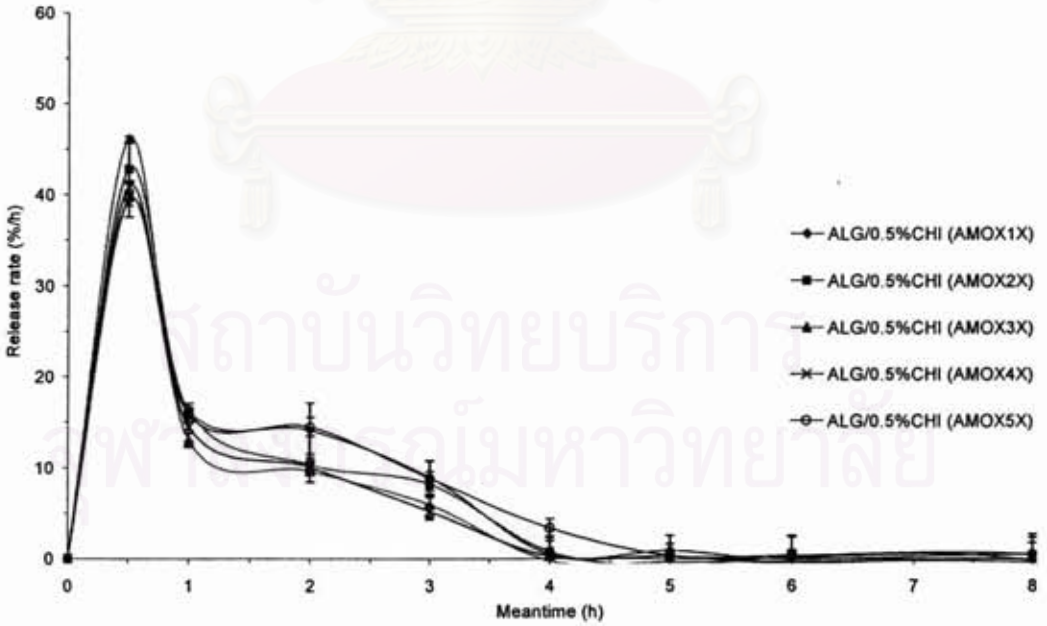


Figure 4.35 The release rate profiles of amoxicillin from ALG/0.5%CHI beads with various drug contents in SIF (pH 7.4 phosphate buffer).

When comparing the *in vitro* release behaviors of amoxicillin between in SGF (pH 1.2 HCl) and in SIF (pH 7.4 phosphate buffer), it was observed that the amoxicillin release rate in SGF was significantly higher than that in SIF. A reason of this phenomenon is mainly due to an inability of calcium alginate to retard the drug release in acidic medium.

CHAPTER V

CONCLUSION AND SUGGESTION

5.1 Conclusion

The gastroretensive dosage forms based on alginate, gelatin and chitosan were developed for effective *H.pylori* eradication. The amoxicillin-loaded alginate beads prepared by solution method and coated with chitosan offered an excellent encapsulation, up to 90% of drug entrapment efficiency. The physicochemical characterization demonstrated that the obvious chemical reaction between the drug and the polymer matrix did not occur.

From the results of floating and mucoadhesive properties studies, the inclusion of gelatin in the amoxicillin-loaded alginate beads resulted in poorer buoyancy and insignificant effect on the mucoadhesiveness of the beads. Conversely, coating the beads with chitosan encouraged the floating ability as well as the mucoadhesive properties. The beads coated with 0.5% (w/v) chitosan showed excellent mucoadhesive properties, comparable to 1% (w/v) chitosan. Varying drug content gave no interference to the floating ability, but a slight reduction of the mucoadhesive ability was observed.

The release of amoxicillin in simulated gastric fluid (SGF, pH 1.2 HCl) was investigated. ALG/0.5%CHI and ALG/1%CHI exhibited the best results for sustained release of amoxicillin for over 6 hours. From the overall studies, ALG/0.5%CHI was summarized as the best formulation, even though the drug content varied up to 10% (w/v). In addition, considering the fact that there was a burst effect of amoxicillin at the first hour in the *in vitro* release test in SGF, it would be a benefit to achieve a quick and effective amoxicillin concentration in gastric tissue. In summary, amoxicillin-loaded alginate beads coated with 0.5% (w/v) chitosan have been proven very useful as a novel alternative for effective clearance of *H.pylori*.

