J. of Supercritical Fluids 116 (2016) 251–263

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

Impregnation of medicinal plant phytochemical compounds into silica and alginate aerogels

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ARTICLE INFO

Article history:
Received 24 February 2016
Received in revised form 31 May 2016
Accepted 2 June 2016
Available online 3 June 2016

Keywords:
Alginate
Silica
Aerogels
Supercritical
Impregnation
Phytochemicals

ABSTRACT

Herbal medicinal plants are important sources of bioactive, medicinal compounds. In this work, impregnation of phytol as a model compound and multicomponent *Clinacanthus nutans* (*C. nutans*) plant extracts into alginate and silica aerogels was investigated, applying two different methods: wet impregnation and supercritical impregnation. Two types of *C. nutans* extracts were prepared by microwave-assisted extraction (MAE) using either ethanol-water solvent mixtures or pure ethanol as solvent. The impregnated compounds were analyzed by Infrared spectroscopy (FTIR), thermogravimetric analysis (TGA) and chromatography (HPLC and UPLC–MS/MS). Results showed that supercritical impregnation method yielded the highest loading content with silica aerogels, with a content of 30.1 ± 0.6 *wt%* of the model compound phytol, and 11.5 ± 0.4 and 23.9 ± 1.0 *wt%* of the extracts obtained with ethanol/water and pure ethanol solvents, respectively. In the wet impregnation method, alginate aerogels exhibited higher loading than the silica aerogels regardless of their surface area properties, indicating that in this case other properties of carrier materials and the nature of compounds have a stronger influence on the compounds loading than the surface properties of the carrier. Impregnation in alginate aerogels yielded higher total phenols and flavonoids contents than in silica aerogels. Plasticized alginate biopolymer was formed when the supercritical impregnation was performed with excess of phytol at 200 bar and 40 °C, indicating that the swelling and/or plasticizing effect of $SCCO_2$ was remarkable combined with the effect of excess solute. Release kinetics results indicate that the alginate aerogel impregnated with the extract obtained with pure ethanol is suitable for controlled drug release whereas the alginate aerogel impregnated with the ethanol/water extract is more appropriate for fast release purposes. The release kinetics results were fitted into several mathematical models: zero order, first order and Higuchi model, to evaluate the mechanism of the release. Results showed that diffusion and erosion and/or swelling control the release of extract from the aerogels.

1. Introduction

Medicinal herbs have received much attention for many years as sources of natural drugs for therapeutic purposes. Herbs with high content of phytochemicals with antioxidant, anti-cancer, anti-inflammatory compounds have applications for diseases treatment and prevention, in chemotherapy and to form good health. *Clinacanthus nutans* Lindau (*C. nutans*) is a popular medicinal plant in South-East Asia. Recently, it has received much attention due to its therapeutic applications related to its antioxidant, anti-inflammatory, antimicrobial and anti-viral properties. In our previous study, *C. nutans* was extracted by Soxhlet extraction, supercritical CO_2 extraction and microwave-assisted extraction (MAE), and it was found that the MAE method provided the highest extraction yield of bioactive compounds in the shorter extraction time. Among various phytochemical compounds identified, phytol was found as the major bioactive compound in the *C. nutans* extracts [1].

Phytol is an acyclic diterpene alcohol. It is an integral part of chlorophyll and it is abundantly found in green plants and planktonic algae. Phytol is commonly used as aromatic ingredient in cosmetic and in food additives. In the medicinal field, it has been claimed that phytol has antibacterial and anti-inflammatory properties, and acts as an excellent adjuvant in vaccine formulation to stimulate humoral immunity response [1–3]. Recent studies have revealed that phytol has several unique therapeutic activities such

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http://dx.doi.org/10.1016/j.supflu.2016.06.002
0896-8446/

as antioxidant and antinociceptive action on the central nervous system (CNS) [4]. This study claimed that phytol showed *in-vitro* antioxidant activity at dosages of 25, 50, 100 and 200 mg/kg. Furthermore, the antibacterial and antiradical properties of phytol could complement new therapies for heart disease, and its antischistosomal effect make it active in the treatment of major endemic diseases [5].

However, these compounds are prone to degradation by ambient conditions. One way to protect the bioactive compounds is by incorporating them into a polymer or biopolymer matrix. For application for oral drug delivery of pharmaceutical compounds, it is necessary to use biodegradable materials with suitable release kinetic characteristics. Alginate is a natural polysaccharide biopolymer mainly derived from brown algae and consists of α-L-guluronic acid and β-D-mannuronic acid (M) residues, linearly linked by 1,4-glycosidic linkage [6]. This natural biomaterial has been accepted in several areas of application as it is biodegradable, low cost, non-toxic and stable. Over many years, alginate has been extensively investigated for food applications, controlled drug delivery, encapsulation and environmental applications [7–10]. Several attempts have been reported on the application of alginate aerogels with desirable therapeutic features for drug delivery either in monolith forms [11] or microsphere beads [9,12]. Hydrogel alginate can be synthesized by inducing the gelation of alginate solution based on two fundamentals methods: (i) diffusion method or (ii) internal setting method. Typically, they can be prepared as hydrogels and dried under ambient condition or by supercritical fluids to produce xerogels and aerogels, respectively. By supercritical drying high specific surface area, large porosity and low density of aerogels can be produced while maintaining the network structure of the polymer or biopolymer. These attractive characteristics make aerogels suitable for delivery of bioactive compounds or drugs for pharmaceutical applications.

