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Dedicated to the memory of
Professor Ecaterina Ciorănescu-Nenitzescu (1909–2000)

DRUG DELIVERY SYSTEMS BASED ON COLLAGEN-TANNIC ACID MATRICES

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Tannic acid cross-links collagen both in acid (pH 3.8) and weakly basic (pH 7.4) medium. The cross-linking does not affect the integrity of triple helical structure of collagen as spectral characteristics, enzymatic degradation and release data of tannic acid from collagen matrices demonstrate. Intermolecular bridging produces changes in the amide III IR band of the collagen-tannic acid matrices. The higher the amount of tannic acid in the matrix, the slower the enzymatic degradation process. The release of tannic acid from the collagen-tannic acid matrices is slower for samples obtained at pH 3.8 than for those resulted at pH 7.4 due to the higher amount of intermolecular bonds formed. The kinetic of tannic acid release from each collagen-tannic acid matrix follows the Peppas mechanism, with a value of the exponent of about 0.6.

INTRODUCTION

Biomaterials play an important role in medicine, being widely used especially as medical devices, artificial implants, drug delivery systems or scaffolds for tissue regeneration and engineering.[1]

Collagen porous dressings are frequently applied in the treatment of different types of wounds and burns, such biomaterials having good adherence to wet wounds, absorbing large quantities of tissue exudates, preserving a moist environment and promoting the growth of new granulation tissue and epithelium on the wound.[2,3]

On the other hand tannic acid (TA) is applied as a drug for the treatment of burns and wounds due to its astringent, haemostatic and antibacterial properties[4]. That is why it is expected that the biomaterials obtained by the combination of collagen with tannic acid have enhanced healing benefit properties.

In order to obtain collagen matrices with controlled biodegradability, improved biological stability and good mechanical properties, the collagen gels are generally chemically crosslinked[5]. But biomaterials requiring chemical crosslinking present disadvantages, due to the toxicity produced by the unreacted or partially reacted crosslinking agent.[6] Tannic acid is used as a natural cross-linking agent in the present study, both to control the biodegradability of collagen and as antibacterial for local drug delivery systems. So far, the tannic acid was used as cross-linking agent only in combination with glutaraldehyde to reduce the aldehyde toxicity.[7] Moreover, its antibacterial and astringent properties were confirmed only in ointment and solution.[8] The local release of antimicrobial non-toxic drugs reduces the contamination and improves the wound healing, which stimulates the research for obtaining new performing drug delivery systems.

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The collagen-TA matrices preparation, their IR spectral characteristics, enzymatic degradation and *in vitro* release of TA are presented in this paper. The drug release data were evaluated using kinetic equations, to establish the profile and drug release.

EXPERIMENTAL

Type I fibrillar collagen hydrogel having a concentration of 2.67% (w/w) was extracted from calf hide by the currently used technology in INCDTP, Division ICPI-Collagen Department.[9] Tannic acid was supplied by Sigma-Aldrich, Germany. Sodium hydroxide and phosphate buffer solution, PBS, (pH = 7.4) were of analytical grade.

The concentration of each collagen gel was 1.2%. TA in concentration of 4, 5, 10 and 20% reported to the dry substance was added into collagen gels and the pH adjusted (1 M sodium hydroxide under mechanical stirring) at 3.8 and 7.4, respectively. The gels were cast in glass dishes of 14.7 cm diameter and 1 cm height at 20^0C and freeze-dried (cooling to -40^0C during 1 h, holding for 3 h, heating to -20^0C within 4 h at 0.12 mbar and holding for 20 h, heating to 20^0C within 8 h at the same pressure, then heating within 4 h to 30^0C at 0.01 mbar and hold for 8 h) using the Christ Model Delta 2-24 KD lyophilizer, Germany.

The composition of the matrices obtained by lyophilisation is given in Table 1.

