THE INFLUENCE OF THE FORMULATION FACTORS ON THE DESIGN AND CHARACTERIZATION OF SOME COLLAGEN-BASED HYDROGELS WITH METRONIDAZOLE

Alice Gianina SIMONCA[1], Mădălina Georgiana ALBU KAYA[2], Ileana RĂU[1], Maria Minodora MARIN[2,3]*, Cristina-Elena DINU-PÎRVU[4], Alina LUPULIASA[4], Mihaela Violeta GHICA[4]

The purpose of this paper was to obtain and characterize some collagen-based hydrogels with different concentrations of metronidazole and strontium ranelate, in order to be used for periodontal therapy. The structural integrity of the hydrogels was evaluated by circular dichroism, and the metronidazole release was investigated using an immersion cell adapted to USP 2 apparatus. The structural analysis revealed that the collagen hydrogels with metronidazole in different concentration preserved the integrity of the collagen triple helix, while the presence of the strontium ranelate in the formulation leads to a structural alteration. The kinetic data obtained for the metronidazole release from the samples containing only the drug, plotted as cumulative drug released percentage as a function of time, and cumulative drug released per unit area, respectively were illustrated. The Higuchi model linearity range for the tested hydrogels was identified and the diffusion coefficients were determined. The kinetic parameters for the collagen hydrogels containing only metronidazole showed the drug concentration influence, the best results being obtained for the sample containing 1.5 % metronidazole. The obtained results suggest that the designed collagen-based hydrogels with metronidazole could be beneficial in the periodontal disease treatment.

Keywords: Collagen, Metronidazole, Strontium ranelate, Hydrogels

1. Introduction

Periodontitis represents an inflammatory illness which injures dental ligaments, gingiva, alveolar bones and cementum, triggers bone resorption, and in the final step all teeth are lost [1,2]. This disease occurs when bacteria from dental

[1] Faculty of Chemical Engineering and Biotechnologies, Bucharest, Department of General Chemistry, University POLITEHNICA of Bucharest, Romania, e-mail: simoncaalice@gmail.com
[2] The National Research & Development Institute for Textiles and Leather, Division of Leather and Footwear Research Institute, Department of Collagen Research, Bucharest, Romania
[3*] Faculty of Applied Chemistry and Materials Science, Advanced Polymer Materials Group, University POLITEHNICA of Bucharest, Romania, correspondence author, e-mail: minodora.marin@ymail.com
[4] Faculty of Pharmacy, Department of Physical and Colloidal Chemistry, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

plaque appears on dental tissues and induces an inflammatory response [3]. The treatment for this illness is difficult, being influenced by numerous factors [4]. Normally, the bacteria responsible for this disease are detached using mechanical washing and topical application of antimicrobial substances such as tetracycline and chlorhexidine [5]. These methods are inefficient for the long term, and in order to overcome these drawbacks many researchers are focused on the development of drug delivery systems, which present many advantages and reduce the collateral effects [6]. Controlled drug delivery systems are a new generation of pharmaceutical treatments that can release bioactive substances with fixed dosages at well-defined periods of time [7,8].

Hydrogels are an important group of biomaterials that are extensively used in various biomedical fields. Due to their exceptional characteristics such as manageable swelling behavior and flexibility, they enhance their broad applicability in tissues regeneration, controlled release of drugs and wound healing [9,10]. Usually, hydrogels are made from natural and synthetic polymers. Among the most used natural polymers considered safe for hydrogel preparation are collagen, alginate, chitosan and carrageenan. All these natural polymers present versatile characteristics like good mechanical stability, excellent biocompatibility and adequate biodegradability, being suitable for tailoring drug delivery systems [11, 12]. Recognized for their exceptional physical strength, necessary flexibility and other crucial properties, synthetic polymers are used for hydrogel fabrication. Examples of these polymers are polycaprolactone, poly (N-vinyl pyrrolidone), poly (hydroxyalkyl methacrylate), N-vinyl-2-pyrrolidone etc. [9,13]. Collagen is often used in medical applications, and particularly for drug delivery systems because of its excellent properties such as biocompatibility, biodegradability and its unique interaction with the human organism [14]. Collagen is the main protein from connective tissues: skin, bones, tendons, ligaments, cartilage, organs, basement membranes etc. It represents a larger family of various genetically different types. Currently, over 28 different types of collagen have been defined as part of vertebrate organisms [15].

