

DETERMINATION OF VISCOSITY-AVERAGE MOLECULAR WEIGHT OF CHITOSAN USING INTRINSIC VISCOSITY MEASUREMENT

PENGUKURAN BERAT MOLEKUL KITOSAN MENGGUNAKAN KAEDAH KELIKATAN INTRINSIK

Norzita Jacob, Norhashidah Talip, Maznah Mahmud, Nurul Aizam Idayu Mat Sani, Nor Akma Samsuddin, Norafifah Ahmad Fabillah
Malaysian Nuclear Agency (Nuclear Malaysia)
Bangi, 43000 KAJANG, MALAYSIA

Abstract

Molecular weight of chitosan can be determined by different techniques such as Gel Permeation Chromatography (GPC), Static Light Scattering (SLS) and intrinsic viscosity measurement. Determination of molecular weight by intrinsic viscosity measurement is a simple method for characterization of chitosan. Different concentrations of chitosan were prepared and measurement was done at room temperature. The flow time data was used to calculate the intrinsic viscosity by extrapolating the reduced viscosity to zero concentration. The value of intrinsic viscosity was then recalculated into the viscosity-average molecular weight using Mark-Houwink equation.

Abstrak

Berat molekul kitosan dapat ditentukan dengan pelbagai teknik yang berbeza seperti Gel Permeation Chromatography (GPC), Static Light Scattering (SLS) dan pengukuran kelikatan intrinsik. Penentuan berat molekul melalui pengukuran kelikatan intrinsik merupakan satu kaedah yang mudah untuk pencirian kitosan. Kepekatan kitosan yang berbeza disediakan dan pengukuran dijalankan pada suhu bilik. Data aliran masa digunakan untuk mengira kelikatan intrinsik dengan mengekstrapolasi penurunan kelikatan kepada kepekatan sifar. Nilai kelikatan intrinsik yang diperolehi kemudiannya digunakan dalam persamaan Mark-Houwink bagi mendapatkan purata kelikatan berat molekul.

Katakunci/keyword : Ubbelohde Viscometer, viscosity average molecular weight, intrinsic viscosity, chitosan

INTRODUCTION

Chitosan is a linear polysaccharide composed of randomly distributed β -(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). The very high molecular weight and therefore a very high viscosity of chitosan precluded its use in several biological applications. However, in some fields (especially in medicine and the food industry) the application of this polysaccharide is limited by its high molecular weight resulting in its low solubility in aqueous media (Minagawa et al. 2007; Biskup et al. 2005). For some specific applications, its degradation products were found to be much more useful. A variety of degradation methods such as chemical (Hasegawa et al. 1993), enzymatic (Ilyina et al. 2000) or radiation (Won-Seok et al. 2002) degradation of high molecular weight polymer are being worked out to generate these degradation products. Degradation by radiation is a simple and environmental friendly as it is free of initiators and side product (Charlesby, 1981).

Molecular weight (MW) is one of the most fundamental parameters in characterizing a polymer. Molecular weight of chitosan can be determined by different techniques. GPC is the most powerful technique for characterizing the molecular weight of polymers. However, it is a relative method and needs molecular weight standards for calibration to obtain the relation between elution volume and molecular weight. One of the simplest and rapid methods for determining the molecular weight of polymer is viscosimetry, although this is not an absolute method and requires the determination of constants.

The intrinsic viscosity, η as function of average molecular weight, M is represented by Mark-Houwink-Sakurada equations

$$\eta = KM^\alpha \quad (1)$$

where K and α are constants for a given polymer–solvent–temperature system. These constants are determined by evaluating a plot of $\log [\eta]$ versus \log molecular weight which the molecular weight has been determined by an absolute method such as light scattering method.

The goals of this study are to determine the effect of radiation on viscosity-average molecular weight and intrinsic viscosity of Ubbelohde viscometer.

EXPERIMENTAL

Materials

Chitosan powder was purchased from Hepe Medical Chitosan GmbH with the following properties, 91.8% of DDA, 6% water content and the viscosity is 1050 mPas 1% in 1% acetic acid at 20°C. Ammonium acetate and acetic acid were obtained from Sigma-Aldrich. All reagents were used as received without any purification.

