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KINETICS OF *IN VITRO* RELEASE OF DOXYCYCLINE HYCLATE FROM COLLAGEN HYDROGELS

Mădălina Georgiana ALBU, [a*] Mihaela Violeta GHICA, [b] Lăcramioara POPA, [b] Minodora LECA[c] and Viorica TRANDAFIR[a]

[a] INCDTP - Leather and Footwear Research Institute, 93, Ion Minulescu, 031215, Bucharest, Roumania
[b] University of Medicine and Pharmacy "Carol Davila", Faculty of Pharmacy, 6, Traian Vuia, 020956, Bucharest, Roumania
[c] University of Bucharest, Faculty of Chemistry, 4-12, B-dul Regina Elisabeta, 030018, Bucharest, Roumania

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The rheological behaviour of collagen hydrogels in the absence and presence of doxycycline hyclate or/and glutaraldehyde shows that both compounds increase their viscosities, the highest viscosity being obtained for the hydrogel containing the highest amount of collagen, doxycycline hyclate and glutaraldehyde. All the hydrogels have pseudoplastic non-Newtonian behaviour. The kinetics of *in vitro* release of doxycycline hyclate from the collagen hydrogels demonstrates that the amount of released drug decrease with increasing collagen concentration. Glutaraldehyde as cross-linking agent has the same effect, so the slowest release was obtained for the most viscous hydrogel. The rheological properties of hydrogels and the rate of doxycycline release can thus be controlled by the concentration of collagen, drug and cross-linking agent.

INTRODUCTION

Collagen, due to its excellent biocompatibility, biodegradability and biological characteristics is a very convenient matrix for drug delivery. It is used mainly in prophylaxis of tissue infection, wound healing or periodontal and ophthalmologic treatment.

Collagen can be processed into a number of forms such as hydrogels, sponges, membranes, injectable solutions, powders, sheets, and tubes, all of these being used in the medical practice.[1]

Causing minimal inflammatory responses and tissue damage,[2] the hydrogels are the most used drug delivery systems at present. Due to their hydrophilic character and cross-linked structure they can contain large amounts of water, that give them physical characteristics similar to soft tissue.

Studies on collagen hydrogel, like injectable gel formulation with 5-fluorouracil, liposome and genes immobilized in collagen gels[3] can be found in the literature. Applications of doxycycline in combination with polymers like chitosan[4] and poly (DL-lactide)[5] are also found. But, to our knowledge, no study has been performed on combination of collagen-doxycycline hyclate as drug delivery system.

Collagen having an important role in regeneration of lost tissues and doxycycline having bacteriostatic properties against a broad spectrum of bacteria and inhibiting the action of collagenase, it is expected that their combination gives a better local drug delivery system for the treatment of skin infections and periodontal disease.

Crosslinking of collagen is compulsory in drug delivery applications because the non-cross-linked hydrogels are mechanically unstable and too soft to handle.[6,7] Consequently it is generally accepted that the clinical and non-clinical performances of hydrogels depend on their rheological properties.[8]

The preparation of non-cross-linked and cross-linked doxycycline hyclate-containing collagen gels, their rheological behaviour and *in vitro* release of doxycycline hyclate are presented in the present study. The drug release data were evaluated using various kinetic equations to establish the drug release kinetics and mechanism.

EXPERIMENTAL

Type I fibrillar collagen hydrogels having a concentration of 2.89% w/w were extracted from calf hide by the currently

* Corresponding author: albu_mada@yahoo.com

used technology in INCDTP Division ICPI-Collagen Department.[9] Doxycycline hyclate was purchased from Sigma-Aldrich, China and glutaraldehyde from Merck, Germany. Sodium hydroxide and phosphate buffer solution, PBS, (pH = 7.4) were of analytical grade.

The composition of hydrogels containing collagen, C, doxycycline hyclate, D, and/or glutaraldehyde, G, is given in Table 1.

Each hydrogel was adjusted at pH = 7.2 with 1M sodium hydroxide under mechanical stirring. The neutral hydrogels were stored for 24 h at 4°C for maturation or cross-linking processes.

Rheological measurements were performed using a rotational viscometer Multi-visc Rheometer-Fungilab equipped with standard spindles and an ultrathermostat ThermoHaake P5.

