Characterisation of gamma irradiated chitosan/pHEMA membranes for biomedical purposes

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Abstract

As a polysaccharide of natural origin, chitosan has the inherent properties of being biocompatible, biodegradable, and non-toxic. These properties make chitosan an ideal candidate for based backbone in copolymeric matrices for use in biomedical applications. Poly(hydroxyethyl methacrylate) is a synthetic hydrogel which possesses a high mechanical strength. The conjunction of these two components results in a new matrix that combines the useful properties of the synthetic pHEMA and natural chitosan. In this work chitosan/pHEMA membranes were obtained and γ-irradiated under nitrogen atmosphere. The effect of various synthesis conditions on the chemical, physical and biological properties was evaluated. The chitosan/pHEMA membranes were characterised using FTIR spectroscopy, scanning electron microscopy and thermal analysis techniques. Its hydration capacity and its antimicrobial properties were also determined. The obtained results showed that the hydration capacity decreases in the irradiated membranes. It was also found that chitosan/pHEMA membranes present good barrier properties against microbes.
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1. Introduction

Natural macromolecular materials such as starch and chitosan have been reported as materials with excellent properties to be used in biomedical applications [1–3]. However they usually

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present weak mechanical properties and are difficult to process as membranes. In this way the modification of natural polymers by introducing branches of a different monomer in the backbone structure becomes a common method to adequate the polymer to each application [4]. Among various kinds of modifications, gamma radiation induced polymerisation has been proved to be an important method, since it makes possible the preparation and sterilisation in one technological step, with no necessity to add initiators or crosslinker agents [5–7].

Chitosan is a partially acetylated glucosamine obtained by deacetylation of chitin, one of the most abundant natural polymers. As a polysaccharide of natural origin, chitosan has many useful features such as non-toxicity, biocompatibility, biodegradability and antimicrobial properties. These characteristics make chitosan an ideal candidate for based backbone in copolymeric matrices for use in biomedical applications. Poly(hydroxyethyl methacrylate) is a synthetic hydrogel which possesses a high mechanical strength and biocompatibility. The conjunction of these two components results in a new interesting matrix that combines the useful properties of the synthetic pHEMA and natural chitosan. However despite the well-known applicability of these polymers as biomaterials, only a few attempts have been reported in trying to prepare chitosan/pHEMA copolymers [8–11]. In this work chitosan/pHEMA membranes were obtained and gamma irradiated disposed under nitrogen atmosphere.

The purpose of the present study has been to evaluate the effect of various synthesis conditions on the physical, chemical and microbiological properties of chitosan/pHEMA membranes.

2. Experimental

2.1. Materials

Chitosan medium molecular weight (1.9×10^5–3.1×10^5 Da) 75–85% deacetylated was obtained from Aldrich Chemical Company, Inc., Milwaukee, USA. This was triturated (500 µm < ϕ < 800 µm) and dried under vacuum at 313 K.

Hydroxyethyl methacrylate (HEMA), stabilised, 98%, was obtained from ACROS Organics, Belgium, and used as received. All other chemical used were of analytical grade.

2.2. Microorganisms

Three bacteria were tested for antimicrobial activity of chitosan/pHEMA membranes. These include a gram-negative bacterium (*Escherichia coli* ATCC 25922) and two gram-positive bacteria (*Bacillus pumillus* and *Staphylococcus aureus* ATCC 29213).

2.3. Preparation of chitosan/pHEMA membranes

The membranes preparation was achieved by mixing different chitosan solutions (1%, 3% and 5% w/V of chitosan in acetic acid 1%) with HEMA monomer (1%, 3% and 5% V/V of the final mixture). The bubble-free aqueous solutions were poured on a clean glass plate in a dust-free atmosphere and allowed to dry at room temperature. The membranes thus formed were washed with NaOH 1% and then with distilled water and carefully peeled off from the glass plate. Irradiation was carried out in Amilon polyamide bags under nitrogen atmosphere at the ^{60}Co portuguese irradiation facility. To avoid the impact of a too high amount of energy over chitosan backbone structure, that would lead to an extended main chain scission [12,13] before HEMA crosslinking could act as protective shield [7], membranes were exposed to 20 kGy instead the traditional sterilising dose of 25 kGy with a dose rate of 0.6 kGy h^{-1}.