5.2 Suggestion for the future work

To obtain a perfect sustained release profile of amoxicillin in the acidic medium, we suggest that other polymers should be further studied. However, there are only few polymers that can be properly formulated especially in the formation of beads. A proper condition of the bead preparation is limited, which depends on the ability of the polymer matrix to form the firm networks with the selected coagulation fluids. To solve this problem, there are several alternatives that we intend to suggest as follows.

- Modify the calcium alginate by crosslinking with a proper molecule that can enhance the effective controlled release of amoxicillin, such as cyclodextrin which has been introduced by coupling with the alginate to retard the release of many substrates.

- Use chitosan directly as the polymer matrix, but the pH of its solution must be adjusted to nearly neural as much as possible prior to mixing with amoxicillin in order to avoid the degradation of drug. Furthermore, it is also possible to crosslink with the crosslinking agents to improve the release behaviors.

- Search for other polymers that can form the beads properly and also achieve the controlled release of drug.

In addition, in this work, the size of beads might not be suitable to be contained in a capsule to achieve an appropriate dose of amoxicillin. In order to overcome this drawback, we suggest that the bead size should be reduced as much as possible. However, the small particles may give the unsatisfied release behaviors. As known, the bead size has an influence on the release behaviors. The smaller size of beads presents a faster drug release and the complete dissolution may take only few hours. Therefore, the optimal size of beads should be concerned with in order not to have an effect on the release behaviors. However, the preparation in the microsphere forms would not be suggested. The microspheres have been reported that their encapsulation efficiency was not satisfactory and, moreover, a floating ability would be lost.

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APPENDICES

APPENDIX A

Calibration curve of amoxicillin

Calibration curve of amoxicillin

The concentration versus peak area of amoxicillin determined by HPLC assay as the same conditions described in Chapter III is presented in Table A1. The plot of calibration curve of amoxicillin is illustrated in Figure A1.

Table A1 Absorbance of various concentrations of amoxcillin determined by HPLC

Concentration (ppm)	Peak area
10	127568
50	634335
100	1232527
200	2502322
500	5964356

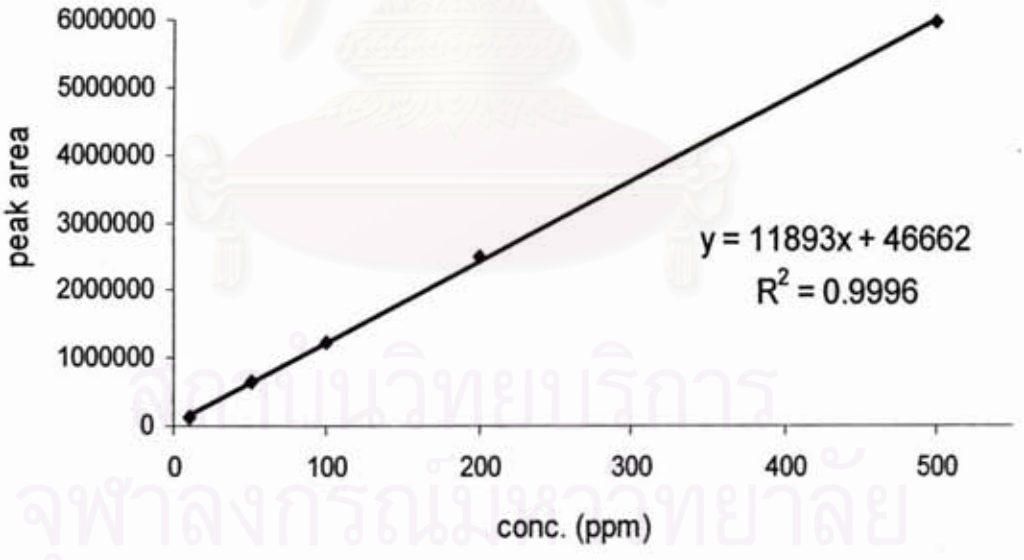

Figure A1 Calibration curve of amoxicillin.

