Supercritical antisolvent precipitation of hybrid carriers for intranasal drug delivery

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ABSTRACT

For an effective intranasal delivery of drugs to the Central Nervous System (CNS) it is necessary the development of mucoadhesive drug delivery systems (DDS) coupled with absorption enhancers. Hydroxypropyl-β-cyclodextrin (HP-β-CD) is a recognised absorption enhancer, being Pluronic F127 and Pluronic F68, poloxamers with mucoadhesive features.
In this work, particles of HP-β-CD containing F68, F127 or a mixture of both, were produced by supercritical antisolvent (SAS) process from an ethanolic solution at mild operating conditions. Quercetin, a flavonoid with bioactive properties, was chosen as model drug to be co-precipitated with the carriers. The hybrid particles produced showed improved mucoadhesive properties, and an encapsulation efficiency of 98% for quercetin-loaded hybrid particles.

INTRODUCTION

During the recent years there has been an increased interest in intranasal drug delivery, not just for the treatment of diseases of the nasal cavity but mainly for brain delivery through the olfactory epithelium and/or via the trigeminal nerves, bypassing the Blood-Brain Barrier (BBB) and minimising systemic exposure [1,2]. With the aim of reach a high extent of drug absorption and bioavailability, various approaches have been proposed, including the administration of micro and nanocarriers with mucoadhesive characteristics so as to maintain the drug on the absorption site. Moreover, the use of absorption enhancers could enhance the transport of APIs through the mucosa [3].

The aim of the present work was the production of mucoadhesive particles with enhanced absorption properties for intranasal delivery of drugs through Supercritical Fluid (SCF) Technology. For that purpose, Hydroxypropyl-β-cyclodextrin, an absorption enhancer [4], and Pluronic F127/ Pluronic F68, poloxamers with mucoadhesive features [5], were chosen as carrier materials to develop hybrid particles for nose-to-brain APIs delivery. Moreover, Pluronics are known to inhibit drug efflux transporters, such as P-glycoprotein, thus increasing their bioavailability. Quercetin was chosen as the model drug since it presents several bioactive properties, such as antioxidant, anticarcinogenic, antiinflammatory and antiviral activities[6,7]. Moreover, its intranasal administration has already proved to produce better effects in comparison with the oral administration [8].

Hybrid particles of HP-β-CD blended with F68, F127 or a mixture of both, were produced by SAS method from an ethanolic solution at mild operating conditions. Particles from individual carrier materials were also precipitated using the same method as well as the co-precipitation with quercetin. The particles produced were analysed by DSC for the knowledge of their thermal behaviour, by FTIR for structural characterisation, by SEM for morphology characterisation and by LD for particle size distribution analysis. Furthermore, the quantification of the HP-β-CD and Quercetin content, the assessment of the remaining ethanol concentration and the evaluation of mucoadhesive properties in the particles were performed.

MATERIALS AND METHODS

Materials

Ethylene oxide–propylene oxide block copolymers Pluronic F127 and F68 were provided by BASF (Germany). KLEPTOSE® HP (HP-β-CD) was a gift from Roquette (Portugal). Anthrone, Quercetin hydrate (Q) with a (purity≥95%) and Mucin from porcine stomach were purchased from Sigma-Aldrich. Ethanol with 96% purity was provided by Panreac (Spain). CO_2 (99.5% purity) was supplied by Carburos Metálicos (Spain).

SAS (Supercritical Antisolvent) precipitation experiments

The flow diagram of the apparatus used for the supercritical antisolvent precipitation is shown in Figure 1.

