

Research Article

Piroxicam-Collagen-Based Sponges for Medical Applications

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Depending on the concentration of the drug and/or the method of administration, drugs could be used in various ways. To take full advantage of the drug beneficial properties in oral medical interventions but also in other types of surgery, like plastic surgery, general surgery, or gynecological surgery, the drug concentration as well as the administration method itself will depend on the wound, type of surgery, and severity of the postoperative pain which can be very different. Generally, the local administration methods are recommended. Piroxicam, a nonsteroidal anti-inflammatory drug (NSAID) of the oxycam class, is generally used to relieve the symptoms of pain and inflammation. Starting from the idea of the special benefit of the interference between collagen-based materials and drug beneficial properties, our work was focused on the synthesis and characterization of new collagen-piroxicam materials. These new collagen-based materials present a good water absorption, and the piroxicam release suggests a biphasic drug release profile whereas the obtained values for the release exponent revealed a complex release mechanism including swelling, diffusion, and erosion.

1. Introduction

Pain represents a high and problematic symptom after any kind of surgery all over the world irrespective of the surgery type, wound dimensions, or the associated pathology of the patient. The current researches in medicine are based on the ongoing development of any potential cures for solving the needs of the patient; the main goal is to reduce and relieve the pain, the inflammation, and the sensation and to heighten the recovery process. All these aspects will contribute to the improvement of patient life quality; this is one of the main reasons for the use of a variety of medication in chronic and acute pain treatment [1, 2].

Piroxicam, an NSAID of the oxycam class of anti-inflammatory drugs, represents a very viable option in pain management. The recent researches in the last decade show that when oral administration of this drug may have negative side effects, one of the usual effects is due to piroxicam gastrotoxicity. When the drug is applied locally, it may be able to bypass this undesired side effect. The recent researches show that by embedding the piroxicam in different types of delivery systems, like gels, scaffolds, polymer-based nanoparticles, liposomes, and even topical, intraligamentary, or intramuscular injection through dermal delivery, the negative gastrointestinal effect may be avoided and at the same time the systemic exposure of the drug is reduced. In this way,

the various negative aspects of the piroxicam are practically reduced. The recent comparative studies on patients with postoperative pain and edema following maxillofacial surgery demonstrated that the NSAID local application represents a better solution by reducing the swelling and the pain in few days [3–7]. According to Desjardins' study [8], piroxicam drug lasts significantly longer (24 hours) and has a better effect compared to aspirin. Because the drug is concentrated in the tissues, local application of piroxicam has also a local analgesic effect, especially in the first few hours after the intervention [9].

One of the main objectives for the use of a wound dressing is the acceleration of the wound healing process. Collagen-based biomaterials are usually the most commonly used biomaterials in the bioengineering of the skin, connective tissue, mucosal tissue (oral and vaginal tissues and even organic tissue), and also in nerve and tendon tissue representing an alternative solution to various types of grafts as wound dressing after surgical defects that occur after removal of benign or premalignant lesions/tumors or reactive proliferations and incisional biopsy lesions. Collagen has some big advantages including resilience, suppleness, dressing property of mimicking the surrounding tissues, and a very good tolerance by tissue itself. It also acts as a strong mechanical barrier, and it helps in hemostasis, relieving pain, and preventing excessive contracture [10–13].

The use of collagen in various forms goes back millennia, but the main collagen application remains for burns and wound cover dressings, alveolar filling, antithrombogenic surfaces, wound healing process speeding, implant therapy by preventing epithelial migration, and allowing cells to regenerate. Collagen sponges when used in burns and ulcers (decubitus ulcers) have the ability to absorb large quantities of tissue exudates, blood, and saliva and present the benefit to adhere to the wet wounds and by clotting to facilitate the dermal and epidermal wound healing. Piroxicam-collagen sponges could also be used as the delivery system for intravaginal wounds or cervical dysplasia [14–18].

Collagen-based biomaterials can be applied in various dental surgeries and dental medicine, including wound dressing and hemostatic abilities, being a biomaterial that can easily help in GTR (guided tissue regeneration) [13]. In maxillofacial surgery, the mix between collagen and NSAIDs represents a very viable option. For example, after removal of an impacted third molar, one of the most frequent procedures in oral and maxillofacial surgery, usually followed by pain, swelling, and trismus (restricted jaw movements) [5], such collagen-based materials could be beneficial. The adhesion between the tissue and the piroxicam-collagen based biomaterial, the mucoadhesion, represents that state where two or more than two elements (from which at least one is biological) may adhere to each other for extended periods of time. The mucoadhesion offers the opportunity to release the drug through different routes of administration like ocular, nasal, vaginal, oral and in some cases intraligamentary, which is a more efficient and safer way of drug administration compared to oral administration [19, 20].

