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Original scientific paper

ALGINATE BASED HYDROGELS STABILITY AND THEIR USE FOR CONTROLLED RELEASE OF KETOPROFEN

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Abstract

Alginate is a biocompatible, non-toxic and biodegradable natural polymer that has found wide application in biomedical science and tissue engineering. Alginate hydrogels are polymers with high water content in their network, which can be used as wound dressings and active substance carriers. Alginate based hydrogels can be obtained in different ways, the most common method to prepare hydrogels from an aqueous alginate solution is to combine the solution with ionic cross-linking agents, such as divalent cations (i.e., Ca^{2+}, Ba^{2+}). The stability of these gels in the physiological environment and the controlled release of active substances from their network are two very important properties that affects the use of these hydrogels for medical purposes. In this research is investigated stability of Ca^{2+} cross linked alginate hydrogels depending on gel-formation agents amount, in this case $CaCO_3$ and GDL. Also it is followed the controlled release of anti-inflammatory drug, ketoprofen that is loaded inside the alginate hydrogels, for different time intervals. Controlled release is followed in two aqueous media with pH=5.3 and pH=7.0, and analyzed through Ritger-Pepass and Pepass-Sahlin equations. The interpolation of obtained values according to these equations and the calculation of the respective coefficients give information on the factors which control the release in alginate hydrogels.

***Keywords**:* alginate, ionic polymerization, wound dressing, hydrogels, controlled release, ketoprofen

1. Introduction

Polymers are the materials that have found wide application in the field of medicine, where they are used in the form of various implants, as wound dressings, carriers of active substances, cells [8]. However, for a polymer to be used for medical purposes, it must meet certain conditions such as biocompatibility, biofunctionality, biodegradability, non-toxicity, etc [9]. As polymers with such properties are alginate hydrogels [1].

Alginate is a biomaterial that has found numerous applications in biomedical science and engineering due to its favorable properties, including biocompatibility and ease of gelation [9]. Alginate can be easily modified via chemical and physical reactions to obtain derivatives having various structures, properties, functions and applications. Alginate-based biomaterials can be utilized as drug delivery systems, wound dressing and cell carriers for tissue engineering [5]. Alginate is typically used in the form of a hydrogel in biomedicine, including wound healing, drug delivery and tissue engineering applications [9]. The most common method to prepare hydrogels from an aqueous alginate solution is to combine the solution with ionic cross-linking agents, such as divalent cations (i.e., Ca^{2+}, Ba^{2+}, Mg^{2+}, Fe^{2+}). The divalent cations are believed to bind solely to guluronate blocks of the alginate chains [9]. One of the most commonly used gel-forming agents during ionic crossbreeding is $CaCl_2$. Due to the solubility of $CaCl_2$ in water, its use as a gel-forming agent is not preferable as the gelling process is very fast and cannot be controlled. $CaSO_4$ and $CaCO_3$ can also be used as gel-forming agents, which due to their low solubility in water give a slow process of gel formation [9]. The speed of the gelling process is an important factor which affects

the uniformity and strength of the gels, the slow process ensures uniform structure and greater mechanical integrity .

Alginate hydrogels are polymers with high water content in their network, which can be used as wound dressings and active substance carriers. The stability of these gels in the physiological environment and the controlled release of active substances from their network are two very important properties, that affects the use of these hydrogels for medical purposes. Recent investigations in the area of controlled drug delivery have the potential to solve the problem of poor bioavailability through polymer-drug combinations that are able to release the active drug in a pre-designed manor [11]. In addition, controlled drug delivery allows for the maintenance of a desired range of drug levels, requires fewer administrations, and optimizes the therapeutic use of the drug. Specifically these delivery systems are ideal for the slow release of water soluble drugs, the fast release of low-solubility drugs, drug delivery to specific sites, drug delivery using nanoparticulate systems, delivery of two or more agents with the same formulation, and systems based on carriers that can dissolve or degrade and be readily eliminated [11].

