INFLUENCE OF THE FORMULATION AND PREPARATION TECHNIQUE ON THE FLUFENAMIC ACID RELEASE FROM DIFFERENT COLLAGENIC SUPPORTS DESIGNED FOR WOUND HEALING

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Drug delivery systems with controlled release are one of the top applications for human health, a new domain in material science with biomedical application. The aim of this study was to obtain and characterize some drug delivery systems based on collagen, dextran and flufenamic acid (matrices, membranes and fibers) for wound healing. The samples were analyzed by FT-IR and *in vitro* release of flufenamic acid. From the FT-IR spectra it can be seen that collagen macromolecule keeps its triple helix structure. The percentage of drug delivered is influenced by the degree of cross linking, the presence of dextran in the designed formulations and the collagen support preparation technique.

Keywords: collagen, dextran, flufenamic acid

INTRODUCTION

Drug delivery systems with controlled release are one of the top applications for human health, a new domain in material science with biomedical application (Vallet-Regi *et al.*, 2007). Natural polymers have been increasingly studied for applications in health care due to their biocompatibility, biodegradability and non-toxicity. Collagen is a potentially useful biomaterial, since it is the major constituent of the connective tissue and allows the controlled drug release within targeted tissues (Helary *et al.*, 2006). Dextran is a non-toxic, hydrophilic homopolysaccharide which can be synthesized from sucrose with dextranase or from maltodextrins with dextrinase (Bachelder *et al.*, 2018). Dextran is commonly used to decrease vascular thrombosis (Booi *et al.*, 2011), to reduce inflammatory response (Gombocz *et al.*, 2017) and to prevent ischemia – reperfusion injury in organ transplantation (Banz *et al.*, 2019), in which dextran acts as a mild reactive oxygen species scavenger and reduces excess platelet activation. Due to its properties dextran has been extensively explored in biomedical and pharmaceutical applications (Robless *et al.*, 2014). Flufenamic acid is a non-steroidal anti-inflammatory drug (NSAID) and a member of the anthranilic acid derivatives (fenamates) (Psomas *et al.*, 2013).

The aim of this study was to obtain and characterize drug delivery systems based on collagen, dextran and flufenamic acid (matrices, membranes and fibers) for wound healing.

MATERIALS AND METHODS

Materials

The type I fibrillar collagen gel having a concentration of 2.54% (w/v) was extracted from calf hide using a technology developed in our institute, Collagen Department (Albu, 2011). Dextran from *Leuconostoc* spp. (Mw 15,000–25,000) was purchased from Fluka (USA), flufenamic acid was purchased from ICN Biochemicals (USA), and Glutaraldehyde (GA) from Merck (Germany). Sodium hydroxide was of analytical grade.

Preparation of the Collagen Composites

Control gels with a 1.2% collagen concentration and a pH value of 7.4 were obtained from the original collagen gel under continuous stirring with distilled water and 1M NaOH solution. 0.5% flufenamic acid (FA) previously dissolved in 1 M NaOH solution along with the dextran in the amounts indicated in Table 1 was added over the 1.2% collagen gel. Some of the hydrogels were cross-linked with glutaraldehyde and left for 24 hours at 4°C for maturation / cross-linking. The amounts of dextran, flufenamic acid and glutaraldehyde were reported to collagen dry substance. Collagen gels with flufenamic acid were obtained with various amounts of dextran, un- and cross-linked, and coded G1-G4.

Table 1. Composition of collagen gels

Sample	Collagen (g%)	Dextran* (g%)	Glutaraldehyde* GA (g%)
G1	1.2	0	0
G2	1.2	0	0.006
G3	1.2	0.6	0.006
G4	1.2	1.2	0.006

*The amounts of dextran and GA were reported to collagen dry substance

The spongy matrices corresponding to previously obtained collagen gels were obtained by lyophilization using the Delta LSC 2-24 Martin Christ, Germany. The collagen matrices were coded according to the composition of the gels from which they were obtained, as follows: M1, M2, M3, respectively M4.

