SYNTHESIS OF ORGANIC BIODEGRADABLE AEROGELS USED IN CONTROLLED DRUG RELEASE

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Abstract: A number of polymers used in the production of carriers for drug delivery applications, possessing high surface area, appropriate surface chemistry and porosity, chemical stability and low cost have been investigated. One of these promising candidates are natural polysaccharides such as alginic acid sodium salt, guar gum, xanthan gum and chitosan due to their outstanding merits. They are similar to extracellular matrix having high chemical versatility, good biological performance and cell or enzyme-controlled degradability.

Alginates were used in the present research for synthesis of organic biodegradable gels by sol-gel process, which were further easily converted to aerogels by supercritical drying. They are safe for use, stable, nontoxic, and derived from renewable sources. Alginates undergo reversible gelation in aqueous solution through interaction with divalent cations such as Ca^{2+}, which create ionic inter-chain bridges. Two fundamental methods of ionic cross-linking were used to prepare alginate hydrogels: the diffusion method and the internal setting method. After producing the hydrogel, alcogels were formed by solvent exchange using 100% ethanol. Ethanol was later replaced by liquid CO_2 with supercritical drying (at 7.375 MPa and 304.14 K). Aerogels made from natural polysaccharides combine both biocharacteristics and aerogel characteristics such as very high porosity and specific surface area, which makes them really attractive in drug delivery applications. The aerogels obtained in present research were therefore studied as drug carriers. The effects of the alginate composition and synthesis on model drug nicotinic acid release were investigated.

INTRODUCTION

There are a lot of synthetic polymers which can be used for controlled drug delivery, however they are not easily accepted by the organism. Also incorporation of drugs into carriers runs under difficult conditions. Therefore scientists have been inclined to use natural-origin polymers, such as proteins and polysaccharides [1]. Polysaccharides have properties that give them advantages over synthetic polymers. They can be extracted in large quantities from renewable sources, such as algae, plants and microorganisms or produced by recombinant DNA technique. They exist in different compositions with characteristics which can not be reached in chemical laboratory by changing preparation parameters. These natural biomaterials have been proved to be safe, stable, non-toxic, renewable and have low costs because of their abundance in nature and ease of processing. Polysaccharidic polymers have been used as thickeners, stabilizers, suspending agents, gelling agents, film formers, emulsifiers, lubricants and in medicine as scaffold materials in tissue engineering and as carriers for drug delivery [2]. Alginate is a well known

polysaccharide, which has been widely used because of his gelling characteristics in water solutions [3]. Alginate is water soluble polysaccharide, extracted from brown see algae. It is a linear copolymer with homopolymeric blocks of (1-4)-linked β-D-mannuronate (M) and its C-5 epimer α-L-guluronate (G) residues, respectively, covalently linked together in different sequences or blocks (MM, GM, GG). Chemical and physical characteristics of alginate strongly depend on ratio, distribution and length of M and G chains. Kinetics of gel formation is usually very fast, final product-gel is strong enough to be used in many industrial and biomedical branches. Alginate characteristics like biocompatibility, mucoadhesion, porosity and simple manipulation has attracted plenty of attention in delivery of proteins, regeneration of tissue and cell encapsulation. To optimize release modeling of active substances from formulations, scientists focused on improvement of mechanical stability and erosion resistance in different organic solvents with modification and extra surface cross-linking.

Alginate based materials are pH dependent. If taken orally, alginate undergoes biodegradation. In the gastrointestinal tract pH varies from acidic conditions in stomach to lighty basic conditions in the intestines [1]. Theoretically alginate shrinks at low pH and encapsuled active substance is not released. At higher pH values alginate becomes degradable and active substance is released. Release of biomolecules from such systems can be therefore predicted and better developed. Biocharacteristics and mild gelling conditions classify alginate on the top of natural-origin polymers, used in tissue engineering and encapsulasion of orally delivered drugs.

Many polysaccharidic hydrogels for drug delivery have been prepared, but one of their weakness is their short life in dry air condition; thus, scientists are developing special coating materials for enhancing their life time to several months or more instead of the current level of several hours. On the other hand, aerogels prepared with supercritical drying of hydrogels after immersion in alcohol are dry and stable materials, which makes them really attractive as a substitute for hydrogels. Aerogels [4] of natural polysaccharides also combine both biocharacteristics and aerogel characteristics like very high porosity (up to 99%) and specific surface areas (≈300 m^2g^{-1}). Higher carrier porosity could increase drug loading capacity. Moreover, release of drugs with low solubility and diffusion would be increased by high surface area of carrier. Spherical alginate aerogels have already been synthesised, but used as precursors of dispersed oxide phases [5].

