CHAPTER III
EXPERIMENTAL

3.1 Materials

Carbazole (Cz; Merck), ammonium persulfate (APS; Sigma Aldrich), and hydrochloric acid (HCl, AR; RCI Labscan) acted as monomer, oxidizing agent, and dopant, respectively, were used in the polymerization of polycarbazole.

- Double-centrifuged natural rubber (DCNR; THAI EASTERN RUBBER CO., LTD.) was successfully utilized to fabricate a DCNR film with Trimethylol-propane tris(3-mercaptopropionate) (TMPTMP; Aldrich), 2-Methyl-4-(methylthio)-2-morpholdinopropiophenone (MMMP; Aldrich) acting as a crosslinking agent and a photoinitiator, respectively.

Indomethacin (IN; Sigma-Aldrich) was used as an anionic drug.

Potassium chloride (KCl), potassium phosphate monobasic (KH_2PO_4), and sodium phosphate dibasic (Na_2HPO_4) were of a biology grade and obtained from Calbiochem. Potassium sodium chloride (NaCl) was purphased from Carlo Erba. All chemicals were employed for preparation of phosphate-buffered saline (pH 7.4).

Ammonia solution (NH_3; EMSURE), Dichloromethane (DCM, ACS; Burdick&Jackson), dimethyl sulfoxide (DMSO; RCI Labscan), hydrogen peroxide (H_2O_2; QRëC), methanol (MeOH, AR; Lobachemie), polyethylene glycol (PEG; Sigma-Aldrich), sodium hydroxide (NaOH, AR; Lobachemie), toluene (AR; QRëC), and distilled water were used as solvents.

3.2 Methodology

3.2.1 Preparation of Indomethacin-loaded Double-Centrifuged Natural Rubber Films (IN-loaded DCNR Films)

DCNR latex (5 mL, 0.0415 mol) was poured into the TMPTMP/MMMP mixture (2: 1 mole ratio) at various crosslink ratios as 0.0008, 0.0032, and 0.0064. After homogeneously stirring, the solution of 0.025 g of indomethacin in PEG (2 mL) was added into latex and continuously stirred for 30 min. Then, latex was casted on a petri dish and cured under UV irradiation at various curing times as 0, 1, 3, 5, and 10 min.

3.2.2 Polymerization of Polycarbazole (PCz)

Cz monomer (60 mM) in DCM (50 mL) was slowly poured along the side of an erlenmeyer flask which contained APS (1.2 M) in HCl (0.5 M, 50 mL). The solution was left for 7 h. Then, it was filtered and washed with distilled water and DCM. Sample was dried at 65 °C for 24 h under vacuum before characterizations.

3.2.3 Preparation of Indomethacin-doped Polycarbazole (IN-doped PCz)

PCz was dedoped with NH_3 solution (0.1 M). The dedoped PCz was stirred with the solution of IN in MeOH (50 mL). The mixture was stirred for 7 h and then filtered. The filtrate was clearly washed with distilled water. The final product was dried in the oven at 65 °C for 24 h.

3.2.4 Preparation of Indomethacin-doped Polycarbazole/ Double-centrifuged Natural Rubber Blend Films (IN-doped PCz/DCNR Films)

IN-doped PCz was added into DCNR latex which was added MMMP and TMPTMP as mentioned in 3.3.1. The IN-loaded PCz/DCNR mixture was poured on a petri dish and then cured under a UV reactor for 5 min.

3.2.5 Preparation of Phosphate-buffered Saline pH 7.4 (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.

3.3 Characterization

3.3.1 Fourier Transform Infrared Spectrometer, FT-IR

The FT-IR spectrum (Nicolet, Nexus 670) was used to identify functional groups of PCz powder, IN powder, IN-doped PCz powder, and IN-loaded PCz/DCNR blend film interaction. Samples were scanned with 64 scans over a wave number period of 400-4000 cm^{-1}. The powder sample was thoroughly grinded with anhydrous KBr. The glassy disk was prepared by pressing the mixture in a die at 7 ton for one minute. For the sample as a film, ATR technique with ZnSe window was applied to investigate functional groups.

3.3.2 Thermogravimetry Differential Thermal Analyzer, TG-DTA

TG-DTA (Perkin Elmer, Pyris Diamond) was used to study the thermal behavior of crosslinked DCNR film, PCz, IN, and IN-doped PCz. The sample was heated from 50 °C to 900 °C at a heating rate of 20 °C/min under nitrogen atmosphere.

