

Production of resveratrol loaded alginate aerogel: Characterization, mathematical modeling, and study of impregnation



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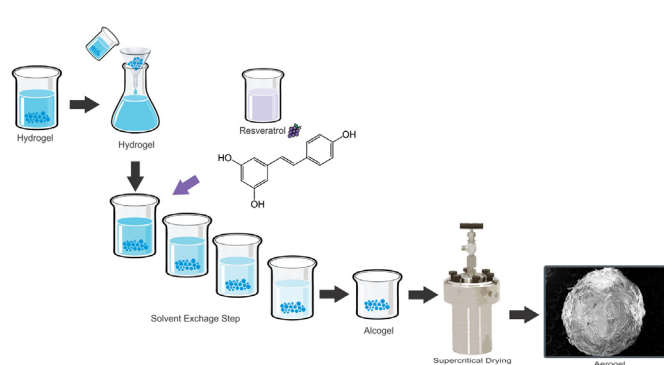
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HIGHLIGHTS

- Aerogel particles were produced by dripping and were impregnated with resveratrol.
- High surface area and pore volume were obtained in atomized particles.
- Gradual solvent exchange produced particles with high surface area and pore volume.
- The resveratrol loading ranged from 6.7 ± 0.4 to $77.1 \pm 7.0\%$.
- Alginate aerogel showed as appropriate material to high resveratrol load.

GRAPHICAL ABSTRACT



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ABSTRACT

Biodegradable polymers have been studied for the production of aerogels, which are specially useful in impregnation processes of target compounds for food and pharmaceutical applications. The aim of this study was to investigate different aspects in the production of alginate aerogels, including the formation of beads (different sizes); the drying kinetics of aerogel in supercritical CO₂; and the loading of a resveratrol by the indirect wet impregnation method. High surface area and pore volume (484 m²/g and 0.70 cc/g) were obtained by the atomization with solvent exchange gradient, while the aerogels produced by dripping without gradient showed surface area of 381 m²/g and pore volume of 0.68 cc/g. Sodium alginate aerogel showed to be an appropriate material to load high amounts of resveratrol, with a loading capacity directly correlated to the concentration of resveratrol solution. Wet impregnation and supercritical drying can be presented as feasible techniques to load bioactive compounds.

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1. Introduction

Trans-resveratrol, 3,5,4'-trihydroxy-*trans*-stilbe, belongs to the stilbene class of polyphenolic compounds and is one of the best-known plant secondary metabolites [1]. In plants, it exists in glycosylated piceid forms (3-O-B-D-glucosides), which protects resveratrol from oxidative degradation [2]. Moreover, glycosylated resveratrol is more stable, soluble and readily absorbed in the

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human gastrointestinal tract [3]. However, it is crucial to develop effective methods to protect the polyphenol from degradation and to promote a controlled release in the site of interest. For this purpose, polysaccharide aerogels have attracted interest in the last decade [4–8].

An aerogel is defined as an extremely light nanoporous structure material derived from a gel whose liquid part has been replaced by a gas [9]. The solid structure may be inorganic (e.g. silica), or organic, when derived from resorcinol-formaldehyde, carbon, polysaccharides or polylactic acid. The main characteristics of Na-alginate aerogel are: open porous structure and high specific surface area (70–680 m²/g). Their porosity ranges from 90 to 99% and its density is low ($\rho = 0.07\text{--}0.46\text{ g/cm}^3$). These properties give then the possibility of providing greater bioaccessibility/bioavailability and improved capacity of carrying target compounds [4,10,11].

The incorporation of active compounds into aerogels has been performed through two main processes: supercritical impregnation or wet impregnation (WI). During the supercritical impregnation process, the saturated mixture containing supercritical CO₂ and the substance diffuses into the aerogel pores, and the impregnation occurs either by precipitation upon depressurization or by chemical adsorption of the solute on the carrier surface. The main driving force for the supercritical impregnation is the substance solubility in supercritical CO₂ [12]. Therefore, the low solubility of target compounds becomes a limiting factor in the process. Alternatively, WI can overcome this problem because target compounds are dissolved in organic solvent and brought into contact with the algogel by soaking for a specific time. Finally by supercritical drying, the aerogel will be impregnated with the target compound [12]. During the supercritical drying, the supercritical CO₂ extracts the organic solvent, causing solute precipitation by an antisolvent mechanism [13,14].

Based on this, the focus of this study was to investigate a preparation method of an aerogel and its loading capacity by wet impregnation. The drying kinetics of the algogel in supercritical CO₂ was studied using sodium alginate (Na-alginate) as polymer. Two methods for formation of Na-alginate beads at different sizes

were used, dripping (resulting in larger particles) and atomization (resulting in smaller particles). Also, the surface area and the pore size distribution of the aerogel particles were determined by nitrogen adsorption and desorption, and at last, resveratrol was used as a model compound to study the loading capacity by the indirect wet impregnation method.

2. Materials and methods

2.1. Materials

Sodium alginate (from brown algae, CAS number 9005-38-3) and calcium chloride (anhydrous, granular, $\geq 93.0\%$, CAS number: 10043-52-4) were used to produce the aerogel and were purchased from Sigma Aldrich (St. Louis, MO, USA). Absolute ethanol ($\geq 99.5\%$, CAS Number: 64-17-5) and carbon dioxide ($\geq 99.0\%$) were used in the solvent exchange and drying processes and were acquired from Synth (Diadema, SP, Brazil) and White Martins S.A (Campinas, SP, Brazil), respectively. Resveratrol 98 %, which was applied in the wet impregnation was donated by Trifa Pharmaceutical Supplies (Richmond, Canada). For chromatographic runs, resveratrol standard ($\geq 99.0\%$, CAS number: 501-36-0) was purchased from Sigma Aldrich (St. Louis, MO, USA). HPLC grade acetonitrile, methanol, and formic acid were purchased from J.T.Backer (Mexico City, Mexico).

2.2. Aerogel production process

A stock solution of sodium alginate was prepared at a concentration of 3% (w/w) dissolving the alginate in distilled water at room temperature (about 24 °C) under stirring overnight (18 h). In a glass vessel, the aqueous solution of calcium chloride (CaCl₂) at 2% (w/w) was kept under agitation at 300 rpm, and the alginate solution was pumped (4 mL/min) into the cross-linking solution (CaCl₂), with a volume ratio of 300 of calcium chloride/cross-linking solution. Two methods were used to obtain alginate beads with different sizes. The larger hydrogel beads were produced by pumping the alginate solution through a nozzle (I.D. 0.57 mm) and dripping into the cross-linking solution. The smaller beads were formed by the atom-