Metronidazole represents a nitroimidazole complex and has been confirmed by many research studies to be bactericidal for the majority of anerobic bacteria such as bacteroides, fusobacteria, and treponemes. The main advantages of the use of this drug are the fact that the active compound is efficient even at low concentration and the fact that the periodontal flora and the health of healthy teeth are not affected when the drug is used [1, 16]. Strontium ranelate is a salt of ranelic acid which is used in several studies to stimulate calcium uptake in bone and for the inhibition of bone resorption [17,18].

The aim of this study was to obtain some collagen hydrogels with different concentrations of metronidazole and strontium ranelate, with applications in periodontal therapy. The investigation of the formulation factors influence on the

triple helix structure of collagen and on the metronidazole kinetics release was also performed.

2. Experimental part

2.1. Materials

Type I fibrillar collagen gel (1% w/v, acid pH) was extracted from calf hide according to the technology currently used in Collagen Department of Division Leather and Footwear Research Institute as previously described [19]. Metronidazole was purchased from Hubei Hongyuan Pharmaceutical technology CO., Ltd., China, Strontium ranelate from OSSEOR, and sodium hydroxide from Merck (Darmstadt, Germany).

2.2. Preparation of collagen/metronidazole/ strontium ranelate hydrogels

The type I fibrillar collagen in gel form with an initial concentration of 1.63% (w/w) and an acidic pH was adjusted at pH 7.4 using 1M solution of sodium hydroxide. The final concentration of collagen gel was 1% and different amounts of metronidazole were added to this gel. Similar samples with strontium ranelate added were also prepared. In Table 1 is presented the composition of the hydrogels investigated.

Table 1.

Composition of obtained hydrogels

Samples	Collagen*, %	Metronidazole*, %	Strontium ranelate *, %
COLL-M1	1	0.75	0
COLL-M2	1	1.5	0
COLL-M3	1	2.25	0
COLL-Sr-M1	1	0.75	1
COLL-Sr-M2	1	1.5	1
COLL-Sr-M3	1	2.25	1

** The amounts of metronidazole, strontium ranelate and collagen are reported to 100 g of gel.*

After preparation all obtained hydrogels were stored for 24 hours at 4°C for stability.

2.3. Characterization methods

2.3.1. Circular Dichroism (CD)

The triple helix structure stability of the collagen hydrogels loaded with metronidazole/strontium ranelate was evaluated by CD. The acquisition of the spectra was recorded on a Jasco Model J-1500 spectrophotometer using a quartz cylindrical container with a path length of 10 mm (2 mL of 0.05 % (w/v) collagen

aqueous solution was placed into the container). CD spectra were obtained by wavelength scans from 190 to 260 nm with a scan rate of 100 nm/min.

2.3.2. In vitro drug release kinetics

In vitro release kinetics of metronidazole from the hydrogels was carried out using an immersion cell adapted to USP 2 apparatus with paddle (Hanson Vision Classic 6, Hanson Research). The immersion cell was fitted with a cellulose acetate membrane (diameter 25 mm and pore size 0.45 μm, Prat Dumas), previously maintained in the receiving medium (phosphate buffer solution pH 7.4). A pre-weighed amount of hydrogel was spread on the membrane surface, the immersion cell being then fixed in the equipment dissolution vessel. During the kinetic experiments the release medium was continuously stirred at 50 rpm and the temperature was kept constant at 37°C±0.5°C. Samples of 5 mL were withdrawn from the dissolution vessels at predetermined time intervals and replaced with equal and fresh volumes of phosphate buffer solution pre-heated at 37°C±0.5°C to maintain the equilibrium constant. The concentration of metronidazole in each aliquot was spectrophotometrically assessed (Perkin-Elmer UV-Vis spectrophotometer) at 319 nm, using the standard calibration curve. The cumulative drug released percentage as a function of time, and the cumulative drug released per unit area were then evaluated.