3.3.3 Scanning Electron Microscope, SEM

SEM was used to investigate the morphology of PCz, dedoped PCz, IN-doped PCz, and the surface of DCNR film and IN-loaded DCNR film before and after the permeation study. Micrographs of the film were obtained using an acceleration voltage of 15 kV at various magnifications in a range of 200x-1000x.

3.3.4 UV-visible Spectrophotometer

A UV-visible spectrum (Tecan, The Infinite® 200 PRO NanoQuant) was used to examine the amount of IN released from the film. A 0.3 mL of sample solution was scanned in a range 230-500 nm.

3.3.5 Two-point Probe Meter

This technique is one common way to measure the resistivity of a semiconductor material. It involves only two equally spaced probes which are in contact with a material of unknown resistance. The probe array is placed in the center of the material. The specific resistivity (ρ) can be calculated using Eq. (3.1):

$$\rho = R_s t = \frac{KVt}{I} \quad \text{.....(3.1)}$$

where: V = the measured voltage (V),

R_s = the sheet resistivity (Ω),

I = the measured current (A),

t = the sample thickness (cm),

K = the geometric corrector factor.

The correction factors can be found in standard two-point probe resistivity test procedure using Eq. (3.2):

$$K = \rho_{ref} / (R_s t) \quad \text{.....(3.2)}$$

where ρ_{ref} is the known specific resistivity of standard silicon wafer (Ω.cm.).

The specific conductivity (σ) can be calculated from the specific resistivity by using this equation.

$$\sigma = 1/\rho \quad \text{.....(3.3)}$$

3.3.6 Swelling and Crosslink Density

The procedure to determine the swelling and the crosslink density of the crosslinked DCNR films followed in ASTM D6814-02. For determination of swelling, the film immediately studied after the crosslinking process and calculated following Eq. (3.4) and (3.5):

$$\text{degree of swelling (\%)} = \frac{M_s - M_d}{M_d} \times 100 \quad \text{.....(3.4)}$$

$$\text{weight loss (\%)} = \frac{M_i - M_d}{M_i} \times 100 \quad \text{.....(3.5)}$$

where: M_s = the weight after submersion in the buffer,

M_d = the weight after submersion for 72 h,

M_i = the initial weight of the sample.

For studying a crosslink density, 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. Eq. (3.6) was used to calculate the crosslink density (Flory-Rehner equation);

$$\nu_e = \frac{-[\ln(1-V_r) + V_r + \chi_1 V_r^2]}{[V_1(V_r^{1/3} - V_r)/2]} \quad \text{..... (3.6)}$$

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. (3.7):

$$V_r = \frac{\text{Weightof dry rubber / Density of dry rubber}}{\left(\frac{\text{Weight of dry rubber}}{\text{Density of dry rubber}}\right) + \left(\frac{\text{Weight of solvent absorbed by sample}}{\text{Density of solvent}}\right)} \quad \text{..... (3.7)}$$

and the density of the dry rubber can be calculated using the Eq. (3.8):

$$\text{Density at } 23 \pm 2\ ^\circ\text{C (g/mL)} = 0.7913 \times \frac{A}{A-B} \quad \text{.....(3.8)}$$

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).

3.3.7 Drug Release Experiments

3.3.7.1 Spectrophotometric Analysis of Model Drug

The solution of IN in MeOH was prepared for a UV-visible spectrophotometer to identify the maximum absorption wavelength. The absorbance at the characteristic peak of IN was used to determine the amount of drug released from the calibration curve.

3.3.7.2 Determination of Drug Content

The IN-loaded DCNR was immersed in hexane. The drug content in each component (0.3 mL) was measured using a UV-visible spectrophotometer at 324 nm. A calibration curve was used to determine amount of the drug in each sample.

3.3.7.3 In Vitro Drug Permeation Study

A modified Franz diffusion cell was used to study the electrically controlled release of the drug from the prepared IN-loaded DCNR film and IN-doped PCz/DCNR blend film at various crosslink ratios. A PBS buffer solution at a pH of 7.4 was used as a receptor component. The cell was stirred continuously with a temperature remain constant at 37 ± 0.5 °C. Another cell, the film as a donor compartment, was placed over a pig's abdominal skin (7 cm^2, 0.2 cm thickness) on the receptor. Electrical potential was applied through the system. The amount of drug which diffused through the screen to the buffer solution was detected by the UV-visible spectrophotometer.