DEVELOPMENT OF LIPID PARTICLES WITH SUPERCRITICAL CARBON DIOXIDE

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The search for suitable carrier systems for different active substances is a major challenge for the pharmaceutical industry to overcome problems related to poorly water-soluble compounds and their bioavailability. This seek is also of great interest for the food and cosmetic industry since it possibles the incorporation of additives regardless of their hydrophobicity. In this study lipid particles loaded with ascorbic acid were produced using the PGSS® concept, with carbon dioxide and GRAS lipid excipients. The particles obtained were analysed by ESEM, DSC, HPLC and single-particle-counting technique.

INTRODUCTION

Over the years it has become evident that the development of new active molecules alone is not sufficient to ensure progress in therapy. By developing the suitable carrier system problems like insufficient concentration due to poor absorption, rapid metabolism and elimination; poor solubility and high fluctuation of plasma levels due to unpredictable bioavailability can be overcome. The in vivo fate of the compound will no longer be determined by its properties, but by the properties of the carrier system. This should allow a controlled and localized release of the active substance to the specific needs of the therapy.[1]

Several research groups are investigating many alternatives for carriers such as emulsions, microemulsions, liposomes, polymeric micro and nanoparticles [2], drug nanocrystals, solid lipid nanoparticles (SLN®) and nanostructured lipid carriers (NLC®) [3]. This study focuses on lipidic structures since not only they have been found appropriate for several administration routes [4], but they also present the advantage of low toxicity due to their composition of physiological lipids when compared to polymeric particles [5], which facilitates the regulatory issues.

The conventional methods for the production of lipid particles include high pressure homogenisation (cold or hot), microemulsions, solvent-emulsification-evaporation or diffusion, w/o/w double emulsion, high speed stirring and ultra sonication [6], all of them multi-step processes, sometimes needing organic solvents. Supercritical fluids, and in particularly carbon dioxide used in the PGSS® technique [7], seem to be a promising method for lipid particle formation allowing the generation of particles from a great variety of substances that do not need to be soluble in supercritical carbon dioxide.[8]. Furthermore, it is a one step process that can be scaled up and designed to assure GMP requirements.

In this work a GRAS lipid excipient, Precirol® ATO5, was used, not only separately but also as a blend with anti-caking agents since this leads to a less ordered solid lipid matrix, a pre-requisite for a sufficiently high load.of the active substance [9]. This product has been used to enhance the bioavailability of sensitive drugs, to protect

active ingredients from light, moisture and oxidation and as controlled release agents [10-11]. Furthermore, the fact that it is made from biocompatible natural matrices decreases the danger of toxicity and makes it a suitable carrier for pharmaceutical, food and cosmaceutical industries.

I - MATERIALS AND METHODS

Materials

Precirol® ATO 5 (glyceryl palmitostearate) was a gift from Gattefossé. Aerosil®200 and Aerosil® R972 (fumed silica) were a gift from Degussa. Ascorbic acid, CAS [50-81-7] (99%) was purchased from Sigma and carbon dioxide, CAS [124-38-9] (99.5%, industrial grade), was obtained from Air Liquide. Chloroform stabilized with ethanol CAS [200-663-8] (99% purity), was purchased from Panreac Quimica SA. All chemicals were used without any further purification.

Experimental apparatus and procedure

The experimental work was performed at a laboratory scale, batch mode, in a PGSS apparatus presented schematically in figure 1.

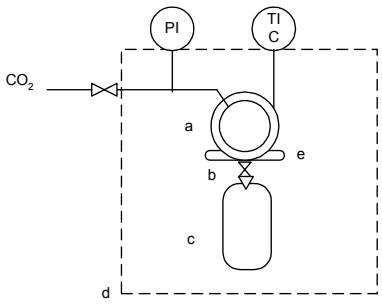


Figure 1 - Experimental apparatus: (a) high-pressure reactor with sapphire windows; (b) high-pressure valve with nozzle; (c) precipitator, (d) termostated air-bath; (e) magnetic stirrer

It consists of a high-pressure stirred reactor with sapphire windows (volume of, 60cm^3) coupled with a nozzle (tested with 300 and 500 μm) through a high-pressure valve. This reactor is placed inside a thermostated air bath heated by means of a controller that maintained temperature within ± 0,1 ºC. The system is pressurised with fresh carbon dioxide until the desired pressure is brought into the cell and the mixture is magnetically stirred. After 1 hour of stirring the system was quickly depressurised and particles were collected in a precipitator. Temperature and pressure operating conditions were chosen according to previous measurements of the solubility of carbon dioxide in Precirol [12].

