Preparation and Characterization of Polyethylene Oxide (PEO)/Gelatin Blend for Biomedical Application: Effect of Gamma Radiation

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Received 6 January 2009; accepted 28 December 2009 DOI 10.1002/app.32034 Published online 6 April 2010 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: polyethylene oxide (PEO)/gelatin blend membranes of four different compositions (w/w) (5/95, 10/90, 20/80, and 30/70) were prepared by solution casting. The films were irradiated by gamma radiation at a total dose of 250 krad (dose rate of 321 krad/h). The X-ray diffractograms demonstrate both the PEO and radiation influences on the blend thus enhancing crystallinity of gelatin. X-ray diffractograms of irradiated blend films containing 30% PEO showed highest integrated intensity. The DTA and TGA study showed that the irradiated blend films. TMA study showed that the incorporation of PEO into gelatin increased melting point of the blend films. The melting

INTRODUCTION

Recently, natural and biodegradable polymers have attracted much attention as suitable biomaterials in environmental conservation. Polymer blending is a useful technique to develop highly functional materials in various industrial domains. With a specific polymer, the number of possible variations in usable properties is limited without resorting to composition changes. Random, block and graft copolymerization, polymer blends, and composites offer significant property diversification. Blending a synthetic polymer with a natural polymer represent a new class of materials which have potential applications in the biomedical field. The success of synthetic polymers as biomaterials relies mainly on their wide range of mechanical properties, transformation processes that allow a variety of different shapes to be easily obtained, and low production costs. Biological polymers present good biocompatibility, but their mechanical properties are often poor, the necessity of preserving biological properties complicates their processability, and their production or recovery costs are very high.¹

point for irradiated gelatin film changes from 52.9°C to 75.6°C and the glass point changes from 60.3°C to 90.6°C. The phase separation and compatibility of the PEO/gelatin blend films were studied by scanning electron microscopy (SEM). The experimental results showed that the blend films exhibit higher thermal stability and improved mechanical properties in dry state, which suggests the occurrence of interaction detected by XRD and DTA among gelatin, PEO, and water molecules in the films. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 117: 2075–2082, 2010

Key words: polyethylene oxide (PEO); gelatin; gamma-ray irradiation; biodegradable

PEO is a biodegradable synthetic polymer, which is also a semicrystalline polymer. PEO is soluble in both aqueous and organic solvents and is, thus, one of the most interesting polymers whose solution properties have been extensively studied both experimentally and theoretically for decades. In recent years PEO has attracted much attention as a polymeric excipient that can be used in formulations for different purposes. For instance, formulations with PEO have been extruded to make different products such as swellable and erodible implants,² scaffolds for tissue engineering,² or in the production of micelles with amphiphilic drugs, when solid dispersions incorporating these drugs are placed in aqueous environments.³ However, PEOs are mostly used to produce controlled release solid dosage forms such as matrices, reservoirs, or coated cores.⁴

Gelatin is a well-characterized protein fragment obtained by partial degradation of water insoluble collagen fiber⁵ and has been widely used in the biomedical field, because of its merits, including its biological origin, biodegradability, hydrogel properties, and commercial availability relatively low cost.⁶ Gelatin is an intriguing candidate for drug delivery and is widely being used as tissue engineering scaffold. Cross-linked gelatin sponges have also been investigated for their application as a component of

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Journal of Applied Polymer Science, Vol. 117, 2075–2082 (2010) © 2010 Wiley Periodicals, Inc.

artificial skin or tissue transplants to promote epithelialization and granulation tissue formation in wound. 7

Gelatin has also been used in medicine as a plasma expander wound dressing, adhesive, and absorbent pads for surgical use.⁸ Recently, gelatin has been demonstrated to exhibit activation of microphage^{9,10} and high-hemostatic effect.¹¹ Consequently, it has been used in a wide variety of wound dressings.^{12,13} The selection of gelatin as a component for preparation of the blend film rest up on two reasons. First, gelatin is a connective tissue protein, well known for its non toxic, nonirritant and biodegradability properties, and good living body compatibility, therefore has been widely used in food, pharmacology, and cosmetic applications.¹⁴ Second, the formation of specific intermolecular interaction through hydrogen bond of two or more polymers is responsible for the observed mixing behavior and properties of the blends.¹⁵ The selection of gelatin is also justified with this point of view.

