PHYSICOCHEMICAL CHARACTERIZATION OF IRRADIATED HIGH MOLAR MASS CHITOSAN

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Abstract

The present study is aimed to determine the bioburden for assessing the sterilization dose and to identify the influence of the absorbed gamma radiation dose on the molar mass and chemical structure of chitosan. The characterization includes the determination of the intrinsic viscosity, deacetylation degree as well as infrared spectroscopy. The obtained results have shown the occurrence of chain cleavage caused by irradiation. It was revealed by a decrease in the intrinsic viscosities of the polymers. The invariance of the infrared spectra of polymers indicated that chain degradation occurs without significant change of the chemical structure. The results obtained have practical implication in the field of radiation sterilization of chitosan used for microencapsulation of mammalian cells.

Key words: sterilization, gamma radiation, radiation doses, copolymers, radiation dose distributions

CARACTERIZACIÓN FÍSICO-QUÍMICA DE QUITOSANA DE ALTO PESO MOLECULAR IRRADIADA

Resumen

El estudio presenta la determinación de la carga microbiana para el establecimiento de la dosis esterilizante y evaluar la influencia de la dosis adsorbida de la radiación gamma sobre la masa molar y la estructura química de quitosana de alto peso molecular. La caracterización de esta muestra incluye la determinación de la viscosidad intrínseca, el grado de desacetilación así como el uso de la espectroscopía infrarroja. Los resultados mostraron la ruptura de la cadena principal por el efecto de la radiación, este efecto se pudo apreciar por el decrecimiento de la viscosidad intrínseca del polímero. La invariabilidad de los espectros infrarrojos indica que la escisión de cadenas tiene lugar sin cambios significativos en la estructura química. Los resultados tienen implicación práctica en la radioesterilización de quitosana para su uso en la microencapsulación de células de mamíferos.

INTRODUCTION

Chitosan, a copolymer from glucosamine and N-acetylated glucosamine, is obtained by means of partial deacetylation of chitin [1,2]. Due to the diverse biological properties of chitosan (ex: antimicrobial, antitumour and anti-immune, among others), this polymer has valuable medical applications [3,4], Some of these applications require sterile materials. For this purpose irradiation techniques seem to have advantages since they do not require any additives, which could contaminate the final sterile product with toxic residuals [5,6]. However, the irradiation can change chitosan characteristics. Therefore, the aim of the present study were to determine the bioburden for assessing the sterilization dose and to identify the influence of the absorbed gamma radiation dose on the molar mass and chemical structure of chitosan. Changes in polymer features were evaluated by comparing the results obtained from the characterization of irradiated and non irradiated chitosan.

MATERIALS AND METHODS

High molar mass chitosan was supplied by Aldrich Chemical Company, Inc. The average molar mass was 853 000 g/mol determined by viscometry, in the buffer solution of 0,05 M sodium acetate trihydrate ($C_2H_3NaO_2 3H_2O$) with 0.05 M acetic acid ($C_2H_4O_2$).

Ionizing irradiation of the samples

Chitosan samples were irradiated in a ^{60}Co gamma source (PX-γ-30), at the Center of Technological Applications and Nuclear Development (CEADEN), Havana, Cuba. The applied doses ranged from 0.5 to 25 kGy.

Assessment of sterilization dose

The initial bioburden was determined using non-sterilized high molecular weight chitosan samples. In order to ensure a sufficient margin of safety, a SAL (Sterility Assurance Level) of 10^{-6} was used, and the unit was taken as Simple Item Portion (SIP). Method 1, described in ISO 111137 for sterilization of health care products [9].

Deacetylation degree determination

The deacetylation degree was analyzed by amino groups potentiometric determination that was made with digital pH-meter (PHM 83 AUTOCAL Radiometer, Norwegian) with differential electrode. For the test 0.1 g of chitosan was dissolved in 100 ml solution of HCl 0.1 M. The titration was carried out with 0.25 M of NaOH.

The acetyl percentage was determined from the relation according to [10]:

$$\% [acetyl] = V \times 0.04305 / w$$

Where: V is the volume of NaOH and w is the weight of the chitosan sample.

