Asian Journal of Chemistry; Vol. 29, No. 4 (2017), 825-828

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2017.20324

Characterization of Chitosan Particles *via* Attenuated Total Reflection Fourier Transform Infrared Spectroscopy, Conductometric Titration, Viscosity Average Molecular Weight and X-ray Photoelectron Spectroscopy

NILUFER YILDIZ VARAN

Department of Textile Engineering, Engineering Faculty, Pamukkale University, Kinikli Kampusu, 20017 Denizli, Turkey

Corresponding author: E-mail: niluferyildizny@yahoo.com

Received: 4 October 2016; *Accepted*: 22 December 2016; *Published online*: 31 January 2017; AJC-18252

This paper reports the characterization of chitosan particles with a 1.0×10^6 viscosity average molecular weight *via* attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy, conductometric titration, viscosity average molecular weight and X-ray photoelectron spectroscopy. The viscosity average molecular weight (M_v) of chitosan was determined based on conductometric titration. The degree of deacetylation which was calculated with the help of acid base titration method was used to determine k and α values. Then the average viscosity molecular weight was calculated with the help of [η], k and α by the Mark-Houwink Sakurada equation. Molecular and packing structures of the chitosan chains in the crystal were observed using polarized light microscopy to evaluate the crystal structure of the anhydrous form of chitosan.

Keywords: Chitosan, Conductometric titration, Viscosity, FTIR, XPS, Packing structure.

INTRODUCTION

Chitosan and its parent compound chitin are naturally occurring β-(1,4)-linked linear aminopolysaccharides. Chitosan, though less prevalent in nature, is a useful and easily accessible derivative of chitin. Both polymers are biodegradable, renewable resources with versatile chemical and physical properties. As such, they are the subject of active scientific and commercial study [1]. Chitosan is the deacetylated derivative of chitin, which is the second most abundant polysaccharide found on earth next to cellulose. Chitin is the main component in the shells of crustaceans, such as shrimp, crab and lobster. It is also found in exoskeletons of mollusks and insects and in the cell walls of some fungi [2,3]. Chitosan is a high molecular weight heteropolysaccharide composed mainly of β-(1,4)-2-deoxy-2-amino-D-glucopyranose units and partially of β-(1,4)-2-deoxy-2-acetamido-D-glucopyranose (Fig. 1).

The physical properties of chitosan arise from its crystalline polymorph and biological activities. Crystal structure of the anhydrous form of chitosan provides knowledge of the molecular and packing structure of the chitosan chains in the crystal. Shear precipitated chitosan fibrids were examined by polarized light microscopy using a Nikon Labophot-Pol microscope (Nikon, Japan, Serial #951848). Photograph was taken with ISO400 mm film using a Nikon N6006AF camera (Nikon, Japan).

Chitin and chitosan are known for their excellent biological properties. Among the most important are biocompatibility with human cells, ordered regeneration of wounded tissues, immune enhancing activity, induction of immediate homeostasis, radical scavenging activity and antimicrobial activity [4].

Chitosan is known to have high affinity for dyes belonging to the acid, direct and fiber reactive dye classes. Chitosan or modified chitosan is dyeable with vat sulfur and disperse dyes. Basic dyes are the only dye classes that have inherent low affinity for chitosan due to charge repulsion. The C (2) basic amine group in each glucosamine group of chitosan is a

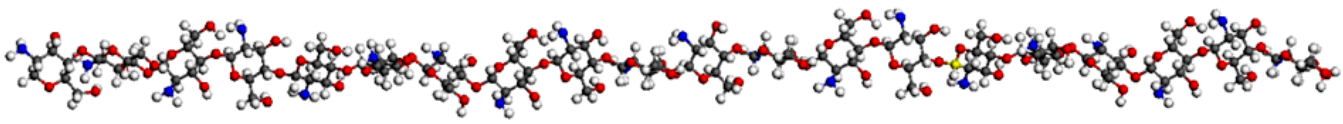

Fig. 1. Molecular structure of chitosan

potential site for ionic interaction with acidic functional groups. Protonation of the basic amine group makes chitosan soluble in dilute aqueous organic and mineral acid solutions. In some cases the ionic interaction is strong enough to render the salt (especially, chitosan sulfate and sulfite salts insoluble in aqueous solution, though the effect can sometimes be overcome by heat or treatment with an excess of other acids [5]. Chitosan ionic interactions have been characterized in terms of binding behaviour and ammonium salt complex formation [6].