Table 1

Compositions of collagen matrices

Sample codes	Collagen, %	pH	TA*, %	Sample codes	Collagen, %	pH	TA*, %
MA	1.2	3.8	0	MB	1.2	7.4	0
CT-4A	1.2	3.8	4	CT-4B	1.2	7.4	4
CT-5A	1.2	3.8	5	CT-5B	1.2	7.4	5
CT-10A	1.2	3.8	10	CT-10B	1.2	7.4	10
CT-20A	1.2	3.8	20	CT-20B	1.2	7.4	20

*Tannic acid concentration reported to dry substance

Spectral characteristics of the matrices were determined using a FT-IR 6000 spectrophotometer with ATR reflection system MKII Golden Gate Single (Jasco) in IR (MID and NIR) region making a number of 164 spectral acquisitions.

The enzymatic degradation was carried out with type I collagenase obtained from *Clostridium histolyticum* in physiological conditions (PBS having pH 7.4, 37^0C). This proteolytic enzyme was purchased from Sigma-Aldrich, Germany. About 1g matrix was incubated in 0.5 mL collagenase solution at 37^0C during 36 h. The degradation reaction was stopped out by cooling the samples at 0^0C. What resulted was centrifuged 15 min, the supernatant freeze-dried and reported to the initial weight of samples (g/g).

In vitro release of tannic acid was determined in triplicate at 37 ± 5^0C using a modified USP paddle method ("sandwich" device).[10] The stirring paddle was rotated with a speed of 50 rpm. The phosphate buffer having the pH 7.4 was used as release/dissolution medium (200 mL). Aliquots of 5 mL were witdrawn from the medium at different times and the medium was completed with the same volume of fresh pre-heated phosphate buffer. TA concentration was measured spectrophotometrically, at 276 nm. The cumulative amounts of TA released from matrices were determined using the calibration curve shown in Figure 1.

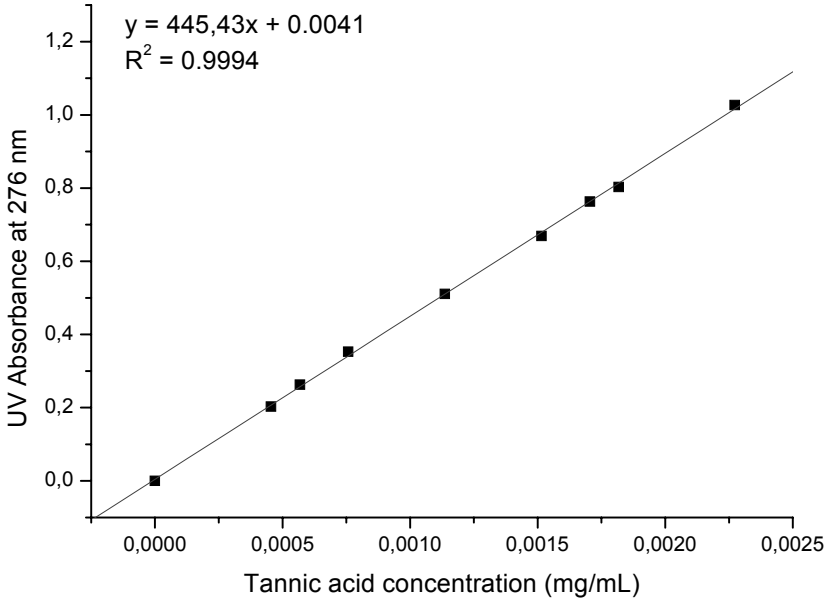


Fig. 1 – Calibration curve of tannic acid in PBS (pH 7.4).

RESULTS AND DISCUSSION

Both collagen and tannic acid contain a lot of reactive groups. TA reacts both with the polar side groups and with those from the main chains of collagen polypeptide forming hydrogen, covalent and electrovalent bonds which stabilize the entire collagen structure.

