Alginate Multi-membrane Spherical Aerogels for Ionic and Non-ionic Drug Release

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ABSTRACT

The aim of the present study was to compare the influence and efficiency of two gelling ions Ba^{2+} and Ca^{2+} when forming alginate multi-membrane spherical aerogels that retain, protect, and deliver model drugs to the gastrointestinal tract. In this research we used a multi-step gelation process for generating complex organic biodegradable aerogels with three-dimensional multi-membrane onion-like architectures with potential applications as carriers in oral drug delivery. After obtaining multi-membrane hydrogels, the alcogels were formed by solvent exchange using ethanol. The ethanol was later removed by supercritical drying using CO_2 at 103 bar and 40°C.
The effect of the number of membranes was investigated regarding the loading and release of the model drugs nicotinic acid and theophylline. Moreover, the efficiencies of Ba^{2+} and Ca^{2+} metal ions for forming tridimensional networks that retain and extend drug release were also investigated. Nicotinic acid release was prolonged by adding membranes around the core and using Ca^{2+} for cross-linking. However, retarded theophylline release was only obtained by using Ba^{2+} for cross-linking. Namely, by increasing the number of membranes and $BaCl_2$ concentration drug release became linear versus time in all studied cases. In the case of nicotinic acid loading increased by adding membranes around the core, however, for theophylline the opposite results were obtained due to the different nature of the model drugs.

INTRODUCTION

Significant naturally-originating polymers, such as proteins and polysaccharides, have been successfully used in oral drug delivery. They should possess appropriate surface chemistry and porosity, chemical stability, and have low production costs. Over recent years many polysaccharidic hydrogels for drug delivery have been prepared, but their short lives under dry air conditions has led scientists towards developing special coating materials for enhancing their lifetimes. On the other hand, aerogels obtained after sol-gel synthesis followed by supercritical drying are dry and stable materials, and are for this reason really attractive as substitutes for hydrogels. Aerogels of natural polysaccharides combine bio-material characteristics, such as good biological compatibility and cell or enzyme-controlled degradability, with aerogel characteristics such as very high porosity and specific surface areas.
In general, when administering drugs a better absorption rate is achieved by the body by using controlled drug delivery products from biodegradable polymers, thus improving their therapeutic effects. Therefore, the pharmaceutical industry has become interested in the

development of biodegradable drug carriers for oral drug delivery. Moreover, supercritical fluid technology using high pressure and supercritical fluids has already been used to prepare different biodegradable aerogels [1-5]. Aerogels as nano-porous materials exhibit unusual properties, such as high surface area, high porosity and mechanical strength. Higher carrier porosity could increase drug loading capacity. Moreover, the release of drugs with low solubility and diffusion could be increased by higher surface areas of the carriers. Supercritical fluid technology has also many advantages in comparison with conventional processes. The presence of organic solvents in products for medical or pharmaceutical applications is lowered and the technology also guarantees a better removal of these solvents without exposing drugs to high temperatures and resulting degradation, especially when using protein drugs or enzymes. Supercritical carbon dioxide, most frequently used as a solvent during supercritical processes, is also non-toxic, non-flammable, and inexpensive. At the end of the drying process it is completely removed from the dried material by depressurization, and can be recovered and reused.

MATERIALS AND METHODS

Materials. The alginic acid sodium salt obtained from commercial brown algae (Sigma, 250 cP, 2 % (25°C) viscosity); was purchased from Sigma-Aldrich. Calcium chloride and barium chloride were used as ionic cross-linkers. Nicotinic acid and theophylline anhydrous (purchased from Sigma-Aldrich) were chosen as the model drugs.

Synthesis of organic alginate aerogels. Ionically cross-linked spherical cores were obtained by the drop-wise addition of a 1.5 % sodium alginate solution with low viscosity into a 0.2 M $CaCl_2$ or $BaCl_2$ solution. The cores were kept for 1 h in salt solution for better cross-linking. Filter paper was used to absorb the surface water and the cores were later immersed within 0.75 % alginate solution for 1 min under gentle agitation. The resulting spheres were filtered through a 1000-2000 μm sieve and rapidly dropped into the $CaCl_2$ or $BaCl_2$ solution, where they were kept for 5 min. The process described above was repeated as many times as necessary for obtaining a spherical multi-membrane hydrogel with the desired number of membranes. Multi-membrane hydrogels were prepared having 3, 5, or 8 membranes.
The multi-membrane alcogels were formed by solvent-exchange inside multi-membrane spherical hydrogels, using ethanol. The hydrogel spheres were dehydrated by immersion within a series of successive ethanol-water baths at increasing alcohol concentrations (10, 30, 50, 70, 90, and 100 %). We avoided using 100 % ethanol immediately because of the risk of affecting the gel structure. Supercritical drying using CO_2 (103 bar, 40°C); as previously described in detail by [6], was later used to remove the ethanol.

