Effect of Natural Polyphenols on Physicochemical Properties of Crosslinked Gelatin-Based Polymeric Biocomposite

A. A. Haroun,[1] S. A. El Toumy[2]

[1]*Chemical Industries Research Division, National Research Centre, Dokki 12622, Cairo, Egypt*
[2]*Chemistry of Tanning Materials Department, National Research Centre, Dokki 12622, Cairo, Egypt*

Received 29 June 2009; accepted 3 November 2009
DOI 10.1002/app.31736
Published online 28 January 2010 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: This work was aimed to study the effect of natural polyphenols extract (*Acacia nilotica* bark) on physicochemical properties of crosslinked gelatin-poly(acrylamide-*co*-acrylic acid), Gel-poly(AAm-*co*-Ac), polymeric biocomposite film. Gelatin-based composite films have extensive application as biocompatible biomaterial as drug carriers, cosmetics, and agricultural food packaging. The prepared composite films were characterized using Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC), in addition to the swelling and degradation behavior. UV-Vis absorption spectra and scanning electron microscopy (SEM) were also applied to observe the interaction between Gel-poly(AAm-*co*-Ac) and natural polyphenol (catechin). The study has demonstrated that the involvement of hydrogen bonding and hydrophobic interactions as the major forces involved in the stabilization of gelatin-based polymeric biocomposite film by the plant polyphenols (catechin and gallic acid derivatives). Thermal stability studies of crosslinked gelatin-based composite film revealed that *A. nilotica* bark extract stabilizes the gelatin molecules and leads to moderate increase of the denaturation temperatures relative to the uncrosslinked one. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 116: 2825–2832, 2010

Key words: *A. nilotica* bark extract; biocomposites; gelatin; polyphenols

INTRODUCTION

Research in recent years has clarified that hydrophobic binding between proteins and polyphenols, mainly condensed tannins, is significant importance in protein–polyphenol interaction. The efficacy of polyphenols in this complexation process derives from the fact that they act as polydentate ligands acting through several potential sites (phenolic residues) with the protein.[1] Vegetable tannins are classified as hydrolysable and condensed tannins.[2] Vegetable tannins (natural polyphenols) constitute one of the most numerous and widely distributed categories in the plant kingdom, with more than 8000 phenolic structures currently known. Generally, only the plant polyphenols with molecular weight between 500 and 3000 Da are called tannins.[3] Pharmacological properties of tannins have been investigated based on recent advances in the structural study of tannins in medicinal plants and various properties of tannins including antitumor and antiviral effects have been revealed.[4] Gelatin is obtained from collagen by heating above the helix-coil transition temperature. When such heating is carried out in solution, it collapses of the rod like three stranded collagen unit into a random coil. Moreover, there is a partial disaggregation of individual chains leading to α (one chains), β (covalently bonded pair), and γ (three covalently bonded chains) coiled polymeric units. Gelatin films cast from solution at temperatures below the helix-coil transition temperature (cold-cast gelatin) partially rebuild the tertiary structure, whereas, films cast above the critical temperature level (hot-cast gelatin) are completely amorphous.[5] Gelatin has gained more attention as edible films for its abundance and biodegradability.[6] It is unique among hydrocolloids in forming thermo-reversible with a melting point close to body temperature, which is particularly significant in edible and pharmaceutical applications.[7] But gelatin film does not have ideal mechanical properties and water vapor barrier as most protein films, which limit its application as edible film and biomaterial. Chemical and physical treatments can be applied to modify the polymer network through crosslinking of the polymer chains to improve protein film functionality. The chemical agents used for crosslinking include aldehydes, gossypol, calcium salts, and others, while physical treatments are such as UV and γ irradiations.[8] Now, aldehydes which bond very quickly to proteins are usually used to crosslink gelatin, but aldehydes have toxicity, which may not

Correspondence to: A. A. Haroun (haroun68_2000@yahoo.com).

be tolerable in many fields. Though some enzyme such as transglutaminase and natural crosslinking agent genipin have been investigated as crosslinking agents for protein film.[9–11] Over the past few years, edible films as food and druggery packagings have attracted more interest, which may be because of edible films can enhance food quality by acting as moisture, gas, aroma, and lipid barriers and provide protection to a food product after the primary package is opened.[12] Also, such films are biodegradable, which will reduce pollution of traditional nonbiodegradable plastic films.[13] When gels are cooled below 30–35°C the random coil polypeptide chains link up to form collagen-like triple helices for part of their lengths, resulting in a so called physical gel. The shear modulus of gels have been shown to increase with increasing helical content. The modulus can be further enhanced by introducing covalent chemical crosslinks between single strand chain segments.[14] This article investigates the influence of the natural polyphenols extract (*Acacia nilotica* bark) on the physicochemical properties of the crosslinked Gel-poly(AAm-*co*-Ac) polymeric biocomposite film.

