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Supercritical impregnation as a feasible technique for entrapment of fat-soluble vitamins into alginate aerogels

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ABSTRACT

The use of supercritical fluids as solvents allows innovative processing applications that can overcome the limitations of organic solvents. Their suitable properties make them appropriate for applications in the pharmaceutical and food industries and even more as a tool for the entrapment of substances. The aim of the presented work was to entrap two substances: 2-methyl-1, 4-naphthquinone (vitamin K_3) and cholecalciferol (vitamin D_3) within alginate aerogels by using supercritical carbon-dioxide. Supercritical impregnation of aerogels is receiving increasing attention as a green technique for entrapping poorly water soluble substances. The entrapment of vitamin D_3 is a huge challenge due to its very high sensitivity.

Impregnation experiments were carried out at 150 and 200 bar and 40 °C. As the process was shown to be feasible for both vitamins, the adsorption isotherms were measured and fitted with the Langmuir model. The effects of pressure, vitamins' concentrations and the time of impregnation on the loaded aerogels were studied. The time of impregnation was shown to be the critical and more important parameter where the highest loadings were achieved after only 1 h of impregnation. The loaded aerogels were characterised using Fourier transform infrared spectroscopy, X-ray diffraction and scanning electron microscopy. Furthermore, in-vitro dissolution testing for vitamin D_3 was performed and the controlled release of the vitamin over a time span of 6 h was achieved.

1. Introduction

The advantages of supercritical fluids over organic solvents are numerous. As a result of their environmental properties, supercritical fluids are used in the pharmaceutical and food industries. Their high diffusivities and low viscosities allow rapid penetrations of the active substances into high varieties of matrices. To date, supercritical carbon dioxide ($scCO_2$) has been the most exploited supercritical fluid as it is non-flammable, non-toxic, recyclable, naturally abundant, and has a low critical point (31.1 °C and 73.8 bar).

Supercritical fluids have established their place in the production of aerogels by the use of a supercritical drying technique. Due to their outstanding properties such as low densities, large open pores and high inner surface areas, aerogels have been proposed as matrices and carriers for active substances, peculiarly pharmaceuticals. Aerogels made of organic materials such as polysaccharides fulfil the pharmaceutical demands for biocompatible (soluble in body) and biodegradable (suitable for enzymatic decomposition in the body) carriers [1]. Several studies on the production of highly porous alginate aerogels have been reported. To name just a few [1–4], alginate aerogels with specific surface areas as high as 600 m^2/g were reached [2]. The extensive use of this polysaccharide is due to its convenient gelation conditions. It is able to undergo revisable gelation within an aqueous solution in the presence of divalent ions such as calcium ions. Two different forms of alginate aerogels could be distinguished: (1) spherical beads, where the gelation is induced by a diffusion method [5] and (2) monoliths, where the gelation is induced by the internal setting method [1].

Supercritical impregnation (adsorption) is one of the recent promising techniques for improving the encapsulation of insufficiently soluble active substances [6]. It is estimated that a huge part (more than 40%) of newly-developed compounds has very low solubility or is practically insoluble in water [7]. This technique is suitable for pharmaceutical, biomedical, cosmetics and food applications, as the final products are solvent free.

By using supercritical impregnation technique it is possible to achieve controlled drug delivery. Controlled drug delivery provides lower dosages of the drugs, increases their therapeutic effect and improves the patient's compliance. These systems are more effective and safe compared with conventional immediate release dosage forms as they are capable to maintain constant concentration of the active compound in tissues or blood for a longer time [8].

Vitamin D_3 is one of two major forms of fat-soluble vitamin D. This vitamin is of great importance for calcium and phosphorus homeostasis regulation; it has positive effects on the cardiovascular system, cancer prevention and various autoimmune disorders. Vitamin D_3 can be obtained either by synthesis in the skin after exposure to sunlight or

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from foods such as salmon, mackerel, herring, and egg yolk, which have to be consumed in large quantities to avoid deficiencies [9]. Regrettably, many studies have shown that vitamin D deficiency is a huge problem worldwide in all age groups and exceeds pandemic proportions due to the avoidance of sun exposure, usages of sunscreens and low dietary intakes [10]. Therefore, fortification of food such as milk and milk products, cereals and beverages commenced in order to improve diets [11]. Food fortification with vitamin D_3 is a difficult task and represents a huge challenge. Namely, this vitamin has very low solubility, is practically insoluble in water and has high sensitivity to light, air and high temperatures, thus causing the vitamin's degradation [12]. Encapsulation of vitamin D_3 within various carriers such as whey protein isolate nanoparticles [13], β-lactoglobulin complexes [14], casein micelles [12], oleoyl alginate ester nanoparticles [15,16], zein nanoparticles coated with carboxymethychitosan [17], and carboxymethyl chitosan hydrogel beads [18] has already been reported. To the best of our knowledge, there are no reported studies dealing with the encapsulations of vitamin D_3 under high pressures using aerogels as carriers. In the presented work, alginate aerogels were used as carriers for vitamin D_3 encapsulation using a supercritical impregnation technique. By impregnating these vitamins inside the aerogels, protection from those environments that promote degradation or undesirable interactions could be achieved. The aim was to design, study, and optimise the supercritical impregnation of vitamin D_3 as an active substance under various operating conditions. For this purpose vitamin K_3 was used as a model substance. In depth knowledge of the thermodynamic equilibrium and kinetics of vitamins' impregnations into alginate aerogels was required. Therefore, adsorption isotherms and kinetic profiles of both vitamins D_3 and K_3 were made and fitted with empirical models.

