Effects of Au nanoparticles on thermoresponsive genipin-crosslinked gelatin hydrogels

Ana L. Daniel-da-Silva · Ana M. Salgueiro · Tito Trindade

Abstract Gold gelatin hydrogel nanocomposites cross-linked with genipin have been prepared, and the effect of citrate capped Au nanoparticles (NPs) as nanofillers in the crosslinking and swelling of gelatin and release of a model drug (methylene blue) from gelatin nanocomposites have been investigated. The citrate-capped Au NPs prevented the crosslinking reaction between the gelatin and genipin and resulted in less crosslinked hydrogels. Although less cross-linked, the Au gelatin nanocomposites swelled less than the unfilled crosslinked gelatin. The gelatin composites were optically active and thermo-sensitive in a temperature range acceptable for living cells. In vitro release studies demonstrated that the irradiation of the composite gels with monochromatic green light ($\lambda = 532$ nm, 100 mW) increases the release of the encapsulated methylene blue, most likely due to the photothermal effect of Au nanoparticles. This opens the possibility to explore the application of these nanocomposites as carriers in remotely controlled light-triggered drug release.

Keywords Gold nanoparticles · Gelatin · Genipin · Biomaterials

Introduction

Recent years have witnessed growing importance of hydrogel nanocomposites for bio-applications [1,2]. These materials bring together the intrinsic functionalities of inorganic nanoparticles (NPs) and the properties of tridimensional networks offered by hydrogels. These hydrogels can be obtained from biopolymers that in controlled experimental conditions provide hydrophilicity, soft consistency, and ability to accommodate biomolecules. All these properties are attractive to promote the efficient transport of pharmaceuticals in living systems, and as such, biocompatible hydrogels have been widely used in drug delivery procedures. A recent trend in the development of hydrogels for drug delivery has been the implementation of multifunctionality, thus leading to smart drug carriers responsive to external stimuli, such as applied magnetic gradients [3,4], monochromatic light irradiation [5] or temperature effects [6]. These endeavors have relied in large extent on the use of inorganic NPs such as gold NPs that are used as functional nanofillers in the hydrogel matrix.

Gelatin is a thermoresponsive hydrogel derived from collagen that has been widely used in bio-applications, either on its own or combined with inorganic phases [7,8]. For example, gelatin composites filled with calcium phosphate have found application in tissue engineering [7] and gelatin fibers containing Ag NPs have been used in antibacterial wound-dressing materials [8]. Gelatin gels have a triple-helical structure that can be crosslinked to improve the thermal and mechanical stability of the network. Gelatin crosslinking has been achieved mainly by using chemical agents, most commonly glutaraldehyde whose major drawback is toxicity [9]. Alternatively, genipin (Fig. 1) is a chemical crosslinker, with very low toxicity, obtained from the natural occurring product geniposide that is extracted from *Gardenia jasminoides* fruits. Genipin has attracted great attention due to its ability to crosslink polymer chains containing primary amines, namely proteins such as gelatin [10–12] and polysaccharides such as chitosan [10,13]. The cytotoxicity of genipin is ca. 10,000 times lower than that of glutaraldehyde [14] and therefore genipin is extremely valuable in the development of biocompatible materials.

A. L. Daniel-da-Silva (✉) · A. M. Salgueiro · T. Trindade
Department of Chemistry, CICECO, Aveiro Institute
of Nanotechnology, University of Aveiro,
3810-193 Aveiro, Portugal
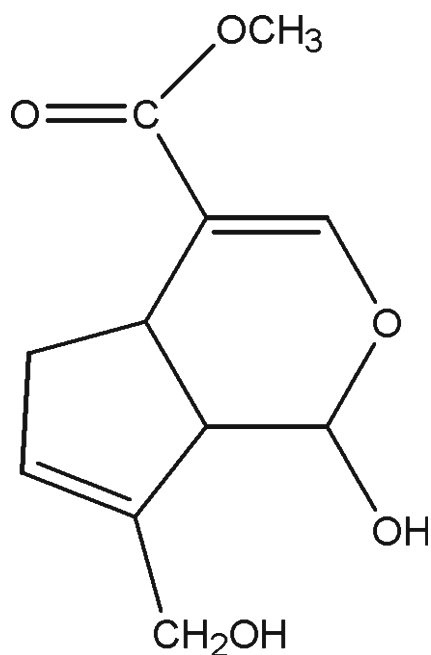e-mail: ana.luisa@ua.pt

Fig. 1 Chemical structure of genipin

The recent interest in Au hydrogel nanocomposites for biomedical applications relies mostly on their optical properties due to the plasmonic behavior of Au NPs dispersed in the matrix. For Au NPs, the surface plasmon resonance (SPR) band is located in the visible and is sensitive to several parameters, including particle size and shape, dielectric constant of the dispersing medium [15]. In addition, Au NPs act as heat dissipators by absorbing light at a frequency matching that one of the SPR band. This effect has been exploited to induce localized temperature gradients that can be used in photothermal therapy [16] and to trigger the release of encapsulated drugs from thermosensitive hydrogels [5,17]. Examples of Au hydrogel nanocomposites are those of chitosan [17,18] and alginate [19]. Although there are a few reports on the preparation of Au gelatin nanocomposites [20,21], the effect of light-irradiated Au NPs on the release properties of the hydrogel has not been investigated. Moreover, there are no reports on the use of genipin for preparing crosslinked Au gelatin nanocomposites, despite its relevance in the context of new formulations for biocompatible hydrogels.

