CHAPTER III
EXPERIMENTAL

3.1 Materials

High molecular weight hydroxyl terminated PDMS's (viscosity 18,000 – 20,000 cSt, Aldrich) were used as the polymer matrices. Tetraethyl orthosilicate (AR grade, Aldrich) and dibutyl thin dilaurate (AR grade, Aldrich) were used as the initiator and the catalyst, respectively. Silicone oil was purchased from Aldrich (viscosity 100 cSt, Aldrich). α,α'-dichloro-p-xylene and tetrahydrothiophene, THT (AR grade, Aldrich), were used to synthesize poly(p-xylylene-bis-tetrahydrothiophenium chloride). Acetone and methanol were used as received.

Salicylic acid, SA (AR grade, Fluka), was used as the model drug. Acrylamide, AAM (AR grade, Fluka), N,N′ methylenebisacrylamide, (N,N′-MBA) (AR grade, Fluka), tetramethylenediamine, TEMED (AR grade, Fisher Scientific), and ammonium peroxodisulfate (AR grade, Fluka) were used as the monomer, crosslinker, catalyst, and initiator, respectively. Sodium acetate (AR grade, Ajax Chemicals) and glacial acetic acid (AR grade, Carlo Erba) were used without further purification.

3.2 Synthesis of Poly(p-phenylene vinylene)

PPV was synthesized via a polyelectrolyte precursor according to the method of Burn *et al.* (1992). To a suspension of 10 g of α,α'-dichloro-p-xylene in 150 mL of methanol, we added 15 mL of tetrahydrothiophene, THT. The resulting mixture was heated in a 50 °C oil bath overnight, and 250 mL of acetone was poured in to precipitate the salt p-phenylene dimethylene bis tetramethylene sulfonium chloride. The mixture was stirred in an icebath for 0.5 hr before filtration. The white solid salt obtained was washed with acetone and dried under vacuum at room temperature until two sequential weighings were the same. The yield was 85% (Burn *et al.*, 1992). Then 1.0 g of the washed and dried salt was dissolved in 7.5 cm^3 of methanol and

then cooled to 0 °C, and was added to 6.3 cm^3 of aqueous sodium hydroxide (0.4M). The reaction mixture was stirred for a duration of 120 min at 0 °C, and was slightly acidified with 1 cm^3 of hydrochloric acid (0.4 M). The solution of 14.8 cm^3 was then dialyzed against a water-ethanol mixture (1:1, 3 x 1000 cm^3) for over three days, after which the solvent was completely removed. The residue was redissolved in methanol. After cooling, the aqueous solution of poly [(p-phenylene) bis(tetrahydrothiophenechloride)] was poured onto a glass dish and allowed to evaporate at room temperature in a free air steam. After 24 hours, the yellowish-green precursor films were heated at 200 °C for 16 hr in vacuum to yield PPV powder. The obtained PPV powder was ground by a jar mill for 2 days.

3.3 Preparation of Salicylic Acid-Doped Poly(p-phenylene vinylene) (SA-doped PPV)

The salicylic acid-doped poly(p-phenylene vinylene) was prepared by the acid-assisted redox doping reaction,as illustrated in eq. 3.1;

$$[PPV]*n + HX*n + H_2O_2*n/2 \rightarrow [PPV^+X^-]*n + H_2O*n \qquad (3.1)$$

In this reaction, hydrogen peroxide (H_2O_2) is chosen as the oxidant; [PPV] represents a repeating unit of the conjugated PPV polymer, and (HX) symbolizes the functionalized salicylic acid. The n is the number of mole of these substances (Sanden, 1997).

3.4 Sample Preparations

3.4.1 Preparation of PDMS Gels and PPV/PDMS Blends

To study the effect of crosslinking ratio, PDMS at various crosslink ratios (N_c/N_m = 0.001, 0.003, 0.005, 0.01, 0.03 and 0.05: where N_c is moles of initiator and N_m is moles of PDMS), were prepared by mixing the high molecular weight hydroxyl terminated PDMS (HO-PDMS), tetraethyl orthosilicate (TEOS), and dibutyl tin dilaurate at various initiator moles. The mixture was cast in a mold

(diameter = 25 mm and height = 1 mm) and dried under vacuum at room temperature (27 °C) for 6 hrs.

PPV/PDMS blend specimens were prepared by the mechanical blending of synthesized PPV particles with HO-PDMS and TEOS at the crosslinking agent and monomer ratio (N_c/N_m) of 0.01, using dibutyltin dilaurate as the catalyst. The mixture was poured into a mold (diameter = 25 mm and height = 1 mm) and allowed to cure under a pressure of 0.6 atm, 27 °C for 4 hr.