MATERIALS

In this research alginic acid sodium salt (Sigma, 250 cP, 2 % (25 °C) viscosity) was used as a source of polysaccharidic monomers in the sol-gel synthesis of hydrogel. Alginate solutions were prepared using distilled water. For the ionic cross-linking calcium chloride, sodium hexa-metaphosphate and $CaHPO_4$ were used. Glucono-δ-lactone was used to reduce pH. For drug dissolution experiments 0.05 M potassium phosphate buffer solution of pH 6.5, adjusted with 2M sodium hydroxide, was prepared. Nicotinic acid (purchased from Sigma-Aldrich) was chosen as a model drug.

METHODS

Alginates were used in the present research to prepare organic biodegradable aerogels. Polysaccharides are biopolymers composed of simple sugar monomers. The macromolecules are linked together by hydrophilic groups such as hydroxyl, carboxyl, and amino groups, which are able to form non-covalent bonds with biological tissues, promoting bioadhesion [6]. Their physical properties depend on monomer composition, chain shape, viscosity and molecular weight, which influence solubility, gelation and surface properties. Polysaccharides can be cross-linked by chemical or physical interaction. Parameters which affect mostly the chemical cross-linking are concentration of cross-linking agent and time. Rapid cross-linking is induced by high cross-linking agent concentration. Chemical cross-linking brings good mechanical stability, but cross-linking agents are often toxic compounds and traces of unreacted agents should be extracted from gel before their application. Thus it is better to use physically cross-linked gels. For physical cross-linking different methods are applied. One of them is cross-linking by ionic interaction. Alginates are able to undergo reversible gelation in aqueous solution through interaction with divalent cations such as Ca^{2+}, Sr^{2+} or Ba^{2+}, which create ionic inter-chain bridges [7], with monovalent cations like Na^{+} gelation does not occur. Alginate is a polysaccharide with mannuronic and guluronic acid residues. The gel is formed because guluronic parts bind to cations and create three dimensional networks. However, some polysaccharides like guar gum need a chemical cross-linker to form a network.

Two methods of ionic cross-linking were used in the present research, resulting in different gel shapes. Using diffusion method ionically cross-linked hydrogel spheres were obtained by dropwise addition of a 1% and 2% sodium alginate solution with low viscosity into a 0.24 M $CaCl_2$ solution. After obtaining hydrogel, the alcogels were formed by solvent exchange using 100% ethanol. The hydrogel spheres were dehydrated by immersion in a series of successive ethanol–water baths of increasing alcohol concentration (10, 30, 50, 70, 90 and 100%). If 100% ethanol was added immediately, the structure of the gel may be affected, because organic hydrogels tend to shrink in organic solvents. Ethanol was later removed by liquid CO_2 with supercritical drying (103 bar, 35°C). Obtained organic biodegradable spheres are shown in Figure 1. The ionic cross-linking by the internal setting method was started with preparation of 2% sodium alginate solution with low viscosity. Then sodium hexa-metaphosphate (1,5 % w/w) was added, the mixture was stirred for 30 min and heated up to 40°C. Then $CaHPO_4$ was added followed by mixing for 60 min (eq.1). After cooling, a solution of glucono-δ-lactone was added to reduce pH. The resulting mixture was poured in cylinder molds and left covered overnight in refrigerator to solidify. The obtained hydrogel matrix was cut in smaller pieces and dehydrated by immersion in a series of successive ethanol–water baths of increasing alcohol concentration (10, 30, 50, 70, 90 and 100%). Ethanol was later removed by liquid CO_2 by supercritical drying (103 bar, 35°C) and aerogel matrix was obtained (Figure 2). Here the gel casting process is controlled by chelator ($(NaPO_3)_6$) and initiator ($C_6H_{10}O_4$). Chelator and Ca ions react with each other and form a three-dimensional network (eq. 2). After addition of glucono-δ-lactone, the complex decomposes and calcium ions are released (eq. 3). Gelation process occurs [8].

$$2\text{Na-alginate} + Ca^{2+} \rightarrow \text{Ca-alginate} + 2Na^{+} \quad (1)$$

$$6PO_3^{-} + 3Ca^{2+} \leftrightarrow Ca_3(PO_3)_6 \quad (2)$$

$$Ca_3(PO_3)_6 + 3C_6H_{10}O_4 \leftrightarrow 6HPO_3 + 3Ca^{2+} + 3C_6H_8O_4^{2-} \quad (3)$$

Figure 1: Alginate aerogels obtained by ionic cross-linking using ionic diffusion method.

