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Controlled release of ascorbic acid from genipin-crosslinked gelatin matrices under moving boundary conditions

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ABSTRACT

The purpose of this research is to investigate swellable genipin-crosslinked gelatin matrices for the controlled delivery of water soluble vitamins (ascorbic acid). The following methods were utilized to describe the physicochemical properties of the system: micro differential scanning calorimetry and small deformation dynamic oscillation in shear. Hydrogel microstructural properties were reported in terms of the average molecular weight between crosslinks and network mesh size. Degree of crosslinking in gels with concentration of genipin crosslinker from 0 to 2.8% (w/w) was measured using ninhydrin assay and UV–vis spectroscopy. Swelling of the gel matrix in aqueous solvent was followed and colorimetric methods were used to measure the diffusion kinetics of ascorbic acid from the gel to the surrounding aqueous phase. Results after treatment of swelling data with improved Fickian theory found matrix swelling was limited by the relaxation of polymer chains. Significance of results lies in the derivation of apparent diffusion coefficients for the transport of water molecules and bioactive compound at 85% and 98% crosslinking that relates the kinetics of bioactive compound release to mesh size of the polymeric network. Thus, there is strong evidence that modulation of the extent of crosslinking impacts on hydrogel morphological characteristics and structural properties, with resulting control of bioactive compound release. Outcomes may be implemented in targeted delivery of bioactive compounds, including vitamins, within the human body, for improved bioavailability.

1. Introduction

Crosslinked biopolymer networks that control active agents for targeted delivery within the human body have long been a point of interest in pharmaceutical and biomedical applications. They combine delivery of therapeutic drugs to the desired location while minimising toxicity and maximising bioavailability in the gastrointestinal tract (Curcio et al., 2013; De Clercq et al., 2016; Gierszewska-Drużyńska & Ostrowska-Czubenko, 2012; Sánchez, Pedraz, & Orive, 2017). Recent advances in food science, specifically within functional foods and nutraceuticals, have applied this school of thought to the delivery of bioactive compounds, including vitamins, polyphenols, essential fatty acids and caffeine. These may already be present within the diet, but encounter challenges upon oral delivery, which may be improved by entrapment or encapsulation within a biopolymer matrix (McClements, 2015; Wani et al., 2015).

Previous studies on food-based biopolymer systems have examined the effect of various experimental parameters affecting bioactive microconstituent delivery from these networks within a stationary boundary, including time (Arcan & Yemenicioğlu, 2014), temperature (Rubilar, Cruz, Zuñiga, Khmelinskii, & Vieira, 2017), biopolymer and co-solute concentration (Panyoyai, Bannikova, Small, & Kasapis, 2016), and fractional free volume or glass transition temperature in high solid preparations (Paramita & Kasapis, 2018). Research regarding swelling biopolymer networks that create a moving boundary focuses on pharmaceutical and biomedical applications, such as targeted delivery of anti-cancer drugs (Mahdavinia, Mosallanezhad, Soleymani, & Sabzi, 2017; Mandal et al., 2017), controlled drug release from wound dressings (Gómez Chabala, Cuartas, & López, 2017) and tissue scaffold engineering for promoting cell growth (Zarrintaj, Bakhshandeh, Rezaeian, Heshmatian, & Ganjali, 2017), where *in vivo* and *in vitro* studies examine drug bioavailability. There is a need to complement these findings by considering the effects of crosslinking on network gel structure in a swellable food-based biopolymer network that creates moving boundary conditions at the polymer-solvent interface. Understanding the kinetics of bioactive compound release under a realistic scenario of swelling of the biopolymer matrix will improve prediction of bioavailability and absorption effectiveness.

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Gelatin is a natural polymer utilised extensively in functional food processing (Gómez-Mascaraque, Lagarón, & López-Rubio, 2015), pharmaceutical (Khan, Shukla, & Bajpai, 2016) and biomedical (Sánchez et al., 2017) applications as a carrier matrix. This is achieved in the form of capsules, microspheres, sealants and would dressings being successful due to its biodegradable, biocompatible, nontoxic and non-carcinogenic nature (Elzoghby, 2013). It is extracted *via* denaturation of hydrolysed collagen with acid or alkaline hydrolysis, which will determine, as well as the source of the protein, a range of structural characteristics, including the molecular weight, isoelectric point and gel strength (Kirchmajer, Watson, Ranson, & Panhuis, 2013). A significant disadvantage of unmodified gelatin is the formation of soft gels that melt at temperatures near 37 °C. This is unsuitable for drug delivery, as the gelatin-based carrier will begin to disintegrate immediately following swallowing (Solorio, Zwolinski, Lund, Farrell, & Stegemann, 2010). To improve the thermal and mechanical stability of the gel, it is necessary to chemically modify gelatin's structure with a crosslinking agent (Pal, Paulson, & Rousseau, 2013; Zhao & Sun, 2018).

