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Production of Curcumin/β-cyclodextrin Inclusion Complexes by Supercritical Antisolvent Process

Iolanda De Marco*, Paola Franco

Università degli studi di Salerno, Dipartimento di Ingegneria Industriale
Via Giovanni Paolo II, 132, 84084, Fisciano (SA), Italy
idemarco@unisa.it

In this work, β-cyclodextrin (β-CD) based inclusion complexes containing curcumin (CURC), a yellow pigment from *Curcuma longa* plants, were obtained through the Supercritical AntiSolvent (SAS) process. Curcumin has been widely used for its antioxidant, antimicrobial, antibiotic and antiviral properties. Recently, curcumin has been proposed as a potential treatment option for patients with coronavirus disease (CoVID-19). In order to protect the active compound, the supercritical antisolvent process was used, to induce the formation of inclusion complexes using β-CD as the carrier. The effect of different parameters, such as pressure, overall concentration of solutes in the liquid solution, and CURC/β-CD molar ratio, on the morphology and size of the composite particles, was investigated. Well-defined microparticles at 1/1 and 1/2 CURC/β-CD molar ratios were produced. The dissolution rate curve of the obtained inclusion complexes was compared to the one of the unprocessed CURC.

1. Introduction

The research of the last year in the pharmaceutical field has been strongly polarized on the search for active compounds that have an effect in contrasting the Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) which cause CoVID-19 that has generated a pandemic, influencing the lives of the entire world population since the end of 2019 (Cascella et al., 2020). In moderate to severe form of the infection, patients may exhibit symptoms, such as difficulty breathing or shortness of breath, severe dyspnea, respiratory distress, and tachypnea (Valizadeh et al., 2020). In some cases, hospitalization in intensive care unit (ICU) is necessary and, unfortunately, the CoVID-19 mortality rate is high. Although the exact mechanism of CoVID-19 pathogenesis is not fully comprehended, aberrant immune responses appear to play a key role in the development of the disease (Ghaebi et al., 2020). Furthermore, many studies carried out in the past year have hypothesized that disease severity may be associated with the production of inflammatory cytokines (Huang et al., 2020).
Curcumin (CURC) is a polyphenol of diacryl heptanoids isolated from *Curcuma longa* plants. For many years, curcumin has been used in health issues for its anti-inflammatory, antioxidant and anti-cancer effects, because of its ability to prevent the progression of tissue damage and the development of inflammatory diseases (Aggarwal et al., 2003). Some studies indicate the potential benefits of CURC against respiratory viral infections in general (Avasarala et al., 2013) and against SARS coronavirus in particular (Barnard and Kumaki, 2011). For this reason, some studies on the use of curcumin in the treatment of SARS-CoV-2 have been published (Soni et al., 2020). CURC has an intense yellow color, is insoluble in water, and is unstable to light, factors that usually limit its application in the pharmaceutical field. These limitations may be overcome coprecipitating the active pharmaceutical ingredient (API) with a hydrophilic carrier (Prosapio et al., 2015) or forming an inclusion complex (Franco and De Marco, 2021). In the former case, it is possible to coprecipitate the API with polymers such as polyvinylpyrrolidone (PVP), in the latter case, cyclodextrins are generally used. Indeed, cyclodextrins (CDs) are cyclic oligosaccharides with a hydrophilic external surface, which determines their water solubility, and a hydrophobic internal cavity, which can hold various molecules. In order to form inclusion complexes, the guest molecule (drug) has to be possess a suitable size, shape, and the right characteristics of hydrophobicity.