Alginate hydrogel controlled release of drugs depends on the cross-linker types and cross-linking methods. In this work is studied the preparation of alginate hydrogels using ionic cross-linking method and controlled release of ketoprofen drug from these hydrogels in two different pH media. Obtained results are interpolated according Ritger-Pepass and Pepass- Sahlin equations and the corresponding coefficient values are calculated.

The transfer of active substances from the hydrogel to the external environment can be described according to Fick's theory of diffusion. However, when the hydrogel is in a physiological environment, other mechanisms such as swelling or erosion of the hydrogels also affect the controlled release. In cases when swelling and degradation of hydrogels affects the speed of the controlled release, the results obtained cannot be expressed through Fick's laws of diffusion. In these cases, other mathematical equations are used to interpolate the results, such as the Pepass equation, the Higuchi equation, the Ritger-Pepass equation or Pepass-Sahlin equation.

Ritger-Peppas equation given by the expression (1), can be used to analyze both, normal diffusion and anomalous diffusion, which occur as a result of the swelling of polymeric networks. Normal diffusion is the diffusion that can be described by Fick's laws, while the anomaly is the diffusion that cannot be described by Fick's laws.

$$\frac{M_t}{M_\infty} = k_1 t^n \tag{1}$$

Where M_t / M_∞ is the release fraction of the substance, n the diffusion exponent and k_1 the kinetic constant which includes the structural and geometric characteristics of the system. The values of exponent n determine the leading mechanism of controlled release, while k_1 represents the degree of network swelling [3].

Peppas-Sahlin equation given by expression (2), involves the release of the active substance from the Fick diffusion and from the II case of transport [14].

$$\frac{M_t}{M_\infty} = k_1 t^n + k_2 t^{2n} \tag{2}$$

where k_1 and k_2 are the kinetic release constants.

From this equation Fick's diffusion is described by the first member with the coefficient k_1, while the coefficient k_2 characterizes the controlled release as a result of the degradation and relaxation of the polymeric network [14].

2. Methods

2.1 Preparation of alginate gels with Ca^{2+} ions

For preparation of alginate hydrogels are used Na-alginic acid as base polymer, and $CaCO_3$ and GDL(D-glucono-δ-lactone) as cross-linking agents. Preparation of alginate hydrogels is based on the method of ionic polymerization, where calcium cations (Ca^{2+}) are used as cross-links between alginate chains.

Preparation of alginate hydrogels is started by dissolving alginic acid in deionised water. Alginate solutions can be prepared in different mass concentrations of alginic acid such as 1%, 1.5%, 2% and 2.5%.

Dilution of alginic acid in deionised water is carried out for 24 hours through continuous oscillations. The resulting solution is homogeneous, odorless and colorless. To form the gel, into the solution are added gel-forming agents $CaCO_3$ and GDL in a certain ratio.

After dissolving process the solution is poured into a test tube, then in the solution is added $CaCO_3$ and vortexed for one minute at high speed and after that in the mixture is added GDL and voretxed for 45 seconds. The mixture prepared in this form is poured into a small petry-dish and left to gelate. Gelation process can vary from 30 minutes to few hours depending on cross-linking agents amount.

Figure 1. Alginate hydrogels obtained by polymerization of alginates with Ca^{2+} ions

The gels obtained in this way have the shape of the container in which the gelling process is performed, are odorless, colorless and transparent. Figure 1. shows two hydrogels obtained by the method described above.

These hydrogels are stored in refrigerator in containers filled with distilled water. If these gels are placed in an open environment without water, after a period of two to three days, they will dry out and become very brittle. Even dried hydrogels are odorless, colorless and translucent.

2.2 Preparation of alginate hydrogels with incorporated gauze

In this paper is described preparation of alginate hydrogels with incorporated gauze. Preparation process of these hydrogels is the same as described above, but at the moment of pouring the mixture on the petry dish, inside the dish is placed cotton gauze. The gauze used consists of cellulose fibers and is very dense. The presence of gauze inside the hydrogel affects its chemical and mechanical properties. In figure 2. is shown an alginate hydrogel with incorporated gauze.