Samples were irradiated in a position parallel to the irradiator and amber and red perspex dosimeters (Harwell) were used to monitor the samples absorbed dose.

2.4. Characterisation of chitosan/pHEMA membranes

2.4.1. Hydration capacity

The hydration capacity of the prepared membranes was determinate by immersing circular pieces of the membrane with average thickness between 17 and 19 µm in physiological solution

(NaCl 0.9% w/V) at 37 °C. Samples were periodically removed after appropriate intervals, blotted free of surface water with a filter paper, weighed and returned to swelling medium. This procedure was repeated until the sample attained the equilibrium hydration degree (constant weight). The hydration percentage of the membrane was calculated by using the following expression:

$$
\begin{aligned}
\mathrm{Hydration}(\%) \\
= \frac{\text{wt of hydrated membrane} - \text{wt of dry membrane}}{\text{wt of dry membrane}} \\
\times 100 \qquad\qquad (1)
\end{aligned}
$$

The dehydration behaviour at 37 °C was determined by thermogravimetric curves in a DuPont 951 TGA thermobalance.

2.4.2. Thermal properties

The membranes thermal properties were evaluated by TGA. The assays were carry out on a DuPont 952 Thermogravimetric Analyser in nitrogen atmosphere at a heating rate of 10 °C/min over the temperature range of 30–800 °C.

2.4.3. Fourier transform infrared spectroscopy

FT-IR spectra of the membranes were obtained by using a Bruker Tensor 27 CSL spectrometer. All spectra were recorded at ambient temperature at the resolution of 4 cm^{-1} and 8 times scanning.

2.4.4. Scanning electron microscopy

The dried non-irradiated and irradiated membranes were coated with a thin layer of gold under reduced pressure and their scanning electron micrographs were obtained using a JEOL (JSM 5310) scanning electron microscope.

2.4.5. Microbiological assays

2.4.5.1. Bioburden evaluation.
It has been previously shown that the growth of several bacterial and fungal strains is inhibited in the presence of chitosan [14,15]. Since the purpose of this work is to prepare chitosan based matrices for use in biomedical applications, this becomes an important issue. To find out if the obtained membranes keep some of the antimicrobial activity of the chitosan backbone, the number of contaminating microorganisms that naturally occur on non-irradiated samples with different chitosan concentrations (1% and 3% w/V) and HEMA 1% (V/V) were evaluated. In this way each sample was suspended in sterile saline solution (NaCl 0.9% w/V) with stirring for 10 min. After that specific volumes of solution were filtered through 0.45 μm pore-size filters. The paper filter of each filtration was then incubated at 36 °C for 7 days on a pre-filled Trypitc Soy Agar (TSA) cassette. All experiments were carry out in triplicate.

2.4.5.2. Microbe penetration test.
The microbial strains in use (*E. coli*, *B. pumilus* and *S. aureus*) were cultured overnight at 37 °C on TSA medium. The bacteria were then suspended in a sterile saline solution (NaCl 0.9% w/V) and as result suspensions of 10^8 cells/ml were obtained for each strain.

The membranes were cut into a size of 1 cm^2 and put on TSA plates. On the upper surface of each sample was dropped 1 ml of one bacterium suspension. This procedure was repeated for each microbial strain. After that the samples were incubated at 36 °C and the observation for bacteria's penetration through the membrane was done for 7 days.