In this paper, piroxicam-collagen-based sponges are studied due to several reasons:

- (i) Local pain is one of the most critical points of patient care in medicine. Therefore, a drug use is mandatory and piroxicam, an NSAID, is a drug used for local pain management where the anti-inflammatory and analgesic effects are beneficial besides the basic commonly used treatment
- (ii) The need to create a local delivery system that bypasses the systemic unwanted toxic side effects
- (iii) Collagen sponges are a very high biocompatible and biodegradable biomaterial. At the same time, they are also well known for their safe and well-characterized supports as drug delivery systems [21–24]

The aim of this study was to obtain materials based on collagen and piroxicam for biomedical purposes, especially for various types of surgeries. The stability of collagen-piroxicam sponges was assured by glutaraldehyde as a cross-linking agent. Water absorption and enzymatic degradation tests were performed. At the same time, the piroxicam release from cross-linked collagen spongy forms was investigated and the kinetic mechanism was discussed.

2. Materials and Methods

Type I fibrillar collagen was obtained using the bovine hide, and, currently, technology from our laboratory (INCDTP-Division Leather and Footwear Research Institute, Collagen Department) was employed. Collagen can be obtained using two main technologies: molecular technology and/or fibrillar technology. In order to obtain molecular collagen, a proteolytic enzyme is used to cleave telopeptides. In this study, we used fibrillar technology to obtain type I collagen by alkaline treatment of bovine dermis with 5–10% sodium hydroxide and 1 M sodium sulphate at room temperature for 2 days in order to remove telopeptidic nonhelical regions. Alkaline treatment was followed by an acidic one with 1 N HCl, and total solubilization of collagen in the undenatured state was performed. The salt precipitation and resolubilisation were carried out in order to purify collagen [25–27]. Collagen in the form of gel was obtained, and its concentration of 2.82% (w/v) was determined by gravimetric methods (dried at 105°C).

Piroxicam drugs were obtained from Alfa Aesar whereas glutaraldehyde, used as a cross-linking agent, was purchased from Merck.

The concentration of the collagen gel was adjusted from concentration of 2.82% and acidic pH to concentration of 1% and 7.4 pH using 1 M sodium hydroxide. The piroxicam (solution) was added to the collagen gel in concentration of 1.0%, 1.5%, and 2.0% (with respect to the dry collagen), and the obtained gels were cross-linked with 0.5% glutaraldehyde (solution) reported to dry collagen. The cross-linked gels were kept 24 hours at 4°C and then freeze-dried using a Delta 2-24 LSC lyophilizer (Martin Christ, Germany) as follows: the gels casted into glass Petri dishes were initially frozen at -40°C during 12 hours followed by main freeze-drying at -40°C and 0.1 mbar for 8 hours. Then, the temperature

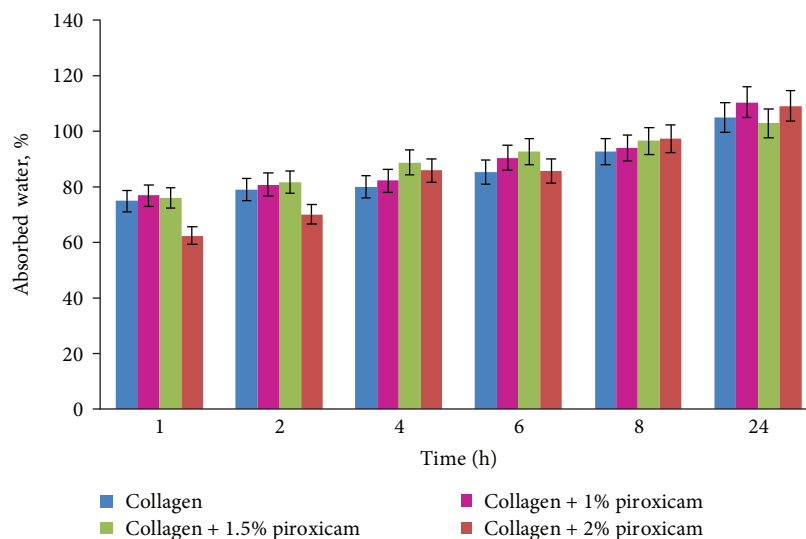


FIGURE 1: Water absorption of piroxicam sponges.

increases in steps to 10°C during 8 hours, to 20°C during another 8 hours, and to 30°C for 8 hours at same pressure, 0.1 mbar. The final freeze-drying lasted 4 hours: 1 hour at 30°C and 0.001 mbar followed by 3 hours until temperature reached 35°C. After 48 hours of freeze-drying, the collagen sponges were obtained and then characterized as we described below.