3.4.2 Preparation of Salicylic Acid-Loaded Polyacrylamide Hydrogel (SA-loaded PAAM Hydrogel)

The 0.2 %w/w SA-loaded PAAM hydrogels (based on the weight of the acrylamide monomer) were prepared by the free-radical polymerization of 2.32 g of acrylamide in an aqueous solution of salicylic acid (0.0125 M) with N, N′ methylenebisacrylamide (MBA) as crosslinker (Lira *et al.*, 2005). Ammonium persulfate and tetramethylenediamine (TEMED) were used as the initiator and the accelerator. To study the effect of crosslinking ratio on the release of SA from SA-loaded PAAM hydrogels, gels at various crosslink ratios (mol $_{MBA}$: mol $_{AAM}$; 0.002, 0.005, 0.010, 0.016, 0.024) were prepared at various amounts of N, N′ methylenebisacrylamide (MBA). Before gelation (typically after 10 min of mixing the reagents at 27 °C), the pre-gel solution was cast in a mold (diameter 8 cm, thickness 0.5 mm). After gelation, the PAAM hydrogel was cut into a disk shape (diameter ≈ 18 mm, thickness ≈ 0.5 mm).

3.4.3 Preparation of Salicylic Acid-Doped Poly(p-phenylene vinylene)/Polyacrylamide Hydrogel (SA-doped PPV-loaded PAAM Hydrogel)

The SA-doped PPV/PAAM hydrogels were prepared by the free-radical polymerization of 2.32 g of acrylamide in an aqueous solution with 7.5 mg of SA-doped PPV, N, N′ methylenebisacrylamide (MBA), and ammonium persulfate and tetramethylenediamine (TEMED), as previously described in the preparation of salicylic acid-loaded polyacrylamide hydrogel. Before gelation (typically after 10 min of mixing the reagents at 27 °C), the pre-gel solution was cast

in a mold (diameter 8 cm, thickness 0.5 mm). After gelation, each SA-doped PPV/PAAM hydrogel was cut into a disk shape (diameter ≈ 18 mm, thickness ≈ 0.5 mm).

3.5 Characterization Method

Fourier-transform infrared (FT-IR) spectra were obtained using a FT-IR spectrometer (Bruker, Equinox 55/ FRA 1065X). Optical grade KBr (Carlo Erba Reagent) was used as the background material. The synthesized PPV powder thoroughly mixed an ground with dried KBr at a PPV:KBr ratio of 1:20 and was compressed into a disc. Scanning electron micrographs were taken with a JEOL, JSM-5200-2AE, using an acceleration voltage of 20 kV and a magnification of 350. A thermogravimetric analyzer (Dupont, TGA 2950) with a heating rate of 10 °C/min under N_2 atmosphere was used to determine the thermal behavior of PPV. A particle sizer (Malvern, Master-sizer X) was used to obtain the PPV particle size distribution and the mean sizes.

To determine the electrical conductivity, PPV disks (25 mm diameter and ~0.2 mm thickness) were prepared from compression with a hydraulic press. Electrical conductivity was measured using a custom-built two-point probe. The specific conductivity, σ (S/cm), was obtained by measuring the resistance, R, and using the following relation: $\sigma = (1/Rt)(1/K)$, where t is the film thickness and K is the geometric correction factor. A geometric correlation factor was obtained by using standard silicon wafer sheets with known specific resistivity values. The measurements were performed in the linear Ohmic regime. The measurements were carried out at 27 °C and repeated at least two times.

To investigate the morphology of the PAAM hydrogel at various crosslink ratios, with and without an electric field, scanning electron micrographs of the hydrogels were taken (JEOL, JSM-5200-2A) using an acceleration voltage of 15 kV and a magnification of 1500. To study the effect of electric field on the PAAM morphology, the hydrogels were swollen in an acetate buffer, at pH 5.5 and attached to a copper electrode. The other electrode was placed elsewhere in the acetate buffer.

Samples were prepared from frozen swollen hydrogels with and without electric field in liquid nitrogen and then dried in vacuum at -50°C.

DSC thermograms of the SA, the PAAM hydrogel, and the SA-loaded PAAM hydrogel were recorded (Dupont, DSC 822) to determine their thermal behavior. The 2-4 mg samples were accurately weighed in an aluminum pan with a sealed cover. The measurements were performed under N_2 atmosphere over 30 – 400°C at heating rate of 10°C/min.

