# โฟมพอลิไวนิลแอซิทาลที่บรรจุยาแอม็อกซิซิลลินสำหรับใช้เป็นเนซัลแพ็ก

นางสาวพรพรรณ พูลแสวง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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# AMOXICILLIN LOADED POLY(VINYL ACETAL) FOAM FOR NASAL PACK APPLICATION

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พรพรรณ พูลแสวง : โฟมพอลิไวนิลแอซิทาลที่บรรจุยาแอม็อกซิซิลลินสำหรับใช้เป็นเนซัลแพ็ก (AMOXICILLIN LOADED POLY(VINYL ACETAL) FOAM FOR NASAL PACK APPLICATION) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : รศ.ดร. ศุภศร วนิชเวชารุ่งเรือง , อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ.นพ.ทรงกลด เอี่ยมจตุรภัทร, 58 หน้า.

ภายหลังจากการผ่าตัดในโพรงจมูกวัสดุเนซัลแพ็ก จะถูกใส่ เข้าไปภายในโพรงจมูกเพื่อดูดซับและห้ามเลือดจากบาดแผลผ่าตัด โดยจะใส่ทิ้งไว้ภายในโพรงจมูกประมาณ 3 ถึง 5 วัน ซึ่งมีปัญหาเรื่องการติดเชื้อแบคทีเรียได้จึงจำเป็นต้องมีการให้ยาปฏิชีวนะ แก่ผู้ป่วยอย่างต่อเนื่อง ดังนั้นวัสดุเนซัลแพ็ก ที่มีสมบัติ ต้านการเจริญของเชื้อแบคทีเรียได้ยาวนานจึงมีประโยชน์อย่างมาก งานวิจัยนี้จึงได้เตรียมวัสดุสำหรับใช้เป็นเนซัลแพ็กจากโฟมพอลิไวนิลแอซิทาล ซึ่งเตรียมได้จากปฏิกิริยาเชื่อมขวางระหว่างพอลิไวนิลแอลกอฮอล์และสารจำพวก แอลดีไฮด์โดยสูตรที่ดีที่สุดสำหรับการเตรียมโฟม คือสูตรที่ใช้สารเชื่อมขวางร่วมกันระหว่าง กลูตารัลดีไฮด์และฟอร์มาลดีไฮด์ โดยใช้เวลาบ่ม 20 ชั่วโมง พบว่าโฟมที่เตรียมได้ มีสมบัติยืดหยุ่น มีโครงสร้างเป็นเซลล์เปิด ขนาดรูพรุนประมาณ 148 ไมโครเมตร นอกจากนี้งานวิจัยนี้ได้ทำการ กักเก็บยาแอม็อกซิซิลลิน ลงในอนุภาคระดับ ไมโคร เมตร ซึ่งเตรียมจาก แคลเซียมอัลจิเนต พบว่า อนุภาค แคลเซียมอัลจิเนต มีประสิทธิภาพการกักเก็บ ยาแอม็อกซิซิลลิน ประมาณ 1 6 % และมีความสามารถในการบรรจุประมาณ 23% โดยอนุภาคที่เตรียมได้สามารถกระจายตัวในชิ้นงานโฟมพอลิไวนิลแอซิทาล โดยใช้พอลิไวนิลแอลกอฮอล์เป็นสารยึดเกาะ โดยยาแอม็อกซิซิลลินถูกปลดปล่อยออกจากโฟมอย่างช้าๆ ในสภาวะเลียนแบบในโพรงจมูก (พีเอช 6.8 และอุณหภูมิ 37 องศาเซลเซียส)

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PORNPAN POONSAVAENG: AMOXICILLIN LOADED POLY(VINYL ACETAL) FOAM FOR NASAL PACK APPLICATION. ADVISOR: ASSOC. PROF. SUPASON WANICHWECHARUNGRUANG, Ph.D., CO-ADVISOR ASSOC. PROF. SONGKLOT AEUMJATURAPAT, M.D., 58 pp.

After nasal cavity surgery, the nasal pack is usually inserted in the nasal cavity to absorb blood and stop bleeding. Bacterial infection may occur since the nasal pack is normally left there for 3-5 days, therefore, oral or instill antibiotic drugs are prescribed. Hence, the nasal pack material with sustained anti-bacterial property will be useful. Here the poly(vinyl acetal) foam was prepared by crosslinking poly(vinyl alcohol) with aldehyde. The optimized formula for foam preparation used the combination of glutaraldehyde and formaldehyde and 20 h of curing time. The obtained foam was flexible and possessed open cell structure with 148 μm diameter approximately. Moreover, amoxicillin was loaded into calcium alginate microparticles. The prepared microparticles showed encapsulation efficiency of 16% and loading capacity 23% approximately. The foam was impregnated with amoxicillin-loaded microparticles through the use of PV(OH) binder. Amoxicillin could be released slowly from the foam when tested in simulated nasal fluid pH 6.8, 37$^{o}$C.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| λ | wavelength |
| % | percent |
| °C | degree Celsius |
| $cm^3$ | cubic centimeter |
| $cm^{-1}$ | unit of wavenumber (IR) |
| h | hour |
| w/v | weight by volume |
| w/w | weight by weight |
| s | second |
| CLFM | confocal laser scanning fluorescence microscopy |
| cP | centipoise |
| µg | microgram |
| µL | microliter |
| ATR-FTIR | attenuated total reflectance-fourier transform infrared |
| kPa | kilopascal |
| mg | milligram |
| min | minute |
| mL | milliliter |
| mV | millivolt |
| MW | molecular weight |
| MWCO | molecular weight cut off |
| PV(OH) | poly(vinyl alcohol) |
| rpm | revolution per minute |
| $cm^{-1}$ | per centimeter |
| SEM | Scanning electron microscope |
| RT | room temperature |
| UV/Vis | Ultraviolet/Visible |
| %EE | encapsulation efficiency |
| %loading | loading capacity |
| DIC | differential interference contrast |

# CHAPTER I
# INTRODUCTION

## 1.1 Nasal pack

Nasal pack is a material used forbleeding control in epistaxis and post-endonasal surgery, for instance, septoplasty, conchotomy, removal polyps and paranasal sinus surgery. It is also applied for internal stabilization after operations involving the cartilaginous-bony skeleton of the nose and for preventing synechiae or restenosis, especially after surgery [1].

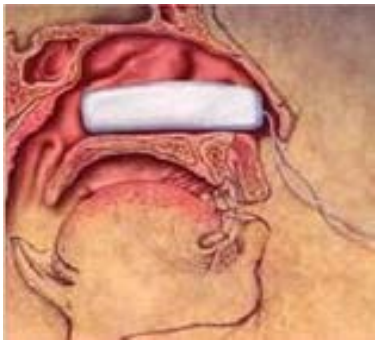


**Figure 1.1** Anterior nasal packing

### 1.1.1 Ideal packing [2]

A packing material for employing in the postoperative of nasal cavity should ideally have the following properties:

- hemostasis
- non-toxic
- non-allergenic
- no foreign body reaction
- no spontaneous dislocation
- effortless insertion and removal
- no patient discomfort or pain
- adaptability to anatomy
- even exertion of pressure on mucosa
- positive influence on postoperative wound healing

- no breathing impairment
- no smell impairment

### 1.1.2 Type of nasal pack [1-3]

*Ribbon gauze packs*

Ribbon gauze pack is open-mesh cotton gauze. It has a low capacity for holding fluid and may harm the soft tissue through its abrasiveness. Since ribbon gauze pack is removed, the abrasive of the gauze pack can lead to trauma and inflammation. Therefore, ribbon gauze packs may be impregnated with soft paraffin or an antibiotic ointment. The ointment component is used to insert or remove of ribbon gauze pack more easily and prevents adherence to soft tissue while the antibiotics are intended to prevent the infection.

**Figure 1.2** Ribbon gauze packs

*Fingerstall packs*

Fingerstall packs are made from latex rubber packed with foam or with gauze. Owing to their non-adherent surface they are easy to insert and remove. The latex pore size is extremely small to prevent ingrowth of viruses or bacteria with the size out of 0.025-0.1 μm range. However, the small pore size lead to obstruction of the nose permits neither breathing nor smelling. In addition, this kind of material is also not suitable for the patients having allergy to latex rubber.

To reduce such limitation, the latex-free fingerstall packs were developed from polyurethane, however, their applications are more difficult.

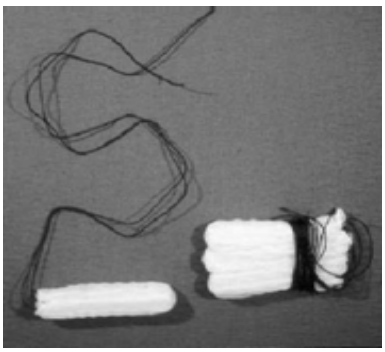


**Figure 1.3** Rubber fingerstall packs

*Foam packs*

Foam packs are favorably used for nasal packing materials. They can be formed into variety of shapes and sizes with great ability to conform to anatomy. The materials also possess high fluid absorption character. In their dry state, the size of the compressed packs is smaller than that at the site of action in the nose after hydration. Water or blood uptake causes a rapid swelling and softening of the foam, this means good absorption of fluid and wound compression at the same time. If the pores of the foam are too large, growth of granulation tissue into the foam will lead to difficult removal, trauma and bleeding. Foam packs can be prepared from various materials such as gelatin, cellulose, polyurethane and poly(vinyl acetal).

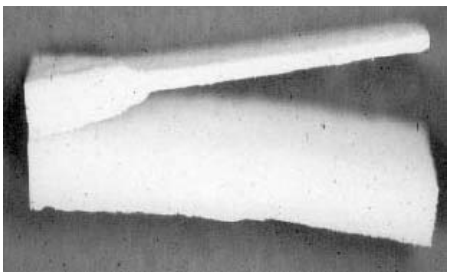
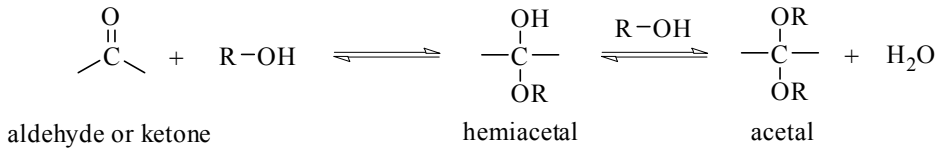


**Figure 1.4** Foam pack: showing under dry condition (upper side) and under moistened condition (lower side)

Poly(vinyl acetal) is prepared by crosslinking between hydroxyl groups of poly(vinyl alcohol) and dialdehydes *via* acetal bond formation. As have been reported, poly(vinyl acetal) is biocompatible, non-toxic, compressible, strong and exceptionally possesses high fluid holding capacity [4].

*Formation of acetal*

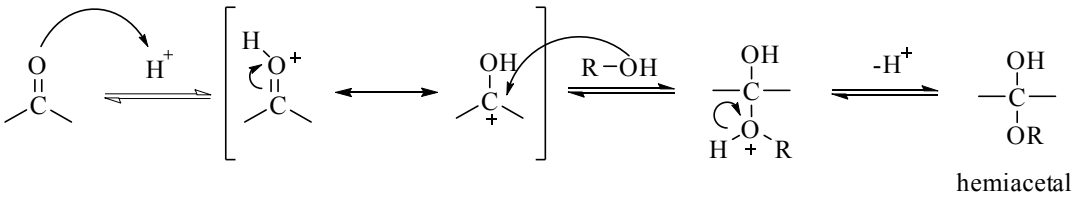
Acetal, two separate oxygen atoms single bonded to a central carbon atom (RO-C-OR), could be obtained from the addition reactions of alcohol with aldehydes/ketones (Scheme 1.1).



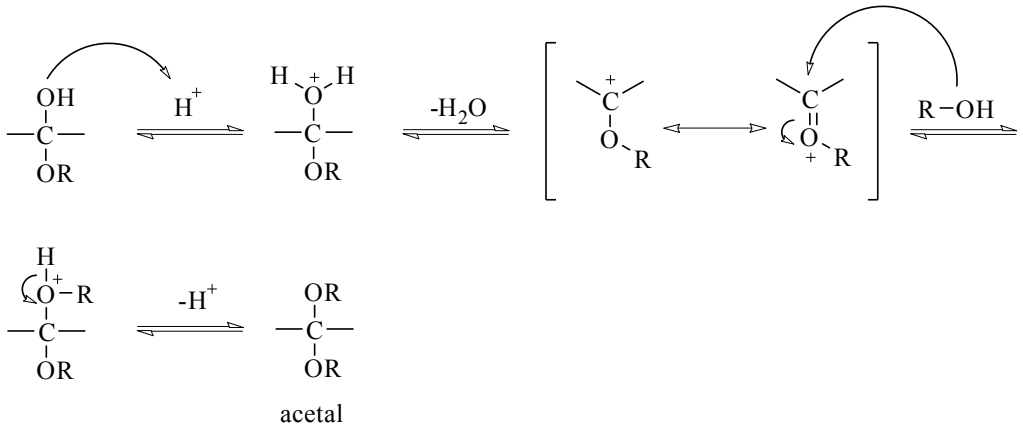

**Scheme 1.1** Acetal formation

Acetal formation occurs *via* acid-catalyzed nucleophilic addition. The mechanism begins with the protonation of the carbonyl oxygen to form resonance-stabilized cation that bears a full positive charge. Then the nucleophile (R-OH) attacks and deprotonation forms the neutral addition product called hemiacetal. After that, an acetal occurs when the hydroxyl group of a hemiacetal becomes protonated and then water losses as a good leaving group leaving a resonance-stabilized cation. Then nucleophilic attack and deprotonation take place to give the acetal (Scheme 1.2) [5-8].

Step 1: Hemiacetal generation



Step 2: Acetal generation




**Scheme 1.2** The mechanism of acetal formation

### 1.1.3 Risk and complication of packing in the post operation

*Infection*

After insertion of nasal pack for 24-48 hours, infection may occur, which lead to toxic shock syndrome (TSS) [9], intracranial abscess [10], foreign body-induced inflammatory, the predisposition toward sinusitis, otitis media because of induced mucosal edema at the openings to the Eustachian tube and the paranasal sinuses, and the bacteraemia and possibly septicemia resulting from the manipulations [11-12]. Hence the oral antibiotic drugs are necessary in the postoperative.

*Toxic shock syndrom (TSS)*

Toxic shock syndrome is a rare, life-threatening complication of bacterial infection. Toxic shock syndrome often results from toxins produced by *Staphylococcus aureus* bacteria, however, the condition may also be caused by toxins produced by group A streptococcus bacteria. The symptoms are a sudden onset with high fever, diffuse rash, vomiting, muscle aches, diarrhea and low blood pressure and can lead to septic shock (Table 1.1). This disease can be fatal and it has been historically associated with the use of contraceptive sponges and superabsorbent tampons. Factors that cause the toxic shock syndrome are the injury of mucous membrane and infected wounds or colonized with the *Staphylococcus aureus* bacteria [2, 9].