One of the drawbacks of gelatin for tissue engineering applications is its solubility in aqueous media; therefore, gelatin-containing structures for long-term biomedical applications need to be crosslinked.¹⁶ The main limitation in the use of chemical crosslinkers for PEO/gelatin blends arises from the presence of some unreacted crosslinker inside the blended matrix and from the risk of formation of toxic products by reaction between gelatin and/or PEO with the crosslinking agent during in vivo biodegradation. Physical crosslinking methods for gelatin include microwave energy,17 gamma radiation, and UV-irradiation.¹⁸ It is reported that, gamma radiation increased the crosslinking between protein chains which increase the mechanical properties of the film.¹⁹Cheorun et al.²⁰ observed that gamma radiation enhance the tensile properties (TS and Eb) of the pectin and gelatin based films. Gamma radiation decontaminates the edible gelatin²¹ and is also capable of increasing the digestible energy of agricultural by-products.²

In this study, PEO/gelatin blend membranes with different ratios of two ingredients were prepared by solution casting method. The morphology and crystallinity of PEO/gelatin blend films were investigated by scanning electron microscopy (SEM) and X-ray diffraction (XRD), respectively. The thermal stability of the PEO/ gelatin films was studied by DTA/TGA and TMA.

EXPERIMENT

Materials

Polyethylene oxide (PEO) used in this work was purchased from BDH, England. The molecular weight of PEO is 6,000 g/mol. Gelatin was purchased from MERCK, Germany. This was Type-B gelatin and has a molecular weight of 10,000 g/mol.

Preparation of PEO/gelatin blend membranes

To prepare the pure gelatin film, 10 g of gelatin was dissolved in 100 mL of distilled water. The gelatin solution was magnetically stirred for 30 min at 50°C. After the complete dissolution of gelatin in water the gelatin solution was kept magnetically stirred for another 20 min at room temperature. The pure aqueous solution of gelatin was cast into a polypropylene sheet, and then the water was evaporated at room temperature. The films of pure gelatin were dislodged carefully, and then subjected to further drying under vacuum desiccators for 2 days. The same process was followed for the preparation of pure PEO film except the solution was stirred magnetically at 70°C.

To prepare the blend films PEO powder with different compositions (w/w) of gelatin was dissolved in 100 mL distilled water. The mixtures of PEO and gelatin with different ratios were magnetically stirred at 50°C for 150 min and then kept at room temperature for 60 min. The initial mixtures were further dispersed by using electric motor for 30 min. The mixture of PEO and gelatin solutions was again stirred magnetically for 2 h at room temperature. Thus most of the water was evaporated, and the original blend films were formed. The mixtures of both polymers in aqueous solutions with different compositions were cast on a polypropylene sheet. The film thickness ranged 50 \pm 10 μ m. The blend films were transferred into vacuum desiccators so as to use them for further study.

Gamma (⁶⁰Co) irradiation of the blend films

The dry PEO/gelatin blend films were irradiated by gamma radiation at a specific total dose of 250 krad (dose rate 321 krad/h). ⁶⁰Co gamma source was used for this purpose.

X-ray diffraction

A Philips PW3040 Pert PRO X-ray diffractometer was used. The films were exposed to CuK α radiation with a primary beam of 40 kV and 30 mA with a sampling pitch of 0.02° and time for each step data collection was 0.1 sec. A 2 θ scan was taken from 15° to 35° to get possible fundamental peaks where Ni filter was used to reduce CuK β radiation. All the data of the samples were analyzed using computer software "X" PERT HIGHSCOPE". The Integrated intensity of the blend films was investigated by using the following equation: Integrated Intensity (I) = Peak Height $(h) \times$ FWHM

where, FWHM = Full Width at Half Maximum

Thermal analysis

The thermal test of the films was taken using computer controlled TG/DTA 6300 system controlled to an EXSTAR 6000 STATION, Seiko Instrument, Inc. Japan. The TG/DTA module uses a horizontal system balance mechanism. All the experiments were performed in the nitrogen atmosphere. Sample weights were 10–15 mg, and heating rate was 10° C/ min within the temperature range of 50–500°C.

Thermo-mechanical analysis

Glass point and linear coefficient of thermal expansion were measured for all the materials using dynamic thermo-mechanical analyzer (DMTA) Liensis 200 with an precision of $\pm 3^{\circ}$ C. The temperature range was 60–120°C.

