CHAPTER II

EXPERIMENTAL

2.1 Instrument and apparatus

1. Analytical balance (Digital scale)
2. ATR-FTIR spectrometer (Nicolet 6700)
3. Cabinet light box (Sumet Labtest, Co., Ltd., Thailand)
4. Centrifuge (Beckman coulter, Avanti tm J-301)
5. ColorQuest XE Spectrophotometer (Hunter Lab, USA)
6. Crock meter (Sumet Labtest, Co., Ltd., Thailand)
7. Dyeing machine in laboratory
8. FTIR spectrometer (Nicolet Impact 410)
9. Grey scale for assessing change: SDC Standard method ISO 105-A03:1993 (Sumet Labtest, Co., Ltd., Thailand)
10. Grey scale for assessing staining: SDC Standard methods ISO 105-A02:1993 (Sumet Labtest, Co., Ltd., Thailand)
11. Hot-plated magnetic stirrer (Coning)
12. Light Fastness testor (Solarbox 1500C)
13. Magnetic stirrer / Magnetic bars
14. Oven (memmert, Sumet Labtest, Co., Ltd.,Thailand)
15. pH meter (Cyberscan 510)
16. Scanning electron microscope (JSM-5410LV JEOL, Japan)
17. Tecator instrument (Kjeltech KD-02, Sweden)
18. Test device, for colorfastness to water and perspiration (Sumet Labtest, Co., Ltd., Thailand)
19. Ubbelohde viscometric tube
20. UV-Vis Spectrophotometer (Jasco V-570)

2.2 Materials and chemicals

1. Blue wool standard No.1 – No.8 (Sumet Labtest, Co., Ltd., Thailand)
2. Chitosan M_v~ 50,000 , % DD ≈ 84 (A.N.Lab, Co., Ltd., Thailand)
3. Cotton rubbing cloth (Sumet Labtest, Co., Ltd., Thailand)
4. 2,4-dinitrophenylhydrazine, analar grade (Fluka, Switzerland)
5. Ethanol, analar grade (Merck, Germany)

6. Glacial acetic acid, analar grade (Merck, Germany)
7. Knitted cotton fabrics (Vission Tex, Co.,Ltd., Thailand)
8. Hydrogen peroxide 50% w/w, commercial grade (L.C.Co.,Ltd., Thailand)
9. Multifiber adjacent fabric, D.W. type (Sumet Labtest, Co., Ltd., Thailand)
10. Multinal H/C (Winimex, Taiwan)
11. Perlavin NEP (Augustchem, Thailand)
12. Polyphos T (V.P.C., Thailand)
13. Potassium periodate, analar grade (Fluka, Switzerland)
14. Potassium polyvinyl sulfate solution (PVSK), analar grade (Wako, Japan)
15. Procion Blue H-ERD dyes, C.I. Reactive Blue 160 (Everlight, Taiwan)
16. Pyridinium chloride (CPC), analar grade (Nacalai tesque, Japan)
17. Remazol Red RB133 dyes, C.I. Reactive Red 198 (Dystar,Thailand)
18. Sodium borohydride, analar grade (Labchem, Australia)
19. Sodium carbonate, commercial grade (Tai-Laing chemical, China)
20. Sodium chloride, commercial grade (KC Salt International, Thailand)
21. Sodium chloride, anal grade (Fluka, Switzerland)
22. Sodium hydroxide, analar grade (Merck, Germany)
23. Sulfuric acid, analar grade (Merck, Germany)
24. Toluidine blue indicator solution, analar grade (Wako, Japan)

2.3 Characterization of chitosan

2.3.1 Molecular weight determination of chitosan by viscometry

Dried chitosan sample (ca, 25 mg or depending on molecular weight of chitosan) was weighed accurately in a 50 mL volumetric flask, dissolved in an aqueous acetic solution (1 M, 5 mL) and water (30 mL), and stirred with a magnetic stirrer overnight. An aqueous NaCl solution (1 M, 10 mL) was added into the chitosan solution and stirred overnight, then the total volume was made up to the mark with distilled water to obtain C_1 solution. A portion of C_1 solution (10 mL) was taken and diluted with a premixed solvent (0.2 M NaCl/0.1 M AcOH, 10 mL) to make C_4 solution.

