CHAPTER 3

EXPERIMENTAL METHODS AND RESULTS FOR CHITOSAN CHARACTERISATION

3.1 Chemicals, Apparatus and Instrument

3.1.1 Chemicals

The various chemicals used in this research project were as shown in Table 3.1.

Table 3.1: Chemicals used in this research project.

CHEMICALS	USAGE	GRADE	SUPPLIER
Acetic acid, glacial	Solvent	99.8 %	BDH Chemicals Ltd.
Chitosan	Polymer	Commercial	Seafresh Chitosan (Lab) Co., Ltd.
Epichlorohydrin	Crosslinking agent	98%	BDH Chemicals Ltd.
Ethanol, absolute	Dehydrating agent	AR Grade	Merck
Glycerol	Plasticiser	Lab reagent	USP
Hydrochloric acid	Degree of deacetylation	AR Grade	Merck
Sodium chloride	Solvent component	AR Grade	Fluka
Sodium hydroxide	Coagulating bath	Lab reagent	Eka Nobel
Urea	Solvent component	AR Grade	Merck

3.1.2 Apparatus and Instruments

The main items of apparatus and instruments used were as given in Table 3.2.

Table 3.2: Apparatus and instruments used in this research project.

APPARATUS AND INSTRUMENTS	COMPANY	MODEL
^{13}C-NMR Spectrometer	Bruker	DPX 300
Falling Ball Viscometer	Haake	Hoppler design
FT-IR Spectrometer	Nicolet	510
Universal Mechanical Testing Machine	Lloyds	LRX+
Automatic Viscosity Measuring System	Schott Gerate	AVS 300
Scanning Electron Microscope	Jeol	JSM-5410
Small-Scale Wet Spinning Apparatus	Bradford University Research Ltd.	-
Vacuum Oven	Eyela	VOS-300SD
Vacuum Oven	Lab-line Instruments	3620-1
X-ray Diffractometer	Jeol	JDX 300

3.2 Characterisation of Chitosan

3.2.1 Chitosan Raw Material

The chitosan raw material used in this project was a commercial product supplied by Seafresh Co., Ltd. (Thailand) in the form of a pale cream-coloured powder. This chitosan product had itself been prepared from chitin (mainly α-chitin) which had been extracted from shrimp waste. The chitosan raw material was used as supplied without further purification.

3.2.2 Infrared Spectroscopy [28]

Infrared spectroscopy (IR) can be used to characterise polymeric materials. When molecular vibrations result in a change in the bond dipole moment, as a consequence of changes in the electron distribution in the bond, it is possible to simulate transitions between energy levels by interaction with electromagnetic radiation of the appropriate frequency. The information obtainable by IR analysis of a polymer enables identification of the major components of the chain structure through their characteristic absorptions. The energies of molecular vibrations for analytical work are expressed conventionally in terms of wavenumber (cm^{-1}).

The infrared spectra of naturally occurring chitin and chitosan have been published by many authors [4, 6]. The most significant peaks in the spectra are those relating to the amide bands at 1665, 1555 and 1313 cm^{-1}, all of which show perpendicular dichroism and which are respectively assigned to C=O stretching, N-H deformation in the CONH plane, and to C-N bending (see Figure 3.1).

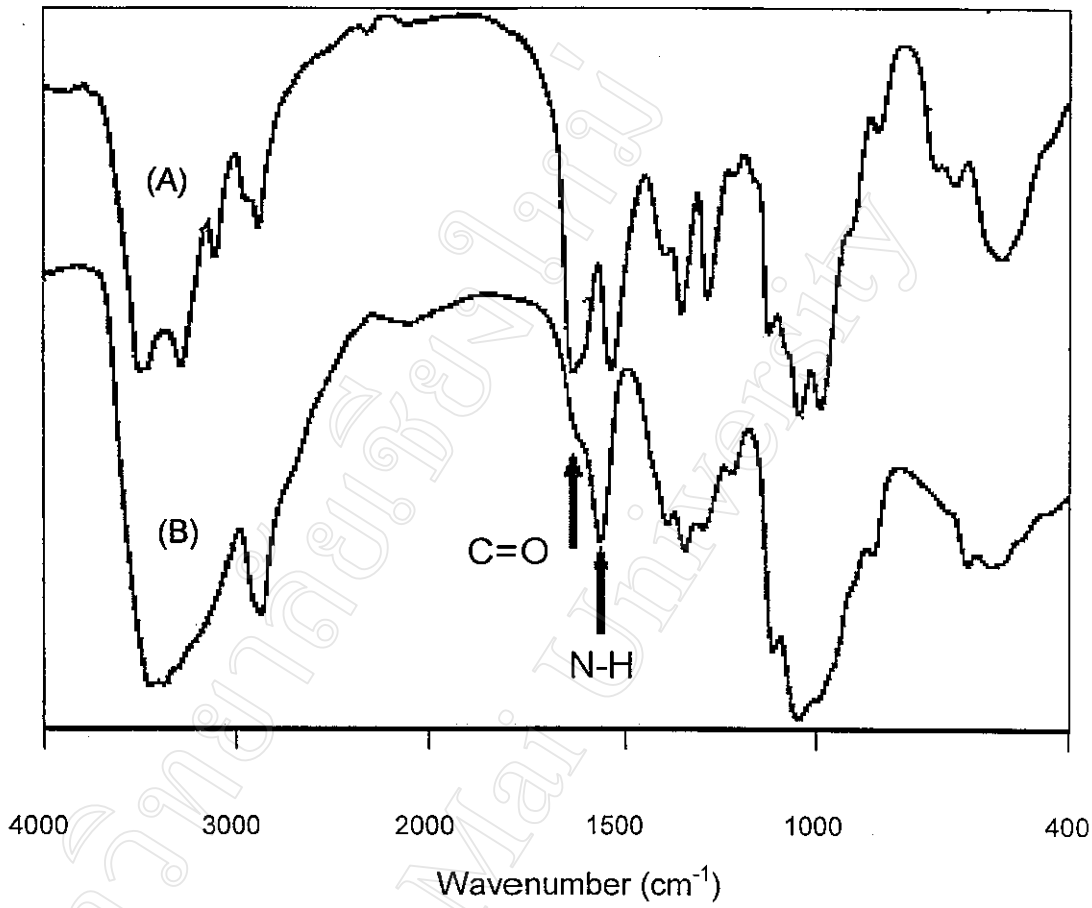


Figure 3.1: Reference IR spectra of (A) chitin and (B) chitosan [29].

As shown in Figure 3.1, the chitosan spectrum differs from that of chitin in that the new amine N-H band at 1590 cm^{-1} predominates over the C=O band at 1665 cm^{-1} while the amide N-H band at 1555 cm^{-1} is absent. These are the most important distinguishing features for practical purposes. The relative intensities of the C=O and amine N-H bands in the chitosan spectrum are dependent on the polymer's degree of deacetylation.

Table 3.3 compares the bond frequencies referred to in the literature and obtained in this research project.

In this research project, a Nicolet Fourier-Transform Infrared Spectrometer was used to record the IR spectrum of the chitosan used. The spectrum, as shown in Figure 3.2, was obtained from a sample prepared in the form of a KBr disc and is seen to be similar to the reference spectrum (B) of chitosan in Figure 3.1 above. The main band frequencies (cm^{-1}) are compared with corresponding data [29] in Table 3.3.