In vitro release of doxycycline hyclate was studied using a Franz diffusion cell in which a standard cellophane membrane was fitted. About 1 g of each hydrogel was applied on its surface. The receptor medium (PBS) was continuously stirred by a rotating Teflon coated magnet stirrer placed inside the cell at 37.0 ± 0.5°C. After different periods of time 5 mL of samples were withdrawn from the receiver compartment and replaced with an equal volume of fresh receptor fluid. The amount of doxycycline hyclate released through the cellophane membrane was spectrophotometrically analyzed at 347 nm. The cumulative percentages of doxycycline hyclate released from hydrogels were determined using a calibration curve, with D concentration ranging from 0.0001 to 0.003 mg/mL (Fig. 1).

Table 1

Compositions of studied collagen hydrogels

Collagen hydrogels codes	Collagen, %	Doxycycline hyclate, %*	Glutaraldehyde, %**
C-1	0.8	0	0
C-2	1.0	0	0
C-3	1.2	0	0
CG-1	0.8	0	0.15
CG-2	1.0	0	0.15
CG-3	1.2	0	0.15
CD-1	0.8	0.2	0
CD-2	1.0	0.2	0
CD-3	1.2	0.2	0
CD-G1	0.8	0.2	0.15
CD-G2	1.0	0.2	0.15
CD-G3	**1.2**	**0.2**	0.15

* Doxycycline hyclate concentration reported at collagen hydrogels weight
** Glutaraldehyde concentration reported at the weight of dry collagen

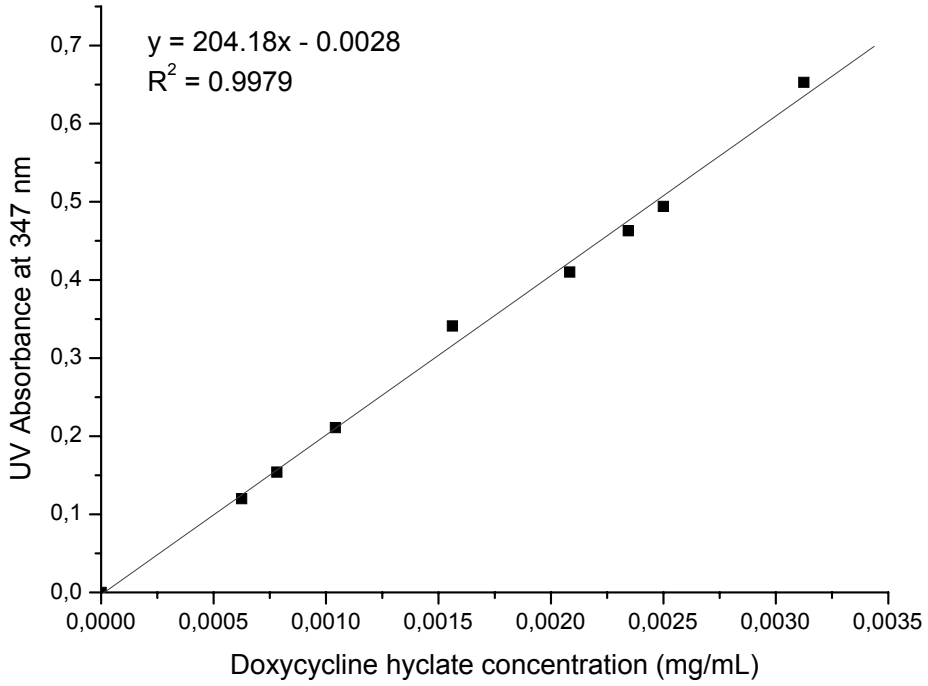

Fig. 1 – Calibration curve of doxycycline hyclate in PBS (pH 7.4).

RESULTS AND DISCUSSION

The rheological properties provide reliable information on hydrogels structure and viscosity, as well as on the hydrogel behaviour at the application site, the drug release from the hydrogel to the tissue surface being strongly influenced by the flow parameters of the system.[10]

The collagen hydrogels, both in the presence and absence of glutaraldehyde were considered as control samples. That is why their rheograms were obtained only at 23°C, the temperature of the hydrogel storage. To simulate the *in vitro* conditions and establish the correlations between the rheological properties and the release parameters of the drug from hydrogels the temperature of 37°C (body temperature) was used.

