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ELECTRON BEAM IRRADIATION AND ADDITION OF POLY(VINYL ALCOHOL) AFFECT GELATIN BASED-FILMS PROPERTIES

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ABSTRACT

Gelatin is a mixture of high molecular weight polypeptides, product of denaturation, and partial structural degradation of collagen, and one of the first materials employed as biomaterials. Aqueous solutions of gelatin (10%), glycerin as plasticizer and poly(vinyl alcohol) (PVA) up to 10% were prepared in a water bath at 70 °C under constant stirring. Films were irradiated with 10 and 20 kGy using an electron beam accelerator, dose rate of 22.4 kGy s^{-1}, energy 1.407 MeV, at room temperature, in the presence of air. After irradiation, mechanical properties, color measurements, water absorption, moisture and film solubility were analyzed. The films showed an improvement in maximum force to rupture the film with increase of the irradiation dose. The higher the puncture force to rupture the lower the elongation at break. Colorimetric tests showed significant differences between samples, and also differences depending of the applied radiation dose, and analyzed color parameter. In water absorption tests a decrease of absorption percentage was found with the increase of the dose for PVA free and 5% PVA samples. The addition of PVA increased the water absorption for all applied doses. The modifications in gelatin colloids can be appointed to radiation-induced crosslinking. Also, the PVA concentration in the samples influenced the resultant material properties.

Keywords: electron beam irradiation, gelatin, poly(vinyl alcohol).

1. INTRODUCTION

Gelatin is a heterogeneous mixture of water-soluble proteins with high average molecular weight deriving from thermal denaturation, and hydrolytic action from collagen. Commercially, this protein comes from mammals' external protective tissues, by boiling skin, tendons, ligaments, bones, etc., with water. It swells up and absorbs 5-10 times its weight of water to form a gel in solutions below 35-4 °C. It is presented as colorless or slightly yellow, transparent, brittle, practically odorless, tasteless sheets, flakes or coarse powder.

The properties of gelatin in aqueous solutions and in the gel state have been extensively studied but they still attract great attention due to the wide range of applications of gelatin colloids, and matrices mainly in the food industry, and film-forming properties. Their uses include not only food (confectionery, jellies, ice cream) and pharmaceutical technology but also manufacturing of rubber substitutes, adhesives, photographic plates, and films, matches, and clarifying agents [1]. Gelatin is produced abundantly, has a relatively low cost, and possesses excellent functional properties.

Biopolymers like proteins and polysaccharides have the capability to produce flexible films with biodegradable character, being gelatin one of the first materials employed in the

biomaterial arrangements [2,3] and has been the focus of many patents. For this reason, gelatin has been studied in film technology both alone and in blends with other biopolymers [4,5].

Poly(vinyl alcohol) (PVA) is a particular carbon-carbon backbone polymer with biodegradable properties under different environment that could be a possible solution to improve mechanical characteristics of protein based-films, under both aerobic and anaerobic conditions [4,6,7]. PVA is produced and widely used in the industrial field, attracting attention as a water-soluble biodegradable polymer as well as a biodegradable segment in the polymer chain. PVA is a non-toxic, water-soluble, synthetic polymer, which has been employed in biomaterial technology for the pharmaceutical and biomedical areas due to its excellent film forming, emulsifying, and adhesion properties [8]. Despite its synthetic character, this polymer is recognized as biodegradable [9]. There are reports about ways to develop biodegradable polymer-related PVA, such as incorporation of a vinyl alcohol block into a non-biodegradable functional polymer chain, grafting functional oligomers into the PVA chain, and modification of the PVA chain.

In the present work, gelatin films containing up to 10% PVA were irradiated with ionizing radiation coming from an electron beam accelerator. After irradiation, texture analysis, color measurement, and water absorption tests were carried out in order to investigate radiation effects.

2. EXPERIMENTAL

2.1. Materials

Bovine Gelatin type B, 240 Bloom/10 mesh, glycerol PA ACS, cod. 15375, and poly(vinyl alcohol) (PVA) were supplied by Gelita do Brasil Ltda, Casa Americana de Art. Lab. Ltda (CAAL), and Pharma Special, respectively.

2.2. Preparation of Gelatin Films

Gelatin aqueous solutions were prepared by dissolving bovine skin gelatin (10% w/w), glycerol (5% w/w) in distilled water, and PVA in different percentage: 0, 5, and 10% in a water bath at 70 °C/30 min., PVAf, PVA 5%, and PVA 10%, respectively. Gelatin based solutions were cast on flat glass plate for film formation, and then dried for 48 h under air-circulating oven at 25.0 ± 0.1 ºC, and 50 ± 5% RH.