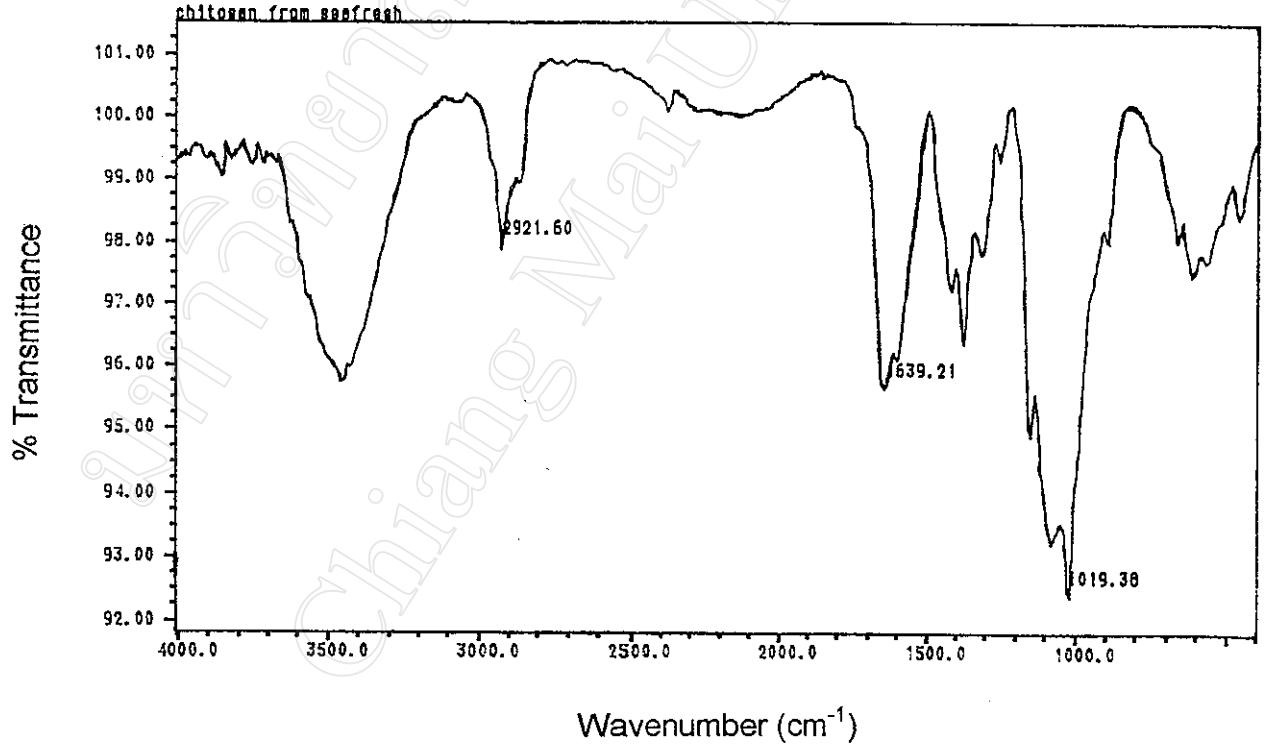
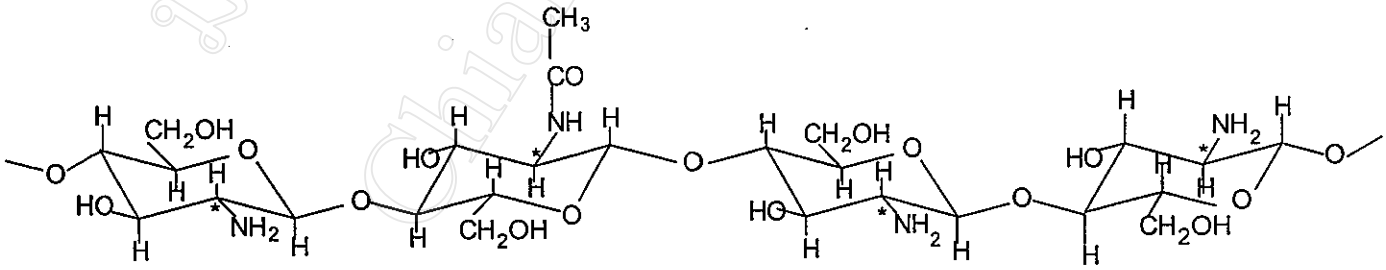


Figure 3.2: IR spectrum of the chitosan (Seafresh) raw material used.

Table 3.3: Vibrational assignments and band frequencies in the infrared spectrum of chitosan.

Vibrational Assignments	Infrared Band Frequencies (cm^{-1})	
	Literature [6]	This Work
N-H,O-H stretching (overlapping)	3400-3600	3500
C-H stretching	2878	2922
C=O stretching (residual)	1665	1648
N-H bending (amine)	1590	1639
C-H bending	1380	1389
C-N bending	1313	1300
C-O bending	1030	1019

CHITOSAN



3.2.3 Moisture Content Determination

The moisture content of the chitosan raw material was determined by drying at 100°C in a heated vacuum desiccator. Weighings were made every 12 hours for a total of 48 hours by which time the weight was judged to have reached a constant level. A total of 3 determinations were carried out. The weight data together with the calculated moisture contents (%) are given in Tables 3.4-3.6.

From the results in Tables 3.4-3.6, the moisture content of the chitosan raw material is seen to be in the range of 7.4-8.0% by weight. This is high for a polymer and is an indication of the hygroscopic nature of chitosan. When left in contact with air, polymers absorb and retain an ***equilibrium moisture content*** which depends primarily on the polymer's own chemical structure and the relative humidity of the environment in which it is stored. Consequently, in any analytical procedure in which the weight of the pure polymer needs to be accurately known, or in which moisture impurities need to be stringently excluded, the polymer should be dried to constant weight beforehand. This applied to the molecular weight determinations described in the following section 3.2.4.

Table 3.4: Weight data and calculated moisture contents of the chitosan raw material. (Run No. 1)

RUN No. 1	Time (hrs)				
	0	12	24	36	48
Chitosan Weight (g)	1.0052	0.9256	0.9303	0.9303	0.9300
Weight Loss (g)	-	0.0796	0.0749	0.0749	0.0752
Moisture Content (%)	-	7.92	7.45	7.45	7.48

Table 3.5: Weight data and calculated moisture contents of the chitosan raw material. (Run No. 2)

RUN No. 2	Time (hrs)				
	0	12	24	36	48
Chitosan Weight (g)	1.0038	0.9256	0.9269	0.9271	0.9266
Weight Loss (g)	-	0.0782	0.0769	0.0767	0.0772
Moisture Content (%)	-	7.80	7.66	7.64	7.69

Table 3.6: Weight data and calculated moisture contents of the chitosan raw material. (Run No. 3)

RUN No. 3	Time (hrs)				
	0	12	24	36	48
Chitosan Weight (g)	1.0034	0.9243	0.9271	0.9268	0.9260
Weight Loss (g)	-	0.0790	0.0790	0.0766	0.0774
Moisture Content (%)	-	7.88	7.60	7.63	7.71

3.2.4 Molecular Weight Determination

3.2.4.1 Principles and Theory of Dilute-Solution Viscometry [25, 30-31]

Measurements of dilute-solution viscosity provide one of the simplest and most widely used techniques for routinely determining polymer molecular weights. However, it is not an absolute method and each polymer-solvent-temperature system must first be calibrated by an absolute molecular weight determination (e.g., by osmometry or light scattering) run on fractionated polymer samples. Dilute-solution viscosities are usually measured at concentrations of up to 1 g per 100 ml of solvent (1% w/v) by determining the ***flow-time*** of a certain volume of solution through a capillary of fixed length. Two typical viscometers are shown in Figure 3.3.