To establish the type of flowing, the data were fitted with the following rheological models:

Bingham $$\tau = \tau_0 + \eta \cdot \dot{\gamma} \quad (1)$$

Casson $$\tau^{0.5} = \tau_0^{0.5} + \eta^{0.5} \cdot \dot{\gamma}^{0.5} \quad (2)$$

Ostwald-de Waele $$\tau = K \cdot \dot{\gamma}^n \quad (3)$$

Herschel-Bulkley $$\tau = \tau_0 + K \cdot \dot{\gamma}^n \quad (4)$$

where τ is the shear stress (Pa), $\dot{\gamma}$ – shear rate (s^{-1}), η – plastic viscosity (Pa.s), τ_0 – yield stress (Pa), K – consistency index ($Pa.s^n$) and n – flow index.

The rheograms of C and CG series hydrogels from Table 1 are shown in Fig. 2.

All the rheograms show a pseudoplastic behaviour, the shear stress increasing with collagen concentrations both for cross-linked and uncross-linked hydrogels.

Glutaraldehyde plays an important role in increasing of the collagen hydrogel viscosity. Thus, the cross-linked C-G1 hydrogel containing 0.8% collagen shows shear stresses values pretty similar with those of the uncross-linked C-3 containing 1.2% collagen. The highest shear stresses were obtained for the hydrogel C-G3 which has the highest concentration of collagen and is cross-linked with glutaraldehyde, as expected.

The hydrogels containing doxycycline hyclate show also pseudoplastic behaviour both at 23 and 37°C, as can be seen from the Figs. 3 and 4.

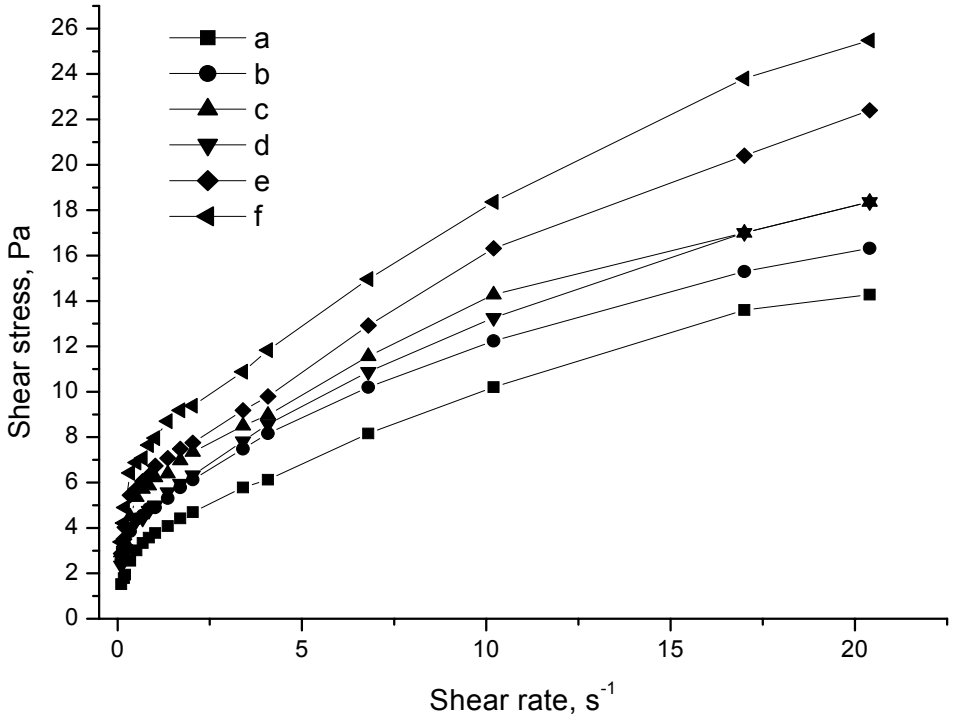

Fig. 2 – Rheograms of the uncross-linked and cross-linked collagen hydrogels at 23°C: a) C-1; b) C-2; c) C-3; d) CG-1; e) CG-2; f) CG-3.

