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Analytical and rheological studies of modified gel dosimeters exposed to X-ray beams☆

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ABSTRACT

The need to know the dose of X-rays to be applied to patients suffering diseases such as cancer requires accurate and stable dosimetric devices. Currently, the use of gelatin-based dosimeters has yielded excellent results but lack adequate thermal stability. In this paper a chemical modification of the gelatin (at concentrations typically used for the preparation of dosimeters) using glutaraldehyde as a cross-linking agent is proposed. Through rheological studies it was found that modified gelatin with glutaraldehyde concentrations between 0.15 and 0.50% w/v shows better thermal stability with an increase in elastic modulus of up to 100 times at 37 °C and convenient reaction times for the preparation of the dosimeters. Subsequently, a mathematical model to easily predict the elastic modulus of materials prepared with different concentrations of gelatin and glutaraldehyde was proposed. The analytical response of modified and unmodified materials was evaluated and no significant alteration of the dosimetric sensitivity was found in the dosimeters (based on itaconic acid and N, N′-methylenebisacrylamide) when an X-ray irradiation dose from 0 to 300 Gy was applied. It was found that the best thermal stability of dosimeters prepared with modified gelatin would decrease the loss of information between the irradiation process and the absorbance reading, thereby improving the stability and linear correlation of data.
Overall, the results indicated that the dosimeters could be modified as proposed and achieve significant improvements regarding to their thermal stability, without changing significantly the usual preparation process.

1. Introduction

Diagnostic radiology and radiotherapy help to detect and propose specific ways of treatment for patients and to effectively treat tumor areas. In these techniques, to know the absolute dose and its spatial distribution in a patient is vital and for that, there are different dosimetric tools. Dosimeters based on polymeric gels indirectly quantify the total absorbed dose after being exposed to ionizing radiation [1]. This is possible due to chemical changes that occur after irradiation, where free radicals formed from water molecules initiate a gelation process [2]. The coexistence of monovinyl- and divinyl-monomers capable of polymerizing produces three-dimensional networks (gels) which retain their initial spatial distribution for long periods thanks to the presence of a gelatin matrix [3]. This matrix preserves the dose distribution, minimizing the diffusion of the monomers and gels. One requirement is that these materials have to be similar to human tissues [4], regarding the response to ionizing radiation. Gelatin is normally used because it is an inexpensive material available from natural sources; also the preparation of these materials is simple and requires only equipment which is commonly available in a chemical laboratory. Gelatin is the denatured form of collagen, which is a structural protein widely distributed in the animal world. Collagen is composed of three polypeptide chains arranged in a triple helix structure by the presence of multiple hydrogen bonds [5]. When gelatin is dissolved in hot water, the denatured polypeptide chains can form a gel after the temperature decreases. This process can be attributed to the formation of intermolecular hydrogen bonds through the formation of infinite polypeptide networks. The balance between the formation of intermolecular hydrogen bridges that are responsible for network formation and intramolecular bonds that can return to the helical polypeptide structure is dependent on pH, temperature and concentration of the protein solution [6]. However, the stability of the gels may not be sufficient when the dosimeters are subjected to conditions of high room temperature, since the degradation of dose information may occur due to the rupture of the physically cross-linked structure of the gelatin matrix. To avoid this problem, one of the used strategies is a chemical cross-linking on the gelatin. Proteins

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that are chemically cross-linked present a significant improvement on their mechanical properties and are much more stable in aqueous media [7]. Among the various cross-linking agents that could form networks with gelatin, it is worthwhile mentioning glutaraldehyde, genipin and carbodiimide [8]. Glutaraldehyde is a widely available cross-linking agent and has been used in areas such as microscopy, biosensors, medicine, pharmacy and industry [9–10]. Glutaraldehyde is an almost colorless dialdehyde, that has high reactivity and low cost. It primarily reacts with the amino groups of the side chains of proteins around neutral pH. Although reactivity with lysine, phenylalanine, tyrosine, tryptophan, cysteine, histidine, proline, serine and glycine has been also reported, being the more reactive group the *e*-amino of lysine residues [11]. These polar amino acids are located on the surface of proteins and thus are readily accessible to glutaraldehyde. Furthermore, it has been shown that the products of the reaction of glutaraldehyde with the amino groups have good stability to extreme temperatures. However, the concentrations of protein and glutaraldehyde must be carefully controlled for an optimum cross-linking [12]. Therefore, a strict control of the reaction conditions must be kept to obtain a reproducible response, due to the structural variability of proteins. One way to follow the crosslinking reaction of macromolecules is by studying the changes of their rheological properties [13]. The rheological behavior of protein solutions depends on the molecular weight, the conformation of the molecule and solvent conditions. Rheology is a method widely used to study the process of gel formation. For example, it has been used in reactions where the main goal was to improve the mechanical properties of hyaluronic acid [14]. In these assays, the sample is subjected to an oscillatory deformation with constant amplitude and constant frequency and the response of the material is measured. The response of viscoelastic materials can be decomposed into two components, one in phase (G') and the other out of phase (G'') with the oscillatory deformation, which correspond to elastic and viscous contributions, respectively. Viscoelastic samples show a phase shift between 0 and π/2. The elastic modulus of a hydrogel depends on the cross-linking degree and the charge density in the polymer network, and thus on the concentration or fraction of cross-linked polymer material after the preparation of the hydrogel [15]. Although there are several theories to predict the elastic modulus and the swelling index of cross-linked materials, the correlation between predictions and experimental data is only qualitative. Furthermore, experimental results show that the elastic modulus of some hydrogels corresponds to a potential functionality of the form presented in equation 1 [16] and not to the linear dependence predicted by elasticity theory of rubber materials [17–18]:

$$G' \propto (\varphi_0)^x \tag{1}$$

with $x = 2.1 \pm 0.1$.