Genipin is a naturally occurring crosslinking agent derived from an iridoid glucoside, geniposide, extracted from the fruit of *Gardinia jasminoides*. It is widely used in herbal medicine and as a food dye as it reacts with amino acids or proteins to form a dark blue colour (Zhang et al., 2014). Genipin is a favourable alternative to other crosslinking agents due to the formation of stable crosslinked networks that display higher biocompatibility and less cytotoxicity when compared to other crosslinking agents, such as formaldehyde, glutaraldehyde and epoxy resins (Yan et al., 2010). Reaction of genipin with gelatin increases the density of intramolecular crosslinks between amino groups of the gelatin molecule and intermolecular crosslinks between adjacent gelatin molecules (Ge et al., 2016; Zhao et al., 2018).

In this study, diffusion of bioactive constituents from the genipin-crosslinked gelatin matrix is followed using ascorbic acid, a water soluble vitamin essential in the human diet. Protection of ascorbic acid within a biopolymer gel will alleviate challenges associated with its delivery, specifically its instability in air, light, oxygen, moisture, basic pH conditions and at high temperatures (Panyoyai et al., 2016). Modelling of swelling and diffusion kinetics leading to a comprehensive understanding of the behaviour of the crosslinked gelatin structure allows for the design of sophisticated targeted delivery systems based on natural polymers present in food systems.

2. Materials and methods

2.1. Materials

Gelatin: Type A porcine gelatin was obtained from Sigma Aldrich (Sydney, Australia). The material has an isoelectric point (pH range of 7.0–9.0, a bloom value of about 225 and a weight average molecular weight of about 75 kDa, as provided by the supplier.

Genipin: It has 98% purity and was purchased from Chengdu King-tiger Pharm-chem. Tech Co. Ltd. (Chengdu, China).

Ascorbic acid: It was obtained from Sigma Aldrich (Sydney, Australia), and is a high purity material (99%) with average molecular weight of 176.12 g/mol.

Ninhydrin assay analytical reagents: Ninhydrin was obtained from Sigma Aldrich (Sydney, Australia). Citric acid, sodium hydroxide, tin (II) chloride dihydrate, ethylene glycol monomethyl ether and isopropanol were purchased from Chem-Supply (Gillman, Australia).

Ascorbic acid analytical reagents: Orthophosphoric acid was obtained from Thermo Fisher Scientific (Scoresby, Australia). Bromine, thiourea and 2,4-dinitrophenylhydrazine (2,4-DNPH) solution were obtained from Sigma Aldrich (Sydney, Australia). Sulphuric acid was obtained from Chem-Supply (Gillman, Australia).

2.2. Methods

Sample preparation: Genipin-crosslinked gelatin matrices were prepared with 40% (w/w) gelatin and crosslinker concentration of 0, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4 or 2.8% (w/w) genipin, with or without the addition of 2% (w/w) ascorbic acid. In doing so, gelatin powder was dissolved in Milli-Q water at 60 °C with stirring for 30 min followed by cooling to 40 °C prior to addition of crosslinker and bioactive compound. This preparation was stirred for a further 7 min to ensure the homogeneous dispersion of genipin and ascorbic acid, and transferred into moulds. The crosslinking reaction was allowed to proceed at 20 °C for 24 h prior to analysis.

2.3. Experimental analysis

Determination of degree of crosslinking: Degree of gelatin crosslinking was measured using a ninhydrin assay to determine the amount of free α-amino groups in each test sample, with genipin crosslinker concentration of 0, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4 and 2.8% (w/w) (De Clercq et al., 2016; Solorio et al., 2010). Ninhydrin solution was prepared by combining solutions A (1.05 g citric acid, 10 mL (1.0 M) sodium hydroxide and 0.04 g tin (II) chloride dihydrate added to deionised water to make 25 mL) and B (25 mL ethylene glycol monomethyl ether and 1 g ninhydrin). The combined ninhydrin solution was stirred for 45 min and stored in a dark bottle. One gram of gelatin-genipin gel was heated with 1 mL ninhydrin solution in a 100 °C water bath for 20 min, cooled to room temperature (15 min) and diluted with 10 mL 10% isopropanol and 5 mL water. Optical absorbance was read at 570 nm using a Lambda 35 UV–vis spectrophotometer (Perkin Elmer, Singapore). Degree of crosslinking was determined compared to uncrosslinked gelatin, given that the optical absorbance of the solution is proportional to the amount of free amino groups present in the crosslinked gelatin sample, with the following equation (Cui, Jia, Guo, Liu, & Zhu, 2014):

$$Degrees\ of\ crosslinking = \frac{Absorbance_{no\ crosslinking} - Absorbance_{crosslinked}}{Absorbance_{no\ crosslinking}} \times 100\% \quad (1)$$

Measurements were carried out in triplicate yielding effectively identical results.

