Sustained release of antibiotics from bone spacer

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Petrochemistry and Polymer Science
Field of Study of Petrochemistry and Polymer Science
Faculty of Science
Chulalongkorn University
Academic Year 2018

การหน่วงการปลดปล่อยยาฆ่าเชื้อโรคจากโบนสเปเซอร์

นายพงษ์พัฒน์ อู่เงิน

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ สาขาวิชาปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2561

Thesis Title	Sustained release of antibiotics from bone spacer
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พงษ์พัฒน์ อู่เงิน : การหน่วงการปลดปล่อยยาฆ่าเชื้อโรคจากโบนสเปเซอร์. (Sustained release of antibiotics from bone spacer) อ.ที่ปรึกษาหลัก : ศ. ดร.ศุภศร วนิชเวชารุ่งเรือง, อ.ที่ปรึกษาร่วม : ผศ. ดร.โรจน์ฤทธิ์ โรจนธเนศ

Bone spacer เป็นวัสดุที่ใช้รักษาผู้ป่วยติดเชื้อทางกระดูก โดยการรักษาจะนำกระดูกเทียมที่ติดเชื้อออกและนำ bone spacer ที่ผสมยาฆ่าเชื้อโรคใส่เข้าไปแทนที่ โดย bone spacer จะปลดปล่อยยาทำลายเชื้อโรคบริเวณดังกล่าว แต่ทางการแพทย์ในปัจจุบันจะใส่ยาฆ่าเชื้อลงไปใน bone spacer โดยตรง ทำให้ยาฆ่าเชื้อถูกปลดปล่อยออกมาความเข้มข้นสูงในช่วงแรก และยาฆ่าเชื้อโรคหมดไปอย่างรวดเร็ว ทำให้บางกรณีเชื้อไม่ถูกกำจัดให้หมดไป ผู้ป่วยจึงสามารถกลับมาติดเชื้อได้อีก งานวิจัยนี้จึงสนใจการกักเก็บยาฆ่าเชื้อโรคก่อนผสมลงใน bone spacer เพื่อหน่วงการปลดปล่อยยา โดยยาละลายน้ำแวนโคมัยซินกักเก็บด้วยอนุภาคข้าวและอนุภาคแคลเซียมซิเตรท ยาไม่ละลายน้ำอีริธโทรมัยซินกักเก็บด้วยอนุภาคเอทิลเซลลูโลสและอนุภาคพอลิแลคติคโคไกลโคลิก แอซิด จากนั้นนำไปผสมขึ้นรูปเป็น bone spacer และศึกษาการปลดปล่อยยาฆ่าเชื้อเป็นเวลา 42 วัน และศึกษาสมบัติความแข็งแรงทางแรงกด จากการศึกษาความสามารถในการกักเก็บยาพบว่า อนุภาคข้าวและอนุภาคแคลเซียมซิเตรทสามารถกักเก็บยาแวนโคมัยซินได้ 84.9 ± 0.3% และ 4.9 ± 3.2% ตามลำดับ อนุภาคเอทิลเซลลูโลสและอนุภาคพอลิแลคติคโคไกลโคลิก แอซิดสามารถกักเก็บยาอีริทโทรมัยซินได้ 52.0 ± 5.0% และ 6.0 ± 0.9% ตามลำดับ เมื่อศึกษาการปลดปล่อยยาฆ่าเชื้อโรคในสารละลายฟอสเฟตบัฟเฟอร์ซาลีน pH 7.4 เป็นเวลา 42 วัน พบว่า bone spacer ที่ใส่อนุภาคแคลเซียมซิเตรทสามารถหน่วงการปลดยาฆ่าเชื้อโรคได้ดีการใส่อนุภาคข้าวและการใส่ยาแวนโคมัยซินโดยตรง ในทางตรงกันข้าม การใส่ยาอีริทโทรมัยซินโดยตรงลงใน bone spacer หน่วงการปลดปล่อยยาฆ่าเชื้อโรคได้ดีกว่าการใส่อนุภาคเอทิลเซลลูโลสและอนุภาคพอลิแลคติคโคไกลโคลิก แอซิด เมื่อศึกษาสมบัติความแข็งแรงทางแรงกด พบว่า bone spacer ที่ใส่ยาลงไปโดยตรงทั้ง 2 ชนิด ไม่ส่งผลต่อสมบัติความแข็งของชิ้นงาน ขณะที่การใส่อนุภาคกักเก็บยา (ยกเว้นอนุภาคข้าว) ส่งผลให้สมบัติความแข็งของชิ้นงานลดลง และเมื่อเปรียบเทียบสมบัติความแข็งของ bone spacer ก่อน-หลัง การปลดปล่อยยา พบว่า bone spacer ที่บรรจุอนุภาคข้าวและยาอีริทโทรมัยซินโดยตรงมีสมบัติความแข็งลดลงภายหลังศึกษาการปลดปล่อยยา

สาขาวิชา	ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์	ลายมือชื่อนิสิต .....
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5972013223 : MAJOR PETROCHEMISTRY AND POLYMER SCIENCE
KEYWORD: bone spacer, drug-loaded particles, sustained drug release, bone infection

Pongpat Oungeun : Sustained release of antibiotics from bone spacer. Advisor: Prof. Supason Wanichwecharungruang, Ph.D. Co-advisor: Asst. Prof. ROJRIT ROJANATHANES, Ph.D.

Bone spacer is a device developed to temporarily insert into the body to treat infected tissue caused by bone replacement. Burst release and short-time release of antibiotics from bone spacer not only limits their ability to clear infection but also causes tissue inflammation. Therefore, here we have fabricated bone spacers containing antibiotic-loaded particles, studied their antibiotic release character, and measured the compressive strength of PMMA cements. Four biocompatible particles were investigated. Vancomycin and erythromycin were used as hydrophilic and hydrophobic model drugs, respectively. Rice granules and calcium citrate particles were loaded with vancomycin and gave the percent loading of 84.9 ± 0.3% and 4.9 ± 3.2%, respectively. Ethyl cellulose and poly(lactic-co-glycolic acid) particles were loaded with erythromycin and gave the percent drug loading of 52.0 ± 5.0% and 6.0 ± 0.9%, respectively. Then the drug-loaded particles were impregnated into poly(methyl methacrylate) bone spacers. Antibiotics released from the obtained bone spacers into PBS buffer pH 7.4 was monitored at 37 ℃. Calcium citrate particles showed improvement in sustaining the release of vancomycin from bone spacer as comparing to rice granules and unloaded drug. In contrast, embedding erythromycin directly into the PMMA gave a better-sustained release of the drug, as compared to the uses of erythromycin-loaded particles. Adding encapsulated drug (vancomycin-loaded rice granules is excluded) into PMMA cement is weaken the compressive strength of PMMA composites. After drug release test, Both PMMA cement loaded with vancomycin-loaded rice granules and PMMA cement loaded with raw erythromycin showed a significant decrease of compressive strength.

Field of Study:	Petrochemistry and Polymer Science	Student's Signature .....
Academic Year:	2018	Advisor's Signature .....
		Co-advisor's Signature .....

ACKNOWLEDGEMENTS

First of all, I would like to express my sincere appreciation and gratitude to my advisor, Prof. Supason Wanichwecharungruang, Ph.D. for her kindness, suggestions, guidance, and patience for my studies and thesis research, and encouragement throughout this work.

In addition, I am sincerely grateful to the other members of my committee, Prof. Pranut Potiyaraj, Ph.D., Duangamol Tungasmita, Ph.D. and Assoc.Prof.Dr. Piya Pinsornsak for reviewing my thesis, technical guideline, and valuable comments given to me especially during my proposal presentation.

Gratefully thank The Halal Science Center, Chulalongkorn University that allows the use of incubator shaker, Center of Petroleum, Petrochemical and Advanced Materials, Chulalongkorn University, and Science Achievement Scholarship of Thailand (SAST).

Finally, I would like to special thanks to my research group for their help and encouragement. Most importantly, I would like to express my gratitude to my family especially my mother, Saowanee Oungeun, who always encourage and support me whatever I do. Without all of them, this thesis would not be succeeded.

Pongpat Oungeun

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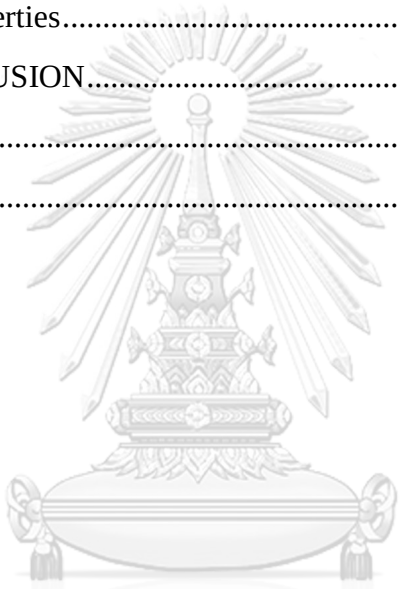

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CHAPTER I
INTRODUCTION

Antibiotic-impregnated poly(methyl methacrylate) spacer (APS) is the material that has been invented to cure the infected tissue after bone replacement [1-3]. APS is produced by mixing of poly(methyl methacrylate) powder with the antibiotics before polymerization casting [1, 4, 5]. To use the APS for treatment of infection, the infected implant must be removed and implanted with antibiotic impregnated bone spacer. Antibiotics will be released from APS gradually and against the microbial in infected tissue. In addition, APS is used to prevent the attachment of tissue and maintain the structure temporarily [6]. When the infection is cleared, the APS will be removed and reimplaned with the new bone replacer [1, 5, 6].

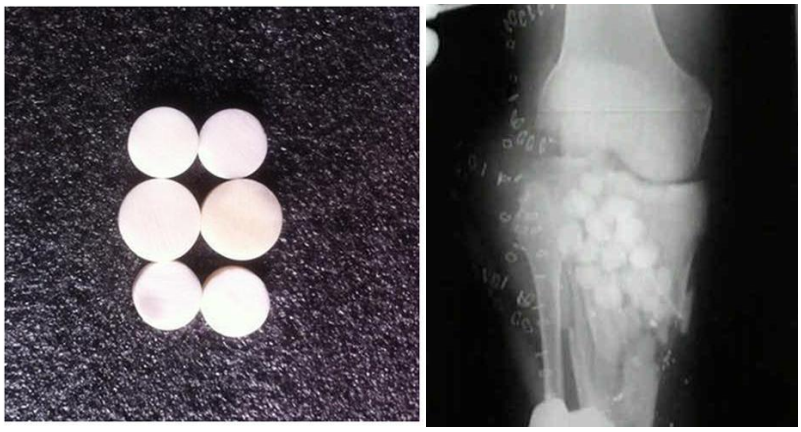
APS is widely used to cure the infection in osteomyelitis. However, the problem of using APS is burst release that is the high concentration of drug is released in the first few days. Burst release can cause the tissue inflammation. In addition, another problem of APS is that not all of the impregnated drug is released from the cement. Many research found that the effective concentration of drug is only 2 weeks for most APS [1, 5, 6], whereas the therapeutic therapy of osteomyelitis requires antibiotics at effective concentration for 4-6 weeks [1, 5]. These problems cause the 10% unsuccessful treatment of osteomyelitis. There patients must be reimplanted with the new APS and this affects the physical and mental health of the patients and also causes money.

Currently, numerous efforts to improve the drug release profiles of APS have been reported. Adding surfactant or additive into the antibiotic-impregnated bone spacer could increase the released-drug concentration in the medium, but came with the cost of compressive strength and burst release [7], adding the combination drug could improve the antibiotic efficacy [8, 9], and using the other antibiotic nanoparticles include silver nanoparticles capped with tiopronin [10], chitosan nanoparticles [11], propylparaben nanoparticles [7], and gold nanoparticles [12] could work against many microbes for weeks.

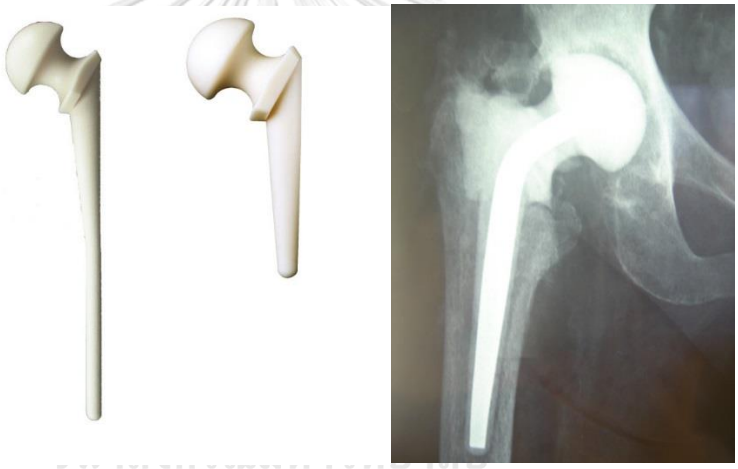
CHAPTER II
LITERATURE REVIEW

2.1 Antibiotic-impregnated poly(methyl methacrylate) spacer

Antibiotic-impregnated bone spacer (ABS) is a material which is invented to cure infection at the site where bone replacer has been removed by orthopedic surgeries [1-3]. One of the popular antibiotic-impregnated bone spacer materials is poly(methyl methacrylate) (PMMA). PMMA is a synthetic polymer, produced from polymerization of methyl methacrylate (MMA). Antibiotic-impregnated poly(methyl methacrylate) spacer is the PMMA spacer added with antibiotics [1-3, 6]. The objectives of using antibiotic-impregnated PMMA spacer are temporal structural maintenance in place of bone replacer, drug carrier, and drug-sustained release [4, 6, 13]. To cure the infected tissue, infected bone replacer have to be taken-out and antibiotic-impregnated bone spacer is to be placed at the site. General therapies take 4 to 6 weeks of the implantation [1, 5]. When the pathogens are eradicated, antibiotic-impregnated bone spacer is removed and the new bone replacer is then placed at the site [1, 5].



(A)



(B)

Figure 2.1 (**A**) Spherical-bead bone spacers. (**B**) Hip spacers

2.2 Properties of antibiotic-impregnated poly(methyl methacrylate) spacer

2.2.1 Physical and mechanical properties

The set and cured bone cement spacer is a white rigid material. Many manufacturers usually add dye such as chlorophyll into the cement

to make the bone spacer green. The aim of adding chlorophyll into the antibiotic-impregnated PMMA spacer is for obvious appearance [14]. The antibiotic-impregnated PMMA spacer used in human body must have good mechanical properties. International Organization for Standardization (ISO) of acrylic for implantation [15] has issued the measurement methods for the mechanical properties of bone spacer. The cement with a diameter of twenty-five millimeter and the height of six millimeters are compressed with removal rod with the diameter of five millimeters at the constant cross-head speed of 20.0-25.4 mm/min. The standard mechanical properties of the set and cured cement are shown in Table 2.1

Table 2.1 Requirement of set and cured PMMA spacer

Mechanical properties	Value (MPa)
average compressive strength	70
bending modulus	800
bending strength	50

Source [15]

2.2.2 Chemical properties

Generally, antibiotic-impregnated PMMA spacer is casted *via* radical polymerization [14]. Poly(methyl methacrylate) cement package consists of powder and liquid components, the powder component and the liquid component [1, 14]. The powder component consists of

poly(methyl methacrylate), dye (e.g. chlorophyll), and barium sulphate or zirconium dioxide. Liquid component consists of methyl methacrylate monomer, initiator, activator, and dye (e.g. chlorophyll). The function of each material are shown in Table 2.2

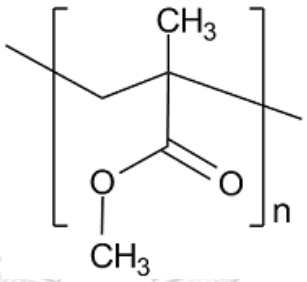


Figure 2.2 Structure of polymethyl methacrylate

Table 2.2 The functions of materials in poly(methyl methacrylate) cement

Complement		Function
powder components	poly(methyl methacrylate)	base
	co-polymer (e.g. MA-MMA)	modify the physical properties of the cement
	barium sulphate or zirconium dioxide	radio-opacifiers
	dye (e.g. chlorophyll)	differentiate cement from bone
	benzoyl peroxide	initiator
liquid components	methylmethacrylate monomer	monomer for polymerization
	N,N-dimethyl-p-toluidine (DMPT)	activator
	hydroquinone	stabilizer preventing premature polymerization
	dye (e.g. chlorophyll)	differentiate cement from bone

Source [14]

2.2.3 Antibacterial properties

Raw poly(methyl methacrylate) cement normally is a non-antibacterial material [5]. The antibacterial property of antibiotic-impregnated PMMA spacer is attained from the added antibiotics. Antibiotic-impregnated PMMA spacer usually releases antibiotics from itself to eradicate the microbial in infected tissue. The type of antibiotics used must be considered for the effective disinfection [16]. Appropriate antibiotics for effective curing are shown in Table 2.3.

Table 2.3 Pathogens and appropriate antibiotics for disinfection

Pathogens	Appropriate antibiotics for disinfection
Staphylococcus aureus	vancomycin, tetracycline, erythromycin, gentamycin, chloramphenicol, streptomycin, cephalosporin
Staphylococcus epidermidis	vancomycin, gentamycin, tetracycline, erythromycin, clindamycin, rifamycin, fluoroquinolones, ceftriaxone
Streptococcus pyogenes	vancomycin, tetracycline, levofloxacin, teicoplanin, spiramycin, azythromycin
Escherichia coli	vancomycin, β-lactams, fluoroquinolones, colistin, cephalosporin, temocillin, pivmecillinam
Haemophilus influenzae	cephalosporin, quinolones, ceftriaxone, β-lactams, amoxicillin-clavulinic acid, tetracyclines, erythromycin
Pseudomonas aeruginosa	β-lactams, quinolones, tetracyclines, chloramphenicol, macrolides
Serratia marcescens	aminoglycosides, cephalosporins, fluoroquinolone, β-lactams, carbapenems
Blastomyces dermatitidis	amphotericin B, itraconazole, voriconazole, fluconazole, posaconazole
Coccidioides immitis	F luconazole, amphotericin B, itraconazole

Source [16-26]

2.3 Antibiotics

2.3.1 Vancomycin

Vancomycin is a glycopeptide antibiotic derived from *Nocardia orientalisused*. Vancomycin is used against a wide range of gram-positive bacteria include staphylococci, group A *β-haemolytic streptococci*, *Streptococcus pneumoniae*, *Corynebacterium spp*. and *Clostridium spp*., and *Clostridium difficile*, but it is not active against gram-negative organisms, fungi, and yeasts [27]. It is recommended for skin infections, intravenous infections, bone and joint infections, endocarditis, meningitis, and methicillin-resistant *Staphylococcus aureus* [28]. The World Health Organization (WHO) has recommended vancomycin as a basic antibiotic for infection treatment.

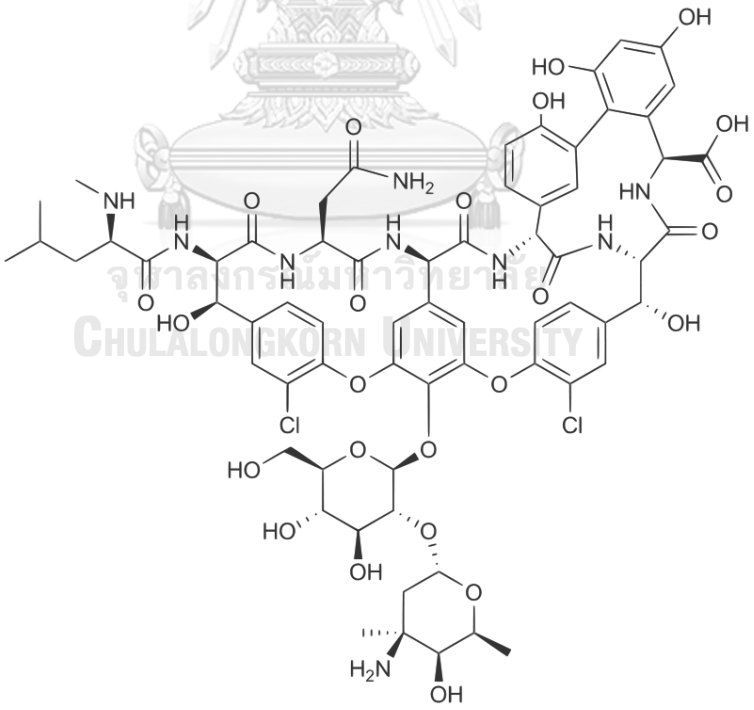


Figure 2.3 Structure of vancomycin

2.3.1.1 Vancomycin for treatment of osteomyelitis

The microorganism which often causes the bone infection is *Staphylococcus aureus* [29]. *Staphylococcus aureus* is a gram-positive bacteria. The first priority antibiotic used to eradicate the gram-positive bacteria is vancomycin. Vancomycin was used to treat the osteomyelitis by Norden et al. in the 1980s [30]. The results showed a good penetration of vancomycin into the bone and vancomycin concentration in the cortical bone was greater than the minimum inhibitory concentration (MIC). In 2017, Mohapatra and Jain reported the implantation of poly(methyl methacrylate) beads loaded with vancomycin into the infected tissue for 10 weeks. Twenty-eight patients out of thirty-two patients (87.5%) were recovered from osteomyelitis and got the secondary stage of reimplantation with replacer [31]. The achievement of using vancomycin to treat osteomyelitis was reported again in 2019, Kurebayashi et al. studied the use of vancomycin-loaded PMMA spacer for 15-61 months in eighteen patients. The results showed a decrease of C-reactive protein values compared to the previous surgery. Decrease of C-reactive protein values indicates the less inflammation and recovery [32]. However, ineffective microbial eradication by vancomycin added PMMA spacer was also reported [33]. Efficacy of vancmycin could be improved via combination with other drugs such as rifampin and nafcillin [30, 33].

2.3.2 Erythromycin

Erythromycin is a macrolide antibiotic that formerly discovered in 1952 [34]. It was used against various gram-positive bacteria, gram-

negative bacteria, and various other organisms. The gram-positive bacteria, include *Streptococcus pneumoniae*, *Staphylococcus aureus, Streptococcus pyogenes*, *Listeria monocytogenes*, *Corynebacterium diphtheria*, *Corynebacterium minutissimum*. The gram-negative bacteria, include *Legionella pneumophila*, *Bordetella pertussis*, and *Haemophilus influenzae*. Other microbes that are eradicated by erythromycin, include *Entamoeba histolytica*, *Treponema pallidum*, and *Mycoplasma pneumonia* [35]. Erythromycin was used to treat some infections caused by bacteria e.g., bronchitis, Legionnaires' disease (a type of lung infection), pneumonia, diphtheria (a serious throat infection), skin infections, sexually transmitted diseases (STD), and bone infections [34, 36].