Sample Preparation And Irradiation

Chitosan powder was weighed about 2g in different plastic bag prior to irradiation process. The irradiation was carried out using electron beam accelerator at irradiation conditions of 2MeV acceleration energy, 10 mA beam current and dose exposure of 2, 5, 10, 25 and 35kGy. Samples of unirradiated and irradiated were then dissolved in 0.15M ammonium acetate and 0.2M acetic acid solvent system for viscosity-average molecular weight and intrinsic viscosity measurement. This solvent should be used for measurement within 3 days. The solution was prepared and continuously stirred for 24 hours before doing the measurement to completely dissolve the chitosan. It can only be used within 24 hours.

Intrinsic Viscosity Determination

Intrinsic viscosity was examined using an automatic system Ubbelohde capillary type viscometer which allows the reading of flow times of the sample taken automatically without using stop watch. The capillary number is 531/10 I and the measurement was conducted at $25 \pm 0.1^\circ\text{C}$. The measurement was started with solvent until five consecutive values differ from their mean value by no more than 0.3s. Next, flow times of five different concentrations of chitosan solution were recorded. Directly before charging the chitosan solution into the viscometer, the solution should be filtered using hydrophilic membrane filter of 0.45 micron size.

The corrected average flow time for a solvent and each chitosan concentration, $t_{i(corr)}$ was calculated by subtracting the Hagenbach correction, $\Delta t_{HC(i)}$ from the average flow time for that concentration, t_i . according to :

$$t_{i(corr)} = t_i - \Delta t_{HC(i)} \quad (2)$$

The Hagenbach correction was provided in the operating instruction for the viscometer. For each chitosan concentration, the following viscosities were determined using the equation given;

$$\text{Relative viscosity, } \eta_{rel} = t/t_s \quad (3)$$

$$\text{Specific viscosity } \eta_{sp} = (t/t_s) - 1 \quad (4)$$

$$\text{Reduced viscosity } \eta_{red} = \eta_{sp}/c \quad (5)$$

$$\text{Inherent viscosity, } \eta_{inh} = \ln \eta_{red} / C \quad (6)$$

The reduced viscosity and inherent viscosity were plotted against chitosan concentration. The value of intrinsic viscosity can be calculated by extrapolating graph of reduced viscosity and inherent viscosity to zero concentration. The average of the two obtained intercept values was calculated.

Viscosity-Average Molecular Weight Determination

Viscosity-average molecular weight was identified by viscometric measurements using an Ubbelohde Capillary Viscometer type 531/10 I. This value was calculated from $[\eta] = KM^a$ equation, where $K = 9.66 \times 10^{-5}$ (dm^3/g) and $a = 0.742$ determined in 0.15M Ammonium Acetate and 0.2M Acetic Acid solution at 25°C.

RESULT AND DISCUSSION

Upon radiation, two main reactions which influence the final properties of polymer: a) scission of main chain, also known as degradation and b) cross-linking, the opposite process to degradation. The former will be followed by reduction in molecular weight while the later will cause an increase in molecular weight. Degradation process usually occurs when natural polymer is subjected to ionizing radiation.

Intrinsic viscosity of chitosan which was irradiated at different doses was demonstrated in Figure 1. It is showed that the intrinsic viscosity dropped dramatically when the chitosan exposed upon radiation at dose of 2kGy. The intrinsic viscosity reduced from 1353ml/g for unirradiated chitosan to 861.5ml/g of irradiated chitosan at dose of 2kGy. For chitosan irradiated at 5kGy and 10kGy, the intrinsic viscosities were 704.5ml/g and 543.5ml/g respectively, whereas at dose of 25kGy the intrinsic viscosity of irradiated chitosan was 330.5ml/g. The intrinsic viscosity was further decreased to 247ml/g of irradiated chitosan at dose of 35kGy. A reduction of intrinsic viscosity was followed by decreasing in viscosity-average molecular weight of chitosan. Changes of viscosity-average molecular weight of chitosan with irradiation dose are depicted in figure 2. A sudden drop of viscosity-average molecular weight was observed at 2kGy where the molecular weight reduced from 390kDa to 210kDa. After 5kGy, the viscosity-average molecular weight of chitosan fell steadily from 160kDa to 40kDa at dose of 35kGy. This reduction was due to the chain scission of chitosan backbone, where degradation process took place.