In this study, chitosan was characterized with FTIR, conductometric titration, viscosity average molecular weight, X-ray photoelectron (XPS) spectroscopy and polarized light microscopy.

EXPERIMENTAL

Commercial samples of chitosan obtained from Sigma-Aldrich Company with a viscosity average molecular weight of 1.0×10^6 was confirmed using intrinsic viscosity and the Mark Houwink Sakurada equation and deacetylation of 70 % measured by acid base titration method. Other reagents used were analytically pure. The solvents used were lactic acid. Hydrochloric acid solution, 0.1 N (N/10) (Certified), both obtained from Fisher Chemical, were used in acid-base titration method to determine the degree of deacetylation. Anhydrous sodium acetate (Fused Crystals/Certified ACS) was obtained from Fisher Chemical. All the reagents and polymers were used as received.

Determination of the degree of deacetylation (DD) of chitosan by conductometric titration: The degree of deacetylation of chitosan was measured by acid-base titration dissolving chitosan in an acidic solution by protonation of its amine groups. In this method, 0.0940 g chitosan completely dissolved in 10 mL of 0.1 N HCl solution and was titrated potentiometrically with a 0.1 N NaOH solution.

Conductivity readings were measured using Orion Benchtop Conductivity Meter, Model 162. The results were obtained within 3-5 s after each addition of NaOH. To the solution, a conductivity probe (Orion Conductivity Cell, Model 013030) was submerged and the solution was stirred until the temperature became constant.

Conductivity readings were noted after adding the NaOH solution. The readings were plotted as volume of NaOH solution *versus* conductivity. The graphs show two deflection points (Fig. 2).

The number of moles of NaOH used between the first and the second deflection points equals to the number of moles of amino groups of chitosan sample, which was calculated by eqn. 1:

$$\text{Number of moles of amino groups} = [M_{NaOH}\,(\text{mol/L}) \times V_{(2\text{-}1)}(\text{mL})\,]/1000 \quad (1)$$

where M_{NaOH} is molarity of standard NaOH solution and $V_{(2\text{-}1)}$ is the difference in volume between the two bending points

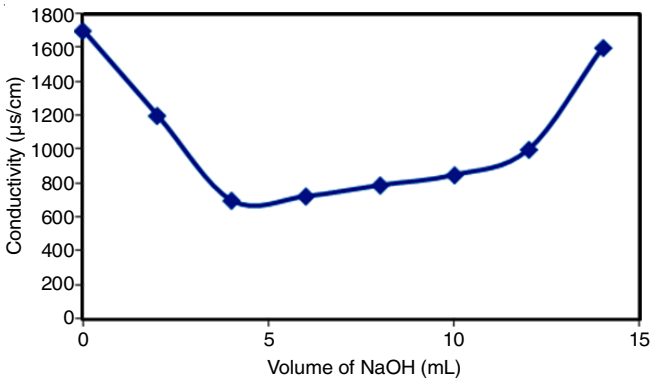

Fig. 2. Conductometric titration curve of deacetylated chitosan

and the degree of deacetylations were calculated using eqn. 2 and the results are presented in Table-1.

Chitosan ($C_6H_{11}O_4N$) molecular weight is 161.16 g/mol.

$$\text{Degree of deacetylation (\%)} = [\text{Number of moles of amino groups (mol)/(Amount of chitosan in g)/(Mw of repeat unit in g/mol)}] \times 100 \quad (2)$$

Viscosity average molecular weight (MW): Although viscometry is not an absolute method for determining the average molecular weight of chitosan, it is one of the simplest and most rapid methods.

