การกักเก็บยาเซฟาโซลินปริมาณสูงในแผ่นไฮโดรเจลโครงร่างตาข่ายแบบแทรกสอดจากยางธรรมชาติและแป้งข้าวเจ้า

จตุพร ประทุมเทศ[1], วริษฎา ศิลาอ่อน[1], อุษณา พัวเพิ่มพูลศิริ[1*]

บทคัดย่อ

การกักเก็บยาเซฟาโซลินปริมาณสูงในแผ่นไฮโดรเจลโครงร่างตาข่ายแบบแทรกสอดจากยางธรรมชาติและแป้งข้าวเจ้า
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บทนำ: ไฮโดรเจลโครงร่างตาข่ายแบบแทรกสอดมีคุณสมบัติดูดซับน้ำหรือของเหลวได้ดี จึงนำมาประยุกต์ใช้เป็นวัสดุทางเภสัชกรรม การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาคุณสมบัติการกักเก็บยาเซฟาโซลินและศึกษาคุณสมบัติทางกายภาพของไฮโดรเจลที่เตรียมจากยางธรรมชาติและแป้งข้าวเจ้า **วิธีการดำเนินการวิจัย:** เตรียมด้วยวิธีฟรีแรคอลดิพอลิเมอไรเซชัน ทำการศึกษาการกักเก็บยาเซฟาโซลินแบบ direct addition ที่ความเข้มข้นเริ่มต้น 10 มิลลิกรัมต่อมิลลิลิตร และคุณสมบัติทางกายภาพ เช่น การบวมพอง, การดูดซับน้ำและโครงสร้างของแผ่นไฮโดรเจล **ผลการศึกษาวิจัย:** พบว่าไฮโดรเจลจากยางธรรมชาติและแป้งข้าวเจ้า ให้ผลการบวมพองและการดูดซับน้ำมากกว่ายางธรรมชาติอย่างมีนัยสำคัญ ($p < 0.05$) โดยไฮโดรเจลให้ผลการบวมพองและการดูดซับน้ำเท่ากับ (70.92 $\pm$ 2.44 % และ 41.49 $\pm$ 0.83 % ตามลำดับ) ยางธรรมชาติให้ผลการบวมพองและการดูดซับน้ำเท่ากับ (5.71 $\pm$ 1.04 % และ 5.39 $\pm$ 0.94 % ตามลำดับ) จากโครงสร้างของไฮโดรเจลโดยกล้องจุลทรรศน์แบบส่องกราดพบว่าภายในแผ่นยางธรรมชาติมีลักษณะพื้นผิวเรียบและปราศจากรูพรุน ขณะที่แผ่นไฮโดรเจลมีพื้นผิวที่ขรุขระและเกิดรูพรุนภายในโครงสร้าง แสดงให้เห็นว่าไฮโดรเจลที่เตรียมขึ้นมีโครงสร้างแบบโครงร่างตาข่ายเกิดขึ้น ดังนั้นจึงทำให้การบวมพองและการดูดซับน้ำเพิ่มขึ้น การศึกษาการกักเก็บยาเซฟาโซลินที่ความเข้มข้นเริ่มต้น 10 มิลลิกรัมต่อมิลลิลิตร พบว่าไฮโดรเจลที่เตรียมจากยางธรรมชาติและแป้งข้าวเจ้าสามารถกักเก็บยาเซฟาลินมากกว่ายางธรรมชาติอย่างมีนัยสำคัญ ($p < 0.05$) โดยมีร้อยละของการกักเก็บยาเท่ากับ 23.75 $\pm$ 1.05 % และ 2.24 $\pm$ 0.76 % ตามลำดับ **สรุปผลการวิจัย:** ไฮโดรเจลจากยางธรรมชาติและแป้งข้าวเจ้ามีคุณสมบัติการบวมพองและการดูดซับน้ำที่ดี และมีโครงสร้างเป็นโครงร่างตาข่ายเกิดขึ้น ดังนั้นระบบไฮโดรเจลนี้จึงสามารถในการนำมาพัฒนาเพื่อใช้เป็นวัสดุทางการแพทย์ต่อไป

คำสำคัญ: ไฮโดรเจลโครงร่างตาข่ายแบบแทรกสอด ยางธรรมชาติ แป้งข้าวเจ้า การกักเก็บ

Abstract

High Dose Cefazolin for Entrapment in Interpenetrating Network Hydrogel Comprising of natural rubber and rice starch

Jatuporn Pratoomted[1], Warisada Sila-on[1] and Utsana Puapermpoonsiri[1*]

[1]Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Warinchamrab, Ubon Ratchathani

***Corresponding author:** Division of Pharmaceutical Chemistry and Technology. Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Warinchamrab, Ubon Ratchathani, Thailand 34190 Tel. 045-353632, e-mail: utsana@gmail.com