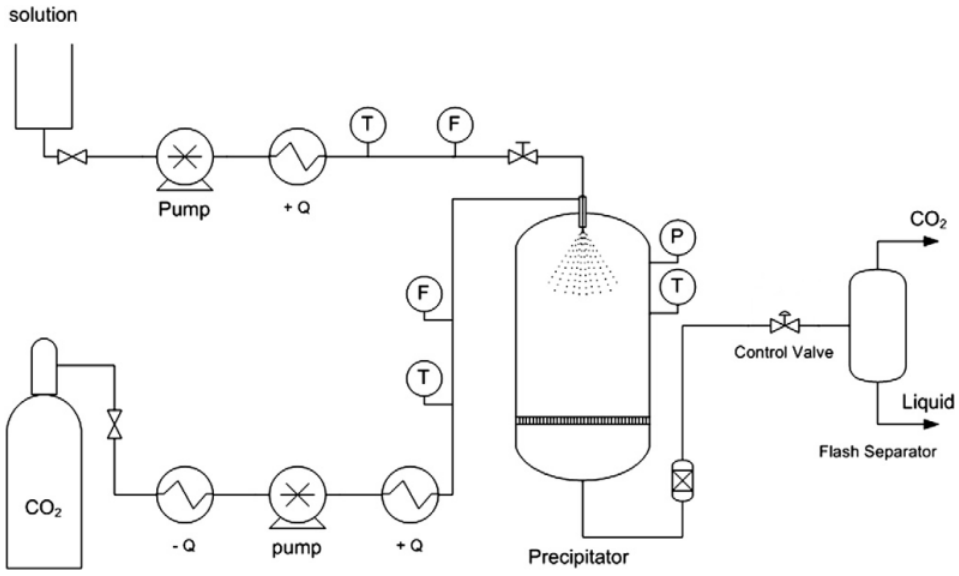

Figure 1: Schematic diagram of the SAS equipment.

The CO_2 used is cooled down before being pressurised with a diaphragm pump (Dosapro, France). Afterwards, it is heated up to the required operating temperature. Carbon dioxide mass flow is measured with a coriolis flow meter. When the mass flow of CO_2 is constant

(2kg/h) and the working pressure and temperature remain stable, the solution is pumped (1mL/min) by a chromatographic pump (GILSON 305) into the precipitator at the desired flow rate. The precipitator is an insulated and jacketed AISI 316 stainless steel vessel of 1.5 L of volume. This precipitator is equipped with Pt-100 thermoresistance with an accuracy of ±0.1 K and with membrane digital pressure meter with accuracy of ±0.25 bar to measure operating conditions. At the bottom of the vessel there is a porous metallic frit with a screen size of 1µm. There is also an external stainless steel filter, which has a screen size of 1µm. The pressure in the precipitator is controlled by a backpressure regulator (BP-66, Go-regulator). Additionally, the valves and the outlet tube are electrically heated to prevent freezing or plugging. A vessel is used to achieve the separation of solvent and CO2 after pressure release. When the desired amount of ethanolic solution (2.5% w/v) has been injected, the liquid pump is stopped and only pure CO_2 is fed for 45 min in the same operating conditions to ensure the complete removal of organic solvent from the precipitator. Finally, the precipitator is depressurised and the particles are recovered. The precipitate is stored at room conditions before its analysis.

Table 1 shows a summary of the experiments performed in this work. All the experiments were performed at 90bar and 35ºC, with the exception of Experiment 7, which was conducted at 90bar and 25°C.

Table 1: Summary of the experiments performed.

Exp	Compounds	Mass ratio
1	HP-β-CD	1
2	F68	1
3	F127	1
4	HP-β-CD:F68	2:1
5	HP-β-CD:F127	2:1
6	HP-β-CD:F68:F127	4:1:1
7	HP-β-CD:F68:F127	4:1:1
8	HP-β-CD:F68:F127:Q	4:1:1:2

Product Analysis

Product yield

Product yield was expressed in % w/w dry basis (db) as the mass (g) of particles recovered in db per 100g of solids in the processed solution.

Morphology

The morphology of the particles was analysed and imaged by Scanning Electron Microscopy (SEM) using a scanning electron microscope model JEOL JSM-820. Particles of representative samples were gold sputtered in an argon atmosphere at room temperature before examination.