Figure 2: Alginate aerogels obtained by ionic cross-linking by the internal setting method using chelator and initiator.

PREPARATION OF DRUG LOADED AEROGELS

Different methods can be used to load aerogels as drug carriers, during sol-gel process or after the supercritical drying [9]. In our experiments, model drug nicotinic acid was added to the solution after mixing sodium alginate with distilled water for 1 h in the case of spherical gels and for 2 h in the case of monolith gels at room temperature. Then the procedure was the same as mentioned above in the synthesis. Nicotinic acid was chosen as a model drug, because of its characteristics, especially solubility, which is 1.67g/100ml in water at 25°C, 0.7g/100ml in ethanol at 25°C and $1.4*10^{-6}$ (mole fraction) in supercritical CO_2 at 100 bar and 35°C.

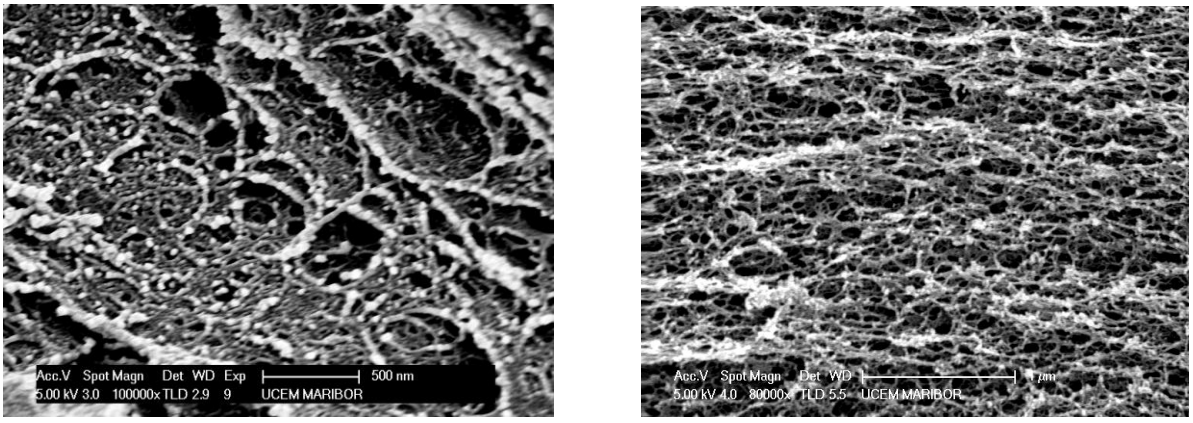
Drug dissolution tests were carried out in an apparatus FARMATESTER-3 (Dema-Ilirska Bistrica). It consists of a covered glass vessel immersed in a thermostated bath, a motor, drive shaft with paddle and a cylindrical stainless steel basket. Weighed amount of alginate gel was placed in the cylindrical basket beneath the paddle to retain the sample on the bottom. The

basket was immersed into the vessel containing 900 mL of buffer solution with pH 6.5 at 37±0.5°C and left under stirring for 24 h. The stirring speed was 50 rpm as it is required by Department of Health and Human Services, Food and Drug Administration. Dissolution testing should be carried out under mild conditions, by the basket method at 50/100 rpm or paddle method at 50/75 rpm [10]. Aliquots of 2 mL were withdrawn and subjected to drug assay by means of UV spectrophotometry at 262 nm using a Varian, Cary 50 Probe spectrophotometer. The taken dissolution medium was replaced with the same amount of fresh buffer solution. The percentage of drug released was calculated and plotted against time. The loading efficiency was calculated by dividing the quantity of drug loaded in the carrier by the mass of the carrier (eq. 4).

