CHAPTER III

EXPERIMENTAL SECTION

3.1 Materials

Commercial grade-chitosan flake (approx. 85% degree of deacetylation) with molecular weight of about 10^6 was purchased from Ebase Co.,Ltd (Thailand). Ethylene diamine (EDA) and methyl acrylate (MA) were purchased from Fluka (Switzerland). Commercial-grade methanol was purchased from TSL Chemical Co.,Ltd (Thailand) and distilled prior to use. Dimethyl sulphate (commercial grade) was kindly provided by Modern Dyestuff & Pigment Co., Ltd., (Thailand). Glacial acetic acid was purchased from Merck Chemicals Ltd. 100% Cotton fabric (plain weave), which was singed, desized, scoured and bleached, was purchased from Bigger Text Co.,Ltd. (Thailand).

3.2 Instruments

CHN analysis was performed on PerkinElmer 2400 series II CHNS/O Analyzer. The infrared (FT-IR) spectra were recorded at the frequency range of 4000-400 cm^{-1} with 64 consecutive scans and 4 cm^{-1} resolution on a Nicolet 6700 FTIR spectrometer. ^1H NMR (300 MHz) spectra were recorded on a Bruker DPX-300 spectrometer. X-ray diffraction was performed using a PW 3710 Philips diffractometer with CuK_α radiation (λ = 0.1542 nm) operated at 40 kV and 30 mA. Thermal gravimetric analysis (TGA) was carried out using a NETZSCH STA 409 C/CD. Morphology of samples was observed using JEOL Model JSM-5410LV. The fabric samples were padded using a Labtec padding machine from Newave Lab Equipment Co., Ltd. (Taiwan). Drying and curing of all treated fabrics was performed using a Rapid stenter oven, Labortex Co.,Ltd. (Taiwan). Depolymerization process was carried out using a Labtec laboratory exhausted dyeing machine. Washing test was carried out using a Gyrowash, James H Heal Co.,Ltd. (England).

PART A: SYNTHESIS OF CATIONIC HYPERBRANCHED DENDRITIC POLYAMIDOAMINE

3.3 Synthesis of hyperbranched dendritic polyamidoamine

Michael addition step was carried out as follows: A solution of EDA (50 g, 0.833 mole) in methanol (200 ml) was added dropwise to a stirred solution of MA (350 g, 4.069 moles) in methanol (200 ml) at 0°C over a period of 2 hours. The mixture temperature was allowed to rise to room temperature and the mixture was continuously stirred for 48 hours. The solvent and excess MA was removed using a rotary evaporator under reduced pressure at 50°C. In this step, methyl ester terminated hyperbranched PAMAM (G-0.5) was achieved.

Amidation step was conducted as follows : A solution of G-0.5 hyperbranched PAMAM (100 g) in methanol (100 ml) was carefully added to a vigorously stirred solution of EDA (60 g, 1 mole or 4 times higher than concentration than the methyl ester content) in methanol (300 ml) at 0°C. The rate of addition was carefully controlled to assure that the temperature did not rise over the room temperature. After completion of addition, the mixture was stirred for 72 hours at room temperature. The solvent and unreacted EDA were removed under reduced pressure at the temperature not higher than 50°C. The excess EDA was removed at azeotropic temperature using toluene to methanol mixture (9:1). G0.0 hyperbranched PAMAM was obtained.

The second round synthesis was then repeated as above except that a two times higher MA (in 100 ml of methanol) was used than that in the first round synthesis. At this step, the G0.5 hyperbranched PAMAM product was obtained.

3.4 Methylation of methyl ester terminated hyperbranched dendritic polyamidoamine (PAMAM-ester)

A typical method was follows: To a solution of G-0.5 hyperbranched PAMAM-ester (20 g, 0.05 mole), 13 g (0.1 mole) of dimethyl sulphate was slowly added. The methylation reaction proceeded immediately at room temperature. The pH value of the system gradually decreased which was instantly raised by the addition

of concentrated sodium hydroxide solution in order to drive the reaction forward. The mixture temperature was controlled at room temperature and continuously stirred. After completion of dimethyl sulphate addition, the mixture was additionally stirred for 30 minutes. The resultant cationic hyperbranched PAMAM-ester was achieved. The cationic hyperbranched PAMAM-ester was employed in the next step without further purification.