Collagen fibers were prepared from the same composition of flufenamic acid gels (Table 1) but, unlike matrices, they were lyophilized at the pH corresponding to the isoelectric point. They were coded as follows: F1, F2, F3 and F4, respectively.

Membranes were obtained by free drying at 25°C of the gels shown in Table 1 and coded Mb1, Mb2, Mb3, Mb4.

The following coding is also used: Series I consisting of: M1, F1, Mb1; Series II: M2, F2, Mb2; Series III: M3, F3, Mb3; Series IV: M4, F4, Mb4.

FT-IR Analysis

FT-IR spectral measurements were performed with a spectrophotometer Jasco FT/IR 4200. All the spectra were recorded at the following parameters: spectral range 4000-600 cm^{-1} , resolution 4 cm^{-1} with 30 acquisitions per each sample.

In vitro Release of Flufenamic Acid from Collagen Composites

The release studies were conducted using a “sandwich” device adapted to a paddle dissolution equipment, as detailed in our previous work (Ghica *et al.*, 2017). The amount of flufenamic acid released at different time intervals was evaluated by UV spectroscopy at $\lambda=288$ nm.

RESULTS AND DISCUSSION

The samples were analyzed by FT-IR and *in vitro* release of flufenamic acid.

Figures 1-3 show the overlapped spectra for M1-M4 matrices, Mb1-Mb4 membranes, respectively for the matrix, fiber and collagen membrane derived from gel G1, corresponding to Series I.

Figure 1. FT-IR spectra for M1, M2, M3 and M4 matrices

Figure 2. FT-IR spectra for membranes Mb1, Mb2, Mb3 and Mb4

Figure 3. FT-IR spectra for matrix, membrane and collagen fiber Series I - M1, Mb1 and F1

From the spectra shown in Figures 1-3 it can be seen that the collagen macromolecule keeps its triple helix structure. Band changes are due to the addition of dextran, which also influences additional cross linking. The composition of the matrices, membranes and fibers being the same, Figure 3 shows that the drying or lyophilization conditioning process does not influence the movement of the bands, and differences can only be observed at their intensities.

The cumulative kinetic profiles corresponding to the flufenamic acid percentage released from the supports corresponding to each series are represented in Figures 4-7.

Figure 4. The cumulative kinetic profiles corresponding to the release of flufenamic acid from the Series I collagen supports (M1, F1, Mb1)

Figure 5. The cumulative kinetic profiles corresponding to the release of flufenamic acid from the Series II collagen supports (M2, F2, Mb2)

Figure 6. The cumulative kinetic profiles corresponding to the release of flufenamic acid from the Series III collagen supports (M3, F3, Mb3)

Figure 7. The cumulative kinetic profiles corresponding to the release of flufenamic acid from the Series IV collagen supports (M4, F4, Mb4)

Figures 4-7 and Table 2 show the influence of the collagen supports preparation process on the percentage of flufenamic acid released during 10 hours of kinetic experiment. Thus for Series I, the percentage of FA released varies as follows: $F1 > Mb1 > M1$, an increase of 11.85% for F1 compared to Mb1, respectively an increase of 16.04% compared to M1 being recorded. For Series II, the order of variation is the same as for Series I, the percentage of AF released being 11.35% lower for M2 in comparison with F2, and 4.22% in comparison with Mb2, respectively. For Series III, the highest % of AF released was obtained for M3, followed by F3 and Mb3. There is an increase of 4.07 % for M3 compared to F3 and 10.63% compared to Mb3, respectively. For IV series, the lowest % of AF was released from F4 support, 8.28% lower than from M4 and 4.00% respectively compared to Mb4 (Table 2). It is also noted the influence of GA