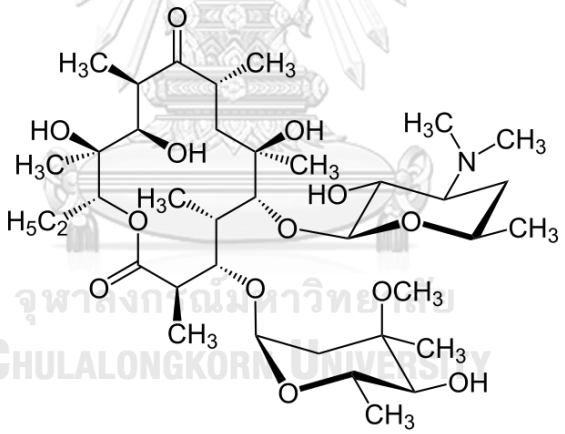


Figure 2.4 Structure of Erythromycin

2.3.2.1 Erythromycin for treatment of osteomyelitis

Erythromycin is one of the antibiotics that has been used to treat osteomyelitis since the 1970s [36, 37]. The pathogens that are eliminated *via* erythromycin include *Staphylococcus aureus*, *Staphylococcus epidermidis, Streptoccocus pyogenes, Streptococcus faecalis, Escherichia*

coli, *Peptococcus anaerobias*, *Peptostreptococcus intermedins*, and *Actinomyces meyeri* [36, 38]. In addition, Erythromycin is one of the antibiotics that can be used during pregnancy [39]. The successes of using erythromycin to treat osteomyelitis had been reported. In 1976, Rosenthal *et al*. used the erythromycin against a variety of aerobic and anaerobic bacterial species (three gram-positive aerobic bacteria and forty-nine anaerobic bacteria). The results indicated the satisfied antibacterial properties of erythromycin, three gram-positive aerobic bacteria were eradicated at 0.23 µg/mL erythromycin or less. Forty-five anaerobic bacteria were inhibited at 6.25 µg/mL erythromycin (96% inhibition) [36]. However, some research reported the failure of using erythromycin to inhibit the pathogens in osteomyelitis. Bystedt *et al*, studied the release of azidocillin, erythromycin, doxycycline, and clindamycin from the human mandibular bone. The results showed the concentration of erythromycin were lower than the MIC inhibit the pathogens. It can be supposed that erythromycin is not appropriate to treat bone infection [40].

To improve the efficacy of erythromycin to treat the osteomyelitis, the drug combination is used. Erythromycin is used with the other drug and give satisfied antibacterial properties. Rosenthal et al. had mixed erythromycin/colistin combination into bone cement and tested bacterial inhibition. The results indicated effective inhibition of erythromycin and colistin that could against 98% of all anaerobic and aerobic bacteria [36].

2.4 Problems of antibiotic-impregnated poly(methyl methacrylate) spacer

2.4.1 Drug burst release

Burst release is the initial high concentration of released drug before the rate of drug release reaches the constant value [41]. High burst release can harm the body. Burst release from antibiotic-impregnated PMMA spacer can cause tissue inflammation [6]. In addition, burst release reduces the effective lifetime of the antibiotic-impregnated PMMA spacer [5, 6].

2.4.2 Short-time release of antibiotics

An ideal of drug release profiles from PMMA cement is six weeks or until the microorganisms are eradicated [1, 3, 5]. However, the process of PMMA casting involves a direct addition of antibiotics into the PMMA cement [1-3, 13]. This caused the higher initial drug release from the resulted PMMA cement. Most research indicated that antibiotic-impregnated PMMA cement can fight against the growth of bacteria for only 2 weeks [1, 5, 6], which is less than half of an ideal drug release period.

2.5 Antibiotics encapsulation technique

Encapsulation is a technique by which one substance or mixture of substance is entrapped or coated with another material or mixture to provide some benefits to the material or mixture inside [42].

Encapsulation has been used in the pharmaceutical industry for prolonged drug release, conversion of the drug from liquids to solids, taste-masking of bitter antibiotics, environmental protection, and extension of the storage period of drug [43]. The current methods for drug encapsulation include physicochemical methods, such as emulsion solvent evaporation and solvent displacement, as well as mechanical methods, such as spray drying [43].

2.5.1 Thermal expansion method

Thermal expansion method is the method applied from the principle that the matter tends to change the shape, area, and volume when it responses to a change in temperature [44, 45]. When the matter is heated, its kinetic energy in molecules increase, the molecules start vibrating and moving more and commonly maintain the greater separation and cause increased space in the substance. The space or cavity in the substance can be used to absorb and store the molecule which fit the cavity inside. However, the thermal expansion method in the pharmaceutical industry is not widespread but can be found in many research related to pharmaceutics [46, 47].

2.5.1.1 Rice granules

Asian rice (*Oryza sativa L.*) is one of the popular economic crops in Thailand [48]. Rice is used widely in food industries and consumer products in many years [49]. One popular way to transform rice is by producing rice flour starch. Rice starch is usually used in food processing,

whereas in medical research this material can be used as the drug-carrier [45, 50, 51]. The particles of rice starch, the rice granules, possess the size of 2-7 μm. The advantages of rice granules are cell compatibility [52], high encapsulation efficiency [53], and biodegradable [54, 55]. Rice starch is sensitive to moisture and temperature. Color of rice granules can change with the time [56]. The encapsulation methods using rice granules include thermal expansion [57], spray-drying [58], anti-solvent precipitation [51], and extrusion [59]. Encapsulation of antibiotics and antioxidants into starch granules had been studied. In 2000, Trindade *et al*. used rice granules for ascorbic acid microencapsulation to prolong the stability of ascorbic acid. Gelatin was also used as a binding agent in the encapsulation process. The results indicate that the unencapsulated vitamin C showed significant losses after 30 days of storage, whereas vitamin C-loaded rice granules with 2% gelatin showed better protected until 90 days [60].

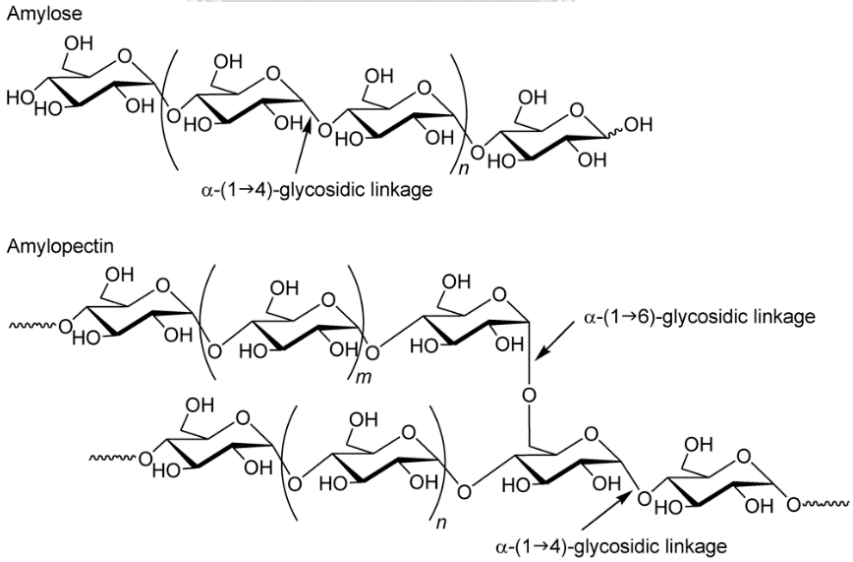


Figure 2.5 Structure of amylose and amylopectin

2.5.2 Co-precipitation method

Co-precipitation is the method involves the simultaneous occurrence of nucleation, growth, coarsening, and agglomeration [61]. Normally, precursors in this process are soluble in the medium under the condition employed. When the co-precipitation occurs, the nucleation of the precipitation is form, agglomerated, and then separated from the solvent. The materials used for drug entrapment can be polymers such as hydroxypropyl methylcellulose, Kollicoat IR® which is a graft copolymer composed with polyethylene glycol and polyvinyl alcohol (PEG: PVA, 1:3), and hydroxypropyl cellulose, organic materials such as sucrose, and ionic compounds such as calcium carbonate and calcium phosphate. Co-precipitation is the method that can be used in many fields include separation and purification in analytical works, nanomaterials, and inorganic precipitation of trace metal [62]. In the fields of cosmetic and food, this method is not popular for encapsulation [63], regardless of its simplicity [64]. In the research of encapsulation of aroma in sucrose crystals, the aroma was added into the supersaturated sucrose syrup at 120 °C to prevent evaporation and preserve the aroma's scent. The obtained aroma particles were very small with the aroma incorporated into sucrose crystals. The aroma particles could be soluble in water [63, 65]. In the fields of pharmaceutical especially drug delivery, solid dispersion prepared by co-precipitation method has proved successful [66]. In 2014, Wang et al. [67] used the calcium carbonate as the gene's carriers and fabricated the gene nanoparticles via co-precipitation method. To fabricate these nanoparticles, DNA plasmid solution was mixed with calcium chloride solution (solution A), whereas cell penetrating peptide (KALA) and protamine sulphate (PS) was mixed

sodium carbonate solution (solution B). Then, solution A was poured into solution B and mixed gently. The gene nanoparticles were then fabricated. The results indicated that these nanoparticles could improve gene delivery efficiency and cellular uptake.

2.5.2.1 Calcium citrate

Calcium citrate is a calcium salt of citric acid. This calcium salt consists of calcium ion and citrate ion with the ratio of calcium: citrate ion equal to 3: 2. The physical properties of calcium citrate are white powder, odorless, and sparingly soluble in water. Calcium citrate is used as a food additive (E333) [68], preservative [69], and modifier of texture [70]. Calcium citrate is sold as supplementary food to solve the osteoporosis [71]. Calcium citrate can be adsorbed in the human body better than calcium carbonate. Calcium citrate not only is easier to digest than calcium carbonate but also produces no adverse effect on stomach acid [72]. Beside calcium citrate, the other forms of calcium salt used as the drug carriers include calcium carbonate, calcium phosphate, and calcium alginate. Calcium carbonate can be used as the anticancer drug carrier [73]. Calcium phosphate was used as a drug carrier in bone and sustained release material in scaffold [74-76]. Calcium alginate is widely used in drug controlled release and drug delivery systems [77-79]. Calcium citrate is not widely used in drug delivery regardless of its effective adsorption in human systems [80].

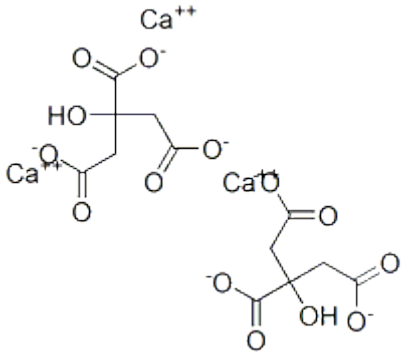


Figure 2.6 Structure of calcium citrate

2.5.3 Solvent displacement method

Fessi *et al*. reported the method that used to manufacture nanoparticles called solvent-displacement [81]. One phase is the solvent phase that usually uses acetone, ethanol, and dichloromethane to dissolve the dissolvable polymers [82]. Another phase is the non-solvent phase (usually water), which may incorporate with a stabilizer. To form nanoparticles, the organic phase is injected into the water phase under continuous stirring. The polymeric particles are formed simultaneously due to the displacement of the solvent [82]. The resulting suspension is evaporated to eliminate organic solvent and centrifuged to receive the drug-loaded nanoparticles. The polymers commonly used in this method are biodegradable polymers such as ethyl cellulose, poly (ε-caprolactone), and other polyesters. The advantages of this technique are simple, economical, rapid, and repeatable. In addition, low-toxic solvents and very high temperatures are not required.

2.5.3.1 Ethyl cellulose polymers

In 1927, ethyl cellulose was originally discovered in Dow Chemical Company while they were synthesizing of cellophane from cellulose [83]. Ethyl cellulose is a derivative polymer from cellulose. The repeating units of ethyl cellulose are glucose units, but some hydroxyl groups are converted into ethyl ether group. Ethyl cellulose are soluble in organic solvents, heat-stable, elastic, good strength, weak-acid and base resistance, and humidity resistance [84]. Initially, ethyl cellulose was used to produce items in luggage, hairbrushes, flashlight cases, and hard hats in 1935. Nowadays, ethyl cellulose is used widely in food and pharmaceutical industries. In food industries, ethyl cellulose is used as a stabilizer and food thickener. Ethyl cellulose can be produced into a non-toxic film which is a safeguard ingredient from water. In pharmaceutical industries, ethyl cellulose is used as a coating agent, tablet binder and filler, flavoring and fragrance fixative, film-former, and viscosity-increasing agent. Nowadays, ethyl cellulose is widely used as a drug carrier and material for sustained-drug release [85]. It also been used as a precursor in the preparation of both water-soluble and sparingly water-soluble drugs using the solvent displacement technique [86].

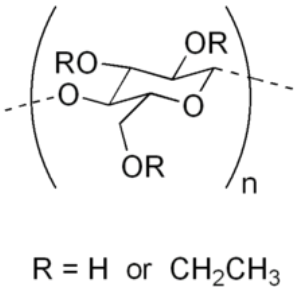


Figure 2.7 Structure of ethyl cellulose

2.5.4 Emulsion solvent evaporation method

Emulsion solvent evaporation method involves the emulsification of an organic solvent (e.g., ethanol, acetone, chloroform, and dichloromethane), containing dissolved polymer and dissolved drug in an excess amount of water with the continuous shaking or stirring. The size and shape of drug particle can be controlled with the concentration of stabilizer [87]. After the drug particles are formed, the organic solvent is evaporated and solid microparticles are recovered from suspension by centrifugation, filtration, or lyophilization [87, 88]. This method has been applied in pharmaceutical industries for many objectives such as controlled drug release, protection of the drug from the environment, and masking the active ingredients [87, 89].

2.5.4.2 Poly(lactic-co-glycolic acid) polymers

Poly(lactic-co-glycolic acid) polymers, PLGA, or PLG was discovered as surgical sutures in the 1960s [90-93]. PLGA is synthesized by ring-opening polymerization using 2 different monomers, the cyclic dimers of glycolic acid and lactic acid. The ratio of lactide to glycolide affects the properties of PLGA. Poly(lactic-co-glycolic acid) polymers which contain glycolic acid less than 50% are soluble in a chlorinated solvent such as chloroform and dichloromethane, whereas poly(lactic-co-glycolic acid) polymers which contain glycolic acid more than 50% are soluble in a fluorinated solvent such as hexafluoroisopropanol [94]. The PLGA that contains more of lactide degrade slower than PLGA that contains more of glycolide [95]. Medical industries used the poly(lactic-co-glycolic acid) polymers in sutures, fixation materials, and drug carriers

[94]. This material can induce the wound healing itself and react with the cell wall of the microbes [96, 97].

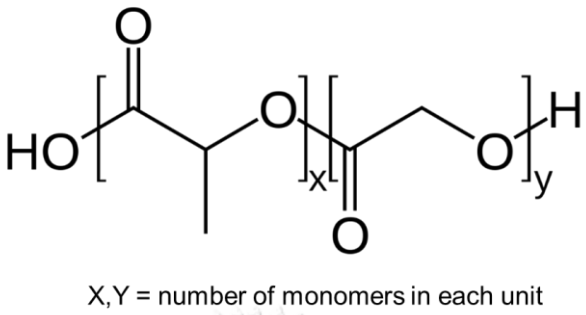


Figure 2.8 Structure of poly(lactic-co-glycolic acid)

2.6 Drug encapsulation in poly(methyl methacrylate) bone spacer

Drug encapsulation has been used in antibiotic-impregnated PMMA cement. The aims of adding of drug-loaded particles into the cement are to prolong drug release and increase the drug concentration in the medium [98]. In 2005, Shi *et al*. used the chitosan nanoparticles to improve the drug release profiles and increase the antimicrobial properties in PMMA cement. The results indicated that the erythromycin was release from erythromycin-chitosan impregnated PMMA spacer for 3 weeks [11]. In 2013, Prokopovich *et al*. used silver nanoparticles as the tiopronin's carriers [99, 100]. PMMA spacer impregnated with silver-tiopronin nanoparticles showed good antibacterial properties, whereas the compressive strength values were not different from the control [10]. Ayre *et al*. used liposome to encapsulate gentamycin. They used phosphatidylcholine and cholesterol as the liposomal matrix and Pluronics as the stabilizer. The resulted indicated the constant release of drug from the gentamycin-liposome impregnated PMMA cement for

more than 30 days. The concentration of released gentamycin increased 22% but came with the cost of mechanical properties [98].

CHAPTER III
EXPERIMENTAL

3.1 Materials, chemical, reagents

Articulated PMMA bone cement was from Palaces® (Richards, Memphis, Tennessee, USA). Vancomycin ($C_{66}H_{76}Cl_3N_9O_{24}$; Mw. 1485.71) was purchased from Hunan HuiBaiShi Biotechnology Co.,Ltd (Hunan, China). Erythromycin was purchased from Greenway Biotech Co.,Ltd (Suzhou, China). Rice flour (New Grade®) was purchased from Thai Wah Public Company Limited (Thailand). Calcium chloride ($CaCl_2$; Mw. 110.98) was purchased from Merck KGaA (Darmstadt, Germany). Trisodium citrate dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$; Mw. 294.10;), ethyl cellulose (viscosity 300 cP, 48% ethoxyl content), poly(lactic-co-glycolic acid) with the ratio of lactic acid to glycolic acid of 50:50 and the Mw of 13,000-23,000, poly(vinyl alcohol) with average of Mw 31,000-50,000, were purchased from Sigma-Aldrich (USA).

3.2 Drug encapsulation

3.2.1 Encapsulation of vancomycin into rice granules

Rice granules were used to encapsulate vancomycin by thermal expansion method at the weight ratio of vancomycin: rice granules of 8: 1. Four hundred milligrams of vancomycin was dissolved in 20 mL of DI water and mixed with 20 mg of rice powder for 30 min with constant stirring. Then, the mixture was heated at 83 °C for 3 h. The mixture was kept at room temperature overnight. The resulting suspension was

centrifuged at 5000 rpm (2348 g) for 30 minutes. The pelleted down vancomycin-loaded rice granules (VAN-RG) were freeze-dried.

3.2.2 Encapsulation of vancomycin into calcium citrate particles

Encapsulate vancomycin into calcium citrate particles were carried out by co-precipitation method [2]. Calcium citrate is the precipitation that can be produced by the reaction between calcium chloride and sodium citrate dihydrate. The mole ratio of calcium ion and citrate ion was studied and listed in Table 3.1

Table 3. 1 the mole ratios of calcium ion: citrate ion

Calcium ion		**Citrate ion**		**Vancomycin (g)**
mmol	g	mmol	g	
1.36	0.055	0	-	0.02
1.19	0.048	0.17	0.033	0.02
1.02	0.041	0.34	0.066	0.02
0.85	0.034	0.51	0.098	0.02
0.68	0.027	0.68	0.132	0.02
0.51	0.021	0.85	0.163	0.02
0.34	0.014	0.102	0.197	0.02
0.17	0.007	0.19	0.229	0.02
-	0	1.36	0.262	0.02

vancomycin was dissolved in DI water. Calcium chloride was weighed and dissolved in DI water. Then, vancomycin solution and calcium chloride solution were mixed homogeneously. The sodium citrate dihydrate was dissolved in DI water and poured into the previous solution and vortexed for 5 minutes. The mixture was shaken in incubator shaker at room temperature for 24 h before centrifugation at 10000 rpm (9391 g) for 30 minutes. The pelleted down vancomycin-loaded calcium citrate particles (VAN-CC) were freeze-dried.

3.2.3 Encapsulation of erythromycin into ethyl cellulose particles

Ethyl cellulose polymers were used to encapsulate erythromycin by solvent displacement method [3] at the weight ratio of erythromycin: ethyl cellulose polymers of 1: 1. Forty milligrams of erythromycin and 40 mg of ethyl cellulose polymers were dissolved in 16 mL of ethanol and mixed homogeneously for 10 minutes. Then, the sixty four mL of DI water was slowly added into organic phase at controlled rate (1.13 mL/min) under constant stirring. The suspension was stirred overnight to remove organic solvent. The resulting suspension was centrifuged at 13000 rpm (15871 g) for 45 minutes. The pelleted down erythromycin-loaded ethyl cellulose particles (ERY-EC) were freeze-dried.

3.2.4 Encapsulation of erythromycin into poly(lactic-co-glycolic acid) particles

Poly(lactic-co-glycolic acid) polymers were used to encapsulate erythromycin by modified emulsion/evaporation method [4] at the weight

ratio of erythromycin: poly(lactic-co-glycolic acid) polymer of 1: 1. Forty milligrams of erythromycin and 40 mg of ethyl cellulose polymers were dissolved in 5 mL of dichloromethane (organic phase). Five percent poly vinyl alcohol solution was prepared: One gram of PVA was dissolved in 20 mL of DI water. Aqueous solution of PVA was added to organic phase and emulsified by sonication for 1 min. Then, the primary emulsion was stirred overnight to evaporate dichloromethane. The resulting emulsion was centrifuged at 13000 rpm (15871 g) for 45 minutes. The pelleted down erythromycin-loaded poly(lactic-co-glycolic acid) particles (ERY-PLGA) were freeze dried.

3.3 Characterization of drug-loaded particles

Morphology of all drug-loaded particles (vancomycin-loaded rice granules (VAN-RG), vancomycin-loaded calcium citrate particles (VAN-CC), erythromycin-loaded ethyl cellulose particles (ERY-EC), and erythromycin-loaded poly(lactic-co-glycolic acid) particles (ERY-PLGA) were characterized using scanning electron microscope (SEM, JSM-7610, JEOL, Tokyo, Japan). The functional groups of precursors and drug-loaded particles were identified using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-IR Nicolet 6700, Thermo Electron Corporation, Madison, WI, USA). The thermal stability and thermal decomposition of precursors and drug-loaded particles were analyzed using thermogravimetric analysis (Netzsch STA 449 F1, Germany).