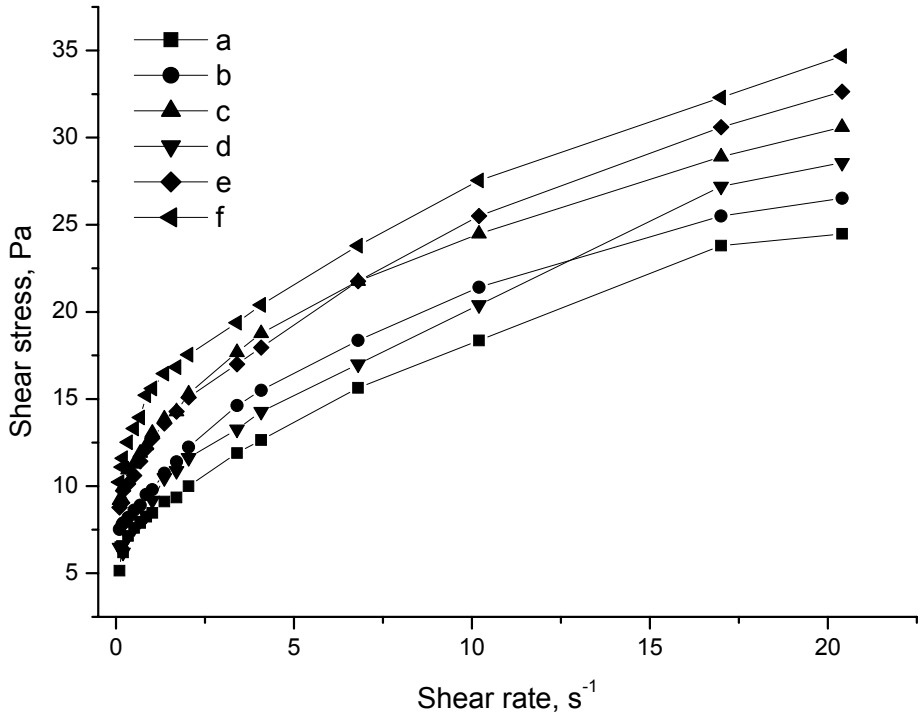

Fig. 3 – Rheograms of uncross-linked and cross-linked collagen hydrogels containing doxycycline hyclate at 23°C: a) CD-1; b) CD-2; c) CD-3; d) CD-G1; e) CD-G2; f) CD-G3.

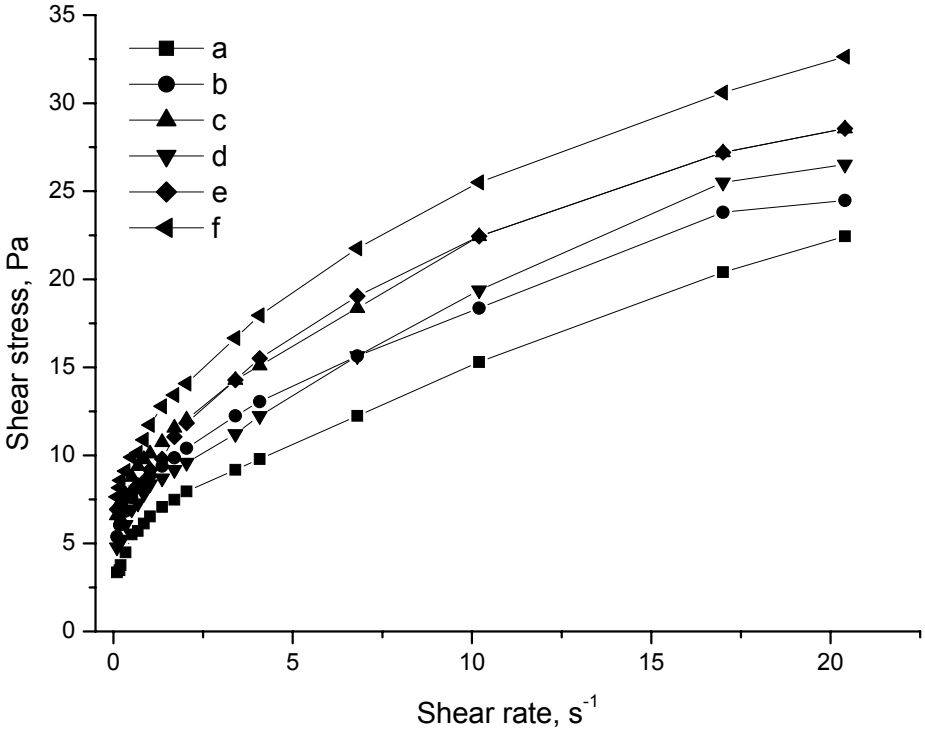

Fig. 4 – Rheograms of uncross-linked and cross-linked collagen hydrogels containing doxycycline hyclate at 37°C: a) CD-1; b) CD-2; c) CD-3; d) CD-G1; e) CD-G2; f) CD-G3.