Preparation of drug-loaded aerogels. The model drugs nicotinic acid and theophylline were added during alginate solution preparation. Next the procedure for the synthesis of hydrogel was the same as that mentioned above. Each of the inner surfaces between core and membrane or between two membranes was covered with the model drug. In this way free inter-membrane spaces were filled. Nicotinic acid and theophylline were chosen as model drugs, because of their characteristics, especially solubility. If a model drug is added during the synthesis of gel, then it is good if it is insoluble or less soluble in ethanol and supercritical CO_2, in order to achieve higher drug loading inside the carrier. The solubility of nicotinic acid in water is 1.67g/100mL at 25°C, in ethanol 0.73g/100mL at 25°C, and in supercritical CO_2

$1.4*10^{-6}$ (mole fraction) at 100 bar and 40°C. The solubility of theophylline in water is 0.76g/100mL at 25°C, in ethanol 0.35g/100mL at 25°C, and in supercritical CO_2 $0.5*10^{-6}$ (mole fraction) at 100 bar and 40°C.

Analytical methods. The dissolution studies of nicotinic acid and theophylline were performed under sink conditions in a FARMATESTER-3 (Dema-Ilirska Bistrica) apparatus at a stirring rate of 50 rpm at 37±0.5°C in 900 ml of buffer solution with pH 6.5 for simulating intestinal fluid. Samples (2 ml) were drawn and subjected to drug assaying by means of UV spectrophotometry at 262 nm (nicotinic acid) and 272 nm (theophylline).
Specific surface area mostly influences diffusion and release of the drug. Therefore specific surface area, total pore volume and pore diameter of monolithic and spherical alginate aerogel samples were determined, namely by nitrogen physisorption at -196 °C using a Micrometrics ASAP 2020MP instrument.
A Sirion 400 NC scanning electron microscope was used for determining the surface morphology of multi-membrane alginate spherical aerogels. The samples were sputter-coated with gold particles and then scanned at an accelerating voltage of 5 kV.

RESULTS

The N_2 adsorption measurements for the spherical core and membrane alginate aerogel samples (Table 1) indicate that for the Ca-derived aerogels the specific surface area was slightly higher (above 400 m2/g) in comparison with Ba-derived aerogels. In the case of Ba cross-linked aerogels the average pore diameter and overall pore volume was higher for the core samples, which was opposite to the results obtained for Ca derived aerogels. A possible explanation could be the ion size. The diameter of the barium ions is larger and therefore more time is needed for them to diffuse inside the viscous alginate solution for interior cross-linking of the core, but after a certain time, stronger and denser core gel structures are formed than with Ca ions, due to alginate's affinity towards the different divalent ions, resulting in higher overall pore volume. It can be concluded from the average size of the pores and the high specific surface area, that alginate multi-membrane aerogel is a mesoporous material.

Table 1: Porosimetric measurement of membrane and spherical core aerogel samples

Aeroge	S_{BET} (m^2/g)	Overall pore volume (cm^3/g)	Average pore diameter (nm)
core (0.2M $CaCl_2$)	402 ± 12	0.66 ± 0.05	8.8 ± 0.4
membrane (0.2M $CaCl_2$)	419 ± 11	2.21 ± 0.04	20 ± 4
core (0.2M $BaCl_2$	359 ± 25	1.73 ± 0.02	22 ± 2
membrane (0.2M $BaCl_2$)	362 ± 10	0.98 ± 0.10	13 ± 3

S_{BET}-specific surface area

Alginate multi-membrane spherical aerogels were loaded with model drugs during the sol–gel synthesis. Spherical aerogels were prepared, having 3, 5, and 8 membranes. The results for drug loading are presented in Table 2. Aerogel loading increased with the number of membranes in the case of samples filled with nicotinic acid. These results suggest that the alginate cross-linked ionic membranes worked as barriers for the ionic model drug. Namely, during solvent exchange and supercritical drying, where the loss of nicotinic acid may appear, the membranes were able to retain most of the drug inside the gel. The same procedure of drug loading was applied for the preparation of theophylline loaded spherical aerogels. The

obtained results show that loading decreased in line with the increasing number of membranes, which is contrary to the results obtained for nicotinic acid loading. The probable explanation could be in the natures of the model drugs, namely nicotinic acid is an ionic drug and theophylline is non-ionic. Gels consisting of ionic polymers such as alginate are expected to interact with ionic drugs, whilst no interaction occurs between the carrier and non-ionic drug, therefore theophylline could have diffused faster from the carrier already during the formulation preparation, thus resulting in lower drug loading.