3.5 Characterizations of cationic hyperbranched dendritic polyamidoamine

3.5.1 FTIR Analysis

The infrared (FTIR) spectra were recorded on a Nicolet 6700 FTIR spectrometer. The hyperbranched dendritic polyamidoamine and cationic hyperbranched dendritic polyamidoamine were scanned at the frequency range of 4000-400 cm^{-1} with 64 consecutive scans and 4 cm^{-1} resolution.

3.5.2 ^1H NMR Analysis

The chemical structure was investigated by ^1H Nuclear Magnetic Resonance Spectroscopy in deuterated chloroform ($CDCl_3$). All of chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. NMR spectra of all samples were recorded on a Bruker DPX-300 spectrometer.

PART B: MODIFICATION OF CHITOSAN WITH CATIONIC HYPERBRANCHED DENDRITIC POLYAMIDOAMINE

3.6 Modification of chitosan with cationic hyperbranched dendritic polyamidoamine-ester

Typical modification method of chitosan with varying amounts of cationic hyperbranched PAMAM-ester was carried-out as follows: A mixture of 5 g chitosan and cationic hyperbranched PAMAM-ester (varied from 10, 50 wt% based on chitosan, and excess amount) was dissolved in 500 ml of 1% (w/v) acetic acid solution and stirred by a magnetic stirrer at about 700 rpm until the dissolution was

complete. The solution pH value was then carefully adjusted to pH 7.5 using dilute NaOH solution. The resultant slurry mixture was continuously stirred at room temperature for 5 days to achieve the reaction between chitosan free amine group and PAMAM methyl ester group. From this procedure, the wet cake products were obtained, in cases of 10 and 50 wt %, and then filtrated off. The filtrate was washed thoroughly with distilled water. The obtained wet cake was kept in a desiccator prior to characterizations. In case of excess amount of cationic hyperbranched PAMAM-ester, the obtained viscous liquid was precipitated in methanol prior to filtration.

3.7 Chitosan and cationic hyperbranched dendritic PAMAM-chitosan film preparation

Chitosan and cationic hyperbranched dendritic PAMAM-chitosan film was prepared by dissolving chitosan and cationic hyperbranched dendritic PAMAM-chitosan powders in 1% w/v acetic acid solution and stirring until the dissolution was complete. Chitosan and cationic hyperbranched dendritic PAMAM-chitosan solution was formed film by casting on a Mylar® polyester film and then dried at room temperature for at 72 hours.

3.8 Characterizations of cationic hyperbranched dendritic PAMAM-chitosan

3.8.1 FTIR Analysis

The functional groups of chitosan and cationic hyperbranched dendritic PAMAM-chitosan film were characterized by Nicolet 6700 FTIR spectrometer. All samples were scanned at the frequency range of 4000-400 cm^{-1} with 64 consecutive scans and 4 cm^{-1} resolution.

3.8.2 ^{1}H NMR Analysis

The chemical structure was investigated by ^{1}H Nuclear Magnetic Resonance Spectroscopy in D_2O/CD_3COOD All of chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. NMR spectra of all samples were recorded on a Bruker DPX-300 spectrometer.

3.8.3 Elemental Analysis

Carbon, hydrogen and nitrogen contents of Chitosan and cationic hyperbranched dendritic PAMAM-chitosan were determined by using a PerkinElmer 2400 series II CHNS/O Analyzer.

3.8.4 SEM Analysis

Morphology of chitosan and cationic hyperbranched dendritic PAMAM-chitosan film was investigated by a scanning electron microscope JEOL Model JSM-5410LV. The scanning electron microscopy was operated at 15 kV to image the samples. The surface of sample was sputter-coated with a thin layer of gold before scanning in order to improve conductivity and prevent electron charging on the surface.

3.8.5 TGA Analysis

Thermogravimetric analysis (TGA) was performed with a NETZSCH STA 409 C/CD. The samples were heated from 30°C to 850°C under oxygen atmosphere at a heating rate of 20°C/min.

3.8.6 X-ray diffraction (XRD) Analysis

X-ray diffraction (XRD) measurements were carried out at room temperature by using a Bruker D8 Advance diffractometer with CuK_{α} radiation ($\lambda = 0.154$ nm) operated at 40 kV and 30 mA. The diffraction patterns were determined over a range of diffraction angle $2\theta = 5$ to 60°.