The CD and CD-G hydrogels series show higher shear stress values compared with C and C-G ones at all the collagen concentration as Figs. 2 and 3 show. This could be explained only by a collagen-doxycycline interaction or by the cross-linking effect of doxycycline on collagen.

The viscosities of all the hydrogels are much higher at 23°C than that at 37°C, as expected.

For establishing the type of rheological behaviour, the mathematical model Herschel-Bulkley describing the non-Newtonian flow, according to which the hydrogels seem to behave, has been investigated.

The rheological parameters obtained for the hydrogels from Table 1 using the Herschel-Bulkley model are presented in Tables 2 and 3.

Table 2

Rheological parameters of uncross-linked and cross-linked collagen hydrogels at 23°C

	Hydrogels					
Rheological parameters	C-1	C-2	C-3	CG-1	CG-2	CG-3
Yield stress, Pa	1.117	1.784	1.911	1.864	2.882	3.004
Consistency index, Pa.s^n	2.425	3.134	3.948	3.108	3.354	4.558
Flow index	0.567	0.512	0.473	0.555	0.583	0.520

Table 3

Rheological parameters of uncross-linked and cross-linked collagen hydrogels containing doxycycline hyclate at 23 and 37°C

	Hydrogels					
Rheological parameters	CD-1	CD-2	CD-3	CD-G1	CD-G2	CD-G3
	23^{0}C					
Yield stress, Pa	4.931	5.460	6.651	5.129	7.040	8.423
Consistency index, Pa*s^n	3.484	4.787	6.361	3.351	5.472	6.582
Flow index	0.581	0.501	0.442	0.594	0.514	0.455
	37^{0}C					
Yield stress, Pa	2.969	4.531	5.238	4.143	4.506	5.491
Consistency index, Pa*s^n	3.076	4.072	4.796	3.623	5.084	6.173
Flow index	0.608	0.533	0.531	0.612	0.525	0.495

The above rheological parameters are in accordance with the rheograms shown in the Figs. 2-4, indicating that all the hydrogels respect the Herschel-Bulkley model for the pseudoplastic behaviour.

The profile and kinetics of drug release are important, they correlating the *in vitro* and *in vivo* drug responses by comparing the results of pharmacokinetics and dissolution profile patterns.[11]

The cumulative *in vitro* release of doxycycline hyclate from both the CD and CD-G collagen hydrogel series at 37°C is illustrated in Fig. 5.

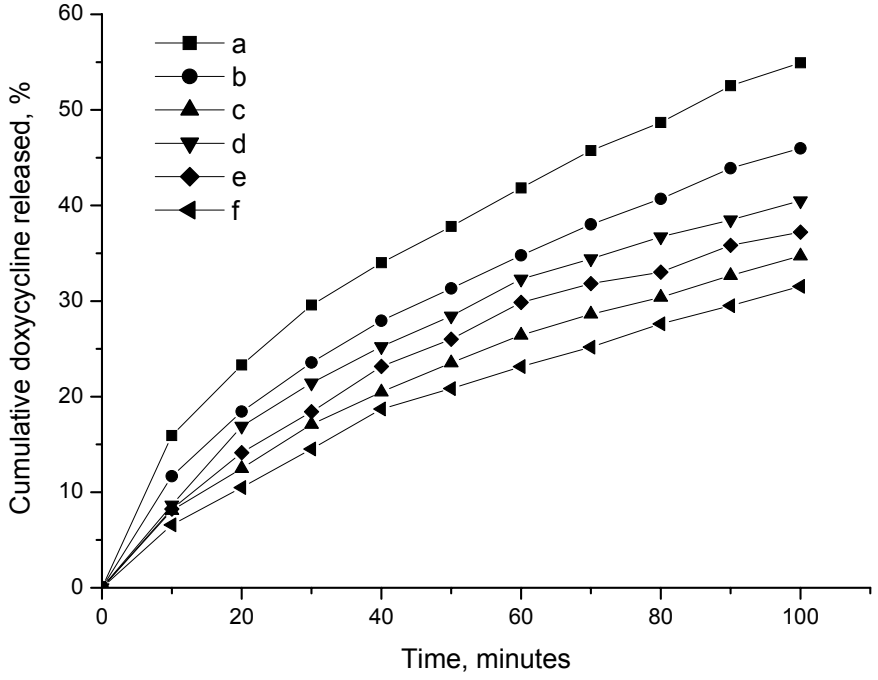

Fig. 5 – *In vitro* release of doxycycline hyclate from collagen hydrogels at 37^{0}C: a) CD-1; b) CD-2; c) CD-3; d) CD-G1; e) CD-G2; f) CD-G3.

