

CHAPTER III
EXPERIMENTAL

3.1 Materials

3.1.1 Preparation of polymer fibers by electrospinning technique

- 1) Nylon6 (particle size 3.00 mm) (Sigma Aldrich, Germany)
- 2) Polystyrene (PS; MW 280,000) (Sigma Aldrich, Germany)
- 3) Polyacrylonitrile (PAN; MW 150,000) (Sigma Aldrich, Germany)
- 4) Formic acid, 85% (CARLO ERBA, France)
- 5) N,N-dimethylformamide (DMF) (Riedel-de Haën, Germany)

3.1.2 Preparation of DNPH-coated polymer fibers

- 1) 2,4-dinitrophenylhydrazine (DNPH) (Rankem, India)
- 2) Acetonitrile (ACN) (Merck, Germany)
- 3) Concentrated hydrochloric acid, 37% (Merck, Germany)

3.1.3 Extraction and elution of aldehydes in water

- 1) Formaldehyde, 37% (Loba Chemie, India)
- 2) Acetaldehyde, >99% (Merck, Germany)
- 3) Propionaldehyde or propanal, 99.8% (Merck, Germany)
- 4) Butyraldehyde or butanal, 99.8% (Merck, Germany)
- 5) Hexanal, 98.3% (Merck, Germany)
- 6) Methanol (Merck, Germany)
- 7) Hexane (Mallinckrodt, USA)



3.2 Methodology

3.2.1 Preparation of electrospun fibrous polymer membrane

The setup for electrospinning process is shown in Figure 3.1. The parameters for preparation of each fibrous polymer membrane were summarized in Table 3.1. Electrospinning process consists of high voltage power supply (230 series, Bertan High Voltage Research), syringe pump (NE-1000, Prosense) and collector. The 3 mL syringe was fitted to a 20G needle, which was connected with high voltage power supply. The collector is simply a copper plate covered with aluminium foil.

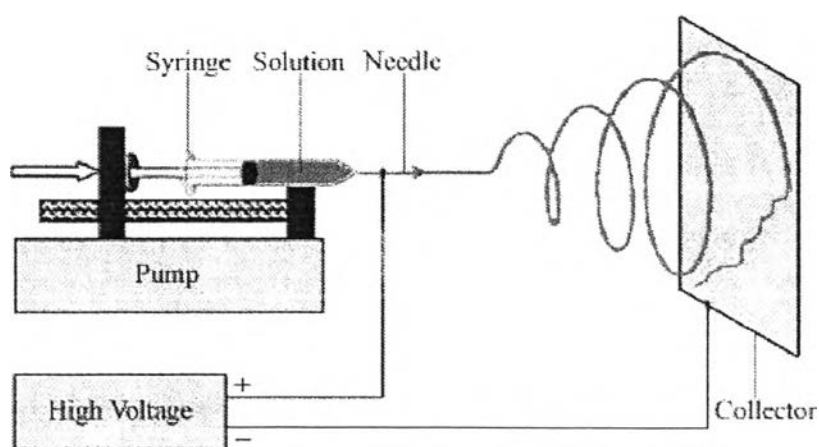


Figure 3.1 Schematic set up of the electrospinning process [62].

3.2.1.1 Preparation of fibrous Nylon6 membrane

32% (w/v) nylon6 solution was prepared by dissolved 1.60 g of nylon6 in 5 mL of formic acid and stirred for 1 hour at 50 °C. A voltage of 23 kV was applied to the needle and the collector. The distance between the needle tip and collector was 10 cm and the flow rate of the solution was 3.3 $\mu\text{L}/\text{min}$. Approximately 0.5 mL of the nylon6 solution was used to fabricate a fibrous nylon6 membrane on the plate. The electrospun fibrous nylon6 membrane was cut in a circular format with a diameter of 13 mm (Figure 3.2) and stored in a desiccator.

3.2.1.2 Preparation of fibrous PS membrane

18% (w/v) PS solution was prepared by dissolved 0.90 g of PS in 5 mL of DMF and stirred for 1 hour at 50 °C. A voltage of 20 kV was applied to the needle and the collector. The distance between the needle tip and collector was 10 cm and the flow rate of the solution was 10 $\mu\text{L}/\text{min}$. About 1.5 mL of the PS solution was used to fabricate a fibrous PS membrane on the plate. The electrospun fibrous PS membrane was cut in a circular format with a diameter of 13 mm (Figure 3.2) and stored in a desiccator.

