

STATIONARY AND DYNAMIC RHEOLOGICAL BEHAVIOUR OF SOME COLLAGEN HYDROGELS FOR WOUND MANAGEMENT CONTAINING MIXTURES OF CHLORHEXIDINE DIGLUCONATE AND TANNIC ACID

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Type I collagen hydrogels having a concentration of 1.1% and containing 5, 10 and 15% tannic acid as well as all the combinations between the above amounts of tannic acid and 1.82, 4.55, 9.09% chlorhexidine digluconate were prepared. Intended to be used for wound healing, they were characterized by stationary and dynamic rheological behaviour, to select the most convenient combination from the point of view of application on wound. The hydrogels behave pseudoplastic, start to flow at very low shear stress, destroy under the action of shear rates higher than about 20 s⁻¹, are thixotropic and elasticity prevails on viscosity in their viscoelastic properties. The most convenient concentrations of drugs to be applied as wound dressing is 4.55% CHDG and 10% TA, the hydrogel having the highest elasticity and viscosity. The hydrogel can be applied by spatula or pouring and, being thixotropic, even using a syringe.

Keywords: collagen, tannic acid, chlorhexidine digluconate, hydrogel.

INTRODUCTION

Hydrogels are three-dimensional networks of natural or synthetic hydrophilic polymers having the capacity to hold large amounts of water or biological fluids within their porous structure, Peppas and Mikos (1986), Brannon-Peppas (1990), Peppas et al. (2000). Polymer insolubility is produced by the crosslinking of backbone or their associates by chemical or physical forces or by the entanglement of chains in the case the polymers are linear and flexible, Hoffman (2002). Thus, the hydrogels can be divided in: permanent – cross-linked by covalent forces and physical – formed by physical interactions (hydrogen bonds, ionic interactions or chain entanglement), Campoccia et al. (1998), Hoffman (2002).

The hydrogels have as specific properties soft consistency, permeability for small molecules and low gel-aqueous solution interface tension, Peppas (1986). The content of water and consistency make them similar to natural tissues, Park (1997), making them very useful in medicine and pharmacy, Peppas and Langer (1994). The high water content contributes to biocompatibility and they can be used as membrane for biosensors, materials for artificial leather, matrices for drug delivery, etc., Park (1997), Peppas (1997).

Type I collagen hydrogels are three-dimensional networks consisting of randomly interwoven mesh of fibrils, with poroelastic/biphasic loose lattice structure filled with about 99% fluid, Abou Neel (2006). The remarkable capacity to hold water is mainly due to its hydrophilic groups: amino, carboxyl and hydroxyl, Charulatha and Rajaram (2003) which also allow the formation of physical bonds between fibrils.

Having low percentage of collagen and loose lattice structure their mechanical properties are weak. Their improving can be realized by increasing of crosslinking density.

The oldest and used tanning agent for leather is tannic acid (TA), the hydrolysable tannin having the highest affinity for collagen, Fueaesaku et al. (1972), Meek and Weiss

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(1979). It does not induce cytotoxicity, reduce biocompatibility or prevent cell remodeling. Moreover, it has antiviral and antibacterial properties, antimutagenic and antigenic activity, Scalbert (1991), induces apoptosis in animal cells, and has antioxidant action, Khan (2000).

Chlorhexidine digluconate (CHDG) is one of the most widely used antimicrobials in clinical practice for skin antiseptics, Karpanen (2008). It is potent even against mutant streptococci, Emilsm (1994). Its efficacy can be improved using phytochemicals, some of them transforming it *in vivo* from bacteriostatic into bactericide, ulea (2011).

The objective of the present paper is the preparation and rheological characterization of acid type I collagen hydrogels, pH 3.8, containing some mixtures of CHDG and TA antibacterials, intended to be used for the wound care, in view of predicting the way they can be applied, their behaviour on application site and delivery of active principles.

