Irradiation of Gelatin. Important Applications for the Development of New Materials

Nélida Lucia del Mastro

Instituto de Pesquisas Energéticas e Nucleares IPEN-CNEN/SP
Av. Prof. Lineu Prestes 2242, 05008-900, Sao Paulo, SP, Brazil
nlmastro@ipen.br

Abstract

Gelatin is obtained from a naturally occurring protein, collagen, by chemical and thermal hydrolysis. As a protein, gelatin is biodegradable. Among biomaterials, gelatin is an interesting material because the dehydrated gelatin is a partially crystalline polymer and has a relatively low melting point. Gelatin melts to a liquid when heated and solidifies when cooled again. Together with water, it forms a semi-solid colloid gel. If gelatin is placed in contact with cold water, some of the material dissolves, their solubility being determined by their manufacturing method. Ionizing radiation acting on aqueous biological systems produces labile intermediates. Macromolecular free radicals may be diffusionally mobile in homogenous solutions but relatively immobile in gelled systems. In this paper, different works, some of them performed in our laboratory, are firstly described showing gelatin irradiation in diverse systems. We have already studied the mechanical properties of gelatin composites prepared with a natural fiber, plasticizer and treated by electron beam irradiation. Departing from that, the range of novel applications for gelatin composites like gelatin nanoparticles as biodegradables and low cell toxic alternative carrier delivery systems are outlined. The potential and the possibilities of using gelatin irradiation for important applications for the development of new materials for medical and food industry are presented.

1. INTRODUCTION

Gelatin or gelatine is a heterogeneous mixture of water-soluble proteins of high average molecular mass not found in nature but derived from the hydrolytic action of collagen - a protein of mammal external protective tissues - by boiling skin, tendons, ligaments, bones and so on with water [1]. There is evidence that gelatin has health-promoting properties like reinforcing resistance of the stomach mucous tunic to stress action and promoting general joint health. It is commonly used as a biological substrate to adherent cell culture and as an ingredient in implantable medical devices.

Although rich in glycine, proline and hidroxyproline, gelatin is, in terms of nutrition, an incomplete protein lacking tryptophan and containing but small amounts of other important

amino acids. But the natural protein gelatin contains glycine and proline in a concentration that is around 10 to 20 times higher than in other proteins. These amino acids perform the important function of building up connective tissue. An insufficient supply of these amino acids can make itself known in the form of painful joints as well as brittle fingernails and hair.

Gelatin is most commonly used as a stabiliser, thickener, or texturiser in foods such as ice cream, jams and yoghurt, and it is also used to improve the mouthfeel of various products. It swells up and absorbs 5-10 times its weight in water to form a gel in solutions below 35-40°C. Commercially, gelatin is presented as colorless or slightly yellow, transparent, brittle, practically odorless, in the shape of tasteless sheets, flakes or coarse powder. Its 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 [2][3].

Gelatin forms a semi-solid colloid gel or hydrocolloid. Depending on the quantity of water available, it occurs in different states, e.g., gel or sol (liquid). Colloidal particles are larger than molecules but too small to be observed directly with a microscope; however, their shape and size can be determined by electron microscopy. Although there are no precise boundaries of size between the particles in mixtures, colloids, or solutions, colloidal particles are usually on the order of 10-7 to 10-5 cm in size; that is, on the order of nanoparticles. As different forces play important roles in the interaction of colloid particles: excluded volume repulsion, electrostatic interaction, Van der Waals forces, entropic forces and steric forces, there are huge possibilities of reaction for gelatin colloid gels.

To improve some desirable properties, and for economical reasons, two or more polymers can be mixed to form polymeric blends. Nano-hydrogels made of bio-compatible hydrophilic polymers can be used in various medical applications such as drug delivery and imaging. Gelatin, we believe, can be included in the list of important natural polymers to be used for the preparation of blends and composites for a wide range of applications.

2. CROSSLINKING, POLYMERIZATION AND CLEAVAGES USING RADIATION TECHNOLOGY

Irradiation of proteins in diluted liquid aqueous solutions had shown to produce cleavages and polymerizations of the peptidic chains. In frozen solutions, fragmentation is observed but polymerization products are absent. Loss of activity occurs in both cases. Electrons within the water molecules closely associated with the protein were considered involved in the processes leading to protein fragmentation [4].