Micro differential scanning calorimetry: The effect of crosslinking on thermal properties of the biopolymer gel was measured using Setaram Micro DSC VII (Setu-rau, Caluire, France). Samples of gelatin with genipin crosslinker concentration from 0 to 2.8% (w/w) of around 360 mg were accurately weighed into cylindrical vessels and sealed. A vessel with equal weight of Milli-Q water served as a reference. Samples were cooled from 20 to 0 °C at a ramp rate of 1 °C/min, followed by heating to 90 °C at the same ramp rate. Analysis revealed the temperature band, from which midpoint melting temperature was obtained, and enthalpy change in the biopolymer matrix as the crosslinker concentration increased. Triplicate runs of overlapping thermograms are averaged here.

Rheological measurements: Small deformation dynamic oscillation in shear was carried out using AR-G2 (TA Instruments, New Castle, DE) with magnetic thrust bearing technology to obtain measurements of the elastic (*G'*; storage modulus) and viscous (*G"*; loss modulus) components of the biopolymer network. Gelatin-genipin samples (40% gelatin, genipin concentration range from 0 to 2.8%) were loaded on to the preheated Peltier plate at 40 °C with 20 mm parallel plate geometry, and exposed edges of the sample were coated with silicone oil (BDH, 50 cS) to prevent moisture loss. Samples were cooled to 20 °C (2 °C/min) with a controlled strain of 0.1% and constant oscillatory frequency of 1 rad/s prior to a 24 h time sweep during which time crosslinking occurred. Parallel plate geometry gap was active, with normal force being maintained at 0.05 ± 0.01 N. At the conclusion of the 24 h crosslinking period, a frequency sweep was performed in the range

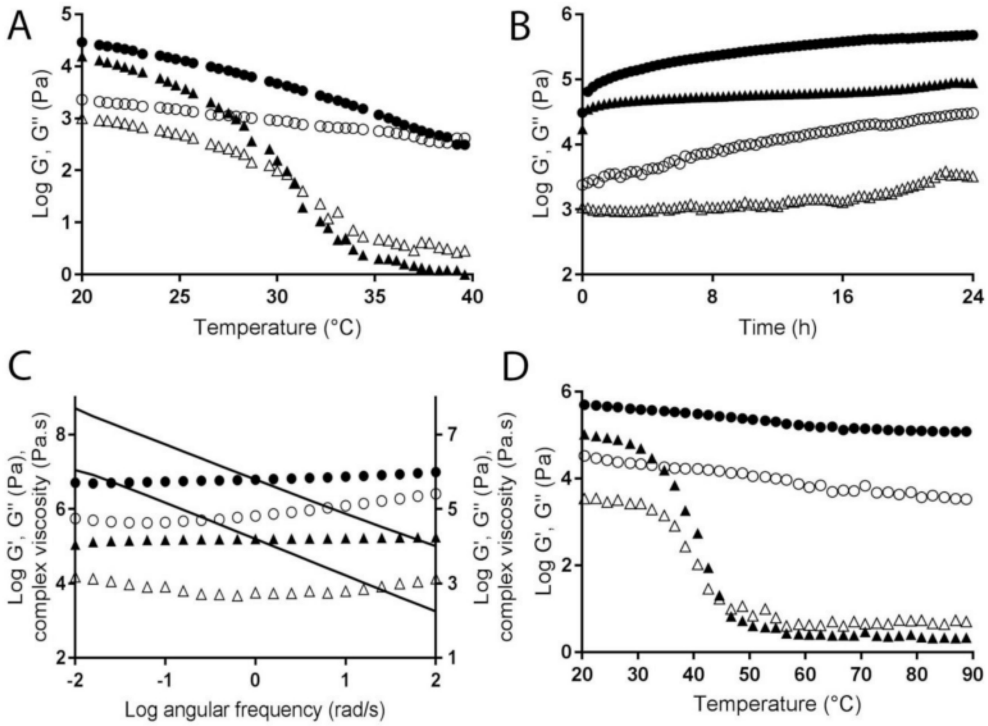

Fig. 3. Rheological profiles of G' (solid symbols) and G'' (open symbols) for uncrosslinked gelatin (triangles) and 2% (w/w) genipin crosslinked gelatin (spheres), (a) cooling profiles from 40 to 20 °C, (b) 24 h time sweep, (c) frequency sweep (uncrosslinked gelatin on left y-axis, crosslinked gelatin on right y-axis, with corresponding complex viscosity as solid lines), (d) heating profiles from 20 to 90 °C.