Introduction: Interpenetrating network (IPN) hydrogel has a swelling and water absorption properties. This IPN hydrogel is possible to develop as a pharmaceutical material. Aim of this work is to study the cefazolin entrapment of IPN hydrogel comprising of natural rubber and rice starch. **Materials and Method:** IPN hydrogel was fabricated by free radical polymerization and evaluated swelling and water absorption properties by different weight method. IPN hydrogel structure was observed by scanning electron microscope (SEM). High-performance liquid chromatography (HPLC) technique was used to determine cefazolin content in this study. **Results:** IPN hydrogel from natural rubber and rice starch provided statistically a greater level of % swelling and % water absorption (70.92 ± 2.44 % and 41.49 ± 0.83 %, respectively) than pure rubber (5.71 ± 1.04 % and 5.39 ± 0.94 %, respectively) ($p < 0.05$). This result related to microscopic morphology of IPN hydrogel. Smooth surface and homogenous matrix with non-porous was seen for pure rubber while roughness surface with internal porous was appeared for IPN hydrogel. This structure of IPN hydrogel will lead to promote swelling and water absorption. With direct addition method for cefazolin entrapment, IPN hydrogel from natural rubber and rice starch provided statistically a greater cefazolin entrapment than pure rubber at the initial loading concentration 10 mg/ml (23.75 ± 1.05 % and 2.24 ± 0.76 %, respectively) ($p < 0.05$). **Conclusion:** IPN hydrogel from natural rubber and rice starch shows high swelling and water absorption due to the presence of the crosslinking network. Thus, IPN hydrogel is able to develop as a medical material.

Keywords: Interpenetrating network hydrogel, natural rubber, rice starch, entrapment

Introduction

Infection is one of the most common causes of problems after the operation. Thus, antibiotic are necessary for treatment of wound infection. Cefazolin is recommended and used widely as antimicrobial prophylaxis after surgical procedures. It is an effective antimicrobial against gram-positive and gram-negative organism. Also, cefazolin can be to use in patient with penicillin hypersensitive (Kusaba, 2009).

Interpenetrating network (IPN) hydrogel is three-dimensional network of hydrophilic polymer which can absorb and retain water or other molecules into structure (Ferrer *et al*., 2010). These properties are useful to enhance drug entrapment. In the study, natural rubber (NR) and starch (S) are good candidates for IPN hydrogel fabrication. Natural rubber was extracted from the bark of the *Hevea Brazilliensis* tree and provides a good elasticity but poor swelling and water absorption properties. Natural rubber have already been used for drug delivery such as metronidazole and nicotin (Herculano *et al*., 2011; Pichayakorn *et al*., 2013) and also promote angiogenesis which is helpful for wound healing process (Ferreira *et al*., 2009). Starch consists of amylose and amylopectin. They related to swelling and water absorption properties (Zou *et al*, 2012) which are useful for exudates absorption from injury. Also, starch has been reported to used for drug delivery such as curcumin and cisplatin (Pereira *et al*., 2013; Likhitkar and Bajpai, 2012) Thus, IPN hydrogel from NR and S are suitable to drug delivery because of elasticity, accelerate wound healing process, water swelling property and water absorption property.

Aim of this work is to study high dose cefazolin for entrapment in IPN hydrogel comprising of natural rubber and rice starch.

Materials and Method

Materials

Natural rubber latex (NRL, 60% natural rubber content) (Luck Four Co., Ltd., Bangkok, Thailand) and rice starch were used for IPN-hydrogel

frabrication. Potassium persulphate (KPS, 97%) (Ajax Finechem Pty Ltd., Aucland New Zealand) and *N,N′*-methylenebisacrylamide (MBA, 99%) (Sigma-Aldrich, Missouri, USA) were used for natural rubber latex polymerization. Maleic acid (Sigma-Aldrich,, MO, Austria) was used as starch crosslinker. Potassium hydroxide (KOH, 85%) (Carlo Erba Co., Ltd., Italy) and Tween® 80 (Sigma-Aldrich,, MO, United Kingdom) were also used in this study.

Methods

1. Preparation of IPN hydrogel

Natural rubber latex (NRL) polymerization was made by KOH, Tween® 80, KPS and MBA with 2 parts per hundred rubber (phr). Following polymerization of NRL, pre-gelatinized starch with maleic acid were added and mixed gently to NRL mixture by ratio of NRL: S 1:2.

2. Cefazolin entrapment of IPN hydrogel

Cefazolin entrapment was performed by direct addition method. The initial loading concentration of cefazolin was 10 mg/ml for 24 hrs and the excess cefazolin was removed by washing with sterile water for injection to reduce the bursting effect.

3. Swelling and water absorption studies

Samples were cut into 2 cm × 2 cm pieces. They were immersed in phosphate buffer solution pH 7.4 (PBS 7.4) for 72 hr at room temperature. Remove the excess media on the surface with filter paper. The weight of swollen sample would be calculated.

4. Microscopic morphology

Surface and internal morphology of sample were observed by scanning electron microscope (SEM) (Cam Scan MX-2000, UK) at magnification 250X.

