ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2010, *55*(9), 543-551

CHARACTERIZATION AND *IN VITRO* RELEASE OF CHLORHEXIDINE DIGLUCONATE COMPRISED IN TYPE I COLLAGEN HYDROGELS

Dorin ŞULEA,[a] Mihaela Violeta GHICA,[b] Marin MICUTZ,[a] Mădălina Georgiana ALBU,[c] Lavinia BRĂZDARU,[a] Teodora STAICU,[a] Minodora LECA[a*] and Lăcrămioara POPA[b]

[a] University of Bucharest, Faculty of Chemistry, 4-12 Regina Elisabeta Blvd, 030018 Bucharest, Roumania
[b] University of Medicine and Pharmacy "Carol Davila", Faculty of Pharmacy, 6, Traian Vuia str., 020956 Bucharest, Roumania
[c] Leather and Footwear Research Institute, Collagen Department, 93 Ion Mincu str., 031215 Bucharest, Roumania

Received March 30, 2010

Three series of drug delivery systems consisting of type I collagen hydrogel containing 0, 0.02, 0.05 and 0.10% chlorhexidine digluconate (CHXD) and having pHs 3.8 and 7.4, the last noncrosslinked and crosslinked with 0.15% glutaraldehyde (GA), were prepared to be used for wound healing. FT-IR spectra show no effect of CHXD on triple helical conformation of collagen irrespective of pH and crosslinking. CD spectra also indicate the absence of CHXD and GA effect on collagen conformation. Rhoogram show pseudoplastic behaviour for all the hydrogels and a decrease of viscosity at zero shear rate with increasing CHXD concentration, regardless of pH and crosslinking, due to its binding to collagen and GA. Dynamic rheological measurements reveal the prevalence of elastic component of viscoelasticity in all the hydrogels and the insignificant effect of CHXD on viscosity in acid medium. CHXD reduces the elastic component in slight basic medium, while GA increases it significantly both in the absence and presence of CHXD, excepting the highest concentration, for which the values are similar with those in acid medium. The acid hydrogels release the lowest amounts of CHXD irrespective of initial concentration, the crosslinked hydrogels release less CHXD than the noncrosslinked ones, but the differences are small and increase a bit with CHXD concentration.

INTRODUCTION

Sustained drug release technology,[1] given its advantages:[2,3] decreasing of systemic toxicity, increased efficiency due to the high concentration of the drug at the damaged site and reduction in side effects, is used for a large variety of drugs, including antibiotics for the prevention and/or minimization of the bacterial infection.[3-6]

The most investigated natural polymer as drug delivery substrate is the type I collagen, due to its remarkable biocompatibility, low antigenicity,[7,8] possibility to control the biodegradability and release rate by crosslinking[8] and to be obtained in different forms: hydrogels, porous matrices or membranes.

The hydrogels are three-dimensional hydrophilic polymeric networks able to hold large amounts of water or biological fluids,[9-12] which vary from 10% to thousands of times the weight of the xerogel.[13]

Depending on the nature of the crosslinking, hydrogels may be permanent – when the crosslinks are covalent bonds or physical – if they form by physical interactions: hydrogen bonding among polymeric chains, ionic interaction or molecular entanglement.[13,14] Given the high water content and soft consistency, which impart unique bulk and surface properties,[15] they resemble natural living tissue more than any other biomaterials,[16] which determine their application in the medical and pharmaceutical sectors,[16-18] including drug delivery.[15-20]

Collagen hydrogels form the basis of many drug delivery systems[15] because they are biocompatible and naturally remodelled by cells, have low immunogenicity, high bioabsorbability

* Corresponding author: minlec@yahoo.com

and capacity to flow. They are obtained from calf hide by fibrillar extraction techniques,[21] which prevent the denaturation of the triple helical structure of collagen molecules which build up the fibrils.[22] These gels are three-dimensional lattice of collagen fibrils formed as monomeric collagen packs into D periodic fibrils in an interwoven random mesh, hold together by hydrogen bonding and ionic interaction among the fibrils and are able to entrap a large excess of fluid.[23,24]

So the collagen has the capacity to heal wounds,[25,26] it is not able to promote by itself the healing process because, being a protein, it serves as a substrate for bacteria. But associated with antiseptics and/or antibiotics and applied topically, collagen hydrogels, sponges or membranes can function as drug delivery systems and are able to control the wound infection by the localized delivery of drugs.[27-29]

CHXD, the selected antimicrobial to be introduced within the collagen hydrogels to control the wound infection, is a powerful antiseptic known to be less toxic to tissues than other antiseptics. It has a high bactericidal activity against a wide spectrum of both gram-positive and gram-negative bacteria including some fungi and viruses[30,31] and resistance to inhibition by blood and organic materials,[32] which make it the most widely used antiseptic in the wound management.[33] Its use has the following advantages: strong binding to skin, ability to adsorb to negatively charged surfaces, persistence, low toxicity and minimal adverse effects on blood and other biological materials.[34-36] It is currently recommended within the Evidence-Based Practice in Infection Control (EPIC)[37] and Healthcare Infection Control Practices Advisory Committee (HICPAC)[38] guidelines for skin antisepsis. However, its antimicrobial efficacy is significantly influenced by pH and organic matter.[39]

The objective of the present paper is the preparation of CHXD containing type I collagen hydrogels to combine the healing capacity of collagen with the high bactericidal activity against a wide spectrum of both gram-positive and gram-negative bacteria in view of controlling the wound infection. The checking of the triple helical structure of the collagen from the prepared 1.1% collagen hydrogels having the pHs 3.8 and 7.4 and the effect of 0.02, 0.05 and 0.10% CHXD, the last ones also crosslinked with 0.15% GA, on this structure by FT-IR and CD, emphasizing of collagen interaction with CHXD as a function of pH and CHXD concentration, as well as with CHXD or/and GA at slight basic pH by rheological measurements and their influence on the kinetics of delivery of CHXD from the above collagen hydrogels are discussed into the present paper.