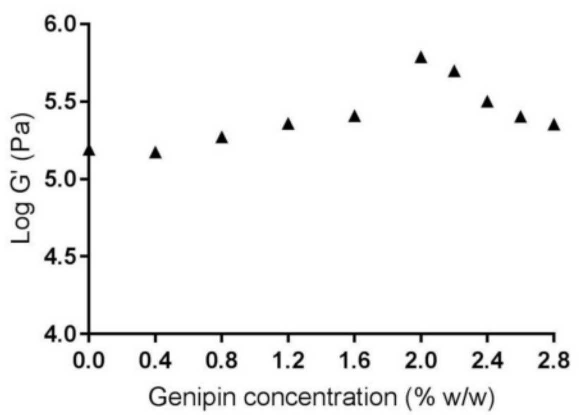

Fig. 4. Storage modulus at 1 rad/s with increasing genipin concentration from 0 to 2.8% (w/w) in 40% (w/w) gelatin gels obtained from the corresponding frequency sweeps in Fig. 3c.

where, l_o is the length of the bond along the polymer backbone, 1.4 Å, as calculated from the arithmetic mean of one C−C bond and two C−N bonds, C_n is the characteristic ratio of the polymer, M_r is the molecular weight of the repeating unit, 94.7 g/mol, l_{per} is the persistence length of the gelatin molecule, 20 Å, and l_s is the linear segment assuming $l_s = l_o$ (Ma et al., 2013; Mark, 2009; Marmorat et al., 2016).

Utilisation of equations (3)–(9) results in derivation of microstructural parameters of the Flory–Rehner theory for our system, as summarised in Table 1. With increasing genipin crosslinking, volume swelling ratio, molecular weight between crosslinks and network mesh size decreased, but polymer volume fraction in the swollen state and crosslink density increased. These results are in qualitative agreement with earlier work, where increasing concentration of crosslinker groups caused a higher crosslinking density, hence a lower mesh size in poly (ethylene glycol)-crosslinked gelatin (Cao, Lee, Peled, & Venkatraman, 2016). This decrease in mesh size with high crosslinking density was confirmed with cryogenic-temperature scanning electron microscopy for transglutaminase-crosslinked gelatin (Marmorat et al., 2016), and it will be used presently to relate to water infusion and ascorbic acid diffusion in genipin crosslinked gelatin hydrogels.

Table 1
Polymer molecular weight between crosslinks and mesh size with varying crosslinker concentration in genipin-crosslinked gelatin gels.

Genipin concentration (% w/w)	Volume swelling ratio	Polymer volume fraction in swollen state	Molecular weight between crosslinks (g/mol)	Crosslink density	Mesh size (nm)
0.0	3.53	0.28	858	87	31
0.4	2.41	0.41	302	248	19
0.8	2.14	0.47	202	372	16
1.2	2.02	0.50	155	484	14
1.6	1.96	0.51	141	531	13
2.0	1.76	0.57	100	749	11
2.4	1.89	0.53	127	589	12
2.8	1.96	0.51	121	621	11

