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Polymer 44 (2003) 5663–5670

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Interaction mechanism of poly (*o*-ethoxyaniline) and collagen blends

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Received 30 April 2003; received in revised form 30 June 2003; accepted 2 July 2003

Abstract

Blend films of poly (*o*-ethoxyaniline) (POEA) and collagen were fabricated by casting under optimized conditions and characterized by Raman scattering and UV–vis absorption spectroscopies. The UV–vis spectra showed that the addition of collagen in the aqueous solution of POEA promotes a dedoping of the POEA. This effect was also observed for the blend films as supported by Raman scattering and a mechanism for the chemical interaction between POEA–collagen is proposed. The influences of different percentage of collagen as well as the pH of stock solutions during the fabrication process of the blend films were also investigated. It was found that the preparation method plays an important role in the flexibility and freestanding properties of the films. Complementary, the surface morphology was studied by atomic force microscopy and the conductivity by dc measurements.

Keywords: Conducting polymer; Collagen; Blend

1. Introduction

Hybrid biomaterials are proposed as one of the promising future directions in biomaterial research in the development of new materials. These systems adopt the advantageous characteristics of their constituent materials and have the promise to circumvent the limitations of present biomaterials [1,2]. We have been looking into the combination of a biopolymer and a conducting polymer in the form of polymeric blends. Blends of collagen, a biological material applied as biomaterial in the medicine field, and conducting polymer can find interesting applications for developing useful materials that require some level of electrical conductivity such as in promoting osteoconductivity or nerve regeneration. This work presents a characterization by Raman scattering and UV–vis absorption spectroscopies, atomic force microscopy (AFM) and dc conductivity of electrically conductive blends containing different percentage of poly (*o*-ethoxyaniline) (POEA) in collagen. The main targets here are to know which are the preparation conditions to get suitable blend film, how the collagen could interfere on the doping state of POEA and what kind of interaction could exist between both materials.

2. Experimental

The POEA was chemically synthesized with ammonium peroxydisulphate in aqueous 1.0 mol l^{-1} HCl at 0 °C, in the doped state using a method described elsewhere [3]. The resulting polymer was then dried under dynamic vacuum for 24 h at room temperature. Anionic collagen was obtained by treating bovine tendon in aqueous alkaline solution in the presence of sulphate and chloride salts of Na^+, K^+ and Ca^{++} for a period of 48 h. Salts in excess were removed by extensive washing with 3% boric acid solution, 0.3% EDTA pH 11.0 and deionized water. The pH was adjusted to a constant value of 3.5 by the addition of acetic acid. The concentration of anionic collagen gel was adjusted to 0.3% [4].

Stock solutions of the conducting polymer (0.3% w/v—in water, adjusted at $pH = 3.5$ with HCl) and collagen (0.3% w/v—in acetic acid, $pH = 3.5$) were prepared separately. The POEA solution with concentration between 0 and 50% was added to the collagen solution and flexible and freestanding films were obtained under optimized conditions by cast. It is important to point out that the $pH = 3.5$ of the mixed solution is the key parameter in terms of getting flexible and freestanding blend films, due to the specific interactions that occurs as it will be explained

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doi:10.1016/S0032-3861(03)00612-8

latter in the interaction mechanism proposed. Cast films are not formed at lower pH while higher pH produces cracked cast films. The films were doped after immersion in HCl 1.0 mol l^{-1} for 2 h (λ maximum 800 nm in UV–vis) or were dedoped with NH_4OH 0.1 N for 2 h (λ maximum 660 nm in UV–vis). Films in dedoped state were immersed in *m*-cresol solution to remove POEA (POEA is soluble in *m*-cresol only when it is in dedoped state) from the blend for some analysis, as it will be described.

The Raman spectra were recorded with 633 nm laser line (He–Ne laser, Spectra Physics, mod 127) using a Renishaw Raman Image equipped with an Olympus or Leica metallurgic microscope and a CCD detector. The UV–vis spectra were collected using an UV–vis Hitachi spectrophotometer. The morphological study was carried out using AFM microscopy, (TMX—2010 Topometrix equipment) operating in 'intermittent contact' using a silicon nitride tip. AFM measurements were carried out on a Topometrix Discoverer TMX 2010 instrument in the intermitent contact mode using 200 μm long v shape cantilevers and Si_3N_4 pyramidal tips integrated onto the underside of the end of the cantilever. The scan rate was 1.22 Hz. The reported AFM images were captured with 500 lines and treated using leveling and shading. The dc conductivity was measured by the four-probe method [5].