3. Results and discussion

3.1. Hydration capacity of chitosan/pHEMA membranes

The hydration capacity or hydration behaviour of a polymeric support has particularly importance when it is to be applied as biomaterial. Its hydration degree influences on the surface and mechanical properties and on the type of solute transport mechanism through the matrix. The chitosan/pHEMA membranes prepared in this study were rather hydrophilic in structure. Fig. 1 shows the hydration behaviour of non-irradiated chitosan and chitosan/pHEMA membranes. As seen from the figure, although the water up-take of the chitosan/pHEMA membranes increase significantly with the increase of HEMA content, all the samples attained the equilibrium hydration degree within few minutes. The same occurs with
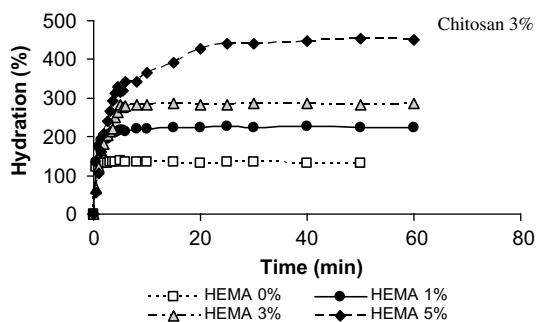
Fig. 1. Hydration capacity of non-irradiated chitosan based membranes at 37 °C in physiological solution as function of time.

Fig. 3. Difference at equilibrium hydration of irradiated and non-irradiated chitosan based membranes.

the increase of chitosan content in which an increase from 1% to 3% of chitosan, keeping HEMA 1%, implies an increase in water up-take from 176% to 214%. This suggests that increasing HEMA (and/or chitosan) content in the matrix promotes an easier penetration of the medium molecules into the polymer network due the introduction of more hydrophilic functional groups (–OH and –NH$_2$) into the polymeric network structure. In fact, depending on the content of HEMA membranes can take up to 11 times more to reach its dry form again (v.d. Fig. 2).

If one compares the hydration capacity of non-irradiated and γ-irradiated membranes it can be observed that although presenting identical profile behaviour, the equilibrium hydration degree of γ-irradiated samples decreases (Fig. 3 shows the differences at the equilibrium). However all irradiated membranes take approximately 15 min to

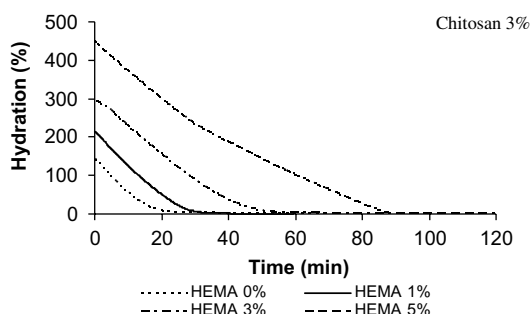come back to its dry form. These results suggest that the capacity to absorb is directly related to the crosslinking occurred during irradiation as reported by other authors with different polymeric matrices [16]. Another explanation may be due to the substantial number of chitosan free amino groups (also responsible for the hydrophilic nature of chitosan) blocked by the growing chains of pHEMA.

3.2. Thermal properties

The TGA thermal analysis was used to provide an indication of the changes in membrane structural network. Fig. 4 shows the thermograms of a γ-irradiated and a non-irradiated chitosan/pHEMA membrane. The behaviour presented was observed for all range of chitosan/HEMA content

Fig. 2. Dehydration behaviour of non-irradiated chitosan based membranes at 37 °C as function of time.

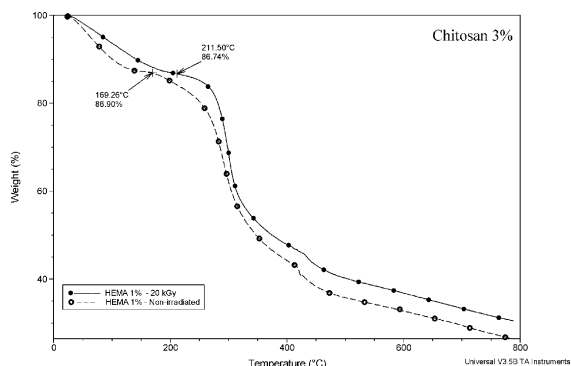Fig. 4. TGA thermograms of an irradiated and a non-irradiated chitosan/pHEMA membrane.

studied and shows an increase in the initial thermal decomposition temperature on the irradiated membranes (from 169.25 to 211.50 °C in chitosan 3%/HEMA 1%). However this improvement is more enhanced in membranes with higher chitosan and HEMA content possibly due to the formation of a more crosslinked network structure (in chitosan 3%/HEMA 5% it goes up from 172.65 to 230.36 °C). Simultaneously, with the increase in HEMA content membrane curves show an increasing similarity with the pHEMA profile curve. This has already been reported for heterogeneous systems [7] which suggest that the length of pHEMA branches grow with the increase of HEMA amount in membranes.