EXPERIMENTAL

The 1.1% control collagen hydrogels, pH 3.8, extracted from calf hide by the current technology used in INCDTP, Division ICPI, Trandafir et al. (2007), was obtained by diluting the initial 2.54% hydrogel, pH 3.21, with distilled water and 1 M NaOH solution under mechanical stirring. The hydrogels containing 1.82, 4.55 and 9.09% CHDG reported to the amount of collagen were prepared using the same components and CHDG solution under manual stirring. TA was added in concentration of 5, 10 and 15% reported in the same way. TA was supplied by Sigma-Aldrich and CHDG by Fargon (Germany) as 20% aqueous solution (w/w). All the hydrogels were matured at 4°C for 24 h.

Stationary rheological behaviour was determined at $23 \pm 0.1^\circ\text{C}$ using a rotational viscometer Hake VT 550 equipped with HV1 sensor system for high viscosities and RheoWin 4 Thermo Fischer Scientific software.

Dynamic (oscillatory) rheological measurements were made at room temperature (23°C) with Micro Fourier Transform Rheometer MRF 2100, GBC-Australia, working under squeezing flow. The angular frequencies were within the 0-1000 rad/s range, 280 discrete frequencies were analyzed simultaneously by a step of 0.5 s^{-1} , 30 spectra acquired for each sample, the gap between the upper and bottom plates was 400 μm and displacement amplitude 0.03 μm , to fall into linear viscoelastic domain. Very small amplitude of pseudorandom squeezing motion allows a continuous monitoring of force transmitted through the viscoelastic sample to the force sensor (bottom plate). By a Fourier transform processing the storage and loss modulus at every individual frequency is obtained.

RESULTS AND DISCUSSION

Various biomedical applications, including controlled delivery systems and wound management, make the rheological characterization of hydrogels a very important task. In the case of the physical hydrogels the rheology can also be used to study the physical interactions between the dispersed phase and the components dissolved into the dispersion medium.

It has been recognized for a long time, Gustavson (1949), that TA interacts with collagen by a variety of forces: hydrogen bonding, hydrophobic and electrostatic, the types of interactions being later confirmed, Heijmen et al. (1997), Cass and Burg (2012). TA interposes between collagen fibrils, by crosslinking the basic side chains groups of polar amino acids of collagen or clustering between, Madhan et al. (2001).

The reaction between collagen and TA is depended on pH: it binds rapidly and quite firmly under acidic conditions – pH 3-4 – and slowly at pH above 6. That is why only hydrogels having an acid pH, of 3.8, were prepared and studied.

Rheological properties of collagen hydrogels are induced especially by the integrity of its fibrils and the fluid movement between the lattice forming collagen fibrils and are governed by the inherent viscoelasticity of the fibrils and their interaction with the fluid, Koob and Hernandez (2002), Park at al. (2002), Elbjeirami at al. (2003), Krishnan (2004).

Giving information on consistency and apparent plasticity, stationary rheological properties are important for predicting the way the hydrogels may be applied (by spatula, pouring, syringe, spray), their behaviour on the application site (entry into intricate spaces versus preventing of flow into critical areas), Moss at all (2006), and delivery of active principles, both properties affecting the kinetics of drug delivery.

The rheograms of the initial collagen hydrogels having the pH 3.8 and of those containing 5, 10 and 15% TA reported to the amount of collagen at increasing of shear rate, $\dot{\gamma}$, are given in Figure 1, where τ is the shear stress.

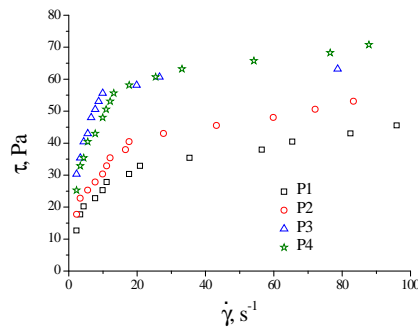


Figure 1. Rheograms of hydrogels crosslinked with: P1 – 0, P2 – 5, P3 – 10, P4 – 15% TA