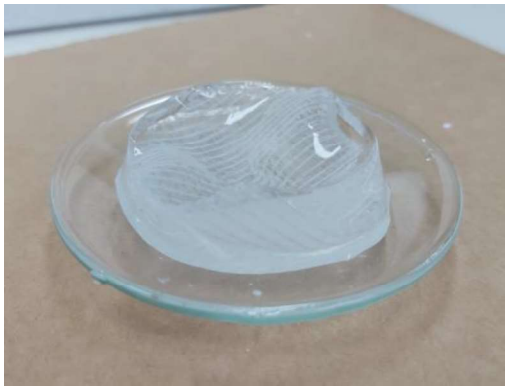

Figure 2. Alginate-based hydrogel with cotton gauze incorporated

2.3 Stability of alginate gels depending on the amount of $CaCO_3$

Gel-forming agents, in our case $CaCO_3$ and GDL, play an important role in the formation of alginate-based gels. During the formation of the gel $CaCO_3$ plays the role of the source of calcium ions (Ca^{2+}) that are responsible for the formation of cross-links between alginate molecules. The amount of $CaCO_3$ added to the alginate solution must provide sufficient ions (Ca^{2+}) to bind all the alginate molecules and gelling process to be complete. The lack of Ca^{2+} ions in the solution causes a portion of the alginate solution to remain unconnected, in this case gelling process will be partial. On the other hand, an excess of $CaCO_3$ in alginate solution will result with brittle hydrogels (solid, like gelatin) that can be crushed by small mechanical actions. Once $CaCO_3$ is insoluble in water, the alginate solution and gel will turn white when the excess amount can be clearly seen.
In this research are included gels with 0.5x, 1x, 2x and 3x $CaCO_3$ / GDL, where the reference value is 1x. During the research it was observed that the solution with 0.5x $CaCO_3$ / GDL did not complete the gelling process and the obtained gel had a non-flat surface. Solutions with 1x and 2x do not differ much from each other and the gelling process is complete, but with a 2x solution the gelling process is faster than with a 1x gel. Whereas, the 3x solution is significantly more rigid and the gelling process is significantly faster, but $CaCO_3$ particles scattered throughout the gel are also observed. This type of gel also begins to break down when trying to remove it from the container where the gelling process was performed.

2.4 Stability of alginate gels depending on the amount of GDL

$CaCO_3$ in alginate solution is added in powder form and as such is not soluble in water and alginate solution. $CaCO_3$ in alginate solution has no effect other than changing the color of the solution from pale yellow to white. For the release of Ca^{2+} ions from $CaCO_3$, is added GDL that changes the pH of the solution and initiates the gelling process.
Alginate gelling process depends directly on both the amount of $CaCO_3$ and the added amount of GDL. In addition, to the sufficient amount of $CaCO_3$, added in alginate solution, it is also necessary that the

amount of GDL to be sufficient to release all the necessary Ca^{2+} ions to begin and complete the alginate gelling process.
During the research, were analyzed these ratios of $CaCO_3$ / GDL, referring to the value 1x, 1:0.5; 1:1; 1:2; 1:3 and 1:4.The 1:0.5 ratio affected a portion of the alginate solution to remain unconnected and to form an hydrogel where exessive amount of $CaCO_3$ can be seen. The 1:1 ratio gave very sensitive hydrogels to mechanical action and somewhat more brittle. The 1:2 and 1:3 ratios do not differ much from each other, however the 1:3 ratio is more resistant to mechanical action. While the 1:4 ratio gave solid like gels glued to the walls of the vessel. It should be noted that the amount of GDL as well as $CaCO_3$ affects the speed of the gelling process, the larger amount results in faster process.