Particle size distribution

Particle size distribution (PSD) was measured with a laser diffractometer (Mastersizer 2000, Malvern). Particles were dispersed in ethyl acetate and measurement was carried out after a gentle rotation of the particles suspension container in order to obtain an ever dispersion of the particles. In this work, particle size measurements are reported as volume distribution and defined as the average diameter ($d_{0.5}$), being the final result the average from 3 measurements. The span value is also reported, that is, the ratio between $d_{0.5}$ and ($d_{0.9}$- $d_{0.1}$); span values near to 1 represent narrow PSD.

Thermal Behaviour

DSC measurements were carried out on a DSC-30 METTLER apparatus with the aim of studying the thermal behaviour of the particles. The samples were placed in an aluminium pan and sealed; the probes were heated from -10 to 350ºC at a rate of 10ºC/min under nitrogen atmosphere (60 mL N2/min).

Structural characterisation

Infrared spectra of the samples and milled pure materials (HP-β-CD, F68 and F127) were recorded on a Bruker ALPHA FT-IR apparatus equipped with a Platinum ATR module including a diamond crystal. The spectra in the range from 4000 to 400 cm^{-1} were the average of 64 scans at a resolution of 2cm^{-1}. The ATR signal was transformed to Transmittance and the obtained spectra was normalised after the baseline correction.

Ethanol content

The residual ethanol content in the particles was determined by HPLC analysis. The chromatography system consists of an isocratic pump (Waters 1515), an automatic injector (Waters 717) and a differential refractive index detector (Waters 410). The column (model KS-802, guard column KS-G from Shodex) was kept at 50°C and flow rate of the mobile phase (0.005 M H_2SO_4) was set at 0.8 ml/ min. The IR detector temperature was 30°C. For the analysis 20 mg of particles were dissolved in 5mL of Milli-Q water. This solution was filtered (0.2 micron pore size) and 20 µL were injected in the HPLC system. The ethanol content was expressed as g of ethanol per 100 g of particles (% w/w).

HP-β-CD content

The determination of HP-β-CD content was performed through the Anthrone method [9,10]. Briefly, particles were dissolved in water (MilliQ) by manual agitation. Samples (1mL) were withdrawn and vortexed for 15s with cold anthrone reagent (5mL), which was prepared fresh daily by dissolving anthrone (0.2% w/v) in a mixture of concentrated sulphuric acid and water (5:2). Then, the mixtures were put in a boiling water bath for 10min, cooled down for 2min under running tap water and finally spectrophotometrically analysed at 625nm (Shimadzu UV-2550). The blank solution was concentrated sulphuric acid and water blend in 2:1 ratio. Standard curve was made for HP-β-CD, which was found to be linear in the range of 20–120mg/L (R^2=0.995). Preliminary tests were performed to assure that the poloxamers did not

interfere with the cyclodextrin–anthrone absorbance at 625nm. Duplicate determinations were performed for each solution.

Quercetin content

The amount of Quercetin loaded inside the particles was determined by spectrophotometric analysis at 375 nm using UV−Vis spectrophotometer (Shimadzu UV-2550) and ethanol as solvent. Calibration was obtained by using standard samples with concentrations between 3 and 12µg/mL ($R^2 = 0.9963$). The analysis was performed in duplicate.

Evaluation of mucoadhesive properties

Mucoadhesive properties of the particles were assessed by the mucin-particle method [11]. This method is based on changes in mucin particle size as a result of interaction between adhesive materials and mucin. Equal volumes of particles aqueous solution (1% w/v) were mixed with mucin aqueous solution (1% w/v), vortexed and incubated at 37°C for 30 min. Changes in particle size were monitored by laser diffraction measurements. Each test was performed in duplicate.