Gamma or electron beam (EB) irradiation are capable of inducing intermolecular crosslinking in different systems [5]. Some authors compared the effects of ultraviolet (UV) and EB radiation on the properties of cured nanocomposite coatings [6]. They found an increase in crosslinking as the radiation dosage increased.

There are reports in the literature about the action of ionizing radiation on gelatin films even before the concept of crosslinking was well understood [7]; some authors, for instance, have focused on the differences found in -COOH and $-NH_2$ content [8]. Radiation-induced

crosslinking on gelatin hydrogel has been described already [9], as well as radiation-induced crosslinking in gelatin solutions [10].

In our laboratory we have made a comparison between the effects of gamma and EB on gelatin. In that work, bovine powder gelatin was submitted to γ-radiation from a ^{60}Co source, dose rate about 7 kGy/h and to EB irradiation, dose rate about 11 kGy/s. The doses applied were up to 50 kGy. The radiation effects were measured following viscosity changes at 40°C in 10% gelatin aqueous solutions. The relationship between the decrease in viscosity of gelatin solutions and the applied radiation dose were comparable for both irradiation processes [11].

In another study, we focused on the effects of ionizing radiation on some gelatin-carrageenan aqueous systems and found anomalous rheological behavior [12]. The comparison of viscosity patterns upon irradiation of gelatin and carrageenan, whether alone or together, suggested the formation of a protein-polysaccharide complex.

Ionizing radiation has shown to be specially useful for the preparation of proteic nanoparticles using the thermosensitive aggregation of a protein and gamma-rays crosslinking [13]. Besides irradiation, natural crosslinking of gelatin using a simple plant-derived phenolic compound caffeic acid has been studied by Kosaraju & Puvanenthiran [14]. Those authors reported a thermo-irreversible gelatin formation at 60°C, and by controlling the crosslinking reaction, they obtained gelatin with modified material properties.

Wang et al. [15] used both, radiation-induced polymerization and a crosslinking agent, a hydrophilic monomer, for the fabrication of hydrogels with well-defined structure and high mechanical strength. In this case, the hybrid gels combine the well-developed structure of biological jellyfish gel and the unique microstructure of the synthetic gel produced by the radiation method, and strong interactions between the two networks were formed. On the other hand, it was described that cross-linked gelatin was used to prepare a coating against corrosion and microbial adhesion [16].

3. DEVELOPING NEW MATERIALS

Among plastic materials for the food industry and pharmaceutical coatings under worldwide development, blends or polymer complexes of edible polymers appear as very promising.

At our laboratory, the changes in gelatin/vegetal fiber/acrylamide composite treated by EB irradiation started to be studied [17]. In another work, we investigated the behavior of gelatin films containing glycerin as plasticizer and poly vinyl alcohol (PVA) in different percentages. After EB irradiation, the films showed an improvement in maximum force to rupture with the increase of the irradiation dose. On the other hand, the PVA addition decreased the maximum force values. We ascribe the modifications to radiation-induced cross-linking, the PVA concentration being also responsible for the resulting material properties [18]. Almost at the same time, another group used gamma-rays to prepare gelatin-PVA blend films and measured some mechanical properties. They concluded that gelatin-PVA blends modified with gamma radiation

had good potential to improve the mechanical and thermal properties of gelatin films to develop new materials combining the desirable properties of both natural and synthetic polymers [19].

There are many different examples of biodegradable polymers prepared for drug delivery or as carriers of active agents [20][21]. In 2004, Zwiorek et al. [22] have shown that cationized gelatin nanoparticles had the potential to be a new effective carrier for nonviral gene delivery. They considered that the major benefit of gelatin nanoparticles was not only their very low cell toxicity, but also their simple production combined with low costs and multiple modification opportunities offered by the matrix molecule. More recently, it was reported that gelatin nanoparticles have been prepared by nanoprecipitation in the presence of an emulsifier [23] or gelatin microparticles by using water-in-water emulsification technique in which aqueous solutions of gelatin and polypropylene glycol (PEG) were employed as dispersed phase and continuous phase, respectively [24].