RESULTS

The FT-IR spectra of the three prepared series of collagen hydrogels show for the ratios A_{III}/A_{1450} and the differences (νA_I - νA_{II}), cm^{-1}, used to evaluate the integrity of collagen triple helical structure, the values given in Table 1.

Table 1

The ratios A_{III}/A_{1450} and the differences (νA_I - νA_{II}), cm^{-1}, for the prepared collagen hydrogels

CHXD, %	pH					
	3.8		7.4			
			Noncrosslinked		Crosslinked	
	A_{III}/A_{1450}	(νA_I - νA_{II}), cm^{-1}	A_{III}/A_{1450}	(νA_I - νA_{II}), cm^{-1}	A_{III}/A_{1450}	(νA_I - νA_{II}), cm^{-1}
0	0.965	101	0.895	102	1.128	103
0,02	1.256	101	1.192	102	1.312	99
0,05	1.318	101	1.219	102	1.343	99
0,10	1.204	101	1.538	102	1.312	101

UV-CD spectra of the control collagen hydrogels and of the corresponding ones containing 0.05% CHXD, able to indicate the preservation of the triple helical structure of collagen are shown in the Figures 1a-c.

The rheograms obtained for the acid and slight basic crosslinked collagen hydrogels can be seen in Figures 2a, b, the noncroslinked ones behaving similar with the last ones, but with lower shear stresses.

The dependence of storage, G', and loss, G", moduli on the applied frequency for the three series of collagen hydrogels are shown in Figure 3a-c.

CHXD cumulative release from all the above mentioned collagen hydrogels is represented in Figure 4.

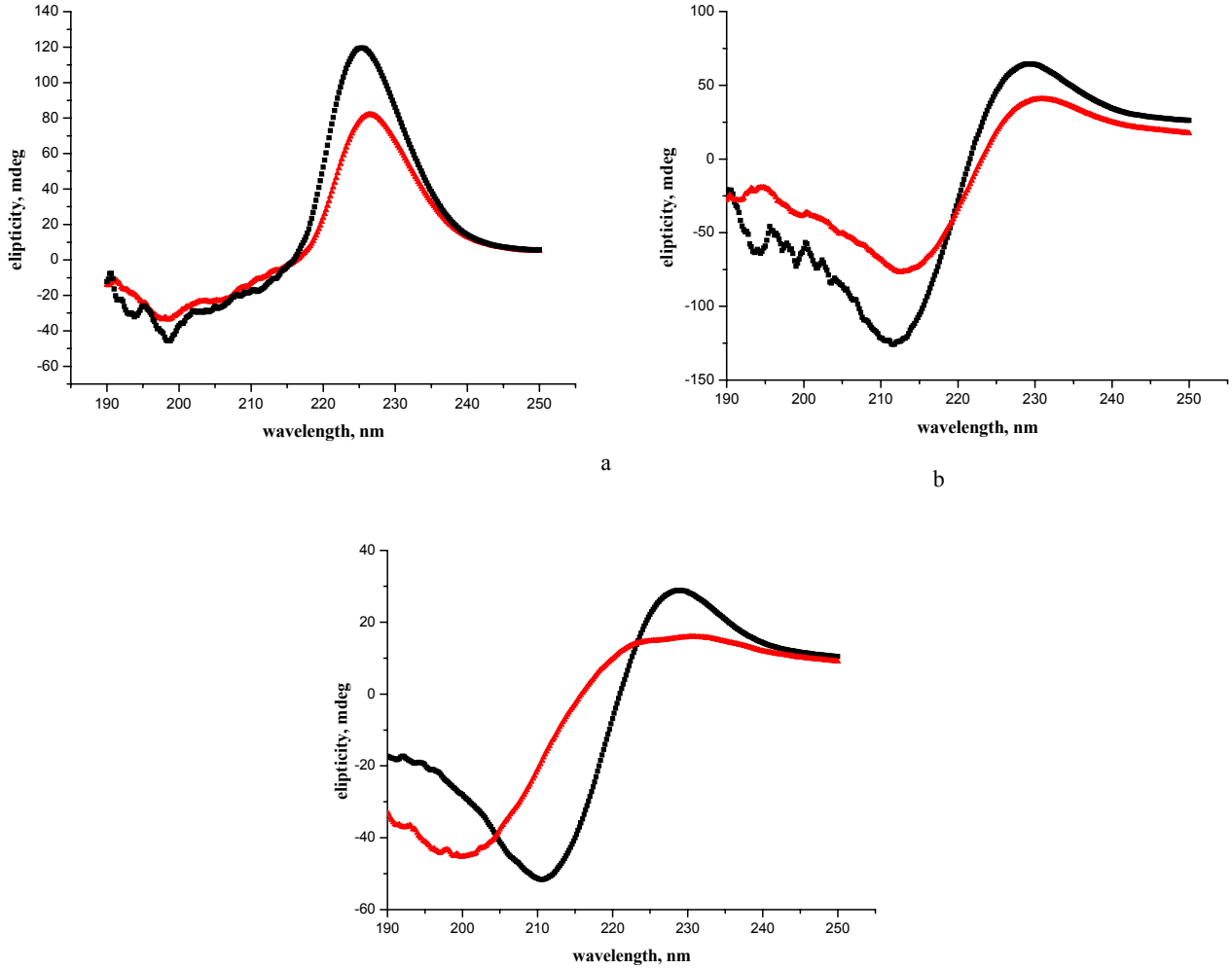

Fig. 1 – CD spectra of collagen hydrogels: a – pH 3.8; b – pH 7.4 uncrosslinked; c – pH 7.4 crosslinked with GA; CHXD concentration: black – 0; red – 0.05%.

