DICLOFENAC SPONGIOUS MATRICES BASED ON COLLAGEN AND ALGINATE FOR RELIEVING INJURY PAINS

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The sport injuries become more and more frequent so there is a need to discover new solutions for increasing the quality of life for people affected by such wounds. The aim of this study was to obtain a novel NSAID drug delivery system, topical diclofenac spongy matrices based on collagen and alginate for the treatment of injuries and relieving of pain. The samples were analyzed by flow analysis and after their freeze-drying were analyzed by FT-IR and in vitro release of sodium diclofenac. The results obtained from analyses confirmed that diclofenac spongy matrices based on collagen and alginate exhibit proper characteristics for the treatment of injuries and relieving of pain.

Keywords: diclofenac, collagen, alginate.

INTRODUCTION

The sport injuries have become more and more frequent so there is a need to discover new solutions for increasing the life quality of people affected by such wounds (Barbour *et al.*, 2017).

The most widely recommended and used drug treatment for the pain associated with these sporting injuries are orally administered drugs, such as nonsteroidal anti-inflammatory drugs (NSAIDs) (Ong *et al.*, 2007). Diclofenac is a member of the aryl alcanoic group of NSAIDs, synthesized by Ciba-Geigy in the 1960's. Development of pharmaceutical forms of diclofenac for application on the skin subsequently followed, and a variety of commercial preparations are currently available (Goh *et al.*, 2014). The chemical name of diclofenac is (2-(2-[(2,6-dichlorophenyl) amino]phenyl) acetic acid) and it contains a phenylacetic acid group, a secondary amino group and a phenyl ring, with chlorine atoms at the two ortho positions. One recent study reported that the current use of any oral NSAID almost doubles the risk for endoscopically confirmed ulcer (Kok *et al.*, 2018). Topically applied NSAIDs could act directly within the area of injury and reduce the inflammatory reaction without unwanted systemic activity (Mason *et al.*, 2004). In the last few decades, wound dressings containing drugs gained popularity as an effective analgesic modality for injuries, owing this to advantages such as ease of application and reduced risk of dose dumping compared with cream and gel

forms of topical delivery. These advantages also lead to better patient compliance (Hsieh *et al.*, 2010).

Collagen, the most abundant protein in the human body, is an excellent material which can be used in applications such as topical drug delivery systems due to its high biocompatibility properties (Ramshaw *et al.*, 2014; Yu *et al.*, 2014). Another material suitable in these applications is alginate, extracted from brown seaweed. Alginate has a unique capability of high absorbency of water, biocompatibility, low toxicity, relatively low cost, and mild gelation by addition of divalent cations such as Ca^{2+} (Chiu, 2008).

The aim of this study was to obtain a novel NSAID drug delivery system, topical diclofenac spongiuous matrices based on collagen and alginate for the treatment of injuries and relieving of pain.

MATERIALS AND METHODS

Materials

The type I fibrillar collagen gel having a concentration of 2.54% (w/v) was extracted from calf hide using technology developed in our institute, Collagen Department (Albu, 2011). Alginic acid sodium salt from brown algae (Alg), Diclofenac Sodium (DFNa) were purchased from Sigma-Aldrich (Germany), and Glutaraldehyde (GA) from Merck (Germany).

Preparation of Collagen Hydrogels

The collagen gel with an initial concentration of 2.54% and acid pH was adjusted using 1M sodium hydroxide at pH 7.4 for better biocompatibility. The final concentration of the collagen gel used was 1% (w/v). Alginate and diclofenac were added in the collagen gels, in different proportions, according to the compositions shown in Table 1, and then the composite gels were cross-linked with 0.025% glutaraldehyde.

Table 1. Composition of collagen gels

code	COLL, %	DFNa, %	ALG,%	GA,%
CDA1	1	0.1	1.5	0.025
CDA2	1	0.1	2	0.025
CDA3	1	0.2	1.5	0.025
CDA4	1	0.2	2	0.025
CDA5	1	-	1.5	0.025
CDA6	1	-	2	0.025

The collagen gels were freeze-dried using Delta 2-24 LSC (Martin Christ, Germany) and spongiuous matrices were obtained.

Methods

Flow Analysis

The rheological behaviour of the tested hydrogels was conducted with a rotational viscometer Multi-visc Rheometer-Fungilab equipped with standard spindle TR 9 and

ultrathermostat ThermoHaake P5. The rheological experiments were performed at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$.

FTIR-ATR Analysis

FT-IR spectral measurements were recorded by 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 Sodium Diclofenac

The *in vitro* kinetics release of DFNa from the designed spongy matrices was determined using a “sandwich” device adapted to a paddle dissolution equipment, as described in our previous studies (Ghica *et al.*, 2017). The amount of released DFNa at different period of time was spectrophotometrically assessed at $\lambda=276 \text{ nm}$.

RESULTS AND DISCUSSION

The samples from Table 1 were analyzed by flow analysis and after their freeze-drying were analyzed by FT-IR and *in vitro* release of sodium diclofenac.

