In vitro Kinetic Release and Flow Behaviour of Some Collagen-Minocycline Topical Hydrogels

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The aim of this work was to develop some topical hydrogel based on collagen-minocycline uncross-linked and cross-linked with glutaraldehyde and to investigate its flow and kinetic behaviour. The rheological measurements were performed using a rotational viscometer. The release studies were conducted using a sandwich device adapted to paddle disolution apparatus. The tested hydrogels showed a pseudoplastic and shear thinning, and also a thixotropic behaviour. Different rheological descriptors were evaluated. The in vitro release data obtained were fitted according to different kinetic models and the highest value for the correlation coefficient was obtained for the power law model. The kinetic and flow properties are strongly influenced by the hydrogel compositions. The quantitative relantionships established between flow and release characteristics indicated a good correlation of these parameters. The results showed that the properties of designed hydrogels allow them to be used for the treatment of soft infected tissues.

Keywords: hydrogel, collagen, minocycline, rheological descriptors, drug release

Nowadays the number of semisolid pharmaceutical dosage forms designed to delivery the antibiotics for topical application in soft infected tissues treatment significantly increased. Among these, the hydrogels were proved to be the systems ensuring a rapid antibiotics release, increased compliance and side effects avoidance compared to the systemic administration [1-4].

The biological characteristics and well defined structure [5-7] recommend the collagen hydrogels as favorable natural carriers for antibiotic topical release [8-10].

Due to its broad spectrum of activity, minocycline is useful in the prevention/treatment of topical infections which can occur in soft tissues (at cutaneous, periodontal levels). Minocycline oral administration induces systemic side effects that can be minimized by using topical formulations [11-14].

Currently the importance of the drug release kinetic modeling from topical semisolid forms is recognized. For a certain concentration of a specific polymer able to form hydrogels, the release kinetic of a drug from polymeric hydrogel depends on both polymer and drug physico-chemical nature and also on their intermolecular interactions. For a specific polymer-drug system, the drug release kinetic can be influenced by concentration, temperature as well as by the presence of some other excipients incorporated in the hydrogel system for biopharmaceutical or technological reasons. Thus, for a specific temperature, the release kinetic from a hydrogel can be modeled through the variation of the components concentration into the formulation [15]. A method of drug release modulation from collagen hydrogels would be using crosslinking agents so that the biodegradability rate could influence the controlled release.

For the valorification of the study on the drug release from hydrogels, the kinetic analysis has to be complemented with their rheological characterization.

For the topical hydrogels, to know the rheological properties is of major importance, because they can highly influence: (i) production technology (incorporation of active or auxiliary substances, consistency, production conditions, extrusion capacity from the conditioning recipient), (ii) quality and stability control during preservation, (iii) usage (ease of application, adhesion to the application site), (iv) therapeutical activity (drug release from semisolid pharmaceutical forms and drug bioavilability) [15-17].

The overall efficiency of an antibiotic topical semisolid system, in terms of adhesion to the treated surface in the time required to ensure the patient compliance and a high drug release rate in order to produce a fast start of the action depends both on the drug pharmacokinetics and the vehicle properties. The formulation should allow a maximum thermodynamic activity of the drug to ensure its highest possible flux [15].

Thus, the purpose of this paper was to design some collagen-minocycline hydrogels uncross-linked and cross-linked with glutaraldehyde as topical drug delivery systems, potentially used in treatment of infected soft tissues, and also to investigate their rheological and kinetic parameters.

Experimental part

Materials and methods

Chemicals

Type I collagen gel (C) having a concentration of 1.99% (w/w) and *p*H 2.1 was extracted from calf hide by the currently used technology in Collagen Department of Division Leather and Footwear Research Institute [18]. Minocycline hydrochloride (MH) was obtained from Sigma (Germany), glutaraldehyde (GA) was supplied by Sigma-Aldrich (Germany), sodium hydroxide, monobasic potassium phosphate and disodium hydrogen phosphate were purchased from Merck (Germany). All the chemicals used in this work were of analytical grade and the water was distilled.