5. Determination of cefazolin content

The cefazolin quantitative was investigated by High-performance liquid chromatography (HPLC) (Waters 717 plus Autosampler) comprising of Water 1525 Binary HPLC pump, column (Thermo® C 18 : 5 µm, 250 x 4.6 mm), Waters 2487 Dual λ Absorbance detector, mobile phase (methanol : 0.1% acetic acid) and flow rate 1 ml/min at 25 ˚C. All data are expressed as mean ± standard deviation (SD). One – way ANOVA analysis of the variance followed by post hoc analysis was used to test the statistical significant of the results at ($p < 0.05$).

Results

Water swelling and water absorption studies

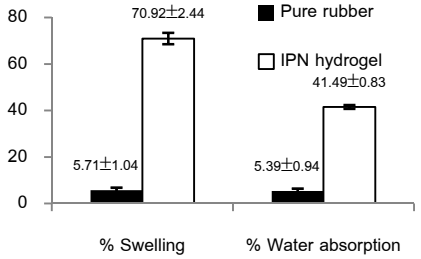

Figure 1 The percentage of water swelling and water absorption of pure rubber (■) and IPN hydrogel (□) from natural rubber and rice starch at MBA amount 2 phr on NRL: S ratio 1:2 (n=3, mean ± SD)

Figure 1, IPN hydrogel from natural rubber and rice starch provides statistically a greater water swelling and water absorption than pure rubber ($p < 0.05$) due to the presence of crosslink-network including high amylose content of rice starch (15.36 %) in IPN hydrogel. Similarly, the percentage of swelling and water absorption increased with increasing amylose content (Zou *et al*., 2012). In addition, these results were supported by scanning electron microscope (SEM) images (Fig 2). SEM shows IPN hydrogel has a higher internal porous and roughness surface. It leads to enhance swelling and water absorption of IPN hydrogel.

Microscopic morphology

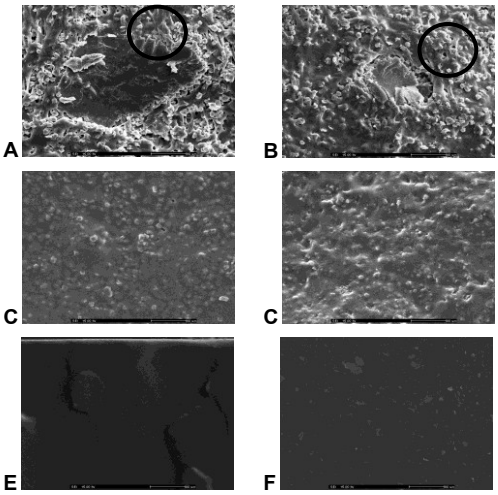

Figure 2 SEM micrographs of IPN hydrogel from natural rubber and rice starch at MBA amount 2 phr on NRL:S ratio 1:2 (A-D) and pure rubber (E and F) at magnification 250X : A and B show a cross section image of IPN hydrogel before and after soak in PBS 7.4, respectively. C and D show a surface section image of hydrogel before and after soak in PBS 7.4, respectively. E and F show a cross section image and a surface section image of pure rubber, respectively.

As Figure 2, SEM shows a homogenous matrix with non-porous and smooth surface structure for pure rubber whereas IPN hydrogel was seen the internal space with roughness surface. It illustrated that the formation of IPN network structure was occurred and facilitates water to diffuse either in or out from IPN hydrogel. It leads to high water swelling and water absorption properties (Liu *et al*., 2012). Thus, IPN hydrogel has a capability to entrap the drug by direct addition method.

Cefazolin entrapment of IPN hydrogel

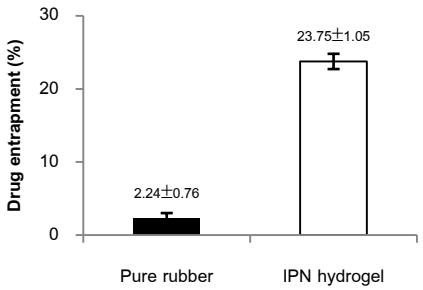

Figure 3 The percentage of drug entrapment of pure rubber (■) and IPN hydrogel (□) from natural rubber and rice starch at MBA amount 2 phr on NRL:S ratio 1:2 (n=3, mean ± SD)

Figure 3 shows IPN hydrogel from natural rubber and rice starch provides statistically a greater cefazolin entrapment than pure rubber (23.75 ± 1.05 % and 2.24 ± 0.76 %, respectively) ($p < 0.05$). It may be due to the internal structure of IPN hydrogel including high amylose content of starch. Amorphous form of amylose was induced by heat in the preparation of IPN hydrogel. Irregular shape of amorphous form also lead to enhance water absorption that is helpful for drug entrapment (Puapermpoonsiri *et al*., 2014; Sila-On *et al*., 2014).

Conclusion

Conclusively, IPN hydrogel from natural rubber and rice starch at MBA amount 2 phr on NRL:S ratio 1:2 shows high swelling and water absorption because of the internal structure from crosslink network and surface area of IPN hydrogel. These properties facilitate absorp drug molecules such as cefazolin within IPN hydrogel structure. Thus, IPN hydrogel is applicable to pharmaceutical material in further study.

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