EXPERIMENTAL

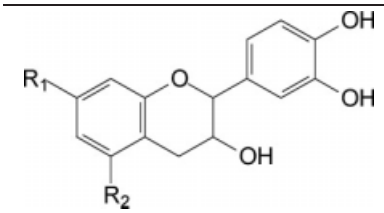
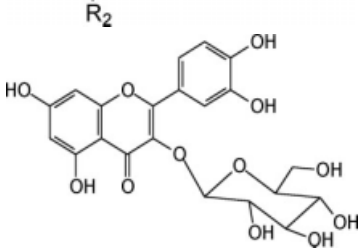
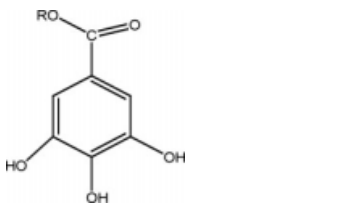
Materials

Edible gelatin obtained from Davis Gelatin (NZ), Christchurch, New Zealand. Poly(acrylamide-*co*-acrylic acid) partial sodium salt, viscosity 50,000 cps, obtained from Aldrich. Bark of *A. nilotica* (*L.*) Delile[15] was collected from Upper Egypt in April 2009. Identification of the plant was confirmed by the Department of Flora Agricultural, Faculty of Science, Cairo University. All other chemicals were of analytical grade.

Extraction and isolation of the natural polyphenols from *A. nilotica* bark

Powder of the air dried bark of *A. nilotica* (2 kg) was defatted with chloroform and extracted with methanol : water mixture (7 : 3) at room temperature. The combined extracts were filtered, evaporated under reduced pressure and lyophilized (300 g). The dry extract was redissolved in 2 L distilled water and extracted with 100% ethyl acetate. After evaporation of the solvent, the ethyl acetate extract and the remaining aqueous phase gave dark brown solids. It was loaded on polyamide 6S column (50 × 3 cm). The column was eluted with distilled water and then water–ethanol mixtures of decreasing polarity. The resulting fractions (1, 2, and 3) were then fractionated. The first one was loaded on Sephadex LH-20 column chromatography using aqueous ethanol for elution to give catechin and catechin-5-*O*-gallate. Fraction 2 was subjected to column chromatography on cellulose using *n*-butanol saturated with water as an eluent to give two major subfractions, then each of them was separately fractionated on Sephadex LH-20 to yield pure quercetin-3-*O*-glucoside and gallic acid. The fraction 3 gave chromatographically pure catechin-7-*O*-gallate and methyl gallate, as shown in Table I.

TABLE I
Phytochemical Screening of *A. nilotica* Bark Extract

Chemical structure	Functional groups	Compounds
	R_1=R_2=OH	Catechin
	R_1=gallic acid R_2=OH	Catechin -7-*O*-gallate
	R_1=OH R_2=gallic acid	Catechin-5-*O*-gallate
		Quercetin 3-*O*-glucoside
	R=H	Gallic acid
	R=CH_3	Methyl gallate

Preparation of the crosslinked gelatin-based polymeric biocomposite films

(1.0) g gelatin was dissolved in 50 mL distilled water with constant stirring at 40°C then add different concentrations of *A. nilotica* bark extract (0, 3, 6, 8, and 10) wt % for 1 h. Followed by addition of aqueous solution of poly(AAm-*co*-Ac) in a different ratios (1 : 1, 2 : 1, and 1 : 2) for further 1 h at 50°C. The products were poured into different Petri dishes, then cooling at −8°C and finally drying at 50°C. Keeping the prepared polymeric biocomposite films for further investigation (Table II).

UV-Vis absorption spectra

The UV spectra of *A. nilotica* bark extract and the crosslinked composite films (III and V) were recorded using Perkin Elmer spectrophotomer. *A. nilotica* (40 mg)bark extract was put into 10 mL volumetric flask. Then distilled water was added to scale. Saturated solution was obtained after sample was shaken for 24 h at 22°C as there still was insoluble part of polyphenol at the bottom of the sample. Saturated solution (2 mL) was taken out and diluted to the extent necessary for UV spectrophotometric measurement. The absorbance value of diluted solutions was determined.

Swelling and degradation properties

The polymeric biocomposite films were weighed in air dried conditions (W_d). They were then immersed in physiological buffer solution (phosphate buffer saline, pH ~7.4) for different periods of time (5, 30, and 60 min). After that, the wet samples were wiped with filter paper to remove excess liquid and reweighed (W_w). All samples under investigation were photographed for visual assessment of the degradation. The swelling percentage (S_w%) was calculated as follows:[16,17]

$$S_w\% = [(W_w - W_d)/W_d]100$$

TABLE II
Composition of the Crosslinked Gelatin-Based Polymeric Biocomposite Films

Sample code	Gel-poly (AAm-*co*-Ac)	*A. nilotica* bark extract conc. (%)
I	1 : 0	0
II	1 : 0	3
III	1 : 1	3
IV	1 : 1	6
V	1 : 1	8
VI	1 : 1	10
VII	2 : 1	3
VIII	1 : 2	3

All samples were placed in sealed glass vials in the dark till the end of the study period.