Intravenously introduced hydrogel-drug conjugate (10 - 200 nm particles) can be effectively accumulated in tissues/organs by prolonged circulation and can be selectively transported into tumor tissues by the enhanced permeability and retention effect. Al-Sheikhly [25] is investigating the radiation-induced synthesis of functionalized polymer nano-hydrogels that can serve as targeted nano-medicine carriers. He is also able to synthesize the nano-gel structure using pulsed electron beam irradiation at high repetition rates, which gives rise to a high intra-chain yield of multiple free radicals. He considers that these free radicals enhance the intra-crosslinking reactions leading to the formation of smaller sized nanogel molecules.

In the trend of novelties, few months ago some authors [26] reported the preparation and characterization of gelatin mediated silver nanoparticles by laser ablation. Also, the use of gelatin as a biorganic reductant, ligand and support for palladium nanoparticles was announced, considering the characteristics of gelatin as a naturally occurring, safe, edible and cheap support [27].

Recently, it was shown that it is possible to develop gelatin microspheres prepared by the emulsification/crosslinking method to be used as carriers for intra-articular administration [28]. Hao et al., [29] have shown that in gelatin microspheres prepared through emulsion chemical-crosslinking method, drug loading increased as loading capacity was increased, but drug encapsulation efficiency tended to rise sharply and then drop.

4. CONCLUSION

Radiation crosslinking is a well established technology with a wide range of applications. As a water-soluble polymer, gelatin can be cross-linked in aqueous solutions to produce gels directly usable in biomedical applications or food coatings. Polymer blends or composites that included gelatin can combine and improve properties of different polymers. Therefore, the use of irradiated gelatin is open to many future developments in various fields.

REFERENCES

1. Windholz, M, 1976. The Merck Index. 9th Edition. Merck & Co, Inc. Rahway, NJ, 4217.
2. Anonymous. "*Gelatin"*. In: Krochwitz J.I., Howe–Grant M. editors. Encyclopedia of Chemical Technology. **11**, 3rd ed. John Wiley & Sons, New York, USA, 711-729 (1980).
3. Jones, N.R. *Uses of gelatin in edible products*. In: Ward A.G., Courts A. editors. The Science and Technology of Gelatin. Academic Press, New York, USA, 365-370 (1997).
4. Audette-Stuart, M.; Houee-Levin, C.; Potier, M. "Radiation-induced protein fragmentation and inactivation in liquid and solid aqueous solutions. Role of OH and electrons". *Radiation Physics and Chemistry*, **72**, p. 301–306 (2005).
5. Lappan, U.; Uhlman, S. "Intermolecular crosslinking of Poly(acrylic acid) in aqueous solution by electron beam irradiation". *J. Applied Polymer Sc.,* **119**, p. 3113-3116 (2011).
6. Salleh, N.G.N.; Yhaya, M.F.; Hassan, A. et al. "Effect of UV/EB radiation dosages on the properties of nanocomposite coatings. *Radiat. Phys. Chem.*, **80**, p. 136-141 (2011).
7. Orlova, M.A.; Nikolskaya, I.I.; Golubtsov, I.V.; Kasanskaya, N.F. "On the action of ionizing radiation on gelatin". *Radiation Effects and Defects in Solids*, **85**, p. 143-149 (1984).
8. Pruzak, L.P.; Sciarrone, B.J. "Effects of ionizing radiation on two gelatin fractions. I. Material preparation, dosimetry, and acid-base behavior". *J. Pharmaceutical Sc.*, **51**, p. 1046-1050 (1962).
9. Bessho, M; Kojima, T.; Furuta, M. et al. "Radiation-induced cross-linking of gelatin hydrogel by using γ-rays". *Nippon Kagakkai Koen Yokoshu*, **86**, 540 (2006).
10. Cataldo, F.; Ursini, O.; Lilla, E.; Angelini, G. "Radiation-induced crosslinking of collagen gelatin into a stable hydrogel. *J. Radioanalytical and Nuclear Chemistry*, **286**, p. 125-131 (2010).
11. Vieira, F.F.; Mastro, N.L.d. "Comparison of gamma-radiation and electron beam irradiation effects on gelatin". *Radiat. Phys. Chem.*, **63**, p. 331-332 (2002).
12. Aliste, A.J.; Mastro, N.L.d. "Anomalous Rheological Behaviour of Gelatin-Carrageenan-Water System Induced by Ionizing Radiation". *Molecular Crystals and Liquid Crystals,* **448**, p. 781-787 (2006).
13. Fujimoto, M.; Takeda, M.; Okamoto, K.; Furuta, M. "Effect o gamma irradiation dose on the fabrication of alpha-elastin nanoparticles by gamma-ray crosslinking". *Radiat. Phys. Chem.*, **80**, p. 142-144 (2011).
14. Kosaraju, S.L.; Puvanenthiran, A.; Lillford, P. "Naturally crosslinked gelatin with modified material properties". *Food Res. Internat.,* **43**, p. 2385-2389 (2010).
15. Wang, X.Z.; Wang, H.L.; Brown, H.R. "Jellyfish gel and its hybrid hydrogels with high mechanical strength". *Soft Matter*, **7**, p. 211-219 (2011).
16. Telegdi, J.; Szabo, T.; Al-Taher, F. et al. "Coatings against corrosion and microbial adhesion". *Materials and Corrosion –Werkstoffe und Corrosion*, **61**, p. 1000-1007 (2010).
17. Inamura, P.Y.; Kasawa, C.T.S.; Colombo, M.A.; Moura,E.A.B.; Mastro, N.L.d. "Changes in gelatin/Brazil nut shell fiber composite treated by electron beam radiation". *Proceedings of the 3th Asia Pacific Symposium on Radiation Chemistry (APSRC) & 10th Biennial Trombay Symposium on Radiation and Photochemistry (TSRP)- 2010*, Bhabha Atomic Research Centre Ed., Lonavala, India, 14-17 Sept, 2010.
18. Inamura, P.Y.; Kraide, F.H.; Moura, E. A.B.; Mastro, N.L.d. "Modification of gelatin properties by radiation induced cross-linking". *Proceedings of the 1st International*