Hydrogels preparation

Reference collagen hydrogels having the concentrations 0.9%, 1.1% and 1.3% and *p*H 7.37 were obtained from the

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Collagen matrices	G1	G2	G3	G4	G5	G6	G7	G8
Collagen, C (g%)	0.9	1.1	1.3	0.9	1.3	1.1	1.1	1.1
Minocycline, MH (g%)	0.0	0.0	0.0	0.4	0.4	0.4	0.4	0.4
Glutaraldehyde, GA (g%)	0.0	0.0	0.0	0.0015	0.0015	0.0	0.0015	0.0030

*the amounts of C, MH and GA are reported to 100 g hydrogel

Table 1
COMPOSITION OF THE INVESTIGATED HYDROGELS

initial collagen gel (C) under stirring with distilled water and NaOH 1M solution. 0.4% minocycline hydrochloride reported to the amount of reference collagen hydrogels was added and some collagen-minocycline hydrogels were obtained. They were cross-linked with 0.0%, 0.0015% and 0.0030% glutaraldehyde at 4°C for 24 h. The composition of the designed hydrogels is summarized in table 1.

Rheological evaluation

The rheological analysis were conducted with a rotational viscometer Multi-Visc Rheometer Fungilab equipped with a standard spindle TR 9. The viscometer allows, for each rotational speed or shear rate specific to the spindle used, the evaluation of the shear stress and the viscosity of the hydrogel tested. Stationary shear rheometry was carried out at two temperatures: 23°C (to investigate the performance at room temperature) and 37°C respectively (to correlate the rheological and the MH release kinetics performances). In order to maintain the temperature strictly constant during the rheological experiments, a ThermoHaake P5 Ultrathermostat was used. To ensure a thermal and mechanical equilibrium and also the results reproductibility, each gel was left unstirred for 10 min , before the experiment start, in the thermostat vessel where the rheological determinations were performed. The rotation speeds limits were chosen between 0.3-60 rpm because they are representative of some operations with biopharmaceutical implications: application of the hydrogel at the administration site, drug release from the formulations designed, as well as the examination of rheograms hysteresis as the upper ranges are more representative for spreading and mixing processes [19]. The ascending rheograms shear stress as function of shear rate, for shear rate values in the range 0.1-20.4 s^{-1} were recorded corresponding to these rotational speeds. The descending rheograms shear stress as function of shear rate were recorded for shear rate values in the range 20.4-0.51 s^{-1}; the lower limit of the shear rate, recordable for the descending curves, corresponds to a rotational speed of 1.5 rpm. Each measurement duration was 10 s. The rheological measurements for each formulation were replicated three times and the average results were reported.

In vitro drug release kinetic

Minocycline release from collagen hydrogels was performed using a sandwich device adapted to a paddle dissolution apparatus (Essa Dissolver, Italy). About 1g of gel was spread by means of a syringe in the sandwich device of the disolution apparatus release vessel. The release medium (phosphate buffer solution *p*H 7.4) was stirred at 50 rpm during the experiment. The working temperature was maintained at 37°C, in order to simulate the administration site temperature. At appropriate time intervals, samples of 5 mL were extracted from the release medium and replaced with an equal volume of fresh, prewarmed buffer solution. The samples concentration in the dissolution medium was evaluated spectrophotometrically at 348 nm (Perkin-Elmer UV-Vis Spectrophotometer), using the calibration curve (fig. 1), linear for concentrations in the range 0-0.00275 g/100mL ($A^{1cm}_{1\%} = 274$, R = 0.9991) [20]. Each experiment was conducted in triplicate and the mean of the results was presented.

The rheological and kinetic parameters specific to the tested hydrogels were determined using the table Curve 2D and Microcal Origin softwares.

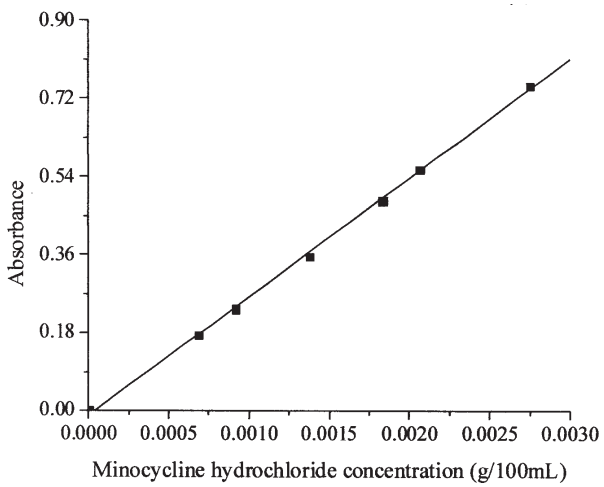

Fig. 1. Calibration curve of minocycline hydrochloride in phosphate buffer solution *p*H 7.4

Results and discussions

Presently it is recognized that the clinical and non-clinical performances of the hydrogels intended for topical application depend on their flow properties [15]. The elaborated study of their rheological behaviour can lead to the potential use of the flow parameters for a drug release optimization from such pharmaceutical systems.