In the sequence of our current work on the development of hydrogel nanocomposites for controlled release [22,23], we report here the preparation of genipin-crosslinked Au gelatin nanocomposites. This work aims to investigate the effect of colloidal gold nanoparticles on the crosslinking, thermal, swelling, and release properties of genipin-crosslinked gelatin hydrogels. Selected hydrogel nanocomposites were then tested for in vitro release of methylene blue, a commonly used model, with and without light irradiation of the gels.

Materials and methods

Materials

Porcine gelatin (type A, 300 bloom, Sigma-Aldrich), genipin ($C_{11}H_{14}O_5$) (98 %, Chengdu King-tiger Pharm-chem. Tech.Co., Ltd), tetrachloroauric acid ($HAuCl_4 \cdot 3H_2O$) (99.9 %, Sigma-aldrich), trisodium citrate dihydrate ($HOC(COONa)(CH_2COONa)_2 \cdot 2H_2O$) (99 %, Sigma-Aldrich), methylene blue (($C_{16}H_{18}ClN_3S$) (Riedel-de-Häen), and phosphate-buffered saline (PBS) solution (pH7.4, Sigma-Aldrich) were used as received without any further purification.

Synthesis of Au nanoparticles

Gold NPs were prepared by reduction of a gold(III) complex using sodium citrate as reducing agent. Typically, 3.76 ml of an aqueous solution of sodium citrate (96.8 mM) was added to a mixture of 95 ml of ultrapure water and 3.7 ml of $HAuCl_4 \cdot 3H_2O$ aqueous solution (12.95 mM) at 80 °C, under vigorous stirring and reflux, and allowed to react over 1 h. A deep-red hydrosol was obtained, which indicated the formation of the gold colloid.

Preparation of Au/gelatin nanocomposites

The nanocomposites were prepared by blending the Au NPs with the gelatin as follows. The colloidal Au (1.25, 2.5, and 3.75 ml) was added to an aqueous solution of gelatin previously prepared, at 45 °C. The volume of water was adjusted to perform a total of 15 mL and the gelatin concentration was 15 gL^{-1}. After achieving a homogenous dispersion of the nanoparticles, 1.25 ml of the selected model drug, methylene blue 0.3 gL^{-1}, was added. The mixture was cool down until 40 °C and 2.25 ml of genipin solution 4 gL^{-1} was added and stirred for 30 min. Afterwards, 2.5 ml of the composite mixture was transferred to a cylindrical glass vial (Ø 17 mm) which was sealed and left for incubation for 48 h at 25 °C. The content of genipin in the composites was 0.4 wt.%, related to the gelatin. The Au NPs load in the composites was calculated assuming complete conversion of the gold complex into metal Au NPs and was found to be 52, 104, and 156 ppm. After incubation, the nanocomposite discs presented a dark blue appearance which is related to the level of crosslinking of gelatin. The gel samples were frozen at −5 °C for 24 h and lyophilized. The final freeze dried discs had approximately 15 mm diameter and 8 mm height.

Swelling studies

The swelling measurements were carried out by immersion of lyophilized hydrogel discs in 50 ml PBS 0.01 M pH7.4 at 37 °C. At the required intervals of time, the samples were removed from the solution and wiped with filter paper to

remove the excess of water before being weighted. The swelling ratio (Q) was calculated from Eq. 1:

$$Q = \frac{W_s - W_d}{W_d} \qquad (1)$$

where W_d and W_s are the weight of the lyophilized and swollen gel, respectively. The equilibrium swelling ratio (Q_{equil}) was determined at the point the hydrated gels achieved a constant weight value. The swelling experiments were performed in triplicate.

Materials characterization

FTIR analysis Fourier transform infrared (FTIR) spectra of the lyophilized gelatin and gelatin nanocomposites were collected using a spectrometer Mattson 7000 coupled to a horizontal attenuated total reflectance (ATR) cell, accumulating 256 scans and using a resolution of 4 cm^{-1}. FTIR spectrum of the Au nanoparticles was collected after drying the colloidal suspension in a KBr pellet. The spectrum was collected with 256 scans and 4 cm^{-1} resolution.

Transmission electron microscopy Transmission electron microscopy (TEM) analysis of Au nanoparticles was performed using a transmission electron microscope JEOL 200CX operating at an accelerating voltage of 30 kV. Samples for TEM analysis were prepared by evaporating dilute suspensions of the nanoparticles on a copper grid coated with an amorphous carbon film.

Differential scanning calorimetry The gel-sol transitions of hydrogels were determined by differential scanning calorimetry (DSC) using a Shimadzu DSC-50 calorimeter. With sample masses of ca. 25 mg, 30-μL aluminum pans were used. Samples were heated from 25 to 80 °C at 2 °C min^{-1}. An empty pan was used as reference.

Zeta potential measurements The surface charge of the Au NPs was assessed by zeta potential measurements, using a Zetasizer Nanoseries instrument from Malvern Instruments (UK).

UV–vis spectrophotometry The optical properties of Au NPs and gelatin Au nanocomposites were investigated by UV–vis analysis of aliquots of the samples. A Jasco V 560 UV/Vis spectrophotometer (Jasco Inc., USA) was used for recording the UV/vis absorption spectra of the aliquots.

In vitro MB release studies

Methylene blue (MB) was used as a model drug and was loaded during the stage of the preparation of the nanocomposites as described above. MB has been used as a model drug namely because it is a water-soluble dye that allows an immediate visual inspection of the test. The release experiments were performed with and without laser irradiation of the samples.