Fourier transform infrared spectroscopy

The composite films were examined using Perkin-Elmer Fourier transform infrared spectroscopy (FTIR) under certain conditions, such as: scan resolution: 4 cm^{-1}, scan rate: 2 mm sec^{-1}, range: 4000–600 cm^{-1}, and mode: transmission.

Scanning electron microscope morphology

The structural features of the prepared composite films were investigated, at high magnification (X1000) and resolution by means of energetic electron beam, using JEOL-5600 LV scanning electron microscope (SEM). Before observation, the fractured surfaces were coated with Au with SEM coating device (Edward spotter coater). Three micrographs were taken from different zones of each surface film under investigation.

Differential scanning calorimetry

Calorimetric measurements were performed using PerkinElmer Diamond differential scanning calorimeter (DSC) equipped with a model PII intercooler. Temperature and enthalpy calibration was performed using high purity standards (benzene and indium). The measurements were carried out on known amounts of crosslinked gel films (3–4 mg of dried sample), which had been stored in a mixed of water/ethanol in the ratio 2 : 3 for 72 h. The wet samples were wiped with filter paper to remove excess liquid and hermetically sealed in aluminum pans (to prevent any loss of liquid during measurements). Heating was carried out at 10°C min^{-1} in the temperature range from −50 to 220°C and another run from 15 to 300°C. Denaturation (T_D) was determined as the peak value of the corresponding endothermic phenomena. The value of denaturation enthalpy (ΔH) was calculated with respect to the weight of air dried gelatin.[18] The renaturation level (X%) was calculated according to the equation:

$$(X\%) = [\Delta H/\Delta H_T]100$$

where $\Delta H_T = 44$ j/g is the melting enthalpy of tendon collagen, which was examined in the same conditions.

RESULTS AND DISCUSSIONS

The phytochemical screening of *A. nilotica* bark extract

The phytochemical investigation of the aqueous ethanol extract of *A. nilotica* bark proved that it

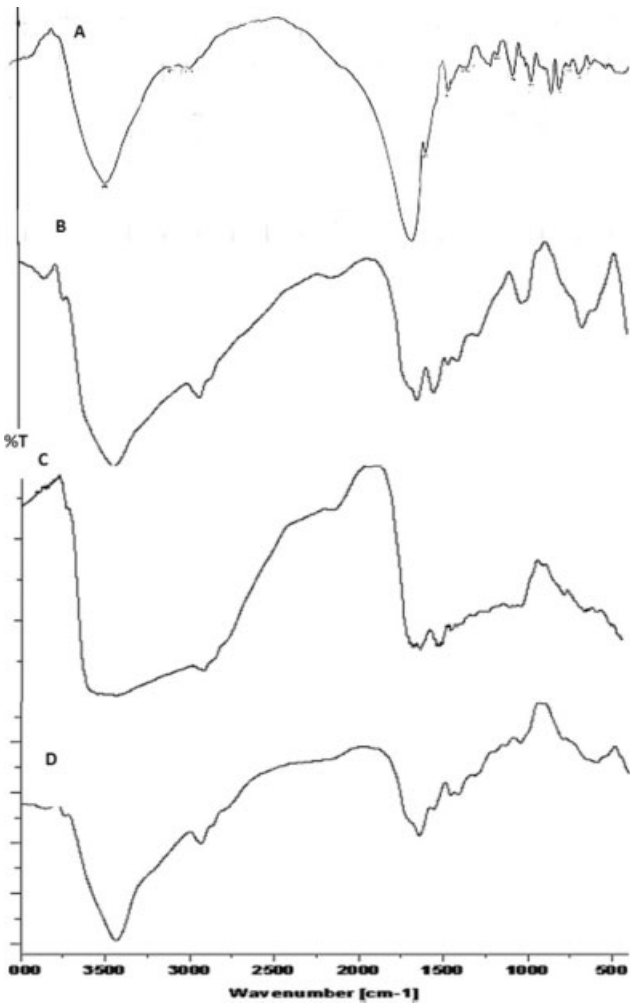


Figure 1 FTIR spectra of (A) gel, (B) crosslinked Gel, (C) crosslinked Gel-poly(AAm-*co*-Ac) at 3 wt % *A. nilotica* bark extract, and (D) poly(AAm-*co*-Ac).

contains catechin, catechin-7-*O*-gallate, catechin-5-*O*-gallate, quercetin 3-*O*-glucoside, gallic acid, and methyl gallate. The structures of the isolated compounds were established through chromatography, as well as conventional chemical and different spectroscopic methods of analysis (UV, Mass and 1/2-D NMR),[19,20] as shown in Table I.