RESULTS

This work started with the precipitation of the single carriers by SAS, followed by the co-precipitation of HP-β-CD with the mucoadhesive polymers in a proportion of 2:1 (mass ratio). Finally, a blend of the three carriers was co-precipitated with quercetin in a proportion of 3:1 w/w (carriers:Q). Table 2 presents a resume of the analysis performed with the products obtained. The process showed a maximum yield of 30% (% w/w db).

Table 2: Product analysis.

Exp	Morphology	$d_{0.5}$ (µm)	Span	% Ethanol (w/w)	% HP-β-CD (w/w)
1	Particles	233.8	3.7	6.0	-
2	Plastification	63.2	1.1	-	-
3	Plastification	457.4	1.8	-	-
4	Particles/Plastification	36.9	1.8	13.2	62.3
5	Particles/Plastification	29.9	1.2	13.1	70.5
6	Particles/Plastification	26.8	1.7	18.6	73.2
7	Particles/Plastification	12.2	13.9	15.2	59.0
8	Particles	5.3	252.3	3.1	62.3

While the isolated SAS precipitation of HP-β-CD produced spherical particles with some tendendy to aggregate, the precipitation of pluronic particles was not achieved. Irregular flat particles were produced both with F68 and F127, suggesting that they were formed by solidification from a polymer melt instead of precipitation by the antisolvent effect [7]. Some pictures of the particles produced can be seen in Figure 2.

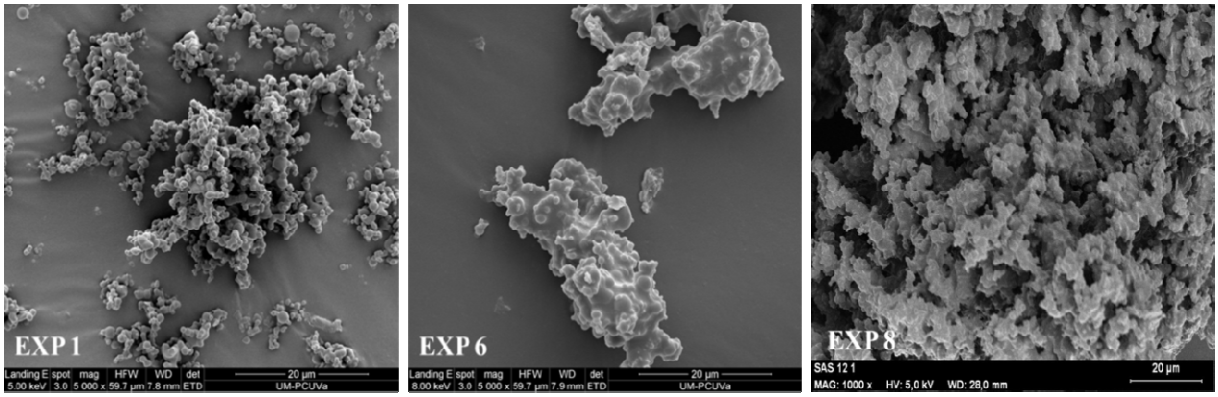

Figure 2: SEM photographs of particles obtained in experiments 1, 6 and 8.

This occurrence was also verified, even less pronounced, in the co-precipitation of mixtures of HP-β-CD with pluronics. Since the melting temperature of F127 decreases more than 15ºC in the presence of CO_2 [12], and probably occurring the same with F68, working near this temperature (37ºC) could be the cause of the plasticisation events. In order to overcome this, experiment 7 was performed at 25ºC; nevertheless the particles obtained were also plasticised with sharp edges.

For the co-precipitation of the carriers with quercetin it was chosen once again to operate near the melting region of pluronics, since it can enhance the encapsulation of Q through the formation of a polymer film over the active compound [7]. Aggregated particles of HP-β-CD:Pluronics:Quercetin were produced, presenting maintenance of the ratios between the compounds (Quercetin encapsulation efficiency of 98% and HP-β-CD present in 62.3% w/w). The DSC thermogram of these particles showed the melting peak of quercetin (data not shown). This result could indicate the presence of segregated quercetin crystals in the particles, and that a lower Quercetin/carriers mass ratio should be used in next experiments [7]. Nevertheless, some complexation or chemical association between the carriers and quercetin may had occurred, since its FTIR spectra (Fig. 3) presents different band shapes observed between 1,500 and 1,800 cm^{-1} when compared with unprocessed quercetin and HP-β-cyclodextrin:Pluronics spectra [13].