Analyzing Fig. 5 it can be seen that the amount of doxycycline hyclate released depends both on concentration of collagen and cross-linking agent during the 100 minutes of observation.

The increasing of polymer concentration produces the decreasing of the percentage of drug released. This can be assigned to the collagen interaction with doxycycline, which produces also the viscosity increase. Thus, the percentage of doxycycline release varies from 54.92% for the uncross-linked hydrogel containing 0.8% collagen (CD-1) to 31.53% for the cross-linked one containing 1.2% collagen (CD-G3) at the end of the 100 minutes.

The cross-linking has the same effect as increasing of collagen concentration, the differences between the amounts of doxycycline released decreasing especially at low collagen concentrations. So only one amount of glutaraldehyde was used, it is expected that this amount will decrease with increasing of the degree of cross-linking.

To study the release kinetics, the data obtained from *in vitro* drug release studies presented in Fig. 5 were fitted with the following kinetics models:

Higuchi:[12] $$\frac{m_t}{m_\infty} = k \cdot t^{0.5} \quad (5)$$

zero order: $$\frac{m_t}{m_\infty} = k \cdot t \quad (6)$$

first order: $$\frac{m_t}{m_\infty} = 1 - e^{-k \cdot t} \quad (7)$$

power law (Peppas): $$\frac{m_t}{m_\infty} = k \cdot t^n \quad (8)$$

where m_t is the amount of drug released at time t, m_∞ – the total drug contents in the designed collagen hydrogels, m_t/m_∞ – the fractional release of the drug, k – the kinetic constant and n – the release exponent, indicating the mechanism of drug release.

The release mechanism is given by the n value in Peppas equation: $n = 0.5$ or less indicates Fickian diffusion, while $0.5 < n < 1$ indicates a non-Fickian model (anomalous mass transfer).

The correlation coefficients of equations (5)-(8) for the hydrogels containing doxycycline hyclate are presented in Table 4.

The release data analyzed on the basis of Higuchi kinetics give the highest correlation coefficients, as can be seen from the Table 4. This means that doxycycline hyclate is released from all the gels – cross-linked or not – by Fickian diffusion that is the diffusion rate of the active compound is much lower than the relaxation time of collagen in gel state. At the same time they demonstrate that the release exponent in the equation (8) is 0.5 or very close to 0.5.

Table 4

Correlation coefficients (r^2) for equations (5)-(8) obtained using the data from Fig. 5

Collagen hydrogels	Higuchi (5)	Zero order (6)	First order (7)	Power law (8)
	r^2 values			
CD - 1	**0.9991**	0.9793	0.9792	0.9960
CD - 2	**0.9980**	0.9773	0.9772	0.9930
CD – 3	**0.9989**	0.9741	0.9742	0.9969
CD - G1	**0.9967**	0.9498	0.9499	0.9887
CD – G2	**0.9943**	0.9566	0.9565	0.9897
CD – G3	**0.9973**	0.9762	0.9763	0.9957

CONCLUSIONS

All the prepared collagen hydrogels – cross-linked or not, containing doxycycline or free of it – present non-Newtonian pseudoplastic behaviour both at 23 and 37°C, following the Herschel-Bulkley rheological model.

Doxycycline has as effect the increase of collagen hydrogels viscosities both in the absence and the presence of the cross-linking agent

glutaraldehyde. The highest viscosity was obtained for the most concentrated hydrogel CD-G3 containing 0.20% doxycycline hyclate and 0.15% glutaraldehyde.

The rate of doxycycline release decreases both with the increasing of collagen concentration in the hydrogel and the presence of the cross-linking agent glutaraldehyde. The slowest release was obtained for the most viscous cross-linked hydrogel CD-G3 and the most rapid for the less viscous uncross-linked one CD-1, containing 0.8% collagen and 0.2% doxycycline hyclate.

The rheological properties of hydrogels and the rate of doxycycline hyclate release can be controlled by the collagen concentration, cross-linking agent and drug content.

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