The infrared spectra of collagen exhibit several features characteristic for the molecular organization of its molecules: amino acids linked together by peptide bonds give rise to infrared active vibration modes amide A and B (about 3330 and 3080 cm^{-1}, respectively) and amide I, II, and III (about 1650, 1550 and 1250 cm^{-1}, respectively).[11]

The amide band A is assigned to the N-H stretching frequency. A free N-H stretching vibration occurs in the range $3400 \div 3440$ cm^{-1}, but when the NH group of a peptide is involved in a hydrogen bond, its position is shifted to lower frequency, usually 3300 cm^{-1}.

Generally, amide I band originates from C=O stretching vibrations coupled with N–H bending ones.

The amide II band arises from the N–H bending vibrations coupled with C–N stretching ones.

The amide III band is represented by N-H bending vibration with significant mixing with the CH_2 wagging vibration from the glycine backbone and proline side chains in collagen.[12]

In the spectrum of the control collagen matrices (M-A and M-B), shown in Figure 2, the five characteristic absorption bands can be observed at 3297, 2931, 1632, 1547 and 1240 cm^{-1}.

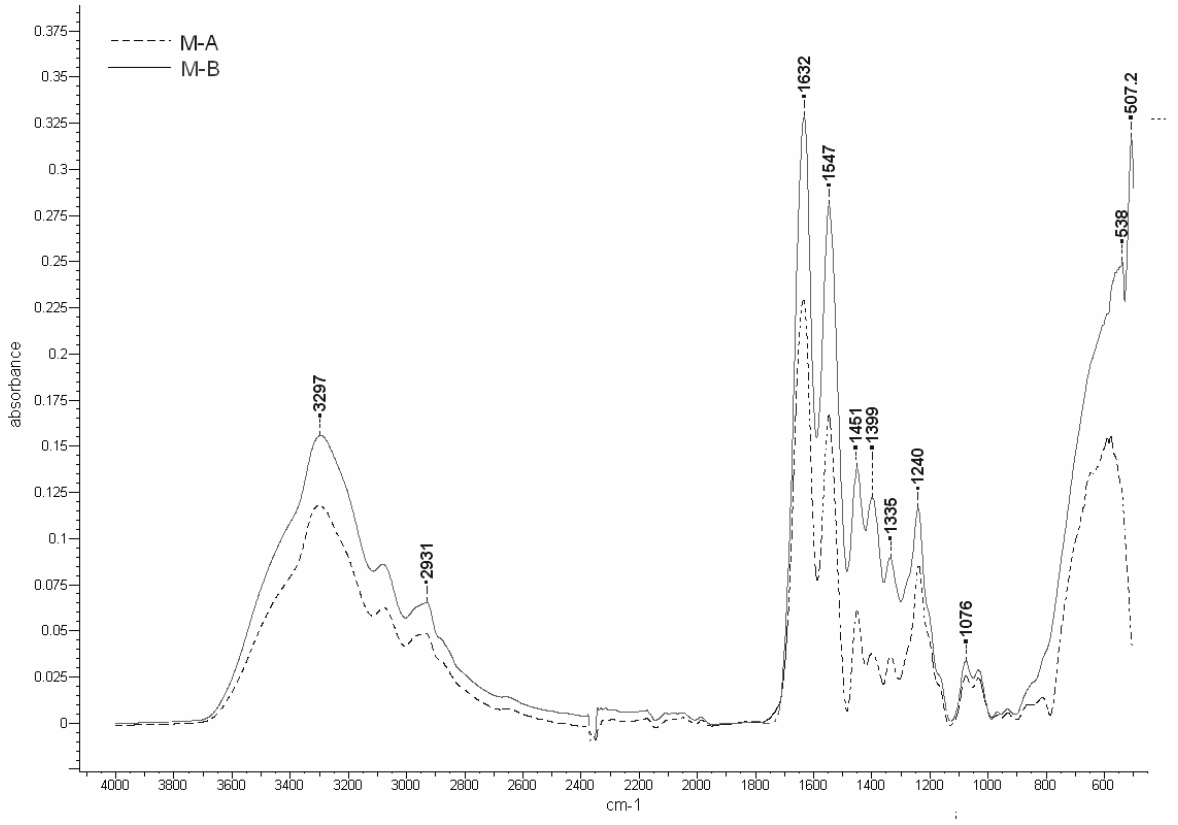


Fig. 2 – FT-IR spectra for collagen matrices.

