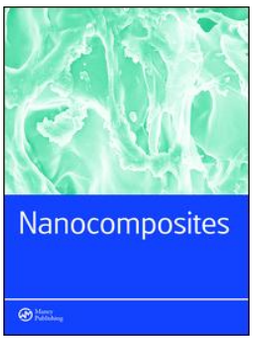

Nanocomposites

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Nanocomposites

Original Research Paper

Nanocomposite hydrogels based on embedded PLGA nanoparticles in gelatin

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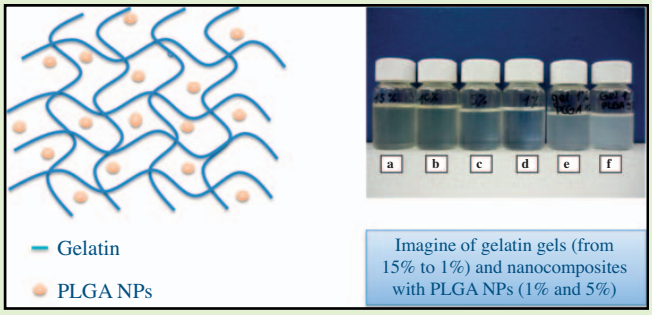
Abstract Novel nanocomposites based on gelatin polymer networks containing poly(lactic-co-glycolide) (PLGA) nanoparticles (PLGA-NPs) were prepared and morphological, chemical and rheological properties were investigated. The successful incorporation of PLGA nanoparticles into the gelatin gels was confirmed by field emission scanning electron microscope (FESEM) and infrared spectroscopy (FT-IR). FESEM microscopy showed also a more homogeneous pore structure of the nanocomposite with respect to the primary gel. The introduction of PLGA nanospheres at a 1% w/v with respect to gelatin weight does not influence the elastic modulus of the pristine gelatin but an increase in the amount of PLGA spheres determine a negative effect on the elastic modulus. The prepared PLGA-NPs gelatin gels with biocompatible and biodegradable properties are very interesting from both applied and fundamental perspectives for a future application in biomedical and food fields for the control release of active molecules.



Keywords Poly (DL-Lactide-co-Glycolide), Biodegradable nanoparticles, Gelatin, Gel and nanocomposite

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Introduction

Gelatin is a natural polymer derived from collagen and is commonly used for pharmaceutical and medical applications because of its biodegradability[1] and biocompatibility in physiological environments.[2] The isoelectric point of gelatin can be modified during the fabrication process to yield either a negatively charged acidic gelatin or a positively charged basic gelatin at physiological pH. This property theoretically allows electrostatic interactions between a charged biomolecule and gelatin of the opposite charge, forming polyion complexes.[2] Various forms of gelatin carrier matrices were reported for controlled release applications while characterization studies have shown that gelatin carriers are able to sorb charged biomolecules such as proteins and plasmid DNA.[3,4] It has been shown that the crosslinking density of gelatin hydrogels affects their degradation rate in vivo leading to a similar profile for the biomolecule release rate from gelatin carriers, suggesting that complexed gelatin/biomolecule fragments are released by enzymatic degradation of the carrier.[5]

The most commonly used biodegradable synthetic polymers for particles formation in biomedical field are saturated poly(α-hydroxy esters), including poly(lactic acid) (PLA) and poly(glycolic acid) (PGA), as well as poly(lactic-co-glycolide) (PLGA) copolymers.[6,7] The chemical properties of these polymers allow hydrolytic degradation through de-esterification. Once degraded, the monomeric components of each polymer are removed by natural pathways. PGA is converted to metabolites or eliminated by other mechanisms, and PLA can be cleared through the tricarboxylic acid cycle. Owing to these properties PLA and PGA have been used in biomedical products and devices, which have been approved by the US Food and Drug Administration.[8] Generally, the co-polymer PLGA is preferred for the fabrication of bone substitute constructs, compared with its constituent homopolymers, as it offers superior control of the degradation properties by varying the ratio between its monomers. PLGA, for instance, has a wide range of degradation rates, governed by the composition of chains, both hydrophobic and hydrophilic balance and crystallinity.[8]

The use of nanocomposites based on a hydrogel matrix and biopolymeric nanoparticles is expanding significantly in recent years for control release applications.