The solvent (0.2 M NaCl/0.1 M AcOH, 10 mL) was transferred into a Ubbelohde tube (**Figure 2.1**). The Ubbelohde tube was placed into a thermostated water bath with the temperature maintained at 25 °C. The liquid was pushed from tube

L to over level (a) of tube N by a rubber bulb. The rubber bulb was removed to allow the solvent fall through level (b). The falling time between level (a) and (b) was measured by a stopwatch in triplicate to give an average t_o.

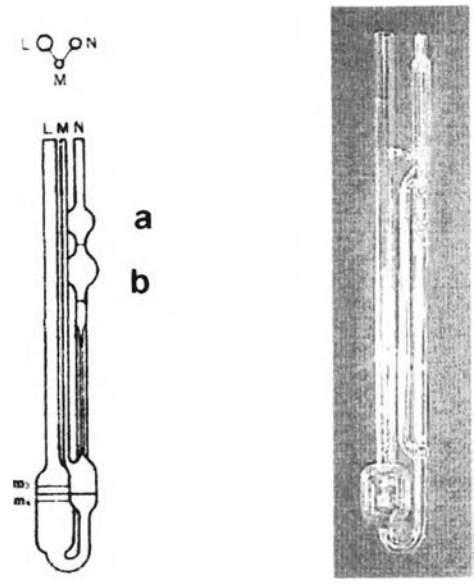

Figure 2.1 Ubbelohde tube.

The Ubbelohde tube was emptied and was rinsed with C_1 chitosan solution (2 X 3 mL) prior to being filled with C_1 chitosan solution (10 mL). The falling time (t_1) of C_1 chitosan solution was measured in triplicate. The solvent (0.2 M NaCl/0.1 M AcOH, 2 mL) was added through tube L and mixed thoroughly to give C_2. The falling time (t_2) was measured again. The solvent (0.2 M NaCl/0.1 M AcOH, 2 mL) was added again to give C_3 and the falling time was measured. The Ubbelohde tube was emptied and rinsed with the solvent (15 mL), water (15 mL) and C_4 chitosan solution (2 X 3 mL). The solution C_4 (10 mL) was filled into the Ubbelohde tube. The falling time was measured as discribed aboved. The solution was diluted to C_5 and C_6 and the falling time was measured according to the procedure described for C_2 and C_3.

A linear plot of η_{sp}/C in Y-axis against C in X-axis was obtained using the linear least square. The intrinsic viscosity $[\eta]$ was obtained from the Y-intercept. The viscosity-averaged molecular weight can be calculated as follows:

$$[\eta] = KM_v^a \quad (K = 1.8 \times 10^{-3}, a = 0.93 \text{ at } 25\ ^\circ C)$$

$$\eta_{sp} = t - t_o / t_o$$

2.3.2 Determination of the degree of deacetylation

Potassium polyvinyl sulfate solution (PVSK) was standardized with a freshly prepared standard solution of cetyl pyridinium chloride (CPC, 0.5% w/v) in aqueous acetic acid solution (0.1 M). The standard CPC solution (5 mL) was placed in a 25 mL beaker, three drops of toluidine blue (0.1% w/v) were added as an indicator, and the mixture was continuously stirred with a magnetic bar. The PVSK solution was added titrimetrically from a burette until the blue color of the indicator changed into reddish purple. The titration was repeated three times. The solvent, 0.1 M aqueous acetic acid solution, was used in place of the standard CPC solution for the blank titration.

The concentration of PVSK solution was calculated according to the following equation:

$$N = 50\ C'/358D$$

Where N = equivalent/L

C' = concentration of CPC (%w/v)

D = difference between CPC and blank titration volumes (mL)

Dried chitosan (~8 mg) was weighed precisely in a 25 mL volumetric flask and the volume was made up to the mark with an aqueous acetic acid solution (0.1 M). The solution (5 mL) was pipetted and placed in a 25 mL beaker and titrated as described above.

