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COLLAGEN/ALBUMIN-BASED MATRICES DESIGNED FOR VAGINAL ADMINISTRATION OF A NON-STEROIDAL ANTI-INFLAMMATORY DRUG

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The aim of this study was to develop some spongious matrices based on collagen and albumin, loaded with a non-steroidal anti-inflammatory drug (NSAID). The formulations were designed as vaginal drug delivery systems with potential applications in some gynaecological conditions associated with inflammation. Spongious matrices were obtained by lyophilization of the corresponding hydrogels, prepared by mixing a type I collagen gel with various proportions of albumin, followed by incorporating aceclofenac as the drug model and glutaraldehyde as a cross-linking agent. The spongious matrices were characterized through various morphological, biological and biopharmaceutical analyses. SEM analysis displayed the specific microstructure of the biopolymers and the presence of aceclofenac in the polymeric network. Swelling capacity and enzymatic degradation revealed good absorption properties and resistance to collagenase for collagenic spongious matrices, while the addition of albumin led to a limited swelling capacity and a rapid degradation profile due to albumin water solubility. The kinetic analysis showed a non-Fickian drug release mechanism for all matrices. By correlating all the obtained results, it can be stated that spongious matrices based on collagen, albumin and aceclofenac can represent a starting point in the development of new therapeutic systems for vaginal administration, considering the introduction of a third polymer in order to improve the resistance and stability of the systems, and therefore their therapeutic performance.

Keywords: collagen/albumin spongious matrices, aceclofenac, vaginal administration

INTRODUCTION

Many gynaecological conditions, which include bacterial vaginosis, endometriosis or gynaecological cancers, are often accompanied by inflammatory processes (Mitchell and Marrazzo, 2014; AlAshqar *et al*., 2021; Garg *et al*., 2023). Moreover, persistent inflammation encountered in such conditions has been associated with infertility and pregnancy complications (Ravel *et al*., 2021).

Regarding the treatment of these pathologies, both systemic and local treatment options are currently available. However, in recent years, localized treatment has received special attention due to a series of advantages such as the minimization of adverse effects and the targeted action of these therapies (Thapa *et al*., 2022). Regarding vaginal drug delivery systems, natural polymers play an important role in the development of such systems, based on distinctive features such as biocompatibility, biodegradability and non-toxicity (Pandey *et al*., 2020; Jain *et al*., 2024).

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Collagen is the main component of the extracellular matrix and is responsible for ensuring tissue strength, being found in connective tissue that forms structures such as the skin, tendons, bones or ligaments (Kong *et al*., 2023). As a biomaterial, it possesses several important characteristics such as biocompatibility, lack of toxicity and biodegradability (Tudoroiu *et al*., 2023), alongside with its versatility which allows its processing in different pharmaceutical forms, such as spongious matrices, films or semi-solid formulations. Moreover, it has a great number of applications in regenerative medicine, tissue engineering, wound healing and controlled drug delivery systems (Marin *et al*., 2018).

Albumin is one of the most important proteins in the body, with important implications in drug transport and blood colloidal osmotic pressure regulation (Belinskaia *et al*., 2021). Moreover, it is a great candidate for developing drug delivery platforms, based on its biocompatibility and non-immunogenicity. An important advantage of albumin compared to other biopolymers is related to the presence of charged amino acids on its surface, allowing the proper loading of hydrophobic drugs. Furthermore, albumin is widely used in drug delivery of anticancer treatments, based on its interaction with some abundantly expressed receptors associated with several cancers (Asrorov *et al*., 2024).

Therefore, the aim of this study was the development and evaluation of some collagen/albumin-based spongious matrices, loaded with a NSAID, with potential applications in the adjuvant treatment of some gynaecological conditions associated with inflammation.

MATERIALS AND METHODS

Materials

The collagen-based hydrogels were obtained by using the collagen gel preparation technique of Collagen Department, Division Leather and Footwear Research Institute, National Research and Development Institute for Textile and Leather, Bucharest (INCDTP). Thus, type I fibrillar collagen gel of approximately 2.30% (w/w) concentration and acidic pH was extracted from calf hide using the established methodology. In order to obtain a collagen gel with a concentration of 1.0% and physiological pH (7.4), 1M sodium hydroxide solution was gradually added to the initial collagen gel, under continuous mechanical stirring.