Influence of *A. nilotica* bark extract on the structural features of the gelatin-based polymeric biocomposite film

Structural diversity of gelatin chain units determines the specific features of gelatin properties. Most of the synthetic polymers show no such features that are typical of the most biopolymers. FTIR spectrum of gelatin [Fig. 1(A)] exhibited characteristic peaks at 1657, 1535, and 1238 cm^{-1} corresponding to amide I band (CO stretching, strong), amide II band (NH bending and CN stretching, weak) and amide III band (CN stretching and NH bending, weak), respectively. Free amino groups (NH_2) stretching band of amide A was overlapped with overtone of NH bending of amide B[21] in the region 3280–3480 cm^{-1}. The characteristic bands of amides IV,V, VI, and out of plane NH bending appeared in the region 640–800 cm^{-1}. FTIR spectra of the crosslinked gelatin with *A. nilotica* bark extract, Gel-poly(AAm-*co*-Ac) and poly(AAm-*co*-Ac), as shown in Figure 1(B–D), respectively, illustrated that the characteristic peaks at 1735 and 1650 cm^{-1} with low intensities, in addition to the broad band in the region 3100–3650 cm^{-1}, which corresponding to the hydrogen bonding the interactions between the hydroxyl groups (—OH) of the polyphenols (catechin, gallic acid) and the amino groups (—NH2) of the gelatin molecules. This indicated that the crosslinking reaction between polyphenols and gelatin could be taken place. To study the interaction of *A. nilotica* bark extract with the gelatin molecules theoretically. Catechin–collagen triple helix interaction has been previously studied[22,23] using the molecular mechanical calculations. Catechin has been placed appropriately near the side chain groups present in catechin and they can act as hydrogen bond donor/acceptor with different side chain groups of amino acids namely, asparagine (Asn), glutamine (Glu), lysine (Lys), and Proline (Pro). Therefore, the bond length and angle between catechin and the mentioned specific binding sites of the gelatin were calculated and reported in Table III. As expected, all the complexes exhibited hydrogen bonding. Multiple hydrogen bonding of the hydroxyl groups of the catechin with the functional groups of the gelatin and the hydrophobic interactions of the benzopyran rings of the catechin with the hydrophobic residues of the gelatin are the major forces involved in the stabilization of gelatin by polyphenol.

Swelling and degradation behavior

Gelatin is soluble in aqueous solution and a few minutes of storage in physiological solution is

TABLE III
Theoretical Prediction of Hydrogen Bond Sites, Distance, Angle for Catechin and Gelatin Triple Helix Complexes

Interaction site of amino acid in gelatin	—OH position in Catechin	Bond length	Bond angle
Asparagin	3	1.111	135
	4^-	1.342	131.1
Glutamine	3	1.111	135
	4^-	1.341	131
Lysine	3	1.111	135
	4^-	1.342	131.1
Proline	3	1.171	146
	4^-	1.311	143.3

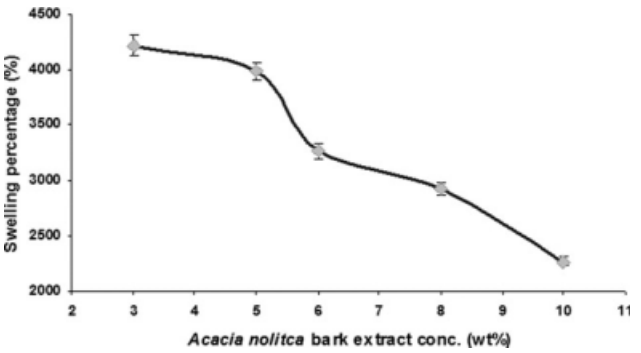


Figure 2 Effect of *A. nilotica* bark extract concentration on the swelling percentage of Gel-poly(AAm-*co*-Ac) polymeric biocomposite.

sufficient to induce considerable swelling of the films, as it can be inferred from the data reported in Figures 2 and 3. The results indicate that swelling reduces on increasing the triple helix content of the samples. Also, it displayed that swelling percentage of crosslinked gelatin films decreased with increase of concentration of *A. nilotica* bark extract. It was proposed that the increase of crosslinking degree resulted in the decrease of gelatin combination with water.