3.2.1.3 Preparation of fibrous PAN membrane

10% (w/v) PAN solution was prepared by dissolved 0.50 g of PAN in 5 mL of DMF and stirred for 1 hour at 50 °C. A voltage of 23 kV was applied to the needle and the collector. The distance between the needle tip and collector was 10 cm and the flow rate of the solution was 8 $\mu\text{L}/\text{min}$. About 2.5 mL of the PAN solution was used to fabricate the fibrous PAN membrane on the plate. The electrospun fibrous PAN membrane was cut in a circular format with a diameter of 13 mm (Figure 3.2) and stored in a desiccator.

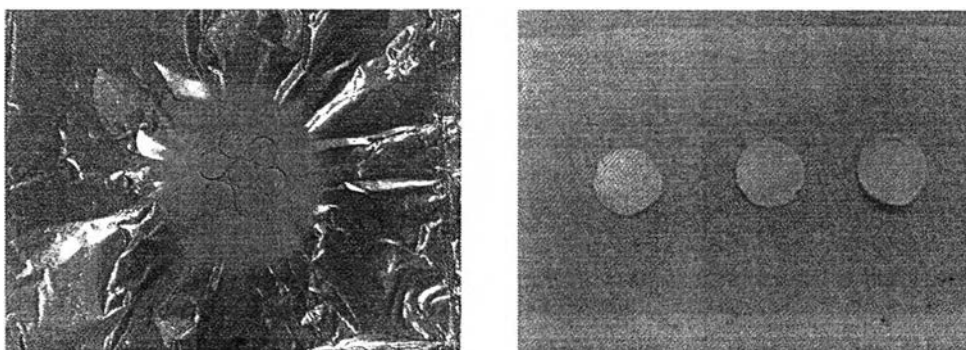


Figure 3.2 Polymer membranes were cut in a circular format with a diameter of 13 mm

Table 3.1 Parameters for fabrication of electrospun fibrous polymer membrane.

	Nylon6	PS	PAN
Polymer solution (% w/v)	32% in formic acid	18% in DMF	10% in DMF
Volume of polymer solution used for electrospinning (mL)	0.5	1.5	2.5
Voltage (kV)	23	20	23
Distance between the needle tip and collector (cm)	10	10	10
Flow rate of the solution ($\mu\text{L}/\text{min}$)	3.3	10.0	8.0

3.2.2 Characterization of electrospun polymer fibers

3.2.2.1 Scanning Electron Microscopy (SEM)

The morphology of electrospun polymer fibers was characterized by a JEOL Scanning Electron Microscopy (model JSM-6480LV). The diameter of fibers was reported as the average values with standard deviation ($n=30$) by Image Tool 3.0 software (Shareware provided by CMEIAS).

3.2.2.2 Thermogravimetric analysis (TGA)

Electrospun polymer fibers were examined and compared between non-DNPH-coating and DNPH-coating by thermogravimetric analysis. Thermogravimetric analysis detects changes of mechanical properties of samples. TGA experiments were carried out with a Pyris 1 TGA (Perkin Elmer, USA). Samples were heated from 50 °C to 600 °C with a heating rate of 25 °C/min. The glass transition temperature was determined by the change of the slope of the obtained curves.

3.2.3 Solution preparation

3.2.3.1 DNPH solutions

DNPH solution in acetonitrile: 1000 mg/L of stock solution was prepared by weighing 100 mg of DNPH into the beaker. Then, 100 mL of acetonitrile



was added with few drops of concentrated hydrochloric acid. The solution was stirred until all the particles were completely dissolved.

DNPH solution in water-acetonitrile: 1000 mg/L of stock solution was prepared by weighing 0.5944 g of DNPH and dissolved in a 50 mL of concentrated hydrochloric acid : milli-Q water : acetonitrile solution with the ratio of 2:4:2 in volume. Then, dilute with milli-Q water to give a concentration of 1000 mg/L.

The 100 – 500 mg/L of DNPH solutions were then prepared by diluting the 1000 mg/L of stock solution for the optimization of DNPH-coated polymer membrane and calibration curve of aldehyde derivatization. DNPH solutions in acetonitrile were diluted the stock solution with acetonitrile and DNPH solution in water-acetonitrile were diluted the stock solution with milli-Q water.