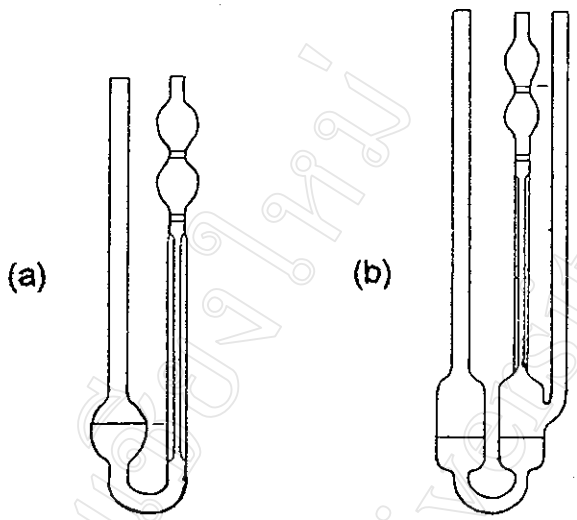


Figure 3.3: Viscometers commonly used in dilute-solution viscometry.
(a) Ostwald viscometer
(b) Ubbelohde viscometer

In dilute-solution viscometry, the following viscosity-related quantities are defined:

(i) the ***relative viscosity*** or the ***viscosity ratio*** (η_{rel}) is given by the ratio of the solution viscosity, η, to the solvent viscosity, η_o, which, in turn, is given by the ratio of the flow-time for the solution (t) to the flow-time for the solvent (t_o)

$$\eta_{rel} = \frac{\eta}{\eta_o} = \frac{t}{t_o} \qquad (3.1)$$

(ii) the ***specific viscosity*** (η_{sp}) is the relative increment in viscosity of the solution over the viscosity of the solvent

$$\eta_{sp} = \frac{\eta - \eta_o}{\eta_o} = \frac{t - t_o}{t_o} = \eta_{rel} - 1 \qquad (3.2)$$

(iii) the ***reduced viscosity*** (η_{red}) is the specific viscosity per unit concentration (c) where the units of c are usually g/dl (g/100ml)

$$\eta_{red} = \frac{\eta_{sp}}{c} \qquad (3.3)$$

(iv) the ***inherent viscosity*** (η_{inh}) is defined as

$$\eta_{inh} = \frac{\ln \eta_{rel}}{c} \qquad (3.4)$$

(v) the ***intrinsic viscosity*** $[\eta]$ is the reduced viscosity or the inherent viscosity extrapolated to infinite dilution (c=0)

$$[\eta] = \lim_{c=0} \eta_{sp}/c \qquad (3.5)$$

$$[\eta] = \lim_{c=0} (\ln \eta_{rel})/c \qquad (3.6)$$

All of these viscosity terms are collected together in Table 3.7. The intrinsic viscosity, $[\eta]$, is a function of the size of the polymer molecules in solution, the polymer-solvent system, and the temperature. Therefore, if flow-time measurements are made at a constant temperature using a specific solvent for a particular polymer, $[\eta]$ should be related to the polymer's molecular weight.

Huggins proposed the following relationship between reduced viscosity and concentration for dilute polymer solutions:

$$\eta_{red} = \frac{\eta_{sp}}{c} = [\eta] + k'[\eta]^2 c \qquad \text{(Huggins Equation)} \quad (3.7)$$

The Huggins Constant, k′, turns out to be equal to 0.3 - 0.5 for a wide variety of polymer-solvent systems. A similar equation to the Huggins Equation is the Kraemer Equation:

$$\eta_{inh} = \frac{\ln \eta_{rel}}{c} = [\eta] + k''[\eta]^2 c \qquad \text{(Kraemer Equation) (3.8)}$$

Table 3.7: Definitions and nomenclature of dilute-solution viscosity quantities [25].

Common Name	**Official Name***	**Quantity**
Viscosity	Viscosity coefficient	η
Relative viscosity	Viscosity ratio	$\eta_{rel} = \frac{\eta}{\eta_o} = \frac{t}{t_o}$
Specific viscosity	Specific viscosity	$\eta_{sp} = \eta_{rel} - 1$
Reduced viscosity	Viscosity number	$\eta_{red} = \frac{\eta_{sp}}{c}$
Inherent viscosity	Logarithmic viscosity number	$\eta_{inh} = \frac{(\ln \eta_{rel})}{c}$
Intrinsic viscosity	Limiting viscosity number	$[\eta] = \lim_{c=0} \eta_{sp}/c$
Intrinsic viscosity	Limiting viscosity number	$[\eta] = \lim_{c=0} (\ln \eta_{rel})/c$

* the **Official Names** are those recommended by the International Union on Pure and Applied Chemistry (IUPAC)

In these Huggins and Kraemer Equations, c is the concentration of the polymer in solution (g/dl), while k′ and k″ are constants for a given polymer in a given solvent at a given temperature and are themselves related by the equation:

$$k' - k'' = 0.5 \qquad (3.9)$$

Thus, the two equations (3.7) and (3.8) should yield linear plots against concentration, c, with their common intercept equal to [η] at c=0, as shown in Figure 3.4. The double extrapolation facilitates the accurate estimation of [η].

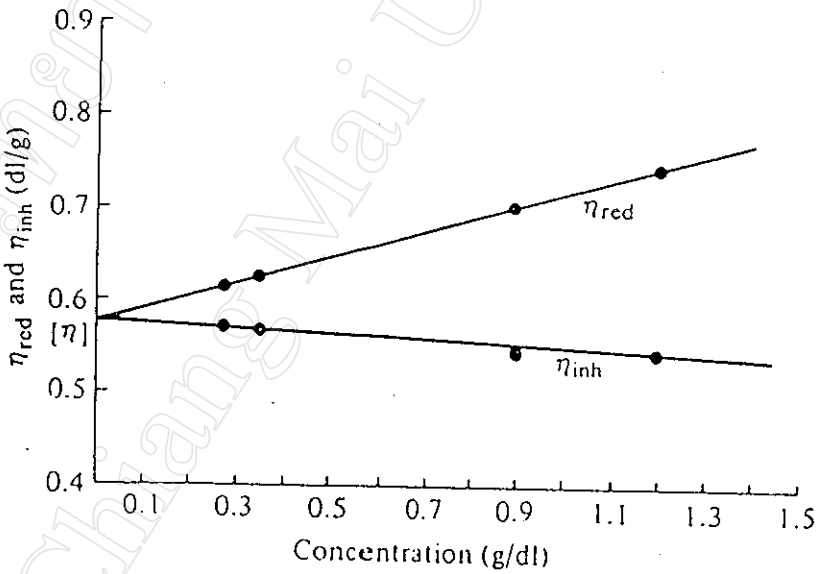


Figure 3.4: Reduced and inherent viscosity-concentration plots for a typical polymer sample [25].

The intrinsic viscosity, [η], of a polymer in solution is related to its molecular weight by the empirical Mark-Houwink-Sakurada Equation:

$$[\eta] = K\bar{M}_v^a \qquad (3.10)$$

where:

K and a are constants for a polymer-solvent pair at a given temperature and are usually obtained from the "Polymer Handbook" [32].

$\bar{M}_v$ is the so-called ***viscosity-average molecular weight***

The exponent 'a' in the Mark-Houwink-Sakurada Equation has a value within the range of $0.5 \leq a \leq 1.0$ and has been used as a molecular conformation parameter. When $a > 0.8$, the polymer molecule has an extended rod shape in solution; for $a = 0.6$-0.8 (the most common value), the molecule is shaped like a random coil; for $a < 0.6$, the molecule is a very compact globular sphere.

In this research project, 0.2 M acetic acid / 0.1 M NaCl / 4 M urea was used as the solvent for chitosan at 25°C for dilute-solution viscosity measurements. The values of the Mark-Houwink-Sakurada constants, K and a, in equation (3.10) for this particular system were obtained from reference [5] as:

$$K = 8.93 \times 10^{-4} \text{ dl/g}$$

$$a = 0.71$$

giving the equation

$$[\eta] = 8.93 \times 10^{-4} \bar{M}_v^{0.71} \text{ dl/g} \qquad (3.11)$$

It has been reported that the addition of the 4 M urea to the solvent helps to prevent the reduction in solution viscosity on standing [33]. This reduction in solution viscosity indicates a decrease in chitosan molecular

weight. This occurs as a result of acid-catalysed hydrolysis in solution of the interconnecting ether glucoside bonds between the repeat units in the polymer chain.

3.2.4.2 Experimental Procedure and Results

In this research project, a Ubbelohde viscometer (type 532 03, size 0c) was used in conjunction with the Schott-Gerate AVS 300 Automatic Viscosity Measuring System (Figure 3.5). Good temperature control was essential with variations in solution temperature during the experiment being kept to within 0.1°C. The flow-times were recorded by the instrument automatically and printed out by a data printer.