Specific viscosity (η_{sp}) was calculated by using eqn. 3:

$$\eta_{sp} = (t - t_s)/t_s \quad (3)$$

where t is a sample flow timer and t_s is a solvent flow time.

The viscosity average molecular weight (M_v) of chitosan can be determined by the following Mark-Houwink Sakurada equation (eqn. 4), where [η] is intrinsic viscosity determined from Huggins plot (Fig. 3) using Huggins equation (eqn. 5) and k and α are empirical coefficients that are dependent on the solvent systems, temperature employed and the degree of deacetylation of chitosan.

$$[\eta] = k\,M_v^{\alpha}\ \text{(Mark-Houwink Sakurada equation)} \quad (4)$$

$$\eta_{sp}/c = [\eta] + k_H\,[\eta]^2 c\ \text{(Huggins equation)} \quad (5)$$

$$[\eta] = \lim_{c\text{-}o}[\eta]/c$$

where [η] is an intrinsic viscosity (mL/g), M_v is the viscosity-average molecular weight, k (mL/g) and are empirical Mark-Houwink parameters, c is the concentration of solution (g/mL), η_{sp} is a specific viscosity, k_H is the Huggins coefficient, respectively.

The viscosity average molecular weight of the chitosan (70 % degree of deacetylation based on the conductometric titration) was determined by the method of Wang *et al.* [8]. Dry chitosan was dissolved in aqueous 0.20 M acetic acid/0.10 M sodium acetate to give an initial polymer concentration of 1.0 mg/mL. Cannon-Ubbelohde semi-micro viscometer (Size 100. L57, Constant = 0.01365 mm^2/s^2 (cSt/s)) was charged with chitosan sample and equilibrated to 30.00 ± 0.05 °C in a constant temperature bath. The solutions were diluted according to the scheme shown in Table-2 and polymer sample flow times

TABLE-1
DEGREE OF DEACETYLATION (DD %) CALCULATED BY CONDUCTOMETRIC TITRATION

Chitosan sample	Reaction time (h)	Amount of chitosan (g)	Difference in volume between two deflection point (mL)	Number of moles of amino groups (mol)	Degree of deacetylation (DD, %)
DD-70	0	0.0976	20.9-16.7=3.8	0.00038	70

TABLE-2
INTRINSIC VISCOSITY MEASUREMENT OF THE AS RECEIVED CHITOSAN (DD-70)

C (g/mL)	0 (solvent)	0.001496	0.000714	0.0004	0.000266	0.000133
Time (s)	98.52	196.076	152.954	123.762	113.66	105.214
η_{sp}		0.990215	0.552517	0.256212	0.153674	0.067946
η_{sp}/C		990.2152	773.617	640.5298	577.7232	510.8691

(η_p) were recorded for 10 replications at each concentration and averaged. The results of viscosity measurement are reported in Tables 3-5. Specific flow times (η_{sp}) were calculated according to eqn. 6, where η_p is a sample flow time and η_s is a pure solvent flow time.

$$\eta_{sp} = (\eta_p - \eta_s)/\eta_s \quad (6)$$

From Huggins plot (Fig. 3) the intrinsic viscosity [η] was determined by extrapolating the linear regression of plots of η_{sp}/C *versus* C to zero concentration. The point at which the linear line touches the η_{sp}/C (y-axis) is the intrinsic viscosity. The degree of deacetylation which was calculated with the help of acid base titration method was used to determine k and a values. Then the average viscosity molecular weight was calculated with the help of [η], k and a by the Mark-Houwink Sakurada equation. The properties of original chitosan are provided in Table-3.