APPENDIX B

Swelling degree

Table B1 The swelling percent of the beads in SGF (pH 1.2 HCl)

Time (min)	Swelling degree ± S.D.			
	Formulation P5	Formulation P6	Formulation P7	Formulation P8
5	3.65 ± 0.68	11.08 ± 0.30	9.91 ± 0.69	11.29 ± 4.53
10	6.77 ± 0.62	14.19 ± 0.98	11.01 ± 0.67	14.13 ± 2.95
30	7.79 ± 0.30	15.36 ± 0.72	12.43 ± 0.64	16.20 ± 2.33
60	8.55 ± 0.33	16.47 ± 0.98	13.33 ± 0.38	18.11 ± 2.57
120	8.55 ± 0.33	16.81 ± 0.52	13.51 ± 0.13	19.44 ± 3.28
180	8.55 ± 0.33	16.97 ± 0.75	13.51 ± 0.13	19.44 ± 3.28
240	8.94 ± 0.30	16.97 ± 0.75	13.51 ± 0.13	19.44 ± 3.28
300	8.94 ± 0.30	16.97 ± 0.75	13.51 ± 0.13	19.44 ± 3.28
360	8.94 ± 0.30	16.97 ± 0.75	13.51 ± 0.13	19.44 ± 3.28

Table B1 (continued) The swelling percent of the beads in SGF (pH 1.2 HCl)

Time (min)	Swelling degree ± S.D.			
	Formulation A	Formulation B	Formulation C	Formulation D
5	13.53 ± 0.75	9.83 ± 1.39	7.76 ± 0.84	11.09 ± 2.05
10	14.91 ± 0.85	10.67 ± 1.63	9.51 ± 0.36	13.67 ± 1.72
30	16.48 ± 0.68	11.72 ± 0.15	10.22 ± 0.15	15.91 ± 0.84
60	17.08 ± 0.66	12.91 ± 0.12	11.31 ± 0.39	18.00 ± 0.25
120	17.56 ± 0.40	13.25 ± 0.59	12.18 ± 0.16	18.98 ± 0.14
180	17.72 ± 0.17	13.59 ± 1.07	12.53 ± 0.17	18.98 ± 0.14
240	17.72 ± 0.17	13.59 ± 1.07	12.53 ± 0.17	18.98 ± 0.14
300	17.72 ± 0.17	13.59 ± 1.07	12.53 ± 0.17	18.98 ± 0.14
360	17.55 ± 0.42	13.59 ± 1.07	12.53 ± 0.17	18.98 ± 0.14

Table B1 (continued) The swelling percent of the beads in SGF (pH 1.2 HCl)

Time (min)	Swelling degree ± S.D.			
	Formulation E	Formulation F	Formulation G	Formulation H
5	4.20 ± 0.74	4.48 ± 0.56	3.85 ± 0.44	4.31 ± 1.21
10	4.90 ± 0.42	4.70 ± 0.23	4.30 ± 0.19	4.87 ± 0.91
30	5.60 ± 0.11	5.37 ± 0.09	4.73 ± 0.21	5.44 ± 0.61
60	5.48 ± 0.27	5.26 ± 0.07	4.95 ± 0.53	5.44 ± 0.61
120	5.48 ± 0.27	5.03 ± 0.39	4.95 ± 0.53	5.24 ± 0.89
180	5.48 ± 0.27	5.03 ± 0.39	4.95 ± 0.53	5.24 ± 0.89
240	5.48 ± 0.27	5.03 ± 0.39	4.95 ± 0.53	5.24 ± 0.89
300	5.48 ± 0.27	5.03 ± 0.39	4.95 ± 0.53	5.24 ± 0.89
360	5.48 ± 0.27	5.03 ± 0.39	4.95 ± 0.53	5.24 ± 0.89

Table B2 The swelling percent of the beads in SIF (pH 7.4 phosphate buffer)

Time (min)	Swelling degree ± S.D.			
	Formulation P5	Formulation P6	Formulation P7	Formulation P8
5	8.35 ± 0.26	6.83 ± 1.23	7.43 ± 0.15	6.49 ± 0.15
10	12.40 ± 0.53	9.11 ± 1.63	9.72 ± 0.13	9.77 ± 0.25
30	16.86 ± 0.25	14.67 ± 1.74	14.42 ± 0.69	14.81 ± 2.04
60	20.56 ± 0.00	20.21 ± 1.15	20.37 ± 0.18	19.61 ± 2.28
120	26.64 ± 1.33	24.50 ± 0.17	25.64 ± 0.70	23.79 ± 1.18
180	30.49 ± 1.35	27.77 ± 0.84	27.94 ± 0.68	26.98 ± 0.65
240	33.62 ± 0.76	29.28 ± 1.52	30.38 ± 0.30	28.43 ± 0.58
300	36.18 ± 0.42	31.56 ± 0.40	31.42 ± 1.21	29.11 ± 0.21
360	37.17 ± 0.42	31.55 ± 1.12	32.81 ± 0.77	29.85 ± 0.02
480	38.52 ± 0.16	32.56 ± 1.10	33.33 ± 0.80	29.98 ± 0.20

Table B2 (continued) The swelling percent of the beads in SIF (pH 7.4 phosphate buffer)

Time (min)	Swelling degree ± S.D.			
	Formulation A	Formulation B	Formulation C	Formulation D
5	4.82 ± 0.74	9.05 ± 1.91	3.60 ± 0.09	6.97 ± 0.22
10	6.59 ± 0.23	10.74 ± 1.96	11.32 ± 0.13	10.83 ± 0.75
30	12.19 ± 1.32	13.74 ± 1.82	14.93 ± 0.19	15.53 ± 0.53
60	17.60 ± 0.68	18.14 ± 2.14	19.27 ± 1.58	19.96 ± 2.31
120	22.58 ± 0.16	23.73 ± 0.78	25.73 ± 0.37	24.91 ± 1.74
180	24.80 ± 0.49	26.83 ± 2.11	28.81 ± 0.43	27.98 ± 0.61
240	26.84 ± 1.38	29.32 ± 1.86	31.13 ± 0.85	29.35 ± 0.27
300	28.71 ± 1.00	30.98 ± 1.15	33.18 ± 0.90	30.20 ± 0.68
360	28.98 ± 1.38	32.09 ± 1.20	34.98 ± 1.31	31.55 ± 0.38
480	29.60 ± 1.25	32.92 ± 1.66	36.01 ± 2.06	31.80 ± 0.03

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Table B2 (continued) The swelling percent of the beads in SIF (pH 7.4 phosphate buffer)

Time (min)	Swelling degree ± S.D.			
	Formulation E	Formulation F	Formulation G	Formulation H
5	8.53 ± 1.78	6.46 ± 1.24	5.75 ± 1.25	4.69 ± 0.35
10	10.66 ± 1.80	7.62 ± 0.26	7.18 ± 1.56	5.30 ± 0.66
30	14.45 ± 1.65	9.52 ± 1.00	8.21 ± 1.27	6.72 ± 0.98
60	18.30 ± 2.25	15.15 ± 1.82	9.66 ± 0.41	8.36 ± 1.00
120	23.49 ± 1.32	21.61 ± 1.95	15.43 ± 1.78	16.12 ± 0.50
180	28.32 ± 2.55	26.80 ± 1.88	21.76 ± 1.45	23.09 ± 1.73
240	29.24 ± 2.10	27.62 ± 2.08	25.01 ± 0.28	23.71 ± 2.61
300	29.70 ± 1.89	27.98 ± 2.59	26.64 ± 1.44	24.45 ± 1.86
360	30.32 ± 1.90	28.71 ± 3.30	26.64 ± 1.44	24.66 ± 1.87
480	30.78 ± 1.70	29.06 ± 2.81	26.64 ± 1.44	24.75 ± 1.73

APPENDIX C

Percentage of drug release

Table C1 Percentage of amoxicillin release in SGF (pH 1.2 HCl)

Time (h)	Percentage of amoxicillin release (%) ± S.D.				
	Commercial capsule	ALG	ALG-GEL	ALG/1%CHI	ALG-GEL/1%CHI
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.5	96.77 ± 0.21	94.80 ± 2.04	85.92 ± 0.97	62.44 ± 2.70	66.49 ± 1.75
1	98.34 ± 3.19	98.32 ± 0.34	95.67 ± 3.36	71.18 ± 0.39	74.28 ± 0.95
2	98.37 ± 4.31	98.94 ± 1.03	96.72 ± 0.63	76.14 ± 0.87	81.40 ± 0.38
3	98.81 ± 3.12	99.17 ± 0.55	97.88 ± 4.03	81.83 ± 2.54	83.15 ± 0.35
4	98.96 ± 3.17	98.98 ± 1.58	99.49 ± 2.74	87.15 ± 1.14	84.93 ± 2.20
5	99.30 ± 2.79	99.39 ± 0.95	100.04 ± 1.89	90.49 ± 1.30	86.44 ± 1.86
6	100.55 ± 2.24	101.00 ± 0.26	101.25 ± 1.49	94.28 ± 0.50	87.87 ± 0.47

Table C1 (continued) Percentage of amoxicillin release in SGF (pH 1.2 HCl)

Time	Percentage of amoxicillin release (%) ± S.D.			
(h)	ALG/0.25%CHI	ALG/0.5%CHI	ALG-GEL/0.25%CHI	ALG-GEL/0.5%CHI
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.5	86.03 ± 0.15	60.60 ± 1.73	66.18 ± 2.01	54.91 ± 9.55
1	90.88 ± 1.81	68.56 ± 0.33	82.83 ± 2.80	74.90 ± 5.54
2	92.05 ± 2.48	75.79 ± 2.82	89.86 ± 2.11	82.40 ± 8.61
3	93.26 ± 1.73	80.03 ± 1.84	88.96 ± 1.02	85.56 ± 5.14
4	95.16 ± 0.33	84.44 ± 1.45	90.25 ± 2.44	85.78 ± 2.35
5	95.93 ± 0.79	87.50 ± 0.10	91.09 ± 5.30	87.91 ± 0.34
6	98.24 ± 1.55	89.49 ± 0.89	91.56 ± 6.16	88.18 ± 1.83

Table C1 (continued) Percentage of amoxicillin release in SGF (pH 1.2 HCl)

Time (h)	Percentage of amoxicillin release (%) ± S.D.			
	ALG/0.5%CHI (AMOX2X)	ALG/0.5%CHI (AMOX3X)	ALG/0.5%CHI (AMOX4X)	ALG/0.5%CHI (AMOX5X)
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.5	56.56 ± 1.89	57.08 ± 5.17	54.60 ± 1.13	54.94 ± 1.47
1	66.92 ± 4.21	67.13 ± 2.89	65.74 ± 0.98	66.93 ± 1.43
2	73.71 ± 5.02	76.47 ± 4.10	75.11 ± 1.60	73.71 ± 3.91
3	78.70 ± 5.88	80.81 ± 3.62	81.99 ± 1.90	81.49 ± 3.16
4	84.04 ± 4.38	84.03 ± 3.14	85.17 ± 0.80	83.61 ± 1.65
5	87.05 ± 1.13	86.96 ± 1.98	87.07 ± 0.84	84.21 ± 1.36
6	87.71 ± 0.47	88.83 ± 0.50	86.92 ± 0.08	84.59 ± 0.59

Table C2 Percentage of amoxicillin release in SIF (pH 7.4 phosphate buffer)

Time (h)	Percentage of amoxicillin release (%) ± S.D.				
	Commercial capsule	ALG	ALG-GEL	ALG/1%CHI	ALG-GEL/1%CHI
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.5	79.45 ± 0.74	50.14 ± 4.68	50.46 ± 1.73	48.72 ± 1.17	46.25 ± 1.68
1	89.89 ± 2.38	70.07 ± 3.02	66.94 ± 1.58	68.89 ± 1.40	61.58 ± 1.44
2	93.79 ± 2.91	92.50 ± 2.36	88.06 ± 1.24	86.32 ± 1.78	87.35 ± 2.55
3	95.41 ± 2.21	96.37 ± 0.22	95.92 ± 0.49	97.40 ± 1.14	94.40 ± 2.63
4	96.47 ± 1.31	98.23 ± 1.36	98.50 ± 1.78	98.02 ± 0.77	96.41 ± 1.81
5	97.99 ± 1.36	97.94 ± 0.96	98.76 ± 0.42	99.11 ± 0.36	98.71 ± 1.75
6	98.97 ± 0.93	98.15 ± 0.48	98.54 ± 0.46	99.09 ± 1.07	98.66 ± 1.45
8	99.68 ± 0.51	99.03 ± 0.19	98.96 ± 0.92	99.72 ± 0.42	99.26 ± 1.19

Table C2 (continued) Percentage of amoxicillin release in SIF (pH 7.4 phosphate buffer)

Time	Percentage of amoxicillin release (%) ± S.D.			
(h)	ALG/0.25%CHI	ALG/0.5%CHI	ALG-GEL/0.25%CHI	ALG-GEL/0.5%CHI
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.5	64.84 ± 1.96	65.32 ± 1.49	55.01 ± 3.58	59.76 ± 4.21
1	80.77 ± 1.46	78.85 ± 1.68	74.09 ± 2.34	76.10 ± 4.33
2	94.33 ± 2.79	91.79 ± 3.45	96.53 ± 0.20	97.87 ± 1.30
3	97.43 ± 0.34	96.53 ± 3.47	98.81 ± 0.47	99.31 ± 0.63
4	99.37 ± 0.28	97.03 ± 2.96	99.73 ± 0.29	98.85 ± 1.37
5	99.57 ± 0.30	97.72 ± 2.23	99.48 ± 0.21	98.66 ± 1.01
6	99.91 ± 0.11	98.10 ± 0.77	99.21 ± 1.19	98.87 ± 0.62
8	99.97 ± 0.05	98.83 ± 1.02	99.14 ± 0.94	99.17 ± 1.43

Table C2 (continued) Percentage of amoxicillin release in SIF (pH 7.4 phosphate buffer)

Time (h)	Percentage of amoxicillin release (%) ± S.D.			
	ALG/0.5%CHI (AMOX2X)	ALG/0.5%CHI (AMOX3X)	ALG/0.5%CHI (AMOX4X)	ALG/0.5%CHI (AMOX5X)
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.5	54.21 ± 3.04	61.61 ± 0.86	57.34 ± 0.64	47.48 ± 0.83
1	74.78 ± 0.37	78.99 ± 0.65	77.66 ± 2.44	66.46 ± 0.97
2	87.87 ± 1.87	91.82 ± 0.33	91.72 ± 0.50	83.88 ± 1.00
3	98.22 ± 1.17	99.70 ± 0.28	99.03 ± 0.97	94.48 ± 0.85
4	99.20 ± 1.23	99.40 ± 0.86	99.41 ± 0.71	98.58 ± 1.39
5	98.21 ± 1.20	98.90 ± 1.17	99.78 ± 0.36	98.98 ± 1.07
6	98.40 ± 2.47	98.55 ± 1.97	99.13 ± 0.15	99.39 ± 0.08
8	98.08 ± 2.71	98.00 ± 2.25	99.02 ± 0.65	99.97 ± 0.05

VITA

Name : Mr.Teerawat Sahasathian

Date of Birth : October 29, 1981

Nationality : Thai

Education : 2000-2004 Bachelor's Degree of Science in Chemistry, Chulalongkorn University, Bangkok, Thailand

2004-2007 Master's Degree of Science in program of Petrochemistry and Polymer Science, Chulalongkorn University, Bangkok, Thailand

Publications : **T. Sahasathian**, T. Kerdcholpetch, A. Chanweroch, N. Praphairaksit, N. Suwonjandee, N. Muangsin, Sustained release of amoxicillin from chitosan tablets, *Archives of Pharmacal Research* 30(4) (2007): 526-531.

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