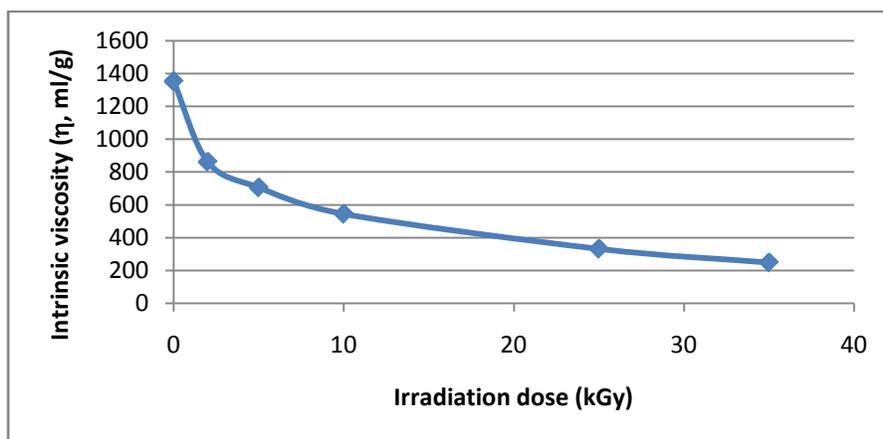


Figure 1: Changes of intrinsic viscosity as function of irradiation dose. Chitosan was irradiated in powder form using electron beam radiation at 2, 5, 10, 25 and 35kGy.

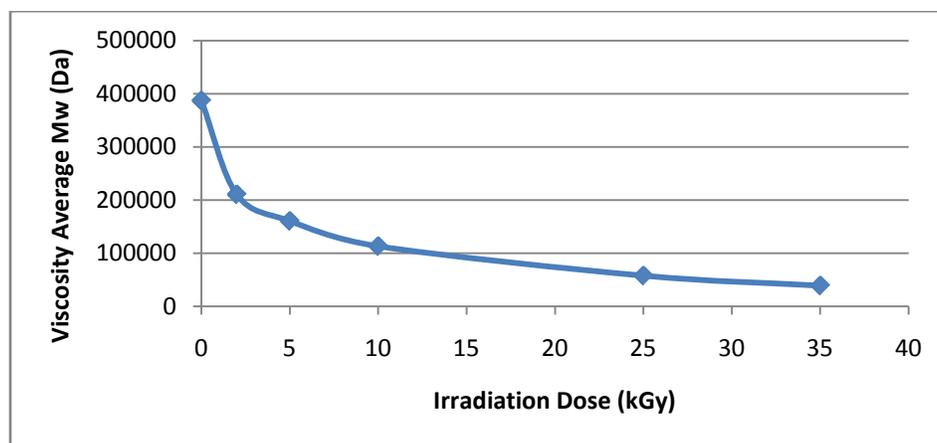


Figure 2: Changes of viscosity-average molecular weight and viscosity as function of irradiation dose. Chitosan was irradiated in powder form using electron beam radiation at 2, 5, 10, 25 and 35kGy.

CONCLUSION

Chain scission or degradation took place in the chitosan when exposed to irradiation at solid state. Both intrinsic viscosity and viscosity-average molecular weight were reduced when the chitosan was exposed to irradiation at dose up to 35kGy. Further, it was concluded that the intrinsic viscosity and viscosity-average molecular weight for chitosan can be determined by Ubbelohde Viscometer.

REFERENCE

1. Minagawa, T., Okamura, Y., Shigemasa, Y., Minami, S. and Okamoto, Y., (2007), Effects of molecular weight and deacetylation degree of chitin/chitosan on wound healing, *Carbohydrate Polymers*, 67: 640–644.
2. Biskup, R. C., Rokita, B., Ulanski, P. & Rosiak, J.M., (2005), Radiation-induced and sonochemical degradation of chitosan as a way to increase its fat-binding capacity, *Nuclear Instruments and Methods in Physics Research B*, 236: 383–390
3. Hasegawa, M., Isogai, A. & Onabe, F., (1993), Preparation of low molecular weight chitosan using phosphoric acid. *Carbohydr. Polym.*, 20, 279–283.
4. Ilyina, A.V., Tikhonov, V.E., Albulov, A.I and Varlamov, V.P., (2000), Enzymic preparation of acid-free-water-soluble chitosan. *Process Biochem.*, 35: 563–568.
5. Won-Seok, C., Kil-Jin, A., Dong-Wook, L., Myung-Woo, B. and Hyun-Jin, P., (2002), Preparation of chitosan oligomers by irradiation, *Polymer Degradation and Stability*, 78: 533–538.
6. Charlesby, A., (1981), Crosslinking and degradation of polymers. *Radiat. Phys. Chem.* 18: 59–66.