Clinical signs of toxic shock syndrome:

- Temperature less than 38.9$^{o}$C
- Diffuse macular or maculopapular rash
- Desquamation of palms or soles 1 to 2 weeks after onset
- Hypotension systolic blood pressure less than 90 mm Hg;
  orthostatic drop in pressure of greater than 15 mm Hg,
  syncope
- Clinical or biochemical involvement of at least 3 organ systems
  - gastrointestinal tract(GIT)
  - Muscular
  - Mucousmembrane
  - Renal
  - Hematologic
  - Central nervous system (CNS)
  - Hepatic
- No other identifiable cause

**1.2 Amoxicillin**

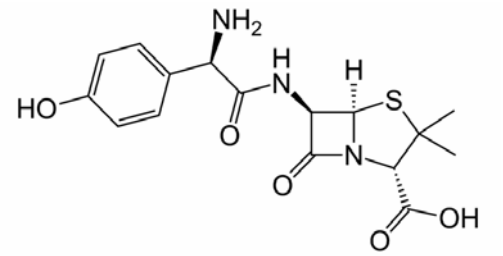



**Figure 1.5** Chemical structure of amoxicillin

Amoxicillin,((2S,5R,6R)-6-[[(2R)-2-Amino-2-(4-hydroxyphenyl)acetyl]amino ]-3,3-dimethyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylic acid), is a penicillin-like antibiotic. It has broad-spectrum of activity including both gram-positive and gram-negative anti-bacteria, and it is used to treat a wide range of bacterial infection such as bronchitis, pneumonia, gonorrhea and ears, nose, throat, urinary tract, and skin infections. It works by inhibiting the synthesis of bacteria cell

wall. Amoxicillin is well absorbed from gastrointestinal tract, it possesses high oral bioavailability (70-90%) with peak plasma levels occurring within 1-2 h [13]. β–lactam ring of amoxicillin may be de-activated by gastric acid and bacterial β-lactamase or penicillinases, leading to an ineffective drug concentration being delivered to the site of infection after oral administration. β-lactamase inhibitor, calvanic acid, is therefore necessary used with high dose of amoxicillin.

Amoxicillin is white or almost white powders, with slight sulphurous odour, compatible with citrate, phosphate and borate buffers. Amoxicillin is soluble in water, sparingly soluble in anhydrous ethanol, very slightly soluble in acetone and has a melting point at 194 °C, molecular formula of $C_{16}H_{19}N_3O_5S$, and molecular weight of 365.40416g/mol. Spectrophotomerically, amoxicillin has a maximum absorption ($\lambda_{max}$) in water at272nm [14].

In the postoperative of nasal cavity, amoxicillin is used not only for oral antibiotic drugs, but also can be used for a topical antibiotic. The topical administration was carried out by soaking the nasal pack in the drug solution before insertion to nasal cavity. However, the patient has to take or instill the drug frequently. This problem could be solved by drug controlled release system.

### 1.3 Controlled release system

Controlled release system offers a sustained release profile compared to the conventional dosage forms. Controlled release system is used in a variety of administration routes, including transdermal, oral and vaginal administration [15].

#### 1.3.1 Advantages of drug controlled release

Controlled release system provides numerous benefits over conventional dosage form. Controlled release dose forms are able to control the rate of drug concentration, the target area of drug administration and maintain therapeutic levels of drug with narrow fluctuations (Figure 1.6). These can reduce toxicity from drug and reduce gastrointestinal side effects. The plasma concentration of drug released from controlled release dosage form fluctuates within the therapeutic range over long period of time. That makes it possible to reduce dosing frequency and decrease total dose of drug administration.

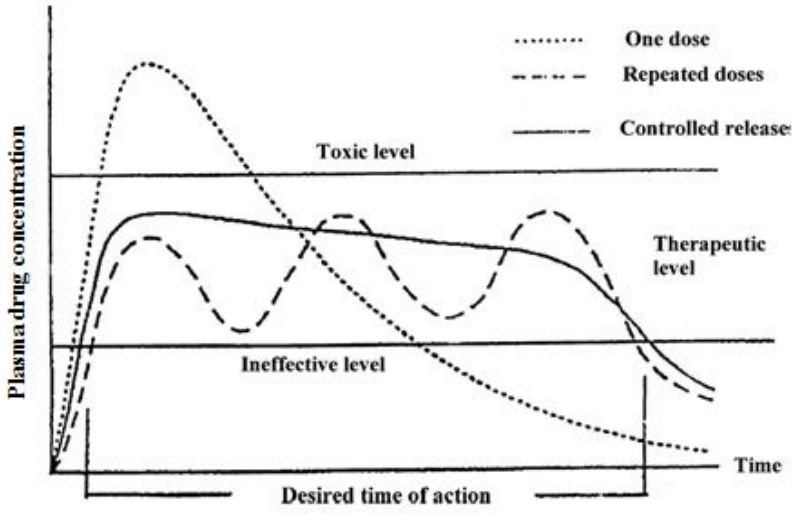



**Figure 1.6** Plasma concentration time profile

### 1.3.2 Controlled release mechanism [15-16]

There are three primary mechanisms by which drugs can be released from a system.

*Diffusion controlled release*

Diffusion release refers to a process by which drug molecules move through the polymer matrix to the external environment, going from regions of higher concentration to lower concentration. However, as the release continues, its rate normally decreases owing to the decrease in the gradient of drug concentration.

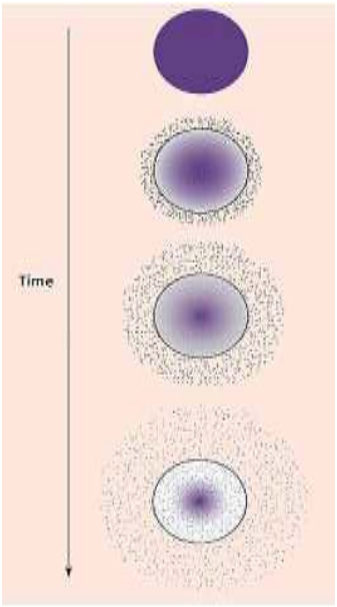



**Figure 1.7** Presentation of diffusion controlled release

*Swelling controlled release*

In this mechanism, swelling of the carrier directly correlates to the drug release. The swelling of the carrier increases the solvent content within the polymer matrix, solubilizing the drugs and enabling them to diffuse through the swollen network into the external environment. Materials popularly used to make swelling carriers are based on hydrogel. The swelling can be triggered by a change in the environment on pH, temperature, ionic strength, etc.

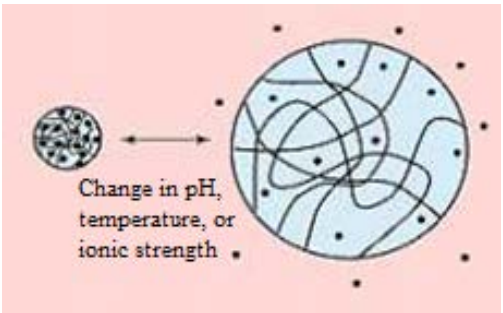



**Figure 1.8** Presentation of swelling controlled release

*Erosion controlled release*

Drug molecules can be released from the matrix owing to erosion of polymers. This mechanism can be classified into 2 types: (1) bulk erosion, the polymer degrades in a fairly uniform manner throughout the polymer matrix (2) surface erosion, the degradation occurs only at the surface of the device.

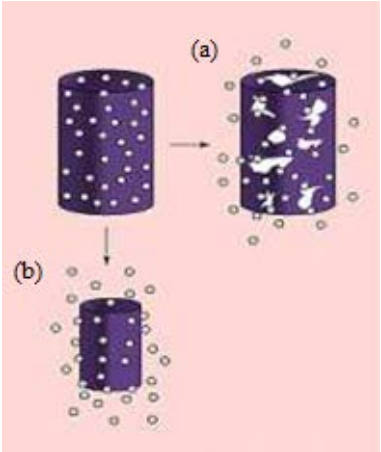



**Figure 1.9** Presentation of erosion controlled release(a) bulk erosion and (b) surface erosion

## 1.4 Alginate

Alginate is a natural polysaccharide polymer isolated from marine brown algae. It is a linear copolymer containing blocks of β-D-manunuronic acid (M) and α-L-guluronic acid (G) residues jointed by 1,4-glycosidic linkages (Figure 1.10) [17-18]. Alginate is widely used in the cosmetic, pharmaceutical and food industry due to its general non-toxicity, biocompatibility, biodegradability, mucoadhesive and gel formation ability. Alginate has been extensively used for biomedical applications such as wound dressing [19], cell culture [20], protein delivery [21], probiotic delivery [22] and drug delivery [23].

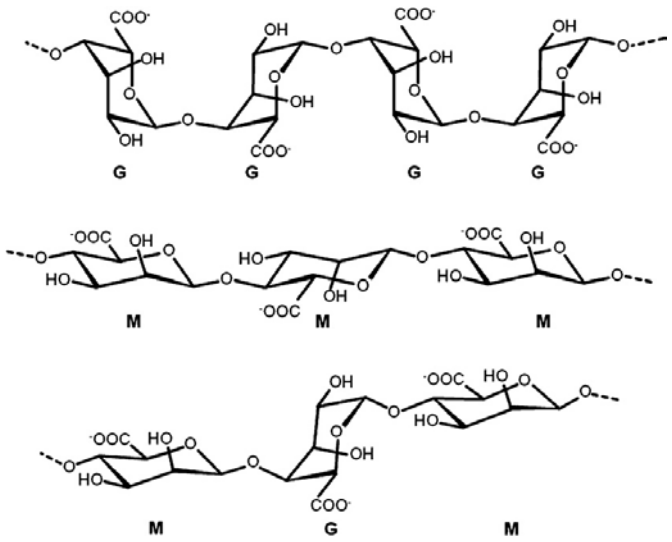
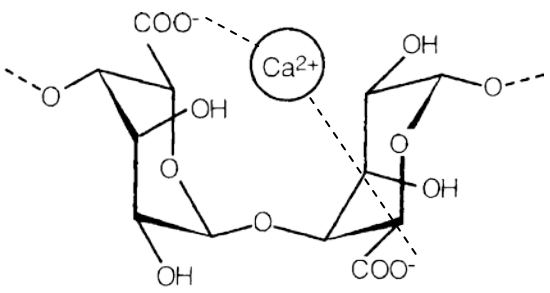



**Figure 1.10** Chemical structures of G-block, M-block, and alternating block in alginate

*Gel formation*

The most important property of alginate is its ability to form gel by addition of divalent or polyvalent cations such as calcium ions ($Ca^{2+}$).This is a result of ionic interaction and intramolecular bonding between the cations and carboxylate groups located on the guluronic acid units, and the self-binding of the guluronic acid units consequently forming a two-dimensional analogue of a corrugated egg-box structure (Figure1.11), resulting in gelation of the solution. The calcium alginate can promote the exchange of calcium ions with sodium ions from body fluid, which enhances the blood-coagulation cascade and cause of hemostasis [24-25].

**(a)**



**(b)**

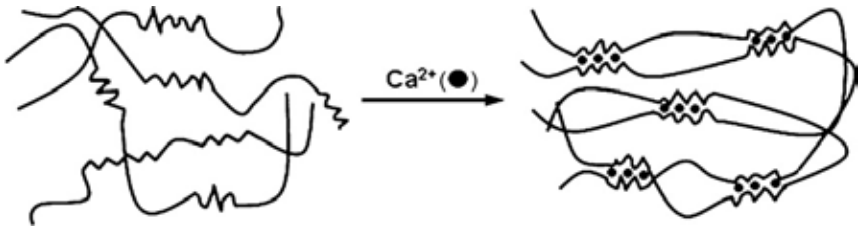



**Figure 1.11** Binding to calcium ion by a G-G block in alginate **(a)** and Egg-box structure of an alginate gel formed by chelation of $Ca2^{+}$ ions**(b)**

## 1.5 Literature reviews of nasal packing materials

*Gelatin foam*

In 1967, Blaug *et al.* [26] fabricated the absorbable foam packing bandage from the gelatin. The gelatin was blended with polyvinyl-pyrrolidone (PVP) to increase the hygroscopic property. The blended solid was dissolved in organic solvent after that, hot water was added to form the absorbable gelatin and the organic solvent was eventually evaporated at the end of the process.

*Polyurethane foam*

In 1985, Lorenz and Ridge [27] prepared the polyurethane-poly (N-vinyl lactam) foam from the linear isocyanate-capped polyether polyol pre-polymer and polyvinyl-pyrrolidone (PVP). The obtained foam was hydrophilic, flexible, and possessed open cell structure. Moreover, the increasing of PVP content gave a high aqueous absorption.

In 2003, Hissink *et al*. [28] fabricated the biodegradable porous absorbent material for packing in antrums or other cavities of human or animal body. The polyether(ester)urethane foam was prepared from 1,4-butanediisocyanate (BDI) and the pre-polymer synthesized by ring opening polymerization of **ε**-caprolactone and glycolide using polyethyleneglycol (PEG) as intiator and stannous octoate as a catalyst. Once the material was employed, it was immediately swell and thus lost its mechanical strength.

*Poly(vinyl acetal) foam*

In 1978, Rosenblatt. [4] prepared the hydrophilic sponge from poly(vinyl alcohol) and formaldehyde. The sponge is biocompatible, non-toxic, compressible, strong and exceptionally high fluid absorptive.

In 2001, Cercone. [29] prepared poly(vinyl acetal) sponge by crosslinking poly(vinyl alcohol) with aldehyde, dialdehyde or dicarboxylic acid. Water absorption could be increased by adding the poly(vinylpyrrolidone)into the reaction during crosslinking process.Non adherent surface was prepared by atomized spray of an poly(vinyl acetate)/poly(vinyl alcohol) copolymer aqueous solution on the poly(vinyl acetal) foam surface.

## 1.6 Literature reviews of alginate microparticles

In 2007, Rastogi *et al*. [23] prepared microspheres containing isoniazid by emulsification method, using sodium alginate as the hydrophilic carrier. The entrapment efficiency was found to be in a range of 40-91%. The increase incrosslinker concentration caused the increase in the entrapment efficiency and extent of drug release. Nearly 26% of isoniazid was released in SGF pH 1.2 in 6 h and 71.25% in SIF pH 7.4 in 30 h. Thus, this method could be useful for encapsulation of hydrophilic bioactives without much loss of the active principle.

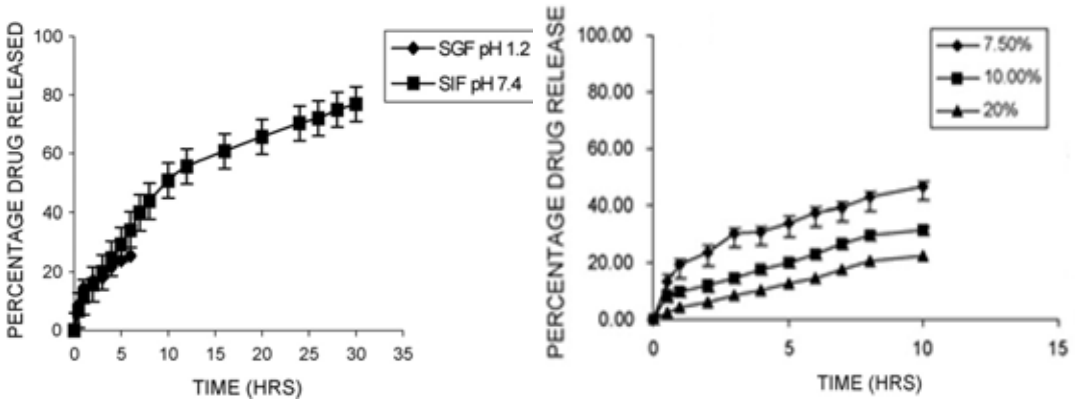



**Figure 1.12** Drug release profile of optimized formulation in SGF pH 1.2 and SIF pH 7.4 (a) and under variable crosslinker concentration in SIF pH 7.4 (b) [23]

In 2008, Maja *et al*. [30] prepared Eudragit®s100-Ca-alginate-chitosan microparticles loading budesonide by one-step spray-drying process. The microparticles were spherical with mean size of 4.05-5.36 μm with narrow unimodal distribution and the particles possessed positive surface charge. The encapsulated budesonide was slowly released in simulated gastric fluid (pH 2.0) and quickly released in simulated intestinal fluid (pH 7.4). The microparticles is a stable candidate for oral delivery of budesonide with controlled release properties for local treatment of inflammatory bowel diseases.