3.3.1 Scanning electron microscopic analysis (SEM)

SEM was performed at the Department of Chemistry, Faculty of Science, Chulalongkorn University, Thailand. A drop of suspension was dropped on the small clean glass slide and dried in a desiccator at room temperature overnight. Then, the sample was mounted on the carbon tape and coated with a gold layer under vacuum at 15 kV for 90 s. The coated samples were observed using SEM at an acceleration voltage of 20 kV.

3.3.2 Fourier transform infrared spectroscopy (FTIR)

FTIR was performed at the Department of Chemistry, Faculty of Science, Chulalongkorn University, Thailand. To prepare the sample of VAN-RG and VAN-CC for FTIR-analysis, the dried-pellets were added with 1 mL of DI water and ground with mortar and pestle for 5 minutes. The pellet was dried in an oven at 60 °C. To prepare the sample of ERY-EC for FTIR analysis, 1 mL of ethanol was added to samples and the mixture was ground with mortar and pestle for 5 minutes. Then, the pellet was left to dry at room temperature. The sample was analyzed using FT-IR with ATR mode. To prepare the sample of ERY-PLGA for FTIR technique, 1 mL of dichloromethane was added and follow the previous preparation.

3.3.3 Thermogravimetric analysis (TGA)

TGA was performed at the Analytical and Testing Service Center, the Petroleum and Petrochemical College, Chulalongkorn University, Thailand. The precursors and samples (all drug-loaded particles) were

analyzed by TGA at 50 °C - 800 °C with the heat rate of 10 °C/min under nitrogen atmosphere.

3.4 Determination of loading capacity and encapsulation efficiency of drug-loaded particles

The amounts of vancomycin loaded into rice granules and calcium citrate particles and erythromycim loaded into ethyl cellulose particles and poly(lactic-co-glycolic acid) particles were determined using UV-Visible Spectrophotometer (CARY 100 Bio, Palo Alto, USA).

One mL of supernatants after centrifugation of VAN-RG and VAN-CC were subjected to quartz glass and determined the absorbance at 283 nm which is the maximum wavelength of vancomycin. In case of erythromycin, the supernatant of ERY-EC was dried in an oven at 60 °C and redissolved in 20 mL of dichloromethane and measured the absorbance at 237 nm. The supernatant of ERY-PLGA was dried in an oven at 60 °C and redissolved in 20 mL of methanol before subjecting to absorbance measurement at 206 nm.

3.4.2 Encapsulation efficiency and loading

Encapsulation efficiency and loading capacity were determined indirectly using the formulas:

$$\text{Encapsulation efficiency (\%EE)} = \frac{D_i - D_f}{D_i} \times 100 \quad (1)$$

D_i = Total amount of drug used

D_f = amount of free drug (unloaded)

$$\text{Loading capacity (\%loading)} = \frac{W_d}{W_p} \text{x}100 \quad (2)$$

W_d = Weight of loaded drug

W_p = Weight of the drug-loaded particles (product)

3.5 PMMA bone spacer casting

The control bone spacer in a cubic shape of 1x1x1 cm^3 were prepared. One gram of PMMA powder (containing poly methyl methacrylate, zirconium dioxide, benzoyl peroxide, and colorant E141) was mixed with 0.5 mL of methyl methacrylate (MMA) liquid monomer (containing methyl methacrylate, N,N-dimethyl-p-toluidine, hydroquinone, and colorant E141). Then, the mixture was put into the silicone mould of 1x1x1 cm^3 and left at room temperature overnight.

A total of 18 bone spacers were prepared, nine with addition of vancomycin and nine with addition of erythromycin. Three different vancomycin-loaded particles types were used in the casting. Group 1: Fifty five mg of vancomycin was mixed with 1 g of PMMA powder and 0.5 mL of MMA liquid monomer homogeneously. The mixture was then immediately put into the silicone mould of 1x1x1 cm^3 and left at room temperature overnight, group 2 and group 3 samples were prepared similarly except that 62.33 mg of the 84.88% vancomycin-loaded rice granules (containing 55 mg of vancomycin) or 407.8 mg of the 4.89% vancomycin-loaded calcium citrate particles (containing 55 mg of

vancomycin) were used in placed of 55 mg of pure vancomycin. Three different types erythromycin-loaded particles were used in casting. Group 1: Fifty five mg of erythromycin was mixed with 1 g of PMMA powder and 0.5 mL of MMA liquid monomer homogeneously. The mixture was then put into the silicone mould of 1x1x1 cm^3 and left at room temperature overnight, group 2 and group 3 samples were prepared similarly except that 110 mg of the 52.01% erythromycin-loaded ethyl cellulose particles (containing 55 mg of erythromycin) or 88.84 mg of the 5.98% erythromycin-loaded poly(lactic-co-glycolic acid) particles (containing 55 mg of erythromycin) were used in placed of 55 mg of pure erythromycin. Figure 3.1 Summarizes bone spacer samples prepared for further studies.

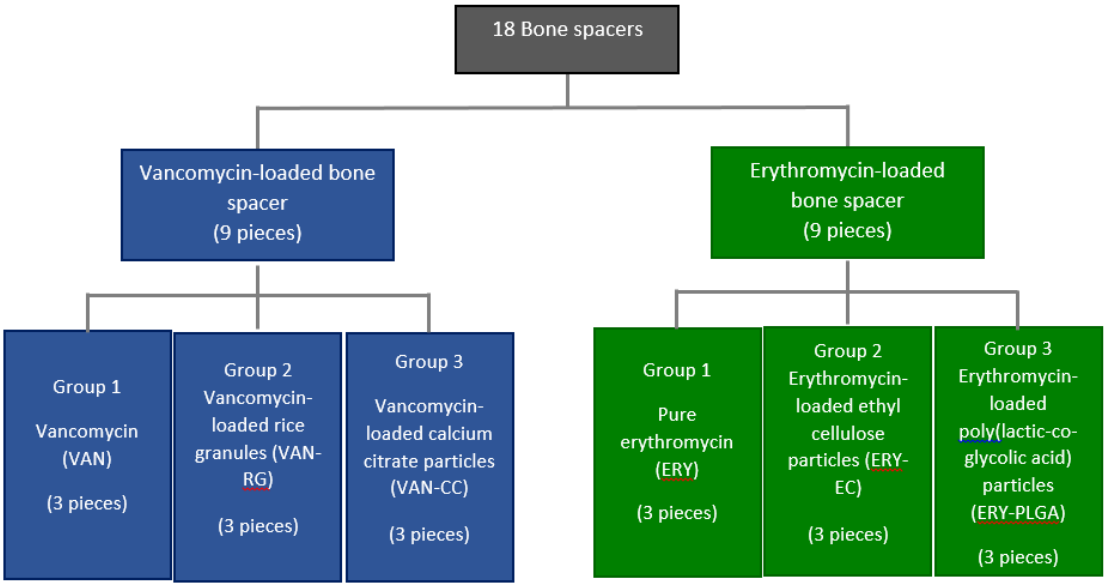


Figure 3.1 Six different types of bone spacers prepared

3.6 Characterization of PMMA bone spacer

The characterization of the prepared bone spacers was carried out using SEM, (JSM-7610, JEOL, Tokyo, Japan) and Universal testing machine (UTM, LR10K, LLOYD Instrument, England).

3.6.1 Mechanical properties

UTM was performed at the Dental Material Science Research Center, Faculty of Dentistry, Chulalongkorn University, Thailand. Bone spacers were dried in a desiccator overnight. The bone spacer piece was analysed using the compressor with diameter 6 mm and the compressive force of 10 kN at the constant cross-head speed of 20.0 mm/min. The machine was stopped when the sample was fractured or passed the upper yield point. The results of compressive strength were shown as the mean value ± standard deviation (SD) of the three samples. One-way analyzes of variance (ANOVA) is the method used to observe the difference of compressive strength of each type of poly(methyl methacrylate) composites.

3.7 Drug release testing

3.7.1 Drug release

The process of acquiring the drug release from the bone cements in phosphate buffer saline pH 7.4 is shown in Figure 3.2. The tested bone spacer (1x1x1 cm^3) with recorded exact weight, was placed into a 50 mL of polypropylene conical tube with screw cap and the tube was filled with 20 mL of 0.01 M PBS, pH 7.4. The tube was then closed and incubated at

37 °C with continuous shaking using water bath shaker (WNE 14 Water Bath with M00 Shaker). At specific times, one mL aliquot of PBS was withdrawn and changed each PMMA cube into 20 mL of fresh PBS. The test was carried out for 6 weeks. The withdrawn aliquot was subjected to drug concentration analysis using UV-Visible spectrophotometer.

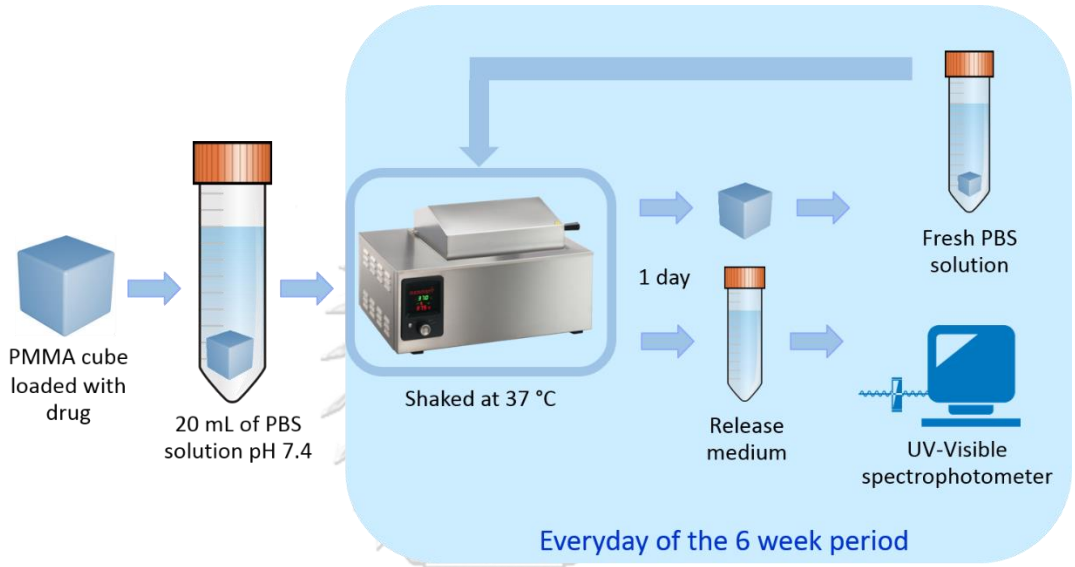


Figure 3.2 Drug release test

3.7.2 Analysis of drug concentration

To quantify the amount of drugs, the withdrawn release medium was subjected to UV-Visible spectrophotometer (at 283 nm and 210 nm for vancomycin and erythromycin, respectively). Both calibration curves of vancomycin standards and erythromycin standards were constructed using standard solutions prepared in release medium at concentration 50 mg/L to 250 mg/L.

CHAPTER IV
Results and discussion

4.1 Drug encapsulation

4.1.1 Encapsulation of vancomycin into rice granules

Vancomycin was encapsulated into rice granules *via* thermal expansion method. To encapsulate vancomycin into rice granules, vancomycin solution was poured into rice granules and the mixture was heated at 83 °C for 3 h. The resulting suspension was cooled and then centrifuged. The pellet of vancomycin-loaded rice granules was freeze-dried, whereas the supernatant was analyzed via UV-Visible spectrophotometry to determine the free vancomycin in the supernatant. The concentration of free vancomycin was used to calculate the encapsulation efficiency and percent loading of vancomycin in the vancomycin-loaded rice granules. The absorbance at 283 nm of each concentration of vancomycin standard solutions was plotted as the standard curve and shown in Figure 4.1.

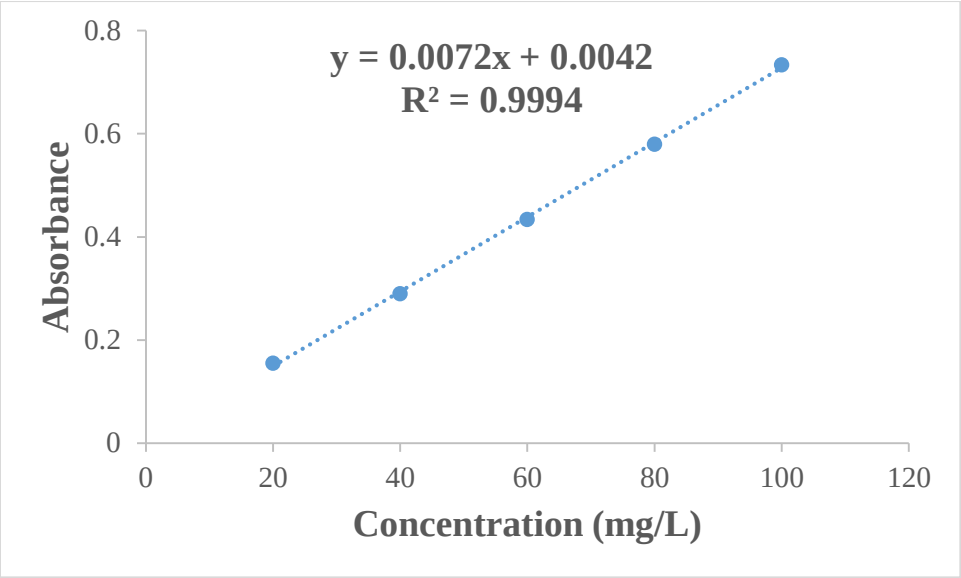


Figure 4.1 Calibration curve of vancomycin standard solutions

The absorbance value of the sample was compared and calculated to find free vancomycin using the formula as follows;

Y	=	0.0072X + 0.0042
0.133	=	0.0072X+0.0042
X	=	17.89 mg/L (In the supernatant)
loaded drug	=	60 mg/L – 17.89 mg/L
loaded drug	=	42.11 mg/L

The concentration of sample was calculated to amount of loaded drug

$$\text{mg of loaded drug} = 42.11 \text{ mg/L} \times \left(\frac{20000 \text{ mg/L}}{60 \text{ mg/L}}\right) \times \left(\frac{400 \text{ mg}}{20000 \text{ mg/L}}\right)$$

$$\text{mg of loaded drug} = 280.74 \text{ mg}$$

The encapsulation efficiency (%EE) was calculated from the amount of loaded drug using the equation as shown below

$$\text{Encapsulation efficiency (\%EE)} = \frac{\text{Total amount of drug used} - \text{amount of free drug (unloaded)}}{\text{Total amount of drug used}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = \frac{280.74\ \text{mg}}{400\ \text{mg}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = 70.18\ \%$$

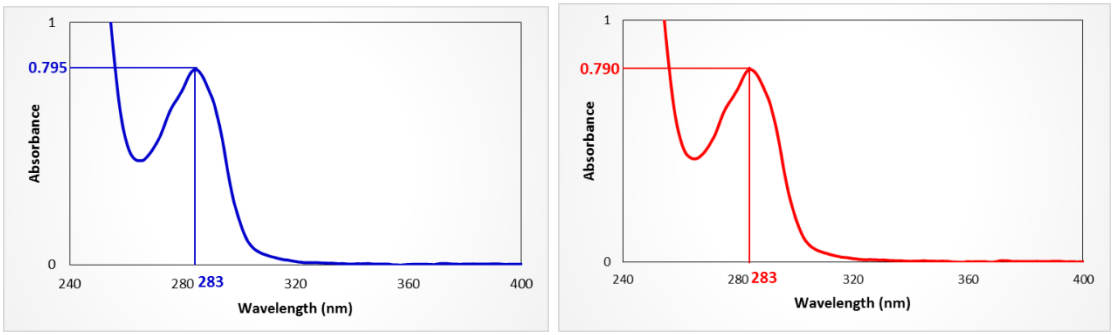
The loading capacity of vancomycin-loaded rice granules was determined using the formula

$$\text{Loading capacity (\%loading)} = \frac{\text{Weight of loaded drug}}{\text{Weight of the drug-loaded particles}} \times 100$$

$$\text{Loading capacity (\%)} = \frac{280.74\ \text{mg}}{330.7\ \text{mg}} \times 100$$; 330.7 mg is the weight of product

$$\text{Loading capacity (\%)} = 84.88\%$$

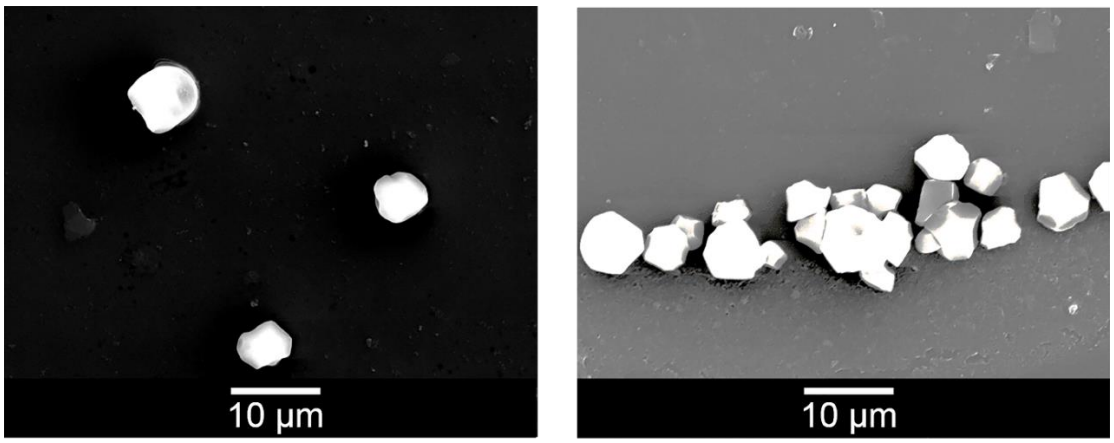
%EE and loading capacity of vancomycin into the rice granules were performed triplicately. Three replication results were shown as an average result (in appendix). The process of loading vancomycin into the rice granules yielded encapsulation efficiency of 70.2 $\pm$ 1.5% and loading capacity of 84.9 $\pm$ 0.3%. Although the process of vancomycin encapsulation into rice granules was performed at high temperature, the degradation of drug was not observed, the stability of heated vancomycin can be confirmed by UV-Visible spectrum (Figure 4.2). The results showed the UV spectrum of heated vancomycin was similar to the UV spectrum of the freshly-prepared vancomycin.



A **B**

Figure 4.2 UV spectrum of (**A**) the freshly-prepared vancomycin solution at 200 mg/L (**B**) the 200 mg/L vancomycin solution after being heated at 83 °C for 3 h

The dried pelleted of vancomycin-loaded rice granules (VAN-RG) was characterized using a scanning electron microscope. The SEM images indicated the encapsulation process did not change the shape and size of original rice granules, polyhedral of multi-pentagonal faces with the average size of 5.9 ± 1.1 µm (Figure 4.3 A), and the vancomycin-loaded rice granules showed the average size of 4.6 ± 0.8 µm (Figure 4.3 B). The surface of VAN-RG was not different from the original rice granules. It implies that most of the drugs may be loaded into rice granules.



A **B**

Figure 4.3 SEM images of (**A**) original rice granules, (**B**) vancomycin-loaded rice granules

The functional groups of vancomycin-loaded rice granules were analyzed via Fourier-transform infrared spectroscopy. Before characterization of VAN-RG with FTIR technique, 1 mL of DI water was added to the VAN-RG and ground with mortar and pestle to release the loaded vancomycin. Then, the pellet was dried in an oven at 60 °C. Compared to the FTIR spectra of initial rice granules and vancomycin (Figure 4.4). Original rice granules (purple line) showed the peak at 3302 cm $^{-1}$ which is stretching of a hydroxyl group (O-H) of amylose and amylopectin and 1646 cm^{-1} which is bending of C-H. The peak at 1000 cm^{-1} which is C-O stretching. FTIR spectrum of vancomycin (red line) showed the peak at 3254 cm^{-1} which is stretching of a hydroxyl group (O-H). The peak at 1651 cm^{-1} indicates bending of N-H. The peaks at 1601 cm^{-1} and 1488 cm^{-1} represent a stretching of C=C (in ring). The peak at 1226 cm^{-1} indicates stretching of C-O-C. FTIR spectrum of VAN-RG

(green line) showed 5 peaks at 3287 cm $^{-1}$, 1638 cm^{-1} , 1600 cm^{-1} ,1498 cm^{-1}, and 1229 cm^{-1}. The peak at 3287 cm^{-1} which is stretching of a hydroxyl group (O-H). The peak at 1638 cm^{-1} indicates bending of N-H. The peaks at 1600 cm^{-1} and 1498 cm^{-1} represent a stretching of C=C. The peak at 1229 cm^{-1} indicates stretching of C-O-C. The FTIR spectrum of VAN-RG showed the similarity to pure vancomycin. The results indicate high loading capacity of vancomycin in the VAN-RG.

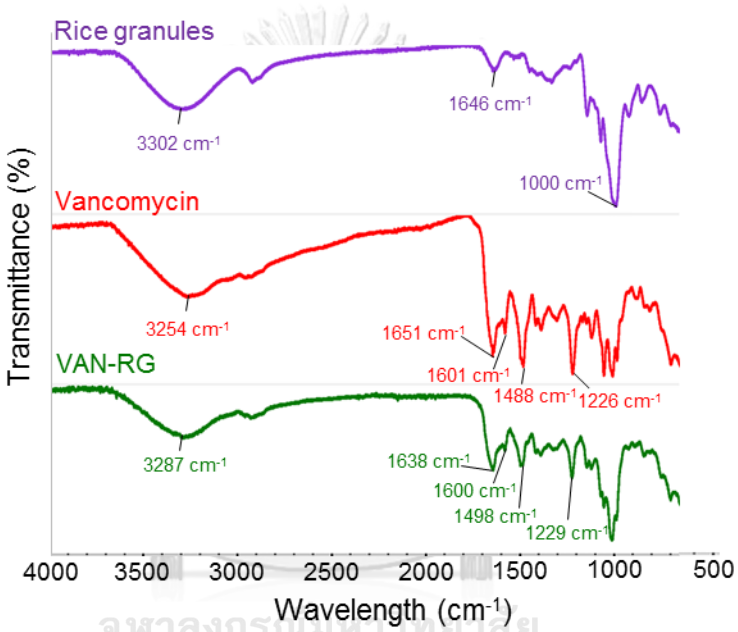


Figure 4.4 FTIR spectra of rice granules (purple line), vancomycin (red line), and vancomycin-loaded rice granules (green line)

The VAN-RG, original rice granules, and vancomycin were analyzed via thermal gravimetric analysis (TGA) to study the thermal stability and thermal decomposition properties (Figure 4.5). Rice granules showed the endothermic peak at around 258-320 °C with the maximum peak at 298 °C (purple line). The thermogram of pure vancomycin showed the endothermic peak at around 205-288 °C with the maximum

peak at 234 °C (red line). VAN-RG showed the much broader endothermic peak at around 203-315 °C with the maximum peak at 237 °C (green line). The disappearance of endothermic peaks of both rice granules and vancomycin, and the appearance of the new endothermic peak indicated the new interactions probably between vancomycin molecules and amylose and amylopectin chains. Vancomycin probably insert and disrupt the arrangement of original amylose and amylopectin chains and form the new interactions with the chains.