Reagents used in the present study were purchased as follows: aceclofenac (ACF) from MP Biomedicals, albumin, glutaraldehyde (GA) and sodium hydroxide from Merck, glacial acetic acid and sodium acetate from Chemical. Distilled water was used both in the preparation stage and in the performed analyses, and all other chemicals used were of analytical grade.

Methods

Preparation of Hydrogels Based on Collagen, Albumin and Aceclofenac

In order to prepare the combined collagen/albumin-based hydrogels, albumin powder was added to the previously obtained 1% collagen gel (pH 7.4), and the mixture was subjected to appropriate homogenization until its complete incorporation. Then, both aceclofenac (0.2 or 0.4%) and the crosslinking agent represented by glutaraldehyde were incorporated. The composition of the prepared hydrogels (Fig. 1) is shown in Table 1.

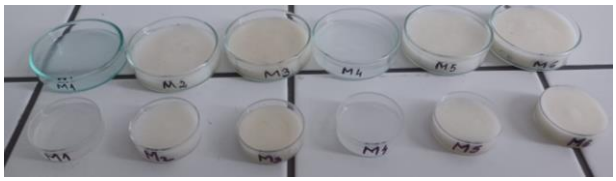


Figure 1. Hydrogels based on collagen, albumin and aceclofenac, crosslinked with GA

Table 1. Composition of the prepared hydrogels and related spongious matrices

Code	Collagen, %*	Albumin, %*	ACF, %*	GA, %*
CA1	1	0	0.2	0.005
CA2	1	2.5	0.2	0.005
CA3	1	5	0.2	0.005
CA4	1	0	0.4	0.005
CA5	1	2.5	0.4	0.005
CA6	1	5	0.4	0.005

* The amount of collagen albumin, ACF and GA are reported to 100 g hydrogel

Obtaining Process of the Spongious Matrices

The spongious matrices were obtained by lyophilization of previously prepared hydrogels, using a Delta LSC 2-24 apparatus (Martin Christ, Germany). The lyophilization program was the one established within the Collagen Department of the Leather and Footwear Research Institute, INCDTP, Bucharest. The lyophilization program lasted 48 hours using a freezing temperature of -40°C during the first 10 hours of the process.

Scanning Electron Microscopy (SEM)

Morphological analysis of the spongious matrices based on collagen, albumin and aceclofenac was performed using a Hitachi Tabletop Microscope TM 4000 Plus (Hitachi, Japan). All samples were analyzed without being coated with a conductive layer and using a voltage of 15 kV. Detection was performed using the back-scattering (BSE) mode.

Swelling Capacity

The evaluation of the swelling capacity of spongious matrices based on collagen, albumin and aceclofenac was performed using a previously described methodology (Tihan *et al*., 2019) and acetate buffer, AB (pH 5.5) as the absorption medium, in order to simulate the conditions encountered at the cervico-vaginal level. The experiment was conducted in duplicate and the swelling capacity was calculated using Equation (1):

$$\text{Swelling capacity (g/g)} = (W_t - W_i)/W \quad (1)$$

where W_t corresponds to the weight of the swollen matrix at specific time points, and W_i is the initial weight of the spongious matrix, in dry form.

Enzymatic Degradation

In order to evaluate the enzymatic degradation of the spongious matrices, a previously reported methodology was used (Tihan *et al*., 2019). The degree of enzymatic degradation was determined by evaluating the weight loss recorded by the spongious matrices, in a predetermined time interval. The experiment was performed in duplicate and weight loss (%) was calculated using Equation (2):

Weight loss (%) = $(W_0 - W_t)/W_0$ x 100 (2)

where W_0 represents the weight of the spongious matrix after soaking in phosphate buffer (initial weight) and W_t – the weight of the matrix soaked in collagenase solution, measured at different time intervals.

In vitro *Release Kinetics of Aceclofenac*

The assessment of the *in vitro* release kinetics of aceclofenac from the spongious matrices was carried out by using an experimental "sandwich" type device, which was attached to a dissolution apparatus with paddles, as previously described (Barbaresso *et al*., 2014), using AB pH = 5.5 at 37°C as release medium. Samples collected at different predetermined time intervals were spectrophotometrically analyzed at a wavelength of 275 nm (representing the absorption maximum of ACF). Furthermore, in order to establish the release mechanism of aceclofenac from the spongious matrices, the experimental data were fitted using the Power Law model (Equation 3) and the Higuchi model (Equation 4):