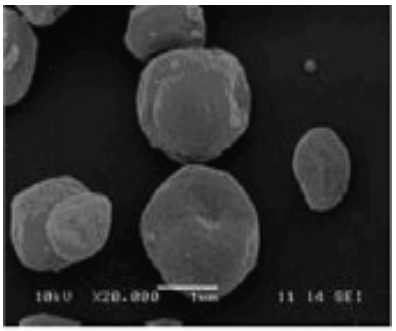
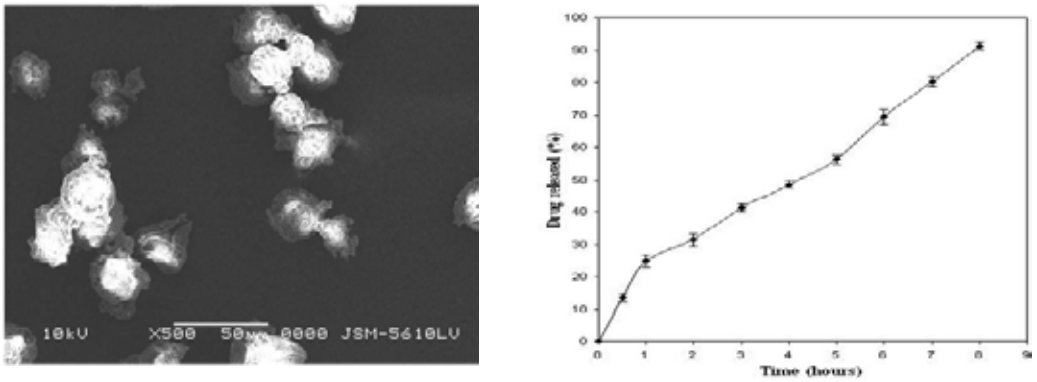


**Figure 1.13** SEM photograph of Eudragit coated chitosan–alginate microparticles Containing BDS [30]

In 2009, Patil *et al.* [31] developed the alginate mucoadhesive microspheres of carverdilol for nasal delivery. The microspheres were prepared by water-in-oil (w/o) emulsification technique. The prepared microspheres were found to be spherical shape with nearly smooth surfaces with the size of 26.36-54.32 μm, favorable sizes for intranasal absorption. The encapsulation efficiency was in the range of 36.62-56.18%. *In vitro* mucoadhesion was performed using sheep mucosa and was in a range of 69.25-85.28%. In vitro release study indicated non-Fickian or anomalous type of transport.




**(a)** **(b)**

**Figure 1.14** SEM photographs of drug-loaded sodium alginate microspheres (a) and in vitro release profile of optimized batch (F1) of alginate microspheres (b) [31]

In 2011, Zhang *et al*. [21] prepared narrow size alginate-chitosan microspheres containing insulin by membrane emulsification technique. The insulin-loading capacity was 56.7%. Under the pH condition of gastrointestinal environment, only 32% of insulin was released during the simulated transit time of drug (2 h in the stomach and 4 h in the intestinal). While under the pH condition of blood environment (pH 7.4), insulin release was stable and the insulin concentration was sustained for a long time (14 days).

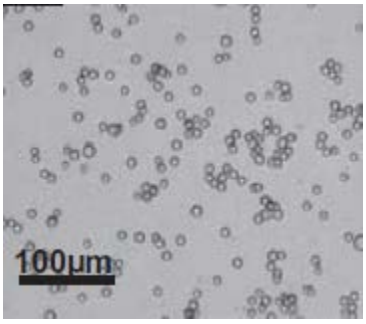
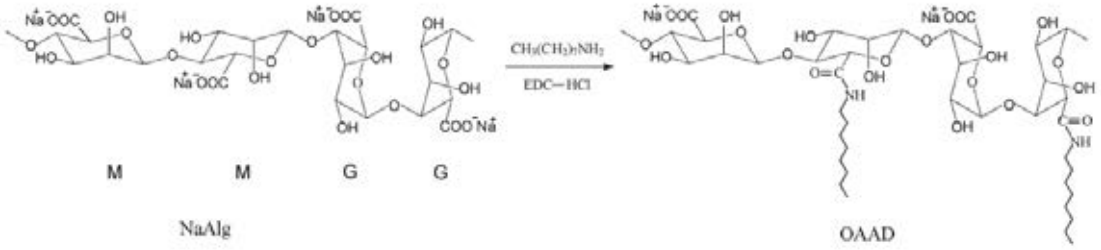



**Figure 1.15** Optical microscope photograph of insulin loaded alginate-chitosan microspheres [21]

In 2011, Yang *et al*. [32] synthesized the amphiphilic alginate derivative by grafting of octylamine *via* coupling reaction (Scheme 1.3). λ-Cyhalothrin was encapsulated with the obtained alginate derivative by emulsification-gelation

technique. The obtained microcapsules were spherical and with λ-cyhalothrin as core and alginate derivative as shell.




**Scheme 1.3** Synthesis ofoctyl-grafted amphiphilic alginate-amide derivative [32]

As presented above, alginate is an appropriate polymer for entrapment and controlled release of drug. Here, amoxicillin, an antibiotic drug, was loaded into alginate polymeric particles. The amoxicillin loaded particles were adhered to poly(vinyl acetal) foam pores and the release of amoxicillin from the foam was investigated.

## 1.7 Research Goal

The goal of this research is to prepare nasal packing material with sustained anti-bacterial property. It was fabricated based on poly(vinyl acetal) foam and the foam was impregnated with amoxicillin-loaded alginate microparticles. The release profile of the amoxicillin from the foam was studied under simulated nasal cavity environment condition pH 6.8 at 37$^{o}$C.

The work included:

1. Preparation of Poly(vinyl acetal) foam
2. Preparation of amoxicillin loaded microparticles
3. Impregnation of microparticles to the foam
4. Controlled release study of amoxicillin from the foam

The model of the product was showed in figure 1.16.

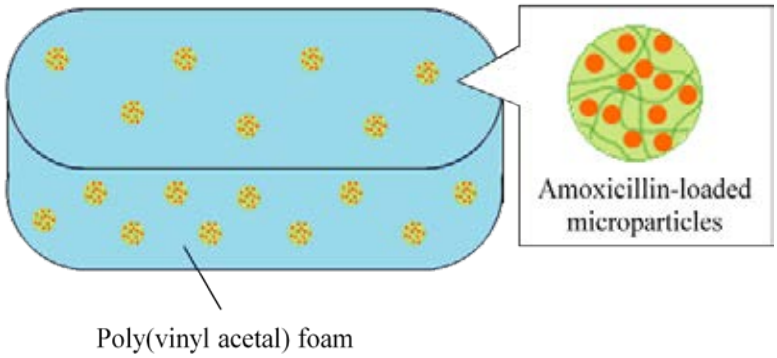



**Figure 1.16** The model of the product

# CHAPTER II

# EXPERIMENTAL

## 2.1 Materials and Chemicals

Amoxicillin was provided by the Government Pharmaceutical Organization (Bangkok, Thailand). Alginic acid sodium salt (viscosity of 2% solution at 25$^{o}$C 250 cps) and poly(vinyl alcohol) (PV(OH)) (MW 31,000-50,000, 87-89% hydrolyzed and MW 124,000-186,000, 87-89% hydrolyzed) were purchased from Sigma Aldrich (Steinheim, Germany). Formaldehyde and glutaraldehyde were purchased from Merck (Darmstadt, Germany). Calcium chloride was purchased from Carlo Erba (Milano, Italy). TritonX-100 was purchased from Acros Organics (Geel, Belgium). Vegetable oil was purchased from Thai Vegetable Oil Public Co., Ltd (Bankok, Thailand). 5(6)-Carboxytetramethylrhodamine (TAMRA) was purchased from FlukaChemie AG (Deisenhofen, Germany). Centrifigal-filtering device (MWCO 100,000, Amicon Ultra-15) was purchased from Merck Millipore (County cock, Ireland). Merocel®, standard nasal pack foam, was purchased from Medtronic Xomed (Jacksonville, U.S.A.). All other solvents and chemicals used were analytical grade and were used without further purification.

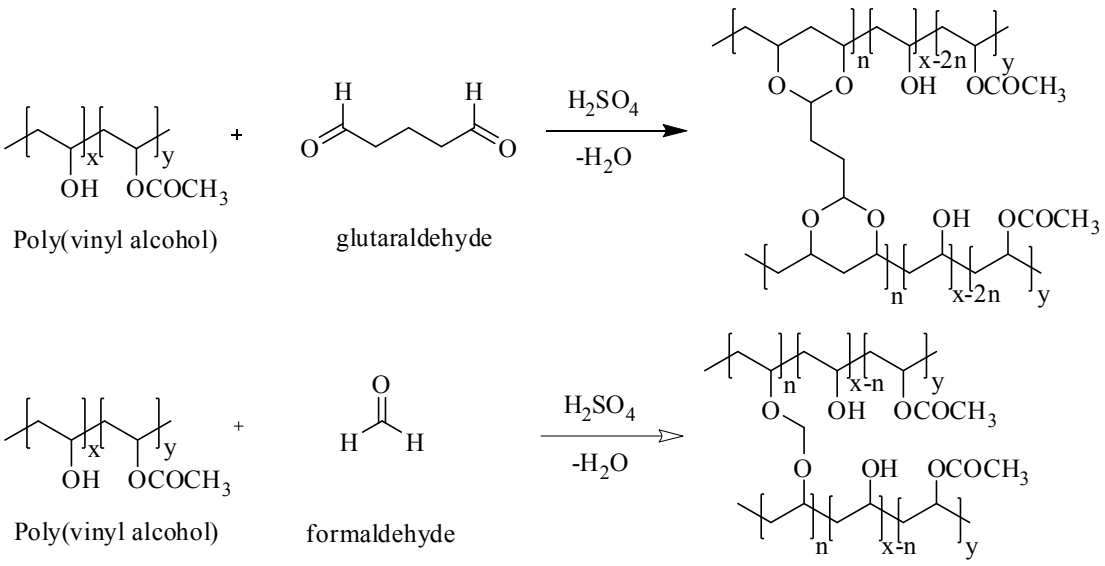
## 2.2 Instruments and Equipments

Attenuated total reflectance-Fourier Transform Infrared (ATR-FTIR) spectra were obtained on a Nicolet 6700 ATR-FTIR spectrophotometer (Thermo Electron Corporation, Madison, WI, USA). The UV absorption spectra were measure with a UV 2500 UV/Vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan) using a quartz cell with 1 cm path-length with operating at 25$^{o}$C. The suspension of particles was freeze-dried using Freeze-Dry/Shell Freeze System Model 7753501 (Labconco Corporation, Kansus, MI, USA). Homogenization was carried out using T25 digital ultra-turrax homogenizer (IKA, Staufen, Germany). SEM images were acquired by JEM-6400 scanning electron microscope (JEOL, Tokyo, Japan). Centrifugation was carried out on GL21M high-speed refrigerated centrifuge (Changsha Yingtai instrument, Hunan, China).

**2.3 Preparation of poly(vinyl acetal) foam**

PV(OH) (MW 31,000-50,000) (4.75 g) was dissolved in 40.5 mL of warmed distilled water using a mechanical stirrer. The PV(OH) solution was then cooled to room temperature. Then 26 μL of Triton X-100 was added into the PV(OH) solution and the mixture was agitated using homogenizer for 10 min. After that, 7 mL of 50% (w/w) sulfuric acid was added, and the mixture was agitated for 10 min. Thereafter, aldehyde solution was added slowly to the mixture and continuously agitated for 10 min. Then the mixture was poured into the mold for curing. The type, concentration and amount of aldehyde, processing temperature and cure time were varied as shown in Table 2.1.

Only F9 formula (Table 2.1) was prepared without heat. In this formula, the poly(vinyl acetal) foam was preparedusingthe combination of glutaraldehyde and formaldehyde. After adding 5 mL of 37% (w/w) formaldehyde solution, 0.75 mL of 5% (w/w) glutaraldehyde solution was slowly dropped into the mixture and continuously agitated. The agitation was stopped when the mixture started to turn viscous, then the mixture was immediately poured into the mold and cure at room temperature for 20 h.




**Scheme 2.1** Synthesis of poly(vinyl acetal)

**Table 2.1** Used conditions for preparing poly(vinyl acetal) foam

| Formula | Aldehyde | Concentration of the adding aldehyde solution(w/w) | Volume of the adding aldehyde solution (mL) | Processing temperature ($^{o}$C) | Cure time (h) | |
|---|---|---|---|---|---|---|
| | | | | | at 60$^{o}$C | at room temperature |
| F1 | glutaraldehyde | 9.25% | 5 | room temperature | 1 | 19 |
| F2 | | 18.5% | 5 | room temperature | 1 | 19 |
| F3 | | 37% | 5 | room temperature | 1 | 19 |
| F4 | formaldehyde | 37% | 5 | room temperature | 1 | 19 |
| F5 | | 37% | 10 | room temperature | 1 | 19 |
| F6 | | 37% | 5 | 60$^{o}$C | 1 | 19 |
| F7 | | 37% | 5 | 60$^{o}$C | 1 | 24 |
| F8 | | 37% | 5 | 60$^{o}$C | 1 | 29 |
| F9 | glutaraldehyde and formaldehyde | 5% glutaraldehyde and 37% formaldehyde | 0.75 mL of glutaraldehyde and 5 mL of formaldehyde | room temperature | - | 20 |

The obtained poly(vinyl acetal) foam was washed with distilled water until the pH of the rinsed water was 5. Then it was dried in oven at 80$^{o}$C for 1 h and kept in dessicator until completely dry. The poly(vinyl acetal) foam was characterized by SEM and ATR-FTIR. Moreover, density and compressive property of prepared foam were also determined.

To determine the density of the foam product 1 $cm^3$ cubic shape piece of the material was weighted and the density was calculated. Three speciments for each foam sample were measured and average.

The compressive property of the foam piece was investigated by the universal testing machine model EZ-S, load cell 500 N and fixed test rate at 5 mm/min (Shimadzu Corporation Kyoto, Japan).