The release experiments carried out without laser irradiation were performed in a thermostatic orbital shaker KS 4000I Control from IKA at the physiological temperature 37 °C and 120 rpm. A lyophilized disc was introduced in a glass beaker containing 50 ml PBS 0.01 M pH 7.4 and 0.05 % (w/v) sodium azide as preserving agent. After predetermined intervals, 1.0 ml of the release medium was drawn and analyzed by UV–vis spectroscopy (λ=663 nm) to determine the amount of MB released at each time point and replaced by 1 ml of fresh PBS to maintain the original volume. The cumulative released fraction at time t (m_t/m_0) was calculated using Eq. 2:

$$\frac{m_t}{m_0} = \frac{50 \times C_n + \sum_{i=0}^{n-1} C_i}{m_0} \qquad (2)$$

where m_t is the cumulative mass of MB released at time t, m_0 is the original mass of MB loaded, C_i is the mass concentration of MB (per milliliter) of the aliquot, C_n is the mass concentration of MB (per milliliter) of the aliquot at time t, and n is the total number of aliquots extracted until time t.

The release experiments carried out with laser irradiation were performed at room temperature (ca. 11 °C). In a typical experiment, the crosslinked gelatines containing methylene blue were prepared in a glass tube ($\varnothing_{int}$=0.6 cm), following the procedure described above. The final volume of the gelatin samples was 0.25 mL per tube. A volume of 0.7 mL of PBS 0.01 M pH 7.4 was added to each tube. The gelatines were irradiated with the laser spot positioned immediately below the interface gel/PBS using a CW diode pumped solid state laser (λ=532 nm, 100 mW) located at a distance of 10 cm from the sample. After irradiation, the PBS solution was analyzed by UV–vis spectroscopy (λ = 663 nm) to determine the amount of MB released. For comparison, release tests were also performed in identical conditions, in the absence of laser irradiation.

Results and discussion

Characterization of Au colloid

Gold NPs were prepared by reduction of a gold(III) complex in aqueous medium using sodium citrate as reducing and stabilizing agent. The resulting aqueous Au colloid
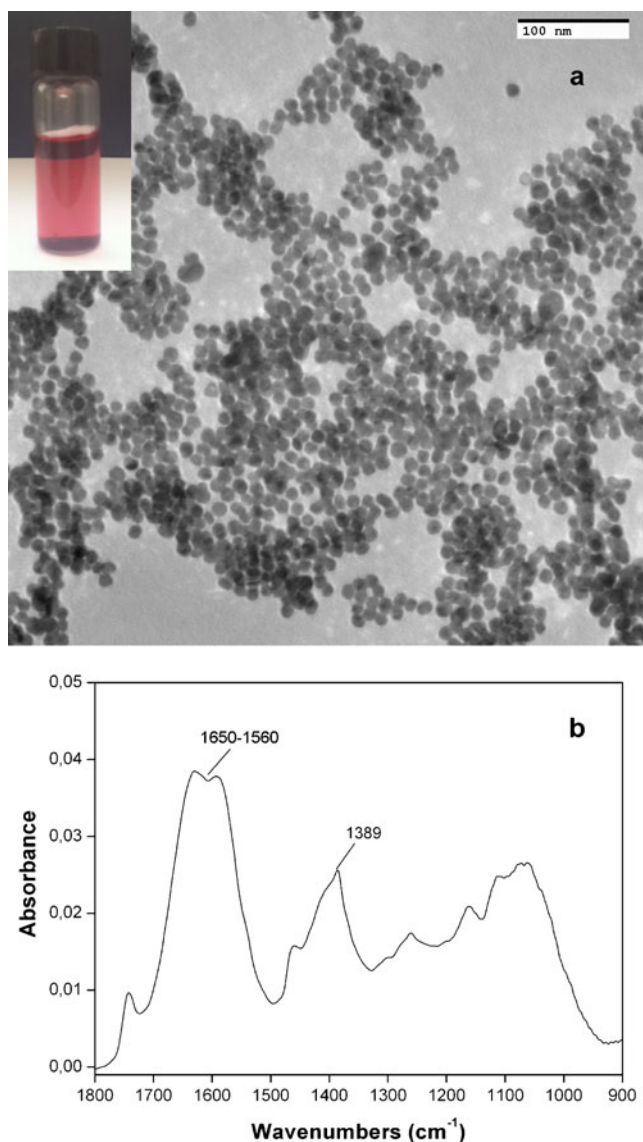
Fig. 2 **a** TEM micrograph and **b** FTIR spectrum of colloidal Au NPs used as dispersed phase

exhibited a red color (Fig. 2a, inset) due to the SPR band peaked about 520 nm. The average particle size for Au NPs was found to be 10±2 nm as determined by transmission electron microscopy (Fig. 2a).

Gold colloids as prepared above are stable because surface chemisorbed citrate ions impart a net negative charge to the particles causing electrostatic repulsion. This was confirmed by zeta potential measurements (−40.1±0.8 mV, pH 6.8) performed on the aqueous Au colloids. FTIR spectrum of Au colloid (Fig. 2b) shows two broad bands, one in the region 1,560–1,650 cm^{-1} and the other centered at 1,389 cm^{-1} corresponding, respectively, to the asymmetric and symmetric stretching vibrations of carboxylate groups [24,25], thus confirming that the Au NPs are capped with citrate anions.

Chemical and thermal characterization of Au and gelatin nanocomposites

The ATR-FTIR spectrum of the non-crosslinked gelatin (Fig. 3) shows the vibrations amide I and amide II characteristic from polypeptides. The band in the amide I region is centered at 1,629 cm^{-1} and corresponds to the C=O stretching vibration in the amide group coupled to the in-phase bending of the N–H bond and the C-N stretching vibration [26]. The band in the amide II region that corresponds to the N–H bending vibration coupled to stretching C–N vibrations was identified at 1,524 cm^{-1}. The spectrum of genipin-crosslinked gelatin is very similar to that of non-crosslinked sample. However, the amide II band is slightly less intense than in the non-crosslinked gelatin. This indicates the reduction of the number of primary amine groups due to the reaction with genipin molecules [27].