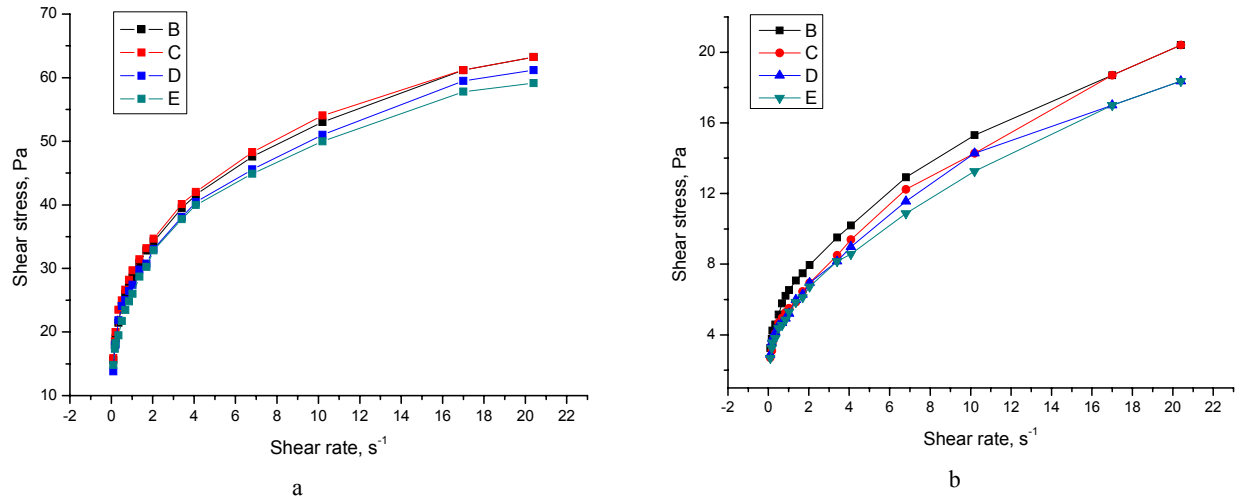

Fig. 2 – Rheograms of collagen hydrogels having the pH: a – 3.8, b – 7.4 crosslinked and CHXD concentrations: B – 0; C – 0.02; D – 0.05; E – 0.10%.