3. Results and discussion

3.1 Circular Dichroism (CD)

To confirm the characteristic triple helix conformation of the collagen samples after metronidazole and strontium ranelate addition, CD investigations were accomplished. CD assessment can be used to establish the integrity of the proteins, a necessary condition before complete structural studies in medical applications [20].

The CD spectra of the samples are shown in Fig. 1.

Collagen is an optically active macromolecule formed by more than 20% proline and hydroxyproline that also creates an exceptional helical structure. In literature data, this configuration is identified as having the polyproline II helix structure in CD spectra with an intersection point at 213-214 nm, a noticeable negative minimum absorption band at 190-200 nm and a positive maximum absorption band at 210-230 nm [21]. Compared with other ordered conformations specific for proteins (α helix and β structures) the triple helix is a compressed conformation [21]. The samples Coll-M1, Coll-M2 and Coll-M3 (loaded only with metronidazole, without strontium ranelate) presented a cross point around 213 nm, a positive maximum peak at 222 nm and a negative minimum peak at 198 nm, suggesting a characteristic triple helix structure [22], while for the samples where

the strontium ranelate was added (Coll-Sr-M1, Coll-Sr-M2 and Coll-Sr-M3), all the characteristic markers for triple helix structure in CD disappeared, indicating that the presence of the strontium ranelate in the polymer matrix destroyed the triple helix structure of collagen.