In this study, a chemical modification of a gelatin matrix is proposed in order to improve its thermal stability using concentrations and conditions commonly used for dosimetric applications. Furthermore, the analytical response to X-ray irradiation of the modified dosimeters is evaluated. For this purpose, it is proposed to modify the gelatin using glutaraldehyde as the cross-linking agent. Then, the dosimetric response and the rheological properties of modified and unmodified dosimeters are analyzed. For a more complete interpretation of the experimental results, a mathematical model is presented. Experimental and mathematical modeling results obtained in this study will allow preparing dosimeters with modified gelatin resulting in a higher thermal stability without significant changes on the dosimetric response to X-ray radiation.

2. Experimental

2.1. Materials

The following chemicals were purchased: N, N′-methylenebisacrylamide (BIS), itaconic acid (ITA) (≥99% purity), glutaraldehyde (GTA) (50 wt.%, in water; density: 1.106 g/mL), from Sigma-Aldrich. Pigskin gelatin (300 Bloom) was obtained from FLUKA and tetrakis hydroxymethyl phosphonium chloride (THPC) from Sigma-Aldrich. Sodium phosphate monobasic and sodium phosphate dibasic with analytical grade were obtained from Anhedra. The buffer solution was prepared with equimolar amounts of both sodium phosphates monobasic and dibasic in water (0.1 M of each).

2.2. Modification of gelatin

The gelatin powder (5.13 g) was added to the buffer solution (100 mL) in a beaker with magnetic stirring at 400 rpm at room temperature and heated up to 50 °C with a hot plate for 30 min. Then, the temperature was decreased to 37 °C maintaining the stirring. Under these conditions, gelatin remained as a low viscosity liquid.

Glutaraldehyde solution was prepared adding different quantities of concentrated GTA (50 wt.%) to buffer solution. Subsequently, an aliquot of gelatin solution was mixed with an aliquot of glutaraldehyde solution (GTA), as shown in Table 1. The final concentrations of GTA used for the modification of gelatin are also presented in this table. Gelatin–GTA mixture was prepared in this manner for all subsequent tests.

2.3. Effect of GTA on gelatin elasticity at different temperatures

To study the effect of GTA on the gelatin elasticity at different temperatures a sample of gelatin solution at 37 °C was rapidly mixed with different amounts of GTA, as depicted in Table 1. An aliquot of the mixture (500 μL) was immediately placed in the inner plate of a circular geometry of 25 mm diameter of an Anton Paar MCR 301 rheometer. During this process, the gap between the plates was set to 20 mm and immediately after loading the sample, the gap was reduced to 1 mm, lowering the upper geometry. Subsequently, a closed chamber was placed to prevent evaporation of the sample to the environment during the assay. For each sample, the elastic modulus (G'), viscous (G'') and tan delta ($tan\ \delta = G''/G'$) of the material was measured from 4 to 65 °C using a temperature ramp of 3 °C min^{-1}, at constant strain of 1% and frequency of 1 Hz. Each sample was studied by triplicate.

2.4. Kinetics of elasticity of modified gelatin with glutaraldehyde

To perform this study a sample of gelatin solution at 37 °C was rapidly mixed with different amounts of GTA (0, 5.0×10^{-2}, 1.0×10^{-1}, 1.5×10^{-1}, 5×10^{-1} and 1.5×10^{0}% w/v) as depicted in Table 1. An aliquot of the mixture (500 μL) was immediately placed in the inner plate of a circular geometry of 25 mm diameter of an Anton Paar MCR 301 rheometer. During this process, the gap between the plates was set to 20 mm and immediately after loading the sample the gap was reduced to 1 mm, lowering the upper geometry. Subsequently, a closed chamber was placed to prevent evaporation of the sample to the environment during the assay. For each sample, the elastic modulus (G'), viscous (G'') and tan delta ($tan\ \delta = G''/G'$) of the material were studied using a constant strain of 1% and frequency of 1 Hz for 120 min at 37 °C.

Table 1
Modification of gelatin with glutaraldehyde.

Test	Gelatin solution mL	GTA solution μL (% w/v)[a]
0	1.00 ± 0.01	20.0 ± 0.2[b] (0)
1	1.00 ± 0.01	20.0 ± 0.2 (5.0×10^{-4})
2	1.00 ± 0.01	20.0 ± 0.2 (5.0×10^{-3})
3	1.00 ± 0.01	20.0 ± 0.2 (5.0×10^{-2})
4	1.00 ± 0.01	20.0 ± 0.2 (1.0×10^{-1})
5	1.00 ± 0.01	20.0 ± 0.2 (1.5×10^{-1})
6	1.00 ± 0.01	20.0 ± 0.2 (5.0×10^{-1})
7	1.00 ± 0.01	20.0 ± 0.2 (1.5×10^{0})

[a] Final concentration of GTA in the sample.
[b] **V**olume of buffer added.