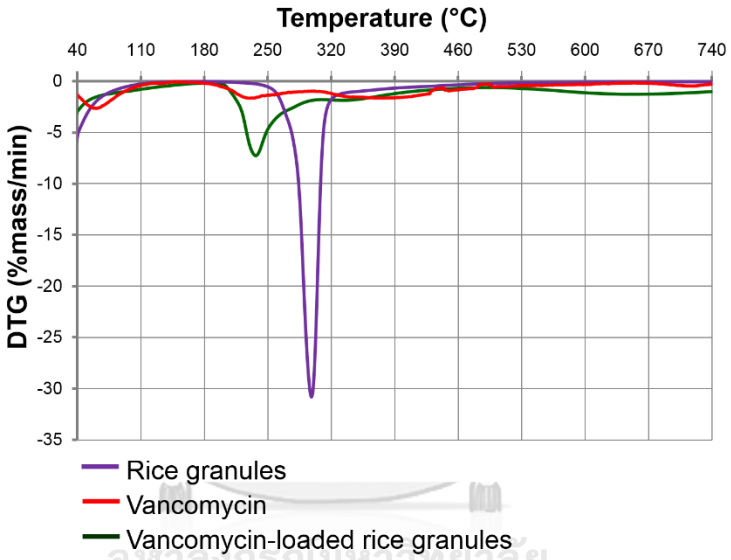


Figure 4.5 TGA thermogram of the endothermic peaks of rice granules (purple line), vancomycin (red line), and vancomycin-loaded rice granules (green line)

4.1.2 Encapsulation of vancomycin into calcium citrate particles

Vancomycin was encapsulated into calcium citrate particles via co-precipitation method. To encapsulate vancomycin into calcium citrate particles, vancomycin solution was mixed with calcium chloride solution

before mixing with sodium citrate solution to prevent calcium citrate precipitation before trapping of vancomycin. The results were listed in Table 4.1

Table 4.1 Percent recovery and amount of entrapped drug in various conditions

Calcium ion		**Citrate ion**		**Vancomycin (g)**	**Product (g)**	**% Recovery**	**Entrapped drug (mg)**
mmol	g	mmol	g				
1.36	0.055	0	-	0.02	0.0223	29.73	ND
1.19	0.048	0.17	0.033	0.02	0.0507	50.20	0.063
1.02	0.041	0.34	0.066	0.02	0.0823	64.80	0.106
0.85	0.034	0.51	0.098	0.02	0.1194	78.55	0.147
0.68	0.027	0.68	0.132	0.02	0.0614	34.30	0.133
0.51	0.021	0.85	0.163	0.02	0.0229	11.22	0.105
0.34	0.014	0.102	0.197	0.02	0.0275	11.90	0.077
0.17	0.007	0.19	0.229	0.02	0.0242	9.45	0.049
-	0	1.36	0.262	0.02	0.0216	7.66	ND

ND = Not detected

The results from Table 4.1 revealed that the mole ratio between calcium ion and citrate ion at 1:1 showed high amount of entrapped drug with small amount of the carrier in obtained product. In this work, the mole ratio between calcium ion and citrate ion at 1:1 was selected for further experiment.

To enhance encapsulation efficiency, the small amount of sodium hydroxide solution was added into the suspension to reduce the solubility of vancomycin and shaking was continued for another 24 h. The resulting suspension was then left in a refrigerator at 4 °C for 5 days. The resulting suspension was centrifuged. The pellet of vancomycin-loaded calcium citrate particles was freeze-dried, whereas the supernatant was analyzed via UV-Visible spectrophotometry to determine the free vancomycin in the supernatant. The concentration of free vancomycin was used to calculate the encapsulation efficiency and percent loading of vancomycin in the vancomycin-loaded calcium citrate particles. The absorbance at 283 nm of each concentration of vancomycin standard solutions was plotted as the standard curve and shown in Figure 4.6.

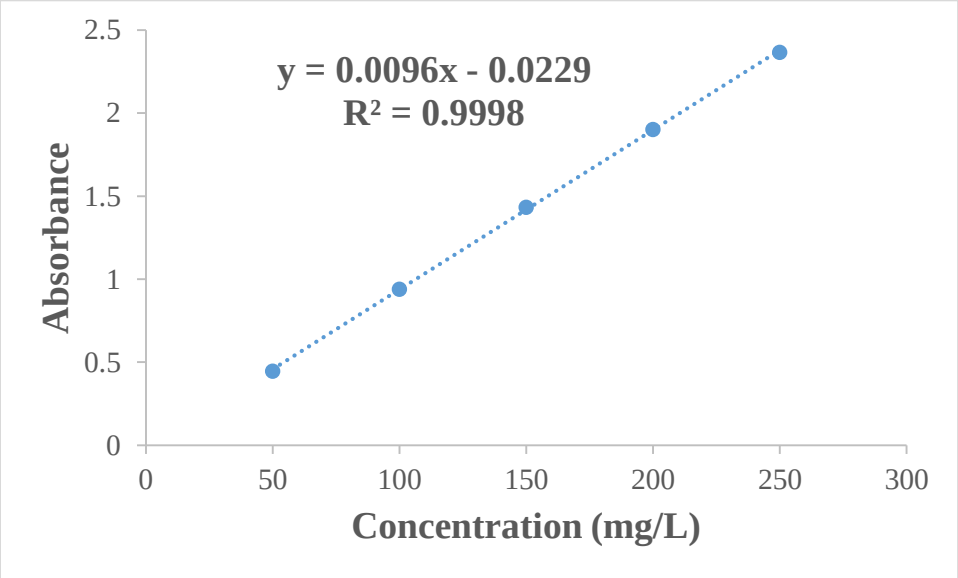


Figure 4.6 Calibration curve of vancomycin standard solutions

The absorbance value of the sample was compared and calculated to find free vancomycin using the formula as follows;

Y = 0.0096X - 0.0229

0.745 = 0.0096X-0.0229

X = 80.00 mg/L (In the supernatant)

loaded drug = 100 mg/L – 80.00 mg/L

loaded drug = 20.00 mg/L

The concentration of sample was calculated to amount of loaded drug

$$\text{mg of loaded drug} = 20.00 \text{ mg/L} \times \left(\frac{100000 \text{ mg/L}}{100 \text{ mg/L}}\right) \times \left(\frac{200 \text{ mg}}{100000 \text{ mg/L}}\right)$$

mg of loaded drug = 40.00 mg

The encapsulation efficiency (%EE) was calculated from the amount of loaded drug using the equation as shown below

$$\text{Encapsulation efficiency (\%EE)} = \frac{\text{Total amount of drug used-amount of free drug (unloaded)}}{\text{Total amount of drug used}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = \frac{40 \text{ mg}}{200 \text{ mg}} \times 100$$

Encapsulation efficiency (%EE) = 20.00 %

The loading capacity of vancomycin-loaded rice granules was determined using the formula

$$\text{Loading capacity (\%loading)} = \frac{\text{Weight of loaded drug}}{\text{Weight of the drug-loaded particles}} \times 100$$

$$\text{Loading capacity (\%)} = \frac{40 \text{ mg}}{1142.9 \text{ mg}} \times 100$$; 1142.9 mg is the weight of product

Loading capacity (%) = 3.5%

%EE and loading capacity of vancomycin into the calcium citrate particles were performed triplicately. Three replication results were shown as an average result (in appendix). The process of loading vancomycin into the calcium citrate particles yielded the average of encapsulation efficiency of 27.9 ± 18.3% and loading capacity of 4.9 ± 3.2%.

The dried pellet of vancomycin-loaded calcium citrate particles (VAN-CC) was characterized using scanning electron microscope. The SEM images showed the shape and size of vancomycin-loaded calcium citrate particles which were spherical with the average diameter of 554.4 ± 220.3 nm (Figure 4.7).

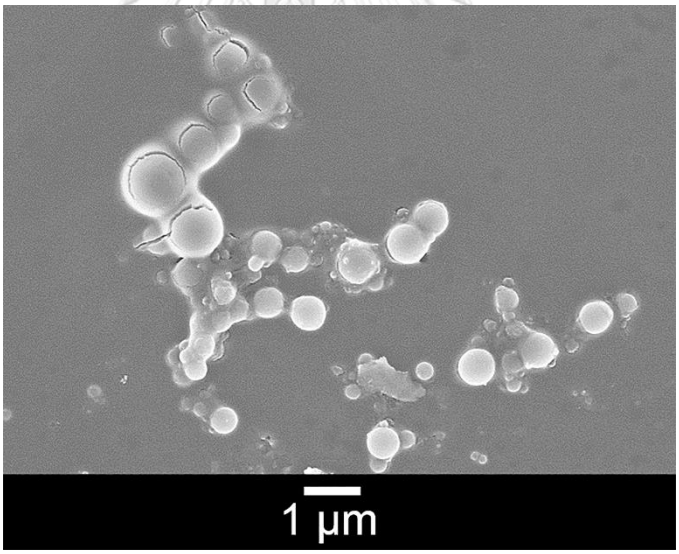


Figure 4.7 SEM images of vancomycin-loaded calcium citrate particles

The functional groups of vancomycin-loaded calcium citrate particles were analyzed via Fourier-transform infrared spectroscopy. Before characterization of VAN-CC with FTIR technique, 1 mL of DI

water was added to VAN-CC and ground with mortar and pestle to break the VAN-CC. Then, the pellet was dried in an oven at 60 °C. Compared to the FTIR spectra of pure calcium citrate and vancomycin (Figure 4.8). Pure calcium citrate (blue line) showed the peaks at 1597 cm^{-1} and 1425 cm^{-1} which are anti-symmetrical and symmetrical vibrations of the COO- group of citrates. FTIR spectrum of vancomycin (red line) showed the peak at 3254 cm^{-1} which is stretching of a hydroxyl group (O-H). The peak at 1651 cm^{-1} indicates bending of N-H. The peaks at 1601 cm^{-1} and 1488 cm^{-1} represent a stretching of C=C (in ring). The peak at 1226 cm^{-1} indicates stretching of C-O-C. FTIR spectrum of VAN-CC (pink line) showed four peaks at 3051 cm^{-1}, 1662 cm^{-1}, 1602 cm^{-1}, and 1425 cm^{-1}. The peak at 3051 cm^{-1} which is stretching of an amine group (N-H). The peak at 1662 cm^{-1} indicates bending of N-H. The peaks at 1602 cm^{-1} and 1425 cm^{-1} represent the anti-symmetrical and symmetrical vibrations of the COO- group of citrates. The FTIR spectrum of VAN-CC showed the FTIR spectrum almost similar to the FTIR spectrum of pure calcium citrate, but appeared the peak at 3051 cm^{-1} and 1662 cm^{-1} that are corresponded to N-H stretching and bending, respectively. This functional group is in the vancomycin molecule. Indicating the existence of vancomycin in the VAN-CC.

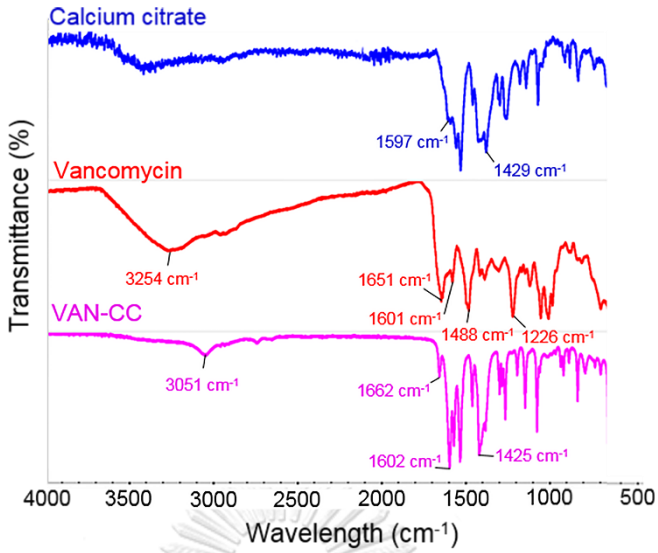


Figure 4.8 FTIR spectra of calcium citrate particles (blue line), vancomycin (red line), and vancomycin-loaded calcium citrate particles (pink line)

The VAN-CC, calcium citrate, and vancomycin were analyzed via thermal gravimetric analysis (TGA) (Figure 4.9). Pure calcium citrate showed three endothermic peaks at 381 °C, 482 °C, and 675 °C. The thermogram of pure vancomycin showed the endothermic peak at around 205-288 °C with the maximum peak at 234 °C. Compared to pure calcium citrate, VAN-CC showed four endothermic peaks at 239 °C, 372 °C, 462 °C, and 676 °C. The appearance of endothermic peaks at 239 °C in the thermogram of VAN-CC indicated that vancomycin molecules probably were trapped in the cavities of calcium citrate particles.

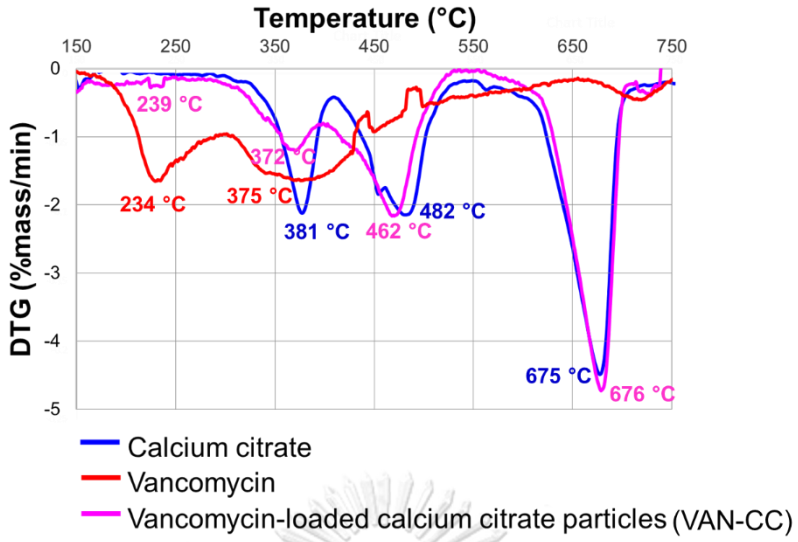


Figure 4.9 TGA thermogram of the endothermic peaks of calcium citrate particles (blue line), vancomycin (red line), and vancomycin-loaded calcium citrate particles (pink line)

4.1.3 Encapsulation of erythromycin into ethyl cellulose particles

Erythromycin was encapsulated into ethyl cellulose particles via solvent displacement method. To encapsulate erythromycin into ethyl cellulose particles, erythromycin and ethyl cellulose polymers were dissolved in ethanol. Then, water was slowly added into organic phase. The transparent mixture would turn to cloudy mixture with the formation of erythromycin-loaded ethyl cellulose particles. The suspension was then stirred under vacuum to evaporate ethanol. The resulting suspension was centrifuged. The pellet of erythromycin-loaded ethyl cellulose particles was freeze-dried, whereas the supernatant was analyzed via UV-Visible spectrophotometry to determine the free erythromycin in the supernatant. The concentration of free erythromycin was used to calculate the

encapsulation efficiency and percent loading of erythromycin in the erythromycin-loaded ethyl cellulose particles.

To determine the free erythromycin in the supernatant, the supernatant was dried in an oven at 60 °C and redissolved in 20 mL of dichloromethane to prevent the interference of dissolved ethyl cellulose polymers when the supernatant was analyzed via UV-Visible spectrophotometry, the absorbance at 237 nm of each concentration of erythromycin standard solutions was plotted as the standard curve and shown in Figure 4.10.

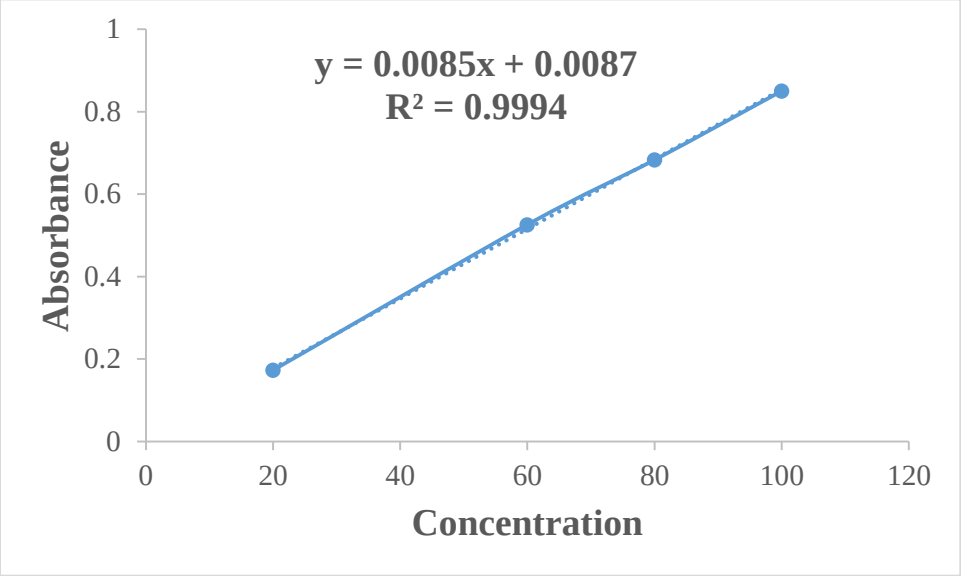


Figure 4.10 Calibration curve of erythromycin standard solutions

The absorbance value of the sample was compared and calculated to find free erythromycin using the formula as follows;

Y	=	0.0085X + 0.0087
0.395	=	0.0085X+0.0087
X	=	45.45 mg/L (In the supernatant)

loaded drug = 80 mg/L – 45.45 mg/L

loaded drug = 34.55 mg/L

The concentration of sample was calculated to amount of loaded drug

$$\text{mg of loaded drug} = 34.55 \text{ mg/L} \times \left(\frac{2000 \text{ mg/L}}{80 \text{ mg/L}}\right) \times \left(\frac{40 \text{ mg}}{2000 \text{ mg/L}}\right)$$

mg of loaded drug = 17.28 mg

The encapsulation efficiency (%EE) was calculated from the amount of loaded drug using the equation as shown below

$$\text{Encapsulation efficiency (\%EE)} = \frac{\text{Total amount of drug used-amount of free drug (unloaded)}}{\text{Total amount of drug used}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = \frac{17.28 \text{ mg}}{40 \text{ mg}} \times 100$$

Encapsulation efficiency (%EE) = 43.19 %

The loading capacity of erythromycin-loaded ethyl cellulose particles was determined using the formula

$$\text{Loading capacity (\%loading)} = \frac{\text{Weight of loaded drug}}{\text{Weight of the drug-loaded particles}} \times 100$$

$$\text{Loading capacity (\%)} = \frac{17.28 \text{ mg}}{37 \text{ mg}} \times 100 \text{ ; 37 mg is the weight of product}$$

Loading capacity (%) = 46.70%

%EE and loading capacity of erythromycin into the ethyl cellulose particles were performed triplicately. Three replication results were shown as an average result (in appendix). The process of loading erythromycin into the ethyl cellulose particles yielded encapsulation efficiency of 56.6 ± 1.3% and loading capacity of 52.0 $\pm$ 5.0%.

The dried pellet of erythromycin-loaded ethyl cellulose particles (ERY-EC) was characterized using scanning electron microscope. The SEM images indicated spherical-shaped with the average diameter of 312.5 $\pm$ 55.3 nm (Figure 4.11).

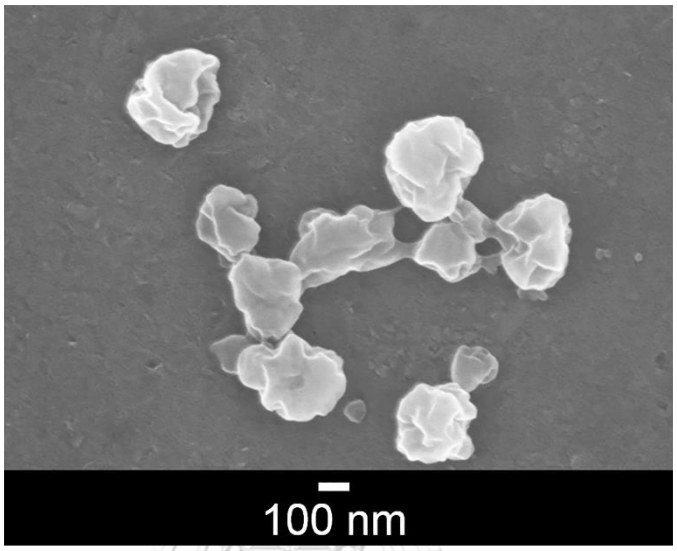


Figure 4.11 SEM images of erythromycin-loaded ethyl cellulose particles

The functional groups of erythromycin-loaded ethyl cellulose particles were analyzed via Fourier-transform infrared spectroscopy. Before characterization of ERY-EC with FTIR technique, 1 mL of ethanol was added to the ERY-EC and ground with mortar and pestle to break the ERY-EC. Then, the pellet was dried in an oven at 60 °C. Compared to the FTIR spectra of ethyl cellulose polymers and erythromycin (Figure 4.12). Ethyl cellulose (green line) showed the peaks at 2975 cm^{-1} and 2867 cm^{-1} related to CH_2 stretching. The peak at 1050 cm^{-1} indicates stretching of C-O-C. FTIR spectrum of erythromycin (pink line) showed the peak at 3517 cm^{-1} which is stretching of a hydroxyl

group (O-H). The peak at 2978 cm^{-1} indicates stretching of C-H. The peaks at 1738 cm^{-1} and 1715 cm^{-1} represent C=O stretching of ester and C=O stretching of aliphatic ketone. FTIR spectrum of ERY-EC (blue line) showed five peaks at 3452 cm^{-1}, 2973 cm^{-1}, 1736 cm^{-1}, 1701 cm^{-1}, and 1048 cm^{-1}. The peak at 3452 cm^{-1} which is stretching of a hydroxyl group (O-H). The peak at 2973 cm^{-1} indicates stretching of C-H. The peaks 1736 cm^{-1} and 1701 cm^{-1} represent C=O stretching of ester and C=O stretching of aliphatic ketone. The peak at 1048 cm^{-1} indicates stretching of C-O-C. The FTIR spectrum of ERY-EC showed the combined peaks of ethyl cellulose polymers and erythromycin components in its spectrum. The peaks at 1736 cm^{-1} and 1701 cm^{-1} could be assigned to C=O stretching, which is the functional group in the erythromycin. These results can prove the existence of erythromycin in the ERY-EC.