cross linking on the percentage of AF released (Series I and II). Obviously, for Series I and II, cross linking with GA results in a decrease in the percentage of drug released after 10 hours from experiment beginning of 2.44% for M2, 5.16% for F2 and 1.81 % for Mb2, compared to M1, F1 and Mb1 respectively (Table 2). The amount of dextran present in the series II ÷ IV has a different influence on the % of AF released. Thus, for matrices, the highest % of AF is obtained for M4, about 1.08 times higher than M3, and 1.14 times than M2. For fibers, the slowest release is recorded for F3, 10.12% less compared to F2 and 2.97% less compared to F4. For membranes, the highest percentage of AF is released by Mb4, about 1.04 times more than Mb2 and 1.14 times more than Mb3 respectively (Table 2). It is noted that in the first hour flufenamic acid release was quite rapid, the percentage of drug released varying between 17.45% (F3 sample) and 43.53% (Mb1 sample). After this rapid release period, the so-called “burst release” stage, it is noted that the non-steroidal anti-inflammatory agent is slowly released during the next 9 hours of the experiment.

The kinetic profiles described above are pursued in the healing of skin lesions for which an important element is the local inflammation. The “burst release” effect, after support contact with the skin lesion and its wetting by the wound exudates, provides a rapid reduction in inflammation and pain associated with lesions of various etiologies. On the other hand, the gradual drug release provides a local anti-inflammatory and analgesic effect over a longer period of time necessary for the wound healing.

In order to establish the drug release mechanism from the collagen supports, the Power law model (eq.1) and its particular case Higuchi ($n = 0.5$) were applied:

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

where, m_t/m_∞ represents the fraction of drug released at time t , k - the kinetic constant, n - the release exponent characteristic for the drug release mechanism.

The highest values of the determination coefficients are obtained for the Power law model (between 0.9577 and 0.9941), showing that this model best describes the mechanism of the drug release from the designed collagen supports. The values of the release exponent ranged from 0.31 to 0.41 for matrices and membranes, and between 0.58 and 0.64 for fibers, indicating a non-Fickian drug transport mechanism from the designed formulations. The values of the parameters specific to Power law model, as well as the determination coefficients specific to this model and for the Higuchi model, are shown in Table 2.

Table 2. Parameter values specific to the Power law model; the determination coefficients specific to the Power law model and the Higuchi model; percentage of drug released

Flufenamic acid support	Higuchi model	Power law model	Release exponent	Kinetic constant (1/minn)	Released percentage (%)
M1	0.9585	0.9788	0.38	0.072	78.63
F1	0.9910	0.9937	0.58	0.023	93.80
Mb1	0.9036	0.9610	0.31	0.116	81.57
M2	0.9698	0.9803	0.41	0.056	76.51
F2	0.9886	0.9924	0.62	0.017	86.53
Mb2	0.9244	0.9757	0.32	0.110	80.09
M3	0.9674	0.9856	0.38	0.074	81.09

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Flufenamic acid support	Higuchi model	Power law model	Release exponent	Kinetic constant (1/minn)	Released percentage (%)
F3	0.9857	0.9933	0.64	0.013	77.77
Mb3	0.9367	0.9698	0.35	0.082	73.30
M4	0.9206	0.9577	0.36	0.099	87.28
F4	0.9866	0.9913	0.62	0.015	80.15
Mb4	0.9286	0.9659	0.34	0.099	83.49

CONCLUSIONS

The percentage of drug released is influenced by the degree of cross-linking, the presence of dextran in the designed formulations and the preparation technique of the collagen supports. Thus, the percentage of flufenamic acid released from collagen drug delivery systems varies in the order: for Series I and Series II - fiber, membrane and matrix, for Series III - matrix, fiber and membrane and for Series IV - matrix, membrane and fiber. For collagen drug delivery systems obtained by the same preparation technique, the percentage of flufenamic acid released varies in the order: (i) for matrices: M4, M3, M1, M2; (ii) for fibers: F1, F2, F4, F3; (iii) for membranes: Mb4, Mb1, Mb2, Mb3.

The flufenamic acid release characteristics show the potential application of the designed collagen supports in wound lesions with different etiologies.

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