3. Results and discussion

3.1. UV–vis spectra

Fig. 1 presents the UV–vis spectra of the aqueous solution containing POEA and collagen recorded along 48 h after mixing both materials. It can be seen that the band at 778 nm is shifted to 672 nm while the band at 425 nm is practically vanished after this period. It is important to point out that the pH was kept at 3.5 despite the shifting in the absorption band maximum. The band at 778 and 425 nm are attributed to the radical cation segments (polarons) that arise due to doping by an acid agent (protonation) while the band at 672 nm is assigned to quinoid species and the band at 350 nm refers to $\pi-\pi^*$ transition in the benzene rings [6,7]. Therefore, it can be concluded that the presence of the collagen in a solution containing doped POEA produces a partial dedoping of the POEA, leading to a more insulating state. The inset in Fig. 1 shows the kinetic of this process by the shifting of λ_{max} for what 48 h is enough to reach the maximum dedoped state of the POEA, which can be achieved under this condition. A possible mechanism for the interaction at this pH (3.5) involves the affinity between the collagen COO^- group and the POEA dopant proton leading to the formation of the COOH group and the dedoping of POEA. As will be shown, these types of interaction only occur under the specific conditions given at pH = 3.5. Another possible mechanism could be the affinity between the collagen NH_2 groups and the POEA dopant protons leading to the dedoping of POEA and the formation of NH_3^+ groups. However, Ashraf et al. [8] have reported that the aminosulfonic acid is not able to promote the doping of the emeraldine base polyaniline because the NH_2 group of the dopant takes the proton from its own acid group (HSO_3) forming a zwitterion structure.

The blend films prepared by casting using the solution containing doped POEA and collagen present some color

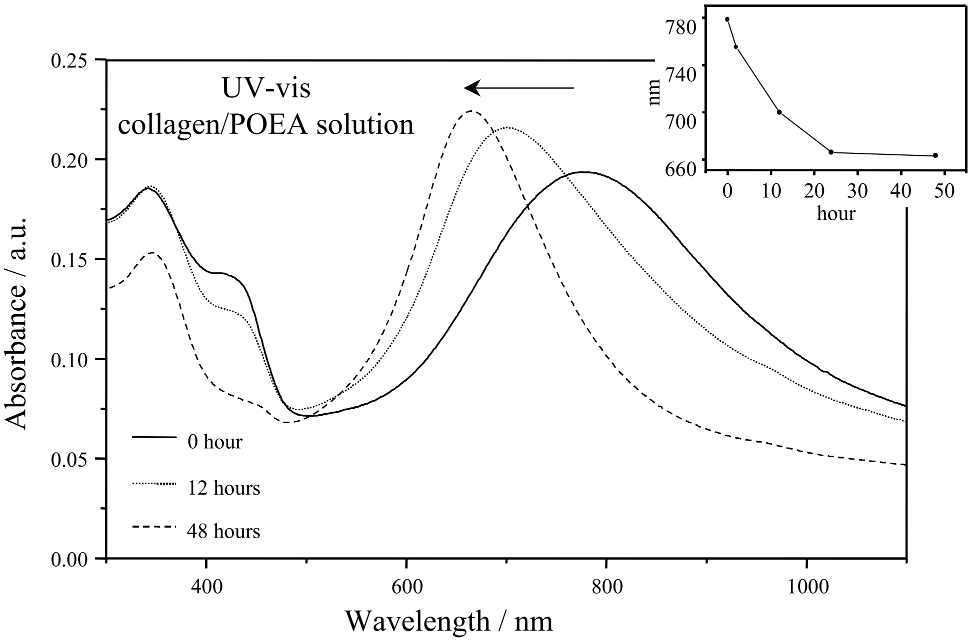

Fig. 1. UV–vis absorption spectra recorded along the time showing the effect of collagen on the aqueous solution containing doped POEA at $t = 0$. The inset shows the shifting of the λ_{max} from 778 to 672 nm due to the presence of collagen in an aqueous solution of doped POEA.