The water absorption tests were performed on collagen sponge half-cubes of 1 cm × 1 cm × 0.5 cm immersed in distilled water at 25°C. Water absorption was calculated at different periods of time using equation (1). Every test was performed in triplicate.

$$\% \text{water absorption} = \frac{w_1 - w_0}{w_0} \times 100, \quad (1)$$

where w_0 is the dry mass and w_1 is the wet mass.

Enzymatic degradation test was performed using collagenase of *Clostridium histolyticum* from Sigma-Aldrich (USA) and a phosphate-buffered saline (PBS) solution with pH 7.4. Sponges with a known weight were immersed in PBS solution and incubated at 37°C overnight. Collagenase (10 µg/mL) was then added, and the test tube was replaced at 37°C. At different time intervals, the degradation was interrupted by removing the scaffold from the degradation solution, squeezing, and weighting again the sponges. The mass loss percentage was calculated as follows:

$$\% \text{collagen mass degraded} = \frac{w_i - w_t}{w_i} \times 100, \quad (2)$$

where w_i is the initial mass and w_t is the mass after time t . Each biodegradation test was performed in triplicate.

Optical microscopy images were registered using a Zeiss Scope A1 digital microscope equipped with a Canon camera (Taiwan).

IR spectra were performed using an Interspec 200-X FTIR equipment from Interspectrum in transmittance mode

in the region 4000–400 cm⁻¹. Collagen samples were prepared for IR measurements by using a manual hydraulic press to form KBr pellets.

The *in vitro* release of piroxicam from the tested formulations was performed using a sandwich device adapted to a paddle dissolution equipment, as previously detailed [28]. The concentration of piroxicam in each sample was investigated by UV spectroscopy at its maximum absorbance corresponding to a wavelength of 353 nm and using the standard calibration curve ($A_{1\%}^{1\text{cm}} = 479$). The cumulative released drug percentage was then assessed. The kinetic data were fitted with the power law model (equation (3)) and its particular cases, Higuchi ($n = 0.5$) and zero-order ($n = 1$) models, and the drug release mechanism was set.

$$\frac{m_t}{m_\infty} = k \times t^n, \quad (3)$$

where m_t/m_∞ is the fractional release of the drug at time t , k is the kinetic constant, and n is the release exponent showing the drug release mechanism. The experiments were conducted in triplicate.

3. Results

Figure 1 presents the results obtained for water absorption experiments. The collagen matrices should possess proper water uptake abilities allowing the biological fluid penetration into the spongy structure and consecutively the drug diffusion within the swollen polymeric network. The obtained data suggest that the amount of piroxicam does not affect the water absorption capacity. At the same time, as expected, the water absorption is increasing in time, being 100% after 24 hours.

The enzymatic collagen degradation measurements as presented in Figure 2 revealed that piroxicam addition, as consequence, decreases the sample degradation. However, after more than 8 hours in collagenase solution, the samples disintegrate, even if they are not completely degraded. The

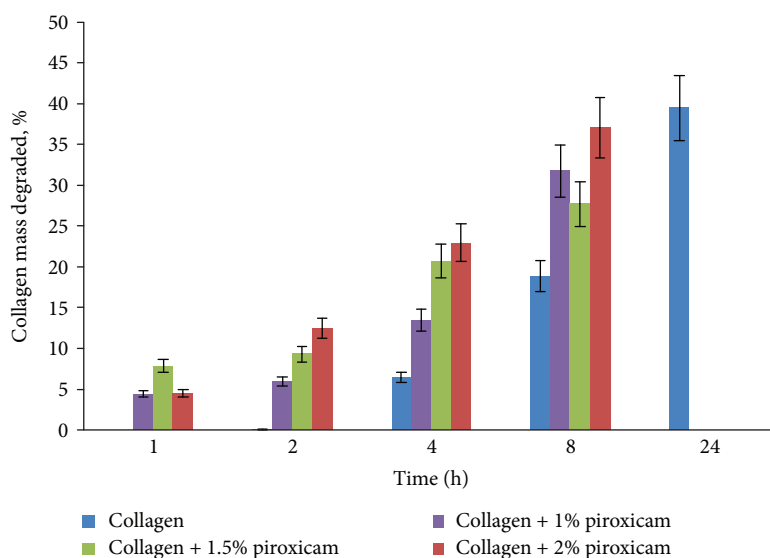


FIGURE 2: Enzymatic degradation of piroxicam sponges.