The results of the rheological experiments carried at 37°C for the control gels (without DFNa) led to the cumulative rheograms plotted as viscosity versus shear rate and illustrated in Figure 1a.

Figure 1. a) Plots of viscosity as a function of shear stress for the collagen hydrogels with sodium alginate evaluated at 37°C ; b) Cumulative release profiles of sodium diclofenac from spongy collagen matrices as a function of time

Figure 1a shows the influence of the composition on the viscosity of the gels tested at 37°C . Thus, the viscosity increases when increasing sodium alginate concentration.

For the graphs shown in Figure 1a it results that the viscosity of the tested systems decreases with increasing shear rate, indicating a non-Newtonian pseudoplastic character. Pseudoplastic behaviour is a desirable requirement for topical systems both in terms of conditioning and of skin application, forming a continuous film at the application site. For the quantification of the pseudoplastic behaviour of the tested

control gels, the Power law model expressing the relationship between viscosity and shear rate is used (eq. 1).

$$\eta = m \cdot \dot{\gamma}^{-n} \quad (1)$$

where m and n are parameters correlated with the formulations of the hydrogels and are determined by linearization of eq. (1) by the double logarithm method. It can be appreciated that the value of m corresponds to the viscosity obtained for the shear rate value of $1 \cdot s^{-1}$ (Ghica *et al.*, 2016; Kaya *et al.*, 2014; Albu *et al.*, 2012). The values of the previously described parameters m and n as well as the R^2 - determination coefficient of the model are listed in Table 2 for the formulations tested at $37^\circ C$.

Table 2. The values of the m and n parameters and the determination coefficient specific to the Power law model applied to control gels evaluated at $37^\circ C$

Gel	m	n	R^2
Gel CDA5	6.953	0.742	0.9998
Gel CDA6	9.523	0.741	0.9997

The values obtained for the determination coefficient R^2 are higher than 0.99, indicating that the experimental data well fitted this model. From Table 2 it can be seen that the rheological parameter m obtained for the control gel with 0.20% sodium alginate recorded a higher value of about 1.37 times than the control gel with 0.15% sodium alginate.

The composite gels were freeze-dried and spongy matrices were obtained. Their results of the FT-IR spectrum are presented in Figure 2.

Figure 2. FT-IR spectra of spongy matrices

From the FT-IR spectrum the typical bands from collagen can be observed: amide A (3297 cm^{-1}), B (3097 cm^{-1}), I (1631 cm^{-1}), II (1550 cm^{-1}) and III (1442 cm^{-1}) (Albu, 2011). Corresponding to alginate, a peak can be observed toward 2931 cm^{-1} , assigned to the C–H antisymmetric stretching vibration and a peak at 1403 cm^{-1} , assigned to the C=O. The peak assigned to stretching vibration modes (3425 cm^{-1}), specific to alginate, doesn't exist, maybe because of the reaction between carboxyl group of alginate and NH of collagen (Fan *et al.*, 2013). Being in a small amount, diclofenac doesn't influence the FT-IR spectrum of the spongy matrices.

The influence of the spongy matrices composition on sodium diclofenac release was analyzed by comparing the kinetic profiles. Thus, the cumulative percentages of the *in vitro* drug release recorded as a function of time, are shown in Figure 1b.

The cumulative diclofenac percentage varies between 71.78% (CDA4 sample with maximum DFNa and maximum sodium alginate) and 92.08% (CDA3 sample with maximum DFNa and minimal sodium alginate) (Table 3). In the first 60 minutes, the release of the sodium diclofenac was rapid (a “burst release” effect), followed by a step in which the anti-inflammatory drug is slowly delivered during the other 7 hours of the experiment. The kinetic profiles described above are pursued in the healing of skin lesions for which an important element is local inflammation. The “burst release” effect, after contacting the skin with the skin lesion and wetting it by wound exudation, provides a rapid reduction in inflammation associated with skin lesions. On the other hand, gradual release of the drug provides a local anti-inflammatory effect over a longer period of time necessary to cure a skin lesion (Ghica et al., 2017).

It is noticed that for the matrices formulated with the same amount of DFNa, the released drug percentage is dependent on the percentage of the biopolymer - sodium alginate. Thus, an amount of 0.2% sodium alginate present in the formulation results in a 13% decrease of released drug percentage for the sample with 0.1% DFNa, and 28% decrease for the sample with 0.2% DFNa. For matrices with a lower sodium alginate content (CDA1 and CDA3), a higher percentage of drug is released when DFNa is 0.2%, while for matrices with a content of 0.2% sodium alginate (CDA2 and CDA4), the sample with a lower drug content releases more compared to the similar sample with DFNa at maximum level.

The experimental kinetic data were analyzed with the Power law model (eq. 2) and its particular case, Higuchi model (n=0.5):

$$\frac{m_t}{m_\infty} = k \cdot t^n \quad (2)$$

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.

Correlation coefficient values as well as kinetic parameters specific to the Power law model are shown in Table 3.