The absorption infrared spectra of the PAAM and SA- loaded PAAM hydrogels were measured by an attenuated total reflection Fourier transform infrared spectrometer (ATR-FTIR;Thermo Nicolet, Nexus 670) to investigate the interaction between the salicylic acid and the polyacrylamide hydrogel. The samples were placed on a zinc selenide (ZnSe) crystal sample holder.

The average molecular weight between crosslinks of the PDMS gels, M_c, was determined from the swelling in HPLC grade toluene at room temperature. The version of the Flory-Rehner equation was used for our PDMS systems:

$$M_c = \frac{v_1 \rho_2 \left(v_{2m}^{1/3} - \frac{v_{2m}}{2} \right)}{-\left[\ln(1 - v_{2m}) + v_{2m} + \chi_1 v_{2m}^{2} \right]} \qquad (3.2)$$

where $$v_{2m} = \frac{w_0}{v_{equil} \times \rho_2} \qquad (3.3)$$

$$v_{equil} = \frac{w_0}{\rho_2} + \frac{w_s - w_0}{\rho_1} \qquad (3.4)$$

$$\chi = 0.34 + \frac{v_1}{RT}(\delta_1 - \delta_2)^2 \qquad (3.5)$$

and where v_1 is the molar volume of solvent (M_w/density), ρ_2 is the polymer density, PDMS equal to 0.97 g/cm^3, ρ_1 is the solvent density or of toluene which is equal to 0.867 g/cm^3, w_o is the original polymer weight, w_s is the swollen polymer weight, χ is the polymer-solvent interaction parameter, R is the universal gas constant, 8.29 N_m/mol.K, T is the absolute temperature, 298 K, δ_1 is the solubility parameter of PDMS which is equal to 19.4 (MPa)$^{1/2}$, and δ_2 is the solubility parameter of toluene which is equal to 18.20 (MPa)$^{1/2}$.

To determine the % swelling and the % weight loss of the PAAM hydrogels at various crosslink ratios, they were immersed in an acetate buffer, pH 5.5, at 37 °C. After 24 h the swollen PAAM hydrogels were removed, gently wiped to clean off the surface water, and then re-weighed. To determine the % weight loss, the swollen PAAM hydrogels were dried in a vacuum oven for 5 days until constant weight values were attained. The % swelling and the % weight loss were calculated using the following equations (Proikis *et al.*, 2006):

$$\text{Degree of swelling (\%)} = \frac{M - M_d}{M_d} \text{ x } 100 \qquad (3.6)$$

and

$$\text{Weight loss (\%)} = \frac{M_i - M_d}{M_i} \text{ x } 100 \qquad (3.7)$$

where M is the weight of a swollen sample, M_d is the weight of swollen sample after drying in vacuum oven, and M_i is the initial weight of the sample (Proikis *et al.*, 2006). All reported data were average values taken from repeated measurements using two specimens.

To determine the mesh size, ξ, the molecular weight between crosslinks, M_c, and the crosslinking density, ρ, of the crosslinked PAAM hydrogels, they were characterized by equilibrium swelling analysis (Lira *et al.*, 2005). Each hydrogel sample (1 cm^2 square) was cut immediately after crosslinking and was weighed in air and heptane (a non-solvent). The particular sample was then placed in deionized water at 37 °C for 5 days for it to swell to the equilibrium size, and was then weighed again in air and heptane. Finally, the sample was dried for 5 days at room temperature and weighed in air and heptane. The average molecular weight between crosslinks of the PAAM gel, M_c, was determined from the swelling data using eq. 3.8 as given by Peppas and Merrill (Peppas *et al.*, 1996):

$$\frac{1}{M_c} = \frac{2}{M_n} - \frac{\frac{\bar{v}}{\bar{V}_1}\left[\ln(1 - v_{2,s}) + v_{2,s} + \chi_1 v_{2,s}^{\,2}\right]}{v_{2,r}\left(\left(\frac{v_{2,s}}{v_{2,R}}\right)^{1/3} - \frac{1}{2}\left(\frac{v_{2,s}}{v_{2,r}}\right)\right)} \qquad (3.8)$$

where M_n is the number-average molecular weight of the polymer before crosslinking (determined by using an Ubelodde tube ~ 36,400 g/mol), $\bar{v}$ is the specific volume of PAAM (0.741 mL/g), and $\bar{V}_1$ is the molar volume of water (18.1 mL/mol). $v_{2,r}$ is the polymer volume fraction in the gel in the relaxed state, $v_{2,s}$ is the polymer volume fraction in the gel in the swollen state, and χ is the interaction parameter of PAAM-water, 0.48 (Peppas *et al.*, 1996).