**2.4 Preparation of amoxicillin loaded in calcium alginate microparticles**

The microparticles were prepared from the emulsification gelation method [23, 33]. Amoxicillin (200 mg) was dispersed into 10 mL of 1% (w/v) aqueous sodium alginate solution. Then the solution was added into 120 mL of vegetable oil containing 10 µL of Triton X-100 and homogenized by homogenizer at 11000 rpm for 30 min. After that, 25 mL of 2 M calcium chloride was added to the continuously stirred mixture and the stirring was continued for 30 min, before being centrifuged at 5000 rpm (2687 g). The water phase containing the microparticles was collected by filtration in vacuum and washed two times with 10 mL of 50% (v/v) ethanol to remove remained oil and one time with 20 mL of distilled water. Dry microparticles were obtained by lyophilization.

The obtained microparticles were characterized by ATR-FTIR, Confocal Laser Scanning Fluorescence Microscopy (CLFM), Zetasizer and UV-Vis spectrophotometer.

The morphology and particle size of microparticles were characterized by CLFM. The microscope images was aquired by Nikon Digital Eclipse C1si Confocal Microscope (Tokyo, Japan) in the differential interference contrast (DIC) mode. A drop of dispersed microparticles was placed on a glass slide and cover with cover slip.

The zeta potential values of microparticles were determinated by a Zetasizer nanoseries model S4700 (Malvern Instruments, Worcestershire, UK) at 25$^{o}$C.

Samples were measured in folded capillary cells integrated with gold electrodes.The number of runs in each measurement was automatically determined by the software.

Entrapment efficiency percentage (% EE) and loading percentage of the microparticles were determined as followed. The all of freshly prepared microparticles were added into 25 mL of 4 M sodium chloride solution and stirred overnight. After that, the dispersion was centrifugally filtered by centrifugal-filtering devices with MWCO of 100,000 (Merck Millipore) at 5,000 rpm (2687 g) for 20 min. The filtered solution was determined for the amount of amoxicillin by UV-Vis spectrophotometer at wavelength 272 nm with the aid of calibration curve. Calibration curve was created from a series of amoxicillin solutions freshly prepared in 4 M sodium chloride solution at concentrations 10, 20, 40, 60, 80 and 100 ppm. The entrapment efficiency percentage and loading percentage were calculated according to the following equations:

$$\% \text{ Entrapment efficiency (\%EE)} = \frac{\text{Weight of entrapped amoxicillin}}{\text{Weight of used amoxicillin}} \times 100$$

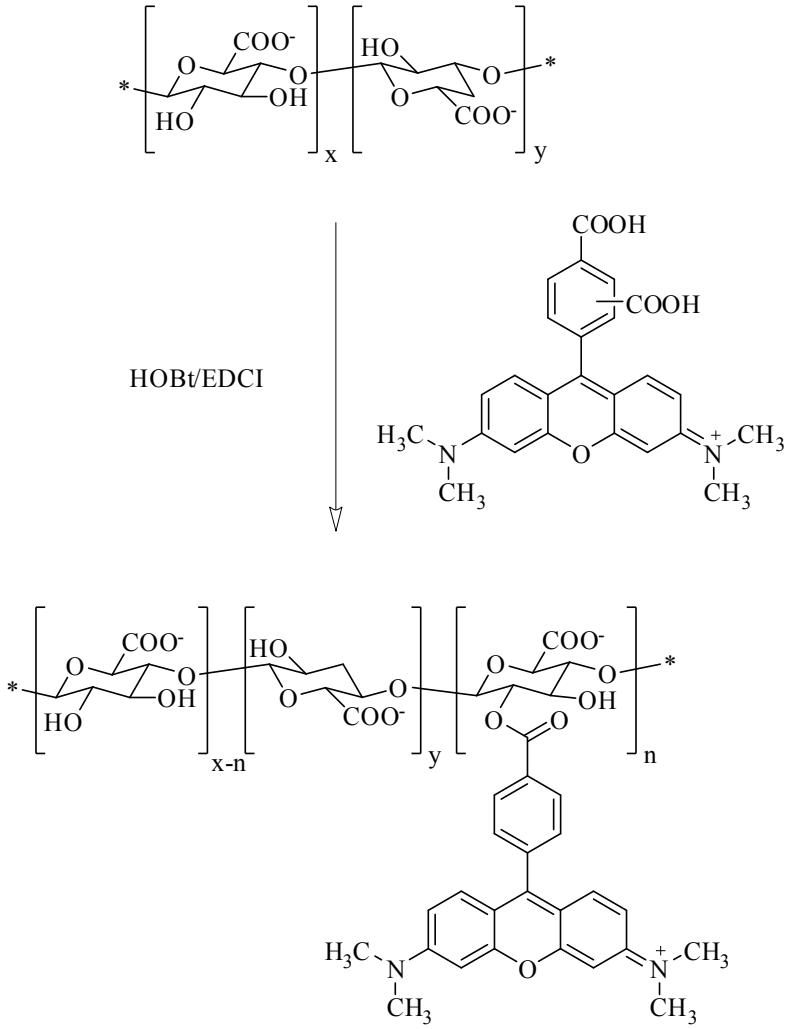
$$\% \text{ Loading} = \frac{\text{Weight of entrapped amoxicillin}}{\text{Weight of entrapped amoxicillin} + \text{Weight of polymer}} \times 100$$

All measurements were made in triplicate for each batch and mean values ± standard deviations were reported.

**2.5 Impregnation of microparticles into the foam**

Fifty milligrams of amoxicillin-loaded alginate microparticles (contain amoxicillin 11.25 mg) were dispersed in 5 mL of 1000 ppm PV(OH) (MW 124,000-186,000) solution. Poly(vinyl acetal) foam piece (4x1x1 $cm^3$) was dipped into the above prepared PV(OH) solution with agitation untill all the dispered microparticles were absorbed into the foam pores. After that, the impregnated foam was dried in vacuum oven at 60$^oC$ for 1 h and kept in dessicator. The obtained product was characterized by SEM. Moreover, density and compressive property of the impregnated foam were also determined.

## 2.6 Fluorescence labeled microparticles




**Scheme 2.2** Synthesis of 5(6)-carboxytetramethylrhodamine-grafted-alginate

To track the location of alginate particles in the foam, the 5(6)-carboxytetramethylrhodamine-graft-alginate (TAMRA-alginate) was synthesized based on the method of Tachaprutinun [34] (Scheme 2.2).The carboxylic group of the fluorophore 5(6)-carboxytetramethylrhodamine (TAMRA) was coupled with the hydroxyl group of the alginate using 1-ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride(EDCI) as a coupling agent. Firstly, EDCI (16.3 mg) in 5 mL of dimethylformamide (DMF) was added into the solution of TAMRA (19.86 mg) in 20 mL DMF under $N_2$ atmosphere. The mixture was stirred at 0°C for 30 min and then poured into the aqueous alginate solution (1.0g in 250 mL water), followed by the

addition of 1-hydroxybenzotriazole (HOBt) (10.0 mg) in 5 mL DMF. After that, the mixture was stirred at room temperature overnight. The mixture was then transferred into the dialysis Cellulose tubular membrane bag (MWCO 12,000-14,000, 7.6 cm. flat width, Sigma-Aldrich) dialyzed against water, freeze-dried and then subjected to ATR-FTIR.

The TAMRA-alginate was used to prepare amoxicillin loaded microparticles and the obtained labeled microparticles were impregnated into the foam as previously described. The obtained microparticles-loaded foam was subjected to CLFM in fluorescent mode. Excitation and detection wavelength were carried out at 561 nm and 595 nm, respectively.

**2.7 Controlled release study of amoxicillin**

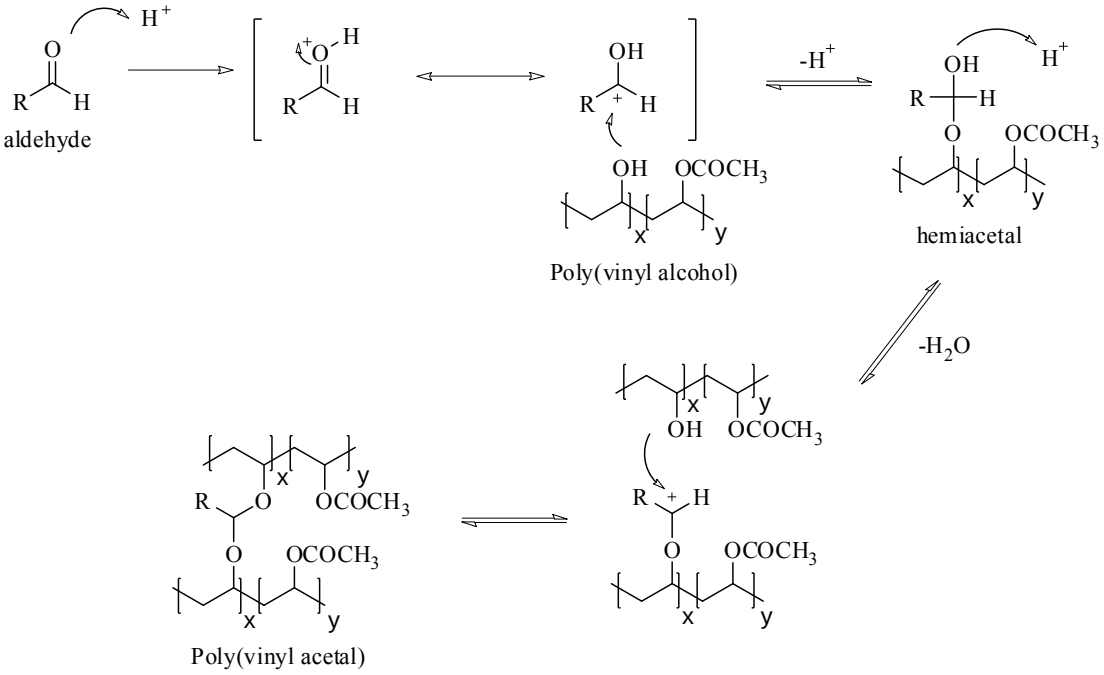
Drug release from the impregnated foam (4x1x1 $cm^3$) was studied in 20 mL of simulated nasal fluid pH 6.8 as the release medium [35]. There lease study was conducted at 37$^oC$ without agitation. Five milliliters sample withdrawn at predetermined time points (0, 1, 2, 4, 6, 24, 30, 48, 72 and 96 h) was filtered (using centrifugal-filtering devices with MWCO 100,000 at 5000 rpm (2687 g) for 20 min) and 5 mL of fresh release medium was added to maintain a constant volume. The concentration of amoxicillin in the aliquots was determined by UV-visible spectrophotometer at 272 nm with the aid of calibration curve. Calibration curve was created from a series of amoxicillin solutions freshly prepared in simulated nasal fluid solution at concentrations 10, 20, 40, 60, 80 and 100 ppm.

# CHAPTER III

# RESULTS AND DISCUSSION

## 3.1 Preparation of poly(vinyl acetal) foam

The poly(vinyl acetal) was prepared by crosslinking of PV(OH) polymer chains with dialdehydes, glutaraldehyde or formaldehyde, under acidic condition. The mechanism of acetal bond formation between PV(OH) and aldehyde is shown in Scheme 3.1.




**Scheme 3.1** The mechanism of acetal bond formation between PV(OH) and aldehyde

To prepare the foam, air bubbles were firstly stably induced into PV(OH) solution with the help of Triton X-100, a foam stabilizer. The PV(OH) solution was agitated with homogenizer to make foam (air droplets in solution). Aldehyde solution was then added into the rich air bubbles mixture to crosslink the polymer chains *via* acetal bond formation in order to solidify or set the foam. The remained crosslinkers, glutaraldehyde and formaldehyde will be toxic, thus, it is vital to get rid of all these excess. Since there compounds are water-soluble and volatile, they could be cleaned

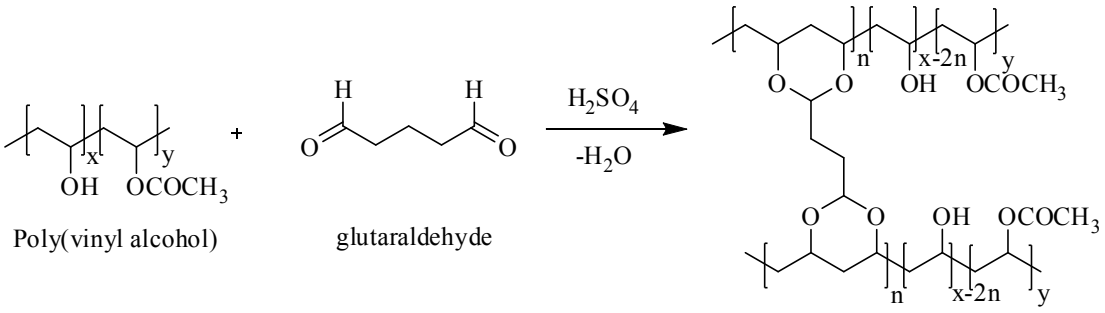
out by simply washing the obtained foam in distilled water until the pH of the rinsed water was almost neutral followed by drying the foam in an oven at 80$^{o}$C for 1 h.

The good nasal packing material should be soft, expandable, fast wicking, non-abrasive and non-friable [4, 29]. Hence, various of poly(vinyl acetal) foam formulas were prepared to get foam with the required characterers. The hardness, porosity, flexibility or compressibility and water absorption of each formula were observed (Table 3.1).

**Table 3.1** Foam properties of nine foam formulations

| Formula | Final concentration of aldehyde (w/w) | Processing temperature ($^{o}$C) | Cure time (h) | | Observation properties |
|---|---|---|---|---|---|
| | | | at 60$^{o}$C | at RT | |
| F1 | 0.84% glutaraldehyde | RT | 1 | 19 | - rigid white foam<br>- soft and friable when soaked in water<br>- broke easily into small pieces when compressed |
| F2 | 1.67% glutaraldehyde | RT | 1 | 19 | |
| F3 | 3.34% glutaraldehyde | RT | 1 | 19 | |
| F4 | 3.43% formaldehyde | RT | 1 | 19 | - non porous product<br>- low liquid absorption |
| F5 | 6.86% formaldehyde | RT | 1 | 19 | |
| F6 | 3.43% formaldehyde | 60$^{o}$C | 1 | 19 | -soft, flexible and compressible when soaked in water<br>- rapid liquid absorption<br>- open cell structure |
| F7 | 3.43% formaldehyde | 60$^{o}$C | 1 | 24 | |
| F8 | 3.43% formaldehyde | 60$^{o}$C | 1 | 29 | |
| F9 | 0.065% glutaraldehyde and 3.43% formaldehyde | RT | - | 20 | -soft, flexible and compressible when soaked in water<br>- rapid liquid absorption<br>- open cell structure with the diameter of 147.78 ± 74.77 µm |

*3.1.1 Using glutaraldehyde as a crosslinker*



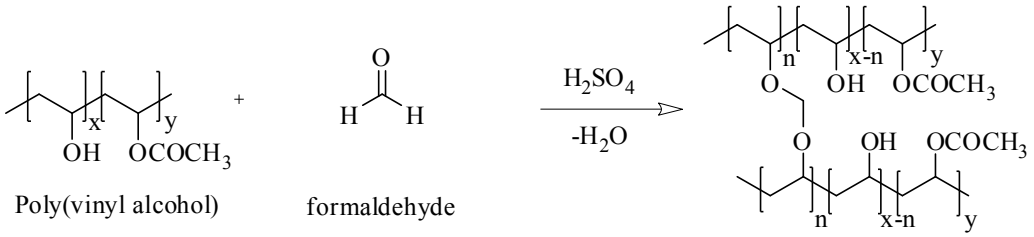

**Scheme 3.2** Synthesis of poly(vinylacetal) using glutaraldehyde

Three formulations using glutaraldehyde as crosslinker were prepared, F1, F2 and F3 with 9.25%, 18.5% and 37% (w/w) glutaraldehyde solution, respectively (Table 2.1). After glutaraldehyde solution was added into the solution containing of PV(OH), Triton X-100 and sulfuric acid, the reaction proceeded very fast as the rich bubbles mixture turned to sticky gel in a short time. The obtained dry foams were white and rigid. Although the obtained foams were soft after soaking in water, they were friable and broke into small pieces easily when being compressed (Figure 3.1).