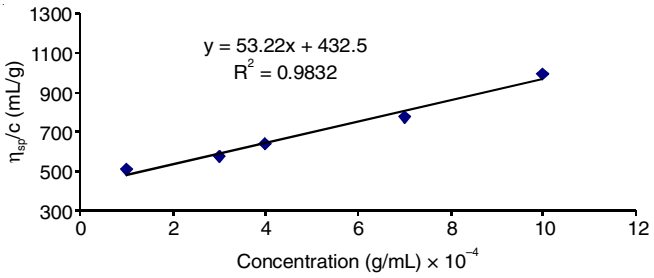

Fig. 3. Huggins plot of η_{sp}/c *versus* concentration for the as received deacetylated chitosan (DD-70) in 02 M CH_3COOH/0.1 M CH_3COONa aqueous solution

Dyeing of chitosan substrates: Acid salts are forming strong salts on chitosan. Chitosan fibrids were dyed separately for 30 min to equilibrium with Acid Red 360 under isothermal conditions ($\Delta T = 0$). Isothermal exhaustion curves of dye on chitosan were obtained to establish the time required to reach equilibrium in the Acid Red 360 experiment (Fig. 4). After dyeing, samples were removed from the dye bath, rinsed briefly in deionized water to remove surface dye and air dried separately with Telon Red AFG (Acid Red 360) Dystar with a concentration of 5 g/L using exhaustion method.

Microscopic images: Shear precipitated dyed chitosan fibrids were examined by polarized light microscopy using a Nikon Labophot-Pol microscope (Nikon, Japan, Serial #951848). Photograph was taken with ISO400 mm film using a Nikon N6006AF camera (Nikon, Japan).

FTIR spectroscopy: Infrared spectroscopy was performed on the samples using the Nicolet Nexus 470 spectrophotometer FTIR infrared analyzer with AVATAR Omni sampler in the attenuated total reflectance (ATR) mode.

Fig. 4. Dyeing procedure of chitosan substrates with Acid Red 360 by exhaustion method

XPS analysis: Surface characterization was performed by X-ray photoelectron spectroscopy (XPS). XPS is based on Einstein's idea about the photoelectric effect, developed around 1905. This analysis is based on a photoelectric effect and it is a quantitative spectroscopic technique.

RESULTS AND DISCUSSION

FTIR: The total reflection infrared spectra of chitosan samples (DD-70) is shown in Fig. 5. FTIR spectra of chitosan shows characteristic bands at 3350 cm^{-1} which refers to O-H stretching and N-H stretching (1° amide). Aliphatic C-H stretching at 2929 and 2874 cm^{-1}. Two absorption peaks at 1650 and 1595 cm^{-1} which refer to C=O (acetyl group) of secondary amide and NH_2 of primary amine. Two absorption peaks at 1425 and 1385 cm^{-1} can be attributed to the C-H bending.

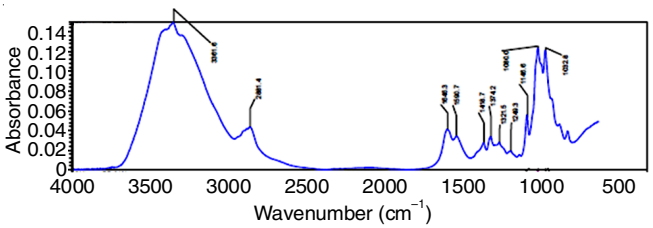

Fig. 5. Infrared spectroscopy of the chitosan sample

XPS analysis: The XPS spectra used in the analysis of materials is obtained by irradiating a specimen with an X-ray beam. As this occurs, the kinetic energy and number of electrons that escape from the top are measured. There are in the range of 1 to 10 nm of the material itself. High-resolution XPS spectra regarding C_{1s}, O_{1s} and N_{1s} are discussed. High-resolution spectra for C_{1s}, O_{1s} and N_{1s} regions were obtained, using a pass energy of 20 eV. Due to their insulating nature, chitosan surfaces became positively charged after the emission of photoelectrons, resulting in a broadening of the spectral lines and drift to ward