The Ubbelohde viscometer needed to be scrupulously clean and dry. Using 0.2 M acetic acid / 0.1 M NaCl / 4 M urea as the solvent, a series of chitosan solutions were prepared by successive dilution with concentrations of 0.025, 0.050, 0.100 and 0.200 g/dl. For flow-time measurements, 15 ml of each solution were accurately pipetted into the viscometer. The viscometer was carefully clamped accurately vertical in the constant temperature water-bath at 25 ± 0.1°C and at least 15 minutes allowed for temperature equilibration before flow-time measurements were made. For the pure solvent and each solution, 5 readings were obtained which agreed to within ±0.2% of their average value. A total of 3 separate molecular weight determinations (Run Nos. 1-3) were carried out and the $[\eta]$ and $\overline{M}_v$ results averaged. The results obtained are shown in the following Tables 3.8-3.10 and Figures 3.6-3.8.

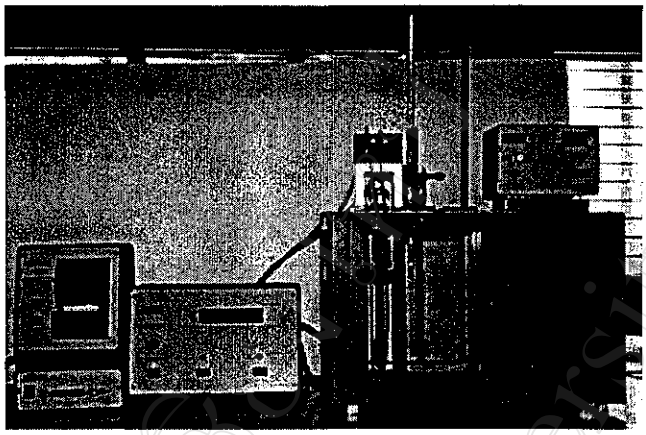


Figure 3.5: The Schott-Gerate AVS 300 Automatic Viscosity Measuring System used in dilute-solution viscometry.

From the viscosity parameters in Table 3.8 for **Run No. 1**, graphs of η_{red} and η_{inh} are plotted against concentration, as shown in Figure 3.6. The common intercept of the graphs at c=0 gives the intrinsic viscosity, $[\eta]$.

Table 3.8: Dilute-solution viscosity data and calculated parameters for chitosan solution at 25.0 ± 0.1°C. (Run No. 1)

Concentration (g/dl)	Flow-time (s)	η_{rel}	η_{sp}	η_{red} (dl/g)	η_{inh} (dl/g)
0	388.8	-	-	-	-
0.025	452.6	1.164	0.164	6.561	6.076
0.050	518.0	1.332	0.332	6.646	5.738
0.100	648.7	1.669	0.669	6.685	5.120
0.200	1062.2	2.732	1.732	8.660	5.025

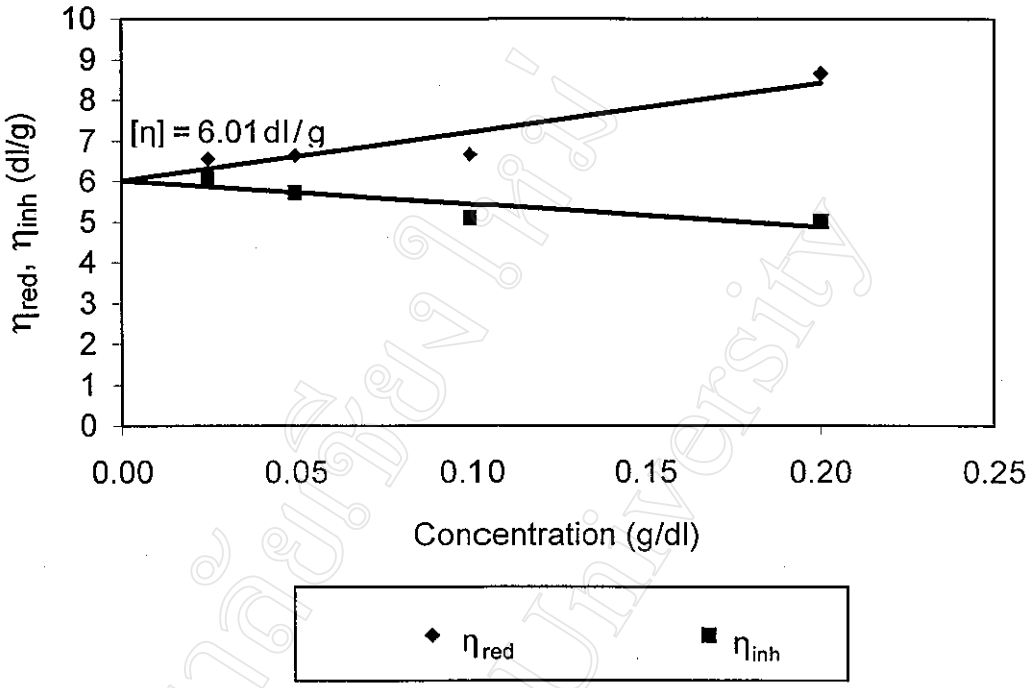


Figure 3.6: Graphs of reduced viscosity, η_{red}, and inherent viscosity, η_{inh}, against concentration. (Run No. 1)

$$[\eta] = (\eta_{red})_{c=0} = (\eta_{inh})_{c=0} = 6.01 \text{ dl/g}$$

From Figure 3.6, the value of $[\eta]$ is estimated to be 6.01 dl/g. Therefore, from

$$[\eta] = K\bar{M}_v^a$$

$$K = 8.93 \times 10^{-4} \text{ dl/g}$$

$$a = 0.71$$

$$[\eta] = 6.01 \text{ dl/g}$$

$$6.01 = 8.93 \times 10^{-4} \bar{M}_v^{0.71}$$

$$\boxed{\bar{M}_v = 2.46 \times 10^5}$$

Table 3.9: Dilute-solution viscosity data and calculated parameters for chitosan solution at 25.0 ± 0.1°C. (Run No. 2)

Concentration (g/dl)	Flow-time (s)	η_{rel}	η_{sp}	η_{red} (dl/g)	η_{inh} (dl/g)
0	388.1	-	-	-	-
0.025	445.0	1.165	0.165	6.595	6.105
0.050	519.8	1.339	0.339	6.788	5.844
0.100	676.5	1.743	0.743	7.432	5.558
0.200	1065.3	2.745	1.745	8.726	5.049

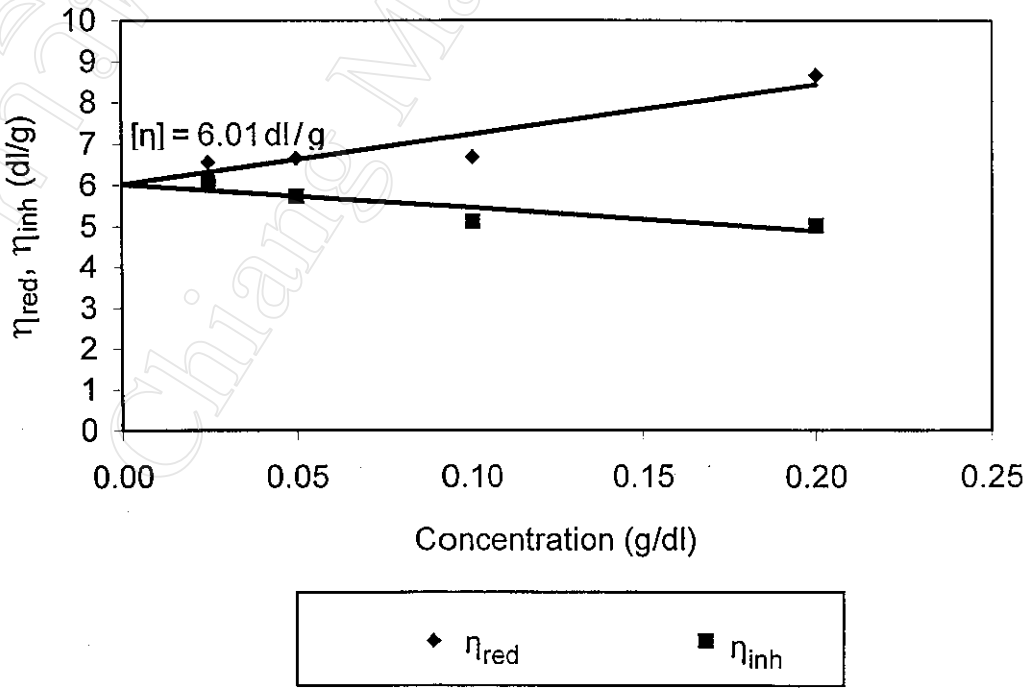


Figure 3.7 Graphs of reduced viscosity, η_{red}, and inherent viscosity, η_{inh}, against concentration. (Run No. 2)

