CHAPTER III
EXPERIMENTAL

3.1 Materials

Double-centrifuge natural rubber latex (DCNR; THAI EASTERN RUBBER CO., LTD.) was used as a matrix of transdermal patch. Trimethylol-propane tris(3-mercaptopropionate) (TMPTMP; Aldrich), and 2-methyl-4'-(methylthio)-2-morpholino propiophenone (MMMP; Aldrich) were used as the crosslinking agent and photoinitiator, respectively. Sodium hydroxide (NaOH; LABAL Chemie), Sodium dodecyl Sulfate (SDS; OmniPure), and TWEEN® 20 (TWEEN® 20; Aldrich) were used as an ingredient for preparing deproteinized natural rubber film.

Indomethacin (IN, commercial glade; Aldrich) was used as an anionic drug model. Ethylene glycol (EG, AR glade; Unilab), propylene glycol (PG, AR glade ; MERCK), and glycerol (GLY, AR glade; CARLO ERBA) were used as plasticizers

Sodium chloride (NaCl; biology glade; Calbiochem), sodium phosphate dibasic (Na2HPO4, biology glade; Calbiochem), potassium chloride (KCl, biology glade; Calbiochem), and potassium phosphate monobasic (KH_2PO_4, biology glade; Calbiochem) were used to prepare a phosphate-buffered saline as the buffer solution pH 7.4.

Methanol (MeOH, AR glade; Lobachemie), hexane (AR glade; ACl Labscan), toluene (AR glade; QRëC), dimethyl sufoxide (DMSO, synthesis glade; MERCK), and distilled water were used as solvents.

3.2 Methodology

3.2.1 Preparation of Deproteinized Natural Rubber

Deproteinized natural rubber (DNR) was prepared for decreasing the amount of protein in the rubber matric via a saponification method. The solution was prepared by mixing between sodium hydroxide (NaOH) (1.5 g per 100 mL DCNR) and sodium dodecyl sulfate (SDS) (2 g per 100 mL DCNR). The solution was continuously stirred for 3 h at 60 °C. Then, the distilled water was added in the

solution with the volume ratio of DCNR: distilled water of 1:5, and stirred for 30 min at room temperature. This solution was centrifuged at 8000 rpm for 60 min to separate the rubber layer and the water layer from each other.

3.2.2 Preparation of Drug-loaded Natural Rubber Films

3.2.2.1 Double Centrifuge Natural Rubber

The crosslinking agent (TMPTMP, 0.3 mL per 100 mL DCNR) and photoinitiator (MMMP, 1 g per 100 mL DCNR) were dissolved in plasticizers (EG, PG, and GLY) at 70 °C for an hour at the volume ratio of plasticizers: DCNR of 1:1, 2:1, and 3:1. IN as an anionic drug was added in the solution and then stirred to obtain a yellow homogenous solution. After that, the yellow homogeneous solution was dropped into 5 mL DCNR and continuously stirred at room temperature for 5 min. The solution was casted in a petri dish (10 cm of diameter) and inserted in a UV curing machine for curing under UV radiation for 10 min.

3.2.2.2 Deproteinized Natural Rubber

The crosslinking agent (TMPTMP, 0.3 mL per 100 mL DCNR) and photoinitiator (MMMP, 1 g per 100 ml DCNR) were dissolved in EG at 70 °C for an hour at the volume ratio of EG:DCNR of 2:1. TWEEN20 was added into the plasticizer (500 mg per 5 mL DNR). IN (200 mg per 5 mL DNR) was added in the solution and then stirred to obtain a yellow homogenous solution. After that, the yellow homogeneous solution was dropped into 5 mL DPNR and continuously stirred at room temperature for 5 min. The solution was casted in a petri dish (10 cm of diameter) and inserted in a UV curing machine for curing under UV radiation for 10 min.

3.2.3 Preparation of Phosphate Buffered Saline (PBS buffer)

NaCl (0.14 M), Na_2PO_4 (10 mM), KCl (2.68 mM), and KH_2PO_4 (1.84 mM) were mixed together in a beaker containing distilled water (800 mL). After complete dissolution, the solution was poured in an erlenmeyer flask. An aqueous HCl (0.1 M) was added into the solution until a pH reached 7.4 and then distilled water was added until the volume was 1000 mL.

3.2.4 Preparation of Pig Skin

The abdominal part of pig skin was used in the membrane permeation. Hair and subcutaneous fat were removed by sharp razor blades until the thickness of the pig skin was about 0.2 cm. The prepared pig skin was cut to circle shape with diameter 2 cm and immersed in PBS buffer pH 7.4 and then kept at room temperature for 24 h before permeation test.

