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Screening design of experiment applied to the supercritical antisolvent precipitation of quercetin

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ABSTRACT

A fractional factorial design of experiment at two levels has been applied to the micro- and nanoprecipitation of quercetin by a supercritical antisolvent process (SAS) in order to identify the factors that are important and their appropriate ranges with the mean particle size used as a response. The model was successfully fitted by multiple linear regressions. In general, higher pressures and temperatures, a lower initial concentration of the solution, and high CO_2/liquid solution flow rate ratios favored the precipitation of smaller particles. The use of a smaller nozzle diameter led to smaller particle sizes in the assayed range but washing time had a negligible effect. The morphologies of the precipitates were analyzed by scanning electron microscopy (SEM). X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) were employed to evaluate the possible loss of crystallinity and activity of the precipitates. Dynamic light scattering (DLS) and image analysis software were used to measure the particle size and particle size distribution. The particle size range of the precipitates was 0.15–1.24 μm and the formation of nanoparticles or microparticles was dependent on the operating conditions of the SAS process. The dynamic light scattering measurements gave higher than expected particle sizes due to possible agglomeration.

1. Introduction

Quercetin is a well-known flavonoid that is found in some plants, foods, and beverages and it has shown pharmacological activities such as antioxidant, anti-inflammatory, anticancer, and antiviral [1]. In a similar way to many drugs and nutraceuticals, quercetin has low solubility in aqueous media [2] and this drawback has limited its absorption upon oral administration. As a consequence, there is considerable interest in processing quercetin to improve its water solubility and even to protect the active groups of the molecule from degradation by applying different coating agents, thus increasing the bioavailability and biological activity of this compound [2]. For example, quercetin has been precipitated at the boundary of the micro- and nanometer range. Sahoo et al. fabricated submicron crystals of quercetin by high-pressure homogenization in a process that involved 20 homogenization cycles at 1500 bar [1]. Quercetin-embedded poly (lactic acid) nanoparticles were synthesized by a novel emulsified nanoprecipitation technique [3] and nanostructured lipid quercetin systems were prepared by a high-pressure homogenization method [4].

Supercritical fluids (SCFs) are increasingly used as an alternative in nanoprecipitation techniques [5–7] for high value added products that can be used in the pharmaceutical, cosmetic, and food industries. The use of SCFs offers advantages such as higher product quality in terms of purity, more uniform dimensional characteristics, a substantial improvement in environmental aspects and, furthermore, their properties can be adjusted continuously by altering the experimental conditions [8–9]. In particular, carbon dioxide (CO_2) under supercritical conditions has been widely used due to its relatively low critical temperature (31.1 °C) and pressure (73.8 bar), which are sufficiently mild to permit the micronization of thermolabile solutes, along with its low toxicity and low cost.

The low solubility of quercetin in supercritical CO_2 [10] led to the choice of a supercritical antisolvent process (SAS) for the micronization of this flavonoid. In the SAS process the solution is sprayed through a nozzle to generate drops of solution in a process that favors the diffusion of the supercritical fluid into these drops, which in turn dissolves them in the solvent and leads to a high supersaturation of the solute solution and the precipitation of a powder.

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Quercetin has previously been processed by an SAS technique and crystalline particles with particle sizes in the micrometer range (1–6 μm) was obtained [5,11–12]. Kakran et al. [13,14] studied some parameters of the SAS process in greater depth, including drug concentration, stirring speed, flow rate, solvent/antisolvent ratio, and how mass transfer between solvent and antisolvent could be enhanced. Particles with sizes in the range 120–450 nm was obtained. Encapsulated quercetin microparticles were recently produced by the extraction of organic solvent from oil-in-water emulsions by supercritical fluid extraction of emulsions [15] and by an SAS process [2].

The supercritical antisolvent extraction of quercetin along with other polyphenols from a range of wastes such as grape seeds [16–17], olive [18], and eucalyptus [19] leaves, amongst others, has been achieved; the coprecipitation by supercritical antisolvent extraction of young tea leaves [20] or rosemary plants [21] has been achieved using polymers. Spherical or quasi-spherical micro- and submicroparticles with potent antioxidant activity were obtained in these processes.

In the work described here the SAS precipitation of raw quercetin was carried out in a comparative study with the aim of obtaining nanoaparticles of quercetin with a narrow particle size distribution. A fractional factorial design at two levels was applied in order to include all the possible parameters involved in SAS process, thus reducing the number of experiments carried out. The main factors evaluated in the supercritical antisolvent process were particle size, size distribution, and morphology of the precipitates.