2.3. Irradiation of Gelatin Based-Films

Gelatin based-films, and added of PVA 5% and 10% were irradiated at 10 and 20 kGy, using an electron beam (EB) accelerator (Dynamitron II, Radiation Dynamics Inc.), at room temperature, in the presence of air, dose rate 22.4 kGy s^{-1}, energy 1.407 MeV, beam current 5.4 mA, tray speed of 6.72 m min^{-1}. Dosimetry was carried out with cellulose triacetate film dosimeters “CTA-FTR-125” (Fuji Photo Film Co. Ltd).

2.4. Mechanical Properties of Films

The mechanical properties of the gelatin based-films were determined by puncture tests through a Texture Analyzer of Stable Micro Systems, TA-XT*Plus*, with a load cell of 50 kg. The measurements of puncture strength (force to rupture) and elongation at break of gelatin-based films, and added of PVA 5%, and 10% were performed using a film support rig (HDP/FSR), and a stainless steel spherical probe (P/0.5S). The test speed was 0.5 mm s^{-1}, and distance of 15 mm.

2.5. Water Absorption

To evaluate the water absorption of gelatin based-films and added of PVA 5% and 10%, specimens (10 x 10 mm) were weight at t = 0 (Mi) and immersed in distilled water, and storage at room temperature for 24 h. At the end of 24 h, the specimens were removed from the water, all surface water wiped off, and weighed (Mf). Tests were performed in triplicates. The water absorption (W) percentage was obtained by equation (1) as follows:

$$\mathbf{W\ (\%) = ((Mf - Mi)/Mi))\ x\ 100} \quad (1)$$

2.6. Moisture Content

Moisture contents of gelatin based-films were determined by measuring the weight loss of specimens (10 x 10 mm), upon drying under air-circulating oven at 105 ± 1 °C until constant weight. The tests were performed in triplicate.

2.7. Film Solubility

Gelatin based-films specimens (10 x 10 mm) were weighed, and dried under air-circulating oven at 105 ± 1 °C for 24 h. Then, the samples were placed in beakers with 50 mL of distilled, and stored at room temperature for 24 h with occasional gentle stirring. After 24 h, the undissolved film were dried under air-circulating oven at 105 ºC for 24 h, and weighed. Film solubility was calculated by the equation (2):

$$\mathbf{FS\ (\%) = ((Wi - Wf)/Wi)\ x100} \quad (2)$$

where Wi was the initial weight of the film expressed as dry matter and Wf was the weight of the undissolved dried film residue. The tests were performed in triplicate.

2.8. Color Measurement

For color measurement, the films were placed on the surface of white standard plate, calibration plate CR-A43, and the color value of L^* (lightness), a^* (greenness), and b^* (yellowness), were measured by Chroma meter CR-400 (Konica Minolta Camera Co.,

Osaka, Japan). The color coordinates ranges were: L^* (0 black to 100 white), a^* (-green to +red), and b^* (-blue to +yellow).

2.9. Statistical Analysis

The data were analyzed using one-way analysis of variant (ANOVA) by means of a Statgraphics Plus 3.1 (Statistical Graphics Corp.). Post hoc analysis was performed by Fisher's LSD test. For all statistical tests the level of significance was set at $P \leq 0.05$. Data are presented as mean ± standard deviation.

3. RESULTS AND DISCUSSION

Present work presents data on using electron beam irradiation for the preparation of gelatin/PVA films, instead of gamma-rays irradiation as other groups had already applied. The mechanical properties of the irradiated gelatin based-films are presented in Tab. 1; i.e., puncture strength and elongation at break. The puncture strength showed its maximum force to rupture the film at the highest dose applied. There was significant difference ($P \leq 0.05$) between samples free of PVA (PVAf) and those containing 10% PVA on the strength improvement with the increase of the dose. The puncture strength improvement with the increase of radiation dose had already detected in similar systems [7]. The elongation at break of the gelatin-base films decreased with the increase of the radiation dose, when comparing PVAf and 5% PVA (Tab. 1). The higher the puncture strength, the lower the elongation at break. Other researchers [10] analyzed gamma cured gelatin-PVA blend films and found that 5% PVA containing gelatin films irradiated with dose under 1 kGy showed the highest tensile strength.

Table 1: Film puncture strength and elongation at break of gelatin based-films with 0 (PVAf), 5 and 10% of PVA irradiated at 10 and 20 kGy.

Properties	Samples	Dose (kGy)		
		0	10	20
Puncture Strength (N)	PVAf	70 ± 10^{Cc}	76 ± 9^{CBa}	78 ± 11^{ABb}
	5%PVA	82 ± 8^{Aa}	77 ± 9^{ABa}	65 ± 7^{Cc}
	10%PVA	81 ± 7^{Aba}	82 ± 8^{Aa}	84 ± 9^{Aba}
Elongation at break (%)	PVAf	54.5 ± 0.7^{Ab}	51.0 ± 0.7^{Bc}	48.3 ± 0.4^{Cc}
	5%PVA	58.3 ± 0.5^{Aa}	58.1 ± 0.5^{ABa}	52.4 ± 0.3^{Cb}
	10%PVA	53.7 ± 0.4^{Abc}	53.6 ± 0.4^{Ab}	54.3 ± 0.6^{Aab}

Means in the same line with different capital letters and means in the same column with different lower case letters are significantly ($P \leq 0.05$) different according to LSD test.

