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

Norzita Yacob, Norhashidah Talip, Maznah Mahmud, Nurul Aizam Idayu Mat Sani, Nor Akma Samsuddin and Norafifah Ahmad Fabillah

Radiation Processing Technology Division, Malaysian Nuclear Agency (Nuclear Malaysia) Bangi, 43000 KAJANG, MALAYSIA

Email: norzita@nuclearmalaysia.gov.my

ABSTRACT

Determination of molecular weight by intrinsic viscosity measurement is a simple method for characterization of chitosan. To study the effect of radiation on molecular weight, chitosan was first irradiated using electron beam at different doses prior to measurement. 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

Penentuan berat molekul melalui pengukuran kelikatan intrinsik merupakan satu kaedah yang mudah untuk pencirian kitosan. Untuk mengkaji kesan sinaran terhadap berat molekul, kitosan terlebih dahulu disinarkan menggunakan alur electron sebelum analisa dijalankan. Kepekatan kitosan yang berbeza disediakan dan pengukuran djalankan pada suhu bilik. Data aliran masa digunakan untuk mengira kelikatan intrinsik dengan mengekstrapolasi penurunan kelikatan kepada kepekatan sifar. Nilai kelikatan intrinsik yang diperoleh kemudiannya digunakan dalam persamaan Mark-Houwink bagi mendapatkan purata kelikatan berat molekul.

Keywords: 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

$$\eta = KM^{\alpha} \qquad (1)$$

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.

MATERIALS AND METHODS

Materials

Chitosan powder was purchased from Heppe 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 ± 0.1°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 :

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

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;

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

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

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

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

The reduced viscosity, η_{red} and inherent viscosity, η_{inh} were plotted against chitosan concentration. The value of intrinsic viscosity, η can be calculated by extrapolating graph of reduced viscosity, η_{red} and inherent viscosity, η_{inh} 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 $[\eta] = KM^{\alpha}$ 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.

RESULTS AND DISCUSSION

Capillary viscometry is simple where the time it takes for a volume of polymer solution to flow through a thin capillary is compared to the time for a solvent flow. The polymer flows under its own weight through a known capillary length with an identified radius. Intrinsic viscosity, η is determined by measuring the relative viscosity at several different concentrations and then extrapolating the reduced viscosity to zero concentration. The intrinsic viscositycan be considered as a measure for the volume demand of the single polymer coil in ideally diluted solution. It reflects the capability of a polymer in solution to enhance the viscosity of the solution. This value will be further used in Mark-Houwink-Sakurada relationship to determine viscosity average molecular weight of chitosan. The intrinsic viscosity of a polymer in a certain solvent can be correlated with the molecular weight through Mark-Houwink-Sakurada empirical equations (Isihar 1992):

$$[\eta] = KM_v^{\alpha} \quad (7)$$

Both the exponent a and K are constants. These constants are dependent on the nature of the molecule and solvent, and on the temperature (Tanglertpaibul & Rao, 1987).

Figure 1 displays the intrinsic viscosity of unirradiated chitosan powder determined in 0.15M Ammonium Acetate and 0.2M Acetic Acid solution. The sample was determined via Huggins and Kremer plot, i.e., plots of reduced viscosity, η_{red} and inherent viscosity, η_{inh} versus concentration, c. Both η_{red} and η_{inh} depend on polymer concentration, so to extract the intrinsic properties of the polymer, one must extrapolate to zero concentration. From the figure, it is explained that the average of the two obtained intercept values which is called intrinsic viscosity, η of chitosan powder in ammonium acetate and acetic acid solution was 1.353 ml/g.