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Rescignano *et al.*[9] described a nanocomposite hydrogel prepared by the incorporation of biocompatible and biodegradable PLLA nanoparticles into semi-interpenetrating polymer hydrogels of a natural polymer such as alginate and thermosensitive poly(N-isopropylacrylamide). This kind of systems has been used to stem different aspects like the double control of release or the vehiculation of nanodevices towards the target of interest. In fact, the addition of polymer nanoparticles into polymer gels also allows the local delivery of hydrophobic drugs when these are loaded into nanoparticles.[10] These nanoparticles have several advantages over the conventional bulk hydrogels for controlling the release of high molecular weight biomolecules. The drug would diffuse out first through the nanoparticles and from them to the hydrogel network; then, they will be further released from the bulk hydrogel network. Therefore, compared with the bulk hydrogel or pure nanoparticles, in the case of a nanocomposite hydrogel, two barriers can control the drug release. The aim of the research reported here is the preparation and rheological characterization of natural gelatin gels containing PLGA nanoparticles. The viscoelastic properties of gelatin gels at different concentrations will be compared with the corresponding gelatin gels containing PLGA nanoparticles.

Experimental part

Materials

Poly(lactic-co-glycolic)acid with a 50/50 ratio (PLA/PGA) was obtained from Absorbables Polymers/Lactel (Durect Corporation, UK) (Mw 91600–120 000 g mol^{-1}). Polyvinyl alcohol (Mw 31 000–50 000 g mol^{-1}, 87–89% hydrolyzed) was used as surfactant and chloroform ($CHCl_3$) from Aldrich was used as solvent. The gelatin used was derived from porcin skin, type A, and was purchased from Sigma-Aldrich. Millipore water (resistivity 18.2 mΩ cm) was used for the preparation of PLGA nanospheres while distilled water (resistivity 15.0 mΩ cm) was used for the preparation of the gelatin gel.

Preparation of PLGA nanoparticles

PLGA nanoparticles were prepared by a double emulsion water/oil/water method with subsequent solvent evaporation. Around 0.125 g of PLGA were dissolved in 5 mL of chloroform for 2 h under magnetic agitation at room temperature. The solution was emulsified with 2.5 mL of Millipore water using a sonicator (SONICS Vibra Cell) at 30% amplitude for 15 min. A PVA aqueous solution (2% w/v) was prepared by dissolving about 0.8 g in 40 mL of Millipore water. This solution was added to the first emulsion and mixed for 15 min with sonication for the formation of the second emulsion. For the solvent evaporation, the second emulsion was transferred in 200 mL of 0.2% w/v PVA aqueous solution in Millipore water and was magnetically stirred over night at room temperature. The nanospheres were collected by centrifugation at 3500 rev min^{-1} for 30 min and then washed four times with Millipore water and finally lyophilized to get a powder.[6]

Preparation of gelatin gels with embedded PLGA nanoparticles

The gelatin was dissolved at various concentrations (5, 10 and 15% (w/v)) in distilled water, by stirring the system for 30 min at 50°C. Then, gels were prepared on Petri dishes (50 mm diameter) and maintained at 4°C for 18 h prior to analysis. For the preparation of nanocomposite hydrogels, aqueous suspensions of PLGA nanoparticles (1, 2.5 and 5% w/w PLGA with respect to gelatin weight) were dispersed into the gelatin aqueous solution (1% w/v) under constant stirring at 50°C during 30 min. Gels were prepared on Petri dishes (50 mm diameter) and maintained at 4°C for 18 h prior to analysis. Nanocomposite hydrogels were denoted as PLGA1-gelatin and PLGA5-gelatin where 1 and 5 denotes the concentration (% w/w) of PLGA with respect to the gelatin weight.

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)

Experiments were made on lyophilized samples with a Perkin Elmer Spectrum One ATR-FTIR spectrometer over a diamond crystal. The wavenumber sweep was 4000–650 cm^{-1} with a resolution of 4 cm^{-1}.