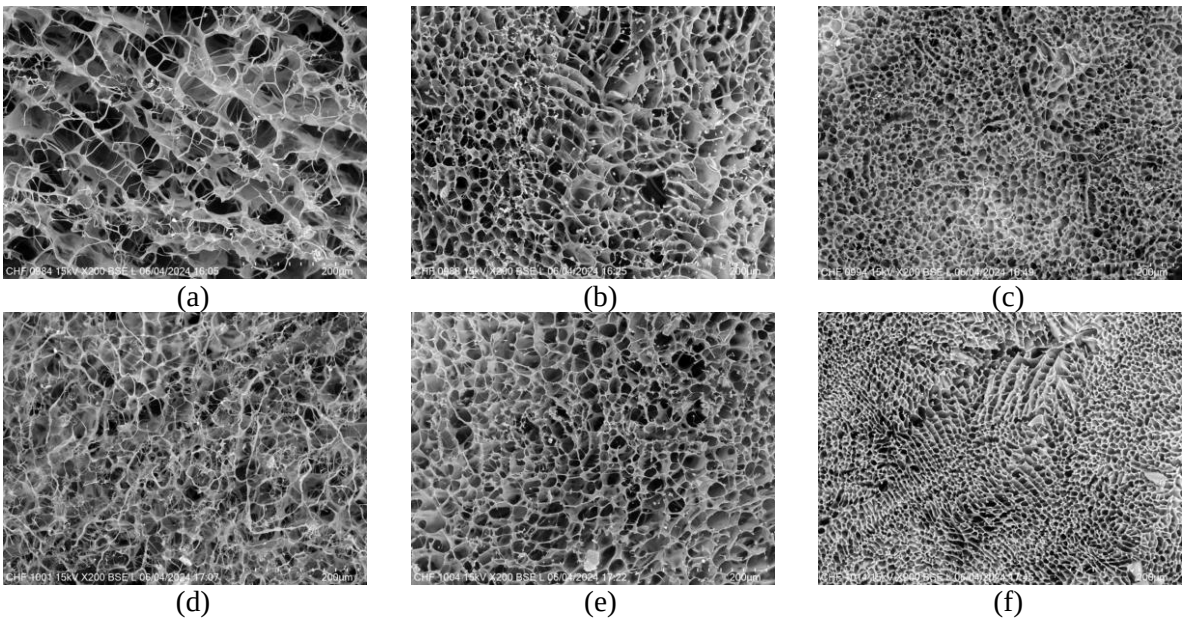
$m_t/m_\infty = k \cdot t^n$ (3)

$m_t/m_\infty = k \cdot t^{0.5}$ (4)

where m_t/m_∞ corresponds to the fraction of drug released at different time points (t), k – the kinetic constant, and n – the release exponent.

RESULTS AND DISCUSSION

SEM analysis revealed the characteristic surface morphology of the designed spongious matrices. Thus, depending on the specific composition of each sample, significant differences can be observed in the structure of the analyzed matrices. The SEM images obtained for the CA1-CA6 spongious matrices are displayed in Figure 2.



(a) (b) (c)

(d) (e) (f)

Figure 2. SEM images of spongious matrices based on collagen, albumin and aceclofenac

Analyzing the obtained SEM images, certain morphological characteristics specific to each polymer can be noticed. The matrices based on collagen and aceclofenac (CA1 and

CA4) showed the characteristic microstructure of collagen matrices, namely, numerous pores having different shapes and sizes and a non-uniform distribution, interconnected by a network of collagen fibers. The addition of albumin generated a series of changes in the microstructure of the matrices, having an increasingly compact appearance, with the increase in the proportion of albumin in the samples. Compared to the collagen matrices, the matrices based on collagen and albumin (CA2, CA3, CA5 and CA6) showed a different pore architecture, presenting an intermediate structure between that of collagen and albumin, the latter being characterized by a denser, lamellar-type polymeric network, with interconnected pores, being more numerous and having smaller sizes with the increase in the proportion of albumin. Regarding the active substance, the images show the presence of aceclofenac, both embedded in the microporous structure and in free form, on the surface of the matrices.

The swelling capacity of the spongious matrices is a very important quality attribute in the case of vaginal administration, since they must quickly hydrate in contact with the limited volume of vaginal fluid available at the site of administration, gradually transforming into the original hydrogel and releasing the drug (Furst *et al.*, 2015). The swelling capacity of all spongious matrices, at different time intervals, is shown comparatively in Figure 3.