From the viscosity parameters in Table 3.9 and the graphs of η_{red} and η_{inh} against concentration in Figure 3.7, the common intercept of the graphs again give the intrinsic viscosity, $[\eta]$, as 6.01 dl/g and therefore the same value of $\bar{M}_v$ as:

$$[\eta] = K\bar{M}_v^a$$

$$K = 8.93 \times 10^{-4} \text{ dl/g}$$

$$a = 0.71$$

$$[\eta] = 6.01 \text{ dl/g}$$

$$6.01 = 8.93 \times 10^{-4} \bar{M}_v^{0.71}$$

$$\boxed{\bar{M}_v = 2.46 \times 10^5}$$

Table 3.10: Dilute-solution viscosity data and calculated parameters for chitosan solution at 25.0 ± 0.1°C. (Run No. 3)

Concentration (g/dl)	Flow-time (s)	η_{rel}	η_{sp}	η_{red} (dl/g)	η_{inh} (dl/g)
0	388.5	-	-	-	-
0.025	451.3	1.161	0.161	6.457	5.986
0.050	516.7	1.330	0.330	6.599	5.703
0.100	670.3	1.725	0.725	7.251	5.453
0.200	1046.7	2.694	1.694	8.470	4.955

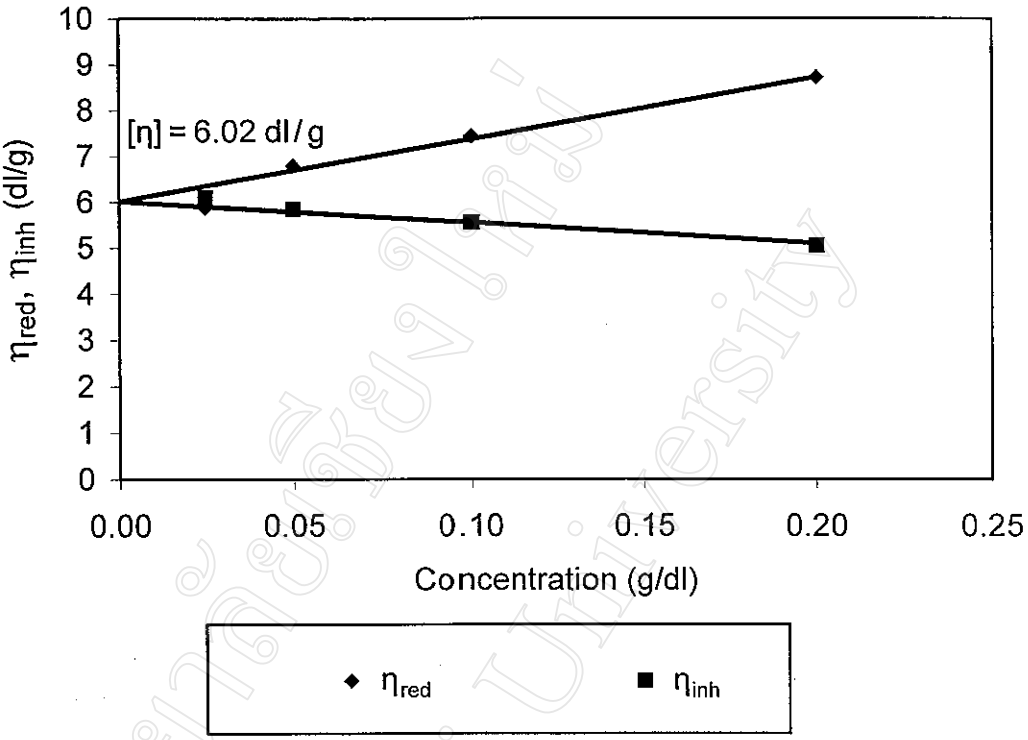


Figure 3.8: Graphs of reduced viscosity, η_{red}, and inherent viscosity, η_{inh}, against concentration. (Run No. 3)

From Table 3.10 and Figure 3.8, the value of $[\eta]$ is estimated as 6.02 dl/g. Therefore:

$$[\eta] = K\bar{M}_v^a$$

$$K = 8.93 \times 10^{-4} \text{ dl/g}$$

$$a = 0.71$$

$$[\eta] = 6.02 \text{ dl/g}$$

$$6.02 = 8.93 \times 10^{-4} \bar{M}_v^{0.71}$$

$$\bar{M}_v = 2.47 \times 10^5$$

3.2.4.3 General Conclusions

From the previous results, the average values of $[\eta]$ and $\overline{M}_v$ for the chitosan raw material used in this research project are calculated as:

$$[\eta] = 6.01 \text{ dl/g}$$
$$\overline{M}_v = 2.46 \times 10^5$$

The high value of $[\eta]$ is a measure of the chitosan's high molecular weight, the stiffness of its main chain, and the strong hydrogen-bonding polymer-solvent interactions in solution. The η_{red} and η_{inh} graphs against c all show good linearity and adherence to the underlying theory and are consistent from Run to Run.

If, for the sake of calculation, we assume that:

$$\overline{M}_n : \overline{M}_v = 1 : [(1+a)\Gamma(1+a)]^{1/a} \qquad (3.12)$$

for a polymer with the *statistically most probable* molecular weight distribution, then we can estimate $\overline{M}_n$ and, therefore, $\overline{DP}_n$, the ***number-average molecular weight*** and ***degree of polymerisation*** respectively. $\Gamma(1+a)$ is the "gamma function" of 1+a as given in the "Handbook of Mathematics" [34].

From $a = 0.71$, $\Gamma(1+a) = \Gamma(1.71) = 0.9106$

$$[(1+a)\Gamma(1+a)]^{1/a} = 1.8658$$

Therefore,

$$\overline{M}_n : \overline{M}_v = 1 : 1.8658$$

$$\boxed{\overline{M}_n = 1.32 \times 10^5}$$

From the molecular formula ($C_6H_{11}NO_4$) of the chitosan repeating unit

repeating unit molecular weight = 161

Thus

$$\overline{DP}_n = \frac{\overline{M}_n}{161} = \frac{1.32 \times 10^5}{161}$$

$$\boxed{\overline{DP}_n \approx 810}$$

From the literature, these $\overline{M}_n$ and $\overline{DP}_n$ values seem to be fairly typical for commercial chitosan made from chitin extracted from shrimp waste.

3.3 Degree of Deacetylation Measurement

3.3.1 Titration Method

3.3.1.1 Experimental Procedure [13]

(a) Preparation of Chitosan Hydrochloride

Approximately 2.5 g of chitosan were weighed out accurately (±0.0001 g) and dissolved with the aid stirring in 100 ml of 10% aqueous acetic acid.

Concentrated hydrochloride acid (HCl) was added dropwise into the viscous solution with continuous stirring until the precipitation of chitosan hydrochloride (CS-HCl) was complete. The white CS-HCl precipitate was then filtered off, washed with isopropanol, and dried in a vacuum oven at 50°C for 24 hours.

(b) Titration of Chitosan Hydrochloride

When completely dry, between 0.5-1.0 g of the CS-HCl was weighed out accurately (±0.0001 g) and dissolved in 100 ml of distilled water in a 100 ml volumetric flask. 10.00 ml of this solution was then titrated with 0.1 M aqueous sodium hydroxide (NaOH) solution using phenolphthalein as indicator. The 0.1 M NaOH was standardized against primary standard grade potassium hydrogen phthalate (KHP), again using phenolphthalein as indicator.

Analysis of the chitosan sample by this titration method was carried out in triplicate and the degree of deacetylation results averaged.