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Among the various CDs, α-cyclodextrin has a too small cavity for most drugs, while γ-cyclodextrin is really expensive. A good compromise is represented by β-cyclodextrin (β-CD), used mainly for pharmaceutical applications since the dimensions of its cavity allow the incorporation of a wide range of drugs (Szejtli, 1990). Different conventional methods, such as kneading, solvent evaporation, sealed-heating, spray-drying, and freeze-drying have been used to prepare guest-host inclusion complexes (Patil et al., 2010). However, these methods suffer from some drawbacks like multistage processing, the high residual content of toxic solvents in the final product, the thermal degradation of the drug due to the high process temperature, and the difficult control of the morphology and size of the produced particles (Sauceau et al., 2008). In order to overcome these limits, supercritical fluids (SCF) assisted processes have been proposed (Baldino et al., 2017). SCF based processes allow to control the morphology and particles size distribution (PSD) in the case of powders production and allow to reduce (Cardea et al., 2013) or in, in the case of some processes, eliminate (Franco et al., 2019) the use of organic solvents; for this reason, they are considered as “green” processes (Franco and De Marco, 2020). Due to its peculiarities, the most used fluid in the supercritical state is the carbon dioxide (Baldino et al., 2015). Among the processes based on the use of the supercritical carbon dioxide ($scCO_2$), the Supercritical AntiSolvent (SAS) technique has been widely employed in different fields to precipitate (De Marco et al., 2013) or coprecipitate with polymers (Prosapio et al., 2018) different active principles. For a successful SAS precipitation, the following prerequisites have to be accomplished: the $scCO_2$ (with the role of the antisolvent) and the liquid solvent have to be completely miscible at the process conditions, whereas the solute/solutes to be processed has/have to be soluble in the selected solvent but insoluble in the binary mixture formed by the solvent and the antisolvent. In the coprecipitation process through SAS, composite microspheres constituted by a polymer and an API are produced; whereas, in the case of the formation of an inclusion complex, the drug is encapsulated into one or two CDs. In the latter case, the obtained microparticles can consist of various units of complexes, which can assume different configurations, from the most frequent 1/1 mol/mol guest/host ratio to 2:1 and 1:2 ratios (Szejtli, 1990). Up to now, a limited number of papers has been devoted to the preparation of β-CD inclusion complexes through the SAS technique. For example, Lee et al. (2010) used the β-CD to mask the bitter taste of an antihistamine drug; Nerome et al. (2013) produced spherical particles by coprecipitating lycopene and β-CD using SEDS (solution-enhanced dispersion by supercritical fluids), a process similar to SAS in which the supercritical carbon dioxide equally has the role of the antisolvent; Jia et al. (2018) used SEDS to obtain microparticles and nanoparticles of berberine/β-CD with improved dissolution properties; Franco and De Marco (2021) used the SAS process to precipitate nimesulide/β-CD and ketoprofen/β-CD inclusion complexes, obtaining microparticles characterized by an accelerated drug release.

Considering the few papers about the preparation of inclusion complexes by the SAS technique despite the advantages brought by the use of supercritical fluids, and considering the growing interest in the use of CURC that can be used in the treatment of CoVID-19, the present work was focused on the formation of inclusion complexes using $scCO_2$ as the antisolvent and β-CD as the carrier, in order to improve the dissolution rate of CURC.

2. Materials and methods

2.1 Materials

β-cyclodextrin (β-CD, purity 99.9 %) and curcumin (CURC, purity > 65 %) were provided by Sigma–Aldrich (Italy). Dimethylsulfoxide (DMSO, purity 99.5 %) was purchased from Carlo Erba (Italy). Carbon dioxide (CO_2, purity 99 %) was supplied by Morlando Group s.r.l. (Italy).

2.2 SAS apparatus and procedure

The experiments were performed in a homemade laboratory plant. Carbon dioxide, which is stored in a tank, is fed to the precipitation chamber with an internal volume of 0.5 L through a high-pressure pump. The liquid solution is prepared by dissolving the solutes (CURC+β-CD) in DMSO. The prepared solution is sprayed into the precipitation vessel through a stainless-steel nozzle with an internal diameter of 100 μm, through a second high-pressure pump. The temperature control into the vessel is ensured by a proportional integral derivative (PID) controller, which is connected with electrically thin bands. Instead, the pressure is measured using a test gauge manometer and regulated by a micrometric valve. The precipitated powder is collected at the bottom of the precipitation vessel on a stainless-steel filter. The mixture consisting of CO_2 and DMSO can pass through the filter, as it has pores of 0.1 μm. Then, the liquid solvent is recovered in a separator located downstream the vessel; the pressure into the separator is fixed at a lower value than that into the precipitation vessel (about 20 bar) through a back-pressure valve. The flow rate and the total amount of delivered CO_2 are measured at the exit of the separator by a rotameter and a dry test meter, respectively. A SAS experiment begins by pumping the CO_2 to reach the desired pressure in the vessel, which is heated up to the selected

temperature. Once the operating conditions are stabilized, the pure DMSO is injected for ten minutes; then, the liquid solution (CURC+β-CD+DMSO) is sent to the vessel with the resulting precipitation of the solute/solutes because of it/their supersaturation. At the end of the injection step, the $scCO_2$ continues to flow to eliminate the residual solvent. After the washing step, the vessel is completely depressurized, the precipitation vessel can be disassembled and the precipitated powder can be recovered and analyzed.