Deacetylation degree (Y) was evaluated using the following equation:

$$Y = 100 - \% [acetyl]$$

Viscometry

The reduced viscosity, η_{red}, was obtained by the expression:

$$\eta_{red} = (\eta_{rel} - 1)/c$$

Where: c is the solution concentration in g/cm^3 and h_{rel} is the relative viscosity. Reduced viscosities data were plotted as a function of concentration and extrapolated to zero concentration to obtain the intrinsic viscosity [h]. The viscosity average molar mass of chitosan was calculated using the Mark-Houwink-Sakurada equation [7].

$$[\eta] = KM^{\alpha}_{v}$$

The reduced viscosity h_{red} of chitosan was measured at 25°C. The Mark-Houwink-Sakurada constants used were K= 1.81x 10^{-3} ml/g and α= 0.93 [8]. The solution viscosity was evaluated using an Ubbehlode capillary viscometer (Viscology TI 1, SEMA Tech, France).
Infrared spectrometry

The infrared spectra were registered using an ATI Mattson Genesis FTIR with a spectral range of 4000- 500 cm^{-1}; employing 10 μg samples.

RESULTS AND DISCUSSION

Bioburden and sterilization dose determination
The results obtained from bioburden determination are shown in table. The initial contamination of samples was found to amount to 102 cfu/g. The verification dose was found to be 6.7 kGy. The result of sterility test was acceptable since none of 100 samples showed positive growth of micro organisms.

Table. Principal parameters employed in the sterilization dose determination

Parameter	
SIP	1.0 g
Bioburden	102 cfu/g
Verification dose. SAL = 10^{-2}	6.7 kGy
Sterilization dose. SAL = 10^{-6}	23.3 kGy
Sterility test result	Non-positive

These results show that a 23.3 kGy dose is totally satisfactory for the sterilization of 1.0 g of chitosan for biomedical application with a SAL of 10^{-6} considering the pharmacopoeia criterion of sterility [9].

Deacetylation degree determination

The degree of deacetylation is an important parameter affecting the physicochemical properties of chitosan. Chitosan with a high degree of deacetylation is a highly charged polycation in solution and is more suitable as a coagulating, chelating or antimicrobial agent. We have the hypothesis that a radiation treated chitosan for capsule formation based on polyelectrolyte complexation can occurs over more physiological pH ranges (up to pH 7.5). In comparison, with non irradiated treated chitosan, which is insoluble at pH above 6.6, irradiated chitosan might have wider application in biotechnology.

In figure 1 are shown two maximum corresponding to the titration inflection points. The first inflection point is related to the neutralization of the excess HCl while the second inflection point corresponds to the complete neutralization of the amino group.

The distance between these two inflections points corresponds to the necessary volume of NaOH for the complete titration of the free amino groups of chitosan; which correspond to 0.2 ml approximately. The deacetylation degree obtained was 91%.

Viscometric studies

The effect of ionizing radiation on the chain length of chitosan was monitored by viscometry. The number average molar mass Mn was correlated with the viscosity average molar mass Mv as follows [7]:

$$\overline{M}n = \overline{M}v\left[(a+1)\sqrt{(a+1)}\right]^{-1/a}$$

Plots of the intrinsic viscosity and number average molar mass as a function of absorbed dose are presented in figure 2.

It shows that the solution viscosity and molar mass decreases exponentially as the absorbed radiation dose increases. It indicates that main change scission is the dominating process. These results can be explained by fact that irradiation of polymers can produce a complex cascade of events such as electron ejection, exited state formation and finally C-C scission [12].

The radiation chemical scission yield was calculated by:

$1/M_n - 1/M_{no} = G(s)D/100\ Na$

Where: M_{no}, M_n denote the number average molar mass before and after irradiation with dose D, respectively; G(S) is the radiation chemical scission, yield and Na is the Avogadro's number.

Figure 3 shows the reciprocal of number average molar mass of chitosan as a function of absorbed dose.