TABLE-3
PROPERTIES OF MEDIUM VISCOSITY AND ORIGINAL CHITOSAN

Chitosan sample	Reaction time (h)	DD (%)	K (mL/g)	a	Intrinsic viscosity [η]	Viscosity average molecular weight (M_v)
Original	0	70	0.000122	1.10141	475.4	1.0×10^6

higher binding energy. Thus, the binding energies of the photoelectron peaks were calibrated, assigning a binding energy of 285.0 eV to the aliphatic carbon (-CH_2-) C_{1s} peaks, present as a carbon surface contaminant. Element atomic percentages were calculated from the integrated intensities of XPS peaks, taking into account the atomic sensitivity factors of the instrument data system.

The survey spectra of chitosan confirmed the presence of carbon, oxygen and nitrogen (Fig. 6). The C_{1s} peak at 285.0 eV was mainly assigned to the carbon surface contaminant -CH_2-, but also to C-NH_2 chemical bindings, given that amines are reported to induce small chemical shifts, namely of 0.6 eV [1]. The peaks at 284.9 eV was assigned to C-O, C-OH and C-N-C=O and the peak at 285.0 eV to O-C-O and N-C=O chemical bindings [9-11].

The resolved N_{1s} spectra of chitosan samples are also shown in Figs. 2 and 3. Two peaks were identified. The peak at 399.9 eV was assigned to N-C=O and NH_2 chemical bindings, while the peak at 400.0 eV was assigned to amino groups in the ammonium form (NH_3^+) [12,13]. Both forms, NH_2 and NH_3^+ were likely to be present in chitosan samples, taking into account the pK_a of chitosan amine groups (about 6.5) [14].

Microscopic images: Chitosan shear precipitated fibrids were birefringent, showed positive birefringence (Fig. 7), indicating that the predominant polymer chain orientation was parallel to the fibrid axis.