Krochta & De Mulder-Johnston [3] considered that gelatin based-films present good mechanical resistance, despite their reduced water vapor barrier. These films are highly susceptible to absorb water from the environment at room temperature and relative humidity

conditions due to the hydrophilic nature of gelatin. Thus, an increasing of the temperature and/or of the relative humidity may reduce the mechanical resistance, and increase the extensibility of this material, for instance. This behavior leads to difficulty in food packaging applications. Several alternatives have been studied to minimize this problem, such as chemical or enzymatic modifications of the gelatin, the use of plasticizer blends, or different plasticizers with different hydrophilicity, and the incorporation of lipids, amongst others. However, these results have not necessarily been satisfactory. The use of ionizing radiation, on the other hand, seems to be one of the most promising tools on this matter [7].

The water absorption, moisture content, and film solubility of films containing 5, and 10% of PVA showed different behavior according to the applied doses (Tab. 2). The water absorption decreased with the increase of radiation dose for PVAf and 5% PVA samples assayed in this work, but not for the 10% PVA samples. Other group [11] found too that the degree of swelling of a gamma-irradiated gelatin-PVA hydrogel was inversely proportional to the gel content and strength. The addition of PVA increased the water absorption of samples, but there were a dose radiation effect ($P \leq 0.05$) for PVAf and 5% PVA. The decrease in water absorption can be ascribed to radiation-induced crosslinking. Salem et al. [12] observed that the use of gelatin-PVA blend, and addition of MWCNT (multiwall carbon nanotubes) enhanced the swelling behavior with time. Cataldo et al. [13] had reported that they induced crosslinking of collagen gelatin derivative from porcine skin, and transformed it into a stable permanent hydrogel by γ-radiation. Using also Co-60 gamma radiation other group obtained crosslinked copolymers of gelatin, and PVA with excellent water absorption, and water retention abilities [14]. Using also gamma irradiation, an improvement of both durability, and mechanical integrity was found when nanoparticles of Ag, gelatin, and PVA hydrogels were prepared [15].

Table 2: Water absorption, moisture content and film solubility of gelatin based-films with 0 (PVAf), 5 and 10% of PVA irradiated at 10 and 20 kGy.

Properties	Samples	Dose (kGy)		
		0	10	20
Water absorption (%)	PVAf	247 ± 2^{Ac}	243 ± 24^{Ac}	239 ± 14^{Ac}
	5%PVA	349 ± 25^{Ab}	328 ± 16^{Bb}	313 ± 10^{CBb}
	10%PVA	370 ± 7^{Cab}	416 ± 14^{Aa}	381 ± 9^{Ba}
Moisture content (%)	PVAf	19.1 ± 0.9^{Aa}	17.1 ± 0.5^{Cb}	17.4 ± 1.0^{BCc}
	5%PVA	16.1 ± 0.6^{Cc}	17.4 ± 0.7^{Bba}	17.6 ± 0.6^{ABc}
	10%PVA	16.4 ± 0.4^{Bbc}	15.9 ± 1.0^{BCc}	18.5 ± 0.2^{Ac}
Film solubility (%)	PVAf	39.5 ± 0.8^{Cb}	42.6 ± 0.7^{Ba}	42.6 ± 0.7^{ABa}
	5%PVA	39.7 ± 0.6^{Aba}	38.6 ± 0.4^{Ab}	39.5 ± 1.0^{Ab}
	10%PVA	37.4 ± 0.6^{Bc}	36.9 ± 0.6^{BCc}	38.4 ± 0.5^{Ac}

Means in the same line with different capital letters and means in the same column with different lower case letters are significantly ($P \leq 0.05$) different according to LSD test.

Strauss & Gibson [16] used plant phenolics as crosslinkers - instead of ionizing radiation - of gelatin gels, and gelatin-based coacervates for use as food ingredients. Gels crosslinked by

these means showed to have greater mechanical strength, reduced swelling, and fewer free amino groups. Dynamic light scattering analyses showed that such crosslinking results in denser polymeric networks. These properties of crosslinked gelatin gels, and gelatin-based coacervates have applications for the development of novel food ingredients.