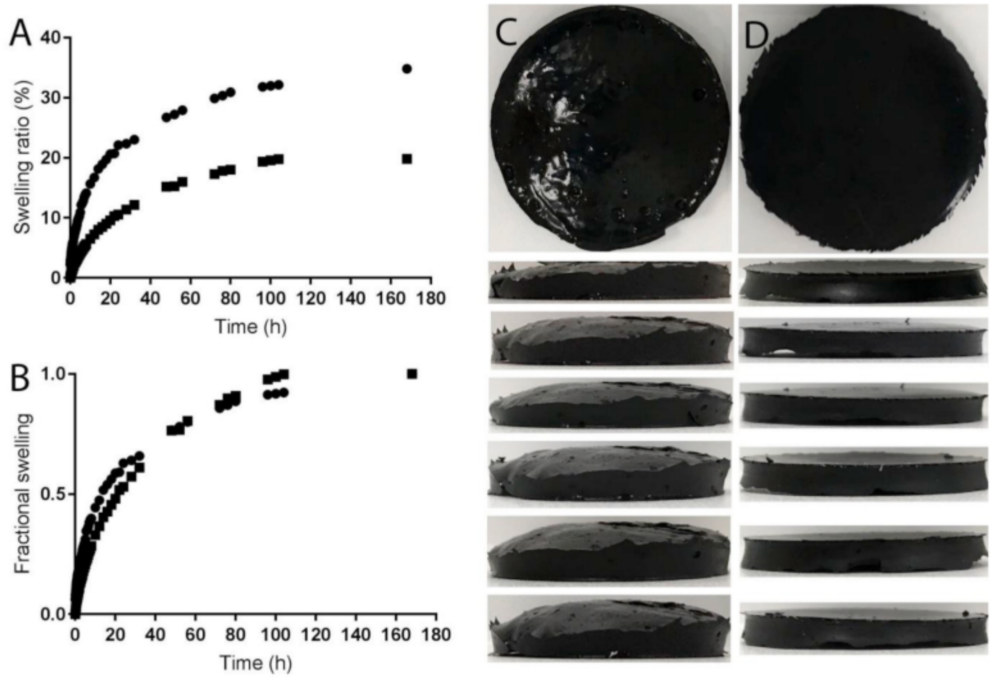

Fig. 5. (a) Swelling ratio of 40% (w/w) gelatin gels with 2.0% (w/w) genipin crosslinker (●) and 2.8% (w/w) genipin crosslinker (■) in water, (b) fractional swelling of 40% (w/w) gelatin gels with 2.0% (w/w) genipin crosslinker (●) and 2.8% (w/w) genipin crosslinker (■) in water, and swelling of gelatin matrices with (c) 2.0% (w/w) and (d) 2.8% (w/w) genipin at 0, 24, 48, 72, 96 and 168 h swelling in water.

3.3. Swelling of the genipin-crosslinked gelatin matrix

Work in the preceding section is often used for network characterisation in the "advanced synthetic polymer research" allowing an extension to the moving boundary conditions of crosslinked gelatin, the archetype of a biological rubber. Understanding the dynamics of swelling and their relation to the mechanism of bioactive compound diffusion from food-based biopolymers affords greater control in targeted delivery systems. Within this context, the mechanism of water transport through the synthesized genipin-gelatin hydrogel needs to be explored first, since it affects directly transport phenomena of ascorbic acid in the composite network.

Fig. 5a reproduces measurements of swelling on the basis of weight change from equation (2) due to water molecule infusion into the biopolymer matrix at two different degrees of crosslinking. Swelling ratio of 40% (w/w) gelatin samples with 2.0% (w/w) genipin crosslinker (85% crosslinking) reached 35% over the duration of the 7 day test at ambient temperature, significantly more than the 20% swelling ratio for samples with 2.8% (w/w) genipin and 98% crosslinking. A biopolymer matrix in contact with a molecularly miscible solvent will swell as its molecules infuse into the network. This swelling is countered by retractive forces induced by crosslinks amongst chain segments (Marmorat et al., 2016; Peppas et al., 2000), hence an increase in crosslinker concentration will diminish swelling capacity. In general, Table 1 shows a volume swelling ratio that is reduced with addition of crosslinker leading to dense intermolecular associations, although the Flory-Rehner theory assumes a crosslinked network comprised of perfectly distributed polymer chains.

Fig. 5b observes mechanistic dynamics in terms of fractional swelling by comparing the swelling ratio at time t to the final equilibrium swelling. This provides insights over the duration of the test period, with the lower degree-of-crosslinking samples swelling more rapidly during the initial stages of experimentation. Estimates from weight changes are accompanied in Fig. 5(c and d) by pictorial evidence on the swelling of gelatin gels. Fig. 5c depicts a genipin-crosslinked gelatin gel with 2.0% (w/w) genipin swelling in Milli-Q water at 20 °C over the time scale of observation (up to 168 h), with images being taken at 24 h intervals (up to 96 h) and after 168 h of swelling. Similarly, Fig. 5d shows the increase in height of the crosslinked gelatin matrix due to water adsorption into the matrix during the swelling study of a genipin-crosslinked gelatin gel with 2.8% (w/w) genipin.

Modeling the experimental observations in Fig. 5(a–d) is afforded with the improved Fickian theory that allows us to determine the rate controlling factor as either molecular infusion or polymer chain relaxation. Quantification of this data is shown in Fig. 6a that follows the initial stages of water adsorption into the gelatin gel. A linear relationship is observed between fractional swelling and the square root of experimental time according to the classical Higuchi equation (Siepmann & Siepmann, 2013):

$$\frac{W_t}{W_\infty} = kt^{1/2} \tag{10}$$

where, W_t is the degree of swelling at time t, W_∞ is the equilibrium degree of swelling, and k is a constant characteristic of the bioactive compound-biopolymer system.