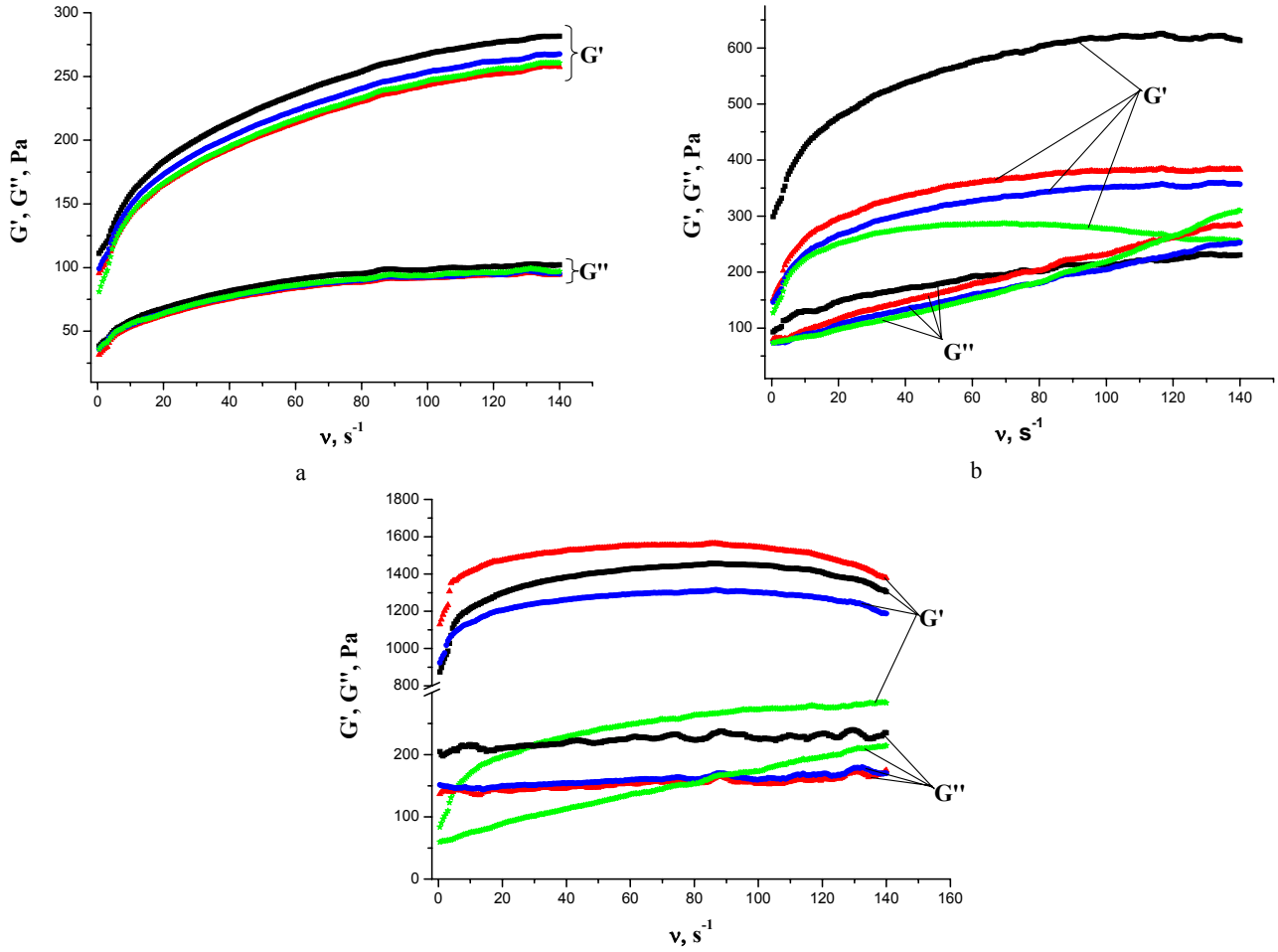

Fig. 3 – Dependence of storage, G', and loss, G", moduli on frequency for collagen hydrogels: a – pH 3.8; b – pH 7.4 noncrosslinked; c – pH 7.4 crosslinked; CHXD concentration: black - 0; blue – 0.02; green – 0.05; red – 0.10%.

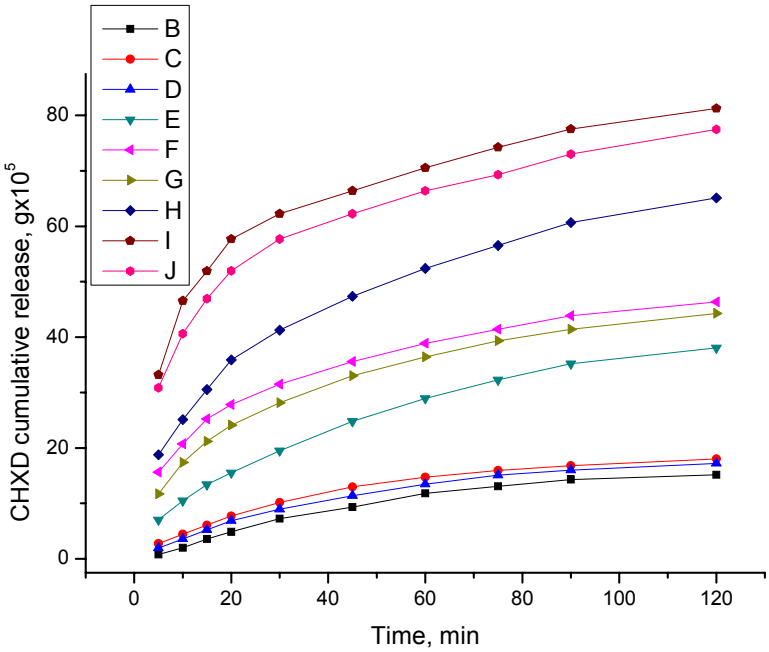

Fig. 4 – CHXD cumulative release from collagen hydrogels containing: $2x10^{-2}$ (pHs: B – 3.8, C – 7.4, D – 7.4 and 0.15% GA); $5x10^{-2}$ (E-G – the same order of curves); 10^{-1}% (H-J– the same order of curves) CHXD.

DISCUSSION

The preservation of the native structure of type I collagen into the hydrogels was checked, it being important for the contribution to the process of wounds healing, especially cell growing.

FT-IR spectrum of the native collagen presents bands characteristics to its specific molecular organization: amide A and B at 3300 and 3080 cm^{-1}, as well as amide I, II and III at 1650, 1554 and 1240 cm^{-1} respectively.[40] Some of these bands can be used to appreciate the preservation of the triple helical conformation of collagen molecule in a given material. Thus, the ratio of absorbance of the band amide III, A_{III}, to that from 1450 cm^{-1}, A_{1450}, serves as a measure of the integrity of the triple helical conformation of collagen, values equal or higher than unity indicating its preservation. At the same time, differences higher than 100 cm^{-1} between the frequencies of the bands amide I, A_I, and amide II, A_{II}, indicates the degradation of collagen.

Amide I, II and III bands appear in the FT-IR spectra of the prepared collagen hydrogels at 1657, 1556 and 1242 cm^{-1} respectively, while the band from 1450 cm^{-1} is shifted to 1458 cm^{-1}. Table 1 shows that the A_{III}/A_{1450} ratios range between 0.895 for the control collagen hydrogel having the pH 7.4 and 1.538 for that with the same pH and 0.1% CHXD, whilst that of the control collagen hydrogel with pH 3.8 is 0.965, very close to unity; the values of (νA_I - νA_{II}) range between 99 and 103, with the lowest value for the crosslinked hydrogel containing 0.02 and 0.05 CHXD and the highest one for the crosslinked control collagen hydrogel. CHXD has as effect the increasing of A_{III}/A_{1450} values with CHXD concentration, excepting the concentration 0.1% both at pH 3.8 and 7.4 if the hydrogel is crosslinked, and the values (νA_I - νA_{II}) remain constant for a given pH, excepting the crosslinked hydrogels, for which a difference of maximum 4 cm^{-1}, equal with the spectral interval between the data points, was obtained. The above data show that CHXD and GA effect on the triple helical conformation of collagen is insignificant irrespective of pH and crosslinking.