Incorporation of active substances into carrier matrices (particularly, polymeric) by impregnation has been applied to protect and preserve valuable compounds from free radicals, oxygen or UV, and for controlled drug release purposes [13,14] or bioavailability enhancement [15]. Among other methods, processes based on supercritical fluids are promising ways to impregnate active substances in a matrix without using organic solvents. Carbon dioxide is the most commonly employed supercritical fluid due to its non-toxic and non-flammable character and because it can be easily separated at ambient conditions leaving negligible residues in the sample [16]. The resulting technology of Supercritical Impregnation (SCI) has been recognized as a promising alternative method to conventional process due to excellent properties of the fluids under supercritical conditions. High density of the fluids encourages the solubilisation of the compounds and thus increases the rate of the impregnation. In addition, low viscosity and high diffusivity enables the fluid mixture to penetrate and impregnate the compounds into the inner parts of the materials without damaging the matrix structure. Another interesting point is that the supercritical fluids could act as a swelling and/or plasticizer agent and cause the polymer or biopolymer carrier matrix to swell, thus facilitating the penetration of the fluid-compounds mixture into the matrix [13,17]. These advantages of the SC fluids properties have been applied in impregnation techniques for drug delivery [18,19], food packaging [20] and dyeing [21,22].

In general, two basic mechanisms of impregnation have been described: (1) physical deposition and, (2) molecular dispersion [17,23]. In the first, the key driving force for the impregnation is the solubility of the substance in the $SCCO_2$. During the process, the $SCCO_2$ solubilize the substance in a fixed time. The saturated $SCCO_2$-substance mixture swells the carriers without dissolving them. The swollen carrier enables the saturated $SCCO_2$-substance to diffuse into the internal part of the carrier, and it is then physically deposited by precipitation upon depressurization. Other factors that influence this type of impregnation include the physical-chemical properties of the carrier materials such as surface area, porosity and chemical functional groups. On the other hand, the second mechanism, called as molecular dispersion impregnation, consists on the chemical adsorption of the solute on the carrier surface at the molecular level, and depends on: interaction of $SCCO_2$-carrier, interaction of solute-carrier (that controls the solubility of solute in the carrier) and solubility of the solute in $SCCO_2$, which determine the corresponding partition coefficients of solute between supercritical fluid and carrier matrix. The saturated $SCCO_2$- solute fluid mixture acts as swelling and/or plasticizing agent and dissolves into the carrier material. The presence of other compounds such as co-solvents and additives may influence the impregnation performance [18,24].

Apart from the SCI method, liquid absorption has also been extensively studied for the impregnation of drugs or active pharmaceutical ingredients. In the conventional method, the drug is dissolved in organic solvent and brought into contact with the carrier material by soaking for a certain time followed by solvent removal. The drawback of this technique is the use of organic solvent and it is only applicable for drugs that have high solubility in the organic solvent [24].

In the case of impregnation of aerogels, impregnation of bioactive compounds or drugs via liquid absorption integrated with $SCCO_2$ drying of the aerogels could eliminate the impregnation step in post-SC drying. Low viscosity, high diffusivity and null surface tension of $SCCO_2$ allows the gel drying while maintaining their porosity without damaging the surface structure of the material. During the SC drying, the $SCCO_2$ extracts the organic solvent, causing solute precipitation by an antisolvent mechanism [25,26]. Upon depressurization, the $SCCO_2$ is released, leaving the solute precipitated and trapped in the matrix pores. Nevertheless, some issues must be taken into account such as undesired reactions, drugs degradation and residual levels of toxic organic solvent in the final product that may lead to low processing efficiency [24,27].

The objective of this study is to impregnate a phytochemical compound in silica and alginate aerogels and to investigate the interaction behavior of the encapsulated compounds with the silica and alginate matrix. The silica aerogels were synthesized via sol-gel methods whilst the alginate hydrogels were prepared via internal setting method. In both cases, aerogels were dried by supercritical CO_2. Pure phytol was chosen as a model herbal compound for this impregnation study, impregnating it into the aerogels and investigating its thermal release characteristics. On the other hand, additional experiments were carried out on the impregnation of multicomponent extracts of the medicinal plant Clinacanthus nutans Lindau (*C. nutans*) into silica and alginate aerogels. Prior to the impregnation, the *C. nutans* extract was obtained by microwave-assisted extraction (MAE) as reported in our previous work (Mustapa et al. [1]). Two impregnation methods were employed: wet impregnation (WI) during the aging process of the alcogel and supercritical impregnation (SC) over preformed, dried aerogel monoliths. Effect of different impregnation methods was discussed and dissolution tests of the phytochemical compounds from the aerogels were also carried out to determine their release kinetics.

2. Methodology

2.1. Materials

Alginic acid sodium salt from brown algae (low viscosity, 4–15 cP), calcium carbonate ($CaCO_3$) and glucono-δ-lactone (GDL), tetramethoxysilane (TMOS), methanol, ammonium hydrox-