3.9 Antimicrobial activity of cationic hyperbranched dendritic PAMAM-chitosan

The antimicrobial activity of chitosan and cationic hyperbranched dendritic PAMAM-chitosan film were evaluated by quantitative method. The test was carried out to against *Staphylococcus aureus* (American Type Culture Collection No. 6538). The microbial reduction was calculated according to the following equation,

$$\text{Reduction (\%)} = \frac{B - A}{B} \times 100$$

Where A and B are the number of bacteria (CFU/ml) from the inoculated treated test samples and blank in the flask incubated for 24 hrs. contact time.

PART C: FABRIC TREATMENT

3.10 Coating of bulk chitosan and in-situ depolymerization of coated chitosan

3.10.1 Treatment of chitosan on cotton fabric

Chitosan was dissolved in 1 % (w/v) acetic acid solution. Cotton fabrics (8.5 g, size 25 x 28 cm.) were padded with a chitosan solution of various concentrations (1, 1.5, 2% w/v) using a Labtec padding machine (Figure 3.1) to provide 80% wet pick up. The padded samples were dried at 100 °C for 10 min and cured at 150 °C for 3 min in a Rapid stenter oven as shown in Figure 3.2. The treated fabric was rinsed in tap water, and subsequently air dried.

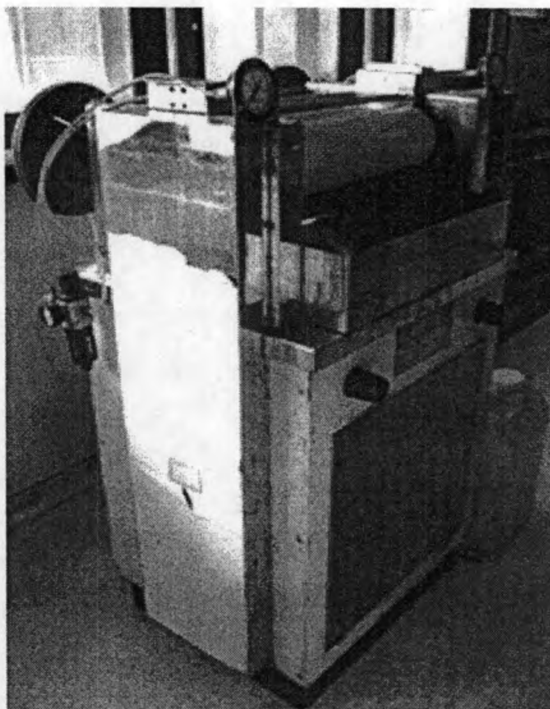

Figure 3.1 Labtec laboratory exhausted dyeing machine

Figure 3.2 Rapid stenter oven

3.10.2 In-situ depolymerization of coated chitosan

The depolymerization of coated chitosan on the fabric was carried out after coated chitosan process. The chitosan coated fabric was put into a sealed stainless steel dyeing tubes containing a solution of sodium nitrite. The sealed stainless steel dyeing tubes were housed in a Labtec laboratory exhausted dyeing machine (Figure 3.3). The treatment was performed at the liquor ratio of 1:25. An effect of sodium nitrite concentration ranging from 20 g/l was investigated. Treatment was carried out at room temperature (30 °C) for 30 mins. Finally, treated fabric was rinsed in tap water, and subsequently air dried.

Figure 3.3 Labtec laboratory exhausted dyeing machine

3.10.3 Washing durability

The laundering test was performed to evaluate the durability of treated fabrics. The washing durability was determined according to ISO 105-C01:1989(E) using a Gyrowash® washing machine. The treated cotton fabric was placed in the stainless steel container and 5 g/l of standard soap solution was add (without optical brightening agent), previously heated to 40°C ± 2°C, to give a liquor ratio of 50:1.

These containers were housed in a washing machine for rotating in a thermostatically controlled water bath at 40°C ± 2°C for 30 min. After that, the sample fabrics were removed, rinse them twice in cold water and then in cold running tap water for 10 min and squeezed them. The samples were air dry by hanging them in air at room temperature.