Morphological characterization

The morphology of the polymeric nanocomposite and nanoparticles was analyzed by field emission scanning electron microscopy (FESEM Supra 25, Zeiss, Germany). Samples were deposited onto FTO (fluorine-doped tin oxide) substrates using a drop casting method, allowing them to dry at room temperature for 24 h and gold coated by an Agar automatic sputter coating.

Rheological characterization

Rheological properties of gels were carried out in a Rheometer AR-1000 using parallel plates of 20 mm. All measurements were carried out at $T = 10$°C. The linear viscoelasticity region was determined by means of oscillatory torque sweeps carried out between 0.1 and 1000 μN m^{-1} at a frequency of 1 Hz frequency sweeps between 10 and 0.1 Hz at a fixed torque in the linear viscoelastic region.

Results and discussion

Morphology of nanocomposite gels

Figure 1 *a–e* shows the FESEM images corresponding to cross-sections performed through the thickness of the gelatin 1% w/v and the nanocomposite hydrogel, PLGA5-gelatin. Figure 1*f* reports an image of the synthesized PLGA NPs. As previously reported,[6] PLGA nanoparticles show a spherical shape. No aggregation phenomena after drying were observed; the average diameter was around 130 nm as previously demonstrated by Rescignano *et al.*[6] A cross-linked morphology can be observed in the images of pure gel and nanocomposites; however, the gelatin 1% w/v is characterized by a more homogeneous and dense pore structure than PLGA5-gelatin. This might suggest that the presence of PLGA nanoparticles interferes somehow with the formation of the gelatin hydrogel as it will be ascertained through rheological measurements. Figure 1*d* shows the image of the