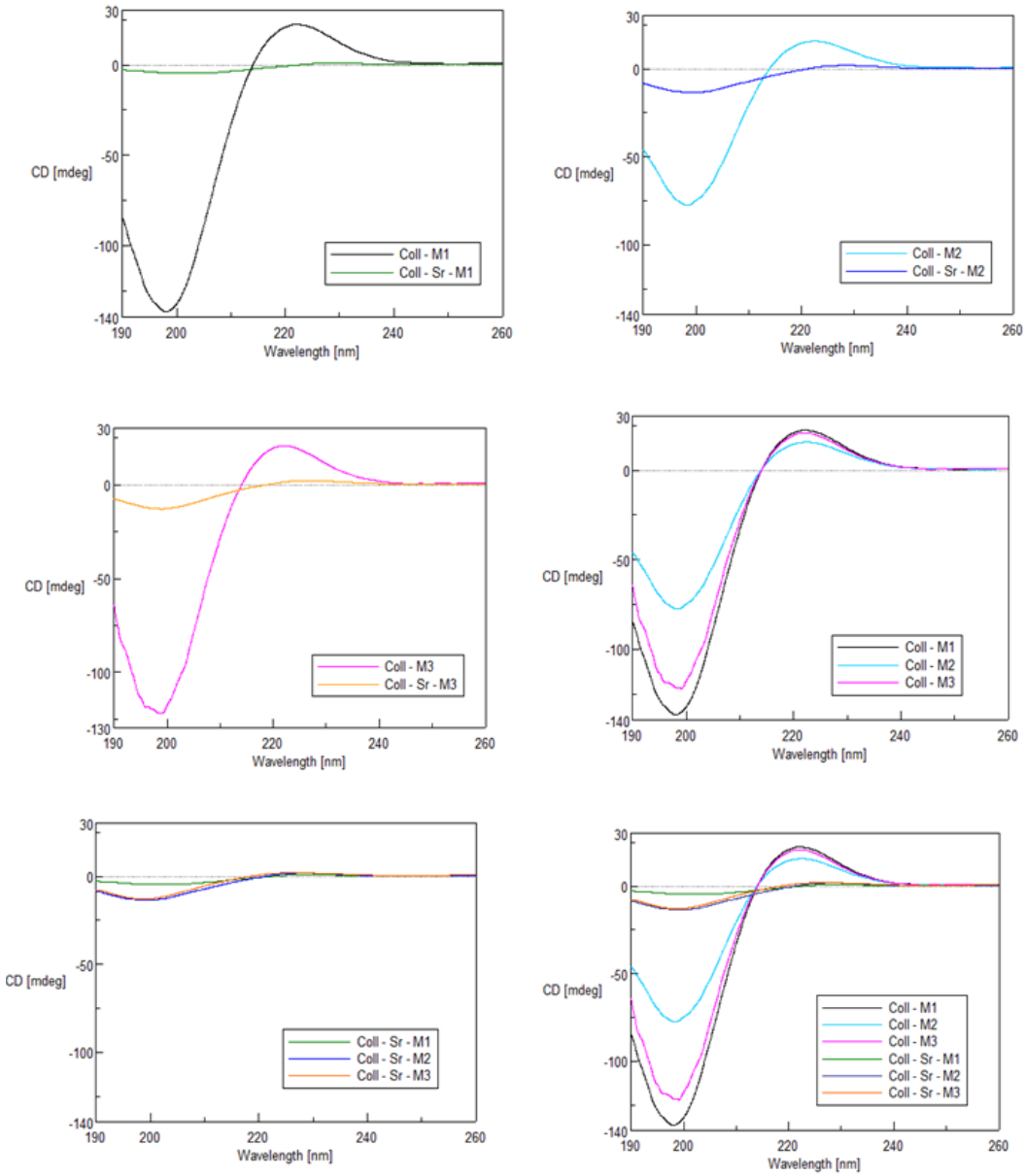

Fig. 1. CD spectra of the obtained hydrogels loaded with metronidazole/strontium ranelate

Based on the achieved results it can be concluded that the triple helix structure of collagen, the highly important point of view for medical applications, was not considerable influenced by the addition of metronidazole, but was significantly altered by the presence of strontium ranelate. Because of this reason we are not recommend as stable biomaterials the one with strontium ranelate.

3.2. In vitro drug release kinetics

Further, taking into consideration the results obtained from circular dichroism analysis, only the samples with metronidazole that preserved the triple helix structure of collagen and without strontium ranelate, were subject to the *in vitro* drug release study.

The results of the metronidazole release kinetics using an immersion cell adapted to USP apparatus 2 are presented in Figs. 2-5. Thus, the MTZ release profiles for hydrogels Coll-M1–Coll-M3 plotted as cumulative drug released percentage (%) versus time (min) were presented in Fig. 2, and the kinetic patterns plotted as cumulative amount of drug released (mg) per unit area (cm^2) versus time (min) were presented in Fig. 3. In Fig. 4 Higuchi release profiles for the same hydrogels were built, and the identification of Higuchi model linearity range for the tested hydrogels was illustrated in Fig. 5.

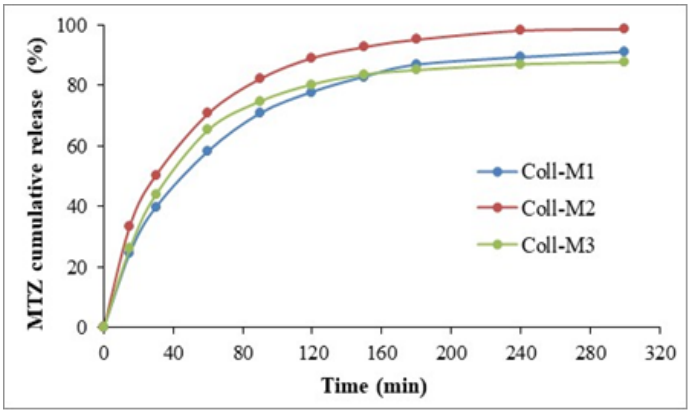

Fig. 2. Time-dependent cumulative release profiles of metronidazole from hydrogels Coll-M1–Coll-M3

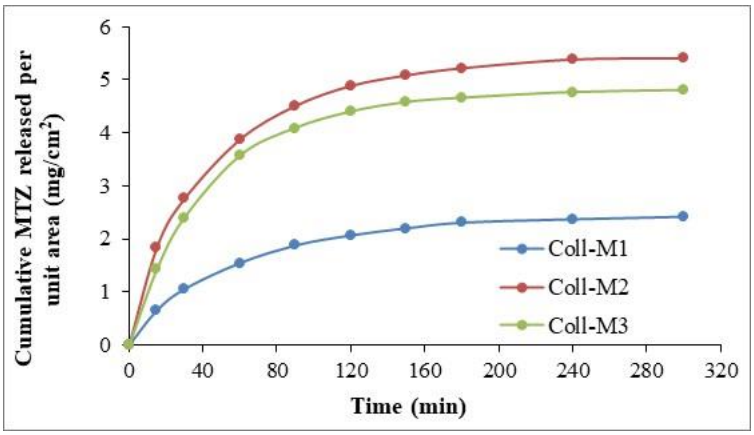

Fig. 3. Cumulative amount of metronidazole released per unit area (cm^2) as a function of time (min)