variations at the macroscopic scale. They present in their majority a bluish matrix, related to a more dedoped state of POEA, and some greenish spots, related to a less dedoped state. However, immersing these original blend films into solutions of HCl (1 mol l^{-1}) or NH_4OH (0.1 N) during 2 h, macroscopically homogeneous more doped (green) and more dedoped (blue) films can be obtained, respectively. Original blend films containing 5, 15 and 50% of POEA in collagen were immersed in HCl and NH_4OH, respectively, to investigate the effect of the amount of collagen on the doping and on the dedoping processes of the POEA. Fig. 2 presents the UV–vis absorption spectra for these films. It can be seen that the amount of collagen does not seem to play an important role on the doping and dedoping processes of the blend films by HCl and NH_4OH, respectively, since the wavelengths for the maximum absorption bands are practically the same when the blend films containing different percentage of collagen are doped (or dedoped). Complementary, dedoped blend films (by immersing in NH_4OH) containing 5, 15 and 50% of POEA in collagen were immersed in *m*-cresol for 2 h (dedoped POEA is soluble in *m*-cresol while the collagen is not) to check the possibility of any chemical interaction between both POEA and collagen in blend films. It has been previously observed [9] that similar polyaniline derivatives (poly (*o*-methoxyaniline)) could be easily extracted from its blends with poly (vinylidene fluoride), under less drastic extraction conditions due to the absence of chemical interaction. In the present case it has been observed that POEA was only partially removed from collagen, which corroborates our hypothesis of chemical interaction between POEA–collagen, as will be confirmed by the Raman analysis shown below.

3.2. Raman scattering

Fig. 3 shows the Raman spectra recorded with 633 nm laser line for 5, 15 and 50% of POEA in collagen blend films, which were doped in HCl 1 mol l^{-1}. The Raman spectra also collected with the 633 nm laser line for doped powder of POEA (HCl 1 mol l^{-1}) and for cast film of pure collagen are given in Fig. 4 as references. Table 1 contains the center of the Raman bands, full width at half maximum (FWHM), relative intensities calculated using a mixed Gaussian–Lorentz function and the assignments [10–13] for the main POEA Raman bands. It is seen that the presence of collagen induces a similar effect observed by Pereira-da-Silva et al. [13] when polyaniline powder doped by protonation (called primary doping) is heated: the growing of the bands around 574 and 1381 cm^{-1} as well as the shoulder at 1643 cm^{-1} with the vanishing of the shoulder at 1620 cm^{-1} when the spectrum of POEA (Fig. 4) is compared with the spectra of the blend (Fig. 3). They have suggested a polyaniline interchain interaction (cross-link forming cyclized structures containing tertiary nitrogen) and/or oxidation (reaction between imine and oxygen) as a result of heating. Complementarily, the influence of the different dopant anions either on the doping process of polyaniline or on its chain conformation is shown by Ashraf et al. [8]. The authors have suggested that the interaction between the NH groups from polyaniline and the C=O groups from the dopant agent can induce a helicoidal conformation of the polyaniline chains, which would be responsible for the observed circular dichroism (CD) of the conducting polymer.

In our case, at pH = 3.5 the POEA and collagen in the blend may present both an electrostatic interaction through

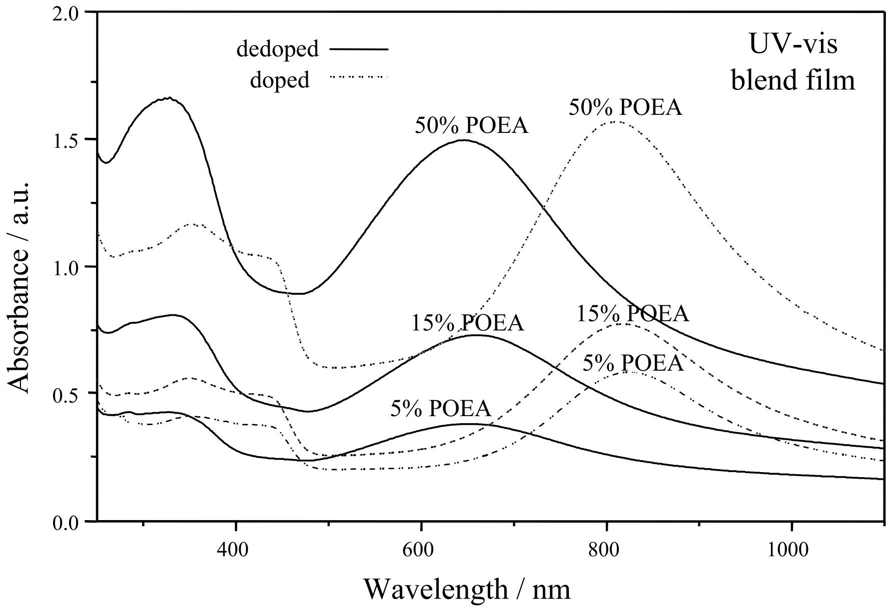

Fig. 2. UV–vis absorption spectra of the blend films containing 5, 15 and 50% of POEA in collagen, which were doped and dedoped by HCl and NH_4OH solutions, respectively.