3.3.1.2 Experimental Results and Calculations

The titration results obtained and the calculated degrees of deacetylation (DD), expressed as percentages (%), are given in Table 3.11. The variability in the DD values is to within ±0.6% of the average value of 91.2%.

Table 3.11: Experimental results and calculated degrees of deacetylation (DD).

Analysis No.	CS-HCl Weight (g)	CS-HCl Soln. Vol. (ml)	Concentration of NaOH (M)	Vol. of NaOH (ml)	DD Value (%)
1	0.5428	10.00	0.0999	2.50	90.87
2	0.5155	10.00	0.0998	2.40	91.76
3	0.5400	10.00	0.0999	2.49	90.97
				Average DD =	91.2

Sample Calculation

From the titration results, the values of the degree of deacetylation (DD) in Table 3.11 were calculated as follows:

$$DD = \% \text{ Deacetylation} = (C \times V_1 \times V_2 \times MW \times 100) / 1000 \times V_3 \times W$$

C = molar concentration of NaOH

V_1 = NaOH volume used in titration

V_2 = made-up volume of CS-HCl solution in water (= 100ml)

MW = mol. wt. of CS-HCl repeat unit (= 197.5)

V_3 = volume of CS-HCl solution used in titration (= 10.00 ml)

W = weight of CS-HCl dissolved in V_2

For Analysis No. 1

$$DD = \frac{0.0999 \times 2.50 \times 100 \times 197.5 \times 100}{1000 \times 10.00 \times 0.5428} \%$$

% DD = 90.87%

The DD values for Analyses Nos. 2 and 3 were calculated in the same way giving an average DD value of:

Average DD = 91.2%

3.3.2 Carbon-13 Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is concerned with the detection of the absorption or emission of electromagnetic radiation by simulated transitions between energy levels in the system under investigation. Since the energy levels are influenced by the environment of the nuclei, the resulting spectrum gives more or less direct evidence regarding the chemical nature of interacting atoms both quantitatively and qualitatively. NMR arises from the interaction of the applied electromagnetic radiation with nuclear spins when the energy levels of the latter are split by an external magnetic field [35].

Reference ^{13}C-NMR spectra of chitin and chitosan [29] are shown in Figure 3.9. It can be seen that the C=O and CH_3 carbon peaks disappear in the chitosan spectrum because of the deacetylation of chitin to chitosan (if complete 100% deacetylation). The spectrum of the chitosan (Seafresh)

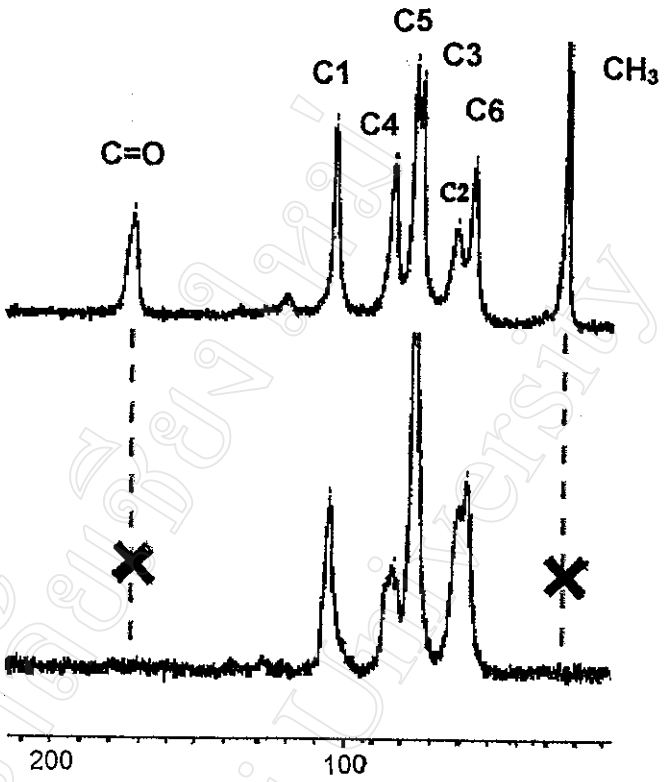


Figure 3.9: Reference ^{13}C-NMR spectra of (A) chitin and (B) chitosan [29].

used in this work is shown in Figure 3.10. Small C=O and CH_3 peaks are still evident in the spectrum at 175 ppm and 24 ppm due to incomplete deacetylation.

In this work, high-resolution solid-state ^{13}C-NMR spectra were obtained using a Bruker DPX 300 ^{13}C-NMR Spectrometer. Chopped chitosan fibres were placed in a zirconium oxide cylinder rotor operating at 4000 MHz under a magnetic field of 7 Tesla and a radio frequency of 300 MHz. The degree of deacetylation (% DD) of the chitosan can be determined from the CH_3 and ring C_1 peak intensities (labelled in Figure 3.10) via the formula given in equation (3.13).

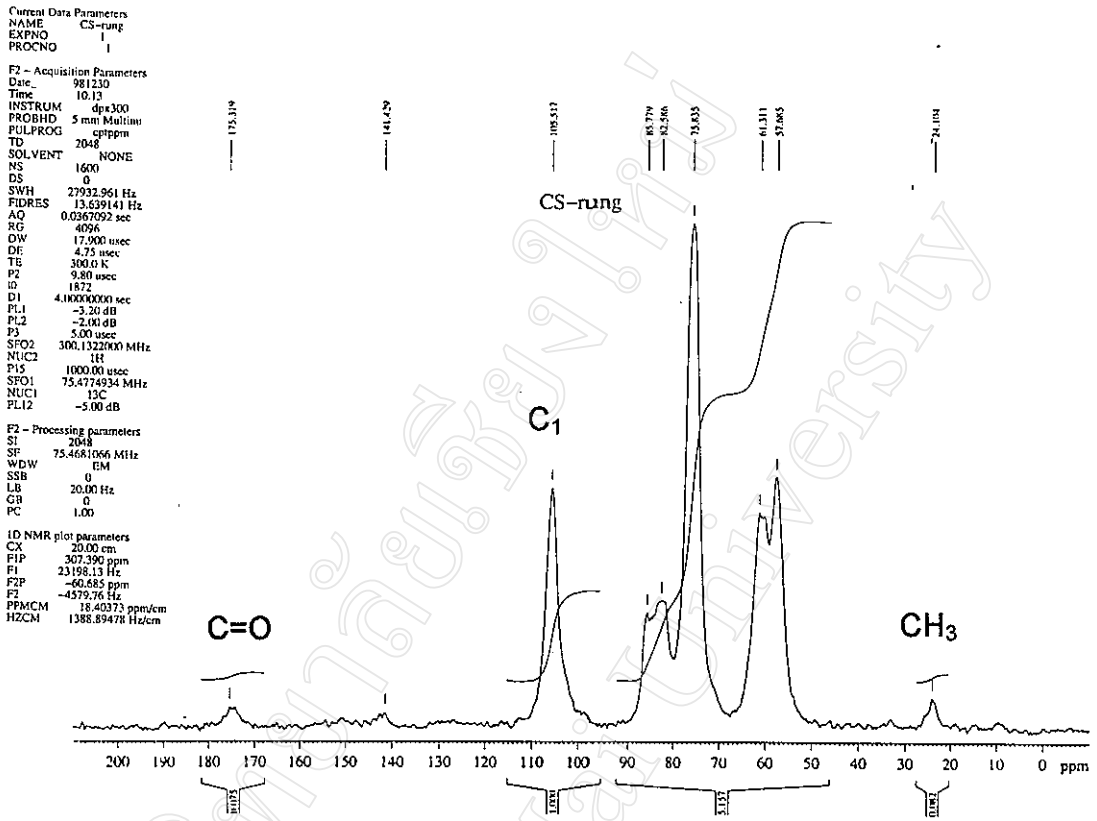


Figure 3.10: ^{13}C-NMR spectrum of chitosan (Seafresh) raw material used in this project.

$$\% \text{ DD} = 100 \times [1 - (I_{CH_3} / I_{C_1})] \qquad (3.13)$$

% DD = % deacetylation

I_{CH_3} = integral of CH_3 peak (24 ppm)

I_{C_1} = integral of ring C_1 peak (105 ppm)

Therefore, from the spectrum in Figure 3.10, the % DD can be calculated as:

$$\% \text{ DD} = 100 \times [1 - (I_{CH_3} / I_{C_1})]$$

I_{CH_3} = 0.082

I_{C_1} = 1.000

% DD = 100 x [1 - (0.082 / 1.000)]

% DD = 91.8%

This % DD value of 91.8% agrees very closely with the previous average value of 91.2% from the titration method. This close agreement is very encouraging because the two methods are fundamentally quite different. Whereas the titration method is a *chemical* method involving a chemical transformation, this NMR method is a *physical* method in which the chitosan is analysed directly.