The spectral characteristics of collagen-TA matrices present modifications due to the intermolecular bonds formed at different pHs and TA concentrations. Thus, the band from 3298 cm^{-1} in M-A control sample shifts to 3296 cm^{-1} for each collagen-TA matrix obtained at pH 3.8 and that from 3297 cm^{-1} in M-B shifts to 3292 cm^{-1} for those obtained at pH 7.4. The higher the amount of TA added in collagen the higher the association by hydrogen bonds, which is more pronounced at pH 7.4.

Some studies demonstrated that the amide I band can be used to determine the secondary structure of proteins. But no differences were observed between the amide I bands of the studied matrices, which proves that the addition of TA preserves the secondary structure.

On the other hand the amide II bands from 1548 and 1547 cm^{-1} for M-A and M-B respectively shift to 1545 cm^{-1} for all the samples containing tannic acid obtained at pH 3.8, as can be seen in Figures 3a, b. In the case of the basic pH the shifting depends on the tannic acid content, having the values 1546; 1546; 1545 and 1544 cm^{-1} for 4; 5; 10 and 20% TA respectively.

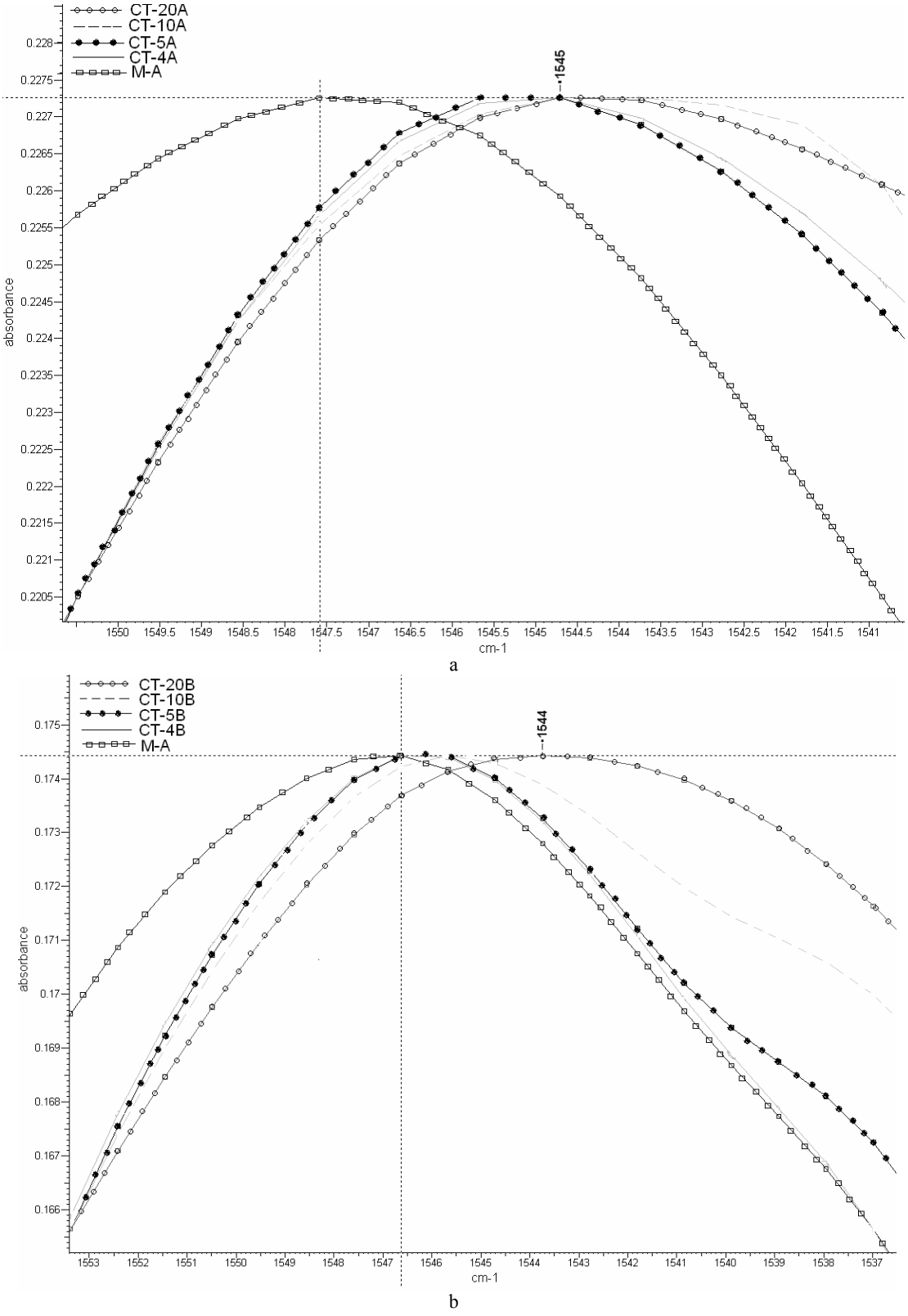


Fig. 3 – Spectral modification of amide II bands of collagen matrices obtained at pH: a –3.8; b – 7.4.