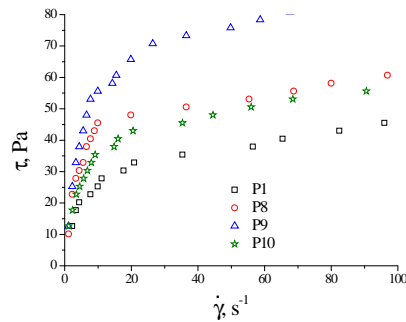


Figure 2. Rheograms of hydrogels with 4.55% CHDG and: P1 – 0, P8 – 5, P9 – 10, P10 – 15% TA

They show that all the hydrogel have pseudoplastic behaviour with practically zero limiting shear stress. The apparent viscosities, η^* , increase with TA amount, excepting 15% TA, for which the values are the same as for 10%, in the limit of experimental errors. This demonstrates that 10% TA is enough to crosslink the collagen. At the same time, the curves show an abrupt decrease of the slopes at $\dot{\gamma}$ exceeding about 20 s^{-1} , which signify that such a shear stress produces a perceptible destruction of gel structure.

The rheograms obtained at decreasing of shear rates, after the hydrogels were sheared until 1000 s^{-1} (not shown), are placed under those resulted with their increasing for each hydrogel. This indicates that the gel structure does not recover even at low shear rates, which means that the hydrogels have a time-dependent behaviour of thixotropic type.

To estimate the measure of crosslinking and gel destruction as a function of TA content, viscosities at zero shear rate, η_0 , for increasing and decreasing of $\dot{\gamma}$, are given in Table 1.

The data in Table 1 show that the η_0 obtained rising $\dot{\gamma}$ increase with TA concentration (crosslinking extent), excepting that of hydrogel containing 15%. This can signify that the amount of TA required by crosslinking was surpassed and the excess has detrimental effect on collagen, according to previous findings, Albu (2009). The

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values obtained when $\dot{\gamma}^*$ decreases are much lower: 2.6 times for the reference and 4.0% for the hydrogel with 10% TA, while for 15% it is 19.6%. This supports the detrimental effect of high amounts of TA.

Table 1. Viscosities at zero shear rate obtained with increasing and decreasing of shear rates

Sample	TA, %	CHDG, %	Increasing of $\dot{\gamma}^*$		Decreasing of $\dot{\gamma}^*$	
			η_o , Pas	n	η_o , Pas	N
P1	0	0	9.80	0.43	3.80	0.46
P2	5	0	13.86	0.35	3.73	0.50
P3	10	0	21.55	0.42	5.37	0.44
P4	15	0	19.17	0.41	0.98	0.79
P5	5	1.82	10.14	0.63	7.55	0.40
P6	10	1.82	15.21	0.48	4.45	0.46
P7	15	1.82	20.72	0.30	3.89	0.47
P8	5	4.55	15.51	0.47	8.02	0.39
P9	10	4.55	16.66	0.55	7.91	0.39
P10	15	4.55	11.93	0.49	1.64	0.62
P11	0	9.09	10.10	0.28	6.62	0.41
P12	10	9.09	14.85	0.42	5.07	0.41
P13	15	9.09	9.11	0.70	3.82	0.51

The rheograms of the collagen gels containing 1.82% CHDG and the same amounts of TA (not shown) look very similar with those in Figure 1, but η_o obtained with increasing $\dot{\gamma}^*$ have lower values, excepting that for 15% TA. This can be explained by the interaction of the two positive charges of molecule of CHDG, Jones (1997), with collagen and/or TA, reducing probably the excess of TA.

Increasing of CHDG at 4.55% gives the rheograms from Figure 2. They show the most reduced destroying of gel structure for 10% TA, while the other two behave similar.

The hydrogels with 15% TA – P11-P13 – give the lowest η_o values (Table 1) due to some denaturizing of the native collagen conformation produced by the excess TA.

The flowing indices, n, which show the easiness of flowing (the lower the flow index the higher the consistency), calculated with Ostwald-de Vaele equation, are given in Table 1. As can be seen, their values are not in accordance with η_o .