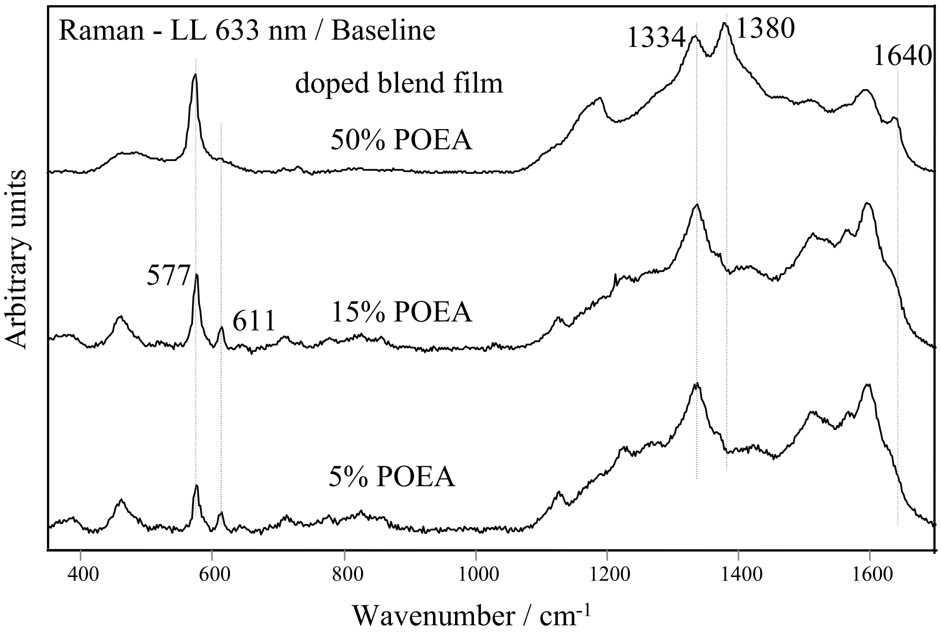

Fig. 3. Raman spectra recorded using the 633 nm laser line for the 5, 15 and 50% of POEA in collagen blend films, which were doped in HCl.

the carboxylic groups (COO^-) from the protein and the imine groups partially protonated (NH^+) from the conducting polymer and hydrogen bonds between COOH from the protein and –N= from the conducting polymer. Fig. 5A shows these interactions at pH = 3.5 for what both imine and carboxylic groups are partially protonated at the specific proportion needed to allow the interaction which leads, therefore, to miscibility and to the production of free-standing films from their blends, what does not happen at other pHs. For instance, Fig. 5B presents both materials at pH = 7.0 for what the interaction is not allowed leading to immiscibility and segregation of the two polymers and consequently a two macroscopic phase material, since the POEA does not contain radical cations and the collagen is not protonated. The molecular structures of collagen and POEA [14,15] shown in Fig. 6 reveal that one collagen molecule has approximately a length of 280 nm containing 376 amino acids (COO^-) spaced by 2.4 nm each other while one molecule of POEA (extended conformation) has approximately a length of 63 nm containing 63 imine groups (NH^+). Therefore, considering this approximation and taking into account the dimensions of the molecules and the spacing between the COO^- and the NH^+ groups itself, it could be speculated that the COO^- would interact with the

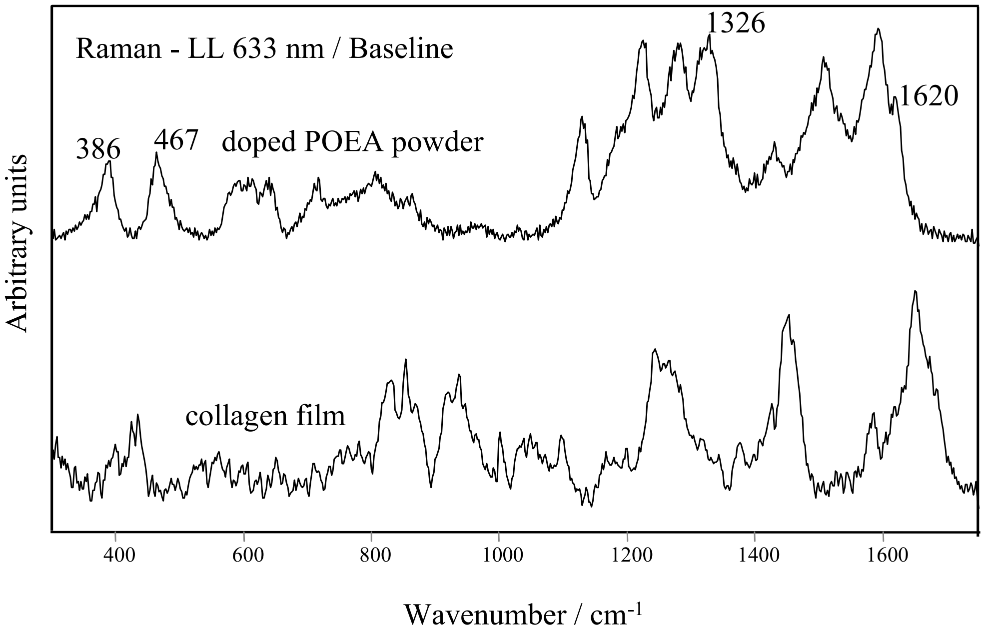

Fig. 4. Raman spectra recorded using the 633 nm laser line for the doped (HCl) powder of POEA and collagen cast films.