As previously indicated in the Experimental part, the viscosity and shear stress of hydrogels were determined as a function of shear rate.

For the reference hydrogels the viscosity determined at 23°C versus shear rate was illustrated for exemplication in the figure 2.

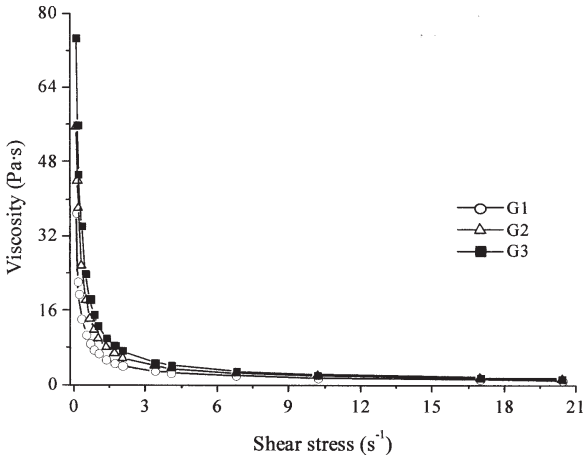

Fig. 2. Flow curves of reference hydrogels analyzed at 23°C

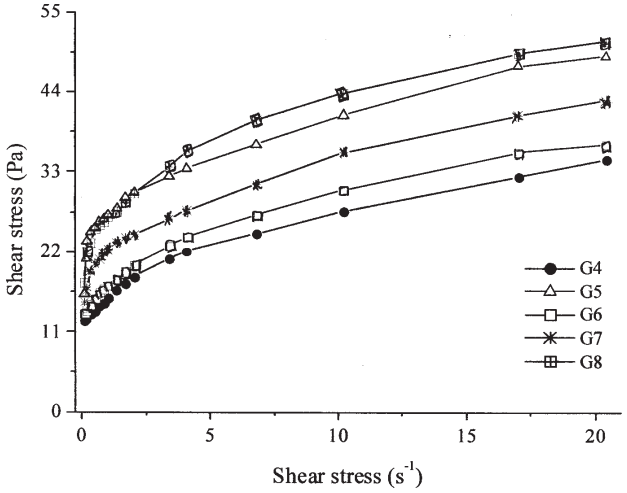

Fig. 3. Flow curves of collagen-minocycline hydrogels analyzed at 23°C

The rheograms presented in figure 2 showed that the viscosity decrease for the shear stress increase, this indicating a non-newtonian behaviour for the reference hydrogels. These results are in accordance with the ones obtained for other reference hydrogels formulated with similar collagen concentrations [21].

The ascending flow curves of shear stress measured as a function of the applied shear rate are presented in the figures 3 and 4 for the minocycline hydrogels collagen-based tested at both temperatures.

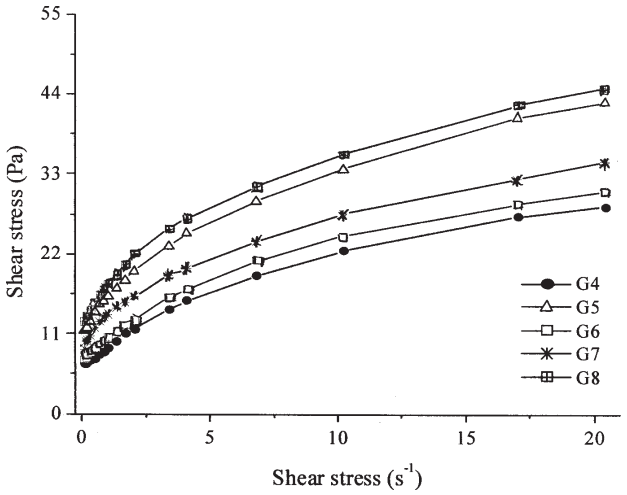

Fig. 4. Flow curves of collagen-minocycline hydrogels analyzed at 37°C

From the rheological profiles presented in figures 3 and 4 we can observe that the hydrogels with MH and collagen exhibited a non-newtonian behaviour at both temperatures, the shear stress increased with shear rate increase.