The ATR-FTIR spectra of the genipin-crosslinked Au gelatin nanocomposites are shown in Fig. 3. These spectra are dominated by the vibrational bands ascribed to gelatin due to the minor amount of Au NPs present in the composite. Nevertheless, a small shift of the amide II band from 1,524 cm^{-1}, in the unfilled crosslinked gelatin, to 1,536 cm^{-1} in the composites is observed in Fig. 3. This band shift suggests that Au NPs interact with amine groups from gelatin, most likely via electrostatic interactions between carboxylate and protonated amine groups. At the pH of the composite mixture (pH5.3), the amine groups from gelatin are protonated and can interact electrostatically with citrate groups capping the Au NPs. These results are in agreement with previous observations that report changes in the IR spectra for the amide II region of different proteins, after the addition of citrate-stabilized Au colloidal nanoparticles [28]. Other workers have proven the electrostatic
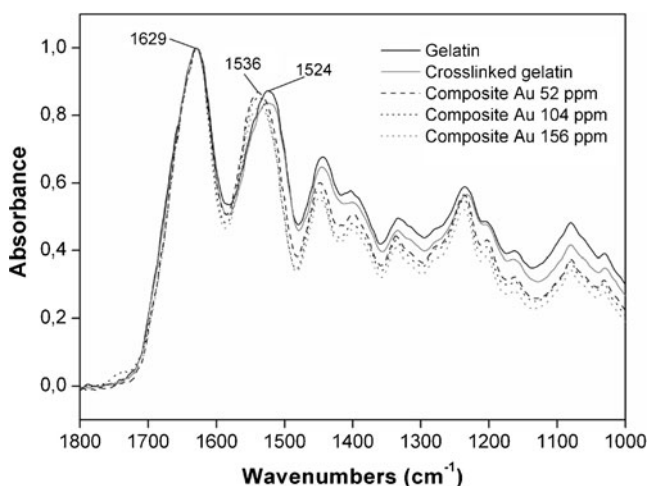

Fig. 3 FTIR-ATR spectra of uncrosslinked gelatin, genipin-crosslinked gelatin, and Au nanocomposites. The intensity of spectra was normalized in relation to the amide I vibration band

binding of bovine serum albumin to citrate-capped gold NPs due to interactions between carboxylate and protonated amine groups using non-spectroscopic techniques [29].

The addition of Au NPs at a concentration of 52 ppm resulted also in the increase of the intensity of the Amide II band in the nanocomposite when compared to the unfilled crosslinked gelatin. This suggests that, due to the interaction of citrate/amine groups, the citrate-capped Au NPs prevent the reaction between the gelatin and genipin, hence restraining the reticulation of gelatin. This is in agreement with the observed decrease of the amide II band for increasing amounts of Au NPs in the nanocomposites (Fig. 3). In fact, similar effects of citrate-capped Au NPs on the chemical reactivity of the amino groups of proteins have been reported for the glycation by fructose [24].

The DSC thermograms of genipin-crosslinked gelatin and derived Au nanocomposites (Fig. 4) show a broad endothermic peak that corresponds to the denaturation (helix-to-coil transition) of the gelatin [11]. The values for the denaturation temperature (T_D), defined here as the temperature of the main peak, are depicted in Table 1. For the neat hydrogel, T_D is 78.9 °C and then decreases by addition of Au NPs to the hydrogel, reaching the value 65.1 °C for the nanocomposite with 156 ppm Au content. Adding Au NPs also resulted in a slight decrease of the area of the endothermic peak, which is directly related to the denaturation enthalpy (ΔH_D) (Table 1).

The decrease of T_D might be ascribed to reduction of covalent crosslinking of the hydrogels, since an increased extent of crosslinking of gelatin implies higher T_D [11]. Thus, the DSC results indicate that the incorporation of Au NPs restrain the formation of covalent crosslinks and are in agreement with the FTIR spectroscopic data presented above. Noteworthy, these observations demonstrate that
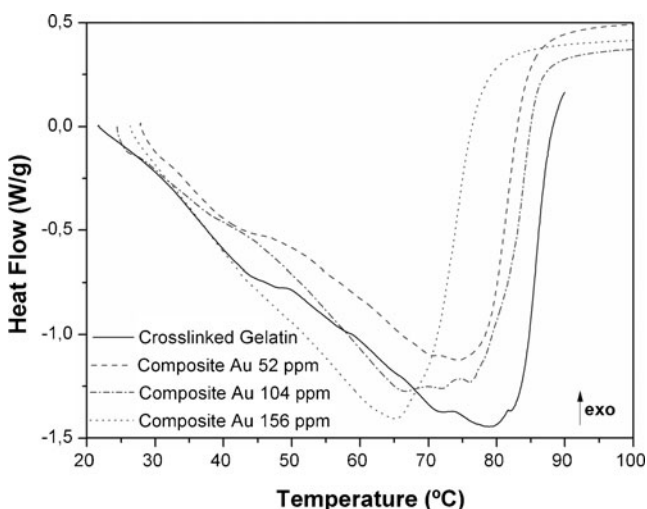
Table 1 Denaturation temperature (T_D) and enthalpy (ΔH_D) of genipin crosslinked gelatin hydrogels and Au nanocomposites calculated from DSC experiments

Au NPs (ppm)	T_D (°C)	ΔH_D (kJ/g)
0	78.9	−2.0
52	74.5	−1.7
104	69.2	−1.8
156	65.1	−1.6

the nanocomposites are thermo-sensitive in the 37–45 °C temperature range, i.e., the temperature range acceptable for living cells. Therefore, these composites have potential for the release of encapsulated molecules triggered by thermal stimuli.