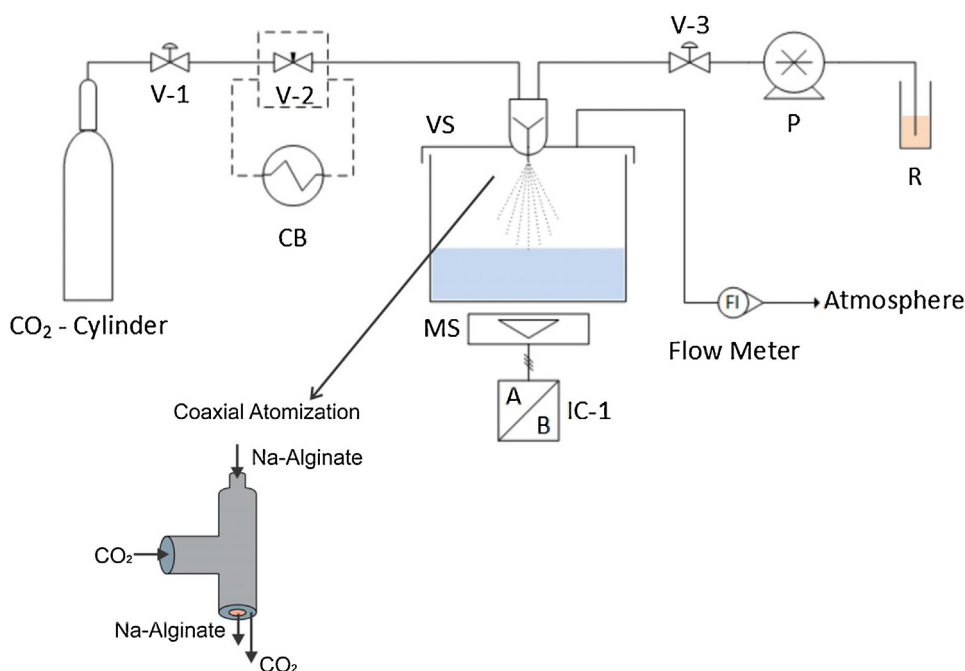


Fig. 1. Experimental set up for the production of hydrogel Ca-alginate particles by dripping or atomization processes (coaxial atomization). V-1 and V-3: control valves; V-2: micrometer valve (Autoclave Engineers, Erie, PA, USA); CB: cooling bath (Marconi S.A., Campinas-SP, Brazil); IC-1: indicators and controller of magnetic stirrer (MS) (IKA, RCT Basic, Staufen, Germany); P: HPLC pump; R: reservoir of alginate solution.

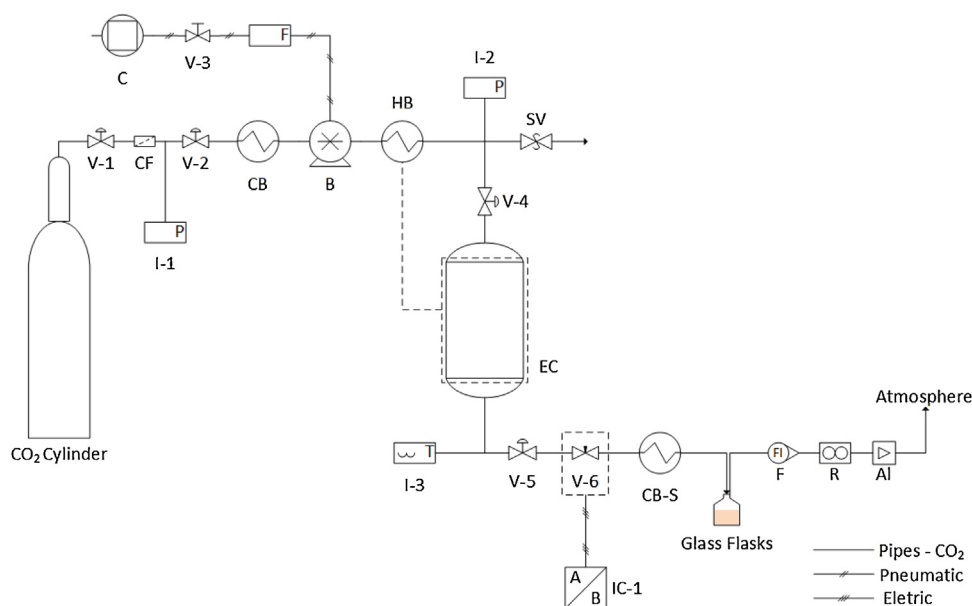


Fig. 2. Diagram of the supercritical drying unit; V-1, V-2, V-3, V-4 and V-5: control valves; V-6: micrometer valve; C: compressor; SV: safety valve; F: compressed air filter; CF: CO₂ filter; CB: cooling bath; B: booster; HB: heating bath; CB-S: cooling bath of coil; I-1 and I-2: pressure indicators; I-3: temperature indicators; IC-1: indicator and controller of temperature of the micrometer valve; EC: extraction column; F: flow meter; AL: alcoholmeter device; R: flow totalizer.

ization of alginate solution using carbon dioxide as atomization gas (Fig. 1). The flow of CO₂ (5 MPa) was controlled by a micrometric valve in a heated bath to avoid the Joule-Thomson effect and monitored by flow meter installed after the precipitation vessel. The flow rate was kept constant at 24 L/min (24 °C, 1 atm). After a certain time, the pumping was stopped and the hydrogel particles were maintained into the gelling solution for 30 min. Posteriorly, the hydrogel particles were filtered using a sieve (180 μm), and submitted to the solvent exchange.

The hydrogel beads were converted to alcogel by solvent exchange steps with ethanol. Two different approaches of ethanol concentration were tested in the exchange step: (1) using an ethanol:water gradient of 10:90, 30:70, 50:50, 70:30, 90:10 and 100:0 (ethanol:water, % v/v) and (2) without the gradient, using 100:0 (% v/v) of ethanol directly. In both cases, the solvent was exchanged every 24 h and 3 exchanges were carried out when using 100% of ethanol. The alcogel beads were kept at ethanol until the SC-CO₂ drying.

The experimental homemade apparatus used in the drying experiments of this study consists of a CO₂ pump (Maximator M-111, Zorge/Germany); a solvent reservoir; a cooling (Marconi S.A.) and a heating thermostatic (Marconi S.A., Campinas-SP/Brazil) bath; manometers (Zurich, São Paulo-SP/Brazil); thermocouples; control valves (Autoclave Engineers); a micrometric valve (Autoclave Engineers, Erie/PA, USA) and a stainless-steel vessel of 50 mL. Fig. 2 shows the schematic flow diagram of the supercritical fluid extraction unit.