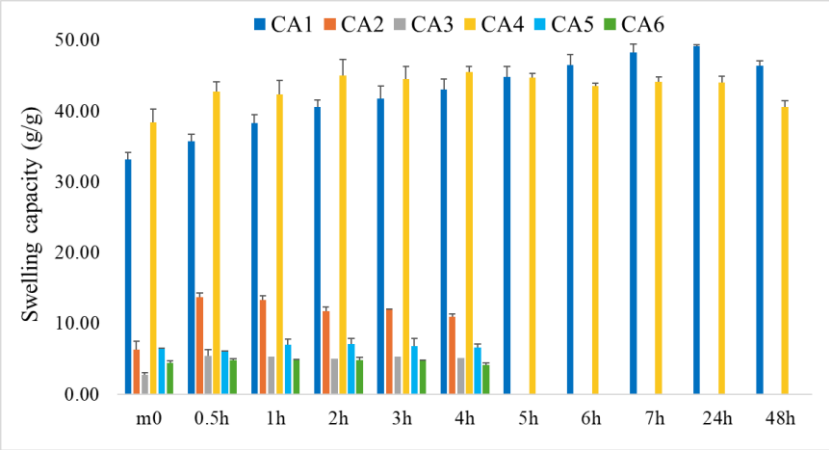


Figure 3. The swelling capacity of the spongious matrices CA1-CA6

The maximum swelling capacity recorded for each individual spongious matrix varied between 4.82 g/g (CA6 matrix) and 49.08 g/g (CA1 matrix). Among the 6 designed spongious matrices, CA1 and CA4, showed the best absorption capacity, showing the characteristic behaviour of collagen matrices. On the other hand, the addition of albumin led to a marked decrease in the swelling capacity, as well as a faster loss of structural integrity, gradually transforming into the original hydrogels, once the threshold of 4 hours is exceeded.

Another important attribute of collagenic spongious matrices is their resistance to enzymatic degradation. Analyzing the obtained results, it can be stated that the CA1 and CA4 matrices, having only collagen and ACF in their composition, had the highest resistance to enzymatic degradation, preserving their structural integrity over a period of 24 hours, compared to matrices that had albumin in their composition, which lost their structural integrity in about 2 hours. However, correlating the results with those obtained in the evaluation of the swelling capacity, the rapid loss of structural integrity can also be associated with the soaking of the samples in the collagenase solution, followed by their gradual disintegration. The weight loss recorded by CA1-CA6 spongious matrices over a period of 24 hours is shown comparatively in Figure 4.

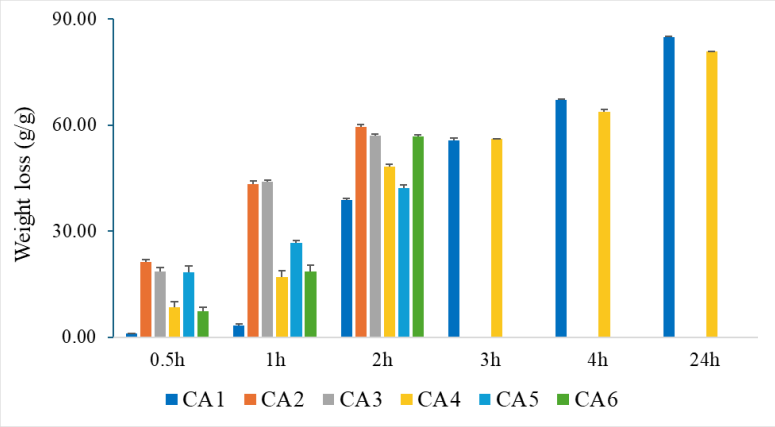


Figure 4. Weight loss (%) recorded by the spongious matrices

The weight loss recorded by CA1 and CA4 samples was comparable between the two matrices, reaching a maximum of 84.94% and 80.78%, respectively, after 24 hours. In this case, doubling the drug concentration in sample CA4 resulted in a decrease in weight loss of approximately 5%. The same trend was observed in the case of collagen/albumin-based matrices, respectively, a slower degradation correlated with the increase of drug concentration in the sample. As for the CA3 and CA6 matrices, no significant influence of drug concentration on the recorded weight loss values was noticed.

For a complete characterization of the biopolymeric drug delivery systems, an analysis of the *in vitro* release kinetics of aceclofenac (model drug) from the designed spongious matrices was performed. Thus, the influence of the spongious matrices' composition on the release kinetics of aceclofenac was studied by comparing the obtained cumulative *in vitro* drug release profiles (Figure 5).