Scanning electron microscopic morphology

SEM was used to examine the morphology of the composite biopolymers. Figure 4 shows SEM-micrographs (1000 X) of (A) crosslinked Gel; (B) crosslinked Gel-poly(PAAm-*co*-Ac) 1 : 1; and (C) crosslinked Gel-poly(PAAm-*co*-Ac) 2 : 1 polymeric biocomposites; at 3% *A. nilotica* bark extract and 60°C. It displayed that both crosslinked gelatin and composite films have smooth surfaces [Fig. 4(A,B)]. It should noted that PAAm-*co*-Ac soluble in aqueous solution. Moreover, the present composite, which contains both gelatin and poly(AAm-*co*-Ac) (1 : 1) has interpenetrating networks. While, At high

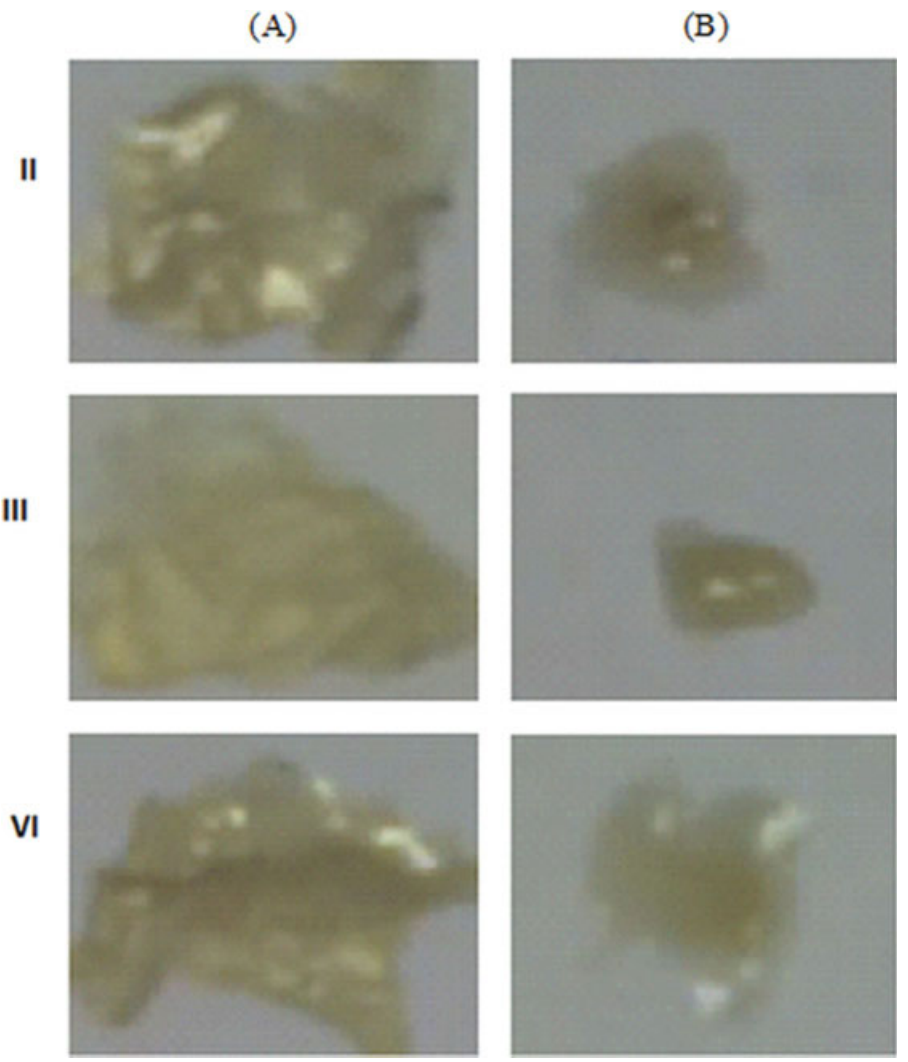


Figure 3 Photographs of the Gel-poly(AAm-*co*-Ac), (A) before and (B) after degradation. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

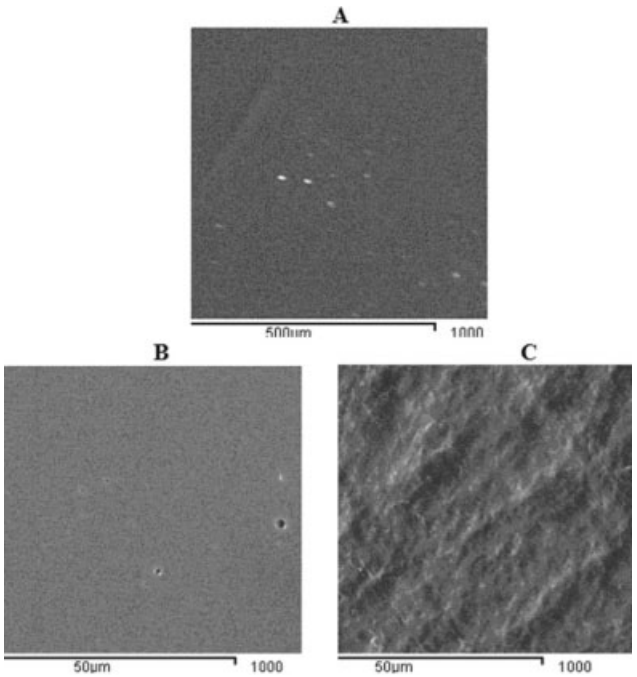


Figure 4 SEM-micrographs (1000 X) of (A) crosslinked Gel, (B) crosslinked Gel-poly(AAm-*co*-Ac) 1 : 1, (C) crosslinked Gel-poly(PAAm-*co*-Ac) 2 : 1 at 3 wt % *A. nilotica* bark extract.

gelatin concentration [Fig. 4(C)], it was supposed that for the native gelatin film and the polymeric matrix presented an orientation in the form of fibers with the presence of discontinuous zones characterized by cracks and randomly distributed along the length of the network. These discontinuous zones could be related to the formation of preferential channels during the process of drying.