Radiation chemical scission yield calculated from the linear correlation was G_s= 2.1 molecules by 100 eV of absorbed energy. This result is in

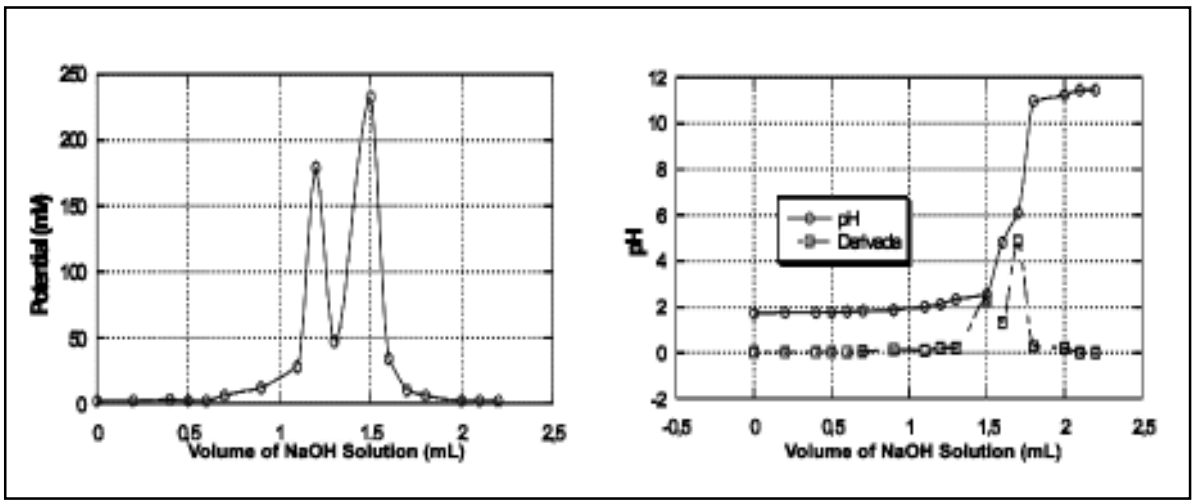

Figure 1. Potenctiometric titration of a chitosan solution in 0.1 M HCl with 0.25 M NaOH.

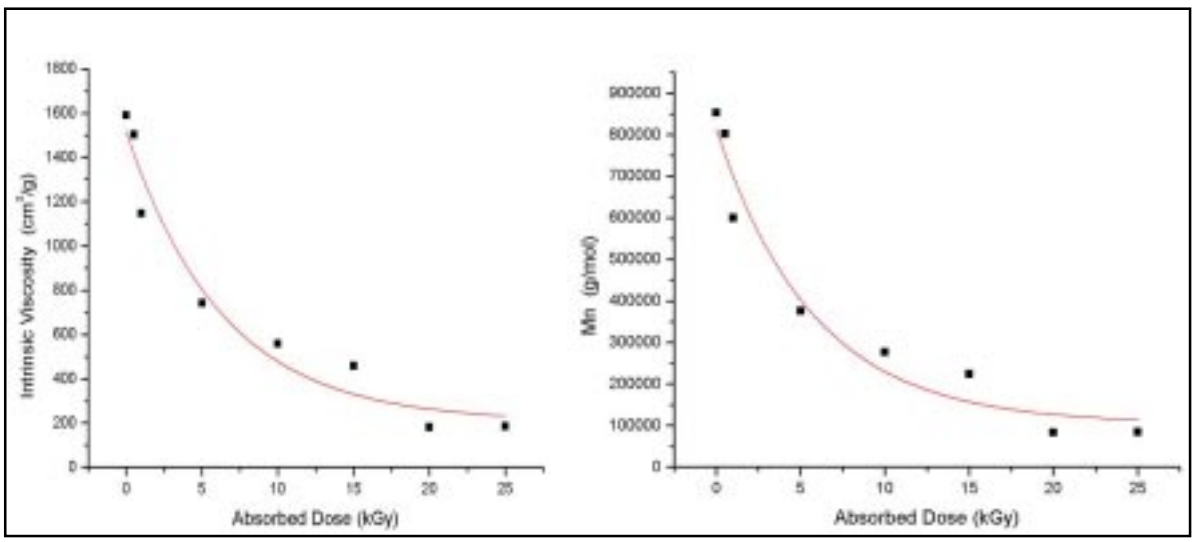

Figure 2. Intrinsic viscosity and number average molar mass of chitosan as a function of absorbed dose.