Table 2: Effect of membrane number on nicotinic acid and theophylline loading of multi-membrane spherical aerogels

Membrane number Ionic cross-linker	Loading (%) nicotinic acid 0.2M $CaCl_2$	Loading (%) theophylline 0.1 M BaCl2
3	53.5	644.8
5	104.2	183.0
8	179.3	71.0

For the drug release studies, nicotinic acid and theophylline as model drugs were entrapped inside the multi-membrane aerogel samples with different numbers of membranes. Both model drugs have good water solubility and high permeability at pH 6.5, therefore the release behaviour is strongly influenced by the nature of the carrier. In our case, drug release occurs through a combination of all three basic mechanisms; release by swelling control, by pure diffusion and by polymer degradation.

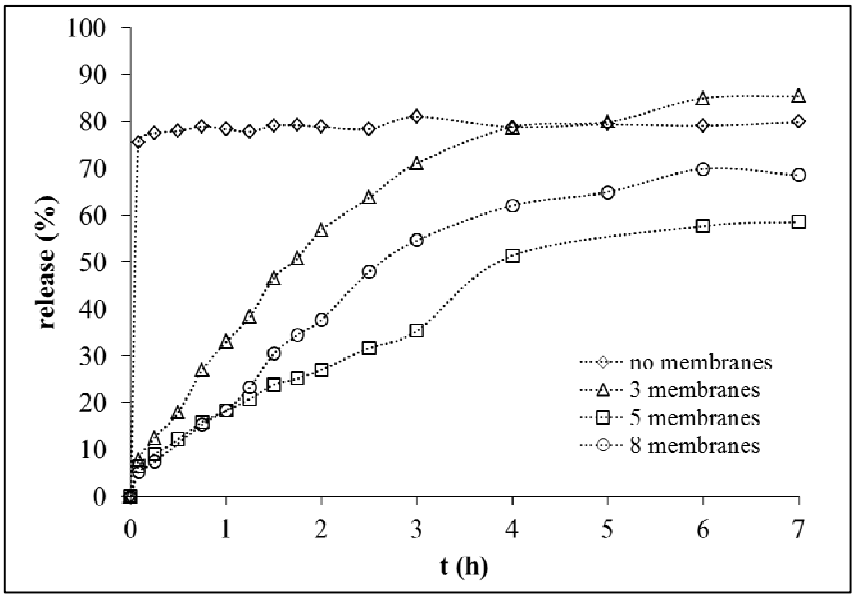


Figure. 1. Nicotinic acid release kinetics from spherical multi-membrane alginate aerogels with 3, 5 and 8 membranes around the core cross-linked using 0.2 M $CaCl_2$.

From Fig. 1 it can be seen that there was a burst of nicotinic acid released from the aerogel carrier. Therefore, the membranes of alginate were synthesised around cores for possible inhibition of the drug release. Fig. 1 also presents nicotinic acid release from multi-membrane alginate aerogels, cross-linked using 0.2 M CaCl2. When comparing the release behaviour of the core and of the multi-membrane aerogel samples, it is obvious that the membranes

prolonged drug release. Moreover, in the case of the 5 membranes, the release became close to linear versus time. However, the samples with 8 membranes exhibited higher release rates after the first hour than the samples with 5 membranes. Obviously at one point, when drug loading was already very high inside the carrier, the efficiency of the membranes as drug barriers was outweighed by the amount of drug. We can conclude that the ionic nanomembranes worked as barriers for ionic nicotinic acid during the drying process, as well as for successfully retaining the drug inside the carrier during the release experiments.

Alginate spherical aerogels cross-linked with Ca^{2+} ions were also researched as carriers for theophylline. Increased number of alginate membranes around the core insignificantly affected theophylline release. Namely, samples with 3 and 5 membranes both reached equilibrium at 4 hours and the release of the drug at that time was 90–95%. These results confirm the fact that non-ionic drugs do not bind onto ionic polysaccharidic gel and are therefore released faster than ionic drugs. To obtain linear theophylline release, alginate carriers had to be prepared some other way than by increasing alginate concentration or number of membranes. Due to the higher affinity of alginate towards divalent Ba^{2+} ions, their efficiency as cross-linking agents for the retention of theophylline inside multi-membrane spherical alginate aerogels was determined in comparison with those spherical aerogels, cross-linked using Ca^{2+} ions.