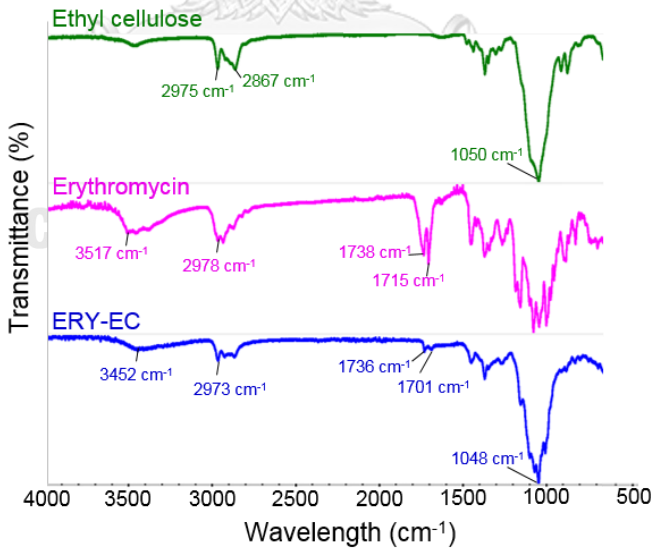


Figure 4.12 FTIR spectra of ethyl cellulose polymers (green line), erythromycin (pink line), and erythromycin-loaded ethyl cellulose particles (blue line)

The ERY-EC, ethyl cellulose polymers, and erythromycin were analyzed via thermal gravimetric analysis (TGA) to study the thermal stability and thermal decomposition properties (Figure 4.13). Ethyl cellulose polymers showed the endothermic peak at around 281-366 °C with the maximum peak at 342 °C. The thermogram of pure erythromycin showed the endothermic peak at around 260-321 °C with the maximum peak at 300 °C. Compared to ethyl cellulose, ERY-EC showed the narrower of endothermic peak at around 300-370 °C with the maximum peak at 349 °C. The disappearance of endothermic peaks of ethyl cellulose polymers and erythromycin, and the appearances of the new endothermic peak suggest the disruption of interaction among erythromycin molecules and among the ethyl cellulose and formed the new interactions between erythromycin and ethyl cellulose in the ERY-EC.

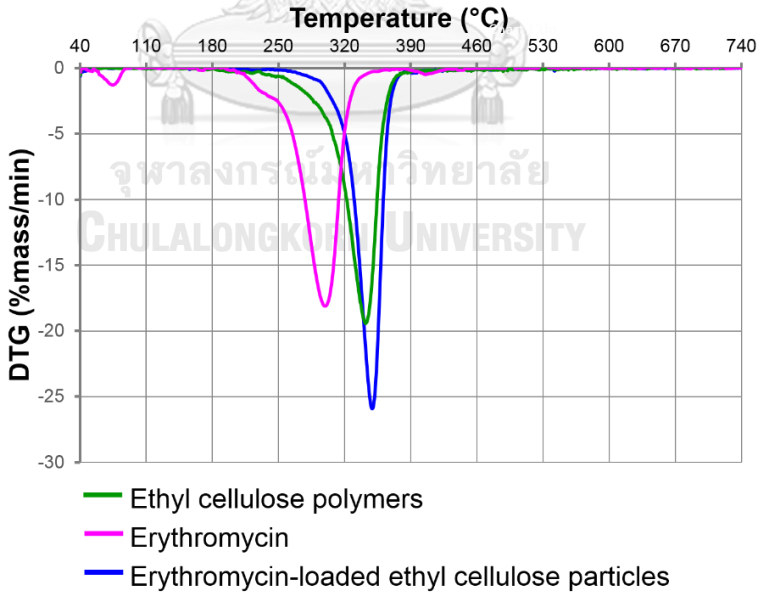


Figure 4.13 TGA thermogram of the endothermic peaks of ethyl cellulose polymers (green line), erythromycin (pink line), and erythromycin-loaded ethyl cellulose particles (blue line)

4.1.4 Encapsulation of erythromycin into poly(lactic-co-glycolic acid) particles

Erythromycin was encapsulated into poly(lactic-co-glycolic acid) particles via emulsion solvent evaporation method. To encapsulate erythromycin into poly(lactic-co-glycolic acid) particles, erythromycin and poly(lactic-co-glycolic acid) polymers were dissolved in dichloromethane and mixed homogeneously. Then, 5% PVA solution was added into the organic phase and emulsified by sonication. After that, the suspension was stirred under vacuum to evaporate dichloromethane. The cloudy suspension of erythromycin-loaded poly(lactic-co-glycolic acid) was then centrifuged. The pellet of erythromycin-loaded poly(lactic-co-glycolic acid) particles was freeze-dried, whereas the supernatant was analyzed via UV-Visible spectrophotometry to determine the free erythromycin in the supernatant. The concentration of free erythromycin was used to calculate the encapsulation efficiency and percent loading of erythromycin in the erythromycin-loaded poly(lactic-co-glycolic acid) particles.

To determine the free erythromycin in the supernatant, the supernatant was dried in an oven at 60 °C and redissolved in 20 mL of methanol to prevent the interference of dissolved poly(lactic-glycolic-acid) when the supernatant was analyzed via UV-Visible spectrophotometry. The absorbance at 206 nm of each concentration of erythromycin standard solutions was plotted as the standard curve and shown in Figure 4.14.

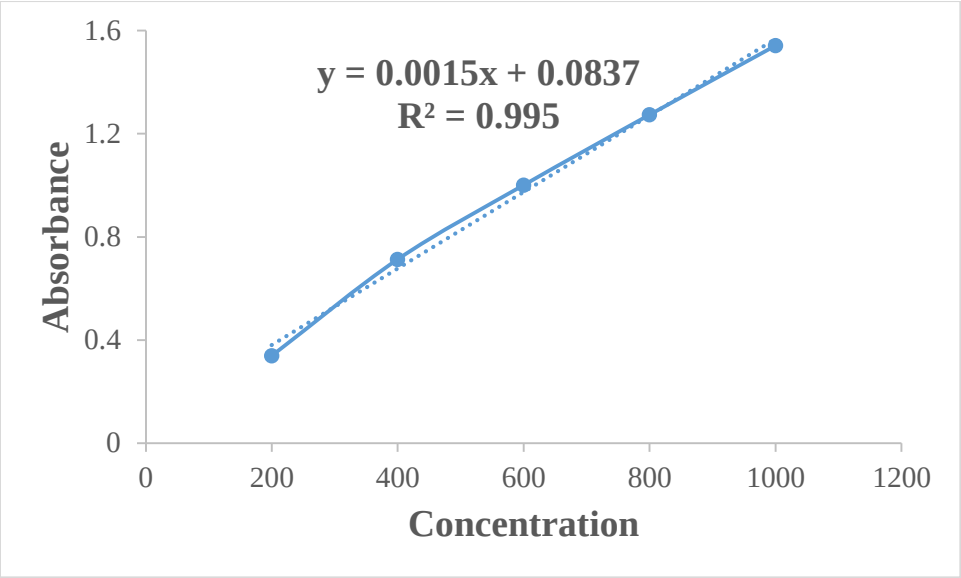


Figure 4.14 Calibration curve of erythromycin standard solutions

The absorbance value of the sample was compared and calculated to find free erythromycin using the formula as follows;

Y	=	0.0015X + 0.0837
0.129	=	0.0015X+0.0837
X	=	30.87 mg/L (In the supernatant)
loaded drug	=	600 mg/L – 30.87 mg/L
loaded drug	=	569.13 mg/L

The concentration of sample was calculated to amount of loaded drug

$$\text{mg of loaded drug} = 569.13 \text{ mg/L} \times \left(\frac{2000 \text{ mg/L}}{600 \text{ mg/L}}\right) \times \left(\frac{40 \text{ mg}}{2000 \text{ mg/L}}\right)$$

$$\text{mg of loaded drug} = 37.94 \text{ mg}$$

The encapsulation efficiency (%EE) was calculated from the amount of loaded drug using the equation as shown below

$$\text{Encapsulation efficiency (\%EE)} = \frac{\text{Total amount of drug used-amount of free drug (unloaded)}}{\text{Total amount of drug used}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = \frac{37.94\text{mg}}{40\text{ mg}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = 94.86\ \%$$

The loading capacity of erythromycin-loaded poly(lactic-co-glycolic acid) particles was determined using the formula

$$\text{Loading capacity (\%loading)} = \frac{\text{Weight of loaded drug}}{\text{Weight of the drug-loaded particles}} \times 100$$

$$\text{Loading capacity (\%)} = \frac{37.94\text{ mg}}{566.9\text{ mg}} \times 100$$; 566.9 mg is the weight of product

$$\text{Loading capacity (\%)} = 6.69\%$$

%EE and loading capacity of erythromycin into the poly(lactic-co-glycolic acid) particles were performed triplicately. Three replication results were shown as an average result (in appendix). The process of loading erythromycin into the poly(lactic-co-glycolic acid) particles yielded encapsulation efficiency of 85.1 $\pm$ 13.2% and loading capacity of 6.0 $\pm$ 0.9%.

The dried pellet of erythromycin-loaded poly(lactic-co-glycolic acid) particles (ERY-PLGA) was characterized by scanning electron microscope. The SEM images indicated spherical shape particles with the average diameter of 11.2 $\pm$ 8.1 μm (Figure 4.15).

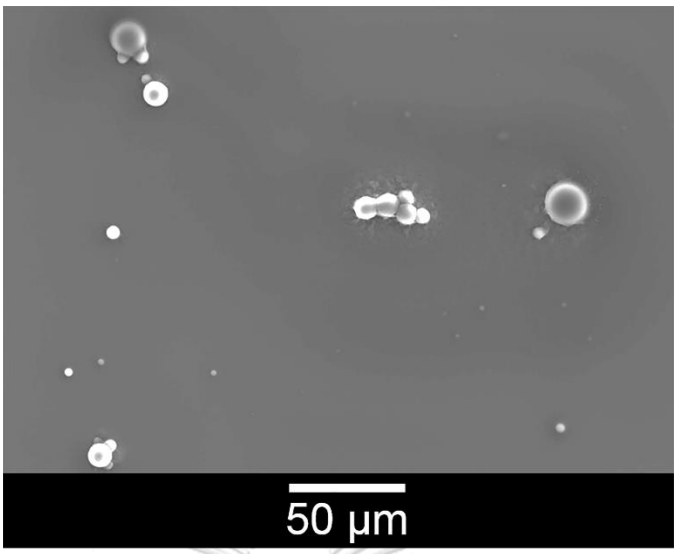


Figure 4.15 SEM images of erythromycin-loaded poly(lactic-co-glycolic acid) particles

The functional groups of erythromycin-loaded poly(lactic-co-glycolic acid) particles were analyzed via Fourier-transform infrared spectroscopy. Before characterization of ERY-PLGA with FTIR technique, 1 mL of dichotomethane was added to the ERY-PLGA and ground with mortar and pestle to break the ERY-PLGA. Then, the pellet was dried in an oven at 60 °C. Compared to the FTIR spectra of poly(lactic-co-glycolic acid) polymers, erythromycin, and polyvinyl alcohol (Figure 4.16). Poly(lactic-co-glycolic acid) polymers (green line) showed the peak at 2948 cm^{-1} indicates stretching of C-H. The peak at 1746 cm^{-1} related to C=O stretching of ketone. FTIR spectrum of erythromycin (pink line) showed the peak at 3517 cm^{-1} which is stretching of a hydroxyl group (O-H). The peak at 2978 cm^{-1} indicates stretching of C-H. The peaks at 1738 cm^{-1} and 1715 cm^{-1} represent C=O stretching of ester and C=O stretching of aliphatic ketone. FTIR spectrum

of polyvinyl alcohol (grey line) showed the peak at 3314 cm^{-1} which is stretching of a hydroxyl group (O-H). The peak at 2942 cm^{-1} indicates stretching of C-H. The peak at 1732 cm^{-1} indicated acetate group remaining in PVA. FTIR spectrum of ERY-PLGA (orange line) showed three peaks at 3331 cm^{-1}, 2938 cm^{-1}, and 1746 cm^{-1}. The peak at 3331 cm^{-1} which is stretching of a hydroxyl group (O-H). The peak at 2938 cm^{-1} indicates stretching of C-H. The peak at 1746 cm^{-1} represent stretching of C=O. The FTIR spectrum of ERY-PLGA showed no difference between pristine PLGA and ERY-PLGA, but quiet similar to FTIR spectrum of PVA. This similarity probably resulted of left PVA on the erythromycin-loaded poly(lactic-co-glycolic acid) particles.

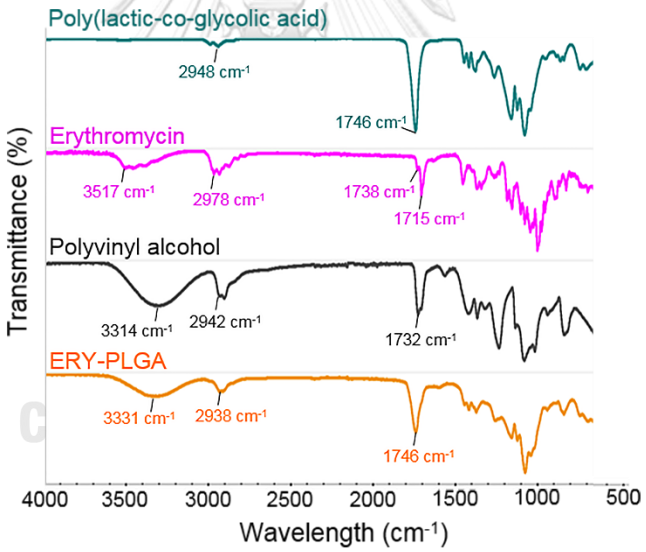


Figure 4.16 FTIR spectra of poly(lactic-co-glycolic acid) polymers (green line), erythromycin (pink line), polyvinyl alcohol (grey line), and erythromycin-loaded poly(lactic-co-glycolic acid) particles (orange line)

The ERY-PLGA, poly(lactic-co-glycolic acid) polymers, erythromycin, and polyvinyl alcohol were analyzed via thermal gravimetric analysis (TGA) (Figure 4.17). Poly(lactic-co-glycolic acid) polymers showed the endothermic peak at around 242-357 °C with the maximum peak at 337 °C. The thermogram of pure erythromycin showed the endothermic peak at around 260-321 °C with the maximum peak at 300 °C. The thermogram of polyvinyl alcohol showed 2 endothermic peaks at around 225-390 °C with the maximum peak at 314 °C and around 390-490 °C with the maximum peak at 425 °C. The thermogram of ERY-PLGA showed three endothermic peaks; the endothermic peak at around 200-350 °C with the maximum peak at 258 °C, the endothermic peak at around 380-460 °C with the maximum peak at 428 °C, and the endothermic peak at around 510-575 °C with the maximum peak at 556 °C. The increase of amount endothermic peaks of ERY-PLGA indicated many components in their particles. The endothermic peak at around 380-460 °C with the maximum peak at 428 °C of ERY-PLGA is similar to the endothermic peak at around 390-490 °C with the maximum peak at 425 °C of PVA. Indicating the existence of PVA on the ERY-PLGA.

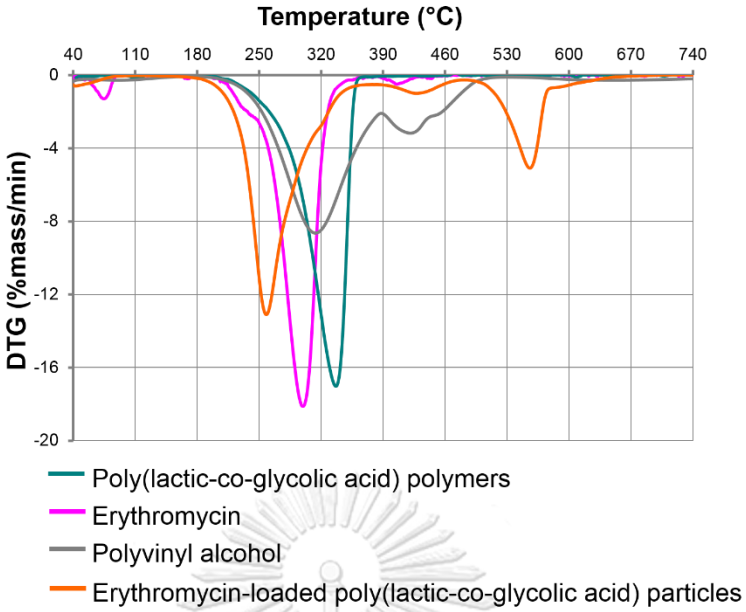


Figure 4.17 TGA thermogram of the endothermic peaks of poly(lactic-co-glycolic acid) polymers (green line), erythromycin (pink line), polyvinyl alcohol (grey line), and erythromycin-loaded poly(lactic-co-glycolic acid) particles (orange line)

4.2 Comparing the four drug-loaded particles

Vancomycin was successfully loaded into rice granules and calcium citrate particle, whereas erythromycin was successfully loaded into ethyl cellulose particles and poly(lactic-co-glycolic acid) particles. The %EE and %loading of all drug-loaded particles are concluded in the Table 4.2

Table 4.2 %EE and %loading of vancomycin-loaded rice granules, vancomycin-loaded calcium citrate particles, erythromycin-loaded ethyl cellulose particles, and erythromycin-loaded poly(lactic-co-glycolic acid) particles

Drug-loaded particles	Drug containing	Size of particles (Mean ± S.D.)	%EE	%loading
VAN-RG	Vancomycin	4.6 ± 0.8 µm	70.2 ± 1.5	84.9 ± 0.3
VAN-CC		554.4 ± 220.3 nm	27.9 ± 18.3	4.9 ± 3.2
ERY-EC	Erythromycin	312.5 ± 55.3 nm	56.6 ± 1.3	52.0 ± 5.0
ERY-PLGA		11.2 ± 8.1 µm	85.1 ± 13.2	6.0 ± 0.9

4.3 PMMA bone spacer casting

The resulted bone spacers after casting into the cubic shape mould is shown in Figure 4.18. The resulted PMMA spacers have a green colour and smooth texture.

Figure 4.18 PMMA composite in a cubic shape of 1x1x1 cm^3

4.3.1 Scanning electron microscopic analysis (SEM)

The morphology of the surface of bone spacers was characterized using scanning electron microscopic analysis (SEM) (Figure 4.19). The SEM images showed the smooth spherical particles of PMMA powder (the main component of PMMA spacer) with the average size of 48.1 ± 21.2 μm. The distribution of raw drug (vancomycin and erythromycin) and drug-loaded particles (VAN-RG, VAN-CC, ERY-EC, and ERY-PLGA) in the matrix of PMMA spacer have been observed. SEM images of Figure 4.19 row B is the results of PMMA loaded with raw vancomycin. This raw drug was used 5.5% (by weight) to fabricate the VAN-PMMA composites. The results indicated the good distribution of vancomycin in the PMMA matrix, but appeared some agglomerates of this drug in some position of the PMMA matrix. Figure 4.19 row C is the results of PMMA loaded with VAN-RG. These drug-loaded particles were used 6.2% (by weight) to fabricate the VAN-RG-PMMA. The results indicated the distribution of VAN-RG, but appeared some agglomerates of this drug in some position of the PMMA matrix. Figure 4.19 row D is the results of PMMA loaded with VAN-CC. These drug-loaded particles were used 40.8% (by weight) to fabricate the VAN-CC-PMMA. The results showed the good distribution of VAN-CC and can be observed apparently in the matrix of PMMA. Adding a lot of VAN-CC in the PMMA composites is resulted in the apparent observation. Figure 4.19 row E is the results of PMMA loaded with raw erythromycin. This raw drug was used 5.5% (by weight) to fabricate the VAN-PMMA composites. The results indicated the good distribution of erythromycin in the PMMA matrix, but appeared some agglomerates of this drug in the matrix of PMMA. Figure 4.19 row F is the results of PMMA loaded with

ERY-EC. These drug-loaded particles were used 11.0% (by weight) to fabricate the ERY-EC-PMMA. The results showed the good distribution of ERY-EC all over the PMMA matrix. Figure 4.19 row G is the results of PMMA loaded with ERY-PLGA. These drug-loaded particles were used 8.9% (by weight) to fabricate the ERY-PLGA-PMMA. The results showed the distribution and some agglomerates of ERY-PLGA in the matrix of PMMA.