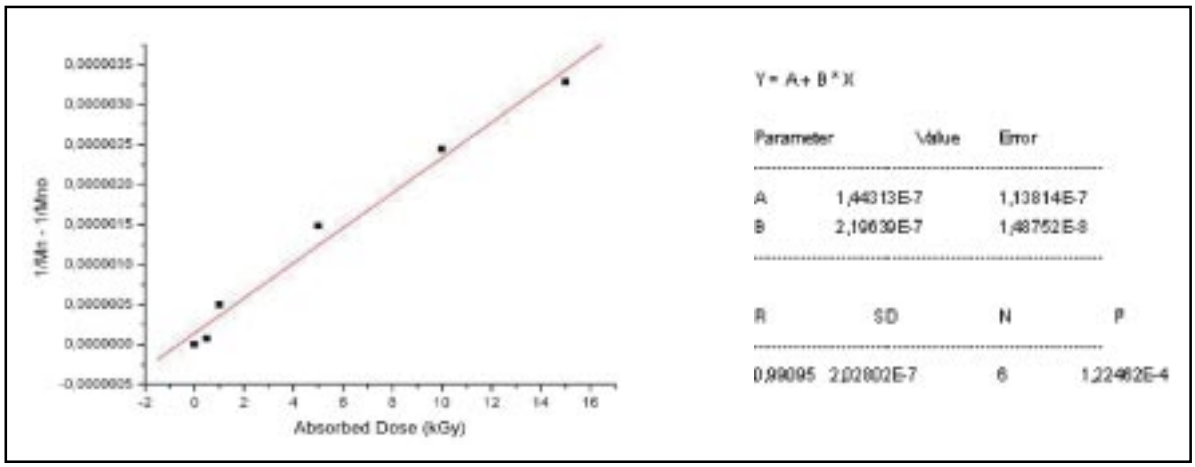

Figure 3. Reciprocal of number average molar mass of chitosan as a function of absorbed dose and linear fit parameters.

agreement with the values reported for other irradiated polysaccharides [8].

Infrared spectroscopy

FTIR spectra for irradiated and non irradiated chitosan samples are shown in figure 4. The IR spectra of chitosan shows a broad O-H absorption band occurring in the region between 3400 and 3500 cm^{-1}. The C-H stretching peak appears at around 2750 cm^{-1}. A significant absorption band at 1082 cm^{-1} is attributed to the characteristic vibration of the C-O bond of the saccharide structure.

Notice that there are no significant differences in the spectral bands, which supports the idea that no remarkable changes in chitosan chemical structure have occurred due to irradiation.

CONCLUSIONS

The value of the minimal absorbed dose required for achieving the sterility of a chitosan with the estimated bioburden was assessed to be 23.3 kGy. Potenciometry, viscometry, and infrared spectrometry are suitable to study the influence of gamma radiation on chitosan samples. Chitosan molar mass decreases exponentially as the radiation absorbed dose increases while the invariance of FTIR spectra of polymer indicates that chain degradation occurs without significant change of the chemical structure. The results obtained have practical implication in the field of radiation sterilization of chitosan used for microencapsulation of mammalian cells.

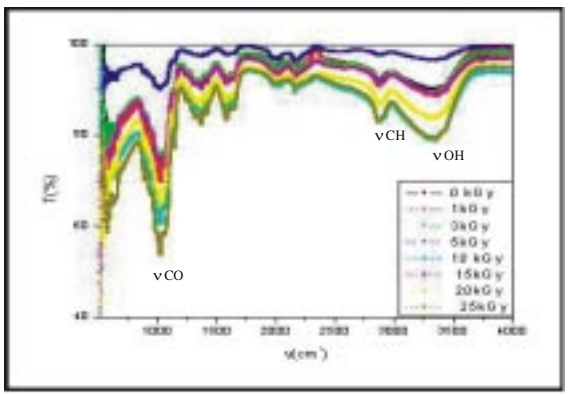

Figure 4. FTIR Spectra of irradiated and non-irradiated chitosan.

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