2. Materials and methods

2.1. Materials and Analytical Methods

Quercetin (C15H10O7) and absolute ethanol (99.8%) were purchased from Sigma–Aldrich (Spain). CO_2 with a minimum purity of 99.8% was supplied by Linde (Spain). The mean particle size of commercial quercetin was about 2.68 μm, as can be seen in Fig. 1.

2.2. Experimental design

The main factors that influence the precipitation of quercetin in a supercritical fluid antisolvent process were investigated using a linear model. To achieve this aim, a fractional factorial design resolution IV at two levels (2^{7-3}) was performed. Seven parameters were studied and three confounding parameters were included, each of which was a pair of associated factors. This model is highly recommended for screening experiments [22]. The use of this fractional design reduces the number of experiments from 128 for a full factorial design (2^7) to 16 (2^{7-3}). In this design, the experimental region is assumed to be a cube and a fraction of all possible corner experiments was selected for experimental study. The complete design consisted of 19 experimental points that included 16 factor points and three replications at the center point (experiments 17–19). Other fractional factorial designs have been successfully applied in several previous cases for screening purposes [23–27].

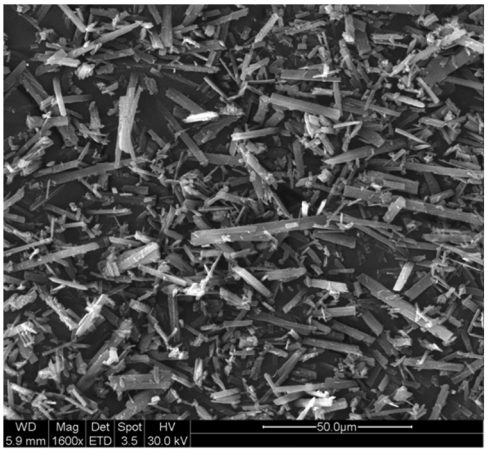

Fig. 1. SEM image of unprocessed quercetin.

The reduction in the number of experiments meant that effects became confounded, i.e., to a certain degree they were mixed up with each other. The choice of confounded factors was made considering that, in statistical terms, the factor that is thought to be the most likely to affect process performance is associated with the weakest interaction. The main effects usually dominate over three-factor interactions [22].

In this work the liquid flow rate, nozzle diameter, and washing time were confounded with the concentration/temperature/pressure, pressure/temperature/CO_2 flow rate, and concentration/temperature/CO_2 flow rate interactions. Thus, it was expected that the effect of the liquid flow rate, nozzle diameter, and washing time would exclude any contribution of the concentration/temperature/pressure, pressure/temperature/CO_2 flow rate, and concentration/temperature/CO_2 flow rate interactions, respectively. The Modde (Version 5.1, Unmetrics, USA) program was used to design the experiments, to analyze the linear model, and to generate the graphics and contour plots to represent the main effects.

Seven factors (pressure, temperature, concentration, CO_2 and liquid solution flow rates, nozzle diameter, and washing time) were identified as possible parameters that could influence the SAS process and thus the particle size and size distribution.

The two levels for each factor are shown in Table 1. The low and high levels of concentration (cc) were 2 and 11 mg/ml; 2 mg/ml was chosen to obtain a sufficient quantity of quercetin for subsequent analysis, and 11 mg/ml was selected due to its low solubility in ethanol at room temperature. The low and high temperature (T) levels were 35 and 65 °C: the low temperature level ensured the presence of supercritical CO_2 and the high level was selected to avoid degradation of quercetin. Pressures (P) of 80 to 250 bar at the aforementioned temperatures would lead to different locations with respect to the mixture critical point (MCP) for ethanol/CO_2 (76.8 bar at 40 °C and 100.7 bar at 60 °C) [28]. In line with common practice, it was assumed that the presence of quercetin did not affect the ethanol/CO_2 equilibrium and therefore an ethanol/CO_2 pseudo-binary mixture was considered rather than a ternary equilibrium [29]. Experiments were carried out below, close to, and far above the MCP. CO_2 and liquid solution flow rates of 10–50 g/ml (Q_{CO2}) and 2–10 mg/ml (Q_L) were included in the design in order to evaluate the effect of flow ratios. Nozzle diameters ($Øn$) from 100 to 200 μm were assayed in an effort to shed light on the hydrodynamics of the process. Washing times (t_w) from 30 to 90 min were chosen on the basis of our previous experience.

Table 1
Twolevel assessment for each factor and calculated effects on PS.

Factors	Low level	High level	Effects
C (mg/ml)	2	11	0.105
T (°C)	35	65	−0.076
P (bar)	80	250	−0.097
Q_L (ml/min)	2	10	0.302
Q_{CO_2} (g/min)	10	50	−0.072
t_w (min)	30	90	−0.003
$Øn$ (μm)	100	200	0.081