3.3 Characterizations

3.3.1 Fourier Transform Infrared Spectrometer, FT-IR

The FT-IR spectrum (Thermo Nicolet, Nexus 670) was used to identify functional groups of the crosslinked NR and drug-blend film interaction. The IN, uncured EG_NR, curing EG_NR, and IN-loaded EG_NR were analyzed with 64 scans over a wave number period of 550-4000 cm^{-1} via attenuated total reflection Fourier transform infrared spectroscopy mode (ATR-FTIR mode).

3.3.2 Thermogravimetry Differential Thermal Analyzer, TG-DTA

TG-DTA (TG-DTA; Perkin Elmer, Pyris Diamond) was used to study the thermal stability of indomethacin, pure NR, uncured EG_NR, cured EG_NR, and indomethacin loaded EG_NR. The sample was heated from 30 °C to 550 °C at a heating rate of 10 °C/min under nitrogen atmosphere.

3.3.3 Scanning Electron Microscope, SEM

The information about surface morphology of IN-EG_NR2 before and after the permeation test was investigated by SEM. Micrographs of the film were obtained using an acceleration voltage of 5 kV at various magnifications in a range of 50-1200x.

3.3.4 CHNS/O Elemental Analyzer

Amount of nitrogen was analyzed by a CHN analyzer (TruSpec Micro model of LECO Company). The calibration curve was created by using ethylenediaminetetraacetic acid (EDTA) which had a certain amount of nitrogen. The calibration curve for determining amount of nitrogen in DPNR was considered and used at the least amount of nitrogen on the curve (at % nitrogen near zero) to receive the highest accuracy of the analysis because the deproteinized natural rubber

had quite a low nitrogen content. Before analyzing the samples, the CHN analyzer was operated in air to eliminate air background (repeating no less than 10 times). The samples were wrapped with a foil cup before inserting into the analyzer. The samples were completely oxidized under oxygen atmosphere at 950 °C. The results are reported as the % nitrogen by weight of the sample.

3.3.5 UV-visible Spectrophotometer

A UV-visible spectrum (Tecan, The Infinite® 200 PRO NanoQuant) was used to determine the amount of IN release from the sample.

3.3.6 Crosslink Density of Natural Rubber Film

The crosslinked NR films were immediately studied after the crosslinking process which followed the ASTM D6814-02 standard. The crosslinked DCNR film was cut to 1 cm^2 and weighed in air and MeOH (non-solvent). The square film was immersed in toluene for 5 days to obtain the equilibrium swelling state. The Eq. (3.1) was used to calculate the crosslink density (Flory-Rehner equation);

$$\nu_e = \frac{-[\ln(1-V_r)+V_r+\chi_1 V_r^2]}{[V_1(V_r^{1/3}-V_r)/2]} \qquad (3.1)$$

where ν_e = the number of chains in a real network per unit volume,

V_1 = the molar volume of solvent (106.29 mL/mol),

V_r = the polymer volume fraction in swollen state,

χ = the Flory interaction parameter of natural rubber (0.391).

V_r can be calculated following Eq. (3.2):

$$V_r = \frac{\text{Weight of dry rubber} / \text{Density of dry rubber}}{\left(\frac{\text{Weight of dry rubber}}{\text{Density of dry rubber}}\right) + \left(\frac{\text{Weight of solvent absorbed by sample}}{\text{Density of solvent}}\right)} \qquad (3.2)$$

and the density of the dry rubber can be calculated using the Eq. (3.3):

$$\text{Density at } 23 \pm 2\ ^{\circ}\text{C (g/mL)} = 0.7913 \times \frac{A}{A\text{-}B} \qquad (3.3)$$

where: A = the weight of specimen measured in air (g),

B = the weight of specimen measured in MeOH (g),

0.7913 = the density of MeOH at 23 ± 2 °C (g/mL).

3.4 Drug Release Experiments

3.4.1 Spectrophotometric Analysis of Model Drug

An aqueous model drug solution was prepared for a UV–visible spectrophotometer to identify the maximum absorption wavelength. The absorbance at the characteristic peak of the model drug was used to determine the amount of drug released from the calibration curve.

3.4.2 Determination of Drug Content

IN-loaded NR was clearly dissolved in hexane and then the solution, 0.1 ml, was poured into a 0.3 ml PBS buffer. The drug content in each component was measured by using the UV-visible spectrophotometer at 324 nm for IN. A calibration curve was used to determine amount of drug in each sample.

3.4.3 *In vitro* Drug Release Study

Modified Franz diffusion cell was used to study the drug permeation of the samples. The PBS buffer solution at pH 7.4 was used as a receptor component. The cell was stirred continuously at a constant temperature at 37 ± 0.5 °C which controlled the temperature by the circulated water. The donor compartment was placed on the prepared pig skin and the sample on the receptor, respectively. Electrical potential (0-9 V) was applied through the system. The amount of drug which diffused through the pig skin to the buffer solution was detected by the UV–visible spectrophotometer.