Optical properties of the nanocomposites

The visible spectrum of the aqueous Au colloid showed a well-defined SPR band centered at 523 nm (Fig. 5a), characteristic of nearly monodispersed Au NPs, whose
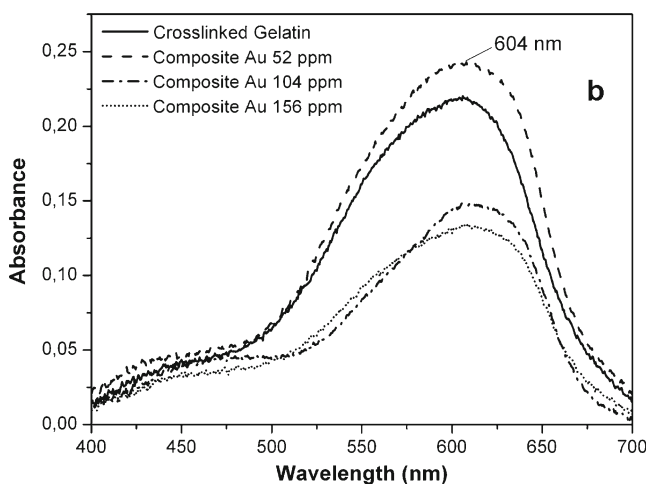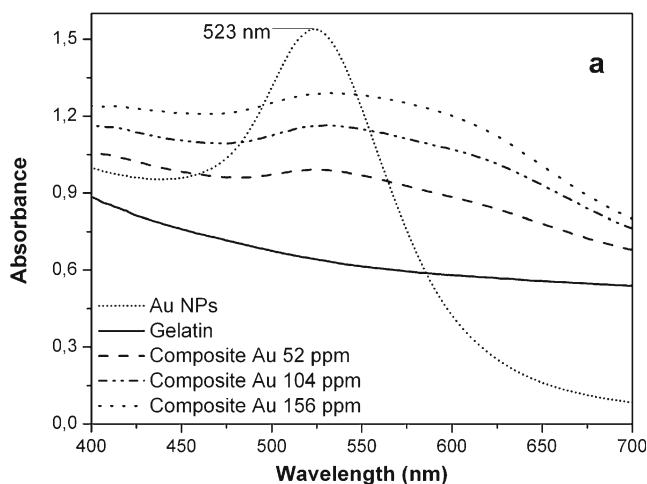

Fig. 4 DSC thermograms of genipin-crosslinked gelatin and derived Au nanocomposites

Fig. 5 Absorption spectra of the gelatin nanocomposites with variable Au NPs content **a** before reticulation with genipin and **b** reticulated with genipin

morphological characteristics are shown in Fig. 2. On the other hand, the visible spectra of gelatin Au composites prior reticulation with genipin (Fig. 5a) display a broader band and a shoulder at ca. 600 nm, the latter becoming more pronounced for increasing Au content. The observation of a shoulder extending to higher wavelength in relation to the SPR band of the original colloid is an indication of Au NPs aggregation, probably mediated by the biopolymer chains, due to interparticle plasmon coupling. This behavior apparently contrasts with the stabilizing effect previously reported for gelatin [20]. However, it should be noted that this stabilizing effect was reported for in situ prepared Au NPs, using gelatin as a reducing and stabilizing agent, thus in the absence of citrate anions [20]. Thus, it might be inferred that the Au NPs aggregation observed in this case arises from the interaction between the gelatin and the citrate capping. These results are in line with previous observations indicating that the adsorption of proteins onto the surface of citrate-capped Au NPs induce their aggregation [29]. At the temperature used for preparing the composites (45 °C), the gelatin polypeptide chains exist predominantly in the form of flexible, unfold coils in solution, and the adsorption of these chains onto the Au NPs surface might also be expectable, thus promoting the NPs aggregation.

For the case of crosslinked gelatin, the optical spectra also evidence the effect of genipin. In fact, these hydrogels exhibit a characteristic blue color that arises from products of the reaction of genipin with amino acids of gelatin [27,30]. The blue pigments are most likely formed through the oxygen radical-induced polymerization and dehydrogenation of intermediate compounds [31]. As a result, the optical spectra of the crosslinked gelatin shows a broad band centered at 600 nm that for the nanocomposites spectra probably have some contribution from the broadening of the SPR band of Au NPs. Figure 5b shows that the overall trend in the composites is the decay of the absorbance peaks at ca. 600 nm with increasing amount of Au NPs. This indicates that less genipin has reacted in the presence of citrate-capped Au NPs and is well in line with FTIR and DSC observations. Crosslinked composites containing 52 ppm Au NPs show however a slight increase of the absorbance peak at 600 nm in relation to the unfilled crosslinked gelatin that contrasts with the overall trend. This can be ascribed to the contribution of the aggregation of the Au NPs that leads to an increase of the absorbance at these wavelengths, as seen in Fig. 5a.