Mechanical testing

Tensile strength (TS) and percent elongation at break (Eb) of the blend Films were measured with a universal testing machine (Hounsfield Series S, UK) using DIN EN 10002-1 method of testing polymer film. The maximum load capacity was 500 N, precision was within $\pm 1\%$. The crosshead speed was 10 mm/min. Gauge length was 20 mm. Four different blends with different concentrations of PEO in gelatin were analyzed using the universal testing machine. The physico-mechanical properties of 5%, 10%, 20%, 30% PEO containing gelatin film were investigated at 65% relative humidity at room temperature to enable identical moisture content.

Morphological study

The morphological studies of the gelatin/PEO blend films were done using a JEOL 6400 SEM at an accelerating voltage of 10 kV. The SEM specimens were sputter-coated with gold.

RESULTS AND DISCUSSION

Characterization of the PEO/gelatin blend films

X-ray diffraction

The X-ray diffractograms of the irradiated and unirradiated blend films are shown in Figure 1. The helical crystalline structure of PEO has been described quite well in the literature.^{18,23,24} The presence of defined X-rays diffraction peaks [Fig. 1(a)] in the interval between 16° and 30° (20) is characteristic



Figure 1 (a) X-ray diffractograms of gamma irradiated pure gelatin, pure PEO and gelatin/PEO blend membranes; (b) Comparison of crystallinity among radiated and unirradiated blend membranes.

of crystalline PEO samples. The more intense crystalline peaks are located at 19.2° and 23.3° (20), which correspond to d-spacings of 0.46 nm and 0.38 nm, respectively.

The pattern of pure gelatin, as shown in Figure 1(a) has a very weak broad shape pattern, indicating amorphous behavior of gelatin.²⁵ If gelatin and PEO have low compatibility, each polymer would have its own crystal region in the blends. No peaks other than those for gelatin and PEO were detected in the patterns of the blend films.²⁵ By increasing the concentration of PEO in the gelatin/PEO blend, the main PEO diffraction peaks exhibited differences in shape and position. The peak displacements for the blends in Figure 1(a) can be considered as an evidence of crystalline structure changes in these materials. It is also evident from the Figure 1(a) that peak intensity is increasing with the increasing content of PEO into gelatin. A clear modification is observed in the XRD of the 30% (PEO) blend in relation to PEO. A sharper peak is at 23.6° (20) for this material, indicating the formation of a more defined lamellar crystalline structure in this blend. Thus, the addition of PEO alters the phase structure of the blend to produce a lamellar structure with a narrow thickness distribution. The blends with high gelatin concentration (i.e., less amount of PEO in the blend) showed a decrease in peak intensity and decrease in width at the half maximum of the peak at $\sim 19^{\circ}$ (20), which may support the participation of gelatin side chains in the PEO crystals in the blends. For the 5% PEO

Gelatin/PEO blends with different compositions (w/w)	(2θ) values (in degree)	Height (counts)	FWHM (2θ) (in degree)	$d = (n\lambda/2\sin\theta)$ (in Å)	Integrated intensity (I)
100/0	_	_	_	_	_
95/5	23.406	517.06	0.3542	3.80071	183.142
90/10	23.142	519.33	0.2755	3.84343	143.075
80/20	19.056	376.13	0.2558	4.65739	
	23.012	584.80	0.7085	3.86484	414.330
70/30	18.7068	1201.04	0.2362	4.74353	
	22.5088	800.95	0.1181	3.95016	
	22.7893	1408.92	0.1574	3.90218	221.764
	23.1210	1129.48	0.2165	3.84694	244.53
0/100	19.199	8973.16	0.2362	4.62299	
	23.036	2254.04	0.1378	3.86093	310.606
	23.301	3467.82	0.1378	3.81762	477.865
	23.645	2841.83	0.2558	3.76277	726.940
90/10 (Non-irradiated)	23.8107	341.30	0.7872	3.73704	268.671
80/20 (Non-irradiated)	23.7932	415.44	0.2755	3.73796	114.453

 TABLE I

 X-Ray Diffraction Results for Pure PEO, Gelatin, and PEO/Gelatin Blend Films

blend, it was observed a very weak typical PEO diffraction line in Figure 1(a). The 10% PEO blend exhibited diffraction lines at 23.14° (2 θ) and the 20% PEO blend showed diffraction lines at 19.05° and 23.14° (2 θ). It is inferred that the integrated intensity of the blend increases with the increasing percentage of PEO (Table I). The significant increase in integrated intensity of the blend films in the 19° and 23° (2 θ) region has been shown in Table I.

