ENCAPSULATION OF PHARMACEUTICALS INTO PECTIN AEROGELS FOR CONTROLLED DRUG RELEASE*

Gabrijela Tkalec, Željko Knez, Zoran Novak**

Faculty of Chemistry and Chemical Engineering, University of Maribor, Maribor, Slovenia

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For this paper pectin aerogels were obtained by the sol-gel process and further supercritical drying with CO_2. The materials were first characterised by nitrogen adsorption and scanning electron microscopy in order to investigate their structures. The highest yet reported surface area on pectin aerogels was achieved during this research (590m^2/g). Then the drug loading was performed as the first step of the sol-gel process. Nicotinic acid was used as a model drug. Membranes were added to the pectin microsphere to control the release of nicotinic acid. The release of the model drug was investigated regarding triple and five membrane-aerogels. It was observed that the triple-membrane aerogel was unable to control the release. By adding the 5- membrane, the release decreased. 50% of the drug was released during the first hour followed by the slow first-order release up to 7h. The results clearly indicate that the addition of membranes on pectin aerogel has a significant impact on the drug release kinetics. The 5-membrane pectin aerogel showed potential to be a suitable carrier of the nicotinic acid after further research.

Keywords: supercritical drying, aerogel, drug delivery, pectin

Introduction

Aerogels are highly advanced materials now used in various applications. They are being used as thermal insulators, capacitors, drug carriers, active food materials in tissue engineering, and many others. They are prepared by the sol-gel process and later supercritical drying. Aerogels have been intensively studied as drug carriers, mostly due to their large surface areas and high porosities. Those properties are suitable either for controlling [1] the release of water-soluble drugs or for increasing the bio-availabilities of low-soluble drugs.

Initially inorganic, mostly silica aerogels were investigated for controlling the release of drugs. Later, organic aerogels were prepared and those aerogels also possess other advantages over silica aerogels, one of them being the biodegradability. In this manner, natural polysaccharide aerogels have been developed and investigated [2–4].

Pectin, as one of natural polysaccharides having gelling and stabilising abilities, is now extensively used in pharmaceutical and food applications. The two types of pectin have different properties and also gel under different mechanisms. Low ester pectin (LM) with a degree of esterification DE<50% gels in the presence of divalent ions [5] by the well-known egg-box model. Pectin is resistant to protease and amylase both of which are active within the upper gastrointestinal (GI) tract, and is digested by micro-flora in the lower GI tract. Therefore, it could work as a drug vehicle from the mouth to the intestines [6]. All these properties are promising for preparing pectin aerogels and using them to control the release of drugs.

Experimental

Materials

Low-methoxyl pectin (*Herbstreith&Fox*). Calcium chloride ($CaCl_2$ – *Kemika*). Absolute ethanol (*Sigma&Aldrich*). CO_2 (*Messer*). KH_2PO_4 (*Sigma&Aldrich*). NaOH (*Sigma&Aldrich*). Nicotinic acid (*Sigma&Aldrich*).

Dissolution media were prepared according to standards [7]. Phosphate buffer solution (PBS) with pH 6.8 was prepared by mixing 250.0mL of 0.2M potassium di-hydrogen phosphate and 112mL of 0.2M sodium hydroxide and diluted to 1000mL with water. Simulated gastric fluid (SGF) was prepared by dissolving 2.0g of sodium chloride and 3.2g of pepsin powder in water. 80mL of 1M hydrochloric acid was added to the solution and diluted to 1000mL with water.

Preparation of pectin aerogel

The diffusion method was used to form wet gels from the pectin solution. Firstly, a 2% pectin solution was prepared and then transferred to an aqueous 0.2 M $CaCl_2$

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Author address: Zoran Novak, Faculty of Chemistry and Chemical Engineering, University of Maribor, Smetanova 17, SI-2000 Maribor, Slovenia
E-mail: zoran.novak@um.si

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solution by using a syringe with a 0.8mm nozzle. Fine spherical particles were formed. Those microspheres were then dehydrated within a series of water-ethanol baths of increasing the ethanol content (10, 30, 50, 70, 90, and 100%), and in this way, shrinkage was prevented during the solvent exchange. The alcogels were then super-critically dried at 40 °C and 100bar. Such prepared aerogels were used for the characterisation.