3.3.3 Comparison of Methods

When comparing the titration and NMR methods for DD determination in terms of their overall precision and reliability, the NMR method should be better, since it is a more direct method with fewer sources of error. It is also more convenient in terms of sample preparation and analysis time.

However, it must also be said that a ^{13}C-NMR spectrometer is a highly sophisticated and expensive piece of equipment which is not always readily available. It is therefore very useful to know, from the results of this work, that the chemical method is also reliable if performed carefully. Although, it is more time-consuming, it has the distinct advantage of only requiring very simple volumetric apparatus. The results here indicate that titration is a reliable method in the event that ^{13}C-NMR is unavailable.

3.4 Storage Stability of Chitosan in Solution : Effects of Temperature and Time

Since chitosan is a cellulose derivative, it is susceptible to acid-catalysed hydrolysis in solution. Consequently, when chitosan in dilute acetic acid solution is left to stand during storage, the chitosan polymer slowly hydrolyses with time. Therefore, the conditions (temperature and time) under which the chitosan solution is stored prior to its use in wet spinning have an important bearing on the quality (especially mechanical strength) of the final fibre product. In this work, the storage stability was studied at both room temperature (25 – 35°C) and at low temperature (<0°C) and the results compared.

3.4.1 Experimental Procedure

3.4.1.1 Preparation of Chitosan Solution

In this research project, dilute-solution viscometry was used to monitor molecular weight changes using the Schott-Gerate AVS 300 Automatic Viscosity Measuring System (Figure 3.5) previously described. Chitosan solutions were prepared by dissolving 3% by weight of chitosan in 1% aqueous acetic acid and were stored in a cupboard at room temperature and in a freezer at <0°C. At <0°C, the solution froze solid. The molecular weights of the chitosan in solution were determined after 0, 1, 2, 3, 5, 7 and 10 days.

3.4.1.2 Molecular Weight Determination

For each time interval, the polymer in solution was precipitated into 5% aqueous NaOH as coagulant, filtered off, washed with water until neutral (checked with litmus) and dried in a vacuum oven at 60°C for 4 hrs. Using 0.2 M acetic acid / 0.1 M NaCl / 4 M urea as solvent, a series of chitosan solutions were prepared by successive dilution with concentrations of 0.025, 0.050, 0.100 and 0.200 g/dl. The flow-times of the solvent and each solution were determined at 25 ± 0.1°C and the viscosity-average molecular weight, $\overline{M}_v$, calculated as described previously in section 3.2.4.

3.4.2 Experimental Results and Calculations

The variations in viscosity-average molecular weight, $\overline{M}_v$, of the chitosan in solution with storage time (days) at room temperature and <0°C are compared in Table 3.12 and Figure 3.11. As expected, $\overline{M}_v$ decreases with time as a result of acid-catalysed hydrolysis of the polymer chain. The $\overline{M}_v$-time profile at room temperature in Figure 3.11 shows a rapid decrease at first followed by a more gradual decrease, typical of a random chain scission process. Also as expected, this rate of molecular weight decrease is slower at the lower temperature of <0°C. The mechanism of the hydrolysis reaction is shown in Figure 3.12. Protonation of the β-(1,4) glycosidic bonds by H_3O^+ leads to bond cleavage, molecular weight reduction, and the eventual formation of short chain chitooligosaccharides [36].

Thus, these results have demonstrated quite clearly that (a) chitosan does degrade quite quickly in 1% aqueous acetic acid solution at room

temperature but that (b) the rate of hydrolytic degradation can be significantly decreased by storage at low temperature. This is useful information since it has a direct bearing on the polymer molecular weight and, hence, on the mechanical properties of the wet-spun fibre. Therefore, the main conclusions to be drawn from this are that:

(1) chitosan solution (spin dope) to be used for wet spinning should be used as soon as possible after preparation
(2) if the solution is to be stored before wet spinning, it should be stored in a freezer at low temperature in order to minimize the rate of hydrolysis and molecular weight decrease

Table 3.12: Comparison of the variations in viscosity-average molecular weight, $\bar{M}_v$, of chitosan with days of storage in solution at room temperature and at <0°C.

Day	Viscosity-Average Molecular Weight, $\bar{M}_v$	
	At Room Temp.	At <0°C
0	2.50×10^5	2.40×10^5
1	2.14×10^5	2.33×10^5
2	1.67×10^5	2.30×10^5
3	1.51×10^5	2.30×10^5
5	1.44×10^5	2.30×10^5
7	1.40×10^5	2.29×10^5
10	1.36×10^5	2.26×10^5