Table 1
Wavenumber, full width at half maximum (FWHM) in cm^{-1} (in parenthesis) and relative intensities got using the mixed Gauss–Lorentz function from the Raman spectra recorded using the 633 nm laser line for doped powder of POEA and different percentage of doped POEA in collagen blend films

POEA powder	POEA 5%	POEA 15%	POEA 50%	Assignments
386 (30) 41	379 (46) 10	372 (55) 13		Ring deformation (in-plane)
467 (33) 40	461 (28) 23	462 (35) 27	481 (82) 20	C–N–C torsion or C–H wagging
	577 (13) 40	577 (15) 65	574 (16) 85	Cyclized struct. or tertiary N
600 (54) 32				Amine deformation (in-plane)
	611 (46) 22	611 (35) 20	615 shoulder	Amine deformation (in-plane)
642 (20) 23				Benzenoid ring deformation (in-plane)
713 (33) 22	714 (26) 9	712 (28) 10	722 (32) 3	C–C ring deformation (out-of-plane)
803 (106) 31	832 (73) 12	823 (50) 12		C–H quinoid deformation (out-of-plane)
1128 (26) 61	1124 (30) 22	1123 (19) 13	1124 (58) 19	C–H deformation (in-plane)
1184 (26) 34	1181 (59) 25		1178 (54) 50	C–H bending (in-plane)
1223 (36) 96	1223 (30) 23	1235 (158) 52		C–N stret. benzene-diamine
1277 (41) 80	1263 (88) 47		1292 (148) 70	Amine deformation (in-plane)
1326 (48) 98	1335 (62) 86	1335 (48) 81	1334 (39) 57	Cation radical (C–N^+ stret.)
	1370 shoulder	1370 shoulder	1380 (43) 100	Cyclized struct. or tertiary N
1427 (50) 31	1421 (111) 33	1420 (98) 54	1420 (46) 54	Benzene ring deformation (in-plane)
1507 (67) 91	1516 (80) 63	1519 (86) 87	1516 (58) 60	N–H bending (in-plane)
1594 (62) 100	1597 (75) 100	1597 (36) 100	1594 (47) 72	C–C benzenoid stret.
1620 (15) 30				C–C benzenoid stret.
	1640 shoulder	1640 shoulder	1640 (28) 46	Cyclized struct. or tertiary N

first and the third NH^+ for instance. Thus, the length of the molecules would be a limiting parameter for the number of interaction sites in this model (linear molecules). Therefore, up to five molecules of POEA could interact with one molecule of collagen as shown in Fig. 5A.

Other evidences that support the chemical interaction between POEA–collagen can be found in Fig. 3 and Table 1

(i) The bands related to the POEA–collagen interaction (577 or 574, 1370 or 1380 and 1640 cm^{-1}) becomes stronger when the percentage of POEA increases from 5 to 50% supporting our model since greater amount of POEA gives more available interaction sites for COO^- and NH^+.