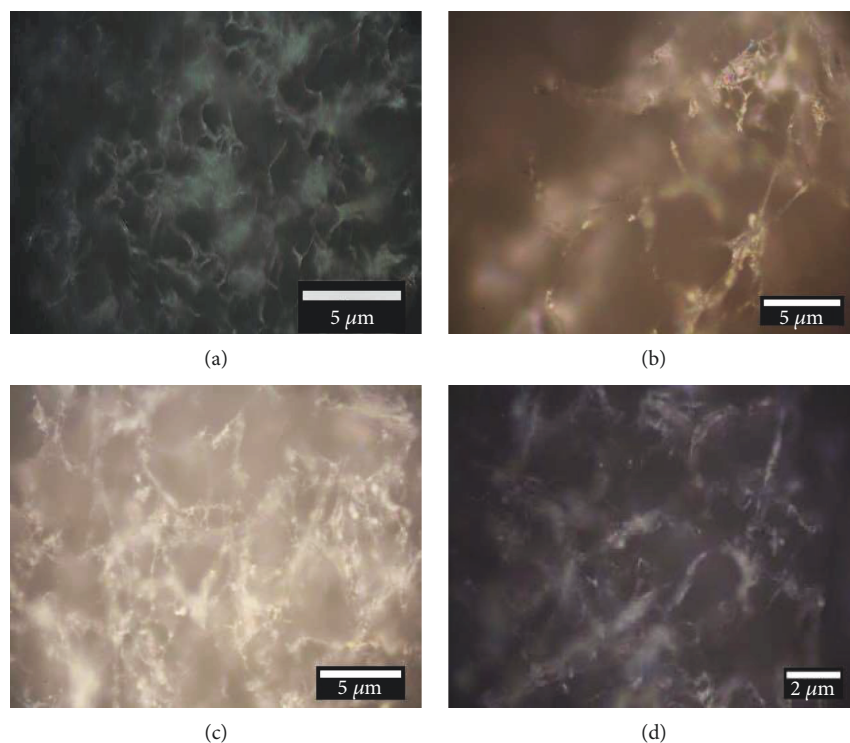


FIGURE 3: Optical microscopy images for collagen sponge (a) (100x), 1% piroxicam collagen (b) (50x), 1.5% piroxicam collagen (c) (50x), and 2% piroxicam collagen (d) (100x).

sponge degradation is relatively slow in the beginning (5–10% in the first two hours). After 8 hours and before disintegration, around 30% of the samples are degraded suggesting that such kind of materials could be useful in oromaxillofacial surgery.

The optical microscopy images (Figure 3) displayed a fibrillar structure with interconnected pores. In all obtained sponges, the light spots prove the piroxicam presence.

The presence of the piroxicam in the collagen sponges was certified also by the IR spectra. Table 1 lists the

assignments for the main absorption peaks obtained for the infrared spectra recorded. As expected, the IR spectra were similar as the characteristic peaks for the main vibrations are the same for collagen and for piroxicam. Nevertheless, some wavenumber shifts were present.

All the above analyses indicate that the designed collagen sponges present adequate properties as drug release supports. Thus, the collagen-piroxicam sponges were further analyzed from the kinetic point of view.

TABLE 1: FTIR spectra peak position and assignments for collagen sponges.

Assignment	Collagen sponge	Wavenumber (1/cm)		
		<i>Collagen</i> + 0.5% piroxicam sponge	<i>Collagen</i> + 1% piroxicam sponge	<i>Collagen</i> + 1.5% piroxicam sponge
N-H stretch (ν_{NH}) of amide A [29, 30]				
Hydrogen bonding of the N-H group with a carbonyl group of the peptide chain [29]	3540	3569	3552	3550
CH ₂ asymmetrical stretch (ν_{asCH_2}) by amide B band [29, 30]	3017	3008	3016	3007
C-O stretching vibration (ν_{CO}) [29]	1681	1681	1680	1680
Amide I C=O stretching [30, 31]				
Hydrogen bond between N-H stretch (ν_{NH}) and C-O (ν_{CO}) [29]	1607	1606	1608	1610
Helical structure of collagen [29]				
Stretching vibration of the C-N group [29]				
C=N stretching of piroxicam [31]	1494	1499	1499	1496
CH ₂ deformation (δ_{CH_2}) [32]				
CH ₃ bend (δ_{CH_3}) [32]	1443	1441	1443	1443
CH ₂ bending vibration (δ_{CH_2}) [30]				
CH ₂ group wagging vibration [29]	1376	1379	1387	1380
NH deformation of amide III (δ_{NH}) [32]	1274	1274	1273	1275
Hydrogen bonding of N-H bending [29, 30]	1241	1237	1240	1240
NH deformation of amide III (δ_{NH}) [32]	1191	1192	1189	1190
S=O asymmetric stretching [31]				
Ester bond [29]	1113	1113	1115	1113
$\nu(\text{C-O})$, $\nu(\text{C-O-C})$ of carbohydrate moieties (collagen) [32]	1062	1063	1062	1063
Skeletal stretching vibrations [30]	899	899	900	900