2. Materials and experimental methods

2.1. Materials

Carbon dioxide (purity 99.5%) was supplied by Merck, GmbH. Ethanol (purity >99.8%) was purchased from Sigma-Aldrich. The alginic acid sodium salt from commercial brown algae (viscosity ≥2.000 cP, 2% (25 °C)) was provided from Sigma-Aldrich. The alginate solutions were prepared using distilled water. Calcium chloride ($CaCl_2$) supplied from Kemika, Zagreb was used in the aqueous solution as an ionic cross-linker. Cholecalciferol – vitamin D_3 (purity 99.9%) and 2-methyl-1, 4-naphthoquinone – vitamin K_3 (purity 99.8%) were purchased from TCI Europe N.V and used as active and model substances, respectively. Dodecyl sulphate sodium salt (SDS, purity >99.0%) from Merck, GmbH was used as a surfactant during the drug dissolution testing.

2.2. Experimental methods

2.2.1. Preparation of alginate aerogel spheres

The preparation of alginate aerogel spheres has previously been described elsewhere [3,5]. Briefly, the alginic acid sodium salt was dissolved in distilled water at a concentration of 2% (*w*/*w*) at room temperature. The alginate aqueous solution was then drop-wise added into a 2% (*w*/*w*) $CaCl_2$ solution. The obtained alginate hydrogel spheres were cured in a salt solution for 1 h. Afterwards dehydration of hydrogels was performed by immersion within a series of successive ethanol–water baths by increasing the ethanol concentrations (10, 30, 50, 70, 90 and 100%). In the final step, alginate alcogel spheres were dried under $scCO_2$ conditions. The extraction of ethanol was performed at 40 °C and three different pressures (100, 120 and 150 bar) with $scCO_2$ flow of 200–300 L/h. 6 h was needed to remove all the ethanol from the alcogels and dried aerogels were obtained in the final step.

2.2.2. Supercritical impregnation of model and active substances

The supercritical impregnation experiments were performed using a model substance, vitamin K_3 and an active substance, vitamin D_3. The main focus in the present study was vitamin D_3. Usage of vitamin K_3 as a model substance was due to its solubility and price. Namely, vitamin K_3 is a fat-soluble vitamin like vitamin D_3 but with a much lower price. Afterwards the experiments were expanded to vitamin D_3.

Supercritical impregnation of aerogels was performed using a static method (batch mode). This method consisted of placing alginate aerogels and one of the vitamins within the impregnation cell. The alginate aerogel samples were placed at the bottom of the impregnation cell whilst the vitamins were at the top so that the $scCO_2$ could come in contact first with the vitamins and then with the alginate aerogels. Aerogels and vitamins were separated by wrapping them into the filter bags to avoid direct contact between them. $ScCO_2$ was slowly introduced into the impregnation cell and kept for a predetermined time period. The impregnation cell was previously heated up to the desired temperature. At the end of the period, the system was slowly depressurised. The depressurisation rate was approximately 3 bar/min [19]. When all the pressure had been released, the alginate samples were removed from the impregnation cell and the final loading could be measured.

The supercritical impregnation apparatus is schematically presented in Fig. 1. A stainless steel impregnation cell was the main part of the experimental set-up with an internal volume of approximately 33 cm^3. It is closed on the bottom and on the top using two stainless steel clamps. The maximum operating pressure was 250 bar. The impregnation cell was heated by electric wire controlled by a thermo-regulator. The temperature inside the impregnation cell was measured by a thermocouple with an accuracy of ±1 °C. Pressure was measured by a digital manometer with an accuracy of ±1 bar. The system was equipped with a micro-metering valve for manually controlling the pressuring and depressurising of the impregnation cell.

2.2.3. Determination of the vitamins' loadings

In order to determine the final loading, the impregnated vitamins were extracted from the alginate aerogels by absolute ethanol. Firstly, the loaded aerogels were weighted and dispersed in a glass beaker filled with 100 mL of absolute ethanol. The glass beaker was sealed and the solution was left under stirring for at least 12 h after which it was sonicated for 15 min. The solution was then filtered using TEFLON 0.45 μm that does not absorb active substances. The concentration of vitamins in ethanol was measured by UV spectrometry ($\lambda_{vitaminK3} = 251$ nm, $\lambda_{vitaminD3} = 265$ nm) using a Varian, Cary 50 Probe spectrophotometer. The vitamins' loadings were determined from the measured absorbances in the absolute ethanol using a calibration curve.

2.2.4. Drug dissolution test

The USP 1 apparatus (Agilent Technologies 708-DS Dissolution Apparatus) was used for the dissolution studies. Weighted alginate aerogels (50 mg) were placed within a cylindrical basket. The basket was immersed in a vessel containing 900 ml of 0.3% SDS in milli-Q water. The pH and temperature of the solution were 7.3 and 37 ± 0.1 °C respectively. The solution was left under stirring for 24 h at a stirring speed of 75 rpm. Aliquots of 2 ml were withdrawn and subjected to a drug assay by LC-MS to determine the vitamin's concentration. The removed dissolution medium was replaced with the same amount of the fresh SDS solution. The released amount of vitamin D_3 was plotted against time. All LC-MS experiments were performed in triplicate.

2.2.5. Mathematical modelling

The determination of the supercritical impregnation capacity at various concentrations was performed by obtaining the experimental adsorption isotherms. The results can be fitted into different mathematical models: Langmuir and Freundlich.

The Langmuir model is given by Eq. (1):

$$q_e = \frac{q_{max} K C_e}{1 + K C_e} \quad (1)$$