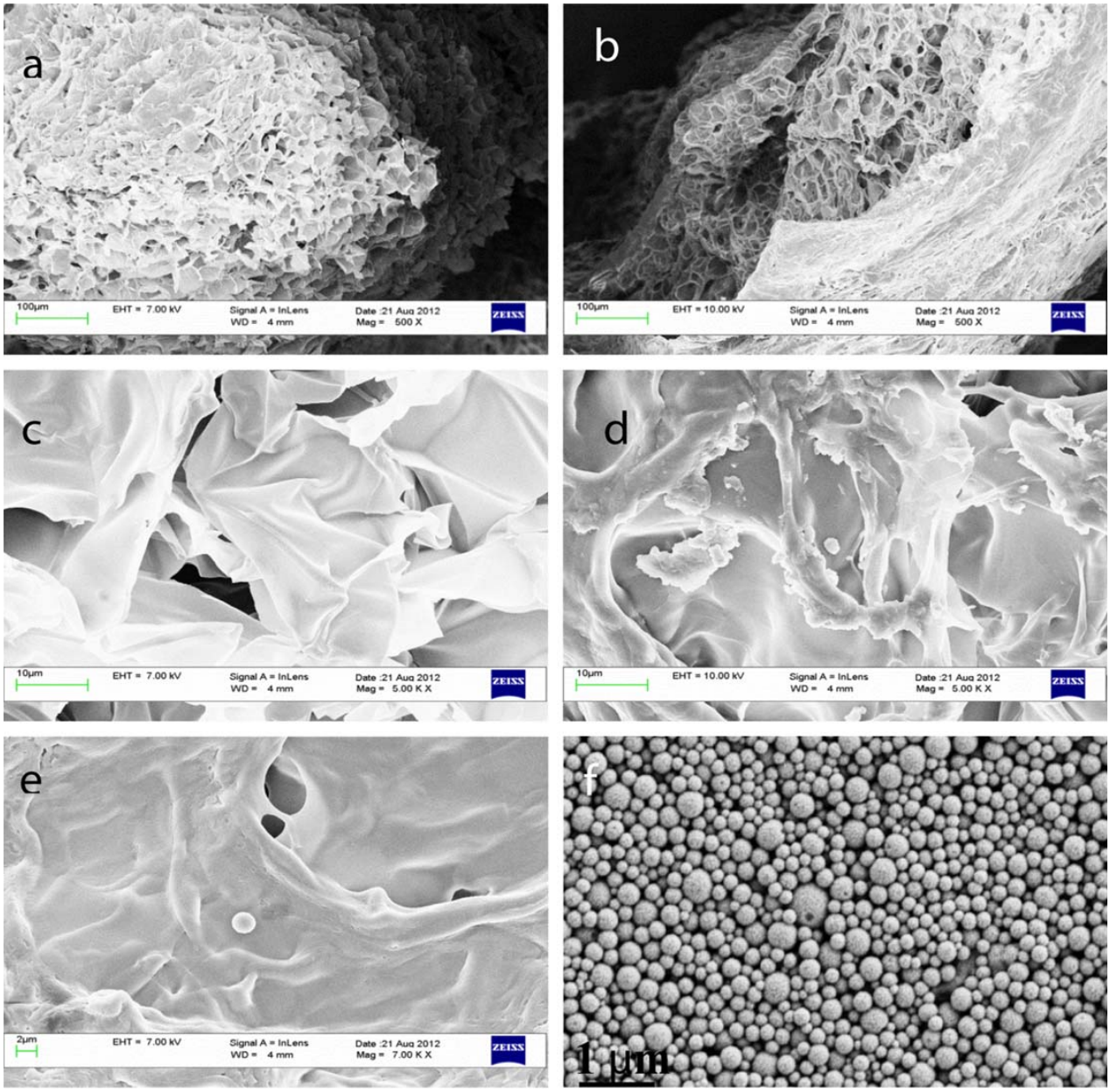

Figure 1 FESEM images of *a*, *c* gelatin gel 1% w/v and *b*, *d*, *e* gelatin gel with 5% PGLA NPs, *f* shows synthesized PLGA-NPs

cross-section of PLGA5-gelatin; several NP aggregates were embedded in the hydrogel matrix. Figure 1*e* shows a magnification image in which a single PLGA nanoparticle can be observed; however, the degree of dispersion in the gelatin matrix could not be assessed from this image.

Chemical characterization by ATR-FTIR

Figure 2 shows the ATR-FT-IR spectra of pure PLGA, gelatin (1% w/v) and two nanocomposite hydrogels prepared at a fixed gelatin concentration (1% w/v) and two different PLGA-NPs concentrations, 1% w/w and 5% w/w. The two nanocomposite hydrogels present the characteristic bands of gelatin (red spectra in Fig. 2), a band situated around 3288 cm^{-1} corresponding to the NH-stretching coupled with hydrogen bonding of the water molecule O—H; C═O stretching around 1630 cm^{-1} for the amide I; the bending vibration of N—H groups and stretching vibrations of C—N

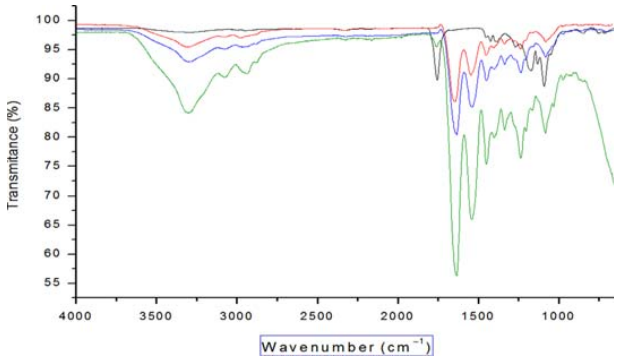

Figure 2 ATR-FTIR spectra corresponding to pure PLGA (black); gelatin 1% (w/v) (red); PLGA1-gelatin (blue) and PLGA5-gelatin (green) nanocomposite hydrogels