(ii) The FWHM of the bands around 577 cm^{-1} does not

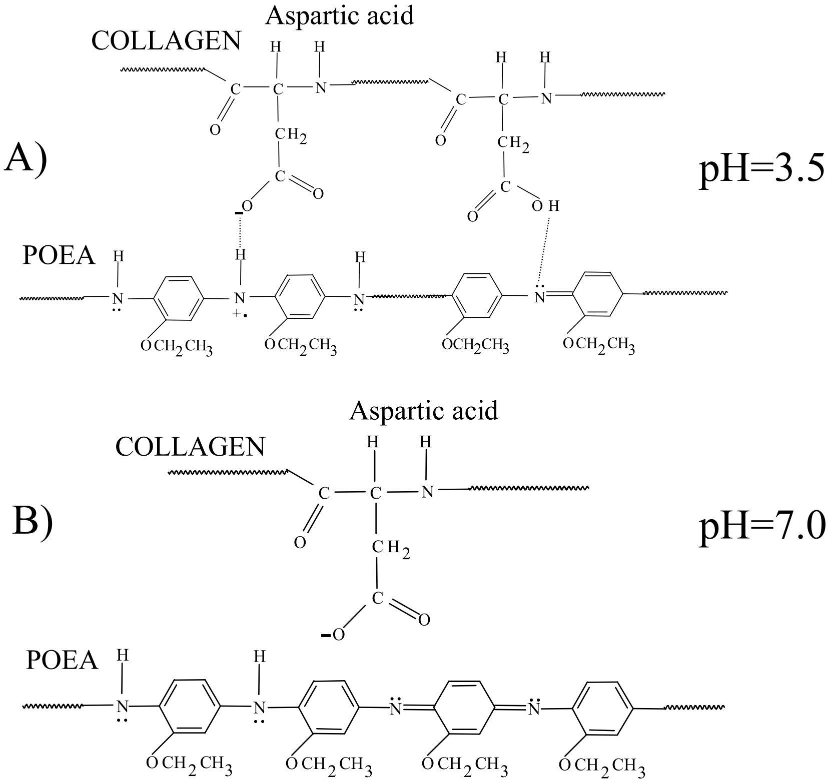

Fig. 5. (A) suggested mechanisms of interaction between POEA and collagen at pH = 3.5. (B) absence of interaction at pH = 7.0.

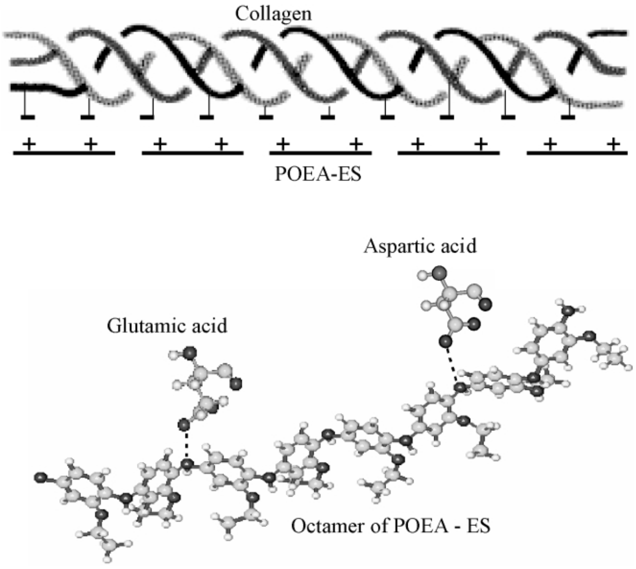

Fig. 6. Geometrical features of the POEA and collagen molecules.

change significantly for the different percentage of POEA in collagen indicating that the increase in intensity of the band at 577 cm^{-1} should not be attributed to a possible better molecular organization around the radiation scatterer due to lower percentage of collagen [16].

(iii) The differences between the spectra of the POEA (Fig. 4) and the blend (Fig. 3) must be attributed to the presence of collagen mainly, which is a strong evidence of the chemical interaction between POEA–collagen (the spectrum of the blend is not a simple superposition of the POEA + collagen spectra).

A final comment from the data in Fig. 3 and Table 1 is related to the increase in intensity of the band at ca 611 cm^{-1} for lower percentage of POEA. Pereira-da-Silva et al [17] working with blends containing conducting polymer have observed that the intensity of the band at ca 611 cm^{-1} is sensitive to the spatial distribution of the polymer in the blend. In our case, the increase of the band could be also an indicative of a better spatial distribution of POEA in collagen, which would be in agreement with our proposed chemical interaction between POEA–collagen as well (otherwise they would form segregated phases), however, this is something that requires further research.

Fig. 7 shows the Raman spectra recorded with the 633 nm laser line for the blend films containing 5, 15 and 50% of POEA in collagen, which were dedoped by NH_4OH. It is observed that the Raman band at ca 1467 cm^{-1} attributed to quinoid structures dominates the spectra as expected. However, the band at 1338 cm^{-1} attributed to the radical cation $C{-}N^+$ (polaron) is still present showing that the films were not completely dedoped. It is important to mention that the spectra for the dedoped films are better defined due to the full resonance between the laser line (633 nm) and the electronic absorption band of the dedoped film.