UV-Vis absorption spectra

Figure 5 shows the UV-Vis absorption spectra of *A. nilotica* bark extract alone and crosslinked gelatin using 3 and 8 wt %, respectively. The results showed that the absorption peak of *A. nilotica* bark extract (catechin) treated gelatin shifted towards lower wavelengths, which were no caused by the polyphenol (catechin) itself. So, it was proposed that the shifts were caused by the formed complex. We could make suggestions about the mechanism of catechin crosslinking gelatin film as follows: Catechin can combine with gelatin, firstly form soluble complex, which can be confirmed by absorption peak of Catechin at 270 nm shifts toward lower wavelength according to UV-Vis absorption spectra. When the degree of combination is suffice, the complex precipitates from the solution. The reaction was reversible and alkali can make the complex analyze to polyphenol (catechin) and protein (gelatin). When polyphenol comes precipitation of gelatin from solution, two situation are envisaged. At low gelatin concentrations, the polyphenol associates at one or more sites on each gelatin chain to give a monolayer, which is less hydrophilic than gelatin itself [Scheme 1(a)]. Aggregation and precipitation then occur where the gelatin concentration is high. The relatively hydrophobic surface layer is formed by combination of different gelatin molecules through multidentate polyphenol [Scheme 1(b)]. From the previous results, it could be concluded that polyphenol (catechin) as crosslinking agent had improved the mechanical properties of gelatin-based film.[24]

Thermal behavior

The DSC data of collagenous materials exhibits an endothermic peak associated to the helix-coil transition of collagen (Table IV). The value of the denaturation enthalpy associated to this peak is related to the relative amount of triple helical structure in the samples and is significantly lower for gelatin with respect to collagen. *A. nilotica* bark extract stabilizes the gelatin molecules and leads to moderate increase of the denaturation temperature. The crosslinked gelatin II has 264.7 and 301.9°C relative to the uncrosslinked one I (223.2 and 276.2°C). The presence of two transition in the polymeric biocomposite films III (-42.1 and 15.4°C) and VI (-21.8 and 40.2°C) suggests that the mixture of poly(AAm-*co*-Ac) and gelatin resulting in two structures with different thermal stability. The first transition at low temperature can be due to the gelatin not crosslinked with the natural polyphenols. This can be explained by the difficulty of the natural polyphenols access to the gelatin molecules in the internal matrix of the film, which could be blocked by the presence of poly(AAm-*co*-Ac). The second transition at higher temperature suggests that the stabilization of the gelatin molecules exposed at the surface of the film by the action of the *A. nilotica* bark extract is a function of its concentration. The denaturation transition has been ascribed both to hydrogen bonds, which break endothermically and to covalent crosslinks, which break exothermically. The previous studies of gelatin films[25–28] have revealed that when films cast at room temperatures and lower, the gelatin macromolecules have mainly a collagen-like helical structure. At the same time, in films prepared from aqueous solutions by evaporating the solvent at temperatures above 35°C, gelatin macromolecules assume the conformation of a statistical coil with no indications of ordering. The kinetic nature of the helix formation process necessitates storage of gelatin gels for a certain period time before drying. During this period, the maximum degree of helicity is attained. The degree of renaturation of the collagen-like helical structure may vary substantial during the casting of the gelatin films and may attain high values in films