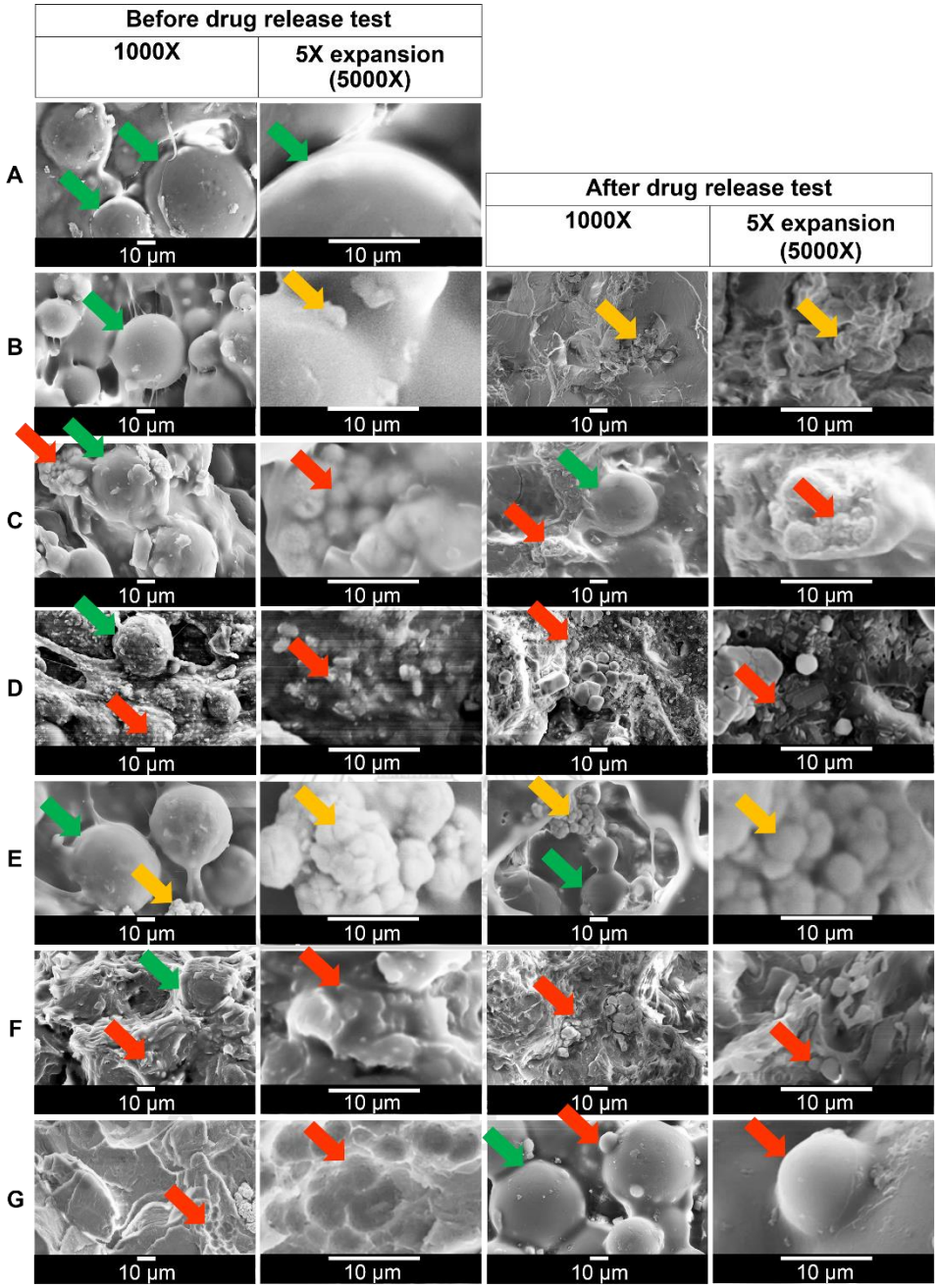


Figure 4.19 SEM images of poly(methyl methacrylate) spacer impregnated with (**A**) none, (**B**) vancomycin (VAN-PMMA), (**C**) vancomycin-loaded rice granules (VAN-RG-PMMA), (**D**) vancomycin-loaded calcium citrate particles (VAN-RG-PMMA), (**E**) erythromycin (ERY-PMMA), (**F**) erythromycin-loaded ethyl cellulose particles (ERY-EC-PMMA), and (**G**) erythromycin-loaded poly(lactic-co-glycolic acid)

particles (ERY-PLGA-PMMA). The green arrows indicate the poly(methyl methacrylate) particles. The yellow arrows indicate the unencapsulated drugs (vancomycin or erythromycin). The orange arrows indicate the encapsulated drug

4.4 Drug release testing

4.4.1 Concentration of drug release (µg/mL)

Figure 4.20 shows the in vitro drug release from bone spacers loaded with different drug-loaded particles. It should be noted here that PBS buffer (50 mL) was changed every day to imitate the daily biological fluid in the body. The results show that concentrations of vancomycin and erythromycin decrease with time. The concentrations of vancomycin dropped quickly during the first five days for all three types of bone spacers. It's likely that the drug was release from the outer surface of the bone spacers. However, the spacer loaded with VAN-CC showed longest sustained release of more than 40 days. The PMMA cube loaded with VAN-CC showed the highest and longest vancomycin release, followed with the PMMA cube loaded with raw vancomycin. We speculate that with low drug loading for the VAN-CC, we had to incorporate higher amount of the drug-loaded particles into the PMMA piece. This higher particle/PMMA ratio probably led to the less homogeneity of the PMMA piece. Thus, it was possible for the loaded drug at the inside of the PMMA piece to find a way to leach out from the piece. The result encourages the use of calcium citrate particles as vancomycin carrier to prolong the antibiotic release from PMMA spacer. It should be noted here that the amount of vancomycin release into the release medium at week 6,

still exceeding the minimum concentration of vancomycin in inhibiting the growth of microbes (2 μg/mL) [101]. The PMMA cubes loaded with all types of erythromycin showed continuous drop of drug concentration until day 10. Interestingly, bone spacers prepared with raw drug (unencapsulated) showed the best sustained drug release. At week 6, the concentration of erythromycin released from all three bone spacers were still exceeding the minimum concentration of erythromycin in inhibiting the growth of microbes (8 μg/mL) [102].

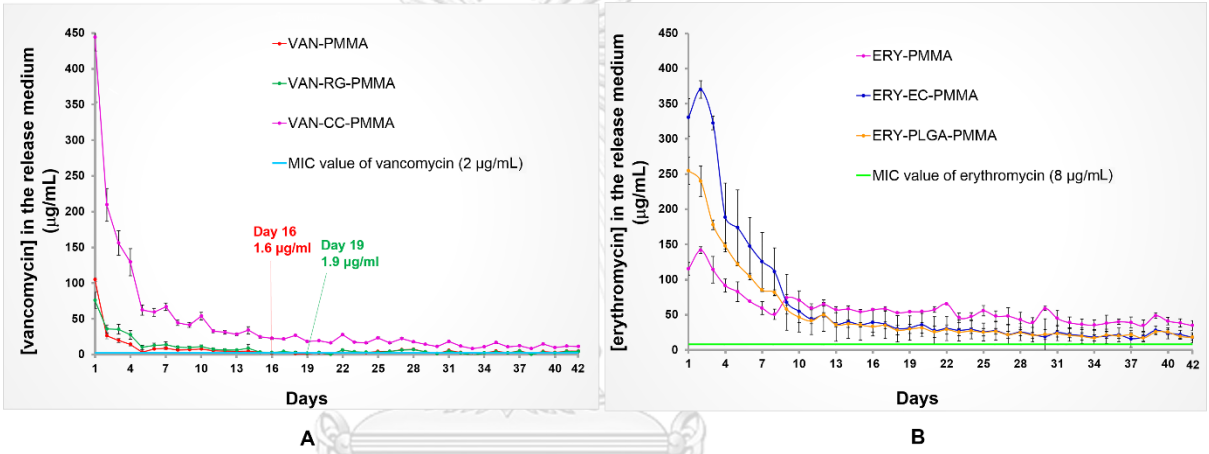


Figure 4.20 Drug release profiles (μg/mL) of (**A**) bone spacers loaded with raw vancomycin, VAN-RG, and VAN-CC and (**B**) bone spacers loaded with raw erythromycin, ERY-EC, and ERY-PLGA

4.4.2 Percentages of accumulated drug released

The drug release of vancomycin from vancomycin-contained composites and erythromycin from erythromycin-contained composites shown as the percentages of cumulative drug released (Figure 4.21). Poly(methyl methacrylate) composites impregnated with the VAN-CC increased the amount of vancomycin release up to 67.4% in 42 days, whereas vancomycin released from PMMA composites impregnated with

VAN-RG and unencapsulated vancomycin released vancomycin of 13.0% and 11.5%, respectively. As mentioned in drug release (μg/mL), incorporating a higher amount of the drug-loaded particles into the PMMA piece let the drug at the inside of the PMMA piece can find a way to leach out from the piece. Similar to the erythromycin-contained composites, ERY-EC produced the best release (96.8%) from ERY-EC-PMMA. Adding other components led to the less homogeneity of the PMMA piece and the way for the drug to disperse into the medium.

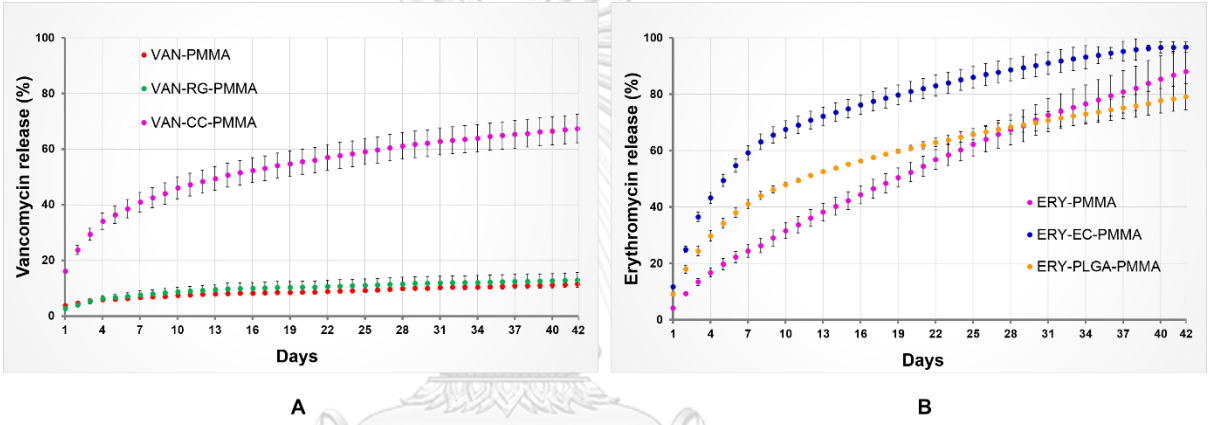


Figure 4.21 Cumulative in percentages of drug release profiles of (**A**) bone spacers loaded with raw vancomycin, VAN-RG, and VAN-CC and (**B**) bone spacers loaded with raw erythromycin, ERY-EC, and ERY-PLGA

4.5 Mechanical properties

The compressive strengths of each bone spacers are shown as the maximum stress values in Table 4.3. The results indicate the slightly decrease of maximum stress of all four bone spacers after the 42 h-drug release. These decrease probably was a result of voids from the eluted drug [14].

Table 4.3 Maximum stress of bone spacers mixed with different type of drug-loaded particles

Drug particles in bone spacers	Average maximum stress (MPa)	
	Before drug release test	After drug release test
PMMA	135.5±10.8	-
VAN-PMMA	92.9±24.1	76.2±6.5
VAN-RG-PMMA	112.7±4.5	76.2±6.5
VAN-CC-PMMA	73.4±17.2	70.0±7.6
ERY-PMMA	120.9±1.9	61.7±7.3
ERY-EC-PMMA	72.2±12.0	66.0±5.6
ERY-PLGA-PMMA	83.6±0.6	68.2±7.6

The difference between the maximum stress values of each bone spacers are evaluated via one-way analyses of variance (ANOVA) which are shown in Figure 4.22. The results showed that adding unencapsulated drug (either vancomycin or erythromycin) into the PMMA composites did not give a significant difference in the compressive strength at the 0.05 level of significance. In contrast, adding encapsulated drug (excluding VAN-RG) produced a significant decrease in the compressive strength at the 0.05 level of significance. Adding VAN-RG did not give a significant difference in the compressive strength. We speculated that rice granules, behave like the additive for the PMMA composites with good mechanical properties to this material [103-105]. After drug release test,

among the six PMMA composites, VAN-RG-PMMA and ERY-PMMA produced a significant decrease in compressive strength. Decreasing of compressive strength of VAN-RG-PMMA can be presumed that rice powder, which is a good moisture barrier, absorb a lot of moisture after the 42-day-release test, affect the compressive strength was decrease [106]. Although all of PMMA composites were dried in a desiccator for weeks. Decreasing of compressive strength of ERY-PMMA can be speculated that good erythromycin release character in ERY-PMMA was allowed the erosion of polymer matrix and drug particles. These erosions may be resulted in cavities in the composite and decreased the maximum strength of ERY-PMMA, whereas ERY-EC-PMMA and ERY-PLGA-PMMA were impregnated with polymers, which could not leach out from the piece and retain the strength of their composites.

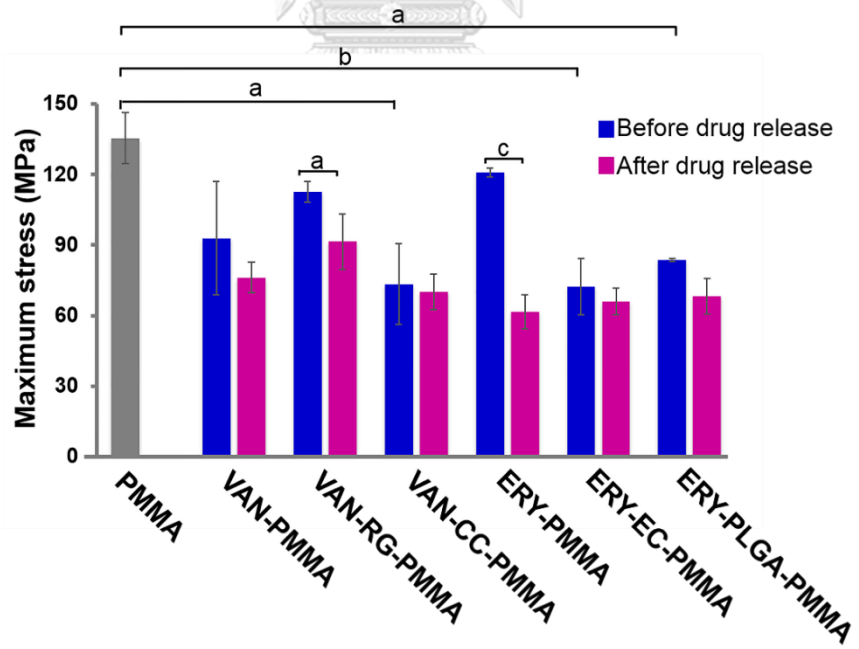


Figure 4.22 Compressive strengths of all PMMA composites measured in maximum stress (MPa) before and after drug release test for 42 days. Significant differences between the tested groups are labeled with a, b, and c for level of certainty of 0.05, 0.01, and 0.001, respectively.

CHAPTER V
CONCLUSION

Here we showed 1) the use of four drug carriers to encapsulate vancomycin and erythromycin 2) the subsequent incorporation of the obtained encapsulated drugs into PMMA bone spacer 3) the monitoring of the drug release from the resulted spacers and 4) the measuring of the compressive strength of all bone spacers. Vancomycin, the representative of the hydrophilic drug, was loaded into rice granules and calcium citrate particles. The vancomycin encapsulation process gave particles of 84.9 ± 0.3% and 4.9 ± 3.2% loading contents and the encapsulation efficiency of 70.2 ± 1.5% and 27.9 ± 18.3% for rice granules and calcium citrate particles, respectively. Erythromycin, the representative of hydrophobic drug, was loaded in to ethyl cellulose particles and poly(lactic-co-glycolic acid) particles, the process gave particles with drug loading of 58.1 ± 5.3% and 6.0 ± 0.9% at the encapsulation efficiency values of 56.6 ± 1.3% and 85.1 ± 13.2%, for ethyl cellulose particles and poly(lactic-co-glycolic acid) particles, respectively. The average sizes of rice granules, calcium citrate particles, ethyl cellulose particles, and poly(lactic-co-glycolic acid) particles were 4.58 ± 0.84 μm, 554.4 ± 220.3 nm, 312.5 ± 55.3 nm, and 11.2 ± 8.1 μm, respectively. Bone spacers fabricated with vancomycin-loaded calcium citrate showed significantly higher drug release for the prolonged period of more than 1 month, comparing to those fabricated with free vancomycin and vancomycin-loaded rice granules. In contrast, hydrophobic drug release (erythromycin in this case) was faster when the drug encapsulation was used. PMMA spacers

fabricated with free erythromycin showed the better sustained release of the drug, comparing to the PMMA spacers fabricated with erythromycin-loaded ethyl cellulose particles and erythromycin-loaded poly(lactic-co-glycolic acid) particles. The compressive strength of bone spacers impregnated with unencapsulated drug did not reduce the compressive strength of PMMA composite, whereas adding encapsulated drug (vancomycin-loaded rice granules is excluded) produced a significant decrease in the compressive strength. After drug release test, PMMA spacers impregnated with vancomycin-rice granules and raw erythromycin produced a significant decrease in the compressive strength.

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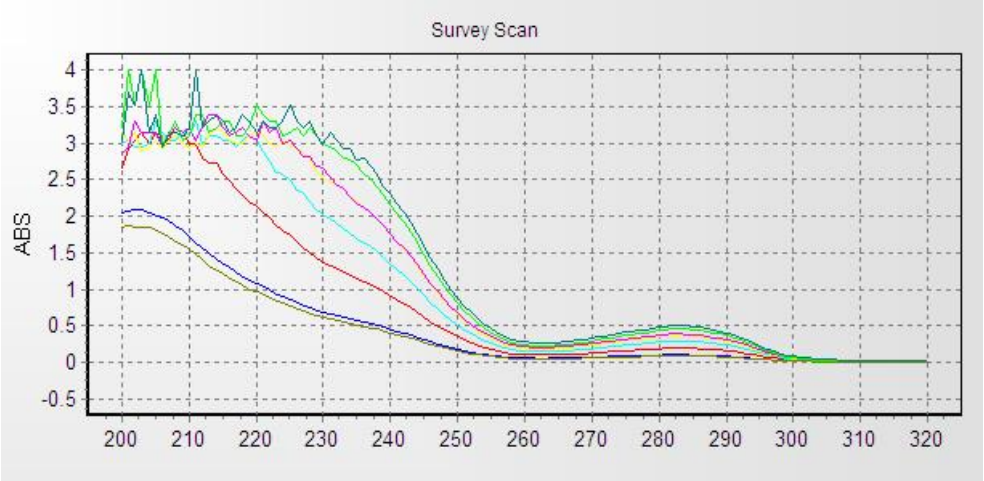
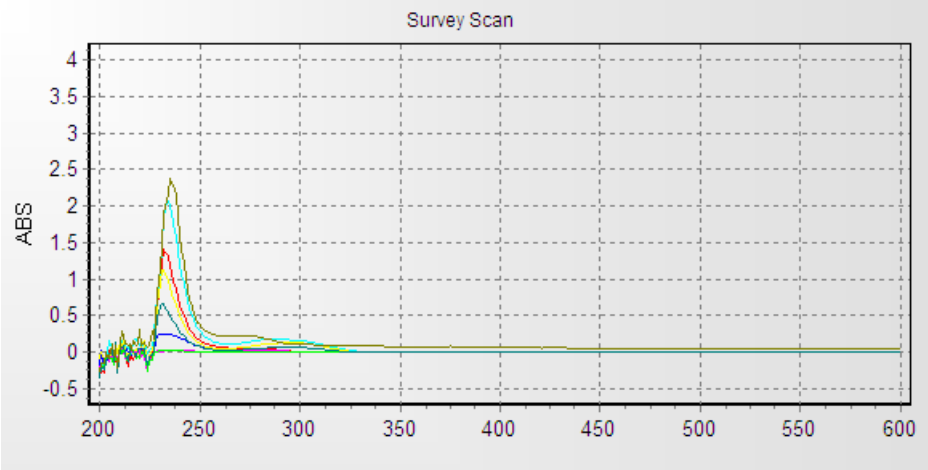
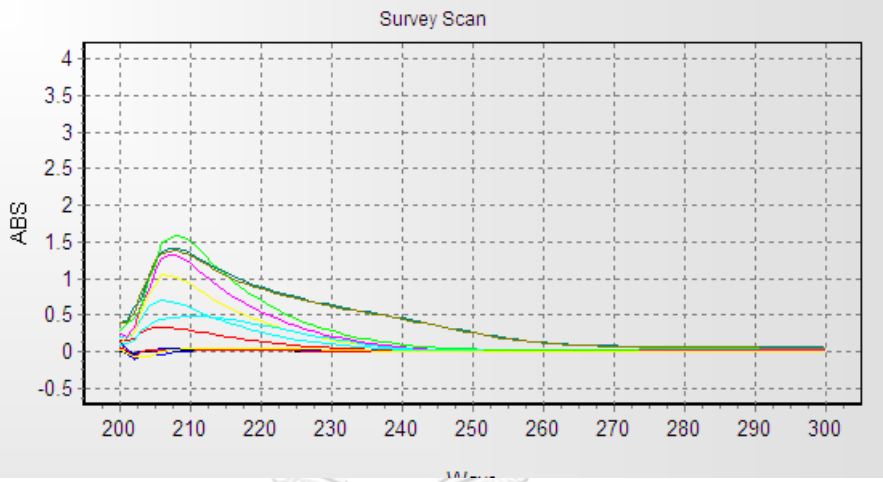
APPENDIX



UV spectra of vancomycin in water



UV spectra of erythromycin in dichloromethane



UV spectra of erythromycin in methanol

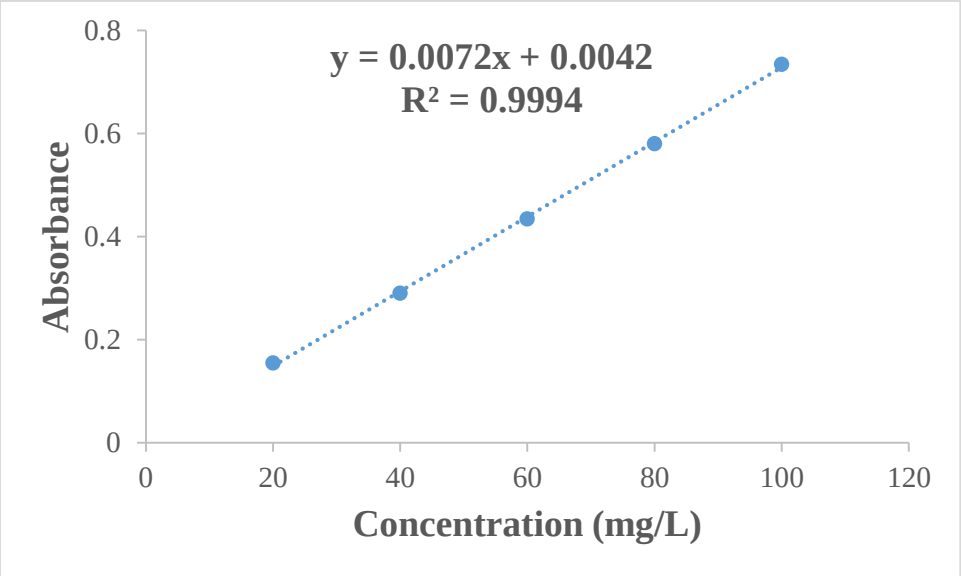
1. **Calculation of %EE and loading capacity of vancomycin in VAN-RG**

Experimental 1

The absorbance of vancomycin standard solution and the diluted sample.