Symposium on Colloids and Materials – New Scientific Horizons, Elsevier Ltd, Amsterdam, The Nederlands, 8-11 May, 2011 (2011).
19. Mubarak, A.K.; Rahman, N.; Rahman, M. "Preparation and characterization of gamma radiation cured gelatin-PVA bio-blend". *Advanced Materials Res*., **123-125**, p. 347-350 (2010).
20. Mortain, L.; Dez, I.; Madec, PJ. Development of new composites materials, carriers of active agents, from biodegradable polymers and wood. *Comptes Rendus Chimie*, **7**, p. 635-640 (2004).
21. Lu, Y.; Chen, SC. Micro and nano-fabrication of biodegradable polymers for drug delivery. *Advanced Drug Delivery Reviews*, **56**, p.1621-1633 (2004).
22. Zwiorek, K.; Kloeckner, J.; Wagner, E.; Coester, C. "Gelatin nanoparticles as a new and simple gene delivery system". *J. Pharm. Pharmaceut. Sci*., **7**, p. 22-28 (2004).
23. Lee, E.J.; Khan, S.A.; Lim, K.H. "Gelatin nanoparticles preparation by nanoprecipitation". *J. Biomaterials Sc. Polymer Edition,* **22**, p. 753-771 (2011).
24. Kong, Y.Q.; Li, D.; Wang, L.J.; Adhikan, B. "Preparation of gelatin microparticles using water-in-water (w/w) emulsification technique". *J. Food Engineering,* **103**, p. 9-13 (2011).
25. Al-Sheikhly, M. "Synthesis and modification of functional polymer nano-hydrogels using pulsed electron beams", *Proceedings of the 2010 LAS / ANS Symposium,* RJ, Brazil, June 21-25, 2010.
26. Darroudi, M.; Ahmad, M.B.; Zamiri, R. et al. "Preparation and characterization of gelatin mediated silver nanoparticles by laser ablation". *J. Alloys & Compounds*, **509**, p. 1301-1304 (2011).
27. Firouzabadi, H.; Iranpoor, N. Ghaderi, A. "Gelatin as a bioorganic reductant, ligand and support for palladium nanoparticles. Application as a catalyst for ligand- and amine-free Sonogashira-Hagihara reaction. *Organic & Biomolecular Chemistry*, **9**, p. 865-871 (2011).
28. Saravanan, M.; Bhaskar, K.; Maharajan, G. "Development of gelatin microspheres loaded with diclorofenac sodium for intra-articular administration". *J. Drug Targeting*, **19**, p. 96-103 (2011).
29. Hao, Z.H.; Wang, Y.L.; Tang, S.S. et al. "Optimization of the process of gelatin-ceftiofur sodium microspheres". *J. Wuhan University of Technology-Materials Sc. Edition*, **25,** p. 975-978 (2010).