2.3 Analytical methods

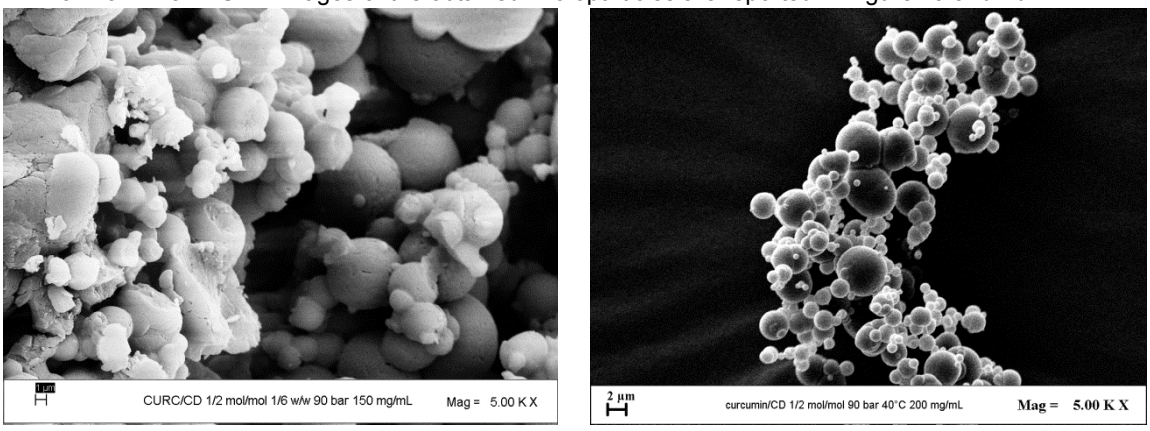
The morphology of the precipitated complexes was determined by Field Emission Scanning Electron Microscopy (FESEM, mod. LEO 1525, Carl Zeiss SMT AG, Oberkochen, Germany). Aluminium stubs, on which carbon tabs were previously stuck, were used to collect samples from different parts of the precipitator. The powders were covered with gold-palladium (layer thickness 250 Å) using a sputter coater (mod. 108 A, Agar Scientific, Stansted, United Kingdom). The diameters of about 1000 particles for each sample were measured from FESEM photomicrographs by using the Sigma Scan Pro image analysis software (release 5.0, Aspire Software International Ashburn, VA). From these data, Microcal Origin Software (release 8.0, Microcal Software, Inc., Northampton, MA) allowed the determination of the particle size distributions (PSDs).
The dissolution kinetics of not processed or released from SAS-prepared samples CURC were studied using a UV/vis spectrophotometer (model Cary 50, Varian, Palo Alto, CA). The analyses were performed at a wavelength of 425 nm. In agreement with the literature, the dissolution tests were achieved in phosphate-buffered saline solution (PBS) at pH 6.8 (Rezaei and Nasirpour, 2019). Samples containing 2 mg of equivalent CURC were incubated in 300 mL of PBS at pH 6.8, continuously stirred at 150 rpm and heated at 37 °C.
The following procedure was executed: for the first 10 min, the sample was detected every 0.3 min, in the range 10–20 min every min, in the range 20-40 min every 5 min and for the remaining part of the release every 30 min. Each analysis was stopped at the end of the drug release; i.e., when the plateau was reached, and all the drug was released to the outer phase. Each analysis was performed in triplicate.