	Standard					Sample
Concentration (mg/L)	20	40	60	80	100	60
Absorbance at 283 nm	0.155	0.29	0.434	0.58	0.734	0.133

The absorbance at 283 nm of each concentration of vancomycin standard solutions was plotted as the standard curve and shown in figure below.



Calibration curve of vancomycin standard solutions

The absorbance value of the sample was compared and calculated to find free vancomycin using the formula as follows;

$$0.133 = 0.0072X+0.0042$$

$$X = 17.89 \text{ mg/L (In the supernatant)}$$

$$\text{loaded drug} = 60 \text{ mg/L} - 17.89 \text{ mg/L}$$

$$\text{loaded drug} = 42.11 \text{ mg/L}$$

The concentration of sample was calculated to amount of loaded drug

$$\text{mg of loaded drug} = 42.11 \text{ mg/L} \times \left(\frac{20000 \text{ mg/L}}{60 \text{ mg/L}}\right) \times \left(\frac{400 \text{ mg}}{20000 \text{ mg/L}}\right)$$

mg of loaded drug = 280.74 mg

The encapsulation efficiency (%EE) was calculated from the amount of loaded drug using the equation as shown below

$$\text{Encapsulation efficiency (\%EE)} = \frac{\text{Total amount of drug used} - \text{amount of free drug (unloaded)}}{\text{Total amount of drug used}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = \frac{280.74 \text{ mg}}{400 \text{ mg}} \times 100$$

Encapsulation efficiency (%EE) = 70.18 %

The loading capacity of vancomycin-loaded rice granules was determined using the formula

$$\text{Loading capacity (\%loading)} = \frac{\text{Weight of loaded drug}}{\text{Weight of the drug-loaded particles}} \times 100$$

$$\text{Loading capacity (\%)} = \frac{280.74 \text{ mg}}{330.7 \text{ mg}} \times 100$$; 330.7 mg is the weight of product

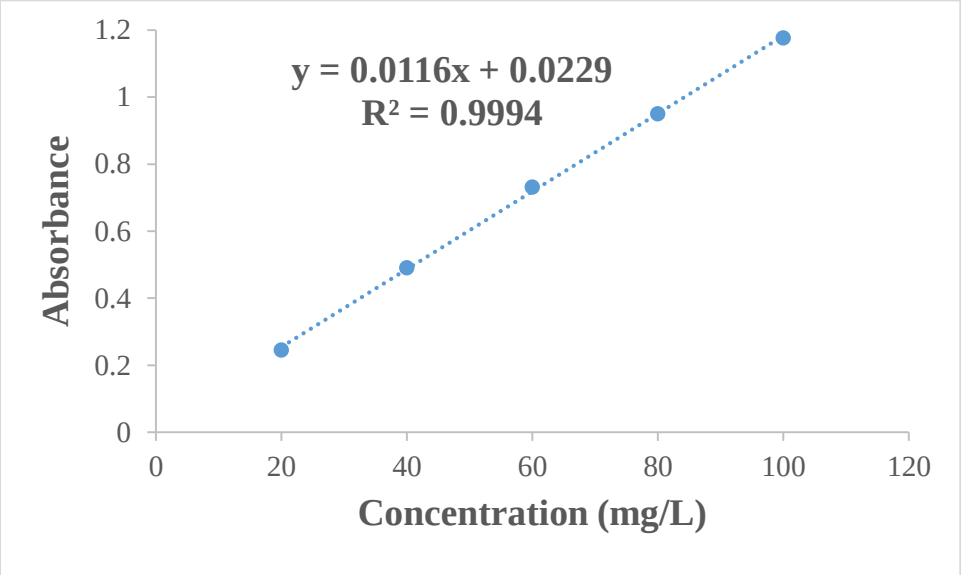
Loading capacity (%) = 84.88%

Experiment 2

The absorbance of vancomycin standard solution and diluted sample.

	Standard					Sample
Concentration (mg/L)	20	40	60	80	100	60
Absorbance 283 nm	0.246	0.491	0.732	0.95	1.177	0.22

The absorbance at 283 nm of each concentration of vancomycin standard solutions was plotted as the standard curve and shown in figure below.



Calibration curve of vancomycin standard solution

The absorbance value of the sample was compared and calculated to find free vancomycin using the formula as follows;

$$Y = 0.0116X + 0.0229$$

$$0.22 = 0.0166X + 0.0229$$

$$X = 16.99 \text{ mg/L (In the supernatant)}$$

$$\text{loaded drug} = 60 \text{ mg/L} - 16.99 \text{ mg/L}$$

$$\text{loaded drug} = 43.01 \text{ mg/L}$$

The concentration of sample was calculated to amount of loaded drug

$$\text{mg of loaded drug} = 43.01 \text{ mg/L} \times \left(\frac{20000 \text{ mg/L}}{60 \text{ mg/L}}\right) \times \left(\frac{400 \text{ mg}}{20000 \text{ mg/L}}\right)$$

$$\text{mg of loaded drug} = 286.72 \text{ mg}$$

The encapsulation efficiency (%EE) was calculated from the amount of loaded drug using the equation as shown below

$$\text{Encapsulation efficiency (\%EE)} = \frac{\text{Total amount of drug used} - \text{amount of free drug (unloaded)}}{\text{Total amount of drug used}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = \frac{286.72 \text{ mg}}{400 \text{ mg}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = 71.68 \%$$

The loading capacity of vancomycin-loaded rice granules was determined using the formula

$$\text{Loading capacity (\%loading)} = \frac{\text{Weight of loaded drug}}{\text{Weight of the drug-loaded particles}} \times 100$$

$$\text{Loading capacity (\%)} = \frac{286.72 \text{ mg}}{336.7 \text{ mg}} \times 100 \text{ ; 336.7 mg is the weight of product}$$

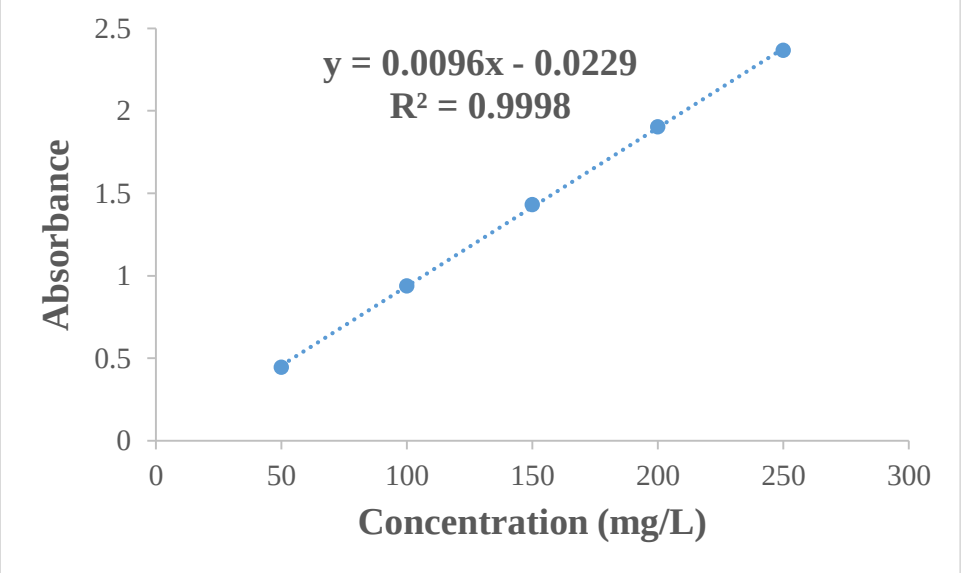
$$\text{Loading capacity (\%)} = 85.15\%$$

Experiment 3

The absorbance of vancomycin standard solution and diluted sample.

	Standard					Sample
Concentration (mg/L)	50	100	150	200	250	60
Absorbance at 283 nm	0.447	0.92	1.433	1.903	2.367	0.157

The absorbance at 283 nm of each concentration of vancomycin standard solutions was plotted as the standard curve and shown in figure below.



The plotted graph of absorbance of vancomycin standard

The absorbance value of the sample was compared and calculated to find free vancomycin using the formula as follows;

$$Y = 0.0096X - 0.0229$$

$$0.157 = 0.0096X - 0.0229$$

$$X = 18.74 \text{ mg/L (In the supernatant)}$$

$$\text{loaded drug} = 60 \text{ mg/L} - 18.74 \text{ mg/L}$$

$$\text{loaded drug} = 41.26 \text{ mg/L}$$

The concentration of sample was calculated to amount of loaded drug

$$\text{mg of loaded drug} = 41.26 \text{ mg/L} \times \left(\frac{20000 \text{ mg/L}}{60 \text{ mg/L}}\right) \times \left(\frac{400 \text{ mg}}{20000 \text{ mg/L}}\right)$$

$$\text{mg of loaded drug} = 275.07 \text{ mg}$$

The encapsulation efficiency (%EE) was calculated from the amount of loaded drug using the equation as shown below

$$\text{Encapsulation efficiency (\%EE)} = \frac{\text{Total amount of drug used} - \text{amount of free drug (unloaded)}}{\text{Total amount of drug used}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = \frac{275.07 \text{ mg}}{400 \text{ mg}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = 68.77 \%$$

The loading capacity of vancomycin-loaded rice granules was determined using the formula

$$\text{Loading capacity (\%loading)} = \frac{\text{Weight of loaded drug}}{\text{Weight of the drug-loaded particles}} \times 100$$

Loading capacity (%) = $\frac{286.72\ mg}{325.1mg}$ x 100 ; 325.1 mg is the weight of product

Loading capacity (%) = 84.62%

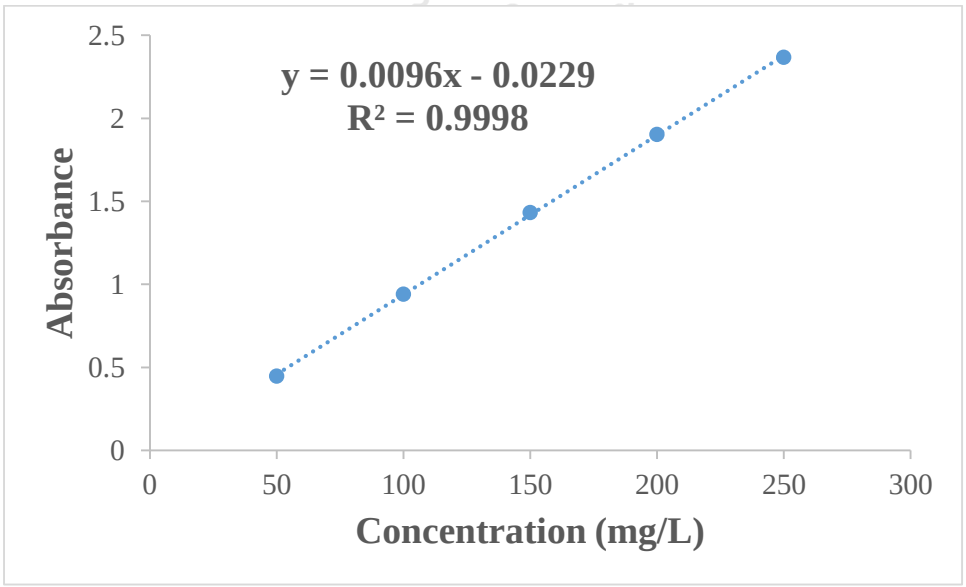
2. Calculation of %EE and loading capacity of vancomycin in VAN-CC

Experimental 1

The absorbance of vancomycin standard solutions and diluted sample.

	Standard					Sample
Concentration (mg/L)	50	100	150	200	250	100
Absorbance at 283 nm	0.447	0.94	1.433	1.903	2.367	0.745

The absorbance at 283 nm of each concentration of vancomycin standard solutions was plotted as the standard curve and shown in figure below.



Calibration curve of vancomycin standard solutions

The absorbance value of the sample was compared and calculated to find free vancomycin using the formula as follows;

$$Y = 0.0096X - 0.0229$$

$$0.745 = 0.0096X - 0.0229$$

$$X = 80.00 \text{ mg/L (In the supernatant)}$$

$$\text{loaded drug} = 100 \text{ mg/L} - 80.00 \text{ mg/L}$$

$$\text{loaded drug} = 20.00 \text{ mg/L}$$

The concentration of sample was calculated to amount of loaded drug

$$\text{mg of loaded drug} = 20.00 \text{ mg/L} \times \left(\frac{100000 \text{ mg/L}}{100 \text{ mg/L}}\right) \times \left(\frac{200 \text{ mg}}{100000 \text{ mg/L}}\right)$$

$$\text{mg of loaded drug} = 40.00 \text{ mg}$$

The encapsulation efficiency (%EE) was calculated from the amount of loaded drug using the equation as shown below

$$\text{Encapsulation efficiency (\%EE)} = \frac{\text{Total amount of drug used} - \text{amount of free drug (unloaded)}}{\text{Total amount of drug used}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = \frac{40 \text{ mg}}{200 \text{ mg}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = 20.00 \%$$

The loading capacity of vancomycin-loaded calcium citrate particles was determined using the formula

$$\text{Loading capacity (\%loading)} = \frac{\text{Weight of loaded drug}}{\text{Weight of the drug-loaded particles}} \times 100$$

$$\text{Loading capacity (\%)} = \frac{40\ \text{mg}}{1142.9\ \text{mg}} \times 100$$; 1142.9 mg is the weight of product

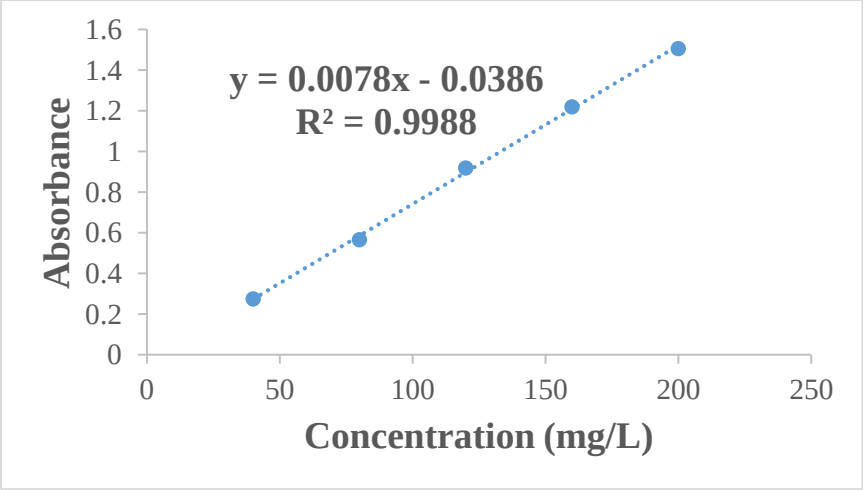
Loading capacity (%) = 3.5%

Experiment 2

The absorbance of vancomycin standard solutions and diluted sample.

	Standard					Sample
Concentration (mg/L)	40	80	120	160	200	120
Absorbance at 283 nm	0.274	0.566	0.918	1.22	1.506	0.44

The absorbance at 283 nm of each concentration of vancomycin standard solutions was plotted as the standard curve and shown in figure below.



Calibration curve of vancomycin standard solution

The absorbance value of the sample was compared and calculated to find free vancomycin using the formula as follows;

$$R^2 = 0.9988$$

$$Y = 0.0078X - 0.0386$$

$$0.44 = 0.0078X - 0.0386$$

$$X = 61.36 \text{ mg/L (In the supernatant)}$$

$$\text{loaded drug} = 120 \text{ mg/L} - 61.36 \text{ mg/L}$$

$$\text{loaded drug} = 58.64 \text{ mg/L}$$

The concentration of sample was calculated to amount of loaded drug

$$\text{mg of loaded drug} = 58.64 \text{ mg/L} \times \left(\frac{100000 \text{ mg/L}}{120 \text{ mg/L}}\right) \times \left(\frac{200 \text{ mg}}{100000 \text{ mg/L}}\right)$$

$$\text{mg of loaded drug} = 97.73 \text{ mg}$$

The encapsulation efficiency (%EE) was calculated from the amount of loaded drug using the equation as shown below

$$\text{Encapsulation efficiency (\%EE)} = \frac{\text{Total amount of drug used} - \text{amount of free drug (unloaded)}}{\text{Total amount of drug used}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = \frac{97.73 \text{ mg}}{200 \text{ mg}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = 48.86 \%$$

The loading capacity of vancomycin-loaded calcium citrate particles was determined using the formula

$$\text{Loading capacity (\%loading)} = \frac{\text{Weight of loaded drug}}{\text{Weight of the drug-loaded particles}} \times 100$$

$$\text{Loading capacity (\%)} = \frac{97.73\ \text{mg}}{534.7\ \text{mg}} \times 100 \text{ ; 534.7 mg is the weight of product}$$

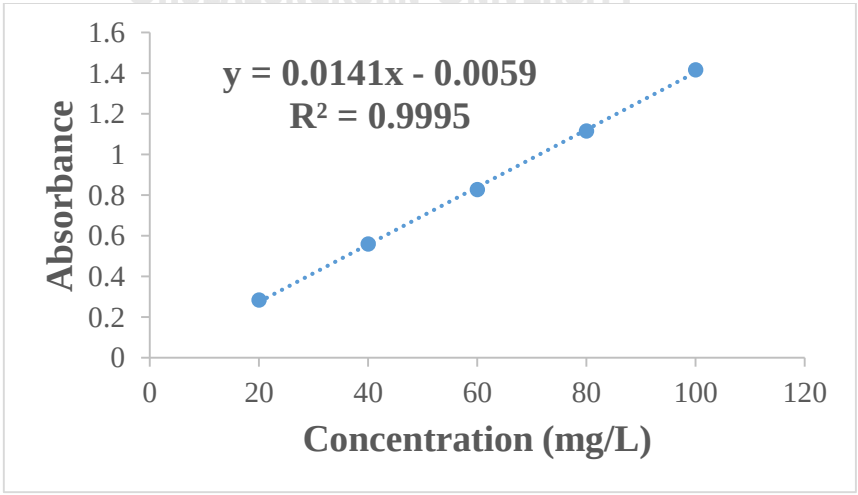
$$\text{Loading capacity (\%)} = 18.31\%$$

Experiment 3

The absorbance of vancomycin standard solution and diluted.

	Standard					Sample
Concentration (mg/L)	20	40	60	80	100	60
Absorbance at 283 nm	0.284	0.559	0.827	1.116	1.416	0.714

The absorbance at 283 nm of each concentration of vancomycin standard solutions was plotted as the standard curve and shown in figure below.



Calibration curve of vancomycin standard solution

The absorbance value of the sample was compared and calculated to find free vancomycin using the formula as follows;

$$Y = 0.0141X + 0.0059$$

$$0.714 = 0.0141X + 0.0059$$

$$X = 51.06 \text{ mg/L (In the supernatant)}$$

$$\text{loaded drug} = 60 \text{ mg/L} - 51.06 \text{ mg/L}$$

$$\text{loaded drug} = 8.94 \text{ mg/L}$$

The concentration of sample was calculated to amount of loaded drug

$$\text{mg of loaded drug} = 8.94 \text{ mg/L} \times \left(\frac{100000 \text{ mg/L}}{60 \text{ mg/L}}\right) \times \left(\frac{200 \text{ mg}}{100000 \text{ mg/L}}\right)$$

$$\text{mg of loaded drug} = 29.81 \text{ mg}$$

The encapsulation efficiency (%EE) was calculated from the amount of loaded drug using the equation as shown below

$$\text{Encapsulation efficiency (\%EE)} = \frac{\text{Total amount of drug used} - \text{amount of free drug (unloaded)}}{\text{Total amount of drug used}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = \frac{29.81 \text{ mg}}{200 \text{ mg}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = 14.90 \%$$

The loading capacity of vancomycin-loaded calcium citrate particles was determined using the formula

$$\text{Loading capacity (\%loading)} = \frac{\text{Weight of loaded drug}}{\text{Weight of the drug-loaded particles}} \times 100$$

$$\text{Loading capacity (\%)} = \frac{29.81\ \text{mg}}{1144.0\ \text{mg}} \times 100$$; 1144.0 mg is the weight of product

$$\text{Loading capacity (\%)} = 2.60\%$$

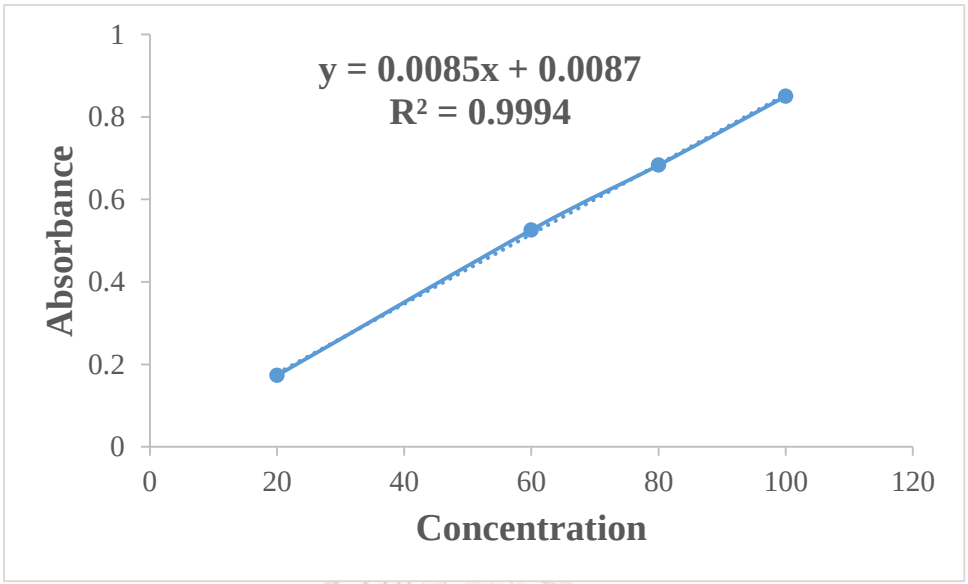
3. Calculation of %EE and loading capacity of vancomycin in ERY-EC

Experimental 1

The absorbance of erythromycin standard solution and diluted sample.