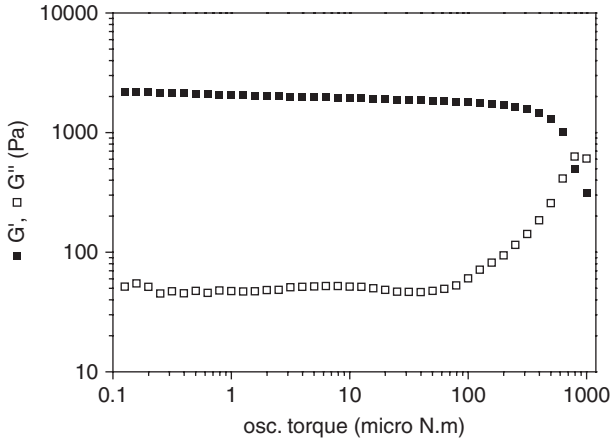

Figure 3 Evolution of G′(■) and G″(□) as function of oscillatory torque for gelatin [10% (w/v)]

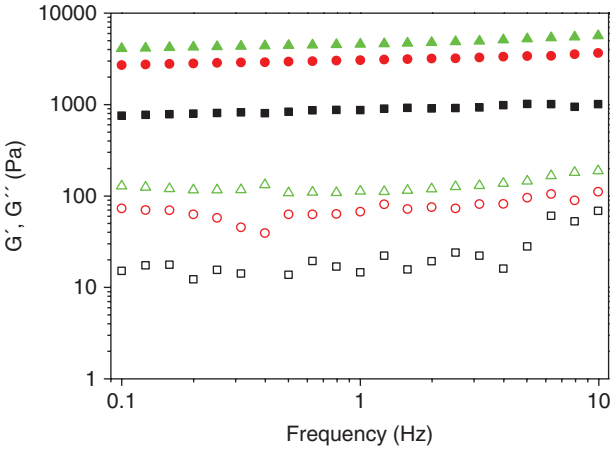

Figure 4 Evolution of G′ (closed symbols) and G″ (open symbols) as function of frequency for gelatin prepared at different concentrations (■) 5% w/v; (●) 10 w/v and (▲) 15% w/v

groups around 1539 cm^{-1} for the amide II; the vibrations in the plane of C—N and N—H groups of bound amide around 1237 cm^{-1}.[11]

Regarding the spectra corresponding to pure PLGA, the characteristic peak located at 1748 cm^{-1} can be attributed to the absorbance of the carbonyl group C=O.[12] This peak was visible only in the spectra corresponding to the nanocomposite hydrogel containing the highest concentration of PLGA nanoparticles (5% w/w). For the nanocomposite hydrogel containing 1% w/w of PLGA nanoparticles, it was not possible to ascertain the presence of PLGA NPs by this technique.

Rheological characterization

The linear viscoelastic region is observed in the low oscillation stress region where both, the elastic modulus and the loss modulus, are independent of the oscillation stress. As a representative example (Fig. 3), the evolution of the elastic and loss modulus with the oscillation torque is shown for gelatin at a 10% w/v concentration. The linear viscoelastic region extends from 0.1 and 100 Pa. A value of 10 Pa was chosen to carry out the rest of the rheological measurements.

As a first step, frequency sweeps were carried out for pristine gelatin gels at different concentrations (5, 10 and 15% w/v) and the results are shown in Fig. 4. It can be observed that, in all cases, the elastic modulus (G′) is higher than the loss modulus (G″) and both remain independent of frequency; this is characteristic of gel behavior.[13]

Figure 4 shows a representation in double logarithmic scale of the values of elastic modulus extracted from frequency sweeps at a frequency = 1 Hz as a function of gelatin concentration. It can be observed that the elastic moduli of gelatin hydrogels increases with the gelatin concentration. In previous studies,[14–16] the viscoelastic results obtained for polymer gels have been analyzed using a theoretical model based on a scaling approach which relates modulus to concentration through the following type of equation

$$G \approx C^{n} \quad (1)$$

where n is an exponent which depends upon the conformation of the chain linking junction points and is related to the fractal dimension ν^{-1} of the object between the junctions through the following equation.[17]

$$G \approx C^{3\nu/(3\nu-1)} \quad (2)$$

Fitting the data represented in Fig. 5 for gelatin hydrogels to equation (2) yields a fractal dimension, $\nu^{-1} \sim 1.5$, which is characteristic of rod-like chains. This result is in agreement with published results regarding gelatin gels considered as entangled networks of rigid rods possibly connected by flexible links.[18]

For the preparation of nanocomposite hydrogels, a concentration of 1% w/v of gelatin was chosen. Figure 6 shows the elastic modulus of gelatin 1% w/v and its nanocomposite hydrogels PLGA1-gelatin and PLGA5-gelatin. It can be observed that, for both nanocomposite hydrogels, G′ is relatively independent from the frequency applied in the studied range so that the incorporation of PLGA nanospheres in gelatin gels does not change the typical gel behavior.