According to Audette-Stuart el al. [17] when proteins are irradiated in bulk liquid phase, most of the damages to polypeptide chains are associated with hydroxyl radicals that are responsible for most of the polymerization, and at least some of the fragmentation observed. Vieira & Mastro [18] studied the effect of two different ionizing radiation sources, a γ-radiation from a ^{60}Co source, and electron beam irradiation, on gelatin protein fragmentation in aqueous media. The relationship between the decrease in viscosity of gelatin solutions, probably due to protein fragmentation, and radiation dose presented comparable values for both irradiation processes. Present results could show a predominance of polymerization instead of fragmentation phenomena that can be ascribed to the fact that the samples were irradiated as aqueous gelatin films also containing glycerin as plasticizer.

Results of the colorimetric tests are presented in Tab. 3, which shows the changes in gelatin based-films color related to the radiation dose applied to the different samples. All samples presented a significant difference between non-irradiated, and irradiated (0 and 20 kGy) samples for *L** (lightness). The addition of PVA (5 and 10%) did not affect the *L** significantly for all studied doses. The *a** factor (greenness) increased significantly ($P \leq 0.05$) with increase of radiation dose for all samples, however, decreasing as increased the PVA percentage. For *b** (yellowness) the samples presented a significant difference between the non-irradiated, and irradiated (10 and 20 kGy) gelatin based-films. Furthermore, the addition of PVA reduced significantly the *b** of irradiated samples as shown at 10 kGy, e.g., PVAf (6.70 ± 0.63) compared to 10% PVA (5.98 ± 0.29). Changes in color properties must be take into account for the development of new irradiated materials.

Table 3: Color measurements of EB irradiated gelatin based-films with 0 (PVAf), 5 and 10% of PVA.

Color parameter	Samples	Dose (kGy)		
		0	10	20
*L**	PVAf	96.64 ± 0.39^{Aa}	96.36 ± 0.43^{ABa}	96.22 ± 0.38^{CBa}
	5%PVA	96.54 ± 0.18^{Aa}	96.45 ± 0.16^{ABa}	96.27 ± 0.12^{Ca}
	10%PVA	96.46 ± 0.23^{Aa}	96.44 ± 0.22^{ABa}	96.27 ± 0.18^{Ca}
*a**	PVAf	-0.42 ± 0.07^{Ac}	-0.96 ± 0.13^{Bc}	-1.18 ± 0.26^{Cc}
	5%PVA	-0.36 ± 0.04^{Ab}	-0.59 ± 0.12^{Bb}	-0.80 ± 0.09^{Cb}
	10%PVA	-0.14 ± 0.04^{Aa}	-0.41 ± 0.07^{Ba}	-0.73 ± 0.06^{Cab}
*b**	PVAf	4.43 ± 0.23^{Cc}	6.70 ± 0.63^{Ba}	8.37 ± 1.16^{Aa}
	5%PVA	4.74 ± 0.18^{Ca}	6.06 ± 0.59^{Bb}	7.30 ± 0.57^{Ac}
	10%PVA	4.60 ± 0.16^{Cb}	5.98 ± 0.29^{Bcb}	7.72 ± 0.52^{Abc}

Means in the same line with different capital letters and means in the same column with different lower case letters are significantly ($P \leq 0.05$) different according to LSD test.

The molecular mechanisms that control functionality in gelatin films are poorly understood. Molecular mobility in water, and glycerol plasticized cold, and hot-cast gelatin films were also studied by some authors [19]. They found that physical crosslinks actually increased the molecular mobility of gelatin on the millisecond time scale, and water, and glycerol, despite their similarity as hydrogen-bonding molecules had quantitatively different effects on gelatin mobility.

A promising application of gelatin is as an edible coating. The literature reports a meat shelf life extension through the use of collagen/gelatin coatings that showed to reduce oxygen, moisture, and oil migration, besides being able to carry antioxidant or antimicrobial agents [20]. This coating can acts as a barrier to water, and oxygen, thereby reducing purge, color deterioration, and aroma deterioration [21]. Collagen itself found commercial success as a protein coating because, even though it is not a good moisture barrier, it is a great oxygen barrier. Collagen also melts when the meat is cooked. Gelatin, the partially denatured product of collagen, can be used to both reduce oxygen, and moisture migration, and as an antioxidant carrier [22]. Gelatin showed to be a better moisture barrier than collagen [6], but it is commonly used as a food additive to improve the texture and increase the water-holding capacity of foods.

4. CONCLUSION

From the behavior of the irradiated gelatin films used in the present study, it can be concluded that radiation affects water absorption of gelatin-based films as it decreased with the increase of electron beam radiation dose, suggesting radiation-induced crosslinking. Puncture strength showed its maximum force to rupture with the highest dose applied. The deformation remained almost constant when 10% PVA was employed, but not for samples PVA free, and containing 5% PVA. There was also some effect of ionizing radiation on color lightness and yellowness. The way ionizing radiation and the PVA content affect these films must be taken in account whenever gelatin derivative materials were employed in a biomaterials arrangement.

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