Aerogel preparation was carried out by the extraction of ethanol from the alcogel using SC-CO₂ as antisolvent. Firstly, a number of gel beads were filtered (about 5 g), placed into the home-made paper filter support (porosity of 3 μm and weight of 1.5 g), and taken to the high-pressure vessel (50 mL, 30.9 cm of internal diameter and 64.7 cm of length). Then, the vessel was filled with 10 mL of ethanol to prevent solvent evaporation from the gel beads and particle shrinking. The vessel was closed and the temperature was regulated at 40 °C. After 10 min of heating, the system was pressurized with SC-CO₂ up to 11.0 MPa and kept for more 10 min at this pressure and temperature. Finally, the SC-CO₂ flow rate was adjusted at 4.15 g/min (100 g CO₂/g alcogel). To avoid ethanol evaporation in the outlet stream, a heat exchanger coupled to a cooling

bath (−2.0 °C) was used. The ethanol fraction was collected in glass vials filled with dry cotton (105 °C for 24 h) and the ethanol contained in the gaseous CO₂ stream was measured by an alcoholmeter device (BFD 100 digital, Instrutherm, São Paulo, Brazil) at determined time intervals (10, 20, 30, 60, 90 and 120 min). The drying kinetics were performed for 2 h and the depressurization step took place at a fixed flow rate (2 L/min). The remained particles bed was collected for further analyses. The supercritical CO₂ was compared to the conventional method of drying (oven), where a number of gel beads were filtered (about 5 g) and placed into a crucible porcelain. Then, the crucible was filled with 10 mL of ethanol to prevent solvent evaporation from the gel beads and particle shrinking. After that, the crucibles contained the particles were taken to the oven. The samples were taken from the equipment for mass measurements at predetermined intervals, until constant weight. All kinetics experiments were performed in duplicate at least.

2.3. Impregnation process

Wet impregnation (WI) was applied in this study, since the target compound has a phenolic structure and is low soluble in SC-CO₂ and soluble in ethanol (about 50 g/L at 25 °C [15]). The absorption into the alcogel large particles was carried out at the end of the solvent exchange period. Solutions of resveratrol in ethanol (1, 5 and 10%, w/w) were prepared and put into contact with the alcogel for 16 h, at room temperature and in the absence of light. The amount of solution used was calculated to obtain a 2:1 mass ratio of resveratrol to aerogel. Subsequently, the solvent was extracted by supercritical CO₂ drying, as described in Section 2.2, leading to the precipitation of resveratrol in the surface and within the pores of the aerogels. The experiments were performed in triplicate and the impregnated particles were stored in the dark at −18 °C.

2.3.1. Loading of resveratrol

The loading of resveratrol (i) was determined as shown in Eq. (1). In this equation, m is the mass of loaded resveratrol and $m_{aerogel}$ is the mass of raw aerogel.

$$\text{Loading}_i = \frac{m_i}{m_{aerogel}} \times 100 \quad (1)$$

The amount of resveratrol was determined gravimetrically and by high performance liquid chromatography (HPLC). The gravimetric method consisted in calculating the mass of resveratrol (m_i) as the difference between the mass of the raw aerogel particles (unloaded) and the mass of loaded particles. The HPLC method was based on washing the particles with methanol and analyzing the washing solvent. Approximately 50 mg of the aerogels were dispersed in absolute methanol, vortexed and storage at 5 °C for 24 h. The process was repeated until the complete removal of the target substance (increase in loading <0.01%), which was seven-time for each sample. The first five washes and the last two washes used 5 mL and 2.5 mL methanol, respectively. The solvent was then filtered through 0.45 μm PTFE (polytetrafluoroethylene) filters and diluted in methanol before the HPLC analysis. The dilutions ranged from 400 times (first wash) to 5 times (last wash).

The quantification of resveratrol in the washing solvent was performed using an Agilent 1200 series (Agilent Technologies, Germany) liquid chromatographer coupled with a diode array detector (DAD G1315D), a G1322A degasser, a G1311A quaternary gradient pump and a G1316A autosampler. A C18 Kinetex 2.6 μm , 3.0 mm i.d., 100 mm column (Phenomenex, California/US) was used and the analysis conditions were obtained from Ji et al. [16]: mobile phase of acetonitrile–water containing 0.2% formic acid (25:75, v/v); column oven at 25 °C.; UV detector wavelength at 306 nm for resveratrol; flow-rate of 0.8 mL/min and an injection volume of 10 μL . The resveratrol was used as analytical standard at concentration from 5 to 75 $\mu\text{g/mL}$. The injections of each wash were carried out in duplicate, and the total amount of resveratrol was obtained accumulating the mass recovered in each wash.

2.4. Chemical and physical analyses

2.4.1. Particle size distribution

The particle size distribution of the smaller particles was determined based on the static light scattering method using a Multi-Angle Static Light-Scattering Mastersizer (Mastersizer 2000; Malvern Instruments, Worcestershire, UK). Ethanol (99.5%) was used as a dispersant agent to aerogel and to algogel particles, while distilled water was used to disperse hydrogel particles. The size of the larger particles of alginate were determined using an optical microscope Multizoom AZ100 (Nikon, Japan), with images recorded by a DS-Ri1 camera (Nikon, Japan). At least 10 images per sample were obtained to assure the reproducibility of the results and at least 100 particles per sample were analyzed using the software NIS-Elements Documentation (Nikon, Japan). Two diameters were measured for each particle, in vertical and horizontal axis, and the mean diameter was expressed as the volume–surface mean diameter (Eq. (2)) and volume mean diameter (Eq. (3)).

$$d_{[3,2]} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (2)$$

$$d_{[4,3]} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (3)$$

where: n_i is the number of droplets with diameter d_i .

The particle size distribution for larger particles was modeled by the Rosin–Rammler distribution model (Eq. (4)). The model was adjusted using the algorithm proposed by Brezání and Zeleňák [17].

$$Q(d) = 1 - \exp \left[- \left(\frac{d}{d_e} \right)^m \right] \quad (4)$$

where: $Q(d)$ is cumulative undersize volume (mass) distribution; d is particle diameter; d_e is reference size constant; and m is distribution constant.

The particle polydispersity was evaluated by PSD (Eq. (5)) and the Span (Eq. (6)).

$$PSD = \frac{d_{(90)}}{d_{(10)}} \quad (5)$$

$$Span = \frac{d_{(90)} - d_{(10)}}{d_{(50)}} \quad (6)$$

where: $d_{(90)}$, $d_{(50)}$ and $d_{(10)}$ are diameters at 90, 50 and 10% of the cumulative number of particles, respectively;

The results of Na–alginate particle characterization (diameters and polydispersity) for dripping and atomization production methods and resveratrol loading were submitted to statistical analysis using the software Minitab 17 with Tukey test ($\alpha = 0.05$).

2.4.2. Nitrogen adsorption/desorption measurements

Nitrogen adsorption/desorption measurements of the larger particles were used to determine the aerogel surface area and the pore size distribution. Approximately 50 mg of aerogel are weighed and heated at 60 °C under vacuum (<1 mPa) for 14 h. The nitrogen adsorption/desorption isotherms were carried out at 77 K (Quantachrome Instruments, Nova 2200e). The specific surface area of the aerogel was calculated with the multipoint BET model in the relative pressure range of 0.06–0.30 (P/P_0). The pore size distribution was calculated with the BJH model using a desorption isotherm for a relative pressure of less than 0.95.