For the evaluation of the MH hydrogels flow behaviour, different rheological model were verified: Bingham, Casson, Ostwald-de Waelle, Herschel-Bulkley [21].

The rheological models are a way of representing a big amount of experimental data under the form of a simple mathematical relation. These equations named constitutive equations are useful for predicting the flow behaviour of the complex systems as hydrogels, for specific shear rates range [19]. The most adequate rheological model depends on the system response at deformation and also on the theoretical model goodness of fit [22].

The values obtained for the correlation coefficients (R) of the above mentioned models are summarized for exemplification for MH hydrogels tested at 37°C in table 2. Comparing the values of R it can be observed that the highest value is obtained for Herschel-Bulkley model for all the prepared formulations (they range from 0.9919 to 0.9989 at 23°C and from 0.9974 to 0.9996 at 37°C respectively) which shows that hydrogels follow this model; the graph of shear stress versus shear rate intersected the shear stress axis at value higher then zero corresponding to the yield stress.

The terms appearing in the previously mentioned rheological model are: τ- 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), n – flow index.

Rheological model	R values for hydrogel formulations				
	G4	G5	G6	G7	G8
Bingham $\tau = \tau_0 + \eta \cdot \dot{\gamma}$	0.9693	0.9655	0.9648	0.9632	0.9600
Casson $\tau^{0.5} = \tau_0^{0.5} + \eta^{0.5} \cdot \dot{\gamma}^{0.5}$	0.9930	0.9939	0.9920	0.9934	0.9922
Ostwald-de Waelle $\tau = K \cdot \dot{\gamma}^n$	0.9900	0.9918	0.9910	0.9927	0.9926
Herschel-Bulkley $\tau = \tau_0 + K \cdot \dot{\gamma}^n$	0.9974	0.9993	0.9977	0.9996	0.9993

Table 2
CORRELATION COEFFICIENTS (R) VALUES OF DIFFERENT RHEOLOGICAL MODELS FOR THE MH HYDROGELS TESTED AT 37°C

Table 3
RHEOLOGICAL PARAMETERS VALUES FOR MH HYDROGELS ANALYZED AT 23°C

Hydrogel	Yield stress (Pa)	Consistency index ($Pa \cdot s^n$)	Flow index	Ascending area ($Pa \cdot s^{-1}$)	Thixotropy area ($Pa \cdot s^{-1}$)	Thixotropy index (%)
G4	10.065	5.789	0.478	540.915	61.223	11.32
G5	13.267	13.466	0.342	808.998	94.179	11.64
G6	10.533	7.015	0.445	595.158	71.116	11.95
G7	12.452	9.235	0.392	691.804	80.763	11.67
G8	13.932	13.739	0.392	851.660	100.798	11.83

Table 4
RHEOLOGICAL PARAMETERS VALUES FOR MH HYDROGELS ANALYZED AT 37°C

Hydrogel	Yield stress (Pa)	Consistency index ($Pa \cdot s^n$)	Flow index	Ascending area ($Pa \cdot s^{-1}$)	Thixotropy area ($Pa \cdot s^{-1}$)	Thixotropy index (%)
G4	4.508	5.024	0.529	426.661	39.874	9.35
G5	7.992	8.329	0.480	652.303	71.447	10.95
G6	4.968	5.875	0.497	463.248	45.287	9.77
G7	6.581	7.070	0.458	527.462	57.716	10.94
G8	8.636	9.513	0.447	692.107	76.053	10.98

The rheological descriptors values specific to Herschel-Bulkley flow model for the semisolid formulations with MH and colagen assessed at both working temperatures are given in tables 3 and 4.

From tables 3 and 4 we can remark that the flow index values are subunitary for all hydrogels tested at both temperatures, highlighting a pseudoplastic and shear thinning behaviour.