2.5 Loading of hydrogels with active substance ketoprofen

Loading hydrogels with the active substance can be done in several ways. One of the most widely used methods is the direct loading. By this method, at first it is prepared a solution with the active substance dissolved in water, then in this solution is added alginic acid and left to dissolve for 24 hours under continuous oscillation. After that, solution is ready for gelling process. The gelling process of active substance-loaded alginates is the same as the pure alginate gelling process.
The use of the direct loading method ensures homogeneous distribution of the active substance in the hydrogel and loading with known concentration.

2.6 Controlled release

The controlled release of the active substance ketoprofen has been investigated in deionized water environment with pH=6.9 and in acidic environment with pH=5.3. For the acidic environment solution of 0.2M CH_3COONa/ CH_3COOH was used.
Controlled release of the active substances ketoprofen was followed by placing 20 ml of alginate gel loaded with the active substance, as described above, in 20 ml of deionized water. Then at certain time intervals the absorbance of the water taken from the vessel was measured in wavelength 260 nm (Filippa, Melo and Gasull 2015). The water taken from the container after the measurement is returned to the container to maintain the initial volume of water. Same way is followed the controlled release of ketoprofen in acidic media.

3. Results

In this paper we are limited on the research of controlled release in samples synthesized with 2% alginate mass concentration and 1x $CaCO_3$ / GDL in ratio 1:3. On the other hand active substance mass concentration is different, taking values 0.05 g / L, 0.04 g / L and 0.03 g / L of ketoprfen. Also in this research is included a hydrogel with incorporated gauze and loaded with concentration of 0.05 g / L ketoprofen. Controlled release for this drug was followed on average of 140 minutes.

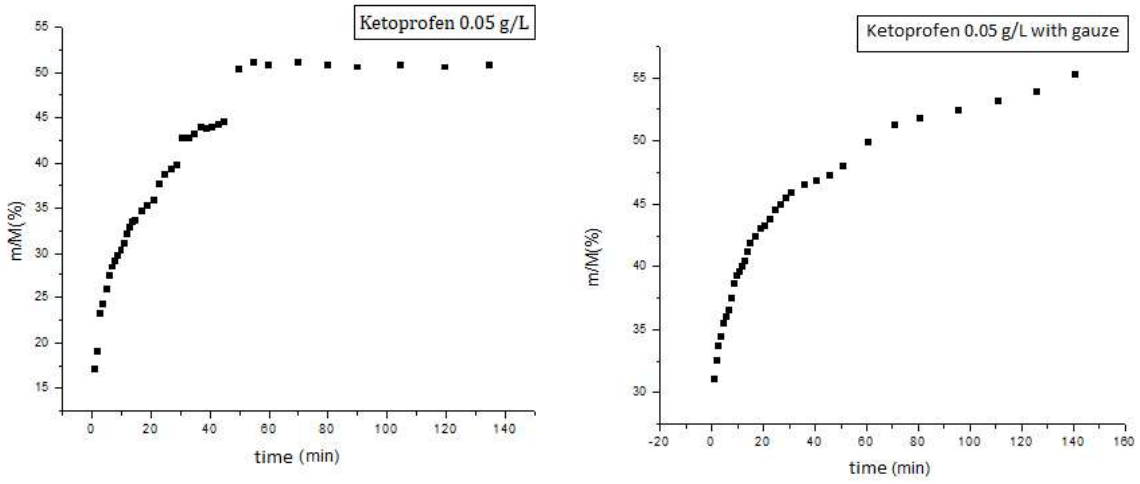

Figure 3. Graphical representation of the controlled release for ketoprofen with concentratin of 0.05 g/L for pure hydrogel and hydrogel with gauze, in aqoueus media with pH=7.In figure 3. is given the graphical representation of the controlled release for ketoprofen with concentration of 0.05 g/L for pure hydrogel and hydrogel with gauze, in aqoueus media with pH=7.0.

From the graphical presentation and interpolation of the values obtained from the controlled release, can be evaluated what are the mechanisms that control the release of active substances from hydrogels. Interpolation of values obtained by controlled release of active substance, ketoprofen, in aqueous and acidic media matches very well with the Ritger-Pepass and Pepass-Sahlin equations.