A molecule of native type I collagen is a poly-L-proline II type triple helix, tertiary structure producing a conformational folding which generates optical activity. Collagen has a characteristic UV-CD spectrum[41-43]: a pronounced negative minimum around 200 nm, a weak positive maximum at about 220 nm and a crossover point at about 212 nm. The degree of helicity of collagen molecules composing the fibrils is measured by the absolute value of the ratio of the intensities of positive and negative peaks, Rpn.[44,45] Literature[46] indicates a Rpn value of 0.12 for the native collagen. Partial denaturing results in changes in the intensity and position of peaks:[44,47,48] decreasing of their absolute intensities and Rpn and a red shifting of the crossover point. Increase of Rpn signifies the decrease of collagen molecule flexibility due to a physical or chemical supermolecular association.

CD spectra from Fig. 1 indicate that pH, CHXD and/or GA have no denaturing effect on collagen. In acid medium the positive peak is significantly higher than the negative one (Fig. 1a), which suggests an increase of intermolecular association. CHXD reduces this association, which is accompanied by an increase of flexibility at the molecular level, which alters to a certain extent the overall gel-like structure of collagen. CD spectra in Figure 1b (pH 7.4) look very similar to those of diluted collagen solutions. The intensity of both peaks decreases in the presence of CHXD, which suggests an increasing of the weight of triple helical structure on unit of volume. This can be due to a slight phase separation induced by CHXD, visually perceptible, that reduces the collagen concentration on unit of volume. No obvious change of flexibility can be seen. GA produces an effect similar with that of CHXD on CD spectrum (Fig. 1c). The simultaneous presence of GA and CHXD seems to increase the flexibility of collagen – the Rpn value is lower for CHXD, or produce a slight denaturation, indicated also by the red shifting of minimum, maximum and crossover point.

Rheological properties, giving information on consistency and apparent plasticity of a hydrogel, are important for the prediction of their behaviour on the application site.[49] Moreover, they influence the kinetics of drug delivery and the stasis time at the application site.

The rheograms in Figure 2a show that 0.02% CHXD produces a very low increase of viscosity of collagen hydrogel having the pH 3.8, but this effect is visible only at lower shear rates ranging between 0.1 and 10.2 s^{-1}, and disappears at 17.0 and 20.4 s^{-1}, which means that the bonds formed between collagen and CHXD are very weak, probably due to the low amount of CHXD. The rheogram of the hydrogel containing 0.05% CHXD is place under the collagen one, showing a decreasing of viscosity, which continues for 0.10% CHXD. This could be explained by the bonding of the positively charged groups of CHXD on the

very few negatively charged ones of the collagen existing even at such a pH, which reduces the hydrogen bonding of collagen fibrils. The effects of CHXD are also reflected in the values of viscosities at zero shear rate, η_o, presented in Table 2.

Table 2

Values of η_o, Pa ×s, for the prepared hydrogels

CHXD, %	pH		
	3.8	7.4	
		Noncrosslinked	Crosslinked
0	28.65	5.75	6.67
0.02	29.45	5.11	5.85
0.05	27.60	4.93	5.81
0.10	16.77	4.70	5.58

When the pH is slightly basic, CHXD reduces viscosity both in the absence and presence of GA, excepting the last two highest shear rates (Fig. 2b). The η_o values also decrease with increasing CHXD concentration, and they are higher if the hydrogels are crosslinked with GA. This might be due to the higher binding of the positively charged groups of CHXD on the high number of negatively charged carboxyl ones of collagen at this pH which, besides the reducing of hydrogen bonding of collagen, may result in small domains in which the collagen fibrils are crosslinked by CHXD, producing a slight discontinuity of the hydrogel. At the same time, CHXD seems to reduce collagen crosslinking by GA, due to consumption of GA by the reaction with CHXD amine groups.

The rheograms in Figure 2a, b show pseudoplastic behaviour for all the hydrogels, so they were analysed with the equations describing this type of relation between shear stress and shear rate:

Ostwald-de Vaele:[50]

$$\tau = \mathrm{K}\dot{\gamma}^n \quad (1)$$

and Herschel–Bulkley:[51]

$$\tau = \tau_0 + \mathrm{K}\dot{\gamma}^n \quad (2)$$

where τ is shear stress, $\dot{\gamma}$ – shear rate, τ_o – limiting shear stress, K – consistency index and n – flowing index.

The values of the determination coefficients obtained with the above equations are shown in Table 3.

Table 3

Values of determination coefficients, R^2, obtained with equations (1) and (2) for the prepared hydrogels

CHXD, %	pH					
	3.8		7.4			
			Noncrosslinked		Crosslinked	
	Eq. (1)	Eq. (2)	Eq. (1)	Eq. (2)	Eq. (1)	Eq. (2)
0	0.9996	0.9998	0.9917	0.9977	0.9935	0.9990
0,02	0.9988	0.9989	0.9877	0.9972	0.9903	0.9986
0,05	0.9980	0.9981	0.9889	0.9976	0.9885	0.9968
0,10	0.9977	0.9978	0.9845	0.9997	0.9898	0.9992

As can be seen from the table, R^2 exceed the value 0.997 in the case of Herschel-Bulkley equation and are a bite lower for Ostwald-de Vaele. The consistency indices have the highest values for the acid hydrogels, and the lowest for the noncrosslinked slight basic ones, which decrease with increasing CHXD concentration, while for the flowing indices the values are inverted an increase with CHXD concentration, as expected.

The values of storage, G', and loss, G", modulus is used to differentiate the elastic and viscous contribution to viscoelasticity of polymer solutions or gels and to make distinction between uncrosslinked and crosslinked systems: high G' values indicate the prevalence of elastic properties,

high G" values preponderance of the viscous ones, while their intersection, known as cross-over point, denotes a gel-sol transition.