The pseudoplastic behaviour with yield stress is a desirable property for the topical systems because at high shear rates, as those for expulsion from the recipient (tube, syringe), the material will flow readily facilitating the clinical administration (cutaneous, periodontal pocket); in case of low shear rates such as spread hydrogel, the material will adopt a higher consistency recovering its original rheological properties before administration [23-26].

The MH hydrogels were also analyzed in terms of thixotropic behaviour.

The thixotropy occurs because the hydrogel needs a finite time period for the initial viscosity recovery, for the restoration of the initial structure destroyed during the continous shear measurement respectively [17,25,27]. The thixotropy study is achieved through the rheogram shear stress versus shear rate corresponding to the direct curve, at increasing shear rates, and to the return curve, recorded at the shear rate values decrease. For the same shear rate, the point on the return curve corresponds to lower shear stress compared to the direct curves, obtaining the hysteresis thixotropy area [24, 28].

In figure 5 the ascending and descending flow curves for the gel G7 at 23 and 37°C are illustrated for exemplification.

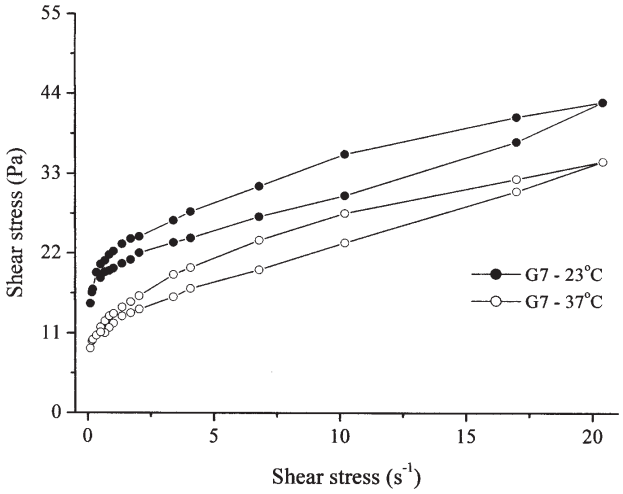

Fig. 5. Flow curves (up and down) for hydrogel G7 analyzed at 23°C and 37°

The thixotropy quantitative evaluation was performed through the determination of the thixotropic area and the thixotropy index.

The thixotropy area (S_{thix}) is the surface between the ascending and descending curves. The area delimited by the ascending curve (S_{asc}) refers to a complete hydrogel rheodistruction being associated with the hydrogel normal manipulation time to expose the drugs incorporated in such formulations to the absorption at the application site (at cutaneous or periodontal level). The descending area refers

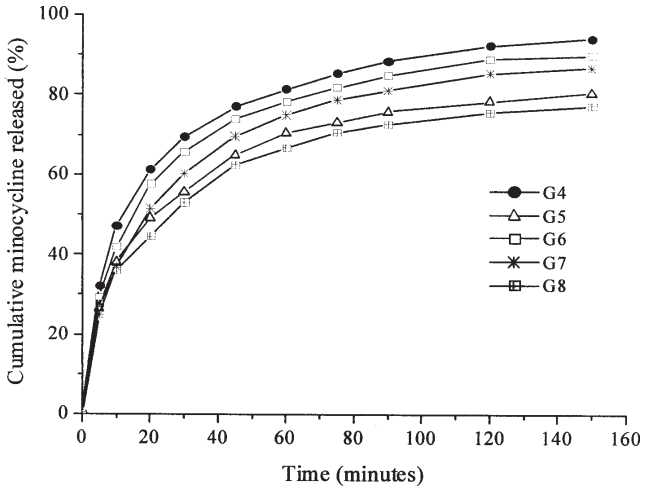

Fig. 6. Cumulative release of minocycline from collagen hydrogels (G4-G8)

to the recovery of the initial structure by the sheared hydrogel [4,23].

The thixotropy index is defined as the ratio between the thixotropy and the ascending areas and represents the percent of rheodistroyed area by the shear stirring [28]. Due to the error in the measurement of the ascending and descending areas only the hydrogels with a thixotropy index of more than 5% are considered thixotropic [15].

The values obtained for the ascending area, thixotropy area and thixotropy index are given in tables 3 and 4. Values higher than 5% recorded for the thixotropy index indicate the thixotropic feature of the formulations tested at both temperatures. The thixotropic feature is also a quality parameter to be targeted in view of transforming an initially viscous hydrogel in a thin product, easy to spread at the administration site.