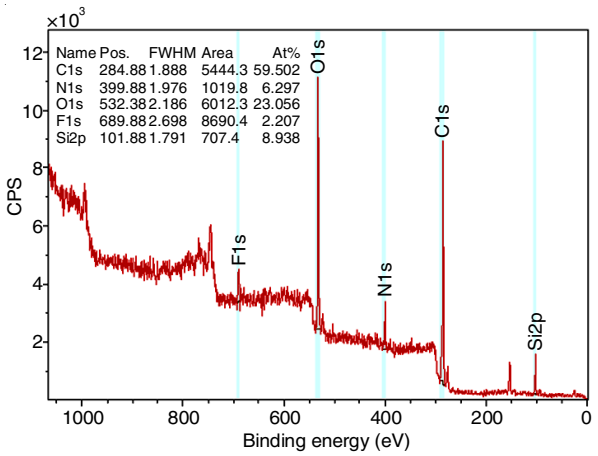

Fig. 6. Chitosan with a degree of deacetylation 70 % bound onto nylon 66 fibers

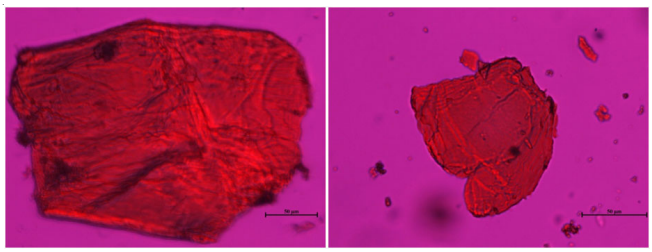

Fig. 7. Chitosan fibrids dyed with fluorescent red

Conclusion

Chitosan was characterized with FTIR, conductometric titration, viscosity average molecular weight, polarized light microscopy and XPS. Glycosidic linkages of C-H stretch at 1156-1152 cm^{-1} show its saccharide structure. Two peaks were identified in the resolved N_{1s} spectra of chitosan samples. The peak at 399.9 eV was assigned to N-C=O and NH_2 chemical bindings, while the peak at 400.0 eV was assigned to amino groups in the ammonium form (NH_3^+). Both forms, NH_2 and NH_3^+ were likely to be present in chitosan samples, taking into account the pK_a of chitosan amine groups (about 6.5). Dissolved chitosan was known to participate in ionic bonds with small water soluble parts. Chitosan-dye ionic interactions were observed to evaluate the inherent affinity of Acid Red 360 for chitosan for the observation of the crystal structure of the anhydrous form of chitosan which provides knowledge of the molecular and packing structure of the chitosan chains in the crystal.

ACKNOWLEDGEMENTS

The author is grateful to Drs. Hudson and Tonelli from North Carolina State University, TECS Department in the College of Textiles for providing facilities and sharing their excellent knowledge and experiences on polymers and also to the Scientific and Technological Research Council of Turkey (TUBITAK) for supporting this work.

REFERENCES

1. S. Salmon and S.M. Hudson, *J. Macromol. Sci. Part C Polym. Rev.*, **37**, 199 (1997).
2. S.M. Hudson and C. Smith, in eds.: D.L. Kaplan, Polysaccharide: Chitin and Chitosan: Chemistry and Technology of their Use as Structural Materials, In: Biopolymers from Renewable Resources, Springer-Verlag: New York, pp. 96-118 (1998).
3. G.A.F. Roberts, Chitin Chemistry; Macmillan Press Ltd.: London (1992).
4. R. Jayakumar, M. Prabaharan and R.A.A. Muzarelli, Chitosan for Biomaterials I, In: Advances in Polymer Science, vol. 243, Springer-Verlag Berlin Heidelberg (2011).
5. M.S. Masri and V.G. Randall, in eds.: R.A.A. Muzzarelli and E.R. Parisher, Chitosan and Chitosan Derivatives for Removal of Toxic Metallic Ions from Manufacturing-Plant Waste Streams, Proceedings of the First International Conference on Chitin/Chitosan, MIT Sea Grant Program, Cambridge, Massachusetts, pp. 277-287 (1978).
6. Y.C. Wei and S.M. Hudson, *Macromolecules*, **26**, 4151 (1993); https://doi.org/10.1021/ma00068a013.
7. T.D. Rathke, Ph.D. Disseration, The Characterization and Utilization of Ammonium Salt Chemistry to Modify the Chemical and Physical Properties of Chitosan films, North Carolina State University, Raleigh, USA (1994).
8. W. Wang, S. Bo, S. Li and W. Qin, *Int. J. Biol. Macromol.*, **13**, 281 (1991); https://doi.org/10.1016/0141-8130(91)90027-R.
9. D. Briggs, D. Briggs and M.P. Seah, Practical Surface Analysis, Wiley, Chichester, vol. 1, p. 437 (1990).
10. G.D.B. Beamson, High Resolution XPS of Organic Polymers, Wiley, Chichester (1992).
11. B.D. Ratnerand and D.G. Caster, in ed.: J.C. Vickerman, Surface Analysis-The Principal Techniques, Wiley, Chichester, p. 43 (1997).
12. C.D. Wagner, A.V. Naumkin, A. Kraut-Vass, J.W. Alisson, C.J. Powell and J.R. Rumble, Jr., NIST-X-ray Photoelectron Spectroscopy Database 20 Version 3.4. National Institute of Standards and Technology, Gaithersburg, MD (2003); Available online: http://srdata.nist.gov/xps.
13. B. Lindberg, R. Maripuu, K. Siegbahn, R. Larsson, C.-G. Gölander and J.C. Eriksson, *J. Colloid Interface Sci.*, **95**, 308 (1983); https://doi.org/10.1016/0021-9797(83)90190-X.
14. A. Domard, in eds.: A. Domard, G. Roberts and K. Vårum: Advances in Chitin Science, Jacques André, Lyon, vol. 2, p. 410 (1997).