Figure 3a shows much higher values for G' than for G", which means that the elastic component prevails in the viscoelastic properties of all the gels in acid media. CHXD has as effect a slight reduction of the elastic component as its concentration increases; the same tendency is observed for G", so it is much reduced. This might indicate a very weak interaction between collagen and CHXD in acid medium. The collagen hydrogels having the same composition but pH 7.4 behave differently as can be seen from Figure 3b: the control collagen hydrogel is by far more elastic than those containing CHXD, which produces a decrease of elasticity and an increase of viscosity as its concentration increases. The same thing is also suggested by the higher values of G" and their pronounced increase with frequency compared with the control hydrogel. A particular behaviour presents the hydrogel containing the maximum amount of CHXD: G' decreases with increasing frequency at values higher than about 50 s^{-1} and a cross-over point is obtained at about 120 s^{-1}, which means that the viscous component becomes higher than the elastic one at higher frequencies. This can have the same explanation as in the case of the corresponding rheograms. If the consistency of domains in which the collagen is crosslinked by CHXD is higher, the viscous characteristic may prevail at the macroscopic level.

Crosslinking with GA has as effect a high increase of G' values in the absence and presence of CHXD as expected, excepting the hydrogel containing the maximum amount of CHXD (Fig. 3c). The addition of 0.02% CHXD increases the storage modulus, but the higher concentrations produce a decrease, which is pretty low for 0.05% and dramatic for 0.10%, when it becomes comparable with that in acid medium. At the same time G" values are almost constant, excepting the last sample, for which an increasing with frequency can be seen, as in the case of noncrosslinked hydrogels. If the effect of the first CHXD concentration is difficult to be explained, the decreasing of G' values for the other two concentrations have the same explanations as in the absence of GA.

The dynamic viscosities of hydrogels, calculated as $G''/2\pi\nu$, show that all the hydrogels are highly pseudoplastic, especially at low frequencies, in accordance with rheograms.

The collagen, as any other protein, is a bioactive substrate carrier able to deliver the active molecules in time and space to the target sites, adjust the concentration-duration relationship, enhance stability and avoid side effect of the drugs.[52,53] Hydrogels act as a moist wound dressing material, absorb and retain the wound exudates along with the foreign bodies, such as bacteria, promote fibroblast proliferation by reducing the fluid loss from the wound surface, protect the wound from external noxae necessary for rapid healing, and help in maintaining a micro-climate for biosynthetic reactions on the wound surface necessary for cellular activities.[54]

One of the first advantages of chlorhexidine digluconate, which bind well to negatively charged surfaces such as epithelial cells, is the persistence of its antimicrobial properties. Used topically, it kills both bacteria and contact and has a lingering residual effect, preventing bacteria of regrowing on the application site.[32]

Drug release of CHXD contained into collagen hydrogels depends on their pH and CHXD concentration, as well as – at constant pH – on collagen crosslinking, as the data in Figure 4 show. Thus, the amounts of CHXD released by the acid hydrogels are the lowest irrespective of its initial concentration, the differences increase with CHXD concentration, the crosslinked hydrogels release less CHXD than the corresponding noncrosslinked ones as expected, but the differences are pretty small and increase a bit with CHXD concentrations, releasing curves are almost parallel for a given concentration, the released percentage amounts are higher for all the hydrogels when they contain 0.05% CHXD. Thus, the released CHXD amount can be modulated by CHXD concentration, pH value and croslinking.

EXPERIMENTAL

Preparation of hydrogels. Type I fibrillar collagen hydrogel having a concentration of 1.83% (w/w) and a pH of 2.1 was extracted from calf hide by the currently used technology in INCDTP, Division ICPI-Collagen Department.[55]

The control hydrogels having the collagen concentration 1.1% and pHs 3.8 and 7.4 were obtained by diluting the initial gel with distilled water and 1M sodium hydroxide solution under mechanical stirring (680 rpm, VELP mechanical agitator). The collagen hydrogels containing 0.02; 0.05 and 0.10% CHXD reported to the amount of gel and having the pHs 3.8 and 7.4 were prepared using distilled water, 1M sodium hydroxide solution and the appropriate amounts of 4% CHXD solution under manual stirring due to the high viscosity. CHXD was supplied by FAGRON, Germany, as 20% aqueous solution (w/w) and glutaric aldehyde from Merck, Germany, sodium hydroxide and phosphate buffer

solution, PBS, (pH = 7.4) were of analytical grade. Crosslinked CHXD containing hydrogels having the pH 7.4 and the above mentioned concentrations were obtained at 4°C by adding the appropriate amounts of CHXD solution under magnetic stirring (1800 rpm, FALC magnetic agitator), followed by 0.15% GA reported to the amount of collagen from the gel after 2 min. All the gels were matured at 4°C for 24 h before the different measurements were made.

FT-IR spectra were obtained using an ABB MB3000 MID-IR spectrometer equipped with a DTGS detector and Horizon software. Data were acquired by ATR technique using a PIKE 45 degree ZnSe trough plate with volatile cover Horizontal ATR. The spectra were corrected for ATR effect and than transformed into absorption ones. Each spectrum is the average of 16 scans, with a spectral interval between data points of 4 cm^{-1}.

UV-CD spectra were determined using a Jasco J-810 spectropolarimeter equipped with a square quartz cuvette (Suprasil) with a 0.02 cm path length. The working parameters were: wavelength range 250-190 nm; scanning speed of spectra – 50 nm/min with 0.2 nm pitch and 2s response time, temperature 23°C (room temperature), average number of spectra accumulation – 4, continuous feeding of measure compartment with high purity nitrogen (5.5) to suppress the oxygen absorption. To create a proper baseline for each of the three series of samples the reference spectra of water as well as CHXD and GA were recorded.