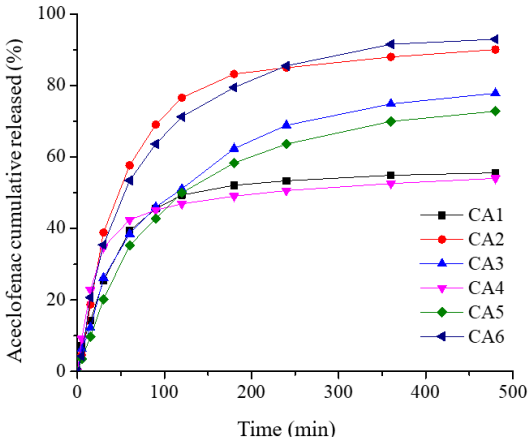


Figure 5. Cumulative release profiles of aceclofenac from CA1-CA6 spongious matrices

Thus, a difference can be observed in the shape of the release profiles of aceclofenac from collagen matrices (CA1 and CA4) compared to those corresponding to collagen/albumin-based matrices (CA2, CA3, CA5, CA6), correlating well with swelling capacity and enzymatic degradations results. However, all release profiles showed a biphasic release mechanism of aceclofenac, highlighting an initial burst-release effect, corresponding to the rapid release of aceclofenac found in free form on the surface of the matrices, in

accordance with the images obtained within the SEM analysis. This phenomenon was obvious within the first 60 minutes of the experiment, and is particularly useful in the rapid control of pain and inflammation at the site of administration. The percentage of drug released in the first 60 minutes varied between 35.30 and 57.65%, the most pronounced burst-release effect being observed for CA2 matrix. After the initial burst-release phase, the release of the drug from the spongious matrices took place gradually during the next 7 hours of the experiment, this second stage of drug release being useful in the long-term control of inflammatory processes associated with various gynaecological conditions with cervico-vaginal localization.

Regarding the maximum percentage of aceclofenac released from the spongious matrices, it varied between 54.03 (CA4) and 92.93% (CA6). The lowest values were recorded for collagen matrices (CA1 – 55.54%; CA4 – 54.03%), these results correlating well with swelling capacity and enzymatic degradation results, where an extended preservation of the structural integrity of these matrices could be observed, which can be associated with a slower degradation of the structural support and a gradual release of the drug. For collagen/albumin-based matrices, the maximum percentage of aceclofenac released was higher, with values between 72.81 (CA5) and 92.93% (CA6), correlated with increased degradation of these matrices and therefore faster release of the incorporated drug.

Subsequently, to establish the release mechanism of aceclofenac from the spongious matrices (CA1-CA6), the experimental data were fitted with the Power Law and Higuchi kinetic models. Considering the correlation coefficients values, it can be observed that the release of aceclofenac from the matrices verified the Power Law model, the coefficients having higher values in this case (0.9534 - 0.9840). Moreover, by analyzing the kinetic parameters, it can be seen that the release exponent (n) recorded values between 0.24 and 0.43 (< 0.5), which denotes a complex (non-Fickian) release mechanism of aceclofenac from the matrices. The values of the correlation coefficients obtained for Higuchi and Power law models, the kinetic parameters (k, n), as well as the maximum percentage amount of aceclofenac released from the matrices, are shown in Table 2.

Table 2. Correlation coefficients for Higuchi and Power law models, kinetic parameters and maximum percentage of aceclofenac released from the spongious matrices

Sample	Correlation coefficient		Kinetic constant, k ($1/min^n$)	Release exponent, n	Drug released, (%)
	Higuchi Model	Power Law Model			
CA1	0.9097	0.9538	0.101	0.30	55.54
CA2	0.9232	0.9534	0.130	0.33	90.06
CA3	0.9763	0.9840	0.068	0.41	77.83
CA4	0.8793	0.9595	0.141	0.24	54.03
CA5	0.9733	0.9786	0.056	0.43	72.81
CA6	0.9526	0.9716	0.110	0.36	92.93

CONCLUSIONS

By correlating all the obtained results, it can be stated that spongious matrices based on collagen, albumin and aceclofenac could represent promising drug delivery systems intended for intravaginal administration in some gynaecological conditions associated with inflammatory processes. However, in order to improve the performance and stability of these systems, future studies will aim to introduce a third polymer, which will lead to an increase in the mechanical strength and stability of the designed matrices, and at the same time, conferring a mucoadhesive character, an important quality attribute which allows extending

the intravaginal retention time of the formulations, thus increasing the therapeutic efficiency of these biopolymeric systems.

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