Gelatin with 2.0% (w/w) genipin is linear on the plot of fractional swelling between 0 and 0.52 (0–14 h), while for 2.8% (w/w) genipin the plot is linear between 0 and 0.8 fractional swelling (0–56 h). This linearity during early stage swelling indicates a Fickian mechanism, where infusion of the solvent molecules is slower than the rate of polymer chain relaxation. Analysis can be further advanced by estimating the apparent infusion coefficient of water molecules into the crosslinked matrix, which for polymeric films or slabs is given by the following equation (Liu et al., 2017):

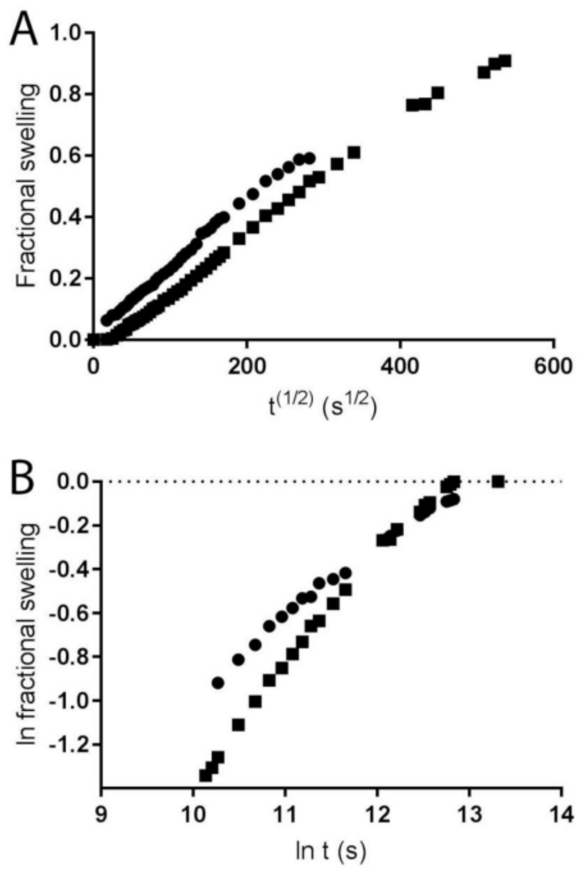

Fig. 6. Fractional swelling of genipin-crosslinked gelatin gels at 2.0% (w/w) (●) and 2.8% (w/w) (■) crosslinker during (a) initial swelling and (b) latter stage swelling.

$$F_s = \frac{w_t}{w_\infty} = 4\left(\frac{D_s t}{\pi h^2}\right)^{1/2} \quad (11)$$

where, F_s is the fractional swelling, D_s is the apparent infusion coefficient, and h is the thickness of gel disc. As shown in Table 2, within the fractional swelling range of Fickian kinetics, equation (11) is able to calculate apparent infusion coefficients, with higher concentration of genipin yielding lower D_s values (2.27×10^{-9} m^2/s).

Latter stage experimentation, defined within the fractional swelling range of 0.40–1.0 (8–168 h) for 2.0% (w/w) genipin and 0.26 to 1.0 (7–168 h) for 2.8% (w/w) genipin in Table 2, does not follow Case I Fickian kinetics. Instead, data are better linearised *via* a power law model that yields a variable diffusion exponent, *n*, according to the below mathematical expression:

$$\frac{W_t}{W_\infty} = kt^n \quad (12)$$

Instead of the Higuchi square root dependence, fractional swelling in the latter stage yields values of infusion coefficient that are below 0.5 in Table 2. For both degrees of crosslinking, transport phenomena were described as less-Fickian (Paramita, Bannikova, & Kasapis, 2015), indicating that water penetration was much slower than the structural relaxation of the gelatin matrix.

3.4. Release kinetics of ascorbic acid from the genipin-crosslinked gelatin matrix

Following quantification of swelling kinetics in the preceding section, it is appropriate to discuss their effect on the diffusion of ascorbic acid due to a concentration gradient differential between matrix and surrounding environment. Modelling assumes perfect sink conditions whereby concentration of the bioactive compound in the surrounding bulk solvent is considered negligible. Furthermore, adequate stirring minimises the thickness of the liquid unstirred boundary layer, eliminating the effect of mass transfer resistance through this layer (Siepmann & Siepmann, 2012).