3. Results and discussion

All the SAS experiments were performed using DMSO as the liquid solvent, a temperature of 40 °C, the flow rates of CO_2 and liquid solution equal to 30 g/min and 1 mL/min, respectively. At the chosen temperature, the selected flow rates permit to work at CO_2 molar fractions approximately equal to 0.98; i.e., on the right of the Mixture Critical Point (MCP) of the binary system $scCO_2$/DMSO. This condition ensures that the operating point lies in the supercritical mixture region (Andreatta et al., 2007). The effect of other operating parameters, such as pressure, overall concentration of CURC+β-CD in DMSO and CURC/β-CD molar ratio on the formation of inclusion complexes was evaluated. In particular, the total concentration was varied from 100 to 200 mg/mL, the pressure from 90 to 150 bar, and the CURC/β-CD molar ratio from 1/2 to 1/1 (corresponding to 1/6 w/w and 1/3 w/w, respectively).

3.1 Precipitation experiments

The first set of experiments was performed to evaluate the effect of the total concentration on the particles' morphology. For this set of experiments, the pressure was fixed at 90 bar and the CURC/β-CD molar ratio at 1/2 mol/mol. Two FESEM images of the obtained microparticles are reported in Figure 1a and 1b.



(a) *(b)*

Figure 1. FESEM images of curcumin/CD microparticles precipitated from DMSO at 40 °C, 90 bar, CURC/β-CD 1/2 mol/mol. Effect of the total concentration (a) 150 mg/mL; (b) 200 mg/mL.

Well-defined spherical microparticles were obtained only at 200 mg/mL, that was fixed for the subsequent sets of experiments.
Therefore, the effect of pressure was evaluated at 200 mg/mL and in correspondence of a CURC/β-CD molar ratio equal to 1/2 mol/mol. In correspondence of all the tested pressures, microparticles were obtained. It was observed that by increasing the pressure, particle size reduced and the degree of coalescence increased. From the point of view of the sphericity of the obtained particles, 90 bar is the best pressure to produce well-defined microparticles. The comparison between the volumetric cumulative particle size distributions is reported in Figure 2.

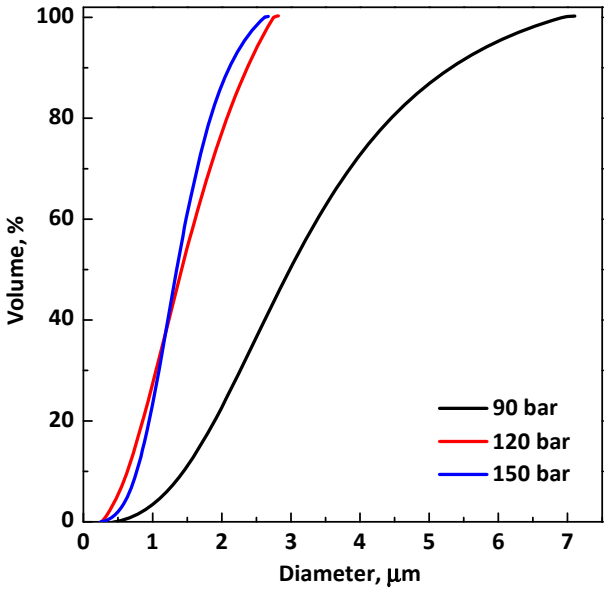
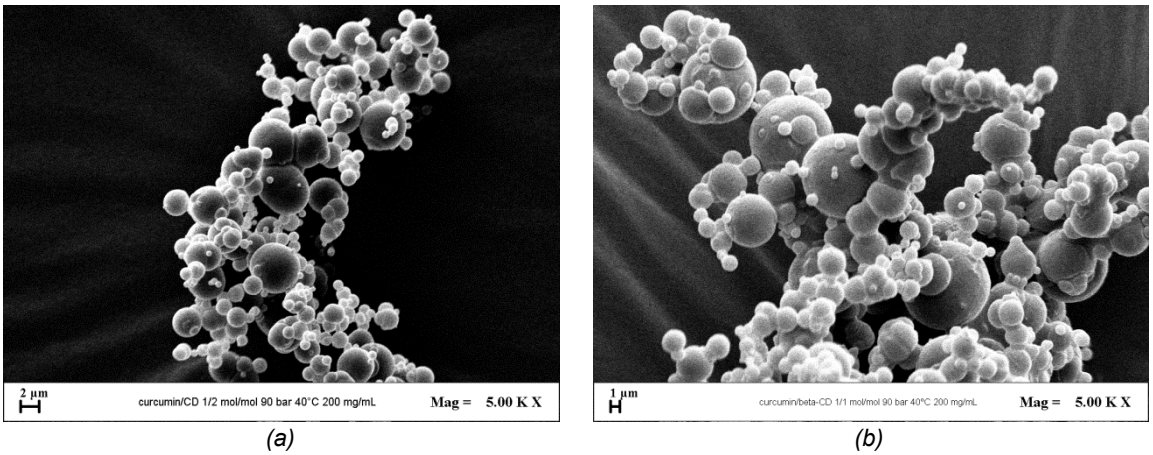