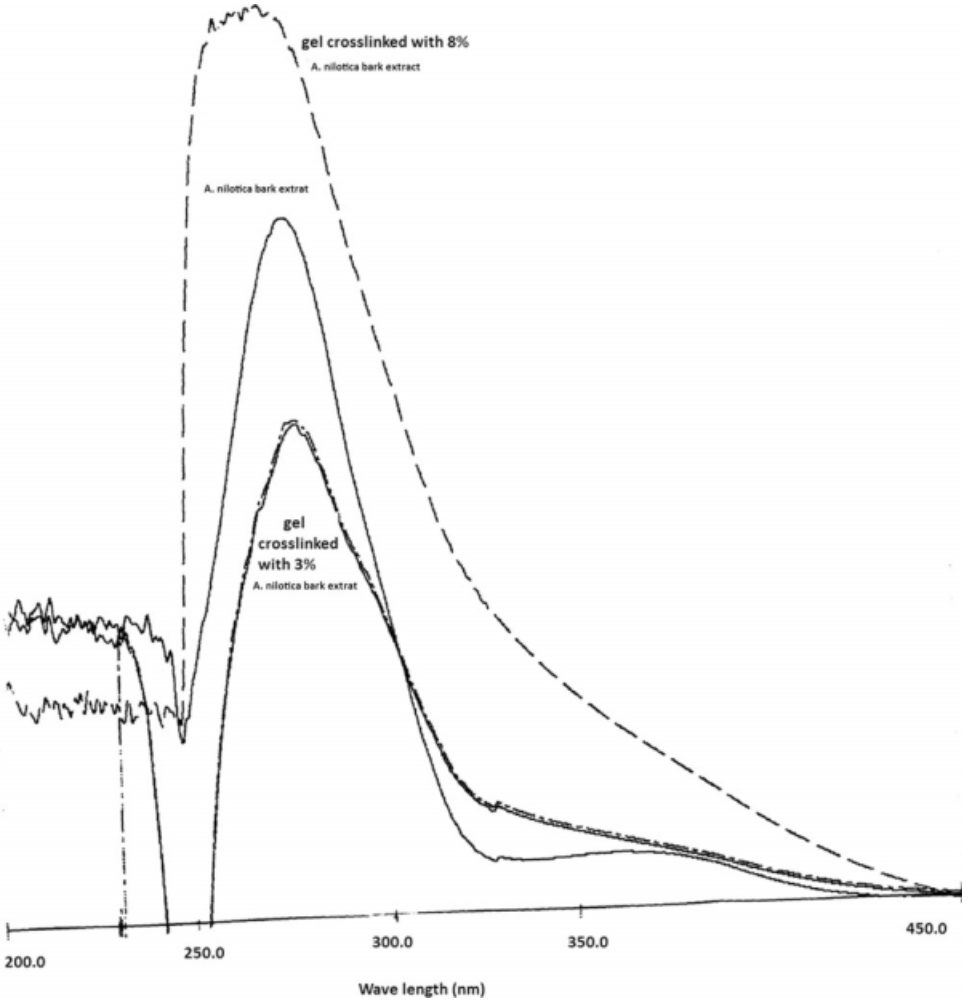


Figure 5 UV-Vis absorption spectra of *A. nilotica* bark extract, Gel crosslinked with 3 and 8 wt % of *A. nilotica* bark extract, respectively.

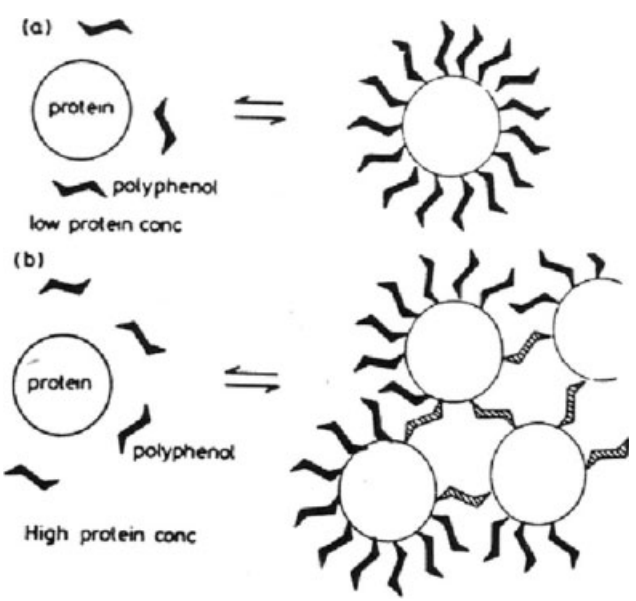
cast from well aged gels. As expected, Figure 6. clearly shows the linear relationship between the different types of gelatin film and the calculated renaturation level (X%).

CONCLUSIONS

A. nilotica bark extract could be used as crosslinking agent, which contains catechin, catechin-5-*O*-gallate, catechin-7-*O*-gallate, quercetin-3-*O*-glucoside, methyl

TABLE IV
DSC Data of the Crosslinked Gelatin-Based Polymeric Biocomposite Films

Sample code	Transition temp (°C)
Poly(AAm-*co*-Ac)	17.2, 120.7, 224.9, 252.3
I	76.0, 223.2, 276.2
II	−4.4, 38.2, 264.7, 301.9
III	−42.1, 15.4
VI	−21.8, 40.2