The un-irradiated gelatin/PEO blend of 90/10 and 80/20 showed no critical change in crystallinity in comparison with the irradiated blend of the same ratios [Fig. 1(b)]. However, it is clear from the Figure 1(b) sharper peaks are obtained from the irradiated blend membranes and this is due to the modified morphology of the blend membranes as well as the increase of crosslinking between gelatin side chains and PEO.^{26,27}

The integrated intensity of pure gelatin, PEO, and blend films changed. The highest integrated intensity of 441 was found for the blend ratio of 80/20 (w/w) but most crystalline structures were formed for the blend ratio of 70/30 (w/w). The change in integrated intensity of the blends, according to Lee et al. analysis²⁸ of PVA/chitosan blend system, means the occurrence of intermolecular hydrogen bonding between two components and this interaction prevents the gelatin from crystallization by comparison of diffraction pattern for pure gelatin with that for the blends.

Thermogravimetric analysis

TGA results are shown in Figure 2. The curves of irradiated pure gelatin showed two zones of weight loss. The first weight loss at approximately 130–150°C is due to the loss of water; the second weight loss started at about 250°C, showing that different

extent of thermal degradation of gelatin protein took place. Kaminska and Sionkowska²⁹ stated that this might be possible after the breaking-up of inter- and intra-molecular hydrogen bonds which are responsible for the maintenance of the polymeric chain order (alpha-helix, beta-structure) in protein films. On the other hand, PEO shows single step of weight loss between 230–430°C.³⁰ With regarding to the mechanism of PEO degradation, Madrsoky and Strauss^{31,32} have established PEO decomposition by random scission of the chain links without chain-endinitiated depolymerization. This supports the single-stage decomposition observed for the PEO.

TGA curves of all blend films show the greatest weight loss in the temperature range of 250–400°C, which are believed due to the disintegration of intermolecular and partial breaking of the molecular structure.³³ As shown in Figure 4, it is understood that the incorporation of PEO to the gelatin increase the thermal stability of the blend film but the thermal stability was the highest for pure PEO film. The



Figure 2 The TGA curves of gamma irradiated pure gelatin, pure PEO, and gelatin/PEO blend films.



Figure 3 The DTA curves of gamma irradiated pure gelatin, pure PEO, and gelatin/PEO blend films.

greatest degradation temperature increased in the sequence of Gel/PEO-90/10 (282.6°C), Gel/PEO-80/20 (328.2°C) and Gel/PEO-70/30 (358.1°C) which is the same as that of their crystallinity, implying thermal stability of the films was improved by their crystalline domains and hydrogen bonding interactions.³⁴

Differential thermal analysis

Figure 3 shows the differential thermal analysis (DTA) curves of irradiated films. The irradiated pure polyethylene oxide (PEO) film shows an endothermic peak at 83.7°C which is the melting point of PEO and this is also attributed to the loss of moisture. The endothermic peak at 405.4°C indicates the decomposition of the polyethylene oxide chain. The irradiated pure gelatin shows endothermic peak at 316.7°C which is attributed to various thermal induced transitions, such as melting of protein chains and beginning of thermal degradation.³⁵

The curves of the gamma irradiated blend films showed the new endothermic peaks appeared in the temperature range of 200-250°C. The gelatin/PEO blend of 70/30 showed a sharp endothermic peak at 78.8°C which marks the melting of the PEO molecules and this melting temperature of PEO is less than that of pure PEO. Homogeneous polymer mixtures with a crystallizable component usually show a decrease in experimental melting points with the addition of the amorphous component, because the interaction of the two polymers reduces crystallite size.^{36,37} Most of the blend films showed endothermic peaks at around 405°C, which resulted from the greatest degradation of the films. Significant changes of DTA curves of the blend films suggest that strong interaction established between gelatin and PEO molecules.38

Thermomechanical analysis

Thermomechanical analysis (TMA) was used to determine gel-melting temperature, which was proven to be accurate and reproducible. The probe position has been plotted versus temperature for each formulation, and the onset point of the transition was taken as film melting temperature (Tm). Pure un-irradiated gelatin film was used as comparison. As shown in Figure 4, incorporation of PEO with gelatin did change the melting temperatures of the blend films and the extent of change depended on the positions of hydroxyl groups on the PEO. The onset temperature of irradiated pure gelatin was 75.6°C which was higher than the onset obtained for the un-radiated pure gelatin film (52.9°C) and this is attributed to the increasing crosslinking among polypeptide chains of gelatin. For the un-irradiated blend film, it can be observed from the Figure 4 that the melting temperature started rising from 51°C and raised up to 69°C and it became almost stable for 30% PEO containing blend films. On the other hand, the irradiated blend film showed peculiar phenomena in melting temperature. The melting point of the irradiated blend film suddenly dropped to 42°C (for 5% PEO containing blend film) from 75.6°C and reached to 70°C (30% PEO containing blend films).