The hydrogel mesh size, ξ, was calculated from the following equation:

$$\xi = v_{2,s}^{-\frac{1}{3}} \left[C_n \left(\frac{2M_c}{M_r} \right) \right]^{1/2} l \qquad (3.9)$$

where C_n is the Flory characteristic ratio for PAAM (8.8), and l is the carbon-carbon bond length (=1.54 Å).

The crosslinking density of the hydrogel was calculated using Eq (3.10) (Peppas *et al.*, 1996):

$$\rho_x = \frac{1}{\bar{v} M_c} \qquad (3.10)$$

Electromechanical properties of PDMS under an oscillatory shear at fixed temperature of 27 °C were measured in order to investigate the effects of crosslinking ratio and electric field strength. Electromechanical properties of PDMS under an oscillatory shear at fixed temperature of 27 °C were measured (Rheometric Scientific Inc., ARES). The dynamic moduli, G' and G", were measured as functions of frequency and electric field strength. The linear viscoelastic regime was determined by the strain sweep test to determine the appropriate strain to be used to measure G' and G". Frequency sweep test was then carried out measure G' and G" as functions of frequency (0.1 -100 rad/s) at fixed strains of 700% and 1% for pure PDMS fluid and for the crosslinked PDMS (crosslink ratios of 0.001, 0.003, 0.005, 0.01, 0.03 and 0.05), respectively. Pre-oscillatory shear at frequency of 1 rad/s, at fixed strains at 700% and 1% for pure PDMS fluid and for the crosslinked PDMS, under electric field (~10 min) was applied to the sample to ensure an equilibrium polarization before each measurement was taken.

3.6 Electromechanical Properties Measurements

The electromechanical properties of the crosslinked PDMS and PPV/PDMS gels under oscillatory shear were measured at a fixed temperature of 27 °C by using a parallel plate fixture (Rheometric Scientific Inc., ARES). The sample diameter was 25 mm with a nominal thickness of 1 mm. Dynamic moduli, G′ and G″, were measured as functions of frequency and electric field strength. Linear viscoelastic regimes were determined in an absent of electric field and under applying electric field by strain sweep tests to determine the appropriate strains used in measuring G′ and G″. A frequency sweep test was then carried out to measure G′ and G″ as functions of frequency (0.1-100 rad/s) at fixed strains of 1%. Pre-oscillatory shears at a frequency of 1 rad/s and fixed strains of 1% were applied to the PPV/PDMS gels under electric field (~ 10 min) to attain equilibrium polarizations before the measurements were taken.

3.7 Deflection Measurement

To study the elastic response of the PDMS and the PPV/PDMS gels to an electric field, films of the PPV/PDMS gels were vertically suspended in silicon oil between a pair of parallel copper electrode plates (40 mm long, 30 mm wide, and 1 mm thick). A rigid plastic clip was used to fix the top position of the gel, as shown in Figure 5 (a). A high DC voltage (UC5-30P HV Power Supply, Gamma High Voltage Research Inc.) was applied in a non-contact mode through the electrodes, which were 10 mm apart. The electric deflection response of the gel was recorded by a video camera, and the deflection properties were analyzed by a digital image analyzer (Panasonic M3000, Japan). Both the voltage and the current were monitored as well. All the measurements were carried out at ambient temperature (27 °C).

3.8 Release of SA from SA-loaded PAAM Hydrogel and SA-doped PPV/PAAM

3.8.1 Preparation of Buffer Solution

To prepare 1000 mL of an acetate buffer solution at a pH of 5.5, 15 mL of glacial acetic acid and 150 g of sodium acetate were added to distilled water in a volumetric flask with a total volume of 1000 mL.

3.8.2 Pigskin Preparation

In-vitro drug released experiments were performed using fresh pigskin obtained from the abdominal part. The skin used in this work was about 1-1.5 mm thick. The whole pigskins were surgically removed and cleaned with normal sterile saline. The subcutaneous fat, tissue, blood vessels, and epidermal hair were carefully removed by blunt section. The skin was free of obvious holes or defects. The complete skin was cleaned with saline and finally with distilled water, cut into a circle, wrapped with aluminium foil, and stored in a frozen state. In the drug release experiment, pigskin was soften at room temperature before use.

3.8.3 Spectrophotometric Analysis of the Model Drug

A UV/visible spectrophotometer (Shimadzu, UV-2550) was used to determine the maximum spectra of model drug. Model drug in aqueous solution was prepared for determining the maximum absorption wavelength. The characteristic peak was observed. The absorbance value at the maximum wavelength of 296 nm of the model drug was read and the corresponding model drug concentrations were calculated from the calibration curve.