2.4.3. Uniaxial compression measurements

The uniaxial compression measurements were carried out using a TA-XT Plus Texture Analyzer (Stable Micro Systems, Godalming, UK) with a 35-mm-diameter cylindrical acrylic plate lubricated with silicone oil to minimize friction between the sample and the probe. The mechanical properties were obtained by compressing four larger particles (dripping method) to 80% of their original height at room temperature (25 °C) using a cross-head speed of 0.5 mm/s. The maximum force (N) needed for compression was calculated by dividing the maximum force by the number of particles (4), which represents the maximal resistance of the surface to compression, besides giving an indication of the hardness of the particles [18].

2.4.4. Scanning electron microscopy (SEM)

The microstructures of the aerogel samples were analyzed using a scanning electron microscope (LEO Electron Microscopy, Leo 440i, Cambridge, UK) with X-ray dispersive energy detector (Oxford, 6070, Cambridge, UK). All samples were gold-sputtered prior to imaging (EMITECH, K450, Kent, UK). Analyses of the sample surfaces were performed under vacuum, using a 15 kV acceleration voltage and a large number of images was obtained in different areas of the samples (at least 20 images per sample) to ensure the reproducibility of the results.

2.4.5. Differential scanning calorimetry (DSC)

A model HP DSC1 (Mettler Toledo, Schwerzenbach, Switzerland) was used for this analysis. Samples were weighed into an empty aluminum pan, and then heated from 25 to 300 °C with a heating rate of 10 °C/min.

2.4.6. X-ray diffraction analysis

X-ray diffraction analysis (Philips Analytical X-Ray, X'Pert-MPD, Almelo, Netherlands) of the alginate aerogels was carried out using a position-sensitive detector and $\text{CuK}\alpha$ radiation within the range of 10 ° to 60 ° \times 2 θ and a step size of 0.037 ° and counting time of 2 s per step.

Table 1

Diameters (d) and polydispersity (PSD and Span) of the particles of the hydrogel, the aerogel, and the alcogel obtained by dripping and atomization with or without solvent exchange gradients.

	Characteristic	Hydrogel	Without gradient		With gradient	
			Aerogel	Alcogel	Aerogel	Alcogel
Dripping	$d_{[3,2]}$ (μm)	3204 ± 106^a	2796 ± 127^b	3094 ± 121^a	3057 ± 5^{ab}	3193 ± 137^a
	$d_{[4,3]}$ (μm)	3208 ± 106^a	2801 ± 128^b	3099 ± 121^a	3065 ± 5^{ab}	3198 ± 137^a
	$d_{(90)}$ (μm)	3306 ± 108^a	2912 ± 117^b	3198 ± 125^{ab}	3191.6 ± 79^{ab}	3313 ± 142^a
	$d_{(10)}$ (μm)	2993 ± 99^a	2611 ± 110^b	2879 ± 112^{ab}	2826 ± 102^{ab}	2949 ± 126^a
	PSD (ad.)	1.10 ± 0.01^a	1.12 ± 0.01^a	1.11 ± 0.04^a	1.13 ± 0.01^a	1.12 ± 0.05^a
	Span (ad.)	0.10 ± 0.01^a	0.11 ± 0.01^a	0.01 ± 0.01^b	0.12 ± 0.01^a	0.01 ± 0.01^b
Atomization	$d_{[3,2]}$ (μm)	281 ± 6^a	235 ± 15^{bc}	251 ± 3^b	236 ± 3^c	293 ± 11^a
	$d_{[4,3]}$ (μm)	442 ± 2^c	410 ± 30^c	475 ± 5^a	460 ± 5^b	478 ± 8^a
	$d_{(90)}$ (μm)	819 ± 1^b	711 ± 2^c	844 ± 0.3^a	697 ± 0.4^d	525 ± 1^e
	$d_{(10)}$ (μm)	126 ± 1^e	161 ± 0.5^b	159 ± 0.1^c	262 ± 0.1^a	132 ± 0.1^d
	PSD (ad.)	6.5 ± 1.0^a	4.4 ± 0.01^c	5.3 ± 0.01^b	2.6 ± 0.01^e	3.9 ± 0.01^d
	Span (ad.)	1.7 ± 0.4^a	1.4 ± 0.1^c	1.5 ± 0.01^b	1.0 ± 0.01^d	1.5 ± 0.01^b

Same superscript letter (in line) indicates no significant difference between the steps of the process ($p \geq 0.05$).

2.5. Mathematical modelling

The drying kinetics of alcogel in supercritical CO_2 was determined by the amount of ethanol extracted at a time t , which was the sum of the ethanol collected in glass vials previously weighed and the ethanol in the outlet gaseous CO_2 stream measured by the alcoholmeter device [5]. The solvent remaining was gravimetrically determined by oven drying at 105°C for 24 h. Based on the mass balance calculated with the amount of ethanol in each stream (vial, gaseous CO_2 and particles), the normalization of drying kinetics was performed as proposed by García-González et al. [5]. In summary, the first normalization considered that the first fraction of ethanol removed in SC- CO_2 drying (at time t_{excess}) corresponded to the ethanol added to the particles bed. So, the drying time (t_{drying}) was defined as the difference between time (t) and the time to remove the excess of ethanol (t_{excess}). The same normalization was performed to the amount of ethanol removed from the particles bed, since this factor is time dependent.

The contribution of diffusion phenomenon to the ethanol removal from alginate particles was evaluated by using the Fick's second law in spherical coordinates, considering that the diffusion is radial and the coefficient (D) is constant (Eq. (7)) [19].

$$\frac{\partial C}{\partial t} = D \left(\frac{\partial^2 C}{\partial r^2} + \frac{2}{r} \frac{\partial C}{\partial r} \right) \quad (7)$$

where: C is the concentration of the diffusing substance (ethanol); r is the spherical coordinate of a certain point; t is the processing time; and D is the diffusion coefficient (m^2/s). The mathematical solution of the Fick's law for a non-steady state diffusion of ethanol out from a spherical particle is given by Eq. (8) [19].

$$\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp\left(-\frac{Dn^2\pi^2 t}{a^2}\right) \quad (8)$$

where M_t is the amount of ethanol (in g) that has left the alginate particle after a certain time t ; M_∞ is the total amount of ethanol (in g) that left the alginate particle after an infinite time; a is the radius of particle (in m); and n is the number of the terms in the series.

The models were fitted to the data using a minimum of constrained nonlinear multivariable function algorithm from the MATLAB software (2014Ra, MathWorks, Natick, MA, USA). The objective function was defined as the average absolute relative deviation (AARD) referred to the amount of ethanol expressed by

the following equation. The coefficient of determination (R^2) also was also determined.

$$ARRD (\%) = \frac{100}{n} \sum_{i=1}^n \left| \frac{x_{i,exp} - x_{i,cal}}{x_{i,exp}} \right| \quad (9)$$

where $ARRD$ is the average absolute relative deviation (%), n is the number of data, $x_{i,exp}$ and $x_{i,cal}$ refer to the experimental and calculated amount of ethanol for the data i , respectively.