The initial drop in melting point may be due to the initial amorphous portion of the blend film and after that the gradual increase in the melting point must be accompanied by increasing crystalline phase in the blend film.³⁹ The blend films also showed change in glass point in the range from 60–90°C. The glass point increased due to the inclusion of PEO into gelatin film. Irradiated blend films showed more increase in glass point that range from 59°C to 91°C (Fig. 5). This must be associated with the increasing crystalline portion of the irradiated blend



Figure 4 Change in melting point with the inclusion of %PEO in gelatin.

Journal of Applied Polymer Science DOI 10.1002/app



Figure 5 Change in glass point with the inclusion of %PEO in gelatin.

films.³⁹ The initial drop in glass point may be associated with the initial amorphous portion.³⁹ The onset of softening exhibited by the blends is followed by the slow and gradual penetration of the probe into the samples reaching its maximum at the final softening point showing characteristic stability of the samples with increasing percentage of PEO. The higher softening temperature range with increase in proportion of PEO in composition accounts for increased stability of the gelatin matrix.

Mechanical properties of the blend films

The ultimate tensile strength of the blends showed a decreasing tendency with increase in percentage of PEO within the range of concentration studied, as shown in Figure 6. The amino group of the gelatin polypeptide chains formed in situ acts as an electron donor and the hydrogen of PEO as an electron



Figure 6 Variation of ultimate tensile strength of the blends with composition.

acceptor.³⁹ This induces dipole–dipole attraction between the two polymeric phases, which is supposed to enhance molecular interaction. The tensile strength (TS) of the un-irradiated and irradiated gelatin film were 28 ± 1.68 (MPa) and 35 ± 0.52 (MPa), respectively. TS of the un-irradiated and irradiated blend film were 17.35 ± 1.42 (MPa) and 27.5 ± 1.37 (MPa), respectively.

The TS values of the irradiated gelatin film are somewhat higher than that of the corresponding blends. This may be due to the consequent interwinding of the individual phases and the formation of crosslinks, which reduces the flexibility and introduces rigidity and stiffness to the existing linear chains of PEO and gelatin. The effect of dipoledipole interaction and H bonding as encountered in blends has been further synergized by the influences of the crosslinks present in the continuous interwined matrices. The percentage elongation at break of the un-irradiated and irradiated blend films were 32.78 and 10.02, respectively. Figure 7 shows percentage elongation at break for different compositions. The incorporation of PEO molecules into the continuous matrix of gelatin disrupts the structural chain regularities of gelatin, which breaks down the molecular packing as evidenced from the XRD curves [Fig. 1(a)], and provides a greater path length (path around the periphery of the dispersed particles) for dissipation of energy before its ultimate rupture. The effect of gamma radiation is clearly observed from the Figures 6 and 7 that irradiated blend films showed improved mechanical property.

Morphological study

PEO and gelatin have different polarity, and while blending them, the phase separation always exists in



Figure 7 Variation of elongation at break of blends with composition.



Figure 8 SEM images of gelatin (a), PEO (b), and gelatin/PEO blend (c) 70/30 (Irradiated), (d) 70/30 (un-irradiated).

the sample no matter whatever methods are used. The degree of phase separation will affect the mechanical strength of blend membrane. The phase distribution of blend membrane is observed using SEM. Differently from PEO, gelatin is a heterogeneous polymer constituted of 20 different amino acids that can be classified as polar or non-polar. Therefore, only half of the gelatin monomers could potentially interact with the functional hydroxyl group of the PEO, explaining the occurrence of phase separation in the blended films.⁴⁰ Moreover, also different from PEO, gelatin is a heterogeneous mixture of single- or multi-stranded polypeptides, each with extended left-handed proline helix conformations, which can form films with physically crosslinked and partially renatured collagen-like structures.⁴⁰ Thus, it could also be suggested that this renatured structure could reduce the potential interactions with the PEO at high concentrations of this polymer.