3.3. AFM analysis

The blend morphology was investigated for cast films containing different compositions of collagen and dedoped or doped POEA. Fig. 8 shows an AFM image of a dedoped blend film containing 15% of POEA and Fig. 9 shows an image of the doped blend film containing 15% of POEA. Comparing Figs. 8 and 9, a globular structure can be found

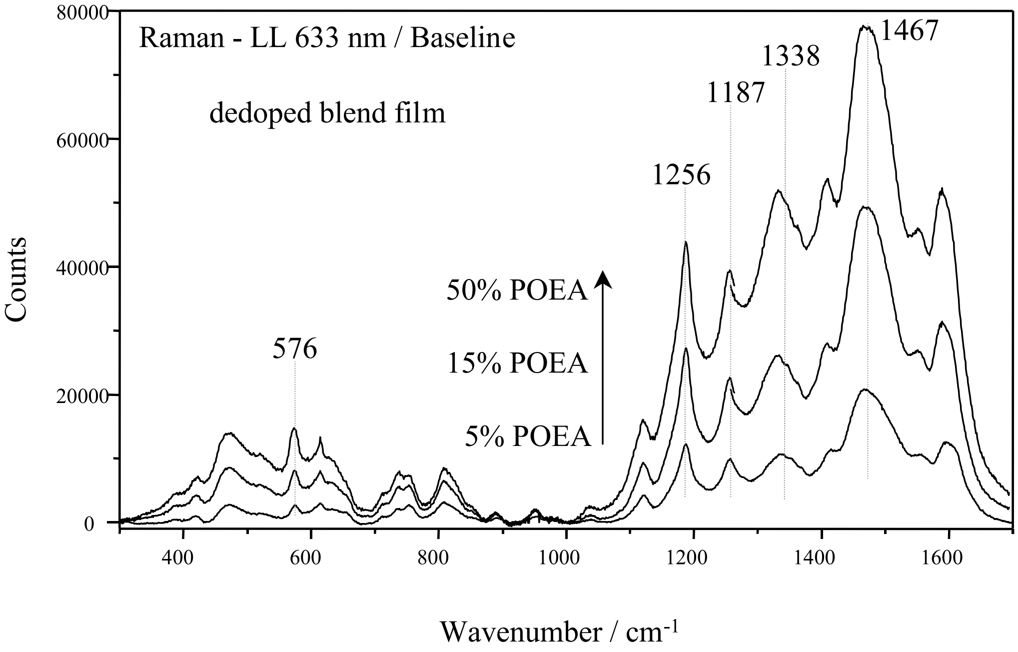

Fig. 7. Raman spectra recorded using the 633 nm laser line for 5, 15 and 50% of POEA in collagen blend films, which were dedoped in NH_4OH.

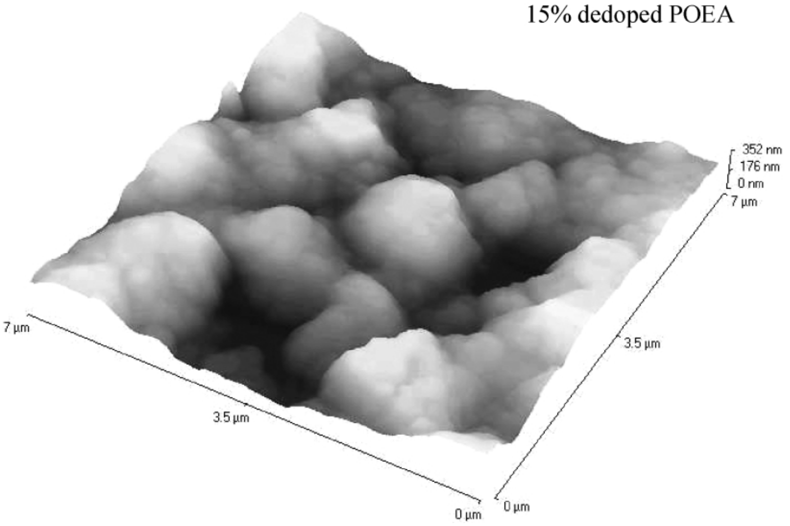

Fig. 8. AFM images of blends containing 15% POEA in collagen dedoped with NH_4OH.

for both films, however, Fig. 9 contains a larger number of smaller globules that arises after doping the film. The later is related to the introduction of the counter ion between the polymer chains [18]. Similar images (not shown) were also obtained for the samples, which were submitted to *m*-cresol extraction, indicating that no significant amount of POEA has been extract, which corroborates the other miscibility evidences. A globular morphology has been also observed in previous work with polyaniline [19] and derivatives [20] prepared under several conditions. The dc conductivity of the doped blend film was carried out using the four-probe method [5] and the results are shown in Fig. 10. It can be seen that the conductivity increases with the increase of the percentage of POEA in the blend, which is in agreement with that observed by UV–vis for those films (Fig. 2). These conductivity values are much higher than those for collagen (insulator material, $\sim 10^{-13}$ S/cm) and comparable to the semiconductor ones. Therefore, despite of the collagen has the tendency of dedoping the POEA, the conductivity can be improved again by immersing the blend films into HCL, which is an important feature in terms of applications.