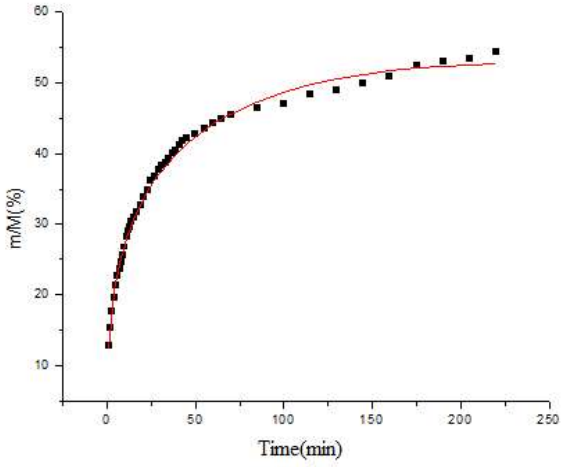

Figure 4. Interpolation of obtained values according to Pepass-Sahlin equation

The graph in figure 4. shows the compatibility of the interpolation of the obtained values with the equation described by Pepass-Sahlin.

Table 1. Coefficients of Pepass-Sahlin equation

Concentration of ketoprofen in the sample	n	k_1	k_2
Ketoprofen 0.05	0.37	15.95	-1.21
Ketoprofen 0.04	0.36	16.83	-1.76
Ketoprofen 0.03	0.16	11.69	5.29
Ketoprofen 0.05 with gauze	0.09	19.62	9.93
Ketoprofen 0.05 in pH=5.3	0.06	-66.53	83.21
Ketoprofen 0.05 with gauze in pH=5.3	0.11	0.86	21.12

Table 1. shows the values of coefficients k_1, k_2 and n from the Pepass-Sahlin equation for the controlled release of active substance ketoprofen from the analyzed networks.

4. Discussion

From the obtained values of the respective coefficients (Table 1.) it can be seen that the controlled release of the active substance ketoprofen is non-Fickian, which means that in the controlled release of this substance besides diffusion other factors such as swelling, erosion, permeation of the environment inside the pores and pore relaxation have also influenced this process. In each sample the values of the constants vary as in different concentrations and different networks one of the controlled release factors predominates, while the other factors are less pronounced. The minus sign in k_2 values means that the amount of water from the environment has penetrated inside the pores.

The values of the coefficient *n* obtained from the interpolation of the controlled release results do not match the predicted values, because the geometric shape of the samples used does not coincide with any of the forms for which this parameter is calculated. In addition to the geometric shape, another limitation for controlled release is the external environment whose volume is taken to be equal to the volume of the hydrogel. Controlled release of the active substance from the hydrogel into the external environment will be carried out until the concentration of the active substance in the external environment becomes equal to the concentration of the substance inside the hydrogel. The small volume of the external environment very quickly achieves saturation which prevents further release of the active substance.

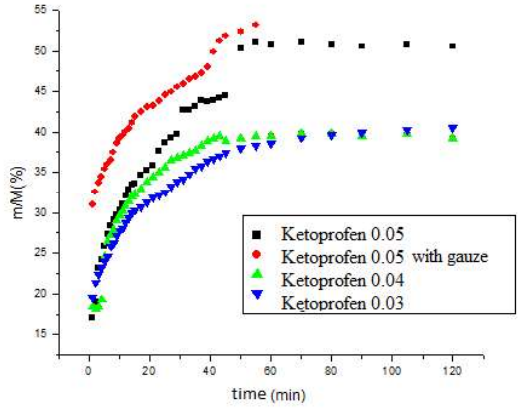

Figure 5. Graphical representation of the dependence of the controlled release for anti-inflammatory substance ketoprofen on the initial amount of the substance that gels are loaded