Figure 3.4 Gyrowash washing machine

3.10.4 Antimicrobial activity

The antimicrobial activity of treated cotton fabrics were evaluated by quantitative method according to AATCC Test Method 100-1999 (Antibacterial Finishes on Textile Material). The test was carried out to against *Staphylococcus aureus* (American Type Culture Collection No. 6538). The microbial reduction was calculated according to the following equation,

$$\text{Reduction (\%)} = \frac{B-A}{B} \times 100,$$

Where A and B are the number of bacteria (CFU/ml) from the inoculated treated test samples in the flask incubated for 24 hrs. contact time and 0 hrs. contact time (immediately after inoculation)

3.10.5 SEM Analysis

Morphology of fabric surface was investigated by a scanning electron microscope JEOL Model JSM-5410LV. The scanning electron microscopy was operated at 15 kV to image the samples. The surface of sample was sputter-coated with a thin layer of gold before scanning in order to improve conductivity and prevent electron charging on the surface.

3.10.6 Nitrogen content

The Kjeldahl method was used to determine nitrogen content of untreated and treated fabrics. The method consists of heating fabrics with sulfuric acid, which decomposes the organic substance by oxidation to liberate the reduced nitrogen as ammonium sulfate. In this step potassium sulfate was added in order to increase the boiling point of the medium (from 169°C to 189°C). Chemical decomposition of the sample was complete when the medium had become clear and colorless (initially very dark).

The solution was then distilled with sodium hydroxide (added in small quantities) which converts the ammonium salt to ammonia. The amount of ammonia present (hence the amount of nitrogen present in the sample) was determined by back titration. The end of the condenser was dipped into a solution of boric acid. The ammonia reacts with the acid and the remainder of the acid was then titrated with a sodium carbonate solution with a methyl orange pH indicator

3.11 Treatment of cationic hyperbranched dendritic polyamidoamine

The cotton fabrics were padded with an aqueous cationic hyperbranched PAMAM dendrimer solution (G2.5, G3.5, G4.5) (5, 10, 15, 20% w/v) to give 80% wet pick up, dried at 100 °C for 10 min and cured at 150 °C for 3 min. The treated cotton fabrics were rinsed several times with tap water, and then air-dried at room temperature.

3.11.1 Antimicrobial activity

The treated cotton fabrics were evaluated antimicrobial activity by quantitative method according to AATCC Test Method 100-1999 (Antibacterial Finishes on Textile Material). The test was carried out to against *Staphylococcus aureus* (American Type Culture Collection No. 6538). The microbial reduction was calculated and presented.

3.12 Combined treatment of cotton fabric with chitosan and cationic hyperbranched dendritic polyamidoamine

Chitosan was dissolved in 1 % (w/v) acetic acid solution. Cotton fabrics (8.5 g, size 25 x 28 cm.) were padded with a chitosan solution of various concentrations (0.1, 0.5, 1% w/v) using a Labtec padding machine to provide 80% wet pick up. The padded samples were dried at 100°C for 10 min and cured at 150°C for 3 min in a Rapid stenter oven.

The treated fabrics were padded again with an aqueous cationic hyperbranched PAMAM solution (G2.5, G3.5, G4.5) (5, 10, 15, 20% w/v) to give 80% wet pick up,

dried at 100 °C for 10 min and cured at 150 °C for 3 min. The treated cotton fabrics were washed several times with tap water, and then air-dry at room temperature.

3.12.1 Antimicrobial activity

The antimicrobial activity was evaluated according to AATCC Test Method 100-1999 (Antibacterial Finishes on Textile Material) to against *Staphylococcus aureus* (American Type Culture Collection No. 6538). The result of antimicrobial activity was reported in term of the microbial reduction percent.

3.12.2 FTIR Analysis

The functional groups of chitosan and cationic hyperbranched dendritic PAMAM on the treated cotton fabrics were characterized by Nicolet 6700 FTIR spectrometer. All fabrics samples were scanned at the frequency range of 4000-400 cm^{-1} with 64 consecutive scans and 4 cm^{-1} resolution

3.12.3 SEM Analysis

Morphology of fabric surface was investigated by a scanning electron microscope JEOL Model JSM-5410LV. The scanning electron microscopy was operated at 15 kV to image the samples. The surface of sample was sputter-coated with a thin layer of gold before scanning in order to improve conductivity and prevent electron charging on the surface.