3.3. Fourier transform infrared analysis

The FTIR analysis was based on the identification of absorption bands concerned with the vibrations of functional groups present in the molecules. Since the irradiation procedure was done in the membrane form, no significant alterations were observed between irradiated and non-irradiated membranes.

3.4. Scanning electron microscopy observation

The SEM micrographs showing the surface morphology of the chitosan/pHEMA membranes before and after irradiation are presented in Fig. 5(a) and (b), respectively. It evidence the reduction of the porous structure due the crosslinking occurred during γ irradiation.

3.5. Microbiological assays

The results from the bioburden evaluation are present at Fig. 5 in colony forming unities (cfu). The data show that the number of contaminating microorganisms that naturally occur on non-irradiated samples is higher for membranes with minor chitosan content. This is in according with the antimicrobial chitosan properties mentioned in literature which reports an interaction between positive charges of chitosan with the electronegatively charged residues of macromolecules at the microorganism cell surface what causes the mem-
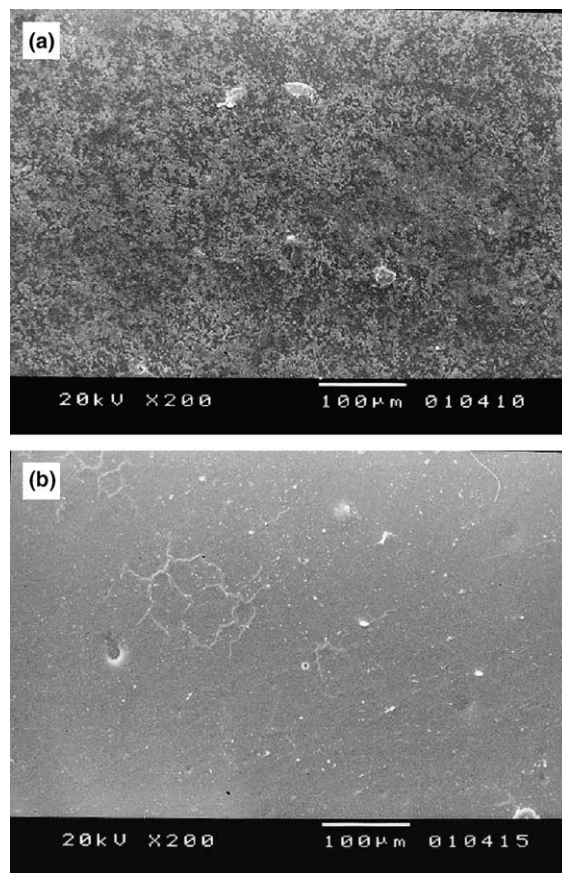

Fig. 5. SEM micrographs of chitosan (3%)/HEMA (1%) membranes surface: (a) before irradiation (b) after irradiation (20 KGy; dose rate of 0.6 kGy h^{-1}).

brane leakage [17]. Simultaneously to bioburden evaluation results, no bacterium was found on the TSA medium under the membranes pads. This evidences the biological activity of the prepared chitosan/pHEMA membranes which can be
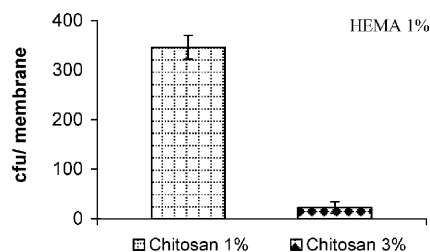

Fig. 6. Bioburden of non-irradiated chitosan/pHEMA mem-branes.

considered as presenting good barrier properties against microbes (Fig. 6).

4. Conclusions

To sum up one can tailor that modifications occurred at the chitosan/pHEMA membranes obtained by gamma irradiation improve its structural arrangement keeping the natural antimicrobial properties of chitosan. In this way, these membranes seem to be a very promising polymeric system to be used as matrix for biomedical applications. However attention must still be drawn to other properties such drug release kinetics and citotocixity, which will be the subject of near work before the realization of *in vivo* studies.

Acknowledgments

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