Drug-loaded aerogels were prepared by the same procedure. An additional step was the addition of the model drug during the first step of the sol-gel process. Nicotinic acid was added to the pectin aqueous solution before the cross-linking. Later during the process of adding membranes, inner surfaces between the core and membrane and between two membranes were filled with the model drug. The further process for obtaining aerogels was the same as described above, including immersion of hydrogels within a series of excessive ethanol baths and later supercritical drying. All the solutions for cross-linking and solvent exchange were saturated with the model drug to prevent diffusion from the carrier.

Characterisation

The prepared aerogels were characterised by swelling experiments, N_2 adsorption, and scanning electron microscopy.

The surface morphologies of the pectin aerogels were determined using a Sirion 400 NC scanning electron microscope (SEM). The samples were sputter-coated with gold particles and then scanned at an accelerating voltage of 5kV.

Nitrogen adsorption was performed for determining the surface areas, pore sizes and pore volumes of the prepared aerogels, using a Micrometrics ASAP 2020MP porosimeter.

For swelling studies, a sample of multi-membrane spherical aerogel was weighted into a beaker containing 40ml of either simulated gastric fluid (SGF) at pH 1.2 or phosphate buffer at pH 6.8, thus mimicking the condition of the gastrointestinal tract. The sample was withdrawn after a pre-selected period of time, blotted dry with tissue paper and weighted. At least three measurements were carried out for each sample in each solution. The swelling ratio was calculated according to equation (1):

$$Swelling\ ratio = \frac{M_t}{M_0} \quad \text{.....(1)}$$

Where M_t is the mass of the swollen gel monolith after time t and M_0 is the initial mass of the dry aerogel monolith.

Determination of the drug content

The drug-loaded aerogel sample was poured into 100ml of phosphate buffer solution. After 10min of sonicating and 6h of stirring at 250rpm and 37±1 °C, the solution was filtered through a TEFLON 0.45μ filter and the amount of a drug was determined spectrophotochemically (Varian, Cary 50 Probe UV spectrophotometer). Drug loading (*DL*) of nicotinic acids was calculated by equation (2), assuming complete drug extraction:

$$DL = \frac{m_d}{m_s} \cdot 100\% \quad \text{.....(2)}$$

Here md is the mass of the drug (mg), obtained by UV analysis and ms is the initial mass of the weighted aerogel sample. Each test was done in triplicate.

Drug dissolution test

In-vitro dissolution tests for both model drugs were performed on FARMATESTER-3 USP 2 apparatus (paddle). The aerogel sample was poured into 900ml of the phosphate buffer (pH6.8) solution that was thermostated to 37±0.5 °C and the rotation speed was 50rpm. The sample was left under stirring for 24h. Aliquots (2mL) of dissolution media were withdrawn at selected time intervals and each time 2ml of the fresh buffer solution was added to the media to maintain the constant volume. The withdrawn samples were passed through a 0.45μm filter and then analysed using UV spectrophotometry (Model Varian, Cry 50 Probe spectrophotometer) at the wavelength of 262nm for nicotinic acid. Cumulated amounts released (in percentages of the initial amounts) were plotted vs. time.

Results and Discussion

Characterisation

The core (2% pectin solution) and membrane (1% pectin solution) from the spherical sample were compared in Figure 1. The core looked more uniformly cross-linked in comparison with the surface of the membrane. This could be due to the longer time of cross-linking and a higher concentration of pectin in the core.