3.8.4 Actual Amount of Drug Content

The actual amount of SA in the SA-loaded PAAM hydrogel (circular disc about 1.8 cm in diameter) was determined by dissolving the sample in 4 ml of dimethyl sulfoxide (DMSO), and 0.5 ml of the solution was added to 8 ml of the acetate buffer solution. The drug amount in the dilute solution sample was measured by using a UV/Visible spectrophotometer at a wavelength of 296 nm and through a predetermined calibration curve. It should be noted that the presence of DMSO in the dilute solution has no apparent effect on the UV absorbance at the wavelength of 296 nm. The data are reported here as an average taken from at least three measurements.

3.8.5 Transdermal Transport Studies

Transdermal diffusion through a hairless pigskin was carried out in order to study the release characteristics of the drug from a SA-loaded PAAM hydrogel. A hairless pigskin (thickness ~ 1-1.5 mm) was placed on top of the acetate buffer solution on a custom built modified Franz diffusion cell. The area available for permeation was 3.14 cm^2. The pigskin was allowed to come into equilibrium contact with the acetate buffer (pH 5.5, ionic strength of 0.001225 M) in the receptor chamber; the buffer was magnetically stirred throughout the experiment period (48 h) at a thermostatically maintained temperature (37 ± 2 °C). The SA-loaded PAAM hydrogel with a particular crosslinking ratio (0.002, 0.005, 0.016 or 0.024) was placed between the copper cathode and the pigskin, which was mounted onto the receptor compartment. To study the effect of electric field strength on the release of the SA from the SA-loaded PAAM hydrogel, the cathode electrode (copper electrode) was connected to a power supply (KETHLEY 1100V Source Meter), which provided various electrical voltgaes (V = 0, 0.01, 0.03, 0.05, 0.07, 0.1, 0.5, 1.0 and 5.0 V) across the SA-loaded PAAM hydrogel, the pigskin, and the buffer solution. The anode electrode pin was positioned in the buffer solution. The duration of applying the electric field to the experiemtntal setup is ~48 h. The donor and receiver compartments were kept in contact by wrapping parafilm at the junction. The total diffusion period investigated was 48 h, 0.3 ml of the buffer solution was withdrawn and an equal amount of fresh buffer solution was added to the cell, every 15 minutes during the first hour, to ensure good contact between the buffer solution and the skin at all times. The amount of the drug in the withdrawn solution samples was determined using a UV spectrophotometer at 296 nm. The data were then analyzed to determine the cumulative amount of the drug released from the samples at each specified diffusion period. The experiments were carried out in triplicate and the data were reported as average values.

3.8.6 Drug Release Studies

The diffusion through a nylon net (mesh size = 2.25 mm^2) was carried out in order to study the release characteristics of the drug from a SA-loaded PAAM hydrogel and SA-doped PPV/PAAM hydrogel. A net was placed on top of

the acetate buffer solution on a custom built modified Franz diffusion cell. The area available for permeation was 2.51 cm^2. The nylon net was allowed to come into equilibrium and in contact with the acetate buffer (pH 5.5, ionic strength of 0.001225 M) in the receptor chamber; the buffer was magnetically stirred throughout the experiment period (48 h) at a thermostatically maintained temperature (37 ± 2 °C). The SA-loaded PAAM and SA-doped PPV/PAAM hydrogel with particular crosslinking ratios (0.002, 0.005, 0.016, or 0.024) were placed between the copper cathode and the net, which was mounted onto the receptor compartment. To study the effect of electric field strength on the release of the SA from the SA-loaded PAAM and SA-doped PPV/PAAM hydrogels, the cathode electrode (copper electrode) was connected to a power supply (KETHLEY 1100V Source Meter), which provided various electrical voltages (V = 0, 0.01, 0.03, 0.05, 0.07, 0.09, and 0.1V) across the hydrogel, the nylon net, and the buffer solution. The anode electrode pin was positioned in the buffer solution. The total duration of the electric field applied to the experimental setup was ~48 h. The donor and receiver compartments were kept in contact by wrapping a parafilm at the junction. The total diffusion period investigated was 48 h; 0.3 ml of the buffer solution was withdrawn and an equal amount of fresh buffer solution was added to the cell every 15 minutes during the first hour to ensure good contact between the buffer solution and the PAAM matrix at all times. The amount of the drug in the withdrawn solution sample was determined using a UV spectrophotometer at 296 nm. The data were then analyzed to determine the cumulative amount of the drug released from the samples at each specified diffusion period. The experiments were carried out in triplicate and the data were reported as average values.