3. Results and discussion

This study was divided into three steps. Firstly, the influence of the solvent exchange method (with or without gradient) to the characteristics of the alcogel and the aerogel produced by dripping and atomization methods was investigated. Then, the supercritical CO_2 and the conventional method of drying were compared and the experimental results were modeled. Finally, the Na-alginate particles obtained by the dripping method were used in the impregnation of *trans*-resveratrol by supercritical CO_2 and ethanol.

3.1. Aerogel particle characterization

Numerous methods have been developed to obtain Na-alginate particles of various size ranges [20]. The dripping method is the most common technique to produce gels with spherical form, but this procedure does not result in particles of micrometric size. The atomization process is an alternative approach to obtain micrometric beads of Na-alginate ($<1000 \mu\text{m}$). In this method, the bulk solution of Na-alginate is broken into droplets, by extrusion through a needle with gas as atomizer fluid. The equipment for atomization is described in Fig. 1, as indicated in Materials and Methods section. Both air or carbon dioxide can be used as atomizer fluids. Table 1 shows the diameters of the alginate particles obtained by different solvent exchange gradients, in each step of the process, by dripping and atomization methods.

Particle shrinkage can be observed in alginate microspheres after supercritical CO_2 drying, by comparing the surface weighted ($d_{[3,2]}$) and the volume-weighted ($d_{[4,3]}$) mean diameters of the alcogels and aerogels. The same behavior was observed for particles produced without gradient in the solvent exchange step. However, the particle polydispersity, *PSD* and *Span*, showed similar values independently of the solvent exchange gradient and the process (dripping or atomization). The fineness ($d_{(90)}$) of particles and range of particle size distributions (*PSD*) are defined differently in different fields of application, but according to Merkus [21], the alginate aerogel beads obtained by the dripping method can be classified as coarse particles (1–10 mm) and as showing a narrow particle size

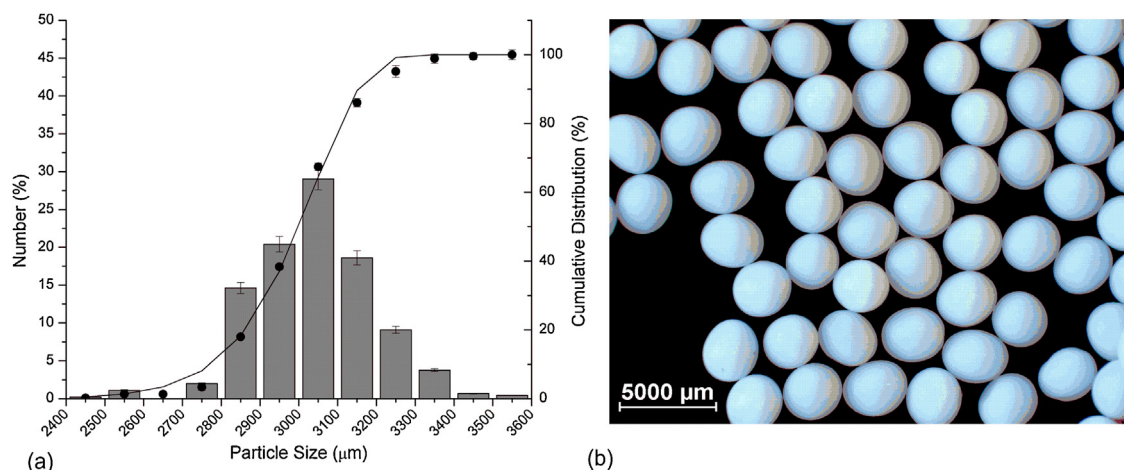


Fig. 3. (a) Particle size (bars), (—) modeled and (●) experimental cumulative distributions, and (b) optical microscopy image of Na-alginate beads obtained by the dripping method.

distributions (PSD between 1.05 and 1.5). However, the particles obtained by the atomization method can be classified as medium particles (10–1,000 μm). Fig. 3 shows the particle size modeled (Supplementary Data Table S1), the experimental cumulative distribution and the optical microscopy image of Na-alginate beads obtained by the dripping method.

Due to the lower affinity of water by the supercritical CO_2 , the replacement of the water present in the Na-alginate hydrogel for a soluble and adequate solvent is necessary [22]. Moreover, the dehydration by solvent exchange and/or supercritical CO_2 causes shrinkage in the Na-alginate fibers and porous network of the particles, as previously reported [4,5,9,22]. It is possible to verify in Table 1 that the shrinkage of the Na-alginate particles is higher after drying than after exchange of the solvent, for both studied processes, and the average particle shrinkage of the particle was about 7% ($d_{[3,2]}$ and $d_{[4,3]}$).

In the atomization method, it is also possible to control the diameter of the Na-alginate aerogel particles by changing the internal diameter of the atomization tubing. Based on this, two different internal diameters were tested and the diameters and polydispersity of the particles were evaluated, as shown in Supplementary Data Fig. S1(b). The decrease in the tube diameter produced particles with smaller diameters, with values distributed around 251.2 or 68.6 μm , to internal diameters of 0.57 and 0.17 mm, respectively. However, the polydispersity, calculated in terms of Span, increased from 1.3 to 2.6, as the internal diameter decreased from 0.57 to 0.17 mm. Moreover, the particles obtained by tubing with internal diameter of 0.17 mm showed a bimodal distribution. In fact, the size of the particles depends on the fluid properties, the jet diameter, and the jet velocity, as demonstrated by Bonhoeffer et al. [23], and the ineffective control of these parameters can lead to a bimodal particle size distribution. Although the possibility of obtaining smaller particle by the atomization process, the dripping method was chosen to produce particles for the impregnation of *trans*-resveratrol experiments due the lowest polydispersity.

The aerogel obtained by supercritical drying and the two processes (atomization and dripping) with and without gradient in the solvent exchange, had their surface area and pore size distribution characterized by N_2 adsorption/desorption, and their maximum force by uniaxial compression measurements. Fig. 4 shows the N_2 adsorption/desorption isotherms and Table 2 shows the estimated structural properties of Na-alginate aerogels obtained by two different processes, two methods of solvent exchange and supercritical CO_2 drying. All the Na-alginate aerogels exhibited adsorption/desorption type IV isotherms with a hysteresis, indi-

cating the existence of mesoporosity in the substrates. In Table 2, it is possible to observe that the average pore size of Na-alginate aerogels beads varied from 6 to 8.1 nm, these values could indicate the presence of mesopores in the aerogel beads.

The surface area of Na-alginate obtained by atomization was higher than that obtained by the dripping method, for both solvent exchange methodologies. This behavior can be explained by the difference between the processes, in which a lower quantity of atomization fluid can be solubilized in Na-alginate solution during the sprinkling, favoring the formation of a porous network in the supercritical CO_2 drying and resulting in large surface area values. Moreover, the surface area observed for the aerogel beads obtained by solvent exchange without ethanol gradient was lower than for aerogels produced under solvent gradient. It demonstrates that the solvent exchange is a crucial step in the porous network formation of Na-alginate beads and, the faster change of water to ethanol may promote the decrease of the aerogel porosity, surface area and, consequently, of the diameter of the beads. Robitzer et al. [9] studying the Ca-alginate aerogels obtained from multistep solvent exchange, affirmed that after solvent exchange from water to ethanol a volume shrinkage of 4% was induced. According to Mehling et al. [7], the main shrinkage of Na-alginate beads occur during the solvent exchange step so that the volume reduction can be significantly influenced by the selection of ethanol concentrations and the number of exchange steps.