Fig. 7a depicts release kinetics with extended time of observation plotted as recorded absorbance following a colorimetric assay of ascorbic acid. This produces data for fractional diffusion (M_t/M_∞) discussed for 2.0 and 2.8% (w/w) genipin-crosslinked gelatin matrices in Fig. 7b. As for network swelling, ascorbic acid diffusion generated two sets of fractional diffusion data that can be treated with a power law model analogous to equation (12):

$$\frac{M_t}{M_\infty} = kt^n \quad (13)$$

where, M_t is the amount of bioactive compound released at time *t*, and M_∞ is the amount of bioactive compound released at infinite time/end of experiment.

Table 3 reproduces the former set of data encompassing a fractional diffusion range of 0–0.47 and 0 to 0.33 for 2.0 and 2.8% (w/w) genipin concentration, respectively, and extending for a longer time period at lower degree of crosslinking (3.3 h). They exhibit good linearity in Fig. 8a as a function of the square root of experimental time yielding a value of 0.5 for the diffusion exponent that argues for a Case I Fickian diffusion, i.e. a process that is not limited by polymeric relaxation in the gelatin-genipin matrix. Similar to calculations of the apparent infusion coefficient of water into the swelling matrix, an apparent diffusion coefficient (D_s) can be calculated for ascorbic acid by using a mathematical expression analogous to equation (11) (Rubilar et al., 2017):

$$\frac{M_t}{M_\infty} = 4\left(\frac{D_s t}{\pi h^2}\right)^{1/2} \quad (14)$$

Utilisation of equation (14) generates values for the apparent diffusion coefficient of ascorbic acid in Table 3 that are an order of magnitude greater than for the infusion of water molecules in Table 2 (about 10^{-8} and 10^{-9}, respectively). This combined result supports the argument for water infusion as the rate-limiting step in the transport phenomena of our system and justifies the detailed investigation on

Table 2
Infusion exponent (*n*) and system characteristic constant (*k*) calculated using power law equation for swelling of genipin-crosslinked gelatin matrices as a result of water infusion.

Genipin concentration (% w/w)	Fractional swelling range of linear graph	Time range of linear graph (h)	k	n	R^2	Type of infusion	Infusion coefficient (m^2/s)
2.0	0 to 0.52	0 to 14	0.0024	0.50	0.9942	Fickian	4.55 × 10-9
2.8	0 to 0.80	0 to 56	0.0017	0.50	0.9824	Fickian	2.27×10^{-9}
2.0	0.40 to 1.0	8 to 168	0.0015	0.31	0.9845	Less-Fickian	
2.8	0.26 to 1.0	7 to 168	0.0090	0.47	0.9775	Less-Fickian	

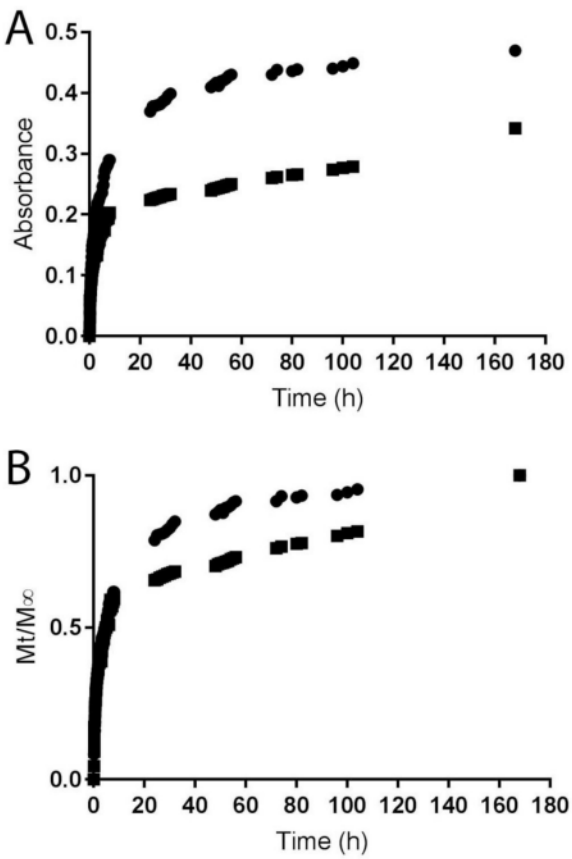

Fig. 7. (a) Absorbance of 2% (w/w) ascorbic acid diffused from genipin-crosslinked gelatin gels into water as a function of crosslinker concentration at 2.0% (w/w) (●) and 2.8% (w/w) (■), (b) fractional release of ascorbic acid diffused from genipin-crosslinked gelatin gels into water as a function of crosslinker concentration at 2.0% (w/w) (●) and 2.8% (w/w) (■).