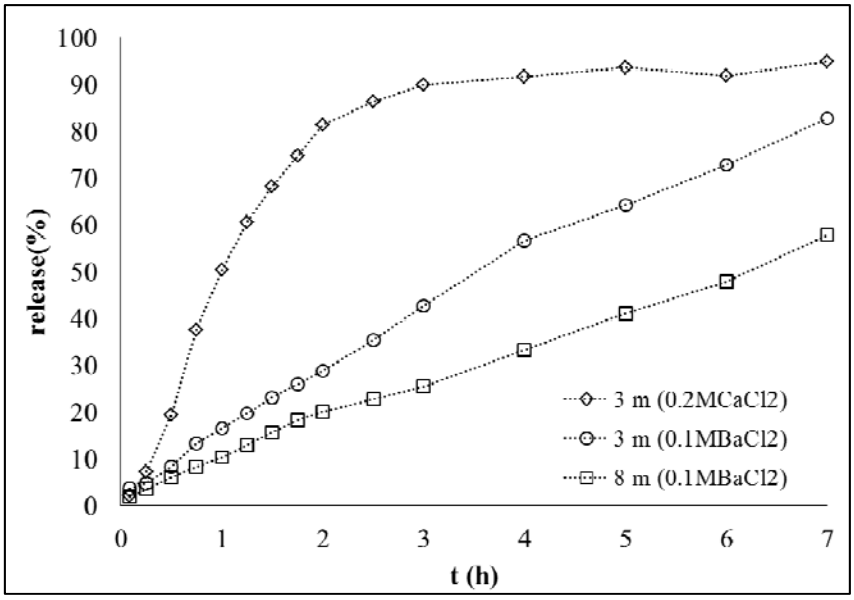
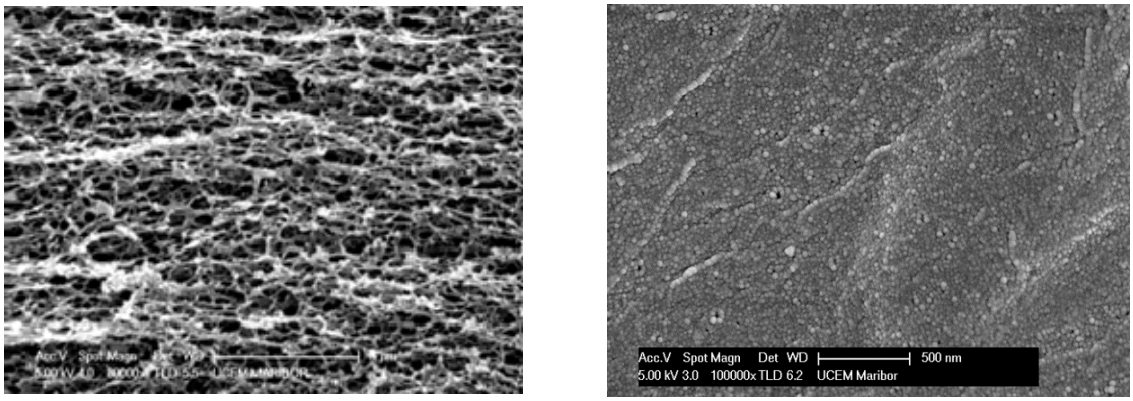


Figure 2. Theophylline release kinetics for spherical multi-membrane alginate aerogels with different number of membranes around the core using different ions (Ca^{2+} or Ba^{2+}) for cross-linking: *3m*, 3 membranes; *8m*, 8 membranes.

The release profiles of theophylline from spherical multi-membrane alginate aerogels with different numbers of membranes around the core and cross-linked by different ions (Ca^{2+} or Ba^{2+}), are presented in Fig. 2. The obtained results show the inhibited and also linear release of the model drug when using Ba^{2+} ions instead of Ca^{2+} ions at even lower salt solution concentrations, used for cross-linking. Thus by the addition of more membranes around the core the release of the model drug was successfully further slowed down. These results were confirmed by SEM images (Fig. 3), where a denser structure was obtained for Ba-derived aerogels.



(a) (b)

Figure 3: SEM images of alginate spherical multi-membrane aerogel sample, cross-linked using (a) 0.2M $CaCl_2$ and (b) 0.1M $BaCl_2$.

CONCLUSION

During the presented research, ionically cross-linked multimembrane spherical aerogels were obtained using a multi-step sol–gel process, they were further used as carriers for ionic and non-ionic drug and the drug release kinetics were studied. Up to 8 membranes were produced around the cores. With higher number of membranes, higher drug loading was obtained in the case of nicotinic acid. Opposite results were obtained for theophylline due to drug characteristics. The drug release kinetics from the spherical core alone was very fast and was successfully prolonged with increasing the number of alginate membranes around the core. When theophylline was incorporated within the carrier, sustained drug release was achieved by using Ba^{2+} ions instead of Ca^{2+} ions, resulting in even more extended release and also linearity, namely 50% of the drug was released in 6 h. Data from the release kinetics indicated that the drug release mechanism is dependent on drug diffusion and the erosion of the polymer matrix. According to these results showing sustained drug release for ionic and non-ionic drugs, alginate multi-membrane spherical aerogels can be safely used as sustained drug carriers.

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