The maximum force supported by aerogel was 26.5 N to beads obtained without solvent gradient, and 23.5 N to beads obtained under the solvent gradient, thus demonstrating that the abrupt solvent exchange may decrease the porous network, increasing the width of Na-alginate fibers and, consequently, increasing the density of the matrix [24]. Moreover, the shrinkage promoted by the abrupt solvent exchange step may approach these structures of Na-alginate and increase the force required to breakdown the Na-alginate beads in about 3 N.

3.2. Mathematical modeling of aerogel drying

The mass transfer mechanism involved in the supercritical drying of aerogels is expected to be influenced by several phenomena, such as liquid swelling and spillage, convective flow and diffusion [5,25,26]. Firstly, the drying is predominantly influenced by convective mass transfer resistances. Finally, as the drying process progresses, the amount of ethanol in the gel decreases and the role of mass transport resistance in the solid substrate (diffusion) becomes predominant [5,27–29]. Fig. 5 shows the kinetics

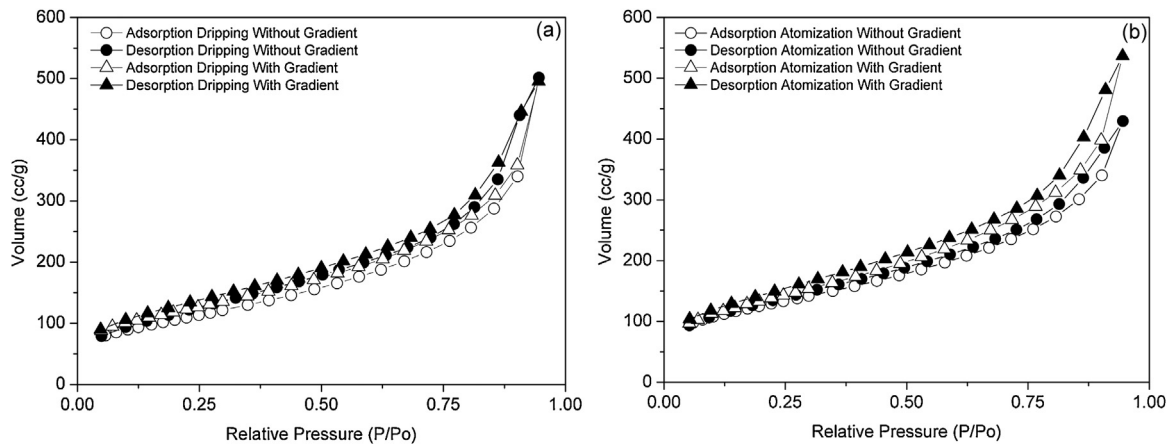


Fig. 4. N_2 adsorption (open)/desorption (filled) isotherms at 77.3 K for alginate aerogel obtained by dripping (a) and atomization (b), with (\blacktriangle) or without (\bullet) gradient at the solvent exchange step.

Table 2

Estimated structural properties of Na-alginate aerogel beads obtained by dripping and atomization, with and without solvent exchange gradient, and supercritical CO_2 drying.

Characteristic	Dripping		Atomization	
	Without gradient	With gradient	Without gradient	With gradient
Surface area (m^2/g)	381.4	424.6	443.4	484.4
Pore volume (cc/g)	0.68	0.65	0.54	0.70
Average Pore Diameter (nm)	8.1	7.2	6.0	6.8
Maximum force (N)	26.5	23.5	n.d.	n.d.

n.d.: not determined.

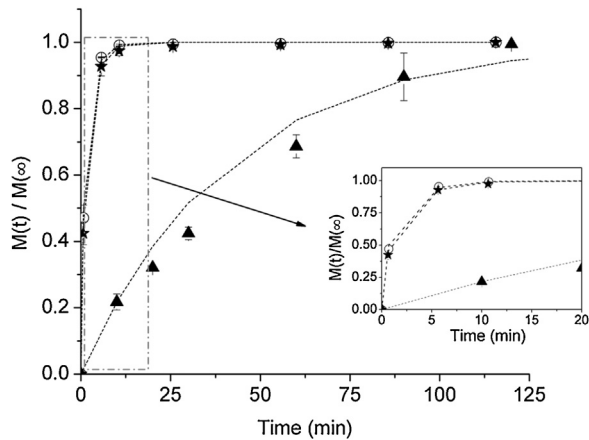


Fig. 5. Comparison of supercritical and conventional drying of alginate aerogels: (\blacktriangle) Conventional drying of dripping aerogel; (\star) Supercritical drying of dropped aerogel; (\circ) Supercritical drying of atomized aerogel. Process conditions: SC- CO_2 at 11.0 MPa and 318 K and oven drying at 318 K.

of ethanol extraction from alginate gel particles by supercritical drying and by conventional drying.

It is possible to observe in Fig. 5 that within the first 10 min of SC- CO_2 drying about 90% of the solvent in the porous gel network was removed, while to a convectional drying only 25% of ethanol was evaporated from gel particles. García-González [5] showed that after 10 min of SC- CO_2 drying more than 60% of the solvent initially in the alginate gel was removed. It is possible to observe in Fig. 5 that the beginning of the drying kinetics (0–10 min) of Na-alginate beads is predominantly influenced by a high dissolution of SC- CO_2 in the liquid into the pore network leading to an expanded liquid. Consequently, occurs the spillage of the excess liquid volume out from the gel particle. These phenomena were described by Selmer et al. Part 1 [25] and Part 2 [26]. Between 10 and 25 min

of the drying, the concentration of CO_2 in the liquid present into the gel pores increases with time until the supercritical conditions are reached for the mixture CO_2 -Ethanol. After 25 min, as the drying process progresses, the concentration of solvent in the gel pore network decreases and diffusion of ethanol in the particle becomes predominant [5]. Table 3 shows the estimated diffusion coefficient for alginate gel particles obtained by dripping and atomization with and without solvent exchange gradient.