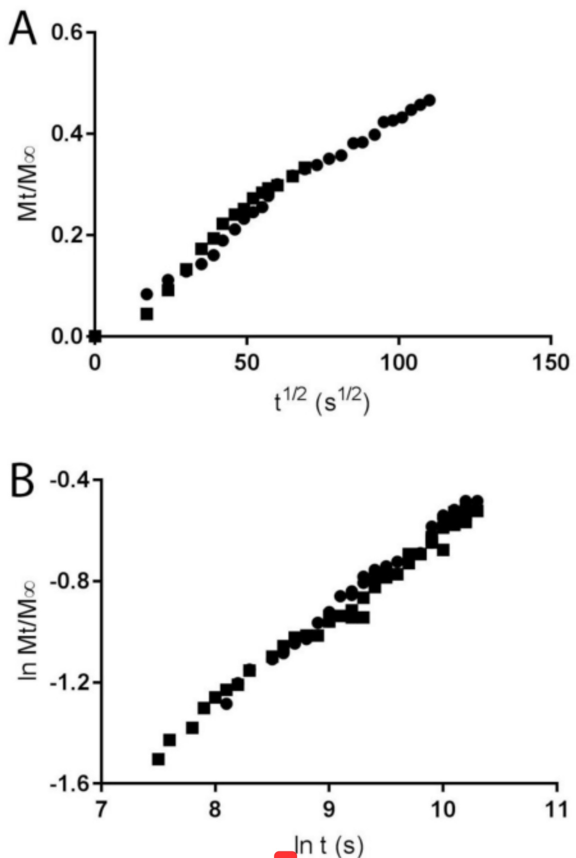

Fig. 8. (a) Initial fractional diffusion of ascorbic acid from genipin-crosslinked gelatin matrices at 2.0% (w/w) (●) and 2.8% (w/w) (■) crosslinker, (b) latter stage fractional diffusion of ascorbic acid at 2.0% (w/w) (●) and 2.8% (w/w) (■) crosslinker.

matrix swelling carried out in Section 3.3. As in these tests, the latter stage of molecular transport of ascorbic acid is also considered covering a fractional diffusion range of 0.28 to 0.62 and 0.22 to 0.60 for 2.0 and 2.8% (w/w) genipin concentration, respectively, in Table 3. Data can be linearised in Fig. 8b using equation (13) with a variable diffusion exponent that falls below the value of 0.5, e.g. $n = 0.34$ for 2.8% (w/w) genipin in Table 3, arguing for a less-Fickian process as molecular rearrangements gradually approach thermodynamic equilibrium.

4. Conclusions

The presented results argue that the swelling kinetics of genipin crosslinked gelatin hydrogels can play a decisive role in the control of ascorbic acid release from the polymeric matrix to the surrounding release medium of water molecules. Initial transport phenomena follow Case I Fickian kinetics but there is a considerable slowdown in the latter stage of diffusion approaching thermodynamic equilibrium. This is manifest in the estimates of the apparent infusion coefficient of water molecules, which becomes the rate determining molecular process in the delivery vehicle. Genipin addition above 2.0% (w/w) induces network formation and maturation, seen in the segment molecular weight between crosslinks, crosslink density and mesh size. This is reflected in overlapping traces of fractional diffusion of ascorbic acid throughout the prolonged timescale of observation at ambient temperature. The above constitutes strong evidence that modulation of the extent of crosslinking impacts on the morphological characteristics and structural properties of genipin crosslinked gelatin hydrogels and ultimately the bioactive compound release behaviour.

Table 3
Diffusion exponent (n) and system characteristic constant (k) calculated using power law equation for ascorbic acid entrapped in genipin-crosslinked gelatin.

Genipin concentration (% w/w)	Fractional diffusion range of linear graph	Time range of linear graph (h)	k	n	R^2	Type of diffusion	Diffusion coefficient (m^2/s)
2.0	0 to 0.47	0 to 3.3	0.0126	0.50	0.9887	Fickian	1.45×10^{-8}
2.8	0 to 0.33	0 to 1.3	0.0188	0.50	0.9789	Fickian	2.20×10^{-8}
2.0	0.28 to 0.62	0.9 to 8	0.0206	0.36	0.9913	Less-Fickian	
2.8	0.22 to 0.60	0.5 to 8	0.0150	0.34	0.9893	Less-Fickian	

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2018.10.026.

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