The morphologies of pure PEO and pure gelatin film are featured in the Figure 8(a,b). The micrograph shows that PEO has crystalline flat lamellae with leaf-like shape. This feature dominated the morphology of pure PEO membrane. Large spaces between the leaf-like structures are available throughout the surface membrane. The crystalline structure is probably due to longer evaporation time taken which enables it to crystallize. Meanwhile, for the scanning electron micrograph of pure gelatin, it is obvious that the surface is rough and uneven. This feature dominates the surface of pure gelatin membrane as shown in Figure 8(a). It is obvious that the phase separation still occurs even at low content of gelatin. When the content of gelatin is 70% [Fig. 8(d)], it is difficult to distinguish the two phases because of the ambiguous phase interface. With further increase of gelatin content up to 90% [Fig. 8(c)], an evident phase variation occurs. The gelatin phase becomes continuous phase and forms beehive-like network structures; PEO phase is dispersed. The degree of phase separation of gelatin in blend membrane has significant effect on the strength of blend membranes. Therefore, it is necessary to decrease the phase separation to avoid excessive loss of strength of blend membranes.

CONCLUSION

Solution casting method was followed to prepare PEO/gelatin blend membranes of four different

compositions. The blend membranes were irradiated to improve the thermal, physico-mechanical, crystalline, and morphological properties of the films. Due to the incorporation of PEO and using gamma radiation, the tensile strength was found to be improved. Also the elongation at break was improved to 5.6%. The thermo-mechanical properties have been drastically improved due to PEO content in films. The DTA/TG study also showed that gamma irradiated 30% PEO blend was more thermally stable than the other blend ratios and this thermal stability is consent with crystalline structure of the 30% PEO blend. The incorporation of PEO also improved the crystallinity of the gelatin film. The blend containing 30% PEO showed the highest integrated intensity. The change in integrated intensity in every blend ratio indicates the intermolecular interaction of PEO side chains to gelatin polypeptide side-chains. The thermal study also showed that the thermal stability of the blend films increased with the increase of crystalline phase in the blend. The SEM study showed the change in the morphological characteristics of the blend membranes. Small phase separation in the blend membrane was observed. The blend containing 30% PEO showed improved porous structure than other blend ratios. The effect of gamma radiation can clearly be observed in every test. The improvement of mechanical properties, thermal stability, and morphological properties along with the radiation induced increased crosslinking can clearly be observed. The incorporation of PEO in gelatin and treating the blend films by gamma radiation increased the crystallinity, thermal stability, and mechanical properties of the gelatin. Therefore, we concluded that the correlation of the phase structure and chemical structure with the phase separation discovered in SEM analysis in this research is important to understand the properties of gelatin/PEO blend as a promising tissue engineering scaffold.

We would like to express our gratitude to Professor Andrea Lazzeri (Department of Chemical Engineering, Industrial Chemistry and Material Science, University of Pisa, Italy.) for his valuable suggestions and cooperation in result and discussion part of the manuscript.

References

- 1. Barbani, N.; Cascone, M. G.; Giusti, P.; Lazzeri, L.; Polacco, G.; Pizzirani, G. J Biomater Sci Polym Ed 1995, 7, 471.
- Adamic, K. J.; Greenbaum, S. G.; Abraham, K. M.; Alamgir, M.; Wintersgill, M. C.; Fontanella, J. J. Chem Mater 1991, 3, 534.
- Deng, Y. J.; Dixon, J. B.; White, G. N. Colloid Polym Sci 2006, 284, 347.
- Chen, E. Q.; Jing, A. J.; Weng, X.; Huang, P.; Lee, S. W.; Cheng, S. Z. D.; Hsiao, B. S.; Yeh, F. J. Polymer 2003, 44, 6051.