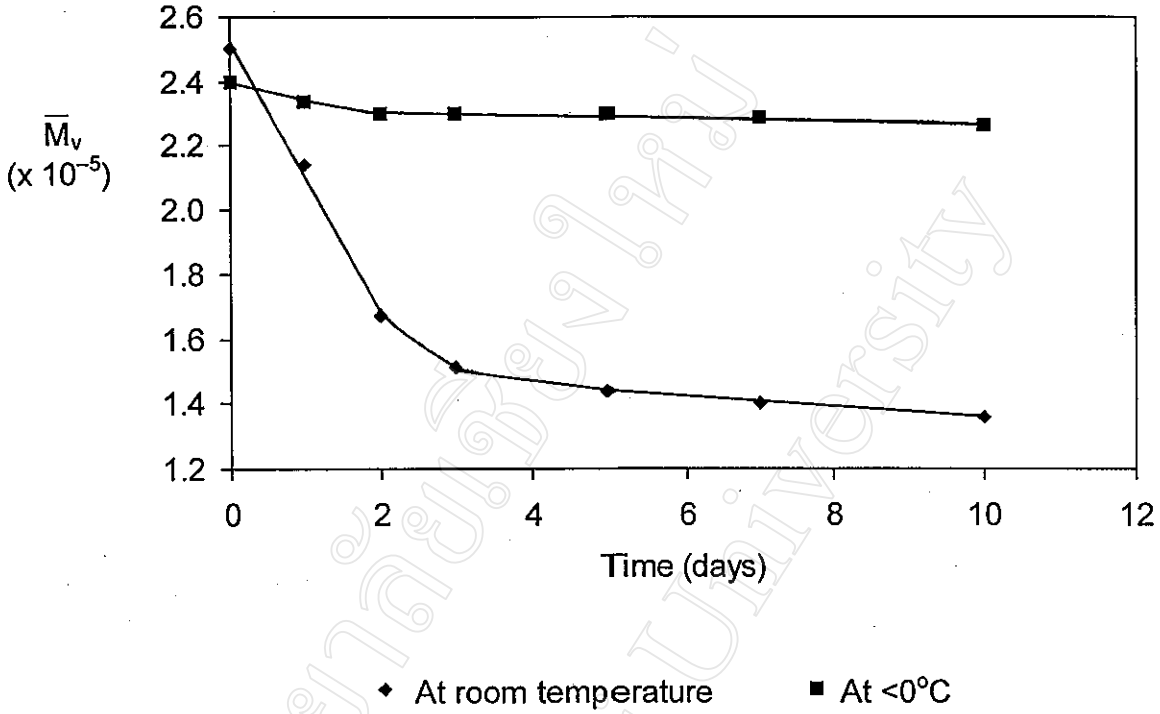
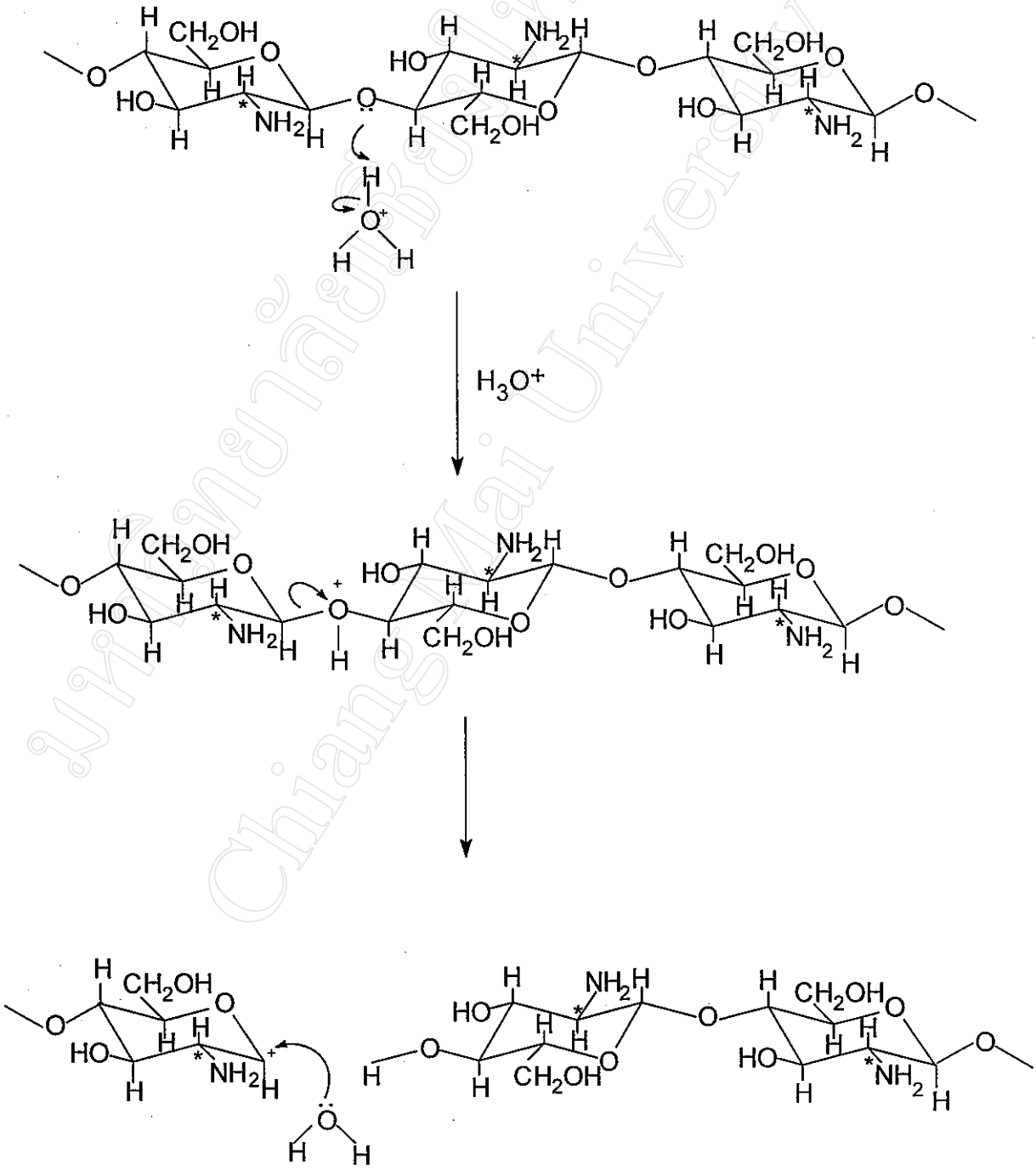


Figure 3.11: Comparison of the $\overline{M}_v$ –time profiles of chitosan in solution at room temperature and <0°C.

ACID-CATALYSED HYDROLYSIS OF CHITOSAN



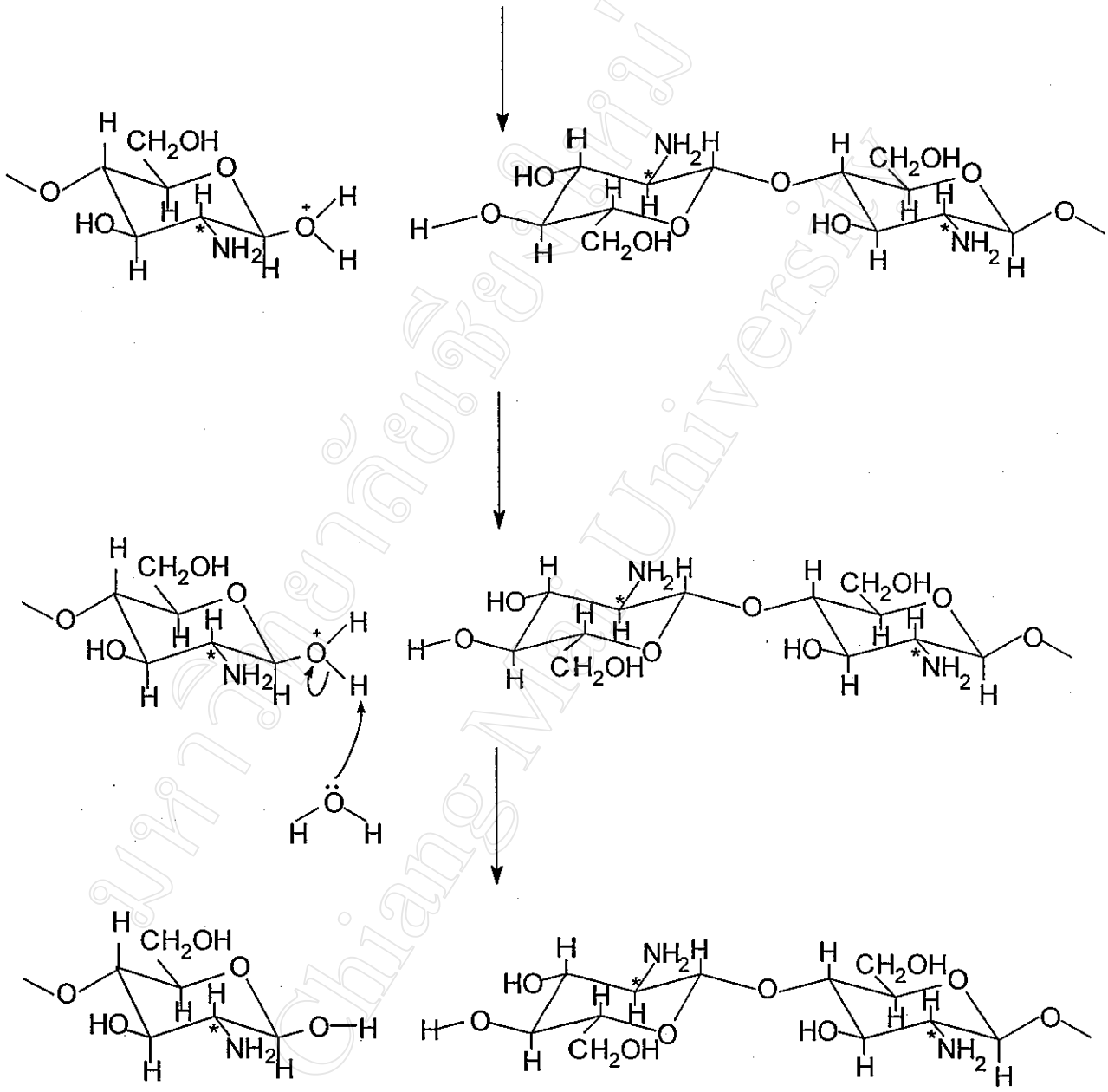


Figure 3.12: Mechanism of the acid-catalysed hydrolysis of chitosan leading to chain scission at the β-(1-4) glycosidic bond [36].