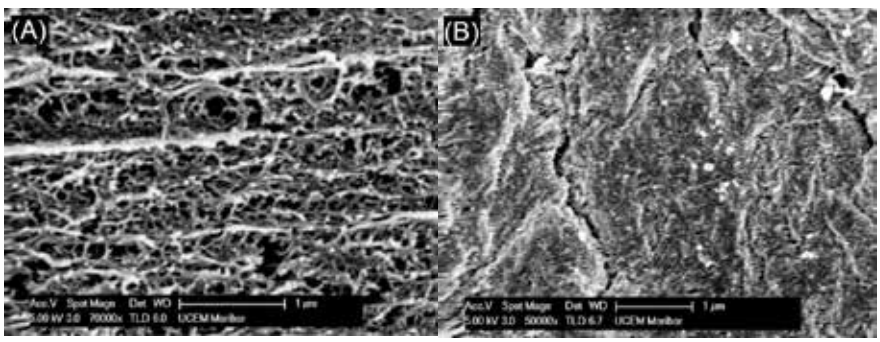

Figure 1. SEM images of pectin (A) membrane and (B) spherical core.

Nitrogen adsorption measurements provided outstanding results on the surface area of spherical aerogels (Table 1). Namely, the surface area of the core was 544m^2/g and that of the membrane even higher at 593m^2/g. This is the highest yet reported surface area on pectin aerogels [8,9]. From the sizes of the pores, as well as from the types of isotherms (class IV), it could be concluded that all the pectin aerogel samples were of mesoporous materials.

Table 1. Effect of the pectin type and concentration on the characteristics of the monolithic and spherical aerogel samples.

Pectin (%)	S_{bet} of aerogel (m^2/g)	Pore volume aerogel (cm^3/g)	Average pore diameter (nm)
Membrane 1.0	593 ± 10	1.64 ± 0.03	12.0 ± 3.3
Core 2.0	544 ± 8	3.27 ± 0.03	26.3 ± 2.3

Swelling experiments were carried out at pH conditions mimicking those of the stomach (pH1.2) and of the intestine (pH6.8). The swelling ratio in the SGF remained near constant during 8h. Oppositely, the swelling of the pectin was strongly influenced by the higher pH of the phosphate buffer leading to a degradation of the sample (Figure 2).

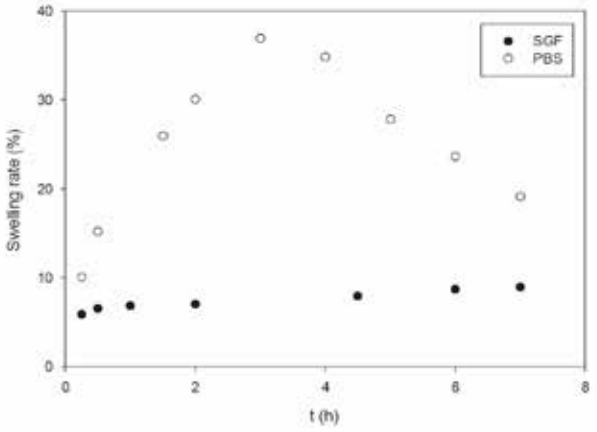

Figure 2. Swelling of spherical samples in simulated gastric fluid (SGF) and in phosphate buffer solution (PBS).

Dissolution of nicotinic acid from multi-membrane pectin aerogels

Pectin aerogels provided 37% loading of the model drug. Nicotinic acid is good-soluble and highly permeable at pH6.8. Thus the carrier characteristics are important to prolonging the releases of the model drugs. Figure 3 displays dissolution profiles of nicotinic acid (NA) from multi-membranes.

It was shown that the 5-membrane aerogels demonstrated better dissolution and more controlled release than the triple-membrane aerogels.