- 5. Kniep, R.; Simon, P. Top Curr Chem 2007, 270, 73.
- 6. Choi, S. S.; Regenstein, J. M. J Food Sci 2000, 65, 194.
- 7. Sarkar, S.; Chourasia, A.; Maji, S.; Sadhukhan, S.; Kumar, S.; Adhikari, B. Indian Acad Sci 2006, 29, 475.
- 8. Klose, A. A.; Machi, E. P.; Hanson, H. L. Food Technol 1952, 6, 308.
- 9. Wainewright, F. W. Physical Tests for Gelatin and Gelatin Products. The Science and Technology of Gelatin; Academic Press: New York, 1977.
- Montero, P.; Gòmez-Guillèn, M. C.; Borderias, A. J. Food Chem 1999, 65, 55.
- Gennadios, A.; Mchugh, T. H.; Weller, C. L.; Krochta, J. M. Edible Coatings and Films Based on Proteins; Technomic Pub.Co.: Lancaster, NC, 1994.
- Veis, A.; Horecker, B.; Kaplan, N. O.; Scheraga, H. A. In The Macromolecular Chemistry of Gelatin; Academic Press: New York, 1964.
- 13. Chatterji, P. R.; Kaur, H. Polymer 1992, 33, 2388.
- Rathna, G. V. N.; Mohan Rao, D. V.; Chatterji, P. R. J Macromol Sci Pure Appl Chem 1996, 33, 1199.
- 15. Rathna, G. V. N.; Mohan Rao, D. V.; Chatterji, P. R. Macromolecules 1994, 27, 7920.
- 16. Wang, W. P. Food Ferment Ind (Chin) 1997, 23, 81.
- Lihong, F.; Yumni, D.; Ronghua, H.; Qun, W.; Xiaohui, W.; Lina, Z. J Appl Polym Sci 2005, 96, 1625.
- 18. Neumann, P. M.; Zur, B.; Ehrenreich, Y. J Biomed Mater Res 1981, 15, 9.
- Ressouany, M.; Vachon, C.; Lacroix, M. J Agric Food Chem 1998, 46, 1618.
- Jo, C.; Kang, H.; Lee, N. Y.; Kwon, J. H.; Byun, M. W. Radiat Phys Chem 2005, 72, 745.
- 21. Fu, J.; Shen, W.; Bao, J.; Chen, Q. Radiat Phys Chem 2000, 57, 345.
- 22. Al-Masri, M. R.; Guenther, K. D. Radiat Phys Chem 1999, 55, 323.
- 23. Chvapil, M. J Biomater Mater Res 1982, 16, 245.
- 24. Hassan, M. M. Polym Eng Sci 2008, 10, 2095.
- 25. Cheng, M.; Deng, J.; Yang, F.; Gong, Y.; Zhao, N.; Zhang, X. Biomaterials 2003, 24, 2871.
- Kim, J. H.; Kim, J. Y.; Lee, Y. M.; Kim, K. Y. J Appl Polym Sci 1992, 45, 1711.
- 27. Pawde, S. M.; Deshmikh, K. J Appl Polym Sci 2008, 109, 3433.
- Mohd Nasir, N. F.; Raha, M. G.; Kadir, N. A.; Sahidan, S. I.; Rampado, M.; Azlan, C. A. Am J Biochem Biotech 2006, 2, 178.
- 29. Paul, D. R.; Barlow, J. W. J Macromol Sci Rev Macromol Chem Phys 1980, 18, 109.
- Sung, K. Y.; Kim, J. Y.; Grainger, W. D.; Okano, T.; Kim, W. S. Korea Polym J 1993, 1, 14.
- 31. Madorsky, L. S.; Strauss, S. J Polym Sci 1959, 36, 183.
- Taboada, M. O.; Sobral, A.; Carvalho, A. R.; Habitante, B. Q. Food Hydrocolloids 2008, 22, 1485.
- Taylor, D. J.; Kragh, A. M. Photographic Gelatin II; Academic Press: London and New York, 1976.
- 34. Zhang, H. L.; Ren, M. Q.; Chen, Q. Y.; Sun, S. L.; Sun, X. H.; Zhang, H. X.; Mo, Z. S. J Polym Sci Part B: Polym Phys 2006, 44, 1320.
- 35. Xiao, C.; Yongshang, L. U.; Jing, Z.; Zhang, L. J Appl Polym Sci 2002, 83, 949.
- Hirabayashi, K.; Suzuki, T.; Nagura, M.; Ishikawa, H. Bunseki Kiki 1974, 12, 437.
- 37. Zhao, Q.; Samulski, T. E. Macromolecules 2003, 36, 6967.
- Xu, X.; Toghiani, H.; Pittman, C. U. J Polym Sci Eng 2000, 40, 2027.
- 39. Pereira, R. P.; Rocco, A. M. Polymer 2005, 46, 12493.
- Yakimets, I.; Wellner, N.; Smith, A. C.; Wilson, R. H.; Farhat, I.; Mitchell, J. Biomacromolecules 2007, 8, 1710.