The inset in Fig. 6 shows a representation of the elastic modulus as a function of the PLGA nanoparticles. The introduction of PLGA nanospheres at a 1% w/w with respect to gelatin weight does not influence the elastic modulus of the pristine gelatin. However, an increase of the

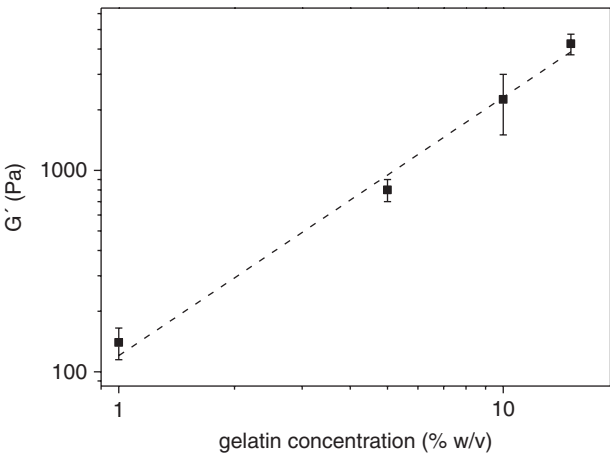

Figure 5 Elastic modulus G′ as function of gelatin concentration: dashed line represents fitting of data to equation (1)

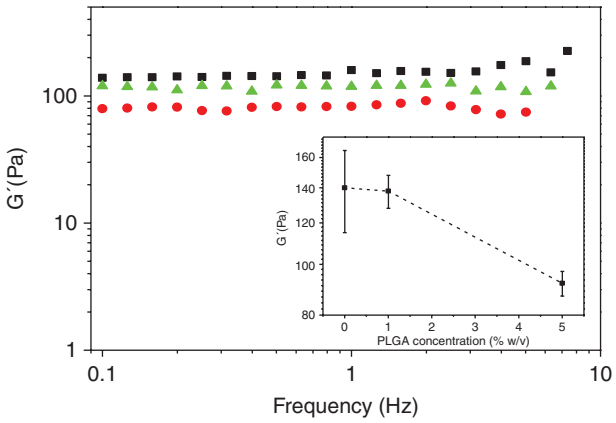

Figure 6 Evolution of G′ as function of frequency for gelatin 1% w/v (■); PLGA1-gelatin (▲) and PLGA5-gelatin (●): inset shows representation of elastic modulus as function of PLGA concentration

concentration of PLGA nanospheres in the gelatin gel to 5% w/w significantly decreases the elastic modulus of the nanocomposite hydrogel with respect to gelatin 1% w/v. This might indicate that the incorporation of PLGA nanoparticles inside gelatin prevents the formation of the gel above a certain concentration, and hence, the degree of crosslinking is lower resulting in a lower elastic modulus.

Conclusions

The inclusion of PLGA nanoparticles in gelatin hydrogels was successfully performed and characterized. The FESEM images revealed differences in the porous structure obtained. Specifically, the presence of PLGA NPs induces a more homogeneous pore structure of the hydrogel nanocomposite than in the case of the neat gel.

The introduction of PLGA nanospheres at 1% w/v with respect to gelatin weight does not influence the elastic modulus of the pristine gelatin but an increase of the amount of PLGA spheres (5% w/v) influences negatively the elastic modulus of the nanocomposite hydrogel with respect to the gelatin 1% w/v.

The successful introduction of PLGA NPs, in the gelatin hydrogel, provides a good tool for future applications in controlled drug release systems and in food technologies since biodegradable polymeric nanoparticles are able to encapsulate different natural and biological systems and release them in a complex and controlled way.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Acknowledgements

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