For the determination of the degree of *N*-acetylation (%DA):

$$\%DA = 100 \times (50C \times 161ND)/(42ND + 50D)$$

Where: N = concentration of PVSK (aquiv./L)

D = difference between chitosan and blank titration volumes (mL)

C = concentration of chitosan (%)

For the determination of the degree of deacetylation (%DD):

$$\%DD = 100 - DA\ (\%)$$

2.4 Treatment of cotton fabric

2.4.1 Oxidation of cotton fabric with potassium periodate

Three pieces of bleached knitted cotton fabrics (0.5 g, 4X5 cm^2 unless specified otherwise) were immersed in an aqueous solution of potassium periodate (0.031 M, 100 mL) at pH 5 (See **Table B1** in Appendix B for recipe of bleached fabric). The solution was shaken at 55 °C for designated period. The cotton fabric samples were washed repeatedly with copious amount of water (10X100 mL) and squeezed. The oxidized fabrics were used for the next reaction without further drying.

2.4.2 Reductive amination with chitosan

A chitosan solution was prepared by dissolving chitosan (1.0 g, 6.2 mmole) in 1% aqueous acetic acid solution (30 mL) at 60 °C. The oxidized fabric samples(0.5 g, 4 X 5 cm^2 unless specified otherwise) were immersed in a chitosan solution (30 min, 1%AcOH, 30 mL) under constant shaking at 60 °C for 2 h and then washed repeatedly with water (10X100 mL) to remove unreacted chitosan. The iminic bonds between the aldehyde group of the oxidized cellulose and amino group of chitosan was reduced by sodium borohydride solution (0.2 M, 30 mL) at room temperature under constant shaking for 2 h. The fabrics were washed with copious amounted water (10X100 mL) to remove excess reductant. The chitosan treated fabrics were allowed to completely dry in the air prior to undergoing the dyeing process.

2.4.3 Dyeing process

The dyeings were carried out in a laboratory dyeing machine with a temperature control unit and 12 shaking units at a liquor ratio of 60:1. The samples of fabrics were dyed at 2% or 4% owf (on weight fabric) with Evercion Blue H-ERD dye and Remazol Red RB133 dye in the presence of varied amount of NaCl (the exact amount used is specified in the results). The dyebath was prepared by dissolving the dye and NaCl in distilled water. The fabrics were added to the dye bath at room temperature. The temperature was raised from room temperature to 80 °C for Evercion Blue H-ERD dye, or 60 °C for Remazol Red RB133 dye and held for 10 min. Sodium carbonate (5 g/L) and sodium hydroxide (0.8 g/L) (2.5 g/L for Remazol Red RB133 dye) were then added and the temperature was held for additional 45 min.

The dyed fabrics were rinsed thoroughly in a soaped solution (1 g/L anionic surfactant Multinal H/C) at 100 °C for 20 min at a liquor ratio of 60:1. The fabrics were rinsed thoroughly with copious amount of water (10X100 mL) and air dried.

2.5 Characterization of fabrics

2.5.1 Determination of the oxidized fabric with 2,4-dinitrophenylhydrazine (2,4-DNP)

Dissolve 3 g of 2,4-dinitrophenylhydrazine in 15 mL of concentrated sulfuric acid. This solution is then added, with stirring, to 20 mL of water and 70 mL of 95% ethanol. This solution is mixed thoroughly and filtered. Fabric samples were immersed into 2,4-dinitrophenylhydrazine reagent about 30 sec. Fabric samples were taken to compare the color of each piece after drying at room temperature for 5 min.

2.5.2 Determination chitosan content

The chitosan contents in the fabrics were determined from the nitrogen percentages. The nitrogen percentages in the fabrics were analyzed by Kjeldahl nitrogen analysis method (Tecator instrument). Samples of fabrics (0.2 g) were suspended in concentrated sulfuric acid (15 mL) and then digested for 150 minutes at 350 °C and the suspension was allowed to cool to room temperature. Distilled water (~80 mL) and excess sodium hydroxide (40 %w/v) was added until the suspension was basic (pH~14). The mixture was heated under reflux until the solution distilled into a receiving flask containing boric acid (4%, 20 mL) and 4-5 drops of the indicator (0.2% bromocresol green in ethanol 0.2% methyl red in ethanol at 5/1 v/v. The mixture in the receiving flask had blue color. A standardized solution of HCl (0.1 N) was added titrimetrically from a burette until the blue color changed into red color. The titration was performed in triplicate. The blank titration was performed with the same procedure but without the fabric sample. The analysis was done twice on different pieces of samples to obtain an average value.

The nitrogen percentage (%N) was calculated from the following equation; $\%N = 1.4007[HCl]\ (V - V_{blank})/W$ where [HCl] is the molar concentration of HCl, V is the sample titration volumes (mL), V_{blank} is the blank titration volumes (mL) and W is the weight of fabric sample (g).