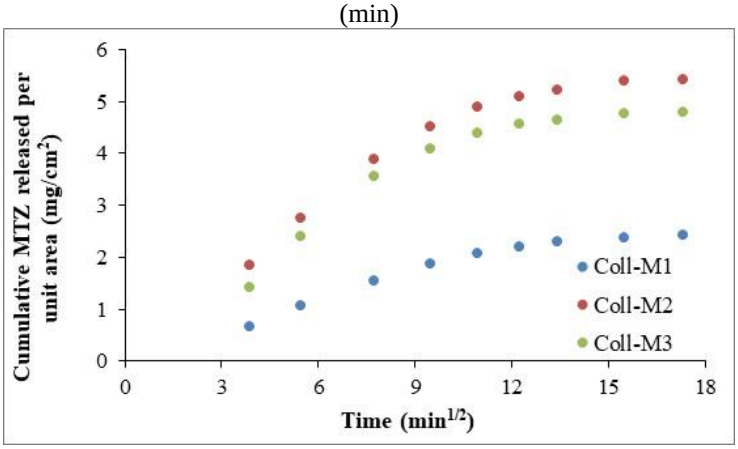

Fig. 4. *In vitro* metronidazole release profiles – Higuchi model application for the hydrogels Coll-M1–Coll-M3

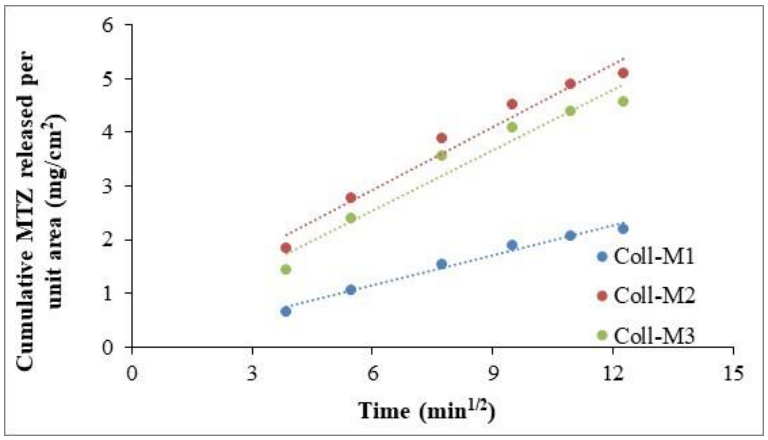

Fig. 5. Identification of Higuchi model linearity range for the hydrogels Coll-M1–Coll-M3

The influence of the MTZ concentration on *in vitro* drug release from the tested hydrogels was analyzed comparing the kinetic profiles recorded in Figs. 2-5. From Fig. 2 one can see similar shapes of the recorded kinetic patterns, with an obvious burst release effect. The drug released percentage after 300 min of experiment varies between 87.86 (Coll-M3) and 98.86 % (Coll-M2), Table 2.

The faster drug release in the first minutes is balanced by a progressive drug delivery in the next hours, ensuring efficient and sufficient metronidazole concentrations to prevent and control the local infection at periodontal tissue level. The cumulative amount of metronidazole released per unit area as a function of time (Fig. 3) as well as the application of Higuchi model (Fig. 4) indicates that the kinetic profiles are strongly influenced by the drug concentration/content, similar results being obtained for the hydrogels with medium and high MTZ content.

The release patterns from Fig. 5 highlighted that the cumulative MTZ released per unit area depends linearly on the square root of time, in the range between 5-150 min. The diffusion coefficients (Table 2) were determined from Fig. 5 and using eq. 1 according to Higuchi model [23].

$$q = 2C_0\sqrt{D \cdot t / \pi} \quad (1)$$

where q is the amount of drug released per unit of membrane surface, C_0 – the initial drug concentration in the hydrogel, D – the drug diffusion coefficient through hydrogel, and t – the drug release time.