3.2.3.2 Aldehydes solutions

Aldehydes stock solution: 1000 mg/L of formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde and hexanal solution was prepared by diluting 60 mL of each compound with milli-Q water. This solution was used for preparation of calibration curve and spiked water sample.

Aldehydes standard solution for calibration curve: The 0.10, 0.30, 0.50, 0.80 and 1.00 mg/L of the aldehydes standard solutions were prepared for the calibration curve. Each concentration was prepared by mixing a certain amount of 1000 mg/L of the aldehydes stock solution with 500 μ L of DNPH solution, with the concentration of 300 mg/L, and then diluting to 3 mL with milli-Q water.

3.2.4 Preparation of DNPH-coated electrospun polymer fibers

3.2.4.1 Procedure for coating DNPH on electrospun polymer fibers

Fibrous polymer membrane was cut in a circular format with diameter of 13 mm and placed in a filter holder (Figure 3.3 (a)). The filter packed with the membrane was preconditioned with 200 μ L of methanol and 200 μ L of milli-Q water respectively. When the membrane was preconditioned, 3 mL of DNPH solution was loaded to coat. After DNPH was completely coated onto the sorbent, the sorbent was purged with nitrogen gas for 3 min or until dry. The filter packed was sealed with parafilm and stored at 4 °C.



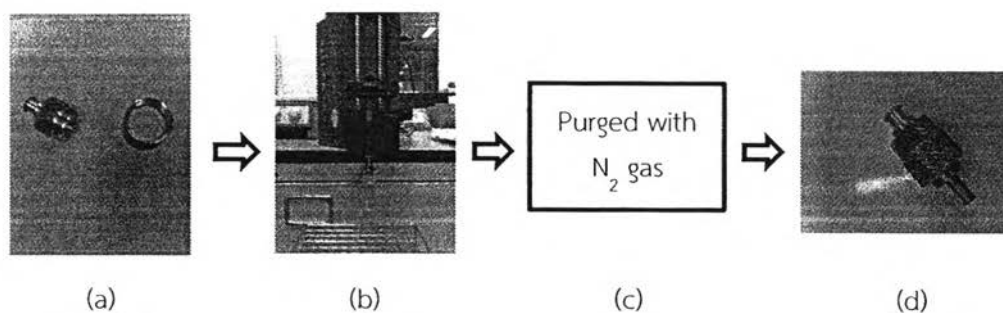


Figure 3.3 Steps of DNPH coating on polymer membrane; (a) placed a fibrous polymer membrane in a filter holder, (b) loading DNPH, (c) purged a membrane with N₂ gas, (d) stored the membrane at 4°C.

To determine which conditions gave the best efficient coating, DNPH on the membrane was eluted out with 1 mL of acetonitrile with the rate of 100 $\mu\text{L}/\text{min}$. The eluted solution was analyzed by HPLC. The peak area of DNPH peak was compared.

3.2.4.2 Types of DNPH solvents

100, 200, 300, 400 and 500 mg/L of DNPH solution in two different solvents: acetonitrile and the mixture of water-acetonitrile, as mentioned in 3.2.3.1 were used to coat the nylon membrane. The electrospun fibrous nylon6 membrane was coated followed the procedure in 3.2.4.1 and DNPH solution was loaded at a flow rate of 100 $\mu\text{L}/\text{min}$. Peak area of DNPH peak is used to determine the optimized solvent for preparing the DNPH solution.

3.2.4.3 DNPH concentration

After the better solvent has been determined and obtained, different concentrations of DNPH were experimented. Various concentrations of DNPH: 100, 200, 300, 400 and 500 mg/L were used to study. In each concentration, coating DNPH onto the electrospun fibrous nylon6 membrane was followed the procedure in 3.2.4.1 and DNPH solution was loaded at a flow rate of 100 $\mu\text{L}/\text{min}$. Peak area of DNPH was used to determine the optimized DNPH concentration for coating the membrane.



3.2.4.4 Coating rate

After the optimum concentration was obtained, a 3 mL of optimized concentration of DNPH solution were used to coat on the nylon6 membrane at various rates: 300, 150, 100, 75, and 60 $\mu\text{L}/\text{min}$. Peak area of DNPH peak was used to determine the optimized rate of coating DNPH on the membrane.

3.2.4.5 Efficiency of DNPH coating on electrospun polymer fibers

After the optimum coating condition on electrospun nylon6 fibers was obtained, the electrospun PS fibers and the electrospun PAN fibers were coated with DNPH at this condition. Peak area of DNPH peak was used to compare the coating efficiency on the membrane.