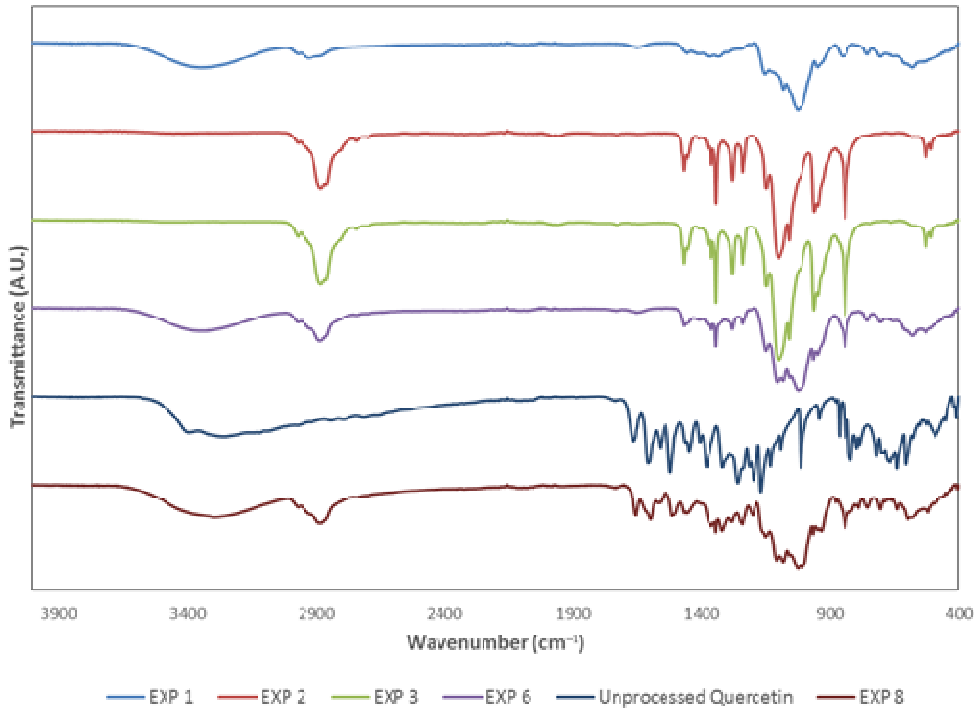

Figure 3: FTIR spectra

Regarding the ethanol content, it is possible to verify that the presence of pluronics blended with HP-β-CD increased the presence of this solvent in the samples. The ethanol content decreased while operating at 25ºC or when Q was co-precipitated with the carriers, being this probably related with the decreased plastification level of the polymers in these experiments.

Finally, the mucoadhesive properties of the hybrid particles produced were assessed. Hybrid particles produced in experiment 6 led to an increase in mucin particle size of 15% while particles composed only of HP-β-CD (exp 1) led to an increase of just 6%. This demonstrates that the blend with pluronics enhanced the mucoadhesive capacity of the final particles.

CONCLUSION

The preparation of HP-β-CD:Pluronics hybrid particles was performed using a supercritical antisolvent process. The precipitation of an ethanolic solution containing a mixture of HP-β-CD with pluronics resulted in particles with enhanced mucoadhesive properties. Moreover, hybrid particles loaded with quercetin were also produced with high encapsulation efficiency (98%). These mucoadhesive particles for nose-to-brain delivery could be a suitable alternative to oral administration of quercetin. Further development in this work will involve the production of particles with different carriers:Quercetin mass ratio values.

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