The band from 1451 cm^{-1}, related to the pyrrolidine ring vibrations of proline and hydroxyproline, is shifted to 1449 cm^{-1} for collagen matrices having 4, 5 and 10% TA and to 1448 cm^{-1} for those containing 20% TA regardless of the pH of the gel from which the matrices were obtained.

The band of M-A from 1238 cm^{-1} splits in two bands: 1237 and 1202 cm^{-1} for collagen-TA samples obtained at pH 3.8, while that of M-B from 1239 cm^{-1} into 1238 and 1201 cm^{-1}, but only when the content of TA is 20% (Table 2 and Figure 4). This can be explained by the bonding of collagen by the tannic acid through intermolecular bridging. Early studies on polyphenol/protein (ex. tannic acid/collagen) binding suggest that polyphenols bound preferentially to proline residues.[13]

The difference between amide I and II bands, $\Delta\nu(\nu I - \nu II)$, is an indication on denaturising of the α-helix of collagen molecule: values higher than 100 cm^{-1} show advanced hydrolysis. Differences ranging between 83 and 92 were obtained that increase with TA content, as Table 2 shows, demonstrating pretty low levels of hydrolysis.

Table 2

FT-IR modifications of collagen-TA matrices

Sample codes	νΔ (cm-1)	νIII(cm-1)	Sample codes	νΔ (cm-1)	νIII(cm-1)
MA	83	1238	MB	83	1239
CT-4A	84	1237, 1202	CT-4B	85	1238
CT-5A	85	1237, 1202	CT-5B	85	1238
CT-10A	87	1236, 1201	CT-10B	89	1236
CT-20A	88	1235, 1201	CT-20B	92	1233, 1201