The specific parameters to Higuchi model are listed in Table 2.

Table 2.

Parameters specific to Higuchi model; the metronidazole released percentage from the hydrogels Coll-M1–Coll-M3

Samples	Slope of regression line (mg/ cm^2·min$^{1/2}$)	Diffusion coefficient D (cm^2/min)	Correlation coefficient R	MTZ released (%)
COLL-M1	0.1869	4.88E-04	0.9954	91.14
COLL-M2	0.3927	5.38E-04	0.9918	98.86
COLL-M3	0.3768	2.20E-04	0.9869	87.86

The values recorded for the correlation coefficient ranging between 0.9869 and 0.9954 showed that Higuchi model well fitted the kinetic experimental data in the time range 5-150 min, this indicating a Fickian diffusion release mechanism, the drug diffusion rate being smaller than the relaxation rate of the polymer.

Concerning the diffusion coefficients, the highest value was obtained for the hydrogel COLL-M2 with a medium content of metronidazole, while for the hydrogel COLL-M1 with the low level of drug a smaller decrease of 10% was

observed. The lowest value of the diffusion coefficient was obtained for the sample with the highest amount of metronidazole, about 2.20-2.45 smaller, compared to the other two hydrogels. It seems that a MTZ concentration higher than 2% induces an increase of the hydrogel viscosity, leading to a resistance of the drug diffusion through the polymeric network.

4. Conclusions

The designed collagen hydrogels with metronidazole in different concentrations preserved the collagen triple helix structure, while the addition of strontium ranelate in the formulation leads to a structural alteration. The kinetic parameters, the cumulative drug released percentage and the diffusion coefficient for the collagen hydrogels containing only metronidazole, are influenced by the drug concentration, the best results being obtained for the sample containing 1.5% metronidazole - medium concentration level. The obtained results showed that the designed collagen-based hydrogels with metronidazole could be beneficial in the periodontal disease management (prevention and treatment).

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R E F E R E N C E S

[1] *N. S. Soysa, H. Waidyarathne, M. Ranaweera, C. N. R. A. Alles*, Clinical efficacy of local application of sustained-release metronidazole in periodontal therapy, Dentistry Review, 2021, **vol. 1**, pp. 100006.

[2] *M. Yar, A. Farooq, L. Shahzadi, A.S. Khan, N. Mahmood, A. Rauf, A.A. Chaudhry, I. U. Rehman*, Novel meloxicam releasing electrospun polymer/ceramic reinforced biodegradable membranes for periodontal regeneration applications, Materials Science and Engineering: C, 2016, **vol. 64**, pp. 148-156.

[3] *L. Popa, M.V. Ghica, C.E. Dinu-Pîrvu*, Periodontal chitosan-gels designed for improved local intra-pocket drug delivery, Farmacia, 2013, **vol. 61**, pp. 240-250.

[4] *˙S. Zupan˙ci˙c, S. Baumgartner, Z. Lavri˙c, M. Petelin, J. Kristl*, Local delivery of resveratrol using polycaprolactone nanofibers for treatment of periodontal disease, Journal of Drug Delivery Science and Technology, 2015, **vol. 30**, pp. 408-416.

[5] *Y. Srithep, T. Akkaprasa, D. Pholharn, J. Morris, S. J. Liu, P. Patrojanasophon, T. Ngawhirunpat*, Metronidazole-loaded polylactide stereocomplex electrospun nanofiber mats for treatment of periodontal disease, Journal of Drug Delivery Science and Technology, 2021, **vol. 64**, pp. 102582.

[6] *G. Greenstein, A. Polson*, The role of local drug delivery in the management of periodontal diseases: a comprehensive review, Journal of Periodontology, 1998, **vol. 69**, pp. 507-520.