Swelling properties

Figure 6 displays the swelling ratio in function of time, for genipin-crosslinked gelatin hydrogels in PBS, and for variable Au NPs load. The unfilled hydrogels swelled smoothly over a period of 60 h, until reaching the equilibrium
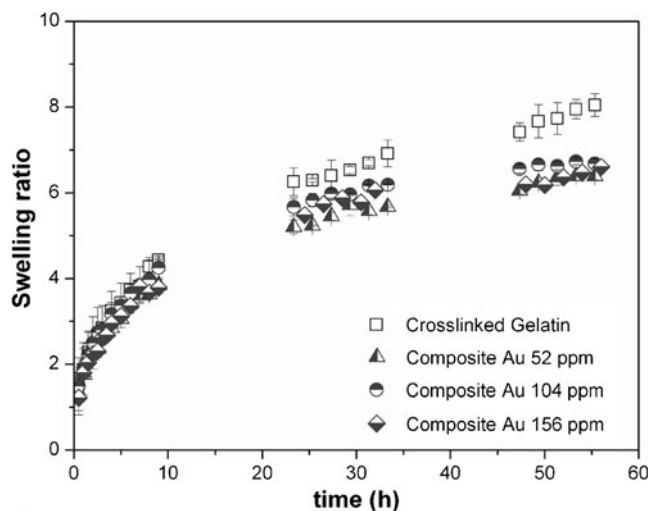

Fig. 6 Swelling ratio (Q) genipin of crosslinked gelatin and derived Au nanocomposites in PBS at 37 °C as a function of time

swelling ratio (Q_{equil}) of 8.1±0.3. The Au composite hydrogels swelled slightly slower than the unfilled gelatin and the incorporation of Au NPs resulted in a decrease of Q_{equil} from 8.1 to ca. 6.5, regardless the Au NPs content.

Gelatin swelling usually decreases with the extension of crosslinking [12,32,33]. The FTIR and DSC results presented above have shown a decrease on crosslinking when Au NPs have been added to the hydrogel. Therefore, more swelling would be expected for these nanocomposites as compared to unfilled gelatin. Indeed, the opposite effect was observed, which indicates that water diffusion into the gel was limited by the Au NPs dispersed in the network. A similar effect has been reported for montmorillonite (MMT) gelatin nanocomposites [34]. Although MMT hinders the formation of chemical crosslinkages, MMT gelatin nanocomposites crosslinked with dextran dialdehyde has been described as swelling less than the unfilled crosslinked gelatin [34]. The reduction on swelling with MMT content was ascribed to a barrier effect of the MMT NPs to the diffusion of solvent molecules into the gelatin [34].

In vitro MB release

In order to assess the potential of the nanocomposites for controlled release procedures, hydrogels with the highest Au NPs content (156 ppm) have been selected for in vitro MB release studies. The amount of MB released was monitored by measuring the absorbance of the release medium at 663 nm, i.e., at the wavelength of maximum absorbance for MB. The release experiments were carried out with and without laser irradiation (λ=532 nm) of the samples.

Figure 7a displays the MB release profiles in PBS from the genipin-crosslinked gelatin and the Au nanocomposite, at 37 °C, in absence of laser irradiation. Both hydrogels
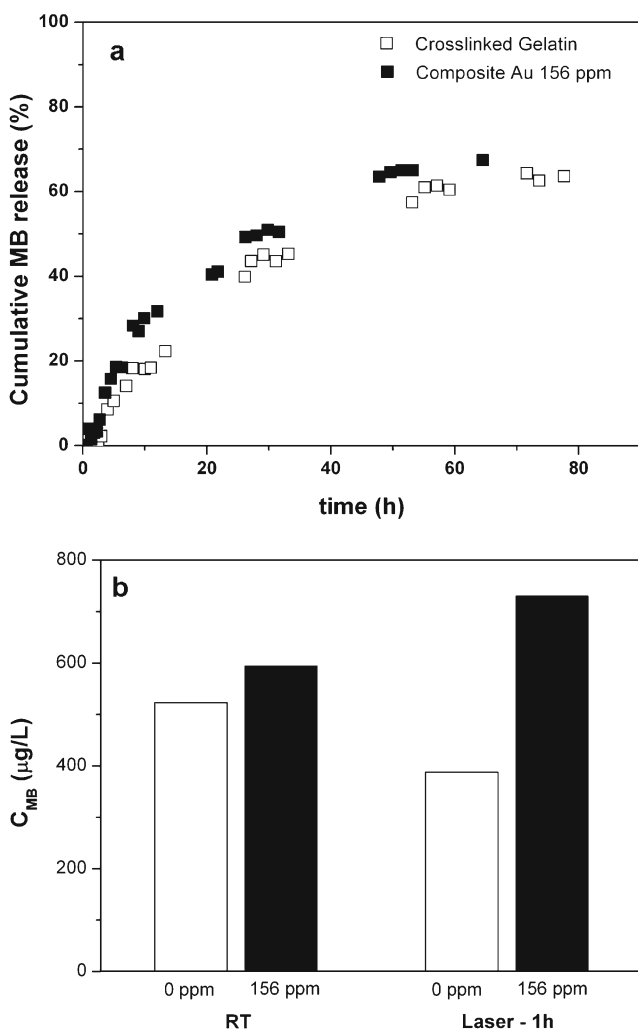
Fig. 7 In vitro MB release in PBS from unfilled crosslinked gelatin (0 ppm) and derived Au nanocomposites (156 ppm): **a** at 37 °C without irradiation, as function of time; **b** at RT, with and without exposure to green light (λ=532 nm) after 1 h

surface, as discussed above. In this case, the gelatin polypeptide chains might enfold the Au NPs and thus prevent the sorption of MB molecules onto the NPs surfaces.