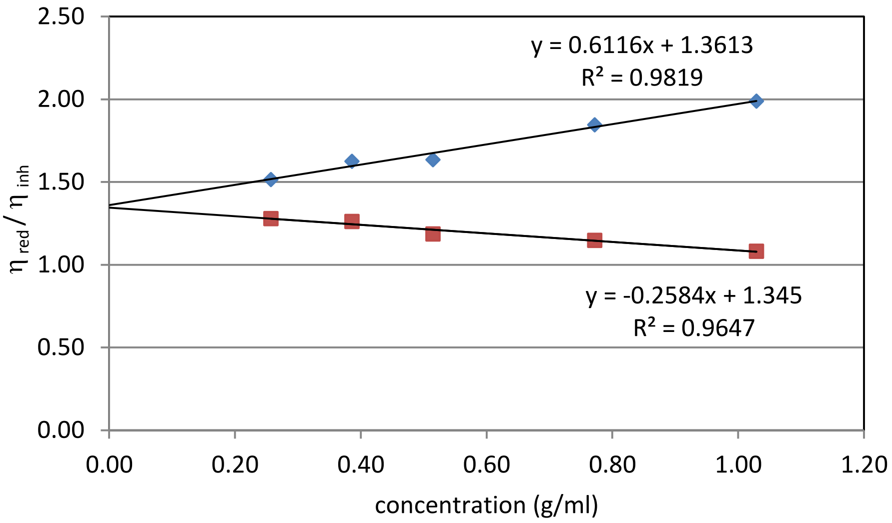

Figure 1: Intrinsic viscosity of chitosan powder in ammonium acetate and acetic acid solution

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 2. It is showed that the intrinsic viscosity dropped dramatically when the chitosan exposed upon radiation at dose of 2kGy. The intrinsic viscosity reduced from 1 353ml/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. The decrease in intrinsic viscosity means a decrease of the hydrodynamic volume of the macromolecular chain (Hirrien et al., 1996).

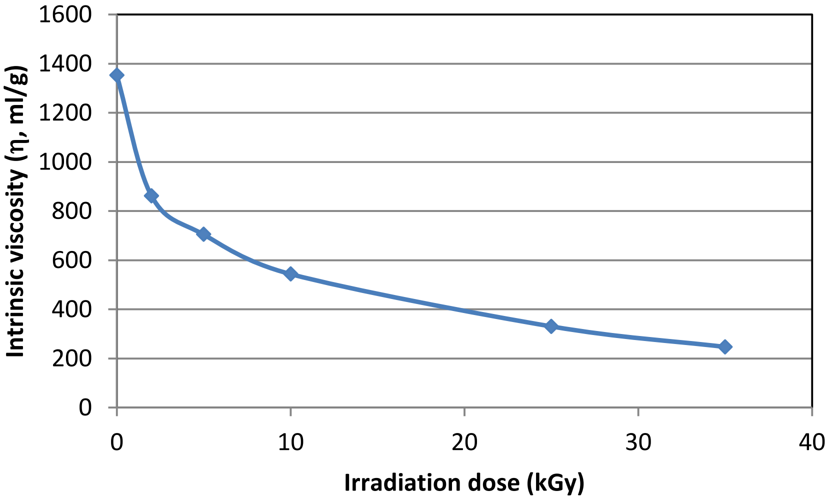

Figure 2: Changes of intrinsic viscosity as function of irradiation dose. Chitosan was irradiated in powder form using electron beam radiation at 2, 5, 10, 25 and 35kGy.

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 3. 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.

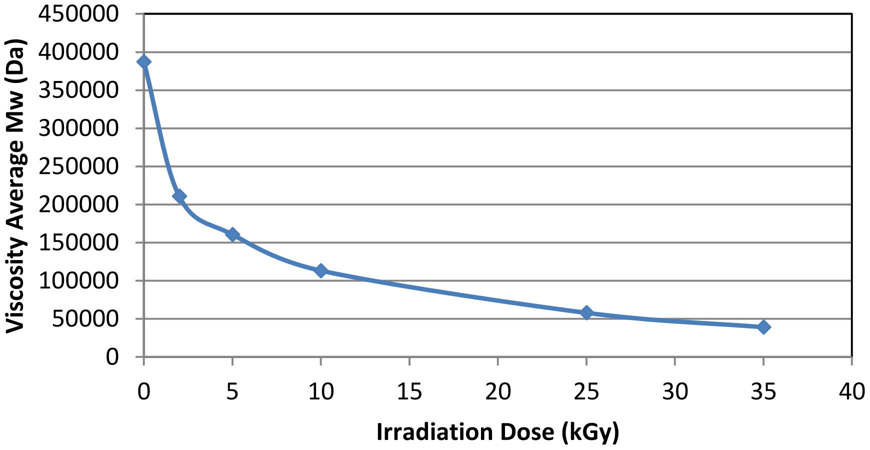

Figure 3: Changes of viscosity-average molecular weight and viscosity as function of irradiation dose. Chitosan was irradiated in powder form using electron beam radiation at 2, 5, 10, 25 and 35kGy.

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.

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