Experiments were performed in the systems described below:

	Components	Pressure (bar)	Temperature (ºC)	Nozzle (μm)
(a)	Precirol	120	60	300
(b)	Precirol + Ascorbic acid solution	120	55	500
(c)	Precirol + Aerosil R972	130	60	300
(d)	Precirol + Aerosil R972 + Aerosil 200 +Ascorbic acid solution	140	60	300

Table 1 - Summary of the experiments performed

Particle Characterization

Scanning electron microscopy (ESEM)

The morphology of the lipid particles was analysed and imaged by environmental scanning electron microscopy (ESEM, Philips XL30, Field Emission Gun) after sputter coating with gold-palladium.
The analysis was carried out in particles obtained in all the experiments.

Particle size and size distribution

The particle size and size distribution was measured by single-particle-counting technique in a TSI particle size analyser, mod. 3603. The measurements were performed with the particles obtained in experiments (a) (b) and (c).

Differential scanning calorimetry

For an estimation of the effect of the anti-caking agents and the ascorbic acid solution in the stability and polymorphism of the Precirol, DSC studies were performed using Perkin Elmer DSC7 coupled with a TAC 7/DX. The samples were sealed in an aluminium DSC pan and the scanning rate was 5ºC/min in a temperature range from 18 to 90ºC.
The measurements were carried out in the following samples: (a), (b), (c) and directly in Precirol supplied.

Chemical analysis

To evaluate the ascorbic acid content of the particles obtained in experiment (d), an HPLC analysis (ThermoFinnigan Surveyor consisting of a quaternary pump, autosampler and diode array detector).was performed. The particles were previously dissolved in chloroform and the ascorbic acid was then extracted with water. Samples were then filtered (0.45mm, milipore filters) and 20μl sample was injected in a Merck Lichrosopher 100 RP-18e column. Quantification was performed 220 nm. The flow rate was 1ml/min. the column was kept at ambient temperature with a mobile phase composed of potassium phosphate and methanol.
The water content analysis was performed in a Karl-Fisher Aquapal titrator apparatus, using hydranal coulomat cg (Sigma ref. 34840) and hydranal (Sigma ref 34836) coulomat ag as titrating solvents.

II – RESULTS AND DISCUSSION

Scanning electron microscopy (ESEM)

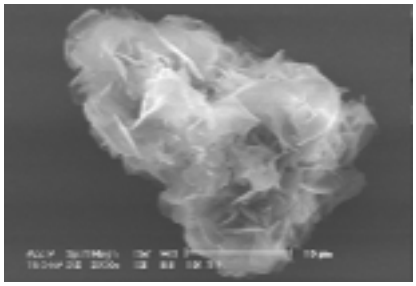


Figure 2 - SEM photo of particles obtained in experiment (a)

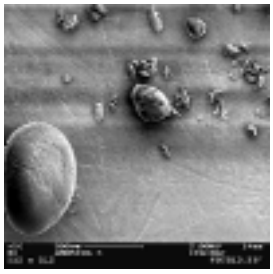


Figure 3 - SEM photo of particles obtained in experiment (b) (* performed in a different microscope, ZAISS 960 Scanning Electron Microscope)

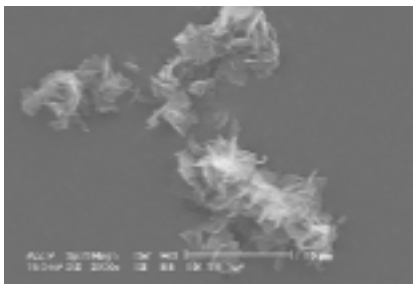


Figure 4 - SEM photo* of particles obtained in experiment (c)

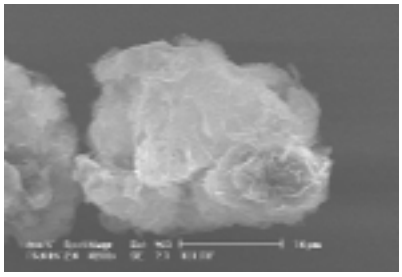