The chitosan content was calculated from the nitrogen percentage using the following equation; chitosan content = $162(N-N_b)/14$ where N is the nitrogen percentage, N_b is the nitrogen percentage of bleached fabric used as a blank.

2.5.3 Color measurement

Dye uptake was studied by measuring %exhaustion of four types of fabrics in solution of Evercion Blue H-ERD dye and Remazol Red RB133 dye. The absorbances of the solution at 615 nm (Evercion Blue H-ERD dye) and 516 nm (Remazol Red RB133 dye) were measured and %exhaustion were calculated from $100(A_0-A)/A_0$ where A_0 is the absorbance before dyeing (in presence of sodium chloride) and A is the absorbance after dyeing. The dyed fabric samples were measured the color yield (K/S) calculated from the reflectance (R) using the following equation: $K/S = (1-R)^2/2R$. The reflectance was measured on a ColorQuest XE Spectrophotometer.

2.5.4 Study of microstructure of fabrics by SEM

Scanning electron microscope (SEM) was used to study the particle and surface morphology of chitosan coated cotton fabrics. The samples of fabrics were coated with gold by sputtering at room temperature. Scanning electron micrographs of fabrics were taken by scanning electron microscope (JSM-5410LV). The instrument was operated at 15 kv.

2.6 Colorfastness testing [30]

Colorfastness tests showed ability of reactive dyes fixed on fabrics were the important requirement which normally test according to ISO standard test. These tests including colorfastness to washing, colorfastness to water, colorfastness to perspiration, colorfastness to rubbing, and colorfastness to light. The test results were expressed in grey scale terms instead of the k/S value. According to the standard grey scale for color change and grey scale for color staining [31], [32].

2.6.1 Sample preparation

Fabric was usually tested in the form of a composite specimen (10X 4 cm.), made up of the test specimen placed in contact with undyed fabric, usually in the form of multifiber strip, of the same size. The purpose of the undyed fabric is to

measure the staining effect of any dye that has been lost from the test fabric. Multifiber fabrics DW was used in this work included acetate, cotton, polyamide, polyester, acrylic and wool. [33]

2.6.2 Colorfastness to washing (ISO 105-C06 B1M: 1994) [34]

A specimen of the fabric was put in contact with multifiber adjacent fabric. The specimen was treated in an aqueous solution containing 4 g/l of ECE detergent at 50 °C for 45 minutes and 50 steel balls. After that, the colorfastness property of the tested sample was evaluated by eyes using the grey scale for color change and grey scale for color staining.

2.6.3 Colorfastness to water (ISO 105-E01: 1994) [35]

The specimen of the fabric in contact with multifiber adjacent fabric was immersed in distilled water, drained and placed between two places under a pressure of 12.5 kPa. Place the test device containing the composite specimen in the oven for 4 hours at 37 °C ± 2 °C. The specimen and the multifiber adjacent fabric were dried by hanging it in the air at temperature not exceeding 60 °C. The change in color of the specimen and the staining of the multifiber adjacent fabric were evaluated by eyes with the grey scales. A test device, an example of which was consists of a stainless steel frame constructed to hold a number of glass or acrylic plates each measuring 60 mm. x 115 mm. The samples of side 40X100 mm. are each placed separately between a pair of these plates in order to keep them moist. A mass of 5 kg was then placed on top of the apparatus so as to apply a pressure of 12.5 kPa to each specimen. The perspiration tester was so constructed that when the mass was removed the specimens remain under pressure.

2.6.4 Colorfastness to perspiration (ISO 105-E04: 1994) [36]

A specimen of the fabric in contact with multifiber adjacent fabric were treated in acid solution and alkaline solution containing histidine, drained and placed between two plates under pressure of 12.5 kPa. Place the test device containing the composite specimen in the oven for 4 h at 37 °C ± 2 °C. The specimens and the multifiber adjacent fabrics were dried separately. After that, the colorfastness property of the tested sample was evaluated by eyes using the grey scale for color change and

grey scale for color staining. Alkaline solution was freshly prepared from 0.5 g histidine monohydrochloride monohydrate, 5 g sodium chloride and 2.5 g disodium hydrogen phosphate dihydrate (Na_2HPO_4 $2H_2O$) per liter of water, brought to pH 8.0 with 0.1 M sodium hydroxide. Acid solution was freshly prepared from containing 0.5 g histidine monolydrochloride monohydrate, 5 g sodium chloride and 2.2 g sodium dihydrogen phosphate dihydrate (NaH_2PO_4 $2H_2O$) per liter of water, brought to pH 5.5 with 0.1 M sodium hydroxide.