As seen in the scheme 3.2, the cyclic 6-membered rings were introduced into the structure of the polymer network and all polymer chains then connected to one another through this 6-membered ring acetal linkage, this lead to the rigid polymer chains. Thus, the obtained products were friable and low flexible. Hence, the products of F1, F2 and F3 formulas were not suitable for using as a nasal pack.

**Figure 3.1** The soaked foam F3 formula after compression foam

*3.1.2 Using formaldehyde as a crosslinker*




**Scheme 3.3** Synthesis of poly(vinyl acetal) using formaldehyde

Formaldehyde was used as a crosslinker in F4 to F8 formulas. F4 and F5 formula were prepared without heat using 5 and 10 mL of 37% (w/w) formaldehyde solution, respectively (Table 2.1). After formaldehyde solution was added into the solution containing PV(OH), Triton X-100 and sulfuric acid, the reaction proceeded slowly. The obtained foams were dense and non-porous (Figure 3.2).

The slow crosslinking reaction led to dense product because the air bubbles collapsed before crosslinking. The dense products were hard and possessed low liquid absorption character. Thus the obtained products of F4 and F5 formulas were not suitable to use as a nasal pack.

**Figure 3.2** The dense product prepared from F4 formula

*Heating and curing time*

Comparing F4 and F6 formula, although both formulas were nearly alike, heating at 60$^{o}$C during foam preparation in F6 formula played an important role to fabricate foam owing to thermal acceleration of the crosslinking reaction. Hence, the PV(OH) could be crosslinked before the collapse of the air bubbles, leading to porous

product. The prepared foam (F6 formula) was soft and it also showed good and rapid liquid absorption.

Further experiment on the formula prepared with heat was carried out. F6, F7 and F8, prepared with different cure time at room temperature of 19, 24 and 29 h, were investigated. All three products possessed similar property such as white foam appearance, fast liquid absorption, soft and flexible soaked foam (Figure 3.3). The cure time did not affect their property much.

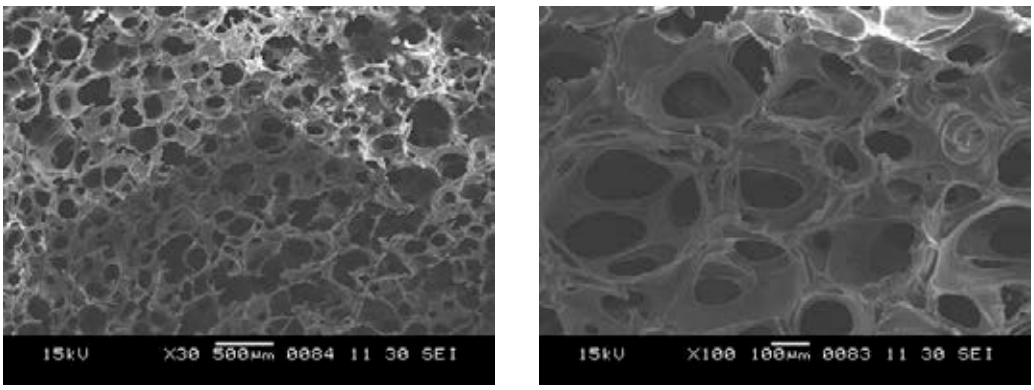
**Figure 3.3** The appearance of foam F6 formula

*3.1.3 Using the combination of glutaraldehyde and formaldehyde as a crosslinker*

F9 formula was prepared without heat but with the combination of glutaraldehyde and formaldehyde. The obtained product was white, soft and compressible with fast liquid absorption.F9 product was similar to F6, F7 and F8 products.

Although F9 foam was similar to F6, F7 and F8 foams, the F9 foam was more convenient to prepare because no required heat was needed during processing and curing. The F9 foam was suitable to be used as a nasal pack because it was soft, porous, flexible and possessed rapid liquid absorption characteristic.

SEM images (Figure 3.4 (a) and (b)) showed that the obtained F9 poly(vinyl acetal) foam had open cell structure with an average diameter of 147.78 ± 74.77 μm (averaged from 100 pores in a SEM images).




**(a)** **(b)**

**Figure 3.4** SEM images of poly(vinyl acetal) foam prepared from F9 formula at 30x (a) and 100x magnification (b)

The ATR-FTIR spectrum of poly(vinyl acetal) foam (Figure 3.5 (a)) showed two peaks at 2777 and 2846 $cm^{-1}$ corresponded to C-H stretching of methylene groups ($-CH_2-$) from the crosslinkers (glutaraldehyde and formaldehyde). The peak at 1168 $cm^{-1}$ and 1007 $cm^{-1}$corresponded to C-O-C stretching and C-O stretching of ether, respectively. Combining to the slight presence of peak at 1088 $cm^{-1}$ assigned to C-O stretching of secondary alcohol, occurrence of acetal formation was confirmed. Moreover, the C=O stretching peak of ester from vinyl acetate group at 1563 $cm^{-1}$ was also absent (Figure 3.5(a)) because ester group was possibly hydrolyzed by sulfuric acid and water during foam preparation.

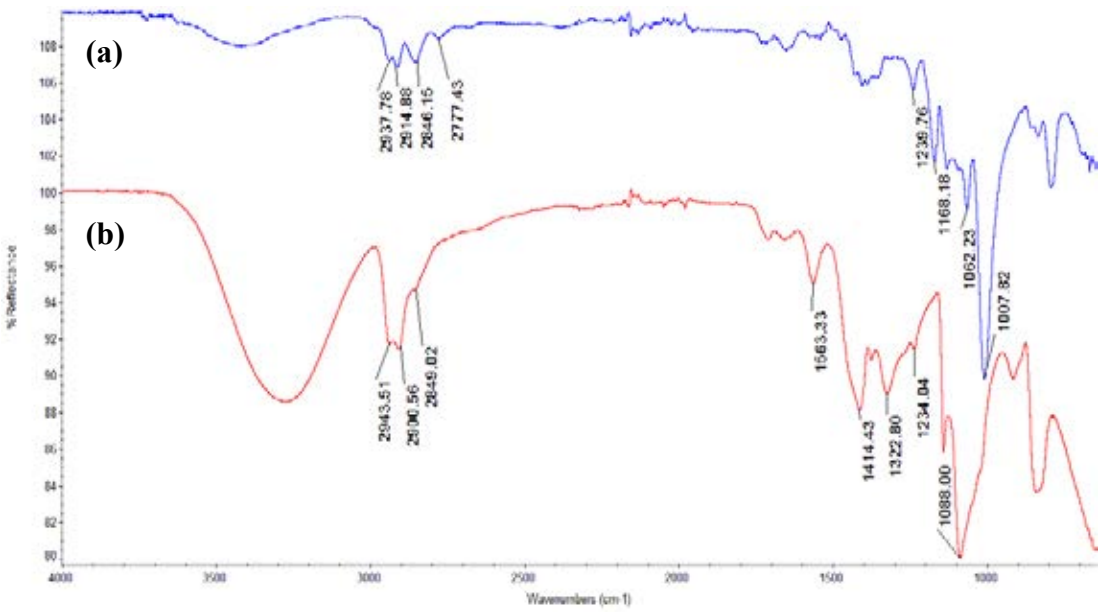



**Figure 3.5** ATR-FTIR spectra of poly(vinyl acetal) foamprepared from F9 formula (a) and poly(vinyl alcohol) (b)

## 3.2 Preparation of amoxicillin loaded in calcium alginate microparticles

### 3.2.1 Entrapment of amoxicillin into microparticles

Amoxicillin was loaded into the calcium alginate microparticles by emulsification gelation method. The amoxicillin was firstly dispersed in sodium alginate aqueous solution. Then the vegetable oil was added into the mixture and homogenized to form aqueous microdroplets containing alginate and amoxicillin in the continuous oil phase. The calcium chloride solution was added into the system. The carboxylate groups ($-COO^-$) of alginate chain were then crossinked with calcium ions ($Ca^{2+}$), and the amoxicillin was entrapped in the calcium alginate microparticles (Figure 3.6).

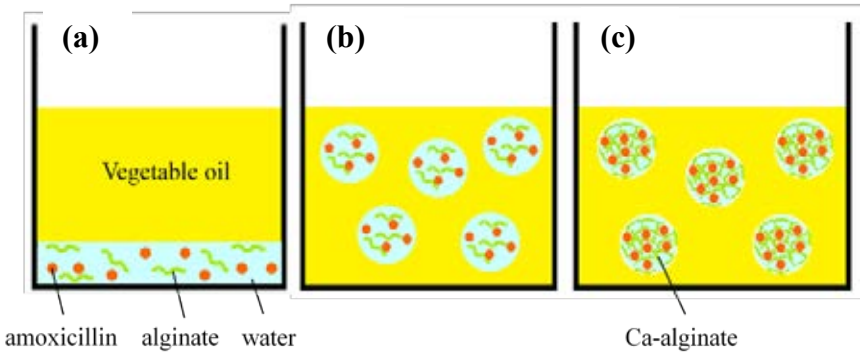



**Figure 3.6** Schematic model showing the microparticles formation steps: separated phase before homogenization (a), under homogenization (b) and after gelation with calcium chloride (c)

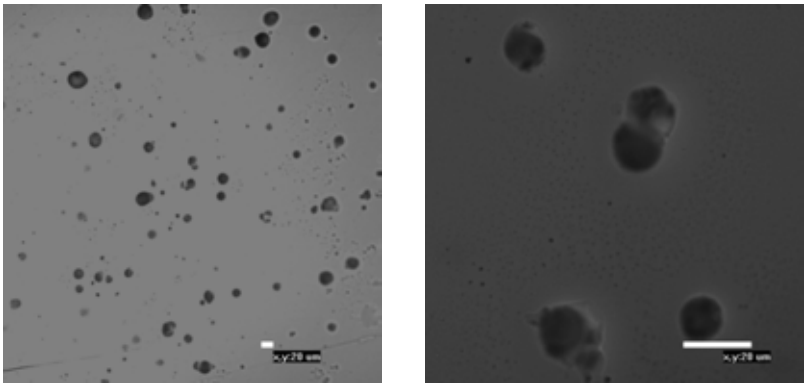
The entrapment efficiency and loading of amoxicillin were obtained by measuring amount of amoxicillin loaded in the microparticles. To completely release amoxicillin from the microparticles, sodium chloride was used. Higher concentration of NaCl, 2 times of the concentration of $CaCl_2$ used in gelation, was used to exchange calcium ions in the crosslinked polymer network with sodium ions. Then the dispersion was filtered by centrifugal-filtering devices with MWCO of 100,000 and the filtered solution was analyzed for the amount of amoxicillin by UV-Vis spectrophotometer at wavelength 272 nm, with the aid of calibration curve. Calibration curve was created from a series of amoxicillin solutions freshly prepared in 4 M sodium chloride solution at concentrations 10, 20, 40, 60, 80 and 100 ppm. The obtained calibration curve was linear (Figure B1 and B2, Appendix B). With the obtained amount of entrapped amoxicillin, % EE and % loading could be calculated. The % EE and % loading were 15.75 ± 4.99% and 22.50 ± 7.13%, respectively.

The low entrapment and loading efficiency are possibly due to the water solubility of amoxicillin. Such property implied that amoxicillin could be eluted by water in the emulsification gelation system during microparticle preparation process. In addition, it was also possible that not all amoxicillin was released from the beads for quantification.

### 3.2.2 Characterizations of amoxicillin loaded microparticles

*Morphology of the microparticles*

The obtained microparticles were observed by confocal microscope in the DIC mode. The microscopic images showed analmost spherical morphology with an average diameter (mean value ± S.D.) of 13.80 ± 4.67 μm (Figure 3.7).



**(a)** **(b)**

**Figure 3.7** Microscopic images of microparticles

*Zeta potential of the microparticles*

The zeta potential was measured by Zetasizer. The zeta potential of obtained microparticles suspended in water was -15.70 ± 5.81 mV.

The negative zeta potential of the microparticles indicated that the amount of the negatively charged alginate exceeded the amount of the positive charge calcium ions, leading to the net negative charge on the particles surfaces. Normally, the highly charged surface of the particles will cause repulsion amongst the particles and retard their aggregations. In case of stable particles suspension, the zeta potentials value should be more positive than +30 mV or more negative than -30 mV [36]. However, the zeta potentials of the obtained microparticles were below -30 mV, indicating the likely hood of particle aggregation in water medium. After leaving the suspension for 1 h, aggregation of the microparticles occurred, however, they could be redispersed back into water medium by agitation or sonication.

*ATR-FTIR spectrum of the microparticles*

The ATR-FTIR spectrum of amoxicillin-loaded microparticles (Figure 3.8 (c)) showed peak at 1583 $cm^{-1}$ corresponded to C=O stretching of carboxylate salt of alginate. Amoxicillin peaks were observed at 1772 $cm^{-1}$ corresponded to C=O stretching of β-lactam, 1683 $cm^{-1}$ corresponded to C=O stretching of amide I and 1517 $cm^{-1}$ corresponded to C=C stretching of benzene ring.

The amoxicillin-loaded microparticles showed their characteristic peaks of both amoxicillin(Figure 3.8 (a)) and calcium alginate (Figure 3.8 (b)), which implied the existence of amoxicillin in calcium alginate microparticles.