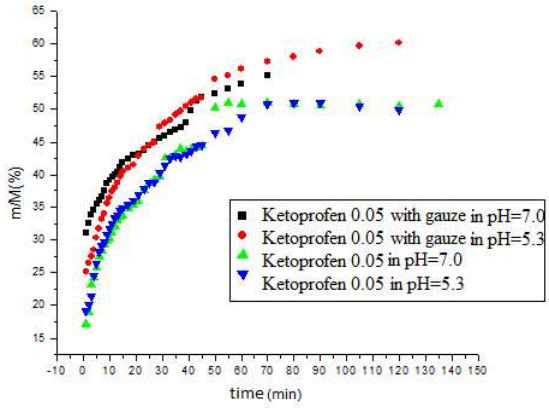

Figure 6. Graphical representation of the controlled release of the substance with initial concentration 0.05 g / L from polymeric networks with and without gauze incorporation, in aquous environment with pH=7.0 and in acidic environment with pH=5.3

Figure 5. shows the controlled release of the active substance ketoprofen from samples with different concentrations of the active substance. From the graph it can be seen that the controlled release is more intense for the time of 60 minutes while later this release is smaller. It is also noted that the amount of active substance released also depends on the initial amount with which the polymeric networks are loaded. The lower the initial concentration, the smaller the amount released, while with the increase of the initial concentration the released amount of the active substance also increases. From the graph we can see that from the sample with mass concentration of the active substance ketoprofen 0.05 g / L for 60 minutes 49% of the initial amount was released, from the sample with concentration 0.05g / L with gauze, for this time, 53%, from the sample with a concentration of 0.04 g / L, 47% of the initial amount was released and from the sample loaded with a concentration of 0.03g / L, 40% of the amount initially incorporated in the hydrogel was released.

Figure 6. shows the dependence of the controlled release of the substance ketoprofen with an initial concentration of 0.05 g/L with and without gauze, in aqueous environment and acidic environment. From the graph it can be seen that the controlled release in the acidic environment is faster and the released quantities of the active substance are higher compared to the quantities released in the aqueous environment, this is also observed for polymeric networks that have incorporated gauze inside them.

The controlled release of the active anti-inflammatory substance from the networks charged with a concentration of 0.05 g / L, located in the environment with pH = 5.3, for a time of 60 minutes has the following values: from the sample ketoprofen 0.05 g/L is released 51% of the initial amount, while from ketoprofen 0.05 g/L sample with gauze 58% of the initial amount incorporate in the sample was released.

5.Conclusion

The results obtained from the research of alginate-based hydrogels synthesis are a good indicator of the properties for these gels and their use for medical purposes. Synthesized hydrogels through the method of ionic polymerization, where $CaCO_3$ was used as a gelling agent, and GDL was used as the initiator of the polymeric process, have shown such properties and stability that enables their use as carriers of active substances and their controlled release.

In addition to the external environment, the amount of gel-forming agents also affects the stability of hydrogels. The small amount of these agents is insufficient for gelling process to be completed, while their excess causes changes in chemical and mechanical properties.

From the graphs above it can be seen that the initial concentration at which the hydrogels are charged and the pH value of the environment are two of the mechanisms which have influenced

the controlled release of active substances from the researched samples. Also it can be seen that the incorporation of gauze inside the hydrogel affects the controlled release of active substances, this effect comes as a result of its interaction with the hydrogel. The gauze used in this research consists of cellulose fibers, which is also a biopolymer, and such an interaction between hydrogel and gauze is natural.

In this research are prepared various alginate based hydrogels with different alginic acid concentrations, different gel forming agents amount and ratio. The best ones for this work are 1% and 2% alginic acid hydrogels with 1x, 1:3 gel forming agents ratio.

Research on the controlled release of the active substance ketoprofen shows that alginate hydrogels are a suitable choice for the incorporation of this substance into the structure of alginate based hydrogels.

From the results of the controlled release, ketoprofen-loaded alginate hydrogels show a rapid release of the active substance, especially in the first hour of investigation, enabling that in further use this drug to come relative quickly to the necessary region of the body, during external application through the skin or as tablets as well.

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