3.2.5 Analysis of aldehydes-DNPH derivative by HPLC

The aldehydes-DNPH derivatives were analyzed by HPLC-UV-Vis. HPLC system (Waters, Waters Corporation) consist of

- Pump and Controller (Waters 600)
- Autosampler (Waters 717 plus)
- Photodiode array detector (Waters 996)

The conditions of HPLC analysis are shown in Table 3.2.

Table 3.2 HPLC conditions [14, 63]

Parameters	Conditions		
Column	C ₁₈ reversed phase-column (100 x 4.6 mm), Phenomenex/Kinetex, particle size: 2.6 μm		
Mobile Phase	Gradient: Time (min)	Acetonitrile	Milli-Q water
	4	55%	45%
	7	90%	10%
	9	100%	0%
	20	100%	0%
	21	55%	45%
Flow rate	0.6 mL/min		
Run time	30 min		
Injection volume	10 μL		
Detection	UV-Vis at the wavelength of 360 nm		



3.2.6 Extraction of aldehydes by DNPH-coated electrospun polymer fibers

3.2.6.1 Extraction procedure

To optimize the condition for extraction and derivatization of aldehydes in water, 10 $\mu\text{g/L}$ of aldehydes solution was used for all studies. First, the electrospun fibrous polymer membrane in a filter holder was pretreated with 200 μL of milli-Q water before extraction. Then, 10 mL of 10 $\mu\text{g/L}$ of aldehydes solution was loaded at certain rate. Later, desorption solvent was loaded at certain volume, where the desorption time was fixed at 10 minutes and the rate would depend on the volume. The eluted solution was collected for further analysis by HPLC. After the HPLC analysis, obtained peak area was used to determine and compare for the optimum conditions. Steps for aldehydes extraction was shown in Figure 3.4.

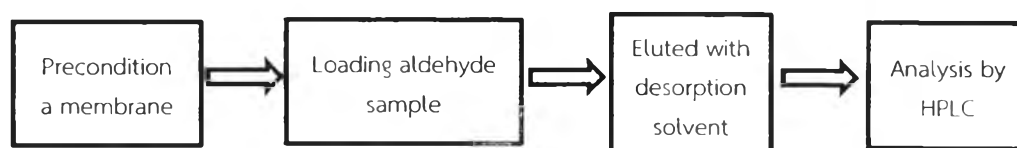


Figure 3.4 Steps of aldehydes extraction

3.2.6.2 Types of desorption solvent

The type of desorption solvent was studied by followed the procedure in 3.2.6.1 and varied the desorption solvent. Acetonitrile, methanol and hexane were selected to study. Peak area of aldehydes-DNPH peak was used to determine the optimized solvent for desorption.

3.2.6.3 Volume of the desorption solvent

After the better desorption solvent has been determined and obtained, the volume of desorption solvent was varied at 100, 200 and 300 μL . The water sample was pass through the sorbent for 10 minutes with the rate of 10, 20 and 30 $\mu\text{L/min}$ for the elution solvent with the volume of 100, 200 and 300 μL respectively.

3.2.6.4 Flow rate of sample

The flow rate of extraction was studied by varying the flow rate of water sample. In each trial, 10 mL of 10 µg/L aldehydes was used. The rates were 2.00, 1.00, 0.50 and 0.33 mL/min (5, 10, 20 and 30 minutes respectively). The extraction procedure was mentioned in 3.2.6.1. 100 µL of acetonitrile was used to desorb aldehydes from the sorbent. Peak area of aldehydes-DNPH peak was used to determine the best extraction time.

3.2.6.5 Recovery of aldehydes extraction

After optimum conditions were obtained, water sample spiked with 10 µg/L aldehydes were experimented for recovery percentage. Since, WHO reported that the formaldehyde concentration in raw water was found in the range of 1-25 µg/L. Thus, the aldehydes was spiked into a water sample at a concentration of 10 µg/L in this study. The concentration of analytes was obtained from reading the calibration curve. The eluted sample volume was 100 µL, the analytes were eluted out with acetonitrile as the desorption solvent.

3.2.6.6 Extraction of aldehydes in drinking water

Drinking water was extracted using the optimized extraction conditions of the SPE sorbent. Aldehydes were detected in the samples. To evaluate the suitability of the proposed method, drinking water was spiked and recoveries were calculated