TABLE 2: Kinetic parameters and correlation coefficients specific to power law model, correlation coefficients specific to Higuchi and zero-order models, and drug release percentage.

Collagen-piroxicam matrices (% of piroxicam)	Kinetic constant (1/min ⁿ)	Release exponent	Correlation coefficient			Drug release percentage (%)
			Power law model	Higuchi model	Zero-order model	
1	0.11	0.39	0.9811	0.9716	0.8761	87.44
1.5	0.12	0.38	0.9860	0.9755	0.8822	96.24
2	0.10	0.41	0.9892	0.9831	0.9009	91.33

From Table 2, it can be seen that the power law model describes well the drug release (correlation coefficient > 0.98, being higher than the ones specific to Higuchi and zero-order models).

The piroxicam release from the designed formulations showed an anomalous drug transport kinetic mechanism, the values obtained for the release exponent being smaller than 0.5.

Figure 4 reveals typical biphasic drug release profiles, with an important piroxicam burst release effect in the first 30 minutes for the collagen matrices with 1%, 1.5%, and 2% piroxicam, ensuring an inflammation rapid diminution, followed by a prolonged release over the next hours of experiments. The obtained values for the release exponent (<0.5) revealed a complex release mechanism including swelling, diffusion, and erosion [28, 33].

Corroborating drug release results with those of collagenase degradation, it can be affirmed that before the sponge disintegration the drug is almost completely released. Moreover, depending on where these materials will be used, the fact that they absorb water suggests that they can absorb oral liquids or sanguinolent liquids and thus reducing the inflammation and the hematoma risk.

4. Conclusions

This study presents new collagen-based materials for biomedical applications, especially in dentistry. These new materials, collagen-based spongy materials, obtained by the lyophilization method, were characterized by various techniques like optical microscopy, IR spectroscopy, water

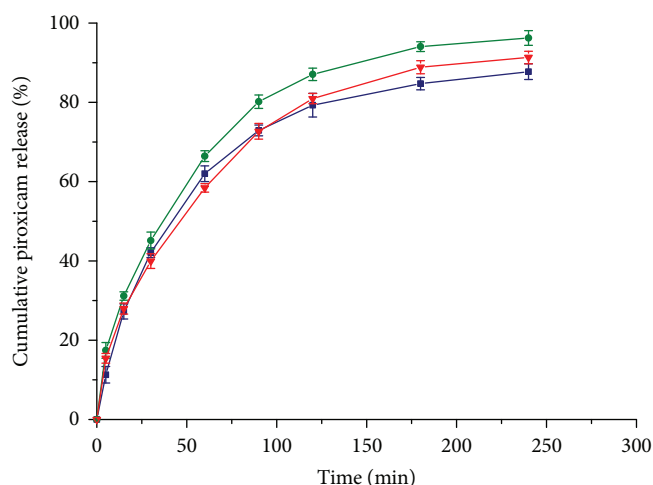


FIGURE 4: Cumulative release profiles of piroxicam from collagen matrices versus time: 1% piroxicam (blue square), 1.5% piroxicam (green circle), and 2% piroxicam (red triangle) ($n = 3$).

absorption, enzymatic degradation, and *in vitro* release kinetics.

The results obtained concerning the hydrolysis and enzymatic degradation showed the inhibitor role of the cross-linking agent, while the piroxicam presence determines an increasing sponge degradation under collagenase leading, at the end, to the sponge disintegration.

The kinetic profiles determined for piroxicam release suggest biphasic drug release profiles whereas the obtained values for the release exponent (<0.5) revealed a complex release mechanism including swelling, diffusion, and erosion.

The results obtained suggest that, in the near future, piroxicam-collagen-based biomaterials would easily represent a viable and modern solution to treat various types of wound that occurs in oromaxillofacial surgery, cumulating the best properties of collagen and the NSAIDs.

Data Availability

All the supporting results are presented in the article.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

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