Scheme 1

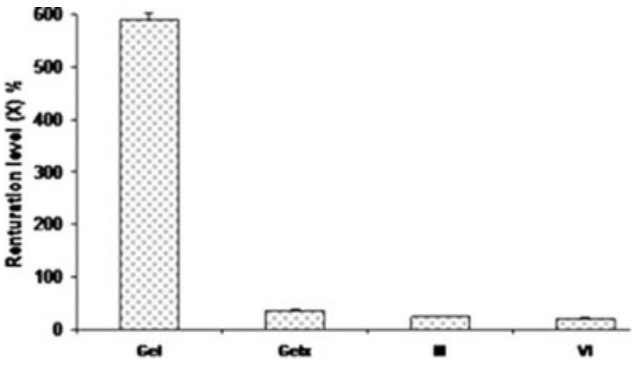


Figure 6 Effect of *A. nilotica* bark extract on the renaturation level of the gelatin-based polymeric biocomposite relative to gelatin (Gel) and crosslinked gelatin (Gel_x).

gallate, and gallic acid. The IR results suggest that the structural features of gelatin were not affected with the presence of poly(AAm-*co*-Ac) and the natural polyphenols. This was by the presence of typical bands in the spectrum for both gelatin and poly (AAm-*co*-Ac). However, the IR spectra for crosslinked Gel-based polymeric composite film showed characteristic broad band, which suggests that the hydrogen bonding and/or the physical interactions may be occur between gelatin, *A. nilotica* bark extract and/or poly(AAm-*co*-Ac). Also, *A. nilotica* bark extract crosslinking of gelatin molecules in the blended networks promotes a reduction in both swelling and degradation of the polymeric biocomposite films. The availability of crosslinked gelatin-based polymeric biocomposite together with the use of natural polyphenols as crosslinking agent, could provide a successful answer to those biomedical problems, where gelatin application is some how hindered by its poor physicomechanical properties.

References

1. Bi, S.; Xianqi, H.; Haslam, E. J Am Leather Chem Assoc 1994, 89, 98.
2. Madhan, B.; Subramanian, V.; Ramasami, T. Int J Biol Macromol 2005, 37, 47.
3. Liao, X. P.; Lu, Z. B.; Shi, B. Ind Eng Chem Res 2003, 42, 3397.
4. Gross, G. G.; Hemingway, R. W.; Yoshida, T. Plant Polyphenols II: Chemistry, Biology, Pharmacology and Ecology; Kluwer Academic Press, Plenum Pub.: NY, 1999.
5. Fraga, A. N.; Williams, R. J. Polymer 1985, 26, 113.
6. Jongjareonrak, A.; Benjakul, S.; Tanaka, M. Food Hydrocolloids 2006, 20, 492.
7. Acht, D.; He, X. W. Polymer 1995, 36, 787.
8. Chambi, H.; Grosso, C. Food Res Int 2006, 39, 458.
9. Bigi, A.; Cojazzi, G.; Roveri, N. Biomaterials 2001, 22, 763.
10. Bigi, A.; Cojazzi, G.; Rubini, K. Biomaterials 2002, 23, 4827.
11. Bigi, A.; Panzavolta, S.; Rubini, K. Biomaterials 2004, 25, 5675.
12. Kim, S. J.; Ustunol, Z. J. Agric Food Chem 2001, 49, 4380.
13. Ou, S.; Kwok, K. C.; Kang, Y. J. Food Eng 2004, 64, 301.
14. Strauss, G.; Gibson, S. M. Food Hydrocolloids 2004, 18, 81.
15. Boulos, L. Flora of Egypt; Al-Hadara Publishing: Cairo, Egypt, 1999; 1, 367.
16. Coradin, T.; Bah, S.; Livage, J. Colloids Surf B 2004, 35, 53.
17. Kenawy, E.; Newehy, M. E.; Ottenbrite, R. M. Biomacromolecules 2007, 8, 196.
18. Bigi, A.; Bracci, B.; Cojazzi, G.; Panzavolta, S.; Roveri, N. Biomaterials 1998, 19, 2335.
19. Malan, E.; Pienaar, D. H. Phytochemistry 1987, 26, 2049.
20. Tobiason, F. L.; Hemingway, R. W. Tetrahedron Lett 1994, 35, 2137.
21. Susi, H.; Ard, J. S.; Carroll, R. J. J Am Leather Chem Assoc 1971, 56, 508.
22. Madhan, B.; Thanikaivelan, P.; Subramanian, V.; Raghava, J.; Nair, B. U.; Ramasami, T. Chem Phys Lett 2001, 346, 334.
23. Madhan, B.; Parathasarathi, R.; Subramanian, V.; Raghava, J.; Nair, B. U.; Ramasami, T. Chem Phys Lett 2003, 369, 131.
24. Cao, N.; Fu, Y.; He, J. Food Hydrocolloids 2007, 21, 575.
25. Robinson, C.; Bott, M. J. Nature 1951, 168, 325.
26. Ramachandran, G. N. Recent Advances in Gelatin and Glue Research; Stiansby, G., Ed.; Pergamon Press: London and NY, 1957; Vol. 32.
27. Robinson, C. Nature and Structure of Collagen; Pergamon Press: London, 1953; p 96.
28. Coopes, I. H. J Polym Sci 1971, 6, 3683.