Rheological behaviour was determined at 23 ± 0.1°C using a rotational viscometer MultiVisc-Rheometer, Fungilab equipped with standard spindle TR 9 and ThermoHaake P5 ultrathermostat. Shear rates ranging between 0.1 and 20.4 s^{-1} (0.3-60.0 rpm), representative for the application on skin,[56] releasing of drug and examination of time-depending rheological properties (thixotropy or rheopexy), were elected as limits.

Dynamic (oscillatory) rheologycal measurements were made at room temperature (23°C) with a Micro Fourier Transform Rheometer MRF 2100, GBC-Australia using the following working regime: squeezing flow, frequency range 0-140 s^{-1}, 280 discrete frequencies simultaneously analyzed in the range by a step of 0.5 s^{-1}, 30 spectra consequently acquired every tested sample, gap between the upper and bottom plates of the rheometer – 400 μm and displacement amplitude – 0.03 μm (to fall into linear viscoelastic domain). Using very small amplitude of a pseudorandom squeezing motion exerted onto a viscoelastic sample, the force transmitted through it to the force sensor (bottom plate) is continuously monitored that both the displacement and force lead, by a Fourier transform processing, to the storage and loss modulus at every individual frequency steps within the employed frequency range.

***In vitro* release of chlorhexidine dicluconate** was determined in triplicate at 37±0.1°C using a modified USP paddle method ("sandwich" device).[57] Phosphate buffer having the pH 7.4 was used as release medium. Aliquots of 5 mL were witdrawn from the medium at different times and the medium completed with the same volume of fresh pre-heated buffer. CHXD concentration was measured spectrophotometrically, at 255 nm. and the cumulative amounts of CHXD released determined using the calibration curve.

CONCLUSIONS

FT-IR spectra of 1.1% collagen hydrogels show the preservation of the triple helical structure of collagen at pHs 3.8 and 7.4 both in the absence and the presence of 0.02, 0.05 and 0.10% CHXD, as well as for the slight basic ones crosslinked with GA.

CD spectra confirm the absence of collagen denaturation, but suggest an increase of intermolecular association in acid medium and a slight phase separation in slight basic one, visually perceptible, produced by CHXD or GA, while the simultaneous presence of GA and CHXD seems to produce increase of collagen flexibility or a very slight denaturation.

The rheograms indicate pseudoplastic behaviour for all the hydrogels and a decrease in viscosities with increasing CHXD concentration irrespective of pH and crosslinking, which is more reduced at pH 7.4 due to CHXD binding to collagen or/and GA.

Dynamic rheological behaviour reveals a high elastic component of hydrogel viscoelasticities and an insignificant effect of CHXD in acid medium, a reducing of elasticity and a decreasing of viscosity with increasing of CHXD concentration in slight basic medium and a considerable increase of elastic component produced by GA both in the absence and presence of CHXD, excepting the highest CHXD concentration for which the values are similar with those in acid medium.

The amounts of CHXD released from all the hydrogels increase with CHXD concentration and have the lowest values in acid medium. The highest amounts of CHXD are released from the noncrosslinked hydrogels with slight basic pH.

REFERENCES

1. G. Greenstein, *J. Periodontol.,* **2006**, *77*, 565.
2. A. T. Florence and P. U. Jani, *Drugs*, **1996**, *10*, 233.
3. 7. A. K. Dash and G. C. Cudworth, *J. Pharmacol. Toxicol. Methods*, **1998**, *40*, 1.
4. J. D. Meyer, R. F. Falk, R. M. Kelly, J. E. Shively, S. J. Withrow, W. S. Dernell, D. J. Kroll, T. W. Randolph and M. C. Manning, *J. Pharm. Sci.,* **1998**, *87*, 1149.
5. V. C. Martins, G. Goissis, A. C. Ribeiro, E. Marcantonio Jr. and M. R. Bet, *Artif. Organs*, **1998**, *22*, 215.
6. C. S. Kwok, T. A. Horbett and B. D. Ratner, *J. Control. Release,* **1999**, *62*, 301.
7. M. Chvapil, Reconstituted collagen in "Biology of Collagen", A. Viidik and J. Vuust. Eds., Academic Press, London, 1980.
8. K. P. Rao and T. Joseph, Collagen graft copolymers and their biomedical applications, in "Collagen Biotechnology", N. M. Nimni Ed., CRC Press, Boca Raton, 1988.
9. N. A. Peppas, A.G. Mikos, Preparation methods and structure of hydrogels, in "Hydrogels in Medicine and Pharmacy", N. A. Peppas Ed., vol. 1, CRC Press, Boca Raton, 1986.