The storage, G' , and loss, G'' , moduli give the elastic and viscous contribution to the viscoelastic behaviour of a polymer gel and allow the distinction between uncrosslinked and crosslinked systems: high G' values indicate the prevalence of elastic properties and high G'' values the prevalence of the viscous ones. For hydrogels, which are highly crosslinked polymer networks, both G' and G'' are very high and are nearly parallel to each other, Barth and Pash (2003).

The dependencies of G' and G'' on angular frequency, ω , for collagen hydrogels from Figure 1 are given in Figure 3.

The Figure shows that the storage moduli are about two times higher than the loss ones for all the hydrogels, which means that the elastic components prevail in their viscoelastic behaviour. TA increase more elasticity than viscosity as expected for a crosslinked polymer. The highest values both for G' and G'' were obtained for the hydrogel containing 10% TA, while for 15% G' is lower and G'' has the lowest values, supporting the hypothesis of a slight denaturizing of collagen produced by the excess TA. G' and G'' increase practically linear with ω within the entire range and the slopes are pretty close. When 1.82% CHDG is added the curves have the same appearance and

placement, but G' and G'' are a little lower over the entire range due to the consumption of a part of the crosslinking agent by CHDG.

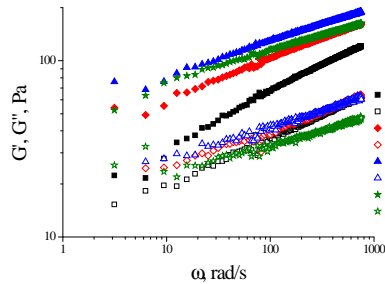


Figure 3. Dependence of G' and G'' on ω for collagen hydrogels from Figure 1

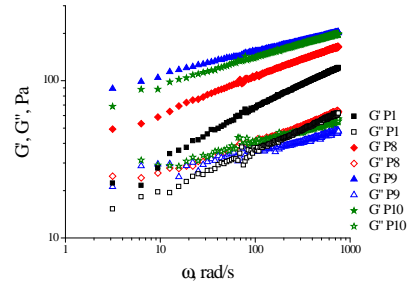


Figure 4. Dependence of G' and G'' on ω for collagen hydrogels from Figure 2

Curves for 4.55% CHDG, presented in Figure 4, show higher values for both moduli, due to the more extended crosslinking. Similar curves are obtained for samples P11-P13.

To make an idea of the values of viscosities at the angular frequency of 1 rad/s (0.16 Hz), the linear fitting of the results were made and the results are given in Table 2.

Table 2. Viscosities of hydrogels at angular frequency of 1 rad/s

Sample	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13
η , Pas	19.1	39.8	56.1	52.8	41.6	43.4	58.2	41.0	81.2	66.7	66.0	76.9	158.6

The viscosities vary with TA and CHDG concentrations in the same way as in Table 1, but all the values are higher than those obtained from stationary measurements, shearing forces, unlike the oscillatory ones, producing the disturbing of gel structure.

The rheological stationary and dynamic measurements show that for a type I collagen hydrogels with concentration 1.1% containing CHDG and TA as antimicrobials, intended to be used as wound dressings, the optimum concentration of CHDG is 4.55% and of TA 10% (sample P9). Such a hydrogel has the highest viscosity and elasticity. Thus, it can be applied both by spatula or pouring, or even using a syringe, given its thixotropic properties.

The drug delivery measurements, which have to be performed, follow to prove the accuracy of the results given by rheology.

CONCLUSIONS

Type I collagen hydrogels with concentration 1.1% and containing 5, 10 and 15% TA as well as all the combinations between the specified amounts of TA and 1.82, 4.55, 9.09% CHDG, intended to be used for wound treatment, were prepared and characterized by stationary and dynamic properties.

The hydrogels have pseudoplastic rheological behaviour, start to flow at very low shear stress, destroy under the action of shear rates higher than about 20 s^{-1} , are thixotropic and elasticity prevails over viscosity in their viscoelastic behaviour.

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The most convenient concentrations of drugs to be applied as wound dressing is 4.55% CHDG and 10% TA, the hydrogels being both the most viscous and elastic. This hydrogel can be applied by spatula or pouring and, being thixotropic, even by syringe.

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