The data presented in tables 3 and 4 revealed that adding glutaraldehyde to collagen hydrogels increased the yield stress and the consistency index of the formulations; the ascending and thixotropy areas also increased at both temperatures. To summarize, for 37°C the increase of the yield stress and thixotropy values is practically double and quite similar for the ascending area compared with the values recorded at 23°C. For the consistency index, the increase is more important at 23°C compared to 37°C (almost double).

Compared with G4, the collagen concentration increase determines at 37°C an increase of yield stress of 1.46 times for G7 and 1.77 times for G5 respectively, while at 23°C the increase is less important, 1.24 times for G7 and 1.32 times for G5 respectively.

We can also notice that for the temperature increase a decrease of the yield stress (about 1.6-2 times) and consistency index values (about 1.20-1.60 times) is recorded. Concerning the thixotropy descriptors the values obtained at 23°C are about 1.23-1.30 times higher compared to the ones obtained at 37°C for the ascending area and 1.32-1.57 times higher for the thixotropy area respectively.

The rheological properties of the semisolid network for each formulation tested depend on the constituents concentration, giving a specific release kinetic profile of the incorporated drug.

Thus the influence of the gel vehicle on the MH release was further examined by comparing the kinetic profiles obtained. The cumulative percent of *in vitro* drug released from the various hydrogel formulations recorded as a function of time is presented in figure 6.

The cumulative MH released percent varied between 77.32 (G8) and 94.04% (G4). For all the semisolid systems more than 75% of drug was released within 150 min.

For the modeling of the drug released from hydrogels various kinetic models [16, 21, 29] were investigated (Power law, Higuchi, Zero-order and First-order). The correlation coefficient R values for the above models are given in table 5.

The signifiance of the terms included in table 5 is as follows: m_t / m_∞, the fraction of drug released at time t, k, the kinetic constant incorporating the system characteristics, and n, the release exponent describing the drug release mechanism.

As it can be seen in table 5, the highest values for R were obtained for Power law model (they range from 0.9875 to 0.9923), proving the fact that this model best describes the MH release mechanism from the hydrogels tested with the sandwich device fitted to disolution apparatus. The values recorded for the release exponent smaller than 0.5 (0.266-0.308) suggested an anomalous (non-Fickian) transport which indicates that the MH delivery from gels was influenced both as drug diffusion through polymeric matrix as well as polymeric chains relaxation.

The quantification of the *in vitro* drug release kinetic was performed through the following parameters: the kinetic constant (k) and the time required for 50% released ($T_{50\%}$) of initial drug loading (specific to Power law model),

Kinetic model	R values for hydrogel formulations				
	G4	G5	G6	G7	G8
Power law $m_t / m_\infty = k \cdot t^n$	0.9899 (n = 0.266)	0.9908 (n = 0.284)	0.9881 (n = 0.278)	0.9875 (n = 0.308)	0.9923 (n = 0.287)
Higuchi $m_t / m_\infty = k \cdot t^{0.5}$	0.9417	0.9525	0.9462	0.9577	0.9564
Zero order $m_t / m_\infty = k \cdot t$	0.8119	0.8301	0.8192	0.8403	0.8377
First order $m_t / m_\infty = 1 - e^{-k \cdot t}$	0.7486	0.7638	0.7531	0.7703	0.7712

Table 5
CORRELATION COEFFICIENTS VALUES (R) FOR MH RELEASE FROM COLLAGEN HYDROGELS OBTAINED USING DIFFERENT KINETIC MODELS

Release parameters	Hydrogel formulations				
	G4	G5	G6	G7	G8
Kinetic constant (min^{-n})	0.266	0.207	0.239	0.199	0.195
$T_{50\%}$ (min)	10.92	22.31	14.19	19.79	26.54
Percent released (%)	94.04	80.56	89.85	86.75	77.32