Figure 7b shows the amount of MB released to the surrounding medium after exposure of the gels to the laser light (λ=532 nm) for a period of 1 h. Under exposure to the green laser, the MB released from the Au composite (156 ppm) is approximately twice than in the unfilled gelatin. For comparison, the MB released was also quantified in identical experimental conditions, without exposing the gels to the green light. In absence of laser exposure, both gels (blank and Au composite) release equivalent amounts of MB. This is in agreement with the results above presented (Fig. 7a) for the release experiments performed at physiological temperature without irradiation. The results show an increment of the MB released from the Au composites in 25 % after exposed to the green light, an effect that might be ascribed to the photothermal conversion of Au nanoparticles. Indeed, the wavelength of the incident light (λ=532 nm) matches the maximum absorption of the SPR band of the Au nanoparticles, and therefore, it is expected that the absorption of this light by the nanoparticles will increase locally the temperature. Since genipin-crosslinked gelatin is sensitive to temperature, as confirmed by DSC results (Fig. 4), it is expected that this heating will induce local transformations in the gel network, thus promoting the release of the encapsulated MB molecules, as observed. It should be noted that, in opposition to the behavior observed in the Au composites, the irradiation of the blank hydrogel seems to decrease the amount of MB released (Fig. 7b), most probably due to photobleaching of some of the MB molecules trapped in the gelatin matrix, as previously reported [38].

Conclusions

The effect of colloidal Au NPs as nanofillers in the crosslinking and swelling of genipin-crosslinked gelatin hydrogels and methylene blue (MB) release from Au gelatin nanocomposites have been investigated. The citrate-capped Au NPs prevent the reaction between the gelatin and genipin and hinder the formation of the gelatin crosslinking network, most probably due to the interaction of carboxylate groups with protonated amine groups from gelatin residues. Although less crosslinked, the Au gelatin nanocomposites swell less than the unfilled crosslinked gelatin, which suggest that Au NPs may act as barrier for the diffusion of solvent molecules into the gel network. Nevertheless, the incorporation of Au NPs did not affect the MB release from gelatin in isothermal conditions. The gelatin composites were thermo-sensitive at the physiological temperatures (37–45 °C) and revealed optical features from Au NPs. The irradiation of the composite gels with monochromatic

exhibited a sustainable MB release for a period of 70 h. Figure 7a shows a profile in which there is a fast release at an initial stage, followed by a slower rate until a steady concentration is achieved. The release profiles are identical in the first 10 h, and minor differences are observed afterwards. Apparently, the decrease of the swelling ratio in the composites does not affect the MB release profile. Also it appears that the Au NPs do not limit the release of MB from the gelatin matrix, conversely to the effect reported for the MB release from Au nanocomposites prepared with silicone elastomers [35] and κ-carrageenan [36]. The decrease of MB release in these composites was ascribe to the interaction between Au NPs and MB, most probably by chemisorption of MB onto Au NPs surface via sulfur atoms [37], that limits the diffusion of MB from the polymer matrix. The absence of this effect on the gelatin composites supports the assumption that the gelatin chains are strongly adsorbed onto the Au NPs

green light (λ=532 nm) in the range of the Au NPs SPR band promoted the release of the encapsulated methylene blue, most probably due to the photothermal effect of Au nanoparticles. Thus, applications of these nanocomposites as carriers in light-triggered drug release can be envisaged.

References

1. Trindade T, Daniel-Silva AL (2011) Nanocomposite particles for bio-applications: materials and bio-interfaces. Pan Stanford Publishing Pte. Ltd, Singapore

2. Schexnailder P, Schmidt G (2009) Nanocomposite polymer hydrogels. Colloid Polym Sci 287:1–11

3. Satarkar NS, Hilt JZ (2008) Magnetic hydrogel nanocomposites for remote controlled pulsatile drug release. J Control Release 130:246–251

4. Brazel CS (2009) Magnetothermally-responsive nanomaterials: combining magnetic nanostructures and thermally-sensitive polymers for triggered drug release. Pharm Res 26:644–656

5. Kang H, Trondoli AC, Zhu G, Chen Y, Chang Y-J, Liu H, Huang Y-F, Zhang X, Tan W (2011) Near-infrared light-responsive core-shell nanogels for targeted drug delivery. ACS Nano 5:5094–5099

6. Daniel-da-Silva AL, Ferreira L, Gil AM, Trindade T (2011) Synthesis and swelling behavior of temperature responsive κ-carrageenan nanogels. J Colloid Interf Sci 355:512–517

7. Chen K-Y, Yao C-H (2011) Repair of bone defects with gelatin-based composites: a review. Biomedicine 1:29–32

8. Rujitanaroj P-O, Pimpha N, Supaphol P (2008) Wound-dressing materials with antibacterial activity from electrospun gelatin fiber mats containing silver nanoparticles. Polymer 49:4723–4732

9. Sisson K, Zhang C, Farach-Carson MC, Chase DB, Rabolt JF (2009) Evaluation of cross-linking methods for electrospun gelatin on cell growth and viability. Biomacromolecules 10:1675–1680

10. Chiono V, Pulieri E, Vozzi G, Ciardelli G, Ahluwalia A, Giusti P (2008) Genipin-crosslinked chitosan/gelatin blends for biomedical applications. J Mater Sci Mater Med 19:889–898

11. Bigi A, Cojazzi G, Panzavolta S, Roveri N, Rubini K (2002) Stabilization of gelatin films by crosslinking with genipin. Biomaterials 23:4827–4832

12. Yao C-H, Liu B-S, Chang C-J, Hsu H-S, Chen Y-S (2004) Preparation of networks of gelatin and genipin as degradable biomaterials. Mater Chem Phys 83:204–208

13. Muzzarelli RAA (2009) Genipin-crosslinked chitosan hydrogels as biomedical and pharmaceutical aids. Carbohyd Polym 77:1–9