4. Conclusion

Blends of POEA and collagen with different compositions were successfully produced by casting, at $\mathrm{pH} = 3.5$, in the form of flexible free-standing films. It is suggested that specific interactions occurs between the blend components when prepared at $\mathrm{pH} = 3.5$: (i) electrostatic interaction between the NH^+ group from POEA and COO^- group from collagen and (ii) hydrogen bond between

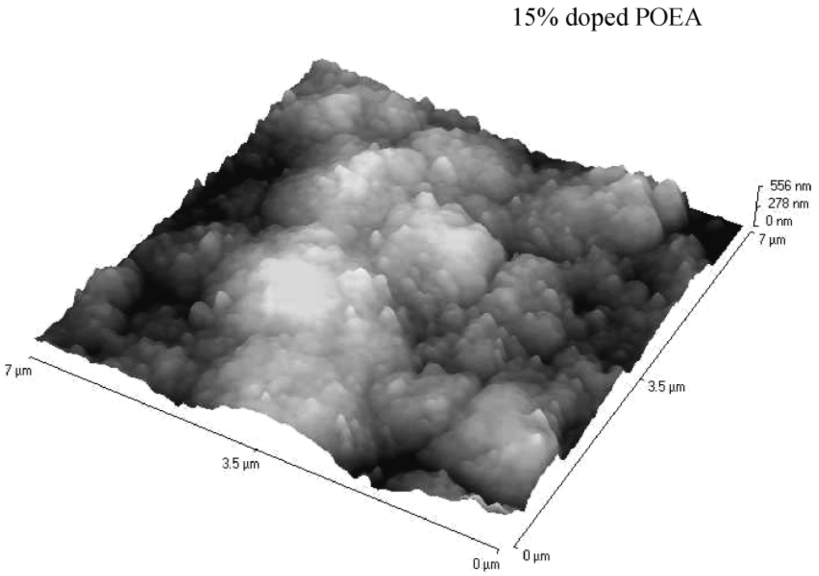

Fig. 9. AFM images of blends containing 15% POEA in collagen doped with HCl.

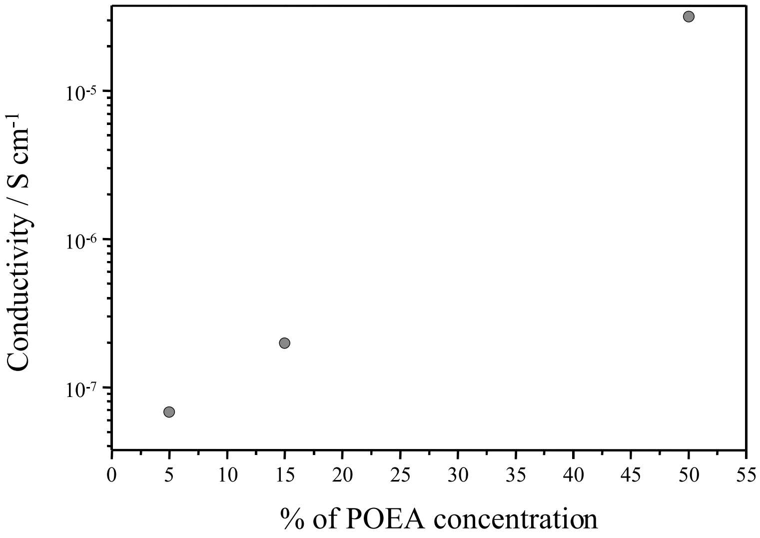

Fig. 10. dc conductivity of the doped blend films.

COOH from collagen and –N= from POEA. These interactions promotes miscibility between POEA and collagen and were evidenced by the following observations: (i) The doped POEA in aqueous solution is partially dedoped due to the presence of collagen, during film preparation, as shown by UV–Vis spectroscopy; (ii) POEA could be only partially removed from collagen when dedoped blend films were immersed in *m*-cresol solution; (iii) Raman spectroscopy showing the increase in intensity of the bands related to cyclized structures or tertiary N with the amount of POEA in the blend. The interaction seems to be stronger for higher percentage of POEA in collagen. These blends present a globular morphology irrespective of the blend composition and doping state, although both leads to changes in surface roughness. The dc conductivity values of blends are in the range of the semiconductor materials, which might open new applications for collagen-based materials.

Acknowledgements

The financial support by FAPESP, CNPq and CAPES is greatly acknowledged. The authors also wish to thank Dra Marcia L. A. Temperini (IQ/USP) and Dr Ricardo Zanatta (IFSC/USP) for providing the Renishaw micro-Raman facilities.

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