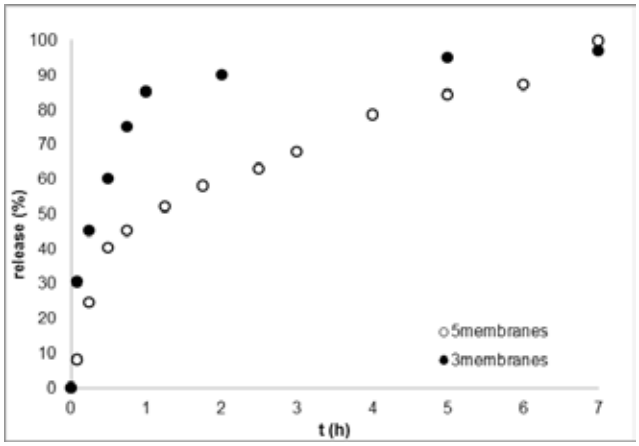

Figure 3. Nicotinic acid releases from triple-membrane and 5-membrane aerogels.

The initial burst release of nicotinic acid happened during the first hour, followed by the slow and sustained drug release noted from the triple-membrane carrier.

Conclusions

In this research, biodegradable low-methoxyl aerogels were prepared by the diffusion method. Nitrogen adsorption gave highly promising results on specific surface areas. The highest specific surface area (593m^2/g) of the spherical pectin aerogels was obtained in comparison with the data reported by other research groups [8,9]. As surface area/volume is one of the key variables in controlling the drug release, different aerogel characteristics can be obtained by changing the types, degrees of esterification or amidation and the concentration of the pectin, thus influencing the drug release. 5-membrane aerogels provided a better dissolution rate with 100% release after 7h. With the addition of membranes, a slower release can be expected due to the longer diffusion pathway.

Acknowledgements

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Abbreviations

DE – degree of esterification
DL – drug loading
GI – gastrointestinal tract
LM – low methoxyl (low ester) pectin
NA – nicotinic acid
S$_{bet}$ – specific surface area, determined by the Brunauer-Emmett-Teller method
SEM – scanning electron microscopy
SGF – simulated gastric fluid

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Izvod

INKAPSULACIJA FARMACEUTSKI AKTIVNIH SUPSTANCI U PEKTINSKIM AEROGELOVIMA U CILJU POSTIZANJA KONTROLISANOG OSLOBAĐANJA

Gabrijela Tkalec, Željko Knez, Zoran Novak

Fakultet za hemiju i hemijsko inženjerstvo, Univerzitet u Mariboru, Maribor, Slovenija

(ORIGINALNI NAUČNI RAD)
UDK 615.2:66.047.3

Za ovu svrhu pektinski aerogelovi su dobijeni sol-gel procesom i u drugom koraku superkritičnim sušenjem pomoću superkritičnog ugljen-dioksida. Materijali su prvo karakterisani adsorpcijom azota i SEM mikroskopijom u cilju određivanja njihove strukture. Tokom ovog istraživanja je postignuta do sada najviša prijavljena specifična površina pektinskih aerogelova (590m^2/g). Za potrebe farmaceutskih aplikacija, aerogelovi su napunjeni aktivnom supstancom u prvom koraku sol-gel procesa. Nikotinska kiselina je korišćena kao model supstanca. Membrane su dodate na pektinske mikrosfere u cilju kontrolisanja otpuštanja nikotinske kiseline. Otpuštanje nikotinske kiseline je istraženo iz tromembranskih i petomembranskih aerogelova. Primećeno je, da tromembranski aerogelovi nisu bili u stanju da kontrolišu otpuštanje supstance. Dodavanjem pet membrani otpuštanje je usporeno. 50% supstance je otpuštno u prvom satu nakon čega je praćeno sporim otpuštanjem do sedmog sata. Rezultati jasno ukazuju, da je dodavanjem membrana na pektinske aerogelove izvršen značajan uticaj na kinetiku otpuštanja supstance. Petomembranski pektinski pokazao je potencijal da, nakon daljnih istraživanja, bude ogovarajući nosač za isporuku nikotinske kiseline u telu.

Ključne reči: superkritično sušenje, aerogel, raspodela lekova, pektin