14. Mi FL, Tan YC, Liang HC, Huang RN, Sung HW (2001) In vitro evaluation of a chitosan membrane crosslinked with genipin. J Biomater Sci, Polym Ed 12:835–850

15. Daniel M-C, Astruc D (2004) Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. Chem Rev 104:293–346

16. Pissuwan D, Cortie CH, Valenzuela SM, Cortie MB (2007) Gold nanosphere-antibody conjugates for hyperthermal therapeutic applications. Gold Bull 40:121–129

17. Choi WI, Kim JY, Kang C, Byeon CC, Kim YH, Tae G (2011) Tumor regression in vivo by photothermal therapy based on gold-nanorod-loaded, functional nanocarriers. ACS Nano 5:1995–2003

18. Matteini P, Ratto F, Rossi F, Centi S, Dei L, Pini R (2010) Chitosan films doped with gold nanorods as laser-activatable hybrid bioadhesives. Adv Mater 22:4313–4316

19. Lim SY, Lee JS, Park CB (2010) *In Situ* growth of gold nanoparticles by enzymatic glucose oxidation within alginate gel matrix. Biotechnol Bioeng 105:210–214

20. Zhang J-J, Gu M-M, Zheng T-T, Zhu J-J (2009) Synthesis of gelatin-stabilized gold nanoparticles and assembly of carboxylic single-walled carbon nanotubes/Au composites for cytosensing and drug uptake. Anal Chem 81:6641–6648

21. Neupane MP, Park IS, Bae TS, Yin HK, Uo M, Watari F (2011) Titania nanotubes supported gelatin stabilized gold nanoparticles for medical implants. J Mater Chem 21:12078–12082

22. Daniel-da-Silva AL, Moreira J, Neto R, Estrada AC, Gil AM, Trindade T (2012) Impact of magnetic nanofillers in the swelling and release properties of κ-carrageenan hydrogel nanocomposites. Carbohyd Polym 87:328–335

23. Daniel-da-Silva AL, Fateixa S, Guiomar AJ, Costa BFO, Silva NJO, Trindade T, Goodfellow BJ, Gil AM (2009) Biofunctionalized magnetic hydrogel nanospheres of magnetite and κ-carrageenan. Nanotechnology 20:355602

24. Singha S, Dasgupta A, Kr DH (2011) Gold nanoparticle induces masking of amines and some therapeutic implications. J Nanosci Nanotechnol 11:7744–7752

25. Coleman MM, Lee JY, Painter PC (1990) Acid salts and the structure of ionomers. Macromolecules 23:2339–2345

26. Hashim DM, Man YBC, Norakasha R, Shuhaimi M, Salmah Y, Syahariza ZA (2010) Potential use of Fourier transform infrared spectroscopy for differentiation of bovine and porcine gelatins. Food Chem 118:856–860

27. Butler MF, Y-F NG, Pudney PDA (2003) Mechanism and kinetics of the crosslinking reaction between biopolymers containing primary amine groups and genipin. J Polym Sci, Part A: Polym Chem 41:3941–3953

28. Brewer SH, Glomm WR, Johnson MC, Knag MK, Franzen S (2005) Probing BSA binding to citrate-coated gold nanoparticles and surfaces. Langmuir 21:9303–9307

29. Lacerda SHDP, Park JJ, Meuse C, Pristinski D, Becker ML, Karim A, Douglas JF (2010) Interaction of gold nanoparticles with common human blood proteins. ACS Nano 4:365–379

30. Lee S-W, Lim J-M, Bhoo S-H, Paik Y-S, Hahn T-R (2003) Colorimetric determination of amino acids using genipin from *Gardenia jasminoides*. Anal Chim Acta 480:267–274

31. Park J-E, Lee J-Y, Kim H-G, Hahn T-R, Paik Y-S (2002) Isolation and characterization of water-soluble intermediates of blue pigments transformed from geniposide of *Gardenia jasminoides*. J Agric Food Chem 50:6511–6514

32. Bigi A, Cojazzi G, Panzavolta S, Rubini K, Roveri N (2001) Mechanical and thermal properties of gelatin at different degrees of glutaraldehyde crosslinking. Biomaterials 22:763–768

33. Goutam T, Analava M, Rousseau M, Basak A, Sarkar S, Pal K (2011) Crosslinking of gelatin-based drug carriers by genipin induces changes in drug kinetic profiles in vitro. J Mater Sci: Mater Med 22:115–123

34. Li P, Zheng JP, Ma YL, Yao KD (2003) Gelatin/montmorillonite hybrid nanocomposite. II. Swelling behavior. J Appl Polym Sci 88:322–326

35. Perni S, Piccirillo C, Pratten J, Prokopovich P, Chrzanowski W, Parkin IP, Wilson M (2009) The antimicrobial properties of light-activated polymers containing methylene blue and gold nanoparticles. Biomaterials 30:89–93

36. Salgueiro AM, Daniel-da-Silva AL, Fateixa S, Trindade T (2013) κ-Carrageenan hydrogel nanocomposites with release behavior mediated by morphological distinct Au nanofillers. Carbohyd Polym 91:100–109

37. Narband N, Uppal M, Dunnill CW, Hyett G, Wilson M, Parkin IP (2009) The interaction between gold nanoparticles and cationic and anionic dyes: enhanced UV–visible absorption. Phys Chem Chem Phys 11:10513–10518

38. Yunus WMM, Sheng CK, Yunus WMZW (2003) Study on photo-bleaching of methylene blue doped in PMMA, PVA and gelatin using photoacoustic technique. J Nonlinear Opt Phys 12:91–100