	Standard				Sample	
					No.1	No.2
Concentration (mg/L)	20	60	80	100	80	80
Absorbance at 237 nm	0.173	0.526	0.683	0.85	0.395	0.314

The absorbance at 237 nm of each concentration of erythromycin standard solutions was plotted as the standard curve and shown in figure below.



Calibration curve of erythromycin standard solutions

The absorbance value of the sample was compared and calculated to find free erythromycin using the formula as follows;

$$Y = 0.0085X + 0.0087$$

$$0.395 = 0.0085X + 0.0087$$

$$X = 45.45 \text{ mg/L (In the supernatant)}$$

$$\text{loaded drug} = 80 \text{ mg/L} - 45.45 \text{ mg/L}$$

$$\text{loaded drug} = 34.55 \text{ mg/L}$$

The concentration of sample was calculated to amount of loaded drug

$$\text{mg of loaded drug} = 34.55 \text{ mg/L} \times \left(\frac{2000 \text{ mg/L}}{80 \text{ mg/L}}\right) \times \left(\frac{40 \text{ mg}}{2000 \text{ mg/L}}\right)$$

mg of loaded drug = 17.28 mg

The encapsulation efficiency (%EE) was calculated from the amount of loaded drug using the equation as shown below

$$\text{Encapsulation efficiency (\%EE)} = \frac{\text{Total amount of drug used} - \text{amount of free drug (unloaded)}}{\text{Total amount of drug used}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = \frac{17.28 \text{ mg}}{40 \text{ mg}} \times 100$$

Encapsulation efficiency (%EE) = 43.19 %

The loading capacity of erythromycin-loaded ethyl cellulose was determined using the formula

$$\text{Loading capacity (\%loading)} = \frac{\text{Weight of loaded drug}}{\text{Weight of the drug-loaded particles}} \times 100$$

$$\text{Loading capacity (\%)} = \frac{17.28 \text{ mg}}{37 \text{ mg}} \times 100$$; 37 mg is the weight of product

Loading capacity (%) = 46.70%

Experiment 2

The absorbance value of the sample was compared and calculated to find free erythromycin using the formula as follows;

Y = 0.0085X + 0.0087

0.314 = 0.0085X+0.0087

X = 35.93 mg/L (In the supernatant)

loaded drug = 80 mg/L – 35.92 mg/L

loaded drug = 44.08 mg/L

The concentration of sample was calculated to amount of loaded drug

$$\text{mg of loaded drug} = 44.08 \text{ mg/L} \times \left(\frac{20000 \text{ mg/L}}{80 \text{ mg/L}}\right) \times \left(\frac{40 \text{ mg}}{20000 \text{ mg/L}}\right)$$

mg of loaded drug = 22.04 mg

The encapsulation efficiency (%EE) was calculated from the amount of loaded drug using the equation as shown below

$$\text{Encapsulation efficiency (\%EE)} = \frac{\text{Total amount of drug used} - \text{amount of free drug (unloaded)}}{\text{Total amount of drug used}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = \frac{22.04 \text{ mg}}{40 \text{ mg}} \times 100$$

Encapsulation efficiency (%EE) = 55.1 %

The loading capacity of erythromycin-loaded ethyl cellulose particles was determined using the formula

$$\text{Loading capacity (\%loading)} = \frac{\text{Weight of loaded drug}}{\text{Weight of the drug-loaded particles}} \times 100$$

$$\text{Loading capacity (\%)} = \frac{22.04 \text{ mg}}{39 \text{ mg}} \times 100 \text{ ; 39 mg is the weight of product}$$

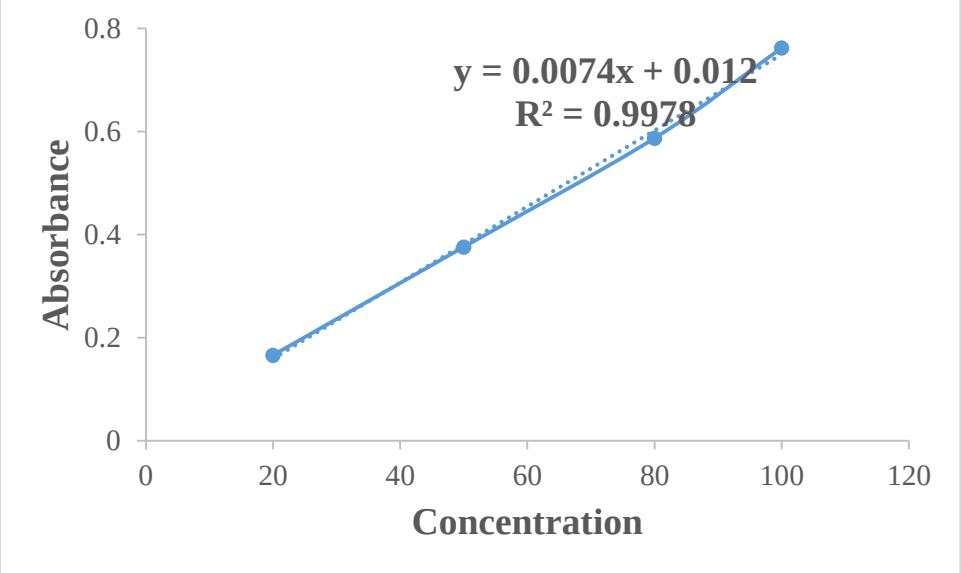
Loading capacity (%) = 56.51%

Experiment 3

The absorbance of erythromycin standard solution and diluted sample.

	Standard				Sample
Concentration (mg/L)	20	50	80	100	50
Absorbance at 237 nm	0.166	0.376	0.587	0.762	0.171

The absorbance at 237 nm of each concentration of erythromycin standard solutions was plotted as the standard curve and shown in figure below.



Calibration curve of erythromycin standard solutions

The absorbance value of the sample was compared and calculated to find free erythromycin using the formula as follows;

$$Y = 0.0074X + 0.012$$

$$0.171 = 0.0074X + 0.012$$

$$X = 21.48 \text{ mg/L (In the supernatant)}$$

$$\text{loaded drug} = 50 \text{ mg/L} - 21.48 \text{ mg/L}$$

$$\text{loaded drug} = 28.52 \text{ mg/L}$$

The concentration of sample was calculated to amount of loaded drug

$$\text{mg of loaded drug} = 28.52 \text{ mg/L} \times \left(\frac{2000 \text{ mg/L}}{50 \text{ mg/L}}\right) \times \left(\frac{40 \text{ mg}}{2000 \text{ mg/L}}\right)$$

$$\text{mg of loaded drug} = 22.82 \text{ mg}$$

The encapsulation efficiency (%EE) was calculated from the amount of loaded drug using the equation as shown below

$$\text{Encapsulation efficiency (\%EE)} = \frac{\text{Total amount of drug used} - \text{amount of free drug (unloaded)}}{\text{Total amount of drug used}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = \frac{22.82 \text{ mg}}{40 \text{ mg}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = 57.04 \%$$

The loading capacity of erythromycin-loaded ethyl cellulose particles was determined using the formula

$$\text{Loading capacity (\%loading)} = \frac{\text{Weight of loaded drug}}{\text{Weight of the drug-loaded particles}} \times 100$$

Loading capacity (%) = $\frac{22.82\ mg}{43.2\ mg}$ x 100 ; 43.2 mg is the weight of product

Loading capacity (%) = 52.82%

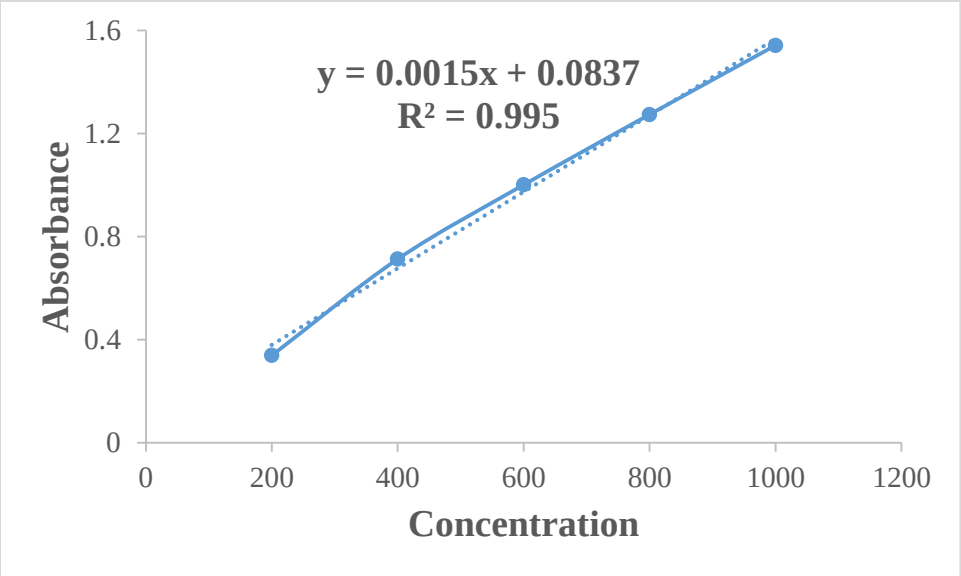
4. Calculation of %EE and loading capacity of erythromycin in ERY-PLGA

Experimental 1

The absorbance of erythromycin standard solution and diluted sample.

	Standard					Sample		
						No.1	No.2	No.3
Concentration (mg/L)	200	400	600	800	100	600	600	600
Absorbance at 206 nm	0.173	0.526	0.683	1.274	0.85	0.129	0.174	0.353

The absorbance at 206 nm of each concentration of erythromycin standard solutions was plotted as the standard curve and shown in figure below.



Calibration curve of vancomycin standard solutions

The absorbance value of the sample was compared and calculated to find free erythromycin using the formula as follows;

$$Y = 0.0015X + 0.0837$$

$$0.129 = 0.0015X + 0.0837$$

$$X = 30.87 \text{ mg/L (In the supernatant)}$$

$$\text{loaded drug} = 600 \text{ mg/L} - 30.87 \text{ mg/L}$$

$$\text{loaded drug} = 569.13 \text{ mg/L}$$

The concentration of sample was calculated to amount of loaded drug

$$\text{mg of loaded drug} = 569.13 \text{ mg/L} \times \left(\frac{2000 \text{ mg/L}}{600 \text{ mg/L}}\right) \times \left(\frac{40 \text{ mg}}{2000 \text{ mg/L}}\right)$$

$$\text{mg of loaded drug} = 37.94 \text{ mg}$$

The encapsulation efficiency (%EE) was calculated from the amount of loaded drug using the equation as shown below

$$\text{Encapsulation efficiency (\%EE)} = \frac{\text{Total amount of drug used} - \text{amount of free drug (unloaded)}}{\text{Total amount of drug used}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = \frac{37.94\text{mg}}{40\text{ mg}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = 94.86\ \%$$

The loading capacity of erythromycin-loaded poly(lactic-co-glycolic acid) particles was determined using the formula

$$\text{Loading capacity (\%loading)} = \frac{\text{Weight of loaded drug}}{\text{Weight of the drug-loaded particles}} \times 100$$

$$\text{Loading capacity (\%)} = \frac{37.94\text{ mg}}{566.9\text{ mg}} \times 100 \text{ ; 566.9 mg is the weight of product}$$

$$\text{Loading capacity (\%)} = 6.69\%$$

Experiment 2

The absorbance value of the sample was compared and calculated to find free erythromycin using the formula as follows;

$$Y = 0.0015X + 0.0837$$

$$0.174 = 0.0015X + 0.0837$$

$$X = 60.2\text{ mg/L (In the supernatant)}$$

$$\text{loaded drug} = 600\text{ mg/L} - 35.92\text{ mg/L}$$

$$\text{loaded drug} = 539.8\text{ mg/L}$$

The concentration of sample was calculated to amount of loaded drug

$$\text{mg of loaded drug} = 539.8 \text{ mg/L} \times \left(\frac{2000 \text{ mg/L}}{600 \text{ mg/L}}\right) \times \left(\frac{40 \text{ mg}}{2000 \text{ mg/L}}\right)$$

$$\text{mg of loaded drug} = 35.99 \text{ mg}$$

The encapsulation efficiency (%EE) was calculated from the amount of loaded drug using the equation as shown below

$$\text{Encapsulation efficiency (\%EE)} = \frac{\text{Total amount of drug used} - \text{amount of free drug (unloaded)}}{\text{Total amount of drug used}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = \frac{35.99 \text{ mg}}{40 \text{ mg}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = 89.97 \%$$

The loading capacity of erythromycin-loaded poly(lactic-co-glycolic acid) was determined using the formula

$$\text{Loading capacity (\%loading)} = \frac{\text{Weight of loaded drug}}{\text{Weight of the drug-loaded particles}} \times 100$$

$$\text{Loading capacity (\%)} = \frac{35.99 \text{ mg}}{567.7 \text{ mg}} \times 100 \text{ ; 567.7 mg is the weight of product}$$

$$\text{Loading capacity (\%)} = 6.34\%$$

Experiment 3

The absorbance value of the sample was compared and calculated to find free erythromycin using the formula as follows;

$$Y = 0.0015X + 0.0837$$

$$0.353 = 0.0015X + 0.0837$$

$$X = 179.53 \text{ mg/L (In the supernatant)}$$

$$\text{loaded drug} = 600 \text{ mg/L} - 21.48 \text{ mg/L}$$

$$\text{loaded drug} = 420.47 \text{ mg/L}$$

The concentration of sample was calculated to amount of loaded drug

$$\text{mg of loaded drug} = 420.47 \text{ mg/L} \times \left(\frac{2000 \text{ mg/L}}{600 \text{ mg/L}}\right) \times \left(\frac{40 \text{ mg}}{2000 \text{ mg/L}}\right)$$

$$\text{mg of loaded drug} = 28.03 \text{ mg}$$

The encapsulation efficiency (%EE) was calculated from the amount of loaded drug using the equation as shown below

$$\text{Encapsulation efficiency (\%EE)} = \frac{\text{Total amount of drug used} - \text{amount of free drug (unloaded)}}{\text{Total amount of drug used}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = \frac{28.03 \text{ mg}}{40 \text{ mg}} \times 100$$

$$\text{Encapsulation efficiency (\%EE)} = 70.08 \%$$

The loading capacity of erythromycin-loaded poly(lactic-co-glycolic acid) was determined using the formula

$$\text{Loading capacity (\%loading)} = \frac{\text{Weight of loaded drug}}{\text{Weight of the drug-loaded particles}} \times 100$$

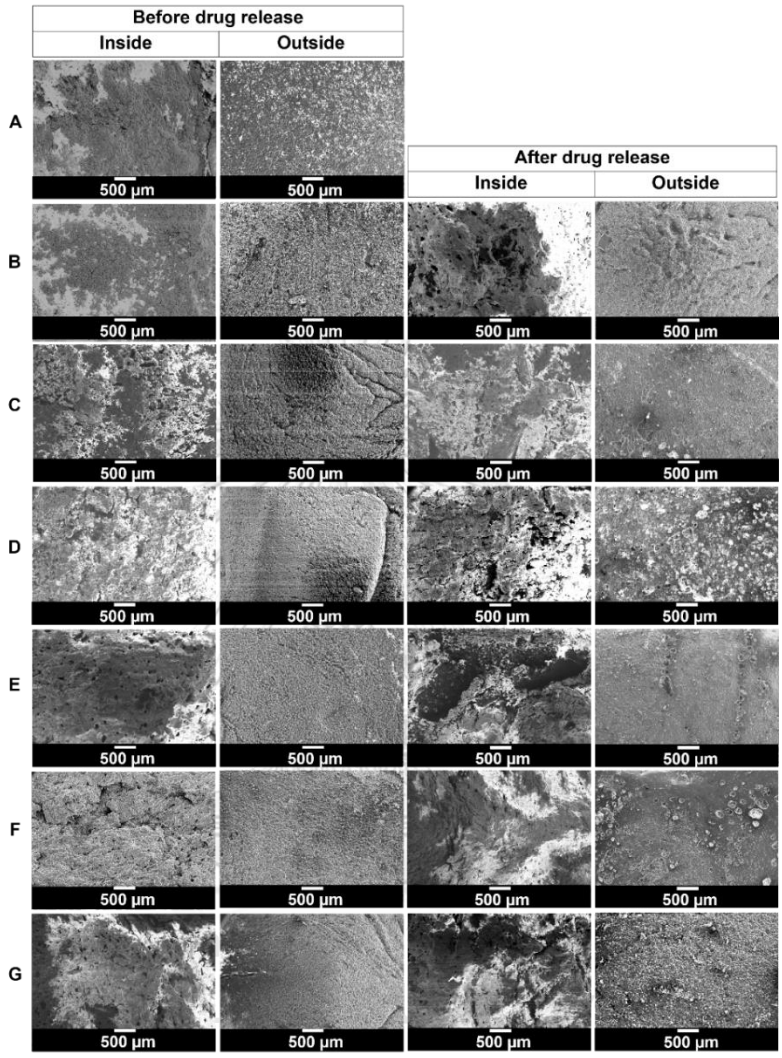
Loading capacity (%) = $\frac{28.03\ \text{mg}}{570.0\ \text{mg}}$ x 100 ; 570.0 mg is the weight of product

Loading capacity (%) = 4.93%

Preparation of phosphate buffer saline pH 7.4

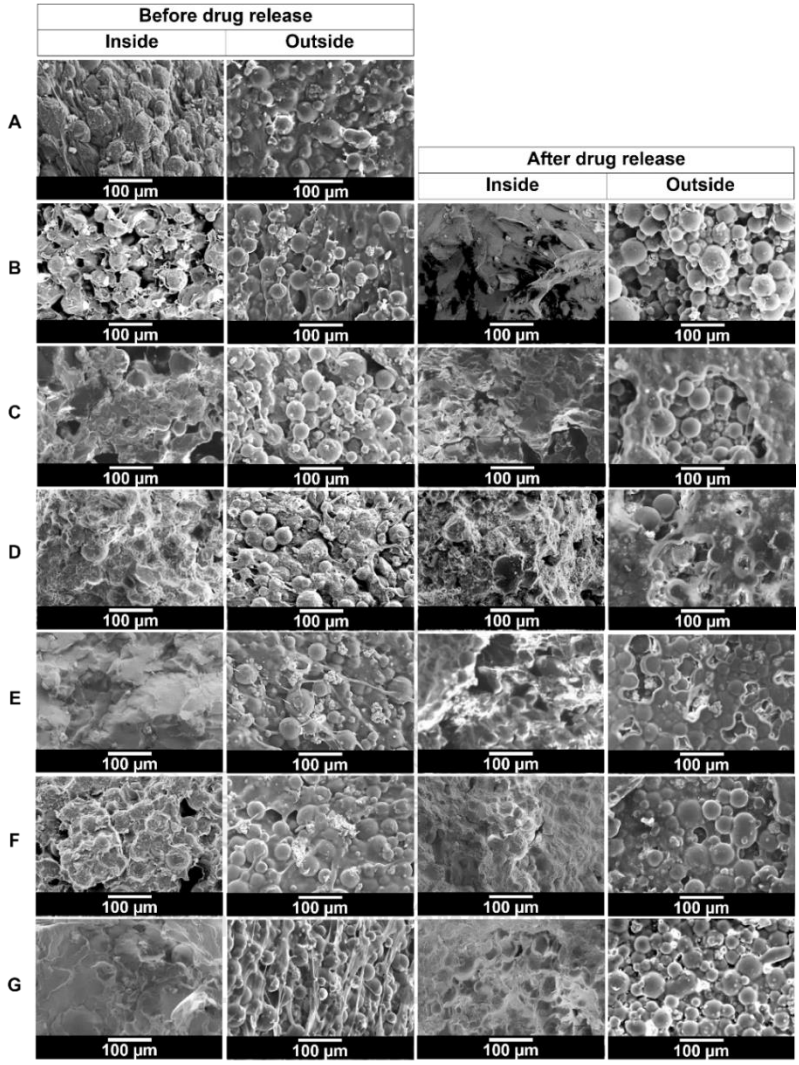
Phosphate buffer saline pH 7.4 (0.01 M) was prepared. Briefly, 1.4 g of disodium hydrogen phosphate, 0.24 g of potassium dihydrogen phosphate, 8 g of sodium chloride, and 0.2 g of potassium chloride were added into water and volume adjusted to 1000 ml. The pH of a solution was measured using pH meter (ST2100-F, STARTER 2100 PH BENCH, OHAUS, Newark, New Jersey, USA) and calibrated using standard buffer pH 4 and pH 7 with accuracy ≥ 96%. The PBS solution were adjusted pH 7.4 using 0.01 M of HCl and 0.01 M of NaOH.

SEM image of bone spacers (30X)



SEM images of poly(methyl methacrylate) spacer impregnated with (**A**) none, (**B**) vancomycin (VAN-PMMA), (**C**) vancomycin-loaded rice granules (VAN-RG-PMMA), (**D**) vancomycin-loaded calcium citrate particles (VAN-RG-PMMA), (**E**) erythromycin (ERY-PMMA), (**F**) erythromycin-loaded ethyl cellulose particles (ERY-EC-PMMA), and (**G**) erythromycin-loaded poly(lactic-co-glycolic acid) particles (ERY-PLGA-PMMA). The green arrows indicate the poly(methyl methacrylate) particles.

SEM image of bone spacer (1000X)



SEM images of poly(methyl methacrylate) spacer impregnated with (**A**) none, (**B**) vancomycin (VAN-PMMA), (**C**) vancomycin-loaded rice granules (VAN-RG-PMMA), (**D**) vancomycin-loaded calcium citrate particles (VAN-RG-PMMA), (**E**) erythromycin (ERY-PMMA), (**F**) erythromycin-loaded ethyl cellulose particles (ERY-EC-PMMA), and (**G**) erythromycin-loaded poly(lactic-co-glycolic acid) particles (ERY-PLGA-PMMA). The green arrows indicate the poly(methyl methacrylate) particles.

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