$$LE = \frac{(drug)g}{(aerogel)g} \cdot 100 \qquad (4)$$

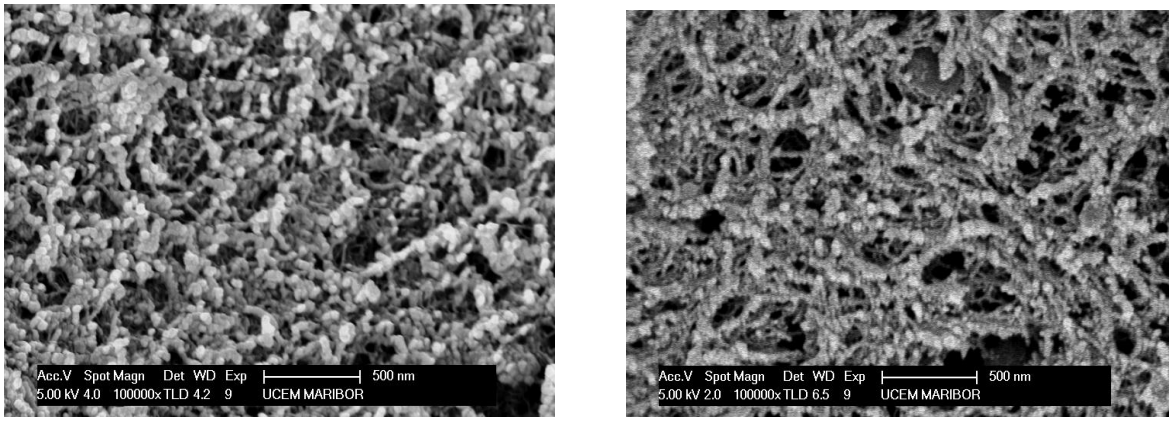
RESULTS

The surface morphology of alginate aerogels was determined using a scanning electron microscope Sirion 400 NC. The samples were sputter coated with gold and then scanned at an accelerating voltage of 3 kV. In the case of spherical aerogels in Figure 3 apparent difference of the gel prepared from 0.75% and 1.5% alginate solution can be observed. The surface of the aerogel made of 1.5 % alginate solution looks uniformly cross-linked in comparison to the surface of aerogel made of 0.75 % alginate solution. Apparently 0.75% alginate solution gives us less cross-linked gel with different nanosized pores and also regions of linear chains. It can be concluded from these results that with increasing the concentration of alginate solution more cross-linked and therefore more compact gels are obtained.



a) b)

Figure 3. SEM images of alginate spherical aerogel samples made of a) 0.75 % alginate solution; and b) 1.5 % alginate solution.



a) b)

Figure 4. SEM images of a) alginate spherical aerogel; and b) monolithic alginate aerogel samples made of 2 % alginate solution filled with nicotinic acid.

Figure 4 presents SEM images of alginate spherical aerogel and monolith alginate aerogel samples made of 2 % alginate solution, filled with nicotinic acid. Monolithic aerogels made of different % of alginate solution gave us similar SEM pictures as in Figure 4b. It seems that by the internal setting method uniformly cross-linked aerogels are obtained irrespective of alginate concentration. During the synthesis Ca^{2+} ions have time to interact with alginate chains and cross-link slowly and controlled. If we compare pictures 4a and 4b, the difference can not be observed in their structure. But results from drug release kinetics in Figure 5 show prolonged release and higher amount of drug released from monolithic than from spherical samples. After 24 h 80 % of drug is released from spherical aerogel and 85 % of drug from monolithic aerogel.