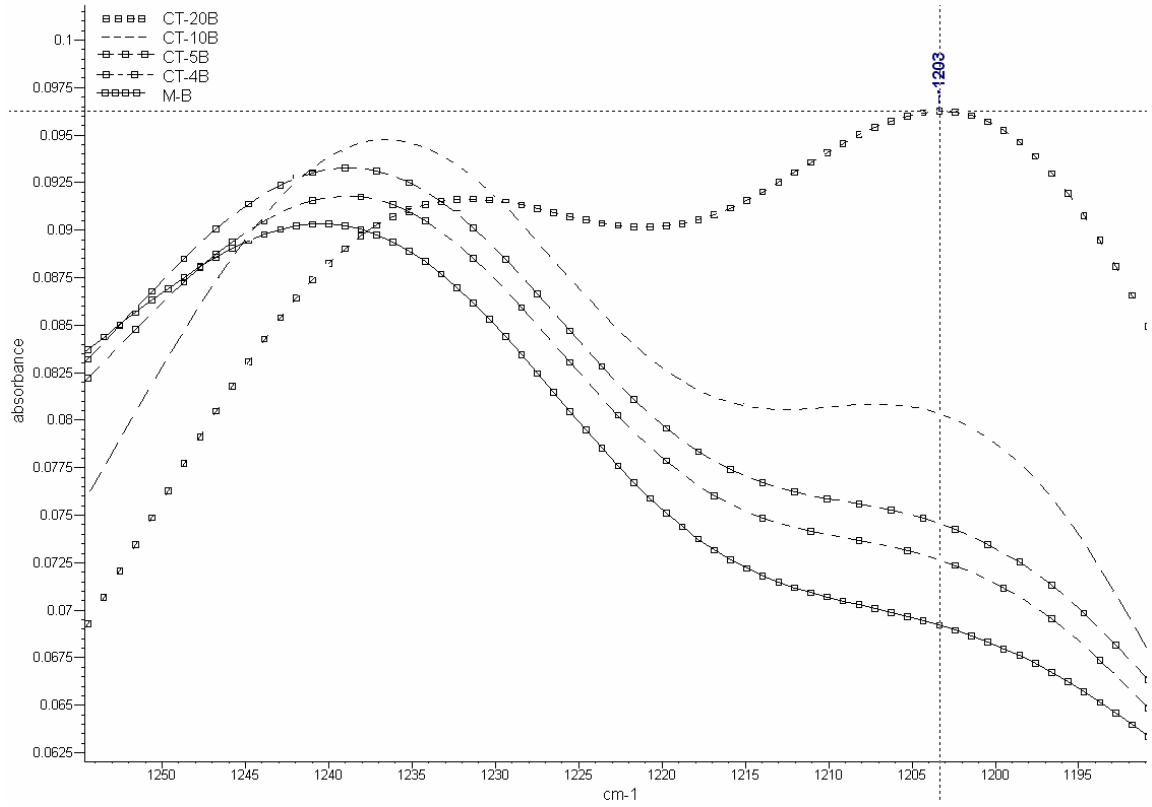


Fig. 4 – FT-IR spectra of the specified collagen matrices.

The preservation of the helical structure of collagen in the collagen-TA matrices was confirmed by the IR absorption ratio of amide III band (1240 cm^{-1}) and that from 1450 cm^{-1} (A_{III}/A_{1450}), which is very close to unity for the all matrices.

The FT-IR investigations reveal that more hydrogen bonds are formed in matrices at pH 7.4 compared with those at pH 3.8. Thus, it can be concluded that crosslinking by hydrogen bonds prevails in weakly basic medium.

The degree of crosslinking of collagen can also be evaluated by biodegradation. Collagenase can cleave only the bonds from the main chains of collagen but not the crosslinks. That is why the digestion resistance to collagenase can reveal indirectly the crosslinking degree of collagen. The resistance to collagenase digestion was studied for collagen-tannic acid matrices and compared with the control matrices obtained at both pHs (Figure 5).