Table 6
THE KINETIC RESULTS OF MINOCYCLINE RELEASE FROM THE DESIGNED HYDROGELS

Kinetic-rheology equations	R	Predictive value				
		G4	G5	G6	G7	G8
$Drug\%released = 140.564 \cdot \tau_0^{-0.269}$	0.9766	93.74	80.36	91.32	84.67	78.70
$Drug\%released = 913.275 \cdot S_{asc}^{-0.376}$	0.9940	93.68	79.86	90.84	86.50	78.10
$Drug\%released = 260.192 \cdot S_{thix}^{-0.276}$	0.9825	94.08	80.09	90.83	84.95	78.72
$T_{50\%} = 1.998 \cdot \tau_0^{1.188}$	0.9843	11.95	23.60	13.41	18.73	25.87
$T_{50\%} = 0.0012 \cdot S_{asc}^{1.514}$	0.9627	11.51	21.89	13.03	15.87	23.94
$T_{50\%} = 0.139 \cdot S_{thix}^{1.205}$	0.9812	11.79	23.82	13.75	18.42	25.69

Table 7
KINETIC-RHEOLOGY EQUATIONS AND PREDICTIVE VALUES FOR THE MH HYDROGELS

and also the drug percent released. The effect of the collagen and glutaraldehyde concentrations on the minocycline released are summarized in table 6.

According to table 6 we remarked that for the same concentration of collagen (1.1.%) and MH (0.4%), the crosslinking agent variation from 0 to 0.0015% (G6 and G7), and to 0.0030% respectively (G6 and G8) leads to a decrease of the drug percent released of about 3.5%, and 14% respectively. In turn, the $T_{50\%}$ value increases about 1.39 and 1.87 times respectively, for the same increase of the crosslinking agent amount.

Compared with G4, the increase of the collagen concentration when the drug and crosslinking agent concentrations are kept constant leads to a decrease of the drug percent released of about 8% for G7 and 17 % respectively for G5 while $T_{50\%}$ increases of about 1.81 times (for G7) and 2.04 times respectively (for G5).

We can remark that the formulation with a lower collagen concentration and a middle level of GA (G4) has smaller values of $T_{50\%}$ (about 23% less) and higher values of the drug percent released (about 4.6% more) compared to the formulation with higher collagen concentration, but uncrosslinked (G6).

These kinetic results are in accordance with the data obtained from the rheological determinations, because the analyzed formulations flow behaviuor dramatically alter the microenvironment where the drug release takes place.

The combined effect of formulation factors influenced both the MH release kinetic profiles from the topical dosage forms and the rheological properties of these pharmaceutical systems [15]. For this reason in the last part of this study we followed the construction of some *rheology – kinetic* quantitative relationships (table 7) using rheological parameters that describe a specific MH release profile. The rheological descriptors with biopharmaceutical implications taken into account were: yield stress, ascending area, thixotropic area (determined at 37°C), and the related kinetic characteristics considered were: drug percent released and $T_{50\%}$.

For this purpose a non-linear estimation method with "user-specified regression" subroutine of Statistica™Stat software was used.

The values of the correlation coefficient R indicate a good predictive power of these equations, proven by the drug percent released and $T_{50\%}$ predictive values (table 7) close to the values mentioned in table 6.

The kinetic-rheology models set can be also used for other hydrogels having the same MH concentration and different collagen and glutaraldehyde concentrations, not designed in this study.

Conclusions

The designed hydrogels showed a pseudoplastic shear-thinnig with yield stress and thixotropic behaviour. The power law model allows the setting of the drug release mechanism. Quantitative relationships between the MH release kinetic parameters and some rheological characteristics (yield stress, ascending area, thixotropic area) of interest from biopharmaceutical point of view concerning the semisolid formulations behaviour at the application site were set. The drug delivery systems obtained as hydrogel forms, uncrosslinked and crosslinked, based on collagen and minocycline, proved a strong correlation between kinetic and rheological properties which demonstrated that drug release depends both on collagen and glutaraldehyde concentrations. Through the simultaneous modulation of the collagen concentration (between the low level of 0.9% and the middle level of

1.1%) and the crosslinking agent (between the low level of 0.0% and the middle level of 0.0015%), adequate results can be obtained concerning both an antibiotic fast release and also adequate rheological characteristics that ensure an appropriate administration at cutaneous or periodontal level.

The process of drug release from hydrogels is a complex process whose analysis should be rigorous for a rational formulations design, the development of tests concerning their quality and stability, the batch to batch control. The rheological analysis is complementing the complex field of the drug release from topical semisolid forms, being an important issue for the potential application of the hydrogels with collagen and minocycline in soft infected tissues treatment.

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