10. L. Brannon-Peppas, Preparation and characterization of crosslinked hydrophilic networks, in "Absorbent Polymer Technology", L. Brannon-Peppas and R.S. Harland Eds., Elsevier, Amsterdam, 1990.
11. N. A. Peppas, P. Buresa, W. Leobandunga and H. Ichikawa, *Eur. J. Pharm. Biopharm*., **2000**, *50*, 27.
12. K. Pal, A. K. Banthia and D. K. Majumdar, *Designed Monomers and Polymers*, **2009**, *12*, 197.
13. A. S. Hoffman, *Adv. Drug Deliv. Rev*. **2002**, *54*, 3.
14. D. Campoccia, P. Doherty, M. Radice, P. Brun, G. Abatangelo and D.F. Williams, *Biomaterials*, **1998**, *19*, 2101.
15. http://www.pharmainfo.net/devisarvani/hydrogels-novel-drug-delivery-system
16. B. D. Ratner and A. S. Hoffman, Synthetic hydrogels for biomedical applications, in "Hydrogels for Medical and Related Applications", J. D. Andrade Ed., ACS Symposium Series, No. 31, American Chemical Society, Washington, 1976.
17. N. A. Peppas, "Hydrogels in Medicine", CRS Press, Boca Raton, 1986.
18. N. A. Peppas and R. Langer, *Sci*., **1994**, *263*, 1715.
19. K. Park, "Controlled Release: Challenges and Strategies", American Chemical Society, Washington, 1997.
20. N .A. Peppas, *Curr. Opin. Coll. Int. Sci.*, **1997**, *2*, 531.
21. J. B. Park, „Biomaterials: Principles and Application", CRC Press, Boca Raton, 2002.
22. D. G. Wallace and J. Rosenblatt, *Adv. Drug Delivery Rev*., **2003**, *55*, 1631.
23. M. K. Keech, *J. Biophys. Biochem. Cytol*., **1961**, *9*, 193.
24. E. A. Abou Neel, U. Cheema, J. C. Knowles, R. A. Brown and S. N. Nazhat, *Soft Matter*, **2006**, *2*, 986.
25. S. D. Gorham, in "Biomaterials", D. Byrom, Ed., Stockton Press, New York, 1991.
26. M. Chvapil, *J. Biomed. Mater. Res*., **1982**, *16*, 245.
27. H. J. T. Rutten and P. H. A., *Eur. J. Surg*., **1997**, *163*, 31.
28. H. Stemberger and H. D. Bader, *Eur. J. Surg*., **1997**, *163*, 17.
29. R. Sripriya, M. S. Kumar and P. K. Sehgal, *J. Biomed. Mater. Res. Part B: Appl. Biomater*., **2004**, *70B*, 389.
30. Crest, General principles of wound care. Recommendations for practice,, October 1998, www.n-i.nhs.uk/CREST
31. R. Sibbald, G. D. Williamson, H. Orsted, L. K. Campbell, D. Keast and D. Sibbald, *Ostomy/Wound Management,* **2000**, *46*, 14.
32. http://www.ehow.com/about_5675328_chlorhexidine-digluconate_.html
33. G. McDonnel and A. D/ Russell, *Clin. Microbiol. Rev.*, **1999**, 147.
34. J. E. Sebben, *J. Amer. Acad. Dermatol*., **1983**, *9*, 759.
35. S. F. Swaim, K. P. Riddell, D. L. Geiger, T. L. Hatchcock and J. A. McGuire, *J. Amer. Vet. Med. Assoc*., **1991**, *198*, 1941.
36. R. Odore, V. Colombatti Valle and G. Re, *Vet. Res. Commun*, **2000**, *24*, 229.
37. R. J. Pratt, C. M. Pellowe and J. A. Wilson, *J. Hosp. Infect*., **2007**, *65 (Suppl. 1)*, 11.
38. N. P. O'Grady, M. Alexander and E. P. Dellinger, *MMWR Recomm. Rep*., **2002**, *51*, 1.
39. G. McDonnell and A. D. Russell, *Clin. Microbiol. Rev*., **1999**, *12*, 147.
40. B. Madhan, V. Subramanian, J. R. Rao, B. U. Nair and T Ramasami, Int. *J. Biol. Macromol.*, **2005**, *37*, 47.
41. Z. Zhang, G. Li and B. Shi, *J. Soc. Leather Technol.Chem.*, **2006**, *90*, 23.
42. Z. Zhang, W. Liu, D. Li and G. Li, *Biosci. Biotechnol. Biochem.*, **2007**, *71*, 2057.
43. R. Usha and T. Ramasami, *Thermochim. Acta*, **2004**, *409*, 201.
44. Y. Feng, G. Melacini, J. P. Taulane and M. Goodman, *J. Am. Chem. Soc.*, **1996**, *118*, 10351.
45. D. Barth, A. G. Milbradt, C. Renner and L. Moroder, *Chem BioChem.*, **2004**, *5*, 79.
46. R. Gayatri, A. K.Sharma, R. Rajaram and T. Ramasami, *Biochem. Biophys. Res. Commun.*, **2001**, *283*, 229.
47. M. L. Tiffany and S. Krimm, *Biopolymers*, **1969**, ***8***, 347.
48. D. D. Jennes, C. Sprecher and W.C. Johnson, Jr., *Biopolymers*, **1976**, *15*, 513.
49. G. P. Moss, D. R. Gullick, A. D. Woolfson and D. F. McCafferty, *Drug Dev. Ind. Pharm*., **2006**, *32*, 163.
50. M. Leca, "Chimia fizică a macromoleculelor", ed. Universităţii din Bucureşti, Bucureşti, 1998.
51. H. A. Barnes, "A handbook of elementary rheology", Institute of Non−Newtonian Fluid Mechanics, University of Wales, 2000.
52. Z. Ruszczak and W. Friess, *Adv.Drug Deliv. Rev*., **2003**, 55, 1679.
53. H.-J. Wang, L. Di, Q.-S. Ren and J.-Y. Wang, *Mater.*, **2009**, 2, 613.
54. http://www.hartmann-online.de/english/produkte/wundbehandlung/wundforum/default.htm
55. V. Trandafir, G. Popescu, M. G. Albu, H. Iovu and M. Georgescu, "Bioproduse pe baza de colagen", Ars Docendi, Bucureşti, 2007.
56. S. Ramachandran, S. Chen and F. Etzler, *Drug. Dev. Ind. Pharm.* **1999**, *25*, 153.
57. D.S. Jones, M.S. Lawlor and D. Woolfson, *Curr. Drug Deliv*, **2004**, *1*, 17.