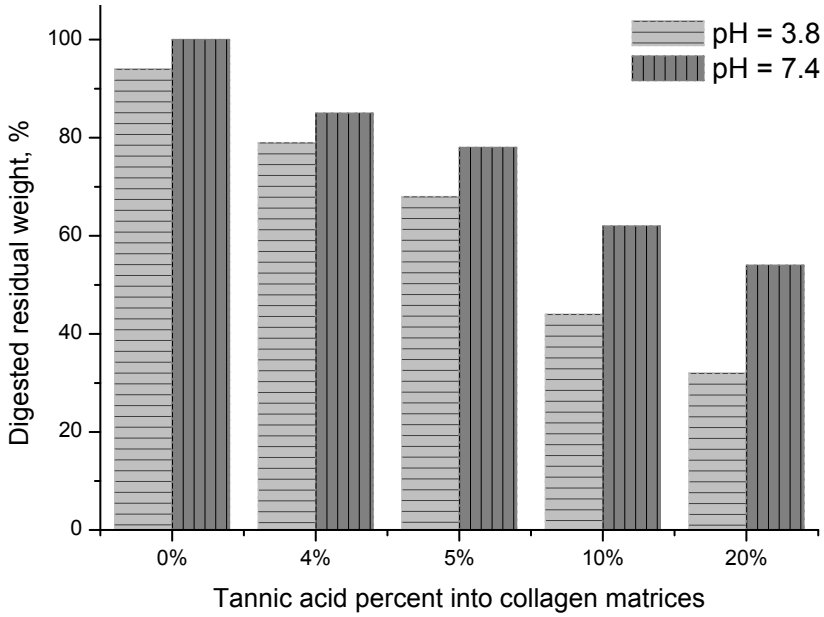


Fig. 5 – Digestion resistance to collagense as a function of TA concentration and pH.

After collagense digestion, the control samples show degradation ranging between 94 and 100%, while those containing tannic acid resulted in 32-85% degradation that is the samples containing TA (the crosslinked ones) are more resistant to collagenase. The higher the TA concentration the greater the digestion resistance.

Analyzing the release of tannic acid from collagen matrices, differences can be observed, as shown in Figure 6a, b: the amount of TA released depends on the pH of the gel from which the matrix was obtained.

As expected, the increasing of TA amount in collagen matrices produces the increasing of percentage of TA released. However there are differences between the matrices containing the same amount of TA but having different pHs: the matrices obtained at pH 3.8 release lower amounts of TA compared with the ones obtained at pH 7.4. Thus, at the end of the 240 min the percentage of TA released from CT-4B is higher with 21.9% than that released from CT-4A, with 18.92% for CT-5B than for CT-5A, with 15.23% for CT-10B than for CT-10A and with 30.91% for CT-20B than for CT-20A. In the case of matrices containing 20% TA the percent of TA released is two times higher than in the case of matrices with 10% TA, which demonstrate that more TA is used for crosslinking at pH 7.4.

These results are in close correlation with the spectral characteristics and enzymatic degradation.

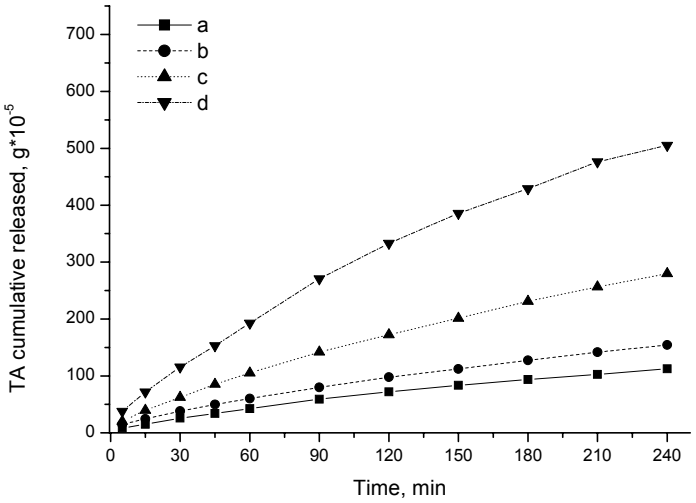
To study the release kinetics, the data obtained from *in vitro* drug release studies, presented in Figure 6, were fitted with the Peppas equation[14]:

$$\frac{m_t}{m_\infty} = k \cdot t^n \qquad (1)$$

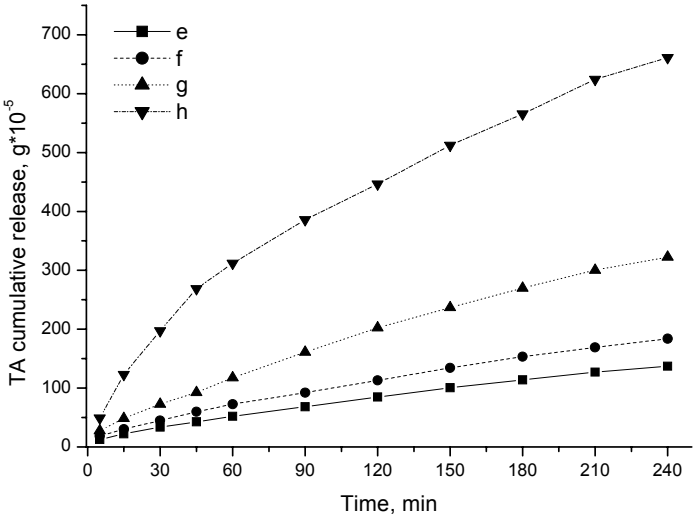
where m_t is the amount of drug released at time t, m_∞ – total drug content in the designed collagen hydrogel, m_t/m_∞ – fractional release of the drug, k – kinetic constant and n – release exponent, indicating the mechanism of drug release.

When the release mechanism is not known or more than one type of release phenomenon (diffusion, swelling or erosion controlled) is involved the Peppas model is used to analyze the drug release.

The value of the release exponent n was calculated as the slope of the straight lines fitting the released data using the least-squares methods. The obtained values are presented in table 3.



a



b

Fig. 6 – *In vitro* release of tannic acid from collagen matrices at 37^0C:
a – CT-4A; b – CT-5A; c – CT-10A; d – CT-20A; e – CT-4B; f – CT-5B; g – CT-10B; h – CT-20B.

Table 3

Release exponent and regression coefficient (r^2) of collagen matrices

Sample codes	Release exponent, *n*	Regression coefficient, r^2	Sample codes	Release exponent, *n*	Regression coefficient, r^2
CT-4A	0.6929	0.9961	CT-4B	0.6330	0.9952
CT-5A	0.6260	0.9948	CT-5B	0.6147	0.9931
CT-10A	0.6912	0.9988	CT-10B	0.6617	0.9928
CT-20A	0.6951	0.9974	CT-20B	0.6521	0.9903

The *n* values higher than 0.5 obtained for all the studied matrices indicate a kinetic release mechanism that tend to be anomalous. The release data analyzed on the basis of Peppas equation give high correlation coefficients, as can be seen from Table 3, which demonstrates that tannic acid is released from all the matrices by the Peppas mechanism (the diffusion rate of the tannic acid is equal with the relaxation rate of polymer).

CONCLUSIONS

The collagen matrices containing or not tannic acid obtained from gels having the pHs 3.8 and 7.4 preserve the integrity of triple helical structure of the native collagen, as the ratios A_{III}/A_{1450} having values higher than unity show. Modifications arise in amide III bands due to the weak intermolecular bridging of collagen by tannic acid. These modifica-

tions are considerable for CT-4A, CT-5A, CT-10A, CT-20A and CT-20B. More hydrogen bonds form in the samples obtained at pH 7.4 than in those obtained at 3.8.

The samples containing TA (crosslinked) are more resistant to collagenase than the uncrosslinked ones. Thus, the control samples show between 94 and 100% degradation, while the matrices containing tannic acid result in 32-85% degradation. The higher the amount of tannic acid in the matrices, the slower the biodegradation process.

The collagen-tannic acid matrices release TA more slowly from samples obtained at pH 3.8 due to the higher number of crosslinks formed.

The kinetic of TA release of all the studied collagen-tannic acid matrices follows the Peppas equation, with a release exponent ranging between about 0.61 and 0.69.

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