The diffusion coefficients obtained to Na-alginate beads processed without solvent exchange gradients (Table 3) were higher than the ones of beads processed using the ethanol gradient. This behavior is probably a consequence of the shrinkage caused by the abrupt solvent exchange, which reduces the bead volume and can obstruct the porous network. Wawrzyniak et al. [29] studied the drying of silica gels and obtained a diffusion coefficient of $5.5 \times 10^{-9} m^2/s$ at 315 K and 0.9 MPa, whereas in the present paper values between 1.8×10^{-9} and $6.3 \times 10^{-11} m^2/s$ at 11.0 MPa and 318 K were obtained. García-González et al. [5], determined a diffusion coefficient of $1.3 \times 10^{-8} m^2/s$ for silica microspheres at 11.0 MPa and 318 K. According to these authors [5], the difference between the diffusion coefficient of pure ethanol in supercritical CO_2 at the drying conditions (Wilke and Chang equation [30]: $4.1 \times 10^{-8} m^2/s$) and the ethanol into the Na-alginate particles is possibly a consequence of the porosity and of the tortuous path that improves the resistance of the gel network.

The diffusion coefficients obtained for atomized particles of Na-alginate beads were lower than the ones obtained for the particles produced by the dripping method, as shown in Table 3. However, the drying kinetics for particles obtained by the different methods had a similar behavior. If the diffusion is influencing the solvent removal process, the drying rate should be controlled by the length of the diffusion path of the material, as described in Eq. (8). So, the difference between the diffusion coefficients can be explained by the smaller path length of the atomized particles as compared to the particles obtained by the dripping method.

Table 3
Estimated diffusion coefficient (D) for alginate gel particles obtained by dripping and atomization with and without solvent exchange gradient.

Method	Parameter				
		$D(\text{m}^2/\text{s})$	ARRD (%)	R^2	
Dripping	Without gradient	SC-CO ₂	1.8×10^{-9}	0.6	0.999
	With gradient	SC-CO ₂	1.5×10^{-9}	0.8	0.999
	With gradient	Oven	6.4×10^{-10}	5.5	0.979
Atomization	Without gradient	SC-CO ₂	6.5×10^{-11}	0.2	0.999
	With gradient	SC-CO ₂	6.3×10^{-11}	0.4	0.999
	With gradient	Oven	n.d.	n.d.	n.d.

n.d.: not determined.

Table 4
Effect of resveratrol solution concentration on loading in alginate aerogel.

Resveratrol solution concentration (% w/w)	Volatile content (%) ^{a,b}	Resveratrol loading (% w/w) ^a	
		Gravimetry	HPLC
0	7.9 ± 0.2^a	–	–
1	6.5 ± 1.2^b	8.8 ± 0.2^{cA}	6.7 ± 0.4^{cB}
5	5.3 ± 0.2^{bc}	44.5 ± 0.1^{bA}	42.3 ± 0.6^{bB}
10	3.5 ± 0.7^d	77.1 ± 7.0^{aA}	63.7 ± 0.9^{aB}

^a Same superscript letter indicates no significant difference ($P \geq 0.05$). Comparisons between lines (small letters) and between columns (capital letters).

^b Volatile content at 60 °C and vacuum of 600 mm/Hg – samples expose for 24 h.

The alcohol gel beads were also submitted to a conventional drying (in oven) and their diffusion coefficients were compared to the values obtained by supercritical CO₂ drying. The conventional drying led to a pronounced shrinkage of the Na-alginate structure, destroying the porous network of the particles and obstructing the solvent extraction from the Na-alginate matrix. This is a very different result in comparison to supercritical CO₂ drying that can preserve the pores network structure [31,32]. Consequently, the surface tension of the remaining liquid into the pore walls will create a capillary pressure gradient, which can collapse the pores and cause the shrinkage the gel, resulting in a xerogel, as shown in Supplementary Data (Fig. S2). This phenomenon reflected in the diffusion coefficient values, once that obtained for oven dried particles (6.4×10^{-10} m/s) was much lower than for the particles from supercritical CO₂ (1.5×10^{-9} m/s). Della Porta [33] compared supercritical CO₂ and air drying for Na-alginate beads and observed a significant shrinking factor of 28% in the air-dried particles compared to than that dried by SC-CO₂. Moreover, the authors showed by SEM images that the SC-CO₂ drying method could preserve the nanostructure while the convention drying collapsed this structure.

3.3. Impregnation of trans-resveratrol

Encapsulation of resveratrol have been performed through several different techniques like as in microparticles spray-dried from emulsions [34,35], in nanoparticles produced by antisolvent precipitation [36] and in by particles from gas saturated solutions-drying [34]. The impregnation of resveratrol in alginate aerogel particles dried by supercritical CO₂ is at this moment first reported here in this work. Wet impregnation was performed using resveratrol as a target compound and large particles of alginate as porous matrices. This is an appropriated technique to load polar target compounds, since they are poorly or not soluble at all in supercritical CO₂. Based on this, the loading capacity of alginate aerogels was evaluated using resveratrol solutions with increasing concentrations after the solvent exchange step. The loading results obtained by gravimetry and by HPLC methods are presented in Table 4, where it is possible to observe that the increase in resveratrol concentration led to an increase in the loading. The highest resveratrol loading measured by HPLC was about 64% (w/w) using a solution containing 10% (w/w) resveratrol. Despite the possible loss resveratrol phenomena, the designed impregnation process allowed to reach high

loading (60–70%), mainly because it is known that resveratrol may be lost during the drying due to resveratrol solubility in supercritical CO₂ increases in of ethanol. Therefore, the maximum possible load depends on several factors as the percentage of resveratrol solution, supercritical CO₂ conditions (temperature and pressure), and composition (resveratrol + ethanol + CO₂) inside the column drying along the drying time.

The results presented in Table 4 also show differences between the two methods: 2.1, 2.2, and 13.4% for 1, 5, and 10% resveratrol, respectively. The most significant difference was observed at 10% resveratrol, i.e., the most concentrated solution. During the experiment using 10% resveratrol, a visual degree of crystallization surrounding the particle was observed after supercritical drying, i.e., this amount of resveratrol was quantified by the gravimetric but not by the HPLC method due to possible loss by the contact with the storage recipient previously to the HPLC analysis. The image presented in Fig. 6 corroborates this visual observation of crystal formation around the particle. The gravimetric method should be used to obtain an estimation of loading, but for precise results, HPLC analysis is more suitable.

Two analytical tools are routinely used to characterize the state of the confined solids into aerogel, i.e., differential scanning calorimetry (DSC) and powder X-ray diffraction (XRD) [37]. DSC thermograms are presented in Fig. 7, where two peaks can be noticed in the raw alginate curve: an endothermic peak close to 113 °C, which may be attributed to the dehydration process; and an exothermic peak at 245 °C that is related to the polymer decomposition, as observed by other authors for raw alginate aerogels [38,39]. The curve for the impregnated alginate aerogel showed a similar profile, as compared to raw alginate, though the first endothermic peak is less deep, and the second exothermic peak (decomposition) is now superimposed to the endothermic peak of resveratrol melting (270 °C).