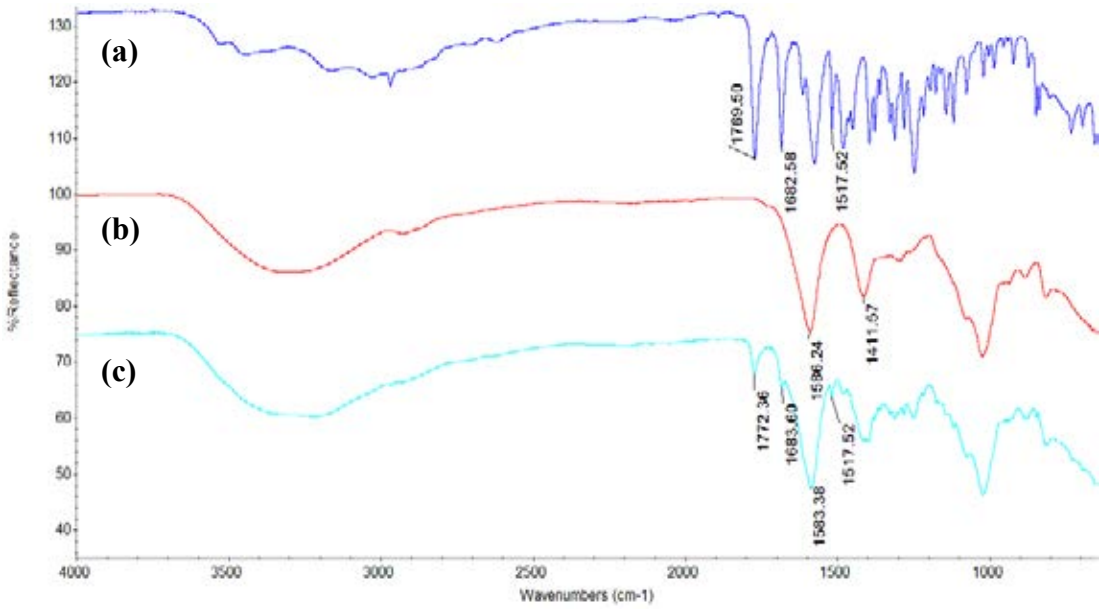
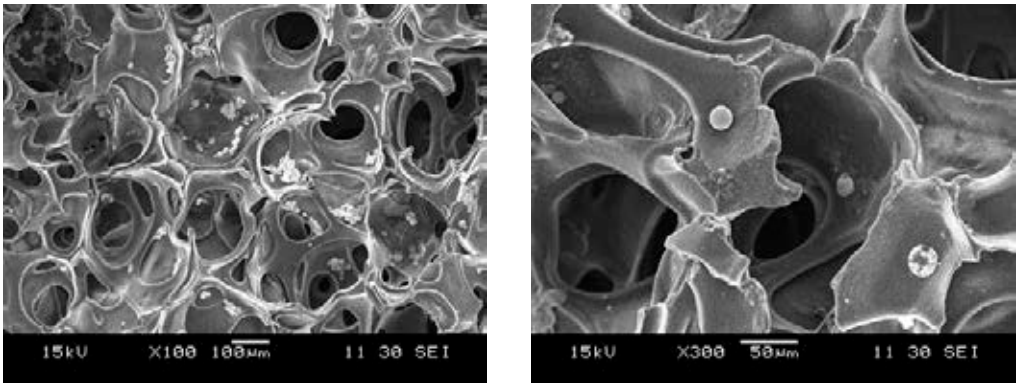



**Figure 3.8** ATR-FTIR spectra of amoxicillin (a), placebo calcium alginate microparticles (b) and amoxicillin-loaded microparticles (c)

### 3.3 Impregnation of microparticles into the foam

Amoxicillin-loaded microparticles were dispersed in PV(OH) solution. Then poly(vinyl acetal) foam piece (4x1x1 $cm^3$) was dipped into the above prepared PV(OH) solution with agitation untill all the dispered microparticles and all the liquid were absorbed into the foam pores. After that, the impregnated foam was dried and characterized by SEM. SEM images (Figure 3.9) showed that the microparticles were successfully loaded into foam pores. The microparticles could penetrate into foam

pores because the size of the microparticles were smaller than the foam cavity. PV(OH) solution was used as a binder to adhere these particles into the foam structure as seen that its pores's surface was thicker than the placebo foam (Figure 3.4) owing to coating of PV(OH). It should be noted here that under wet condition, alginate beads can usually swell to approximately 2 times of their size.




**(a)** **(b)**

**Figure 3.9** SEM images of amoxicillin-loaded microparticles loaded in foam porous at 100x (a) and 300x magnification (b)

### 3.4 Fluorescence labeled microparticles and CLFM analysis

To track the location of alginate microparticles in the foam, the TAMRA-alginate was synthesized owing to non-florescence of the calcium alginate microparticles. The carboxylic group of the fluorophore TAMRA was covalently linked to hydroxyl groups on the alginate chains using EDCI as a coupling agent. After that, the mixture was dialyzed against water to remove the excess reactants and non-grafted TAMRA. The obtained product was pink powder after freeze-drying. The dry product was characterized through ATR-FTIR.

The successful TAMRA moiety grafting was confirmed by ATR-FTIR. ATR-FTIR spectrum of TAMRA-alginate (Figure 3.10 (b)) showed the absorption peak at 1740 $cm^{-1}$ corresponded to C=O stretching vibration, indicating the new ester functionality and the absorption peak at 2851 $cm^{-1}$ corresponded to C-H stretching vibration, indicating the methyl groups of TAMRA.

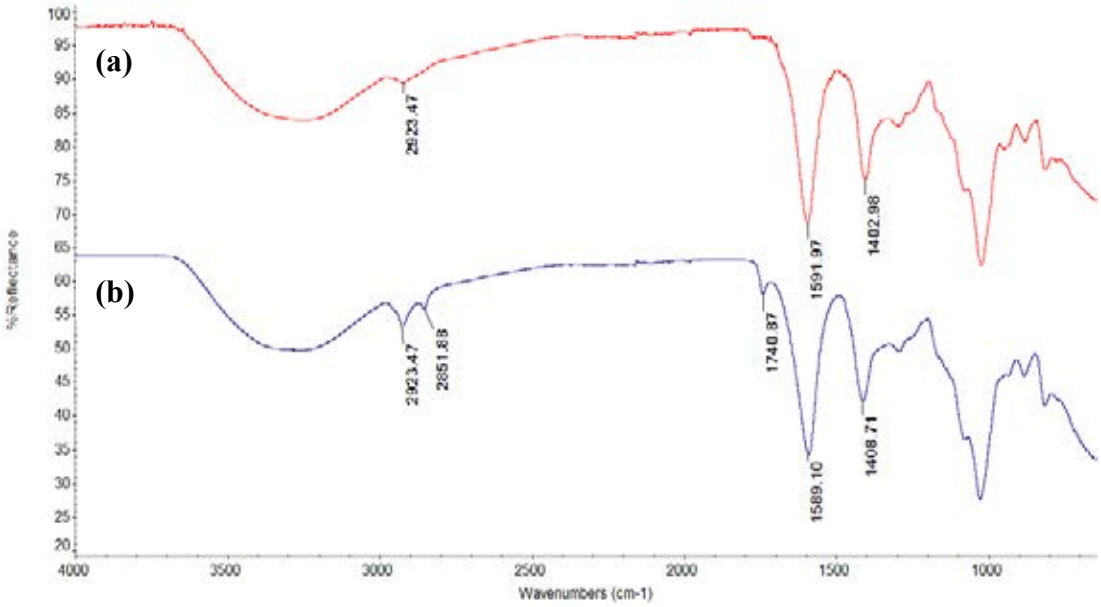
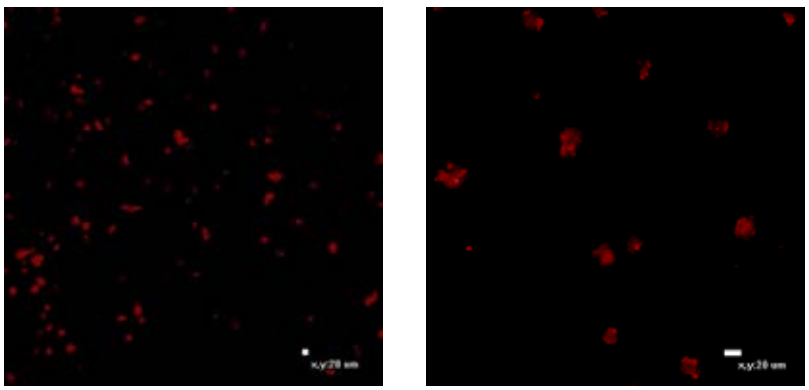



**Figure 3.10** ATR-FTIR spectra of sodium alginate (a) and TAMRA-alginate (b)

The TAMRA-alginate was used to prepare microparticles and the obtained labeled-microparticles were impregnated into the foam as previously described.The obtained microparticles-loaded foam was subjected to CLFM analysis, with the excitation and detection wavelengths at 561 nm and 595 nm. The fluorescent signal clearly confirmedthat the microparticles could penetrate and distribute in foam pores well (Figure 3.11 (b)).



**(a)** **(b)**

**Figure 3.11** The confocal fluorescent microscopic images of TAMRA labeled-amoxicillin loaded microparticles (a) and TAMRA labeled-amoxicillin loaded microparticles loaded in foam pores (b)

### 3.5 Controlled release study of amoxicillin

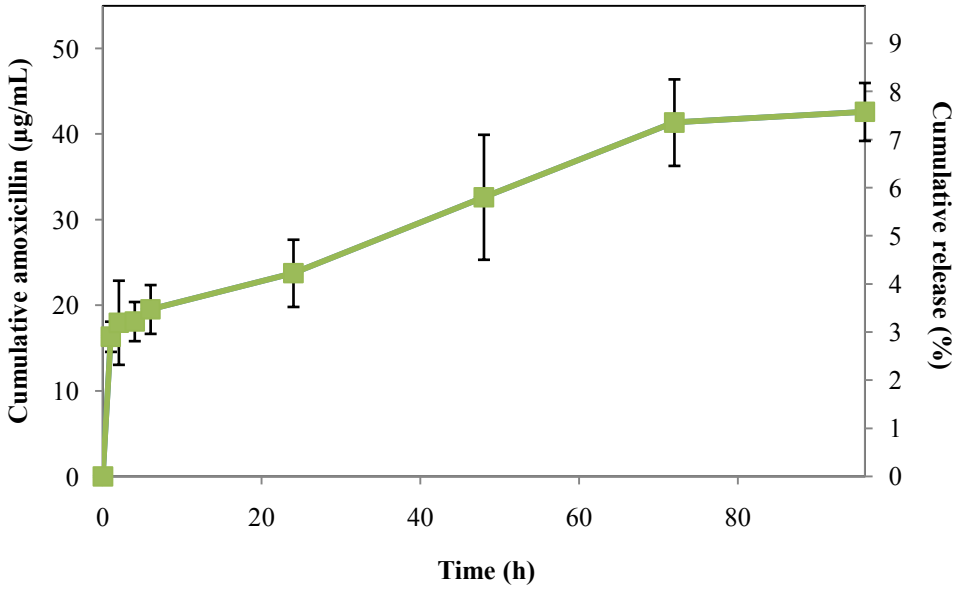



**Figure 3.12** Release profile of amoxicillin-loaded foam. Data are shown as the mean ± S.D. and are derived from 3 independent experiments.

The release profile of loaded amoxicillin from the impregnated foam in simulated nasal fluid at pH 6.8, 37$^{o}$C (Figure 3.12) was investigated and the result showed a biphasic pattern characterized by an initial release and a successive sustained release phase. About 2.90% of amoxicillin (16.33 µg/mL) was released within the first hour, and around 7.58% amoxicillin (42.61 µg/mL) was already released within 96 h.

It was speculated that when the impregnated foam was added into the release medium, the unentraped amoxicillin adhered on the surface of the microparticles and foam was fast dissociated into the release medium, and this resulted in the burst release during the first hour. After that, the penetration of the release medium into microparticles leaded to an ion exchange of calcium ion with monovalent ions from the release medium, consequently, the microparticles swelled. However, the high crosslink density between calcium ions and alginate cause slow swelling of the microparticles. The entrapped amoxicillin was therefore gradually released from the microparticles, resulting in the sustained release of amoxicillin. It should be noted

here that this model system could be used with other drugs. In addition, release rate of the drug should be adjustable by the degree of $Ca^{2+}$ crosslinking was controllable by adjusting the concentration of $CaCl_2$ solution used during the gelation process.

### 3.6 Density and compressive property of foam

The density of foams, merocel (standard nasal pack foam), F9 foam and impregnated foam, were obtained by dividing the dried weight of tested foam sample with the volume. The density of merocel (standard foam), F9 foam and impregnated foam were 97.40 ± 8.67, 110.17 ± 10.36 and 281.93 ± 13.77 mg/cm$^3$, respectively.

Comparing between the merocel and F9 foam, F9 foam's density was slightly higher than the merocel's, owing to the smaller pore size of the F9 foam. The impregnated foam showed the highest density value. The added weight from microparticles and PV(OH) deposited in foam pores was the logical cause of its increased density.

The compressive property of the foams was investigated by the universal testing machine in compressive mode. Statically crushed specimens were tested at a platen speed of 5 mm/min with loaded cell 500 N. The maximum compression on the sample was set to 50% of the original specimen height. Compressive force and displacements were recorded at 50% strain. The compressive properties of merocel, F9 foam and impregnated foam are shown in Table 3.2.

**Table 3.2** Compressive properties of foam

| Samples | stress (kPa) | |
|---|---|---|
| | dry | wet |
| Merocel | 211.59 ± 17.29 | 1.91 ± 0.08 |
| F9 foam | 319.00 ± 68.91 | 2.90 ± 1.05 |
| Impregnated foam | 594.61 ± 61.77 | 1.96 ± 0.33 |

The compressive property indicates the softness of the material when being compressed. Comparing merocel and F9 foam at dry condition, F9 foam gave higher stress value owing to higher density of F9 foam. The impregnated foam gave the highest stress value due to the increasing of strength from microparticles and PV(OH)

binder distributed in foam pores. Interestingly, the three foams, merocel, F9 foam and impregnated foams showed similar compressive property.

# CHAPTER IV

# CONCLUSION

In this research, the nasal packing material with anti-bacterial property was fabricated from poly(vinyl acetal) foam impregnated with amoxicillin-loaded calcium alginate microparticles. Poly(vinyl acetal) foam was prepared *via* acetal bond formation between hydroxyl groups of PV(OH) and aldehhyde groups of glutaraldehyde and formaldehyde. The optimized condition of the poly(vinyl acetal) foam preparation used the combination of glutaraldehyde and formaldehyde as crosslinker without heat in processing and curing step. The obtained foam had open cell structure with the diameter of 147.78 ± 74.77 μm. The foam was fast wicking, soft, flexible and compressible upon contacting with water.

Amoxicillin was successfully loaded into calcium alginate microparticles using emulsification gelation method with % EE and % loading of 15.75 ± 4.99% and 22.50 ± 7.13%, respectively. The amoxicillin-loaded microparticles showed spherical shape with the average size of 13.80 ± 4.67 μm. SEM and CLFM analyses indicated that the prepared microparticles could penetrate and adhere to the foam pores using PV(OH) as a binder.

The release profile of amoxicillin from the foam impregnated with amoxicillin-loaded microparticles in simulated nasal fluid at pH 6.8, 37$^{o}$C, indicated sustained release of amoxicillin.