Figure 2. Volumetric cumulative PSDs of curcumin/CD microparticles precipitated from DMSO at 40 °C, 200 mg/mL, CURC/β-CD 1/2 mol/mol; effect of pressure.

Since the drug/β-CD molar ratio can strongly affect the complex inclusion formation, another set of experiments was performed at different curcumin/CD molar ratios; indeed, this parameter was varied from 1/2 mol/mol to 1/1 mol/mol. It is possible to observe from Figure 3 that well-defined microparticles were obtained in both cases.



(a) *(b)*

Figure 3. FESEM images of curcumin/CD microparticles precipitated from DMSO at 40 °C, 90 bar, 200 mg/mL. Effect of CURC/β-CD molar ratio (a) 1/2 mol/mol; (b) 1/1 mol/mol.

3.2 Dissolution tests

Dissolution tests performed to compare the dissolution rates of unprocessed CURC and CURC/β-CD 1/1 mol/mol are reported in Figure 4. Pure CURC dissolved completely in PBS in about 31 h, whereas the CURC contained in the CURC/β-CD 1/1 mol/mol complex dissolved in about 35 min (the 90 % of the API was dissolved in 20 minutes).

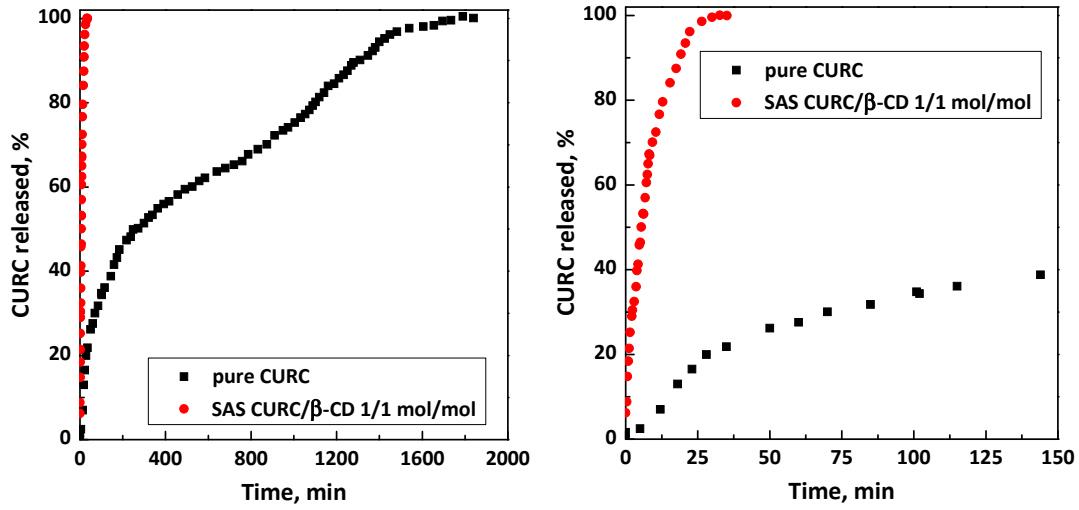


Figure 4: Dissolution tests in PBS at pH 6.8 and 37 °C; (a) entire dissolution profiles; (b) enlargement of dissolution profiles.

4. Conclusions

In this paper, the preparation of inclusion complexes containing curcumin was achieved via SAS process using β-CD as the carrier. The influence of different parameters, such as the total concentration, the operating pressure and the CURC/ β-CD molar ratio on morphology and dimensions of the produced composite particles was investigated. In particular, spherical microparticles were successfully obtained at various CURC/β-CD molar ratios. It was proved that β-CD is an effective carrier to be used for SAS coprecipitation, in order to improve the dissolution and, consequently, the bioavailability of active principles.

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