Table 3. Drug released percentage; kinetic parameters and correlation coefficients for Power law model; correlation coefficient for Higuchi model

Spongy matrices with DFNa	DFNa Released percentage (%)	Kinetic constant (1/minn)	Release exponent	Correlation coefficient (Power law mode)	Correlation coefficient (Higuchi model)
CDA1	87.96	0.080	0.40	0.9862	0.9779
CDA2	77.51	0.068	0.41	0.9843	0.9768
CDA3	92.08	0.119	0.35	0.9782	0.9563
CDA4	71.78	0.064	0.40	0.9836	0.9755

The kinetic data best fitted the Power law model, with correlation coefficients ranging from 0.9782 to 0.9862, higher values than those recorded for the Higuchi model (between 0.9563 and 0.9779, Table 3). Thus, the release exponent between 0.35 and 0.41 indicates a non-Fickian drug transport mechanism.

CONCLUSIONS

The tested gels showed non-Newtonian pseudoplastic flow with shear thinning at a working temperature of 37°C, this being a mandatory requirement for topical systems in terms of conditioning as well as under the appearance of skin and the formation of a continuous film at the application site. Spongy matrices based on collagen, alginate and diclofenac with glutaraldehyde were prepared by lyophilization. The FT-IR analysis showed that collagen kept its structure in all the samples. The recorded kinetic profiles show that the designed spongy matrices could be promising supports for drug delivery systems with potential use in the treatment of injuries and relieving pain.

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REFERENCES

- Albu, M.G. (2011), *Collagen Gels and Matrices for Biomedical Applications*, Lambert Academic Publishing, Saarbrücken.
- Albu, M.G. *et al.* (2012), "Rheological behavior of some collagen extracts", *Leather and Footwear Journal*, 12(3), 193-200.
- Barbour, K.E. *et al.* (2017), "Vital Signs: Prevalence of Doctor Diagnosed Arthritis and Arthritis-Attributable Activity Limitation - United States, 2013-2015", *Morbidity and Mortality Weekly Report*, 66(9), 246-253, <https://doi.org/10.15585/mmwr.mm6609e1>.
- Chiu, C.T. *et al.* (2008), "Development of two alginate-based wound dressings", *Journal of Materials Science: Materials in Medicine*, 19(6), 2503-2513, <https://doi.org/10.1007/s10856-008-3389-2>.
- Fan, L. *et al.* (2013), "Preparation and characterization of sodium alginate modified with collagen peptides", *Carbohydrate Polymers*, 93, 380-385, <https://doi.org/10.1016/j.carbpol.2013.01.029>.
- Ghica, M.V. *et al.* (2017), "Development, Optimization and In Vitro/In Vivo Characterization of Collagen-Dextran Spongy Wound Dressings Loaded with Flufenamic Acid", *Molecules*, 22(9), 1552, <https://doi.org/10.3390/molecules22091552>.
- Ghica, M.V. *et al.* (2016), "Flow and thixotropic parameters for rheological characterization of hydrogels", *Molecules*, 21(6), E 786, <https://doi.org/10.3390/molecules21060786>.
- Goh, C.F., and Lane, M.E. (2014), "Formulation of diclofenac for dermal delivery", *International Journal of Pharmaceutics*, 473(1-2), 607-616, <https://doi.org/10.1016/j.ijpharm.2014.07.052>.
- Hsies, L.F. *et al.* (2010), "Efficacy and Side Effects of Diclofenac Patch in Treatment of Patients with Myofascial Pain Syndrome of the Upper Trapezius", *Journal of Pain and Symptom Management*, 39(1), 116-125, <https://doi.org/10.1016/j.jpainsymman.2009.05.016>.
- Kaya, D.A. *et al.* (2014), "The influence of marine algae and natural plant oils on collagen-based cream properties", *Proceedings of the 5th International Conference on Advanced Materials and Systems (ICAMS)*, 237-242.
- Kok, A.G. *et al.* (2018), "Coprescribing proton-pump inhibitors with nonsteroidal anti-inflammatory drugs: risks versus benefits", *Journal of Pain Research*, 11, 361-374, <https://doi.org/10.2147/JPR.S156938>.
- Mason, L. *et al.* (2004), "Topical NSAIDs for chronic musculoskeletal pain: systematic review and meta-analysis", *BMC Musculoskeletal Disorders*, 5, 28-35, <https://doi.org/10.1186/1471-2474-5-28>.
- Ong, C.K.S., Tan, C.H. and Seymour R.A. (2007), "An evidence-based update on non-steroidal anti-inflammatory drugs", *Journal of Clinical Medicine and Research*, 5(1), 19-34, <https://doi.org/10.3121/cmr.2007.698>.
- Ramshaw, J.A.M., Wekmeister, J.A. and Dumsday, G.J. (2014), "Bioengineered collagens: Emerging directions for biomedical materials", *Bioengineered*, 5(4), 227-233, <https://doi.org/10.4161/bioe.28791>.