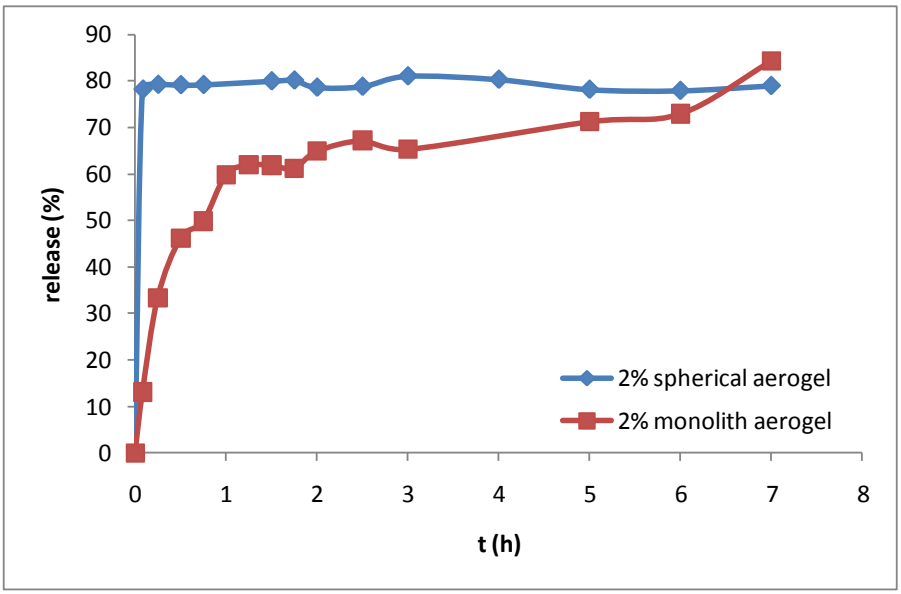


Figure 5. Nicotinic acid release kinetics for a) 2% spherical alginate aerogel and b) 2% monolithic alginate aerogel.

Loading of alginate aerogel samples with nicotinic acid with different alginate concentration was also determined. From Table 1 it can be seen, that loading of the aerogels is higher in the case of spherical samples. When the concentration of alginate in the case of monolithic aerogel increases, loading increases too. Interpretation of these results could be that less shrank aerogels and therefore larger surface areas and overall pore volumes are obtained at higher alginate concentration (Table 2), which could retain more drug even after immersion in 100% ethanol and after supercritical drying. According to T. Mehling [9] the main shrinkage of alginate hydrogel occurs during solvent exchange, but it hasn't been studied yet how physical parameters influence volume decrease. As it was described by D. K. Rassis [11] fillers can be used to strengthen mechanical properties of prepared gels. If we compare shrinkage of hydrogels, synthesized with and without drug incorporation (Table 2), gel shrinkage decreases with addition of drug. In the case of 1.5% alginate concentration, shrinkage of gel decreases from 63 %, when gel is synthesised without filler, to 36 %. It can be concluded that drug in our case works as filler. Moreover, higher the concentration of alginate, more stable cross-linked networks are obtained and resulting shrinkage is smaller.

Spherical aerogels drug loading decreases with alginate concentration. It would be expected the same as in the case of monoliths. Spherical aerogel sample shrinkage is smaller at the end of supercritical drying in comparison to monoliths. That could be the reason for higher drug loading.

Table 1. Experimental data for the loading (%) of monolithic and spherical aerogels of different alginate concentration with model drug nicotinic acid.

	Alginate conc. (%)	Loading (%)
monolith	1.5	2.2
	2	2.8
	2.5	4.1
sphere	1.5	21.0
	2	16.4
	2.5	11.6

Table 2. Effect of alginate concentration (250 cP) on shrinkage of gel synthesised using the internal setting cross-linking method after immersion in 100 % ethanol.

Alginate c. (%)	Shrinkage of hydrogel without drug (%)	Shrinkage of hydrogel, when drug added (%)	S_{bet} of aerogel (m^2/g)	Overall pore volume of aerogel (cm3/g)
1.5	63.3±5	36±6	138.6005 ± 1.2744	0.840 ± 0.003
2	65.4	32±4	151.8110 ± 1.2596	1.14 ± 0.005
2.5	44.6±5	32±4	218.8842 ± 2.1119	1.549±0.006

CONCLUSION

In this work, biodegradable alginate aerogels with different shape and ionic cross-linking were produced and further used as drug carriers. With regard to results we can conclude, that ionically cross-linked aerogels obtained by synthesis using diffusion method exhibited higher

loading in comparison to monolithic ionically cross-linked aerogels obtained by the internal setting method. However their drug release kinetics is too fast. That is why monolithic alginate aerogels seem to be more suitable in controlled drug release. With increasing alginate concentration during the synthesis in both types of synthesis more compact and cross-linked aerogels were obtained.

It was also proved, that incorporation of drug during sol-gel synthesis of monolithic alginate hydrogels decreased alginate gel shrinkage (from 63% to 36% in the case of 1.5% alginate concentration). The effect was stronger than if the drug was added at the end of synthesis.

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