[7] *V. S. B. Brey Gil, C. S. Brey Gil, G. A. Castro Goulart, R. L. Oréfice*, Multi-drug hybrid delivery systems with distinct release profiles based on gelatin/collagen containing vesicles derived from block copolymers, International Journal of Biological Macromolecules, 2019, **vol. 139**,

pp. 967-974.
[8] *G.T. Tihan, C. Ungureanu, R.C. Barbaresso, R.G. Zgârian, I. Rău, A. Meghea, M.G. Albu, M.V. Ghica*, Chloramphenicol collagen sponges for local drug delivery in dentistry, Comptes Rendus Chimie, 2015, **vol. 18**, pp. 986-992.
[9] *P. Kesharwani, A. Bisht, A. Alexander, V. Dave, S. Sharma*, Biomedical applications of hydrogels in drug delivery system: An update, Journal of Drug Delivery Science and Technology, 2021, **vol. 66**, pp. 102914.
[10] *Y. Liu, W. He, Z. Zhang, B.P. Lee*, Recent developments in tough hydrogels for biomedical applications, Gels, 2018, **vol 2**, pp. 46.
[11] *H. Madolia, S.D. Aurya*, Preparation and evaluation of stomach specific IPN hydrogels for oral drug delivery: a review, Journal of Drug Delivery and Therapeutics, 2013, **vol. 3**, pp. 131-140.
[12] *S. Mondal, S. Das, A.K. Nandi*, A review on recent advances in polymer and peptide hydrogels, Soft Matter, 2020, **vol. 16**, pp. 1404-1454.
[13] *S. Chatterjee, P.C. Hui, C.W. Kan*, Thermo-responsive hydrogels and their biomedical applications: special insight into their applications in textile based transdermal therapy, Polymers, 2018, **vol. 10**, pp. 480.
[14] *D.I. Udeanu, M.G. Albu Kaya, M.V. Ghica, Ș. Marin, M.M. Marin, D.A. Kaya, L. Popa, C.E. Dinu-Pîrvu*, Anti-inflammatory drug-loaded biopolymeric spongious matrices with therapeutic perspectives in burns treatment, Farmacia, 2018, **vol. 66**, pp. 783-790.
[15] *V. Trandafir, G. Popescu, M. G. Albu, H. Iovu, M. Georgescu*, Bioproducts based on collagen. Ars Docendi. 2007, pp. 84-85, București, România.
[16] *G. Greenstein*, The role of metronidazole in the treatment of periodontal diseases, Journal of Periodontology, 1993, **vol. 64**, pp.1-15.
[17] *J. E. Fonseca, M. L. Brandi*, Mechanism of action of strontium ranelate: what are the facts?, Clinical Cases in Mineral and Bone Metabolism, 2010, **vol. 7**, pp. 17-18.
[18] *J. Buehler, P. Chappuis, J. Saffar, Y. Tsouderos, A. Vignery*, Strontium ranelate inhibits bone resorption while maintaining bone formation in alveolar bone in monkeys (Macaca fascicularis), Bone, 2001, **vol. 29**, pp. 176–179.
[19] *M.G. Albu*, Collagen gels and matrices for biomedical applications, Lambert Academic Publishing, Saarbrücken. 2011.
[20] *S. M. Kelly, T. J. Jess, N. C. Price*, How to study proteins by circular dichroism, Biochimica et Biophysica Acta, 2005, **vol. 1751**, pp. 119-139.
[21] *M.M. Marin, M.G. Albu-Kaya, C.E. Stavarache, R.R. Constantinescu, C. Chelaru, J. Ghitman, H. Iovu*, Extraction and studies on the properties of type II collagen as potential biomaterial in cartilage repair. UPB Scientific Bulletin, Series B, 2021, **vol. 83**, pp. 229-238.
[22] *R. Gayatri, A. K. Sharma, R. Rajaram, T. Ramasami*, Chromium (III)-induced structural changes and self-assembly of collagen, Biochemical and Biophysical Research Communications, 2001**, vol. 283**, pp. 229-235.
[23] *M.V. Ghica, M.G. Albu, D.A. Kaya, L. Popa, Ş. Öztürk, L.C. Rusu, C. Dinu-Pîrvu, C. Chelaru, L. Albu, A. Meghea, C. Nitipir*, The effect of Lavandula essential oils on release of niflumic acid from collagen hydrolysates, Korean Journal of Chemical Engineering, 2016, **vol. 33**, pp. 1325-1330.