Fig. 8 presents the diffractograms of resveratrol and of raw and impregnated alginate aerogels. The raw alginate particles (Fig. 8b) present an amorphous halo, without crystalline peaks, in accordance with its amorphous nature, and showing that the supercritical drying did not affect its original structure. The diffractogram of loaded particles presented peaks from pure resveratrol influenced by the amorphous nature of the alginate matrix. According to Bauer et al. [40], in the context of drug delivery, it is essential that polymorphs and amorphous states have different solubility

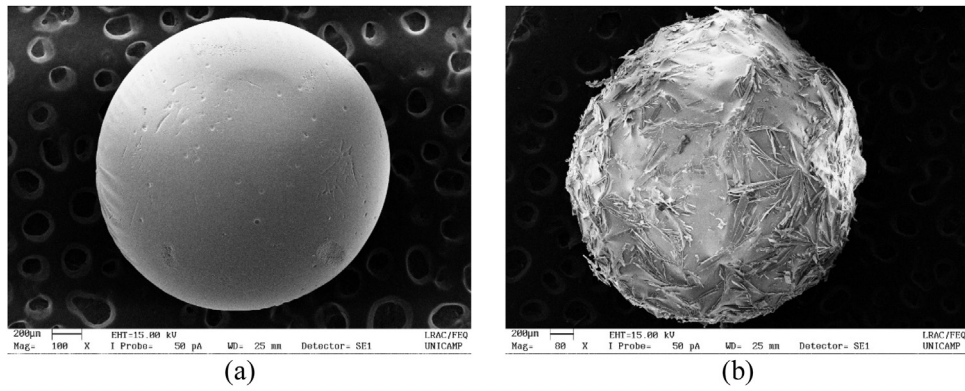


Fig. 6. Surface morphology of (a) raw alginate particle and (b) loaded alginate particle. Scale bar of 200 µm.

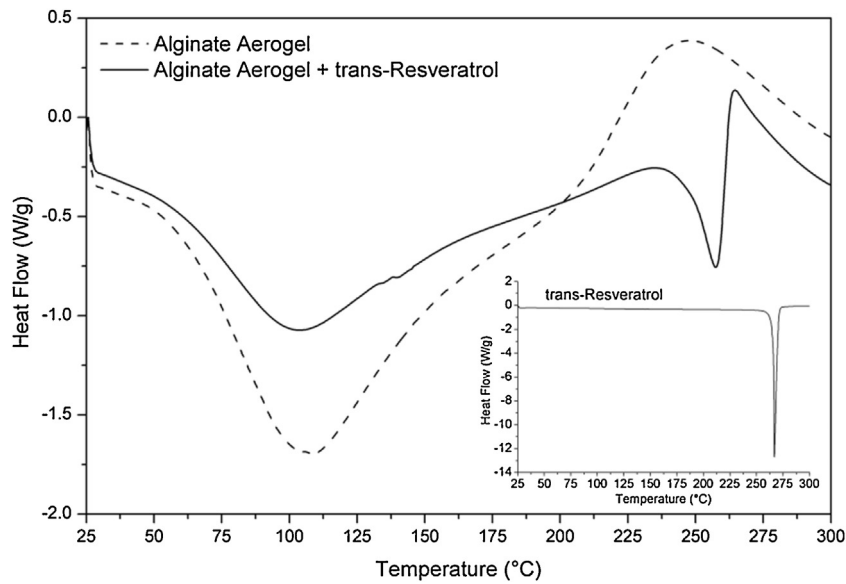


Fig. 7. DSC thermograms of raw alginate aerogel, alginate aerogel impregnated with resveratrol (solution of 10%), and pure resveratrol.

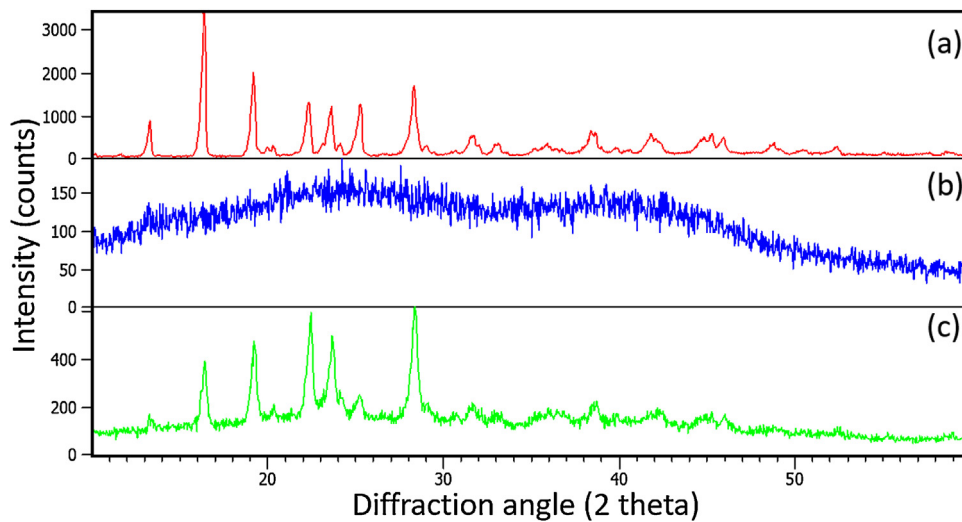


Fig. 8. X-ray diffraction patterns for: (a) pure resveratrol; (b) raw alginate aerogel; (c) alginate aerogel impregnated with resveratrol (solution of 10%).

and thus different therapeutic activity, consequently, amorphous state has gained a great deal of attention in recent times in both pharmaceutical science and industry [37].

In summary, it is possible to obtain Na-alginate aerogel beads loaded with resveratrol in a short processing time, particularly under supercritical drying, in which the time to reduce 98% (at

least) of the ethanol concentration is about 25 min. Moreover, it was demonstrated that the use of several steps (with gradient) or one-step (without gradient) of solvent exchange (ethanol) had few influences on the particle characteristics. These results are important since they can impact directly on the cost of the process.

4. Conclusions

Shrinkage of the particle size of alginate microspheres was observed after supercritical drying when comparing the surface weighted and the volume-weighted mean diameters of the aerogels and aerogels. The same behavior was observed for particles produced without gradient in the solvent exchange step. However, no significant differences in the polydispersity of the particles were observed. Within the first 25 min of SC-CO₂ drying, about 98% of the solvent in the porous gel network was removed, while to a conventional drying only 30% of the ethanol was evaporated from the gel particles. High surface area (484 m²/g) and pore volume (0.70 cc/g) were obtained for the atomization with exchange solvent gradient. Aerogels obtained by dripping without gradient of ethanol showed surface area of 381 m²/g and pore volume of 0.68 cc/g.

The resveratrol loading ranged from 6.7 ± 0.4–77.1 ± 7.0% and increased with the concentration of the resveratrol solution. The supercritical CO₂ does not change the amorphous structure of the alginate on drying and that the resveratrol crystal structure is preserved in the impregnation. Alginate aerogel showed to be an appropriate material to load high amount of resveratrol, and wet impregnation and supercritical drying can be presented as feasible techniques to load polar compounds into an aerogel. However, other factors should be studied such as pressurization/depressurization rate, effect of atomization gas and drug release from impregnated beads, before performing the cost evaluation of the process and further applications.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.supflu.2020.104882>.

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