Thoroughly wet one composite specimen in the alkaline solution at pH 8 at a liquor ratio of 50:1 and allow it to remain in the solution at room temperature for 30 min. Wipe excess liquid off the specimen between two glass rods and place the specimen between two plates of the perspiration tester, with a pressure of 12.5 kPa. Repeat with the other composite specimen in the acid solution using a separate perspiration tester. Place the perspiration tester in an oven at 37 °C ± 2 °C for 4 h and then removed specimen, open out allow to dry by hanging it in the air at temperature not exceeding 60 °C. Both specimens were then assessed for color change of the tested fabric and staining of the multifiber fabric.

2.6.5 Colorfastness to rubbing (ISO 105-X12: 2001) [37]

The specimens of the fabric were rubbed with a dry rubbing cloth and with a wet rubbing cloth. For dry rubbing, With the dry rubbing cloth flat in place over the end of the finger of crock meter rub to end fro in a straight line along a track 10 cm long on the dry specimen, 10 times to and fro in 10 seconds with a drawn ward force of a Newton. For wet rubbing, repeat the test described in dry rubbing with a fresh dry specimen and with a rubbing cloth that has been wetted with water, using take-up of about 95-100%. After rubbing, dry the cloth at room temperature. The staining of the rubbing cloths is evaluated by eyes with the grey scale.

2.6.6 Colorfastness to light (ISO 105-B02: 1994) [38]

This test measures the resistance to fading of dyed textiles when exposed to daylight. The test was of importance to the dyestuff manufacturer, the dyer and the retailer. The xenon arc light was used for this test. The xenon arc was a much more intense source of light which has a very similar spectral content to that of daylight so that the test is speeded up considerably. Because of the large amount of heat generated by the lamp an efficient heat filter to be placed between the lamp and

specimen and the temperature was monitored. This was in addition to a glass filter as above to remove the ultra-violet radiation.

The essence of the test was to expose the sample under test to the light source together with eight blue wool reference standards. The sample and blue wool standards were partly covered so that some of the material fades and some was left unfaded. A rating was given to the sample which was the number of the reference standard which shows a similar visual contrast between exposed and unexposed portions as the specimen. This means that the specimen will be given a grade between one (poor light fastness) and eight (highly resistant to fading). If the result was in between two blue wool standards, it is given as for example 3 - 4.

The sample under test and a set of blue wool reference standards were arranged on a suitable backing. The middle third of the strips was covered with opaque card (A). The assembly was then exposed to light until the specimen just shows a change in shade (4 – 5 on the grey scale). The number of the standard showing a similar change is noted. The exposure was continued until the contrast in the specimen was equal to grey scale 4, at which point a second segment of the specimen and standard was covered with another pieced of opaque card (B). The exposure is again continued until the contrast between the exposed and unexposed parts of the specimen is equal to grey scale 3, at which point the exposure was terminated. When the cards are removed the specimen and standards will show two areas that have been exposed for different lengths of time together with an unexposed area. The specimen was given the rating of the standard which shows similar changes. If the exposed areas have different ratings then the overall rating was the mean of the two ratings.

2.7 Determination of dye uptake in exhaustion step

Tested sample consist of chitosan powder, BF, RCOF, RCBF. Dye uptake was studied by measuring %exhaustion of various amounts of tested samples in a solution of dye (0.007%w/v) in the absence and presence of sodium chloride salt (1.225 g/L) under constant shaking at room temperature (30 ^{o}C). After 1 h, the absorbance of the solution at 615 nm (for Evercion Blue H-ERD dye) or 516 nm (for Remazol Red RB 133 dye) was measured and %exhaustion was calculated from $100(A_0-A)/A_0$ where A_0 is the initial absorbance and A is the absorbance after 1 h. The effect of salt

concentration on dye uptake was also investigated by varying the concentration of the sodium chloride salt. Dye uptake of chitosan powder was also studied in comparison to the cotton fabrics using similar procedure.