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# APPENDICES

# APPENDIX A

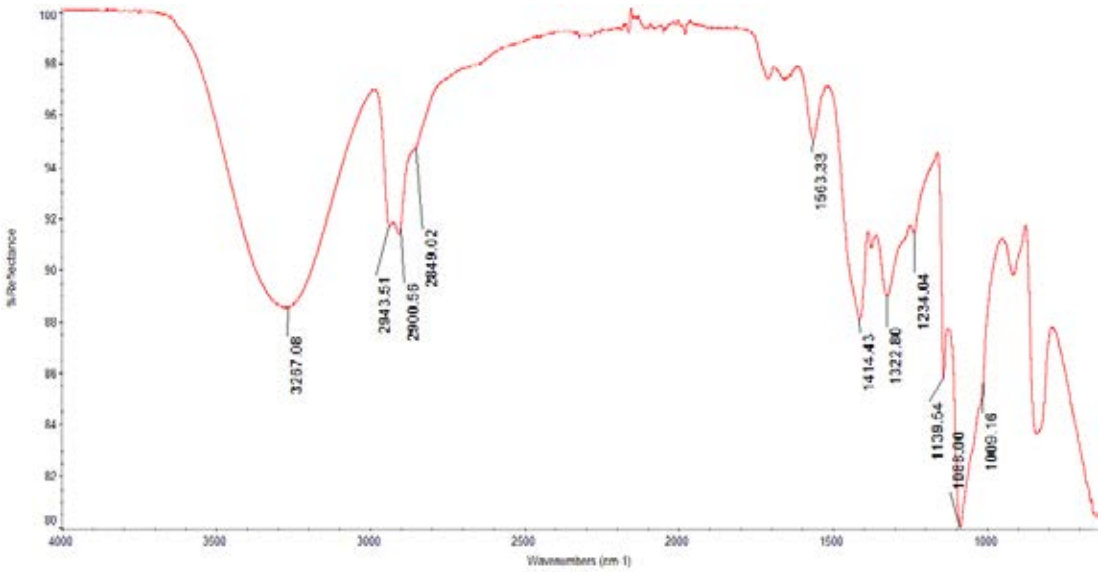



**Figure A1** ATR-FTIR spectrum of poly(vinyl alcohol)

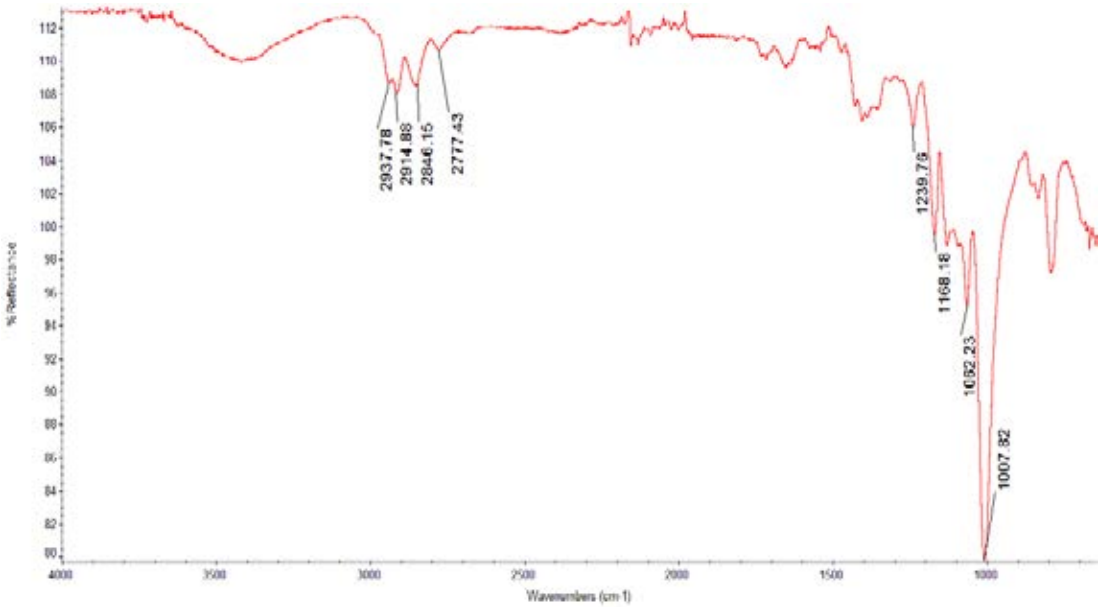



**Figure A2** ATR-FTIR spectrum of poly(vinyl acetal) foam

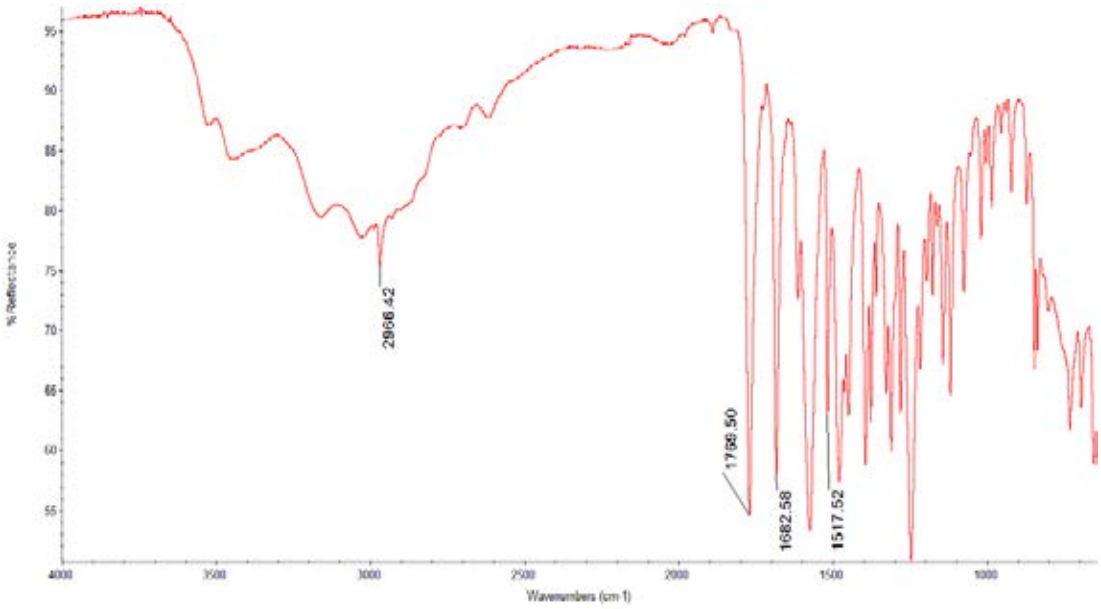



**Figure A3** ATR-FTIR spectrum of amoxicillin

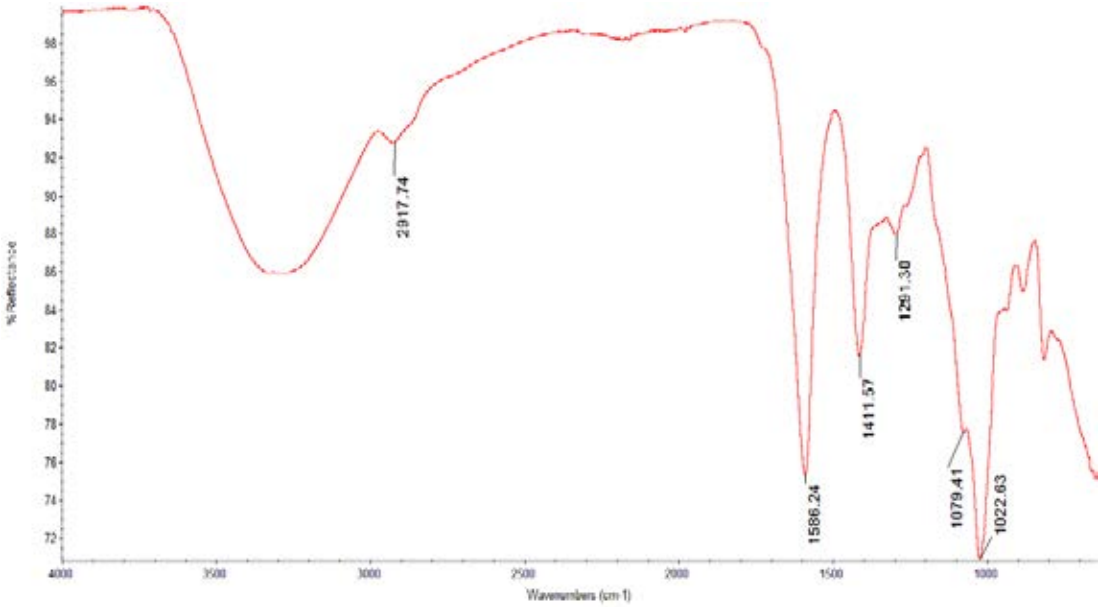



**Figure A4** ATR-FTIR spectrum of placebo calcium alginate microparticels

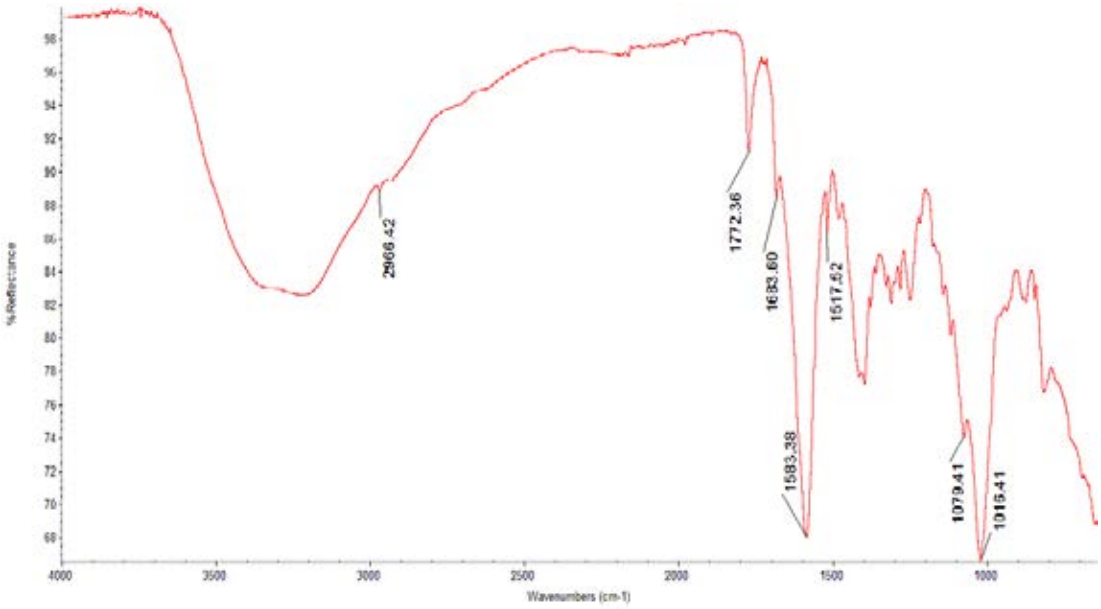



**Figure A5** ATR-FTIR spectrum of amoxicillin-loaded microparticles

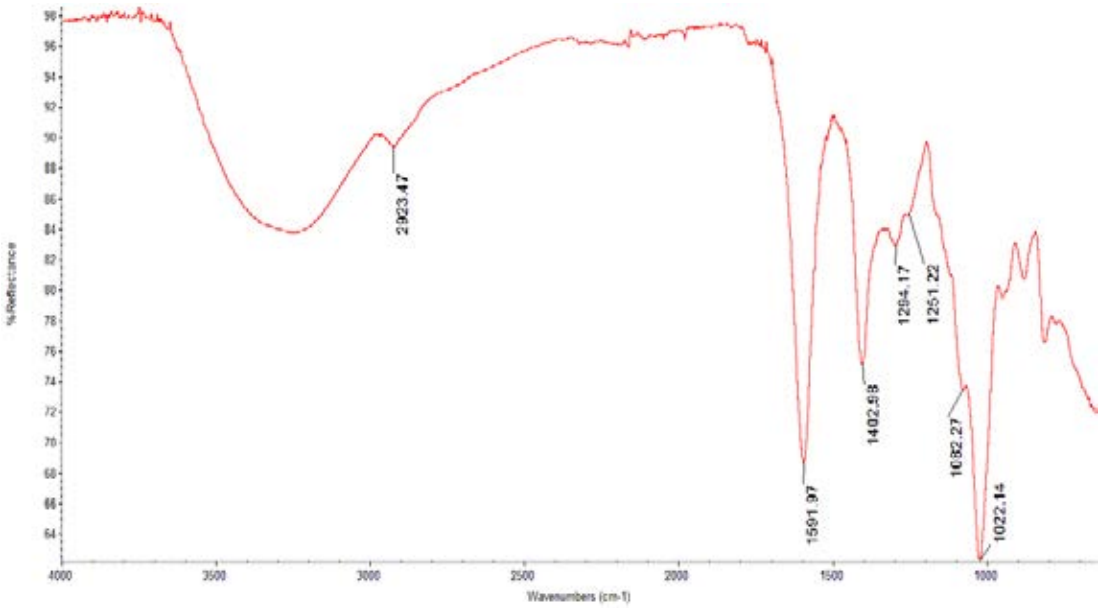



**Figure A6** ATR-FTIR spectrum of sodium alginate

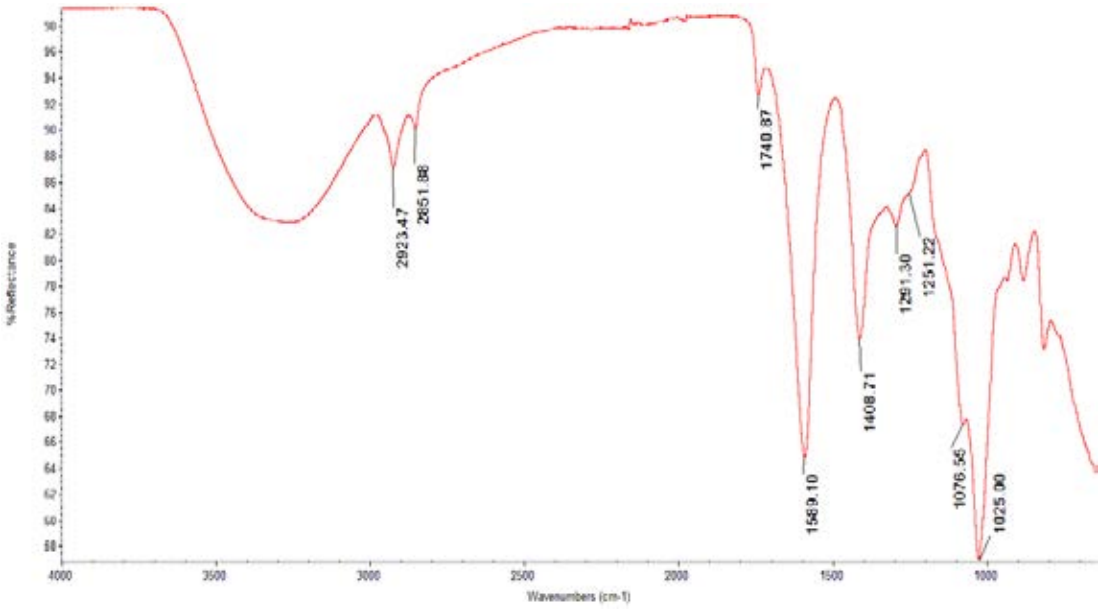



**Figure A7** ATR-FTIR spectrum of TAMRA-alginate

# APPENDIX B

## 1. Calculation of % entrapment efficiency and loading content of amoxicillin loaded into microparticles

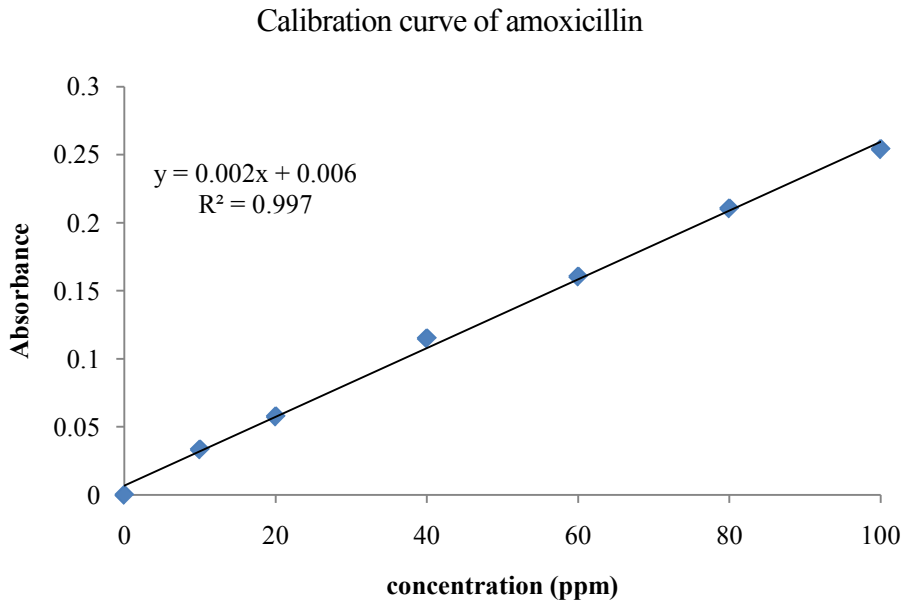



**Figure B1** Calibration curve of amoxicillin at 272 nm

By plotting a graph between absorbance and concentrations of amoxicillin solutions, a linear relationship was obtained and used for calculation of concentration of amoxicillin.

From the equation of calibration curve;

$$Y = 0.002X + 0.006, R^2 = 0.997 \qquad (1)$$

The amount of amoxicillin loaded into the microparticles was calculated by equation (1);

$$0.086 = 0.002X + 0.006$$

$$X = 40 \text{ ppm} = 40 \text{ mg/L}$$

The amoxicillin diluted forty-fold, so the amount of amoxicillin was

$$40 \times 40 = 1{,}600 \text{ mg/L}$$

In final volume of 25 mL had amoxicillin of (1,600 x 25)/1000 = 40 mg

Weight of employed amoxicillin and alginate were 200 and 100 mg

$$\text{Entrapment efficiency (\%EE)} = \frac{\text{Weight of entrapped amoxicillin}}{\text{Weight of used amoxicillin}} \times 100$$

$$= (40 / 200) \times 100$$

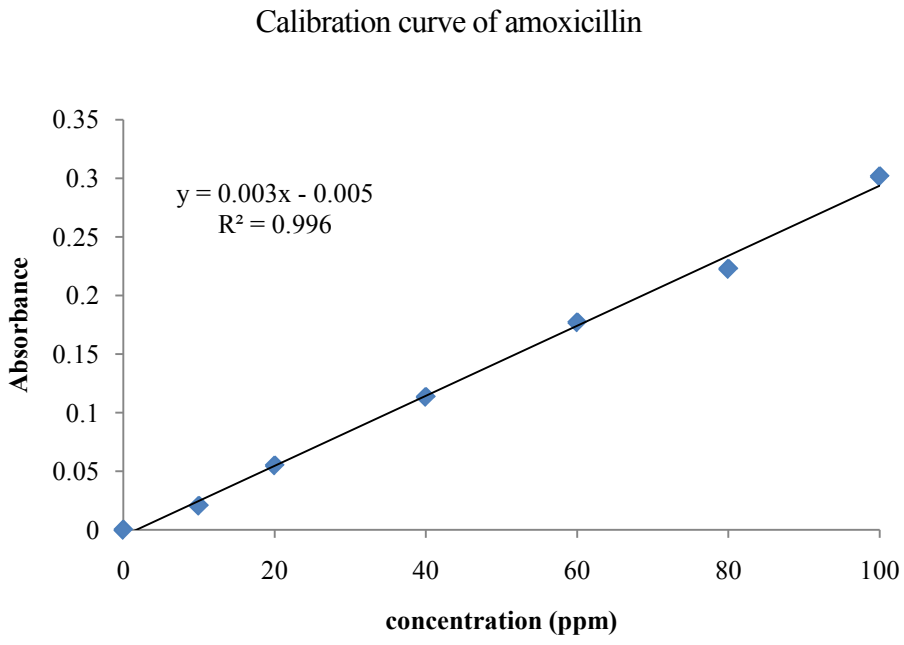
$$= 20.00\%$$

$$\text{\% Loading} = \frac{\text{Weight of entrapped amoxicillin}}{\text{Weight of entrapped amoxicillin} + \text{Weight of polymer}} \times 100$$

$$= (40 / (40 + 100) \times 100$$

$$= 28.57\%$$

**2. Controlled release study**




**FigureB2** Calibration curve for release study of amoxicillin at 272 nm

By plotting a graph between absorbance and concentrations of amoxicillin solutions, a linear relationship was obtained and used for calculation of concentration of amoxicillin.

From the equation of calibration curve;

$$Y = 0.003X-0.005, R^2 = 0.996 \quad (2)$$

The amount of released amoxicillin from the impregnated foam

*- Amoxicillin in the stimulated nasal fluid medium at 1 h*

From equation (2) $0.042 = 0.003X - 0 .005$

$X = 15.667$ ppm = 15.667 mg/mL

Weight of amoxicillin in 5 mL of sampling = (15.667 x 5)/1000
= 0.078 mg

Weight of amoxicillin in 20 mL of release medium = (15.667 x 20)/1000
= 0.313 mg

Cumulative release of amoxicillin1 h = 0.313 mg

Weight of amoxicillin in the microparticles 50 mg = (22.50*50)/100
= 11.25 mg

∴Cumulative release of amoxicillin1 h = (0.313*100)/11.25
= 2.79%

*- Amoxicillin in the stimulated nasal fluid medium at 2 h*

From equation (2) $0.031 = 0.003X - 0 .005$

$X = 12.000$ ppm

Weight of amoxicillin in 5 mL of sampling = (12.00 x 5)/1000
= 0.06 mg

Weight of amoxicillin in 20 mL of release medium = (12.00 x 20)/1000
= 0.240 mg

Cumulative release of amoxicillin 2 h = 0.240+0.078
= 0.318 mg

Weight of amoxicillin in the microparticles 50 mg = (22.50*50)/100
= 11.25 mg

∴Cumulative release of amoxicillin2 h = (0.318*100)/11.25
= 2.83%

*- Amoxicillin in the stimulated nasal fluid medium at 4 h*

From equation (2) $0.023 = 0.003X - 0.005$

$X = 9.333$ ppm

Weight of amoxicillin in 5 mL of sampling = (9.333 x 5)/1000
= 0.047 mg

Weight of amoxicillinin 20 mL of release medium = (9.333 x 20)/1000
= 0.187mg

Cumulative release of amoxicillin 4 h = 0.187+0.078+0.060
= 0.325 mg

Weight of amoxicillin in the microparticles 50 mg = (22.50*50)/100
= 11.25 mg

$\therefore$ Cumulative release of amoxicillin 4 h = (0.325*100)/11.25
= 2.89%

*- Amoxicillin in the stimulated nasal fluid medium at 6 h*

From equation (2) $0.027 = 0.003X - 0.005$

$X = 10.667$ ppm

Weight of amoxicillin in 5 mL of sampling = (10.667 x 5)/1000
= 0.053 mg

Weight of amoxicillin in 20 mL of release medium = (10.667 x 20)/1000
= 0.213 mg

Cumulative release of amoxicillin 6 h = 0.213+0.078 + 0.06 + 0.047
= 0.398 mg

Weight of amoxicillin in the microparticles 50 mg = (22.50*50)/100
= 11.25 mg

$\therefore$ Cumulative release of amoxicillin 6 h = (0.398*100)/11.25
= 3.54%

*- Amoxicillin in the stimulated nasal fluid medium at 24 h*

From equation (2) $0.027 = 0.003X - 0.005$

$X = 15.000$ ppm

Weight of amoxicillin in 5 mL of sampling = (15.000 x 5)/1000
= 0.075 mg

Weight of amoxicillin in 20 mL of release medium = (15.000 x 20)/1000
= 0.300 mg

Cumulative release of amoxicillin 24 h = 0.300 + 0.078 + 0.06 + 0.047 + 0.053
= 0.46 mg

Weight of amoxicillin in the microparticles 50 mg = (22.50*50)/100
= 11.25 mg

∴ Cumulative release of amoxicillin 24h = (0.460*100)/11.25
= 4.09%

*- Amoxicillin in the stimulated nasal fluid medium at 48 h*

From equation (2) $0.027 = 0.003X - 0.005$

$X = 20.667$ ppm

Weight of amoxicillin in 5 mL of sampling = (20.667 x 5)/1000
= 0.103 mg

Weight of amoxicillin in 20 mL of release medium = (20.667 x 20)/1000
= 0.413 mg

Cumulative release of amoxicillin 48 h = 0.413 + 0.078 + 0.06 + 0.047 + 0.053 + 0.075
= 0.588 mg

Weight of amoxicillin in the microparticles 50 mg = (22.50*50)/100
= 11.25 mg

∴ Cumulative release of amoxicillin 48 h = (0.588*100)/11.25
= 5.23%

*- Amoxicillin in the stimulated nasal fluid medium at 72 h*

From equation (2) $0.027 = 0.003X - 0.005$

$X = 18.667$ ppm

Weight of amoxicillin in 5 mL of sampling = (18.667 x 5)/1000
= 0.093 mg

Weight of amoxicillin in 20 mL of release medium = (18.667 x 20)/1000
= 0.373 mg

Cumulative release of amoxicillin 72 h = 0.373 + 0.078 + 0.06 + 0.047 + 0.053 + 0.075 + 0.103
= 0.790 mg

Weight of amoxicillin in the microparticles 50 mg = (22.50*50)/100
= 11.25 mg

∴ Cumulative release of amoxicillin 72 h = (0.790*100)/11.25
= 7.02%

*- Amoxicillin in the stimulated nasal fluid medium at 96 h*

From equation (2) $0.027 = 0.003X - 0.005$

$X = 20.333$ ppm

Weight of amoxicillin in 5 mL of sampling = (20.333 x 5)/1000
= 0.102 mg

Weight of amoxicillin in 20 mL of release medium = (20.333 x 20)/1000
= 0.407 mg

Cumulative release of amoxicillin 96 h = 0.407 + 0.078 + 0.06 + 0.047 + 0.053 + 0.075 + 0.103 + 0.093
= 0.917 mg

Weight of amoxicillin in the microparticles 50 mg = (22.50*50)/100
= 11.25 mg

∴ Cumulative release of amoxicillin 96 h = (0.917*100)/11.25
= 8.15%

# APPENDIX C

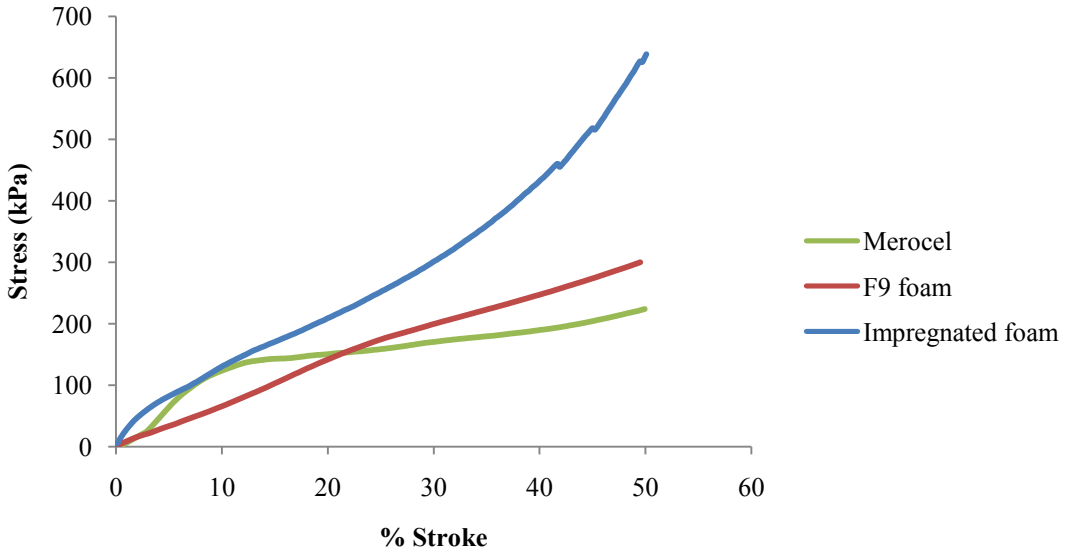



**Figure C1** Compressive property of dry foams

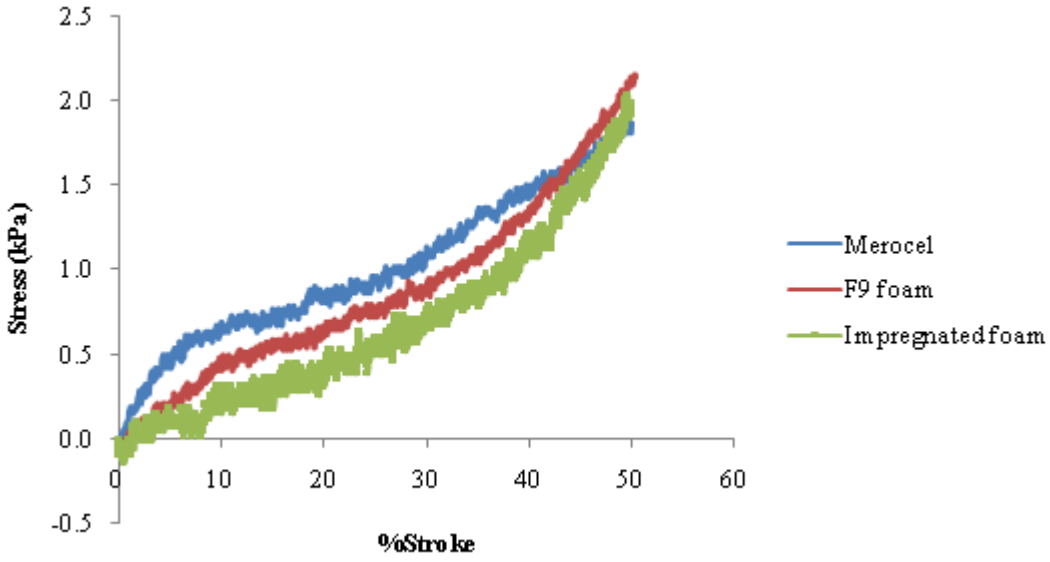



**Figure C2** Compressive property of wet foams

# VITAE

Miss Pornpan Poonsavaeng was born on October 19th, 1987 in Chonburi, Thailand. She obtained a Bachelor's Degree of Science in Industrial Chemistry from King Mongkut's Institute of Technology Ladkrabang in 2009. After that, she started her Master's degree in the Program of Petrochemical and Polymer Science at Chulalongkorn University. During master degree study, she had an opportunity to present her work in poster session in the topic of "Nasal pack with controlled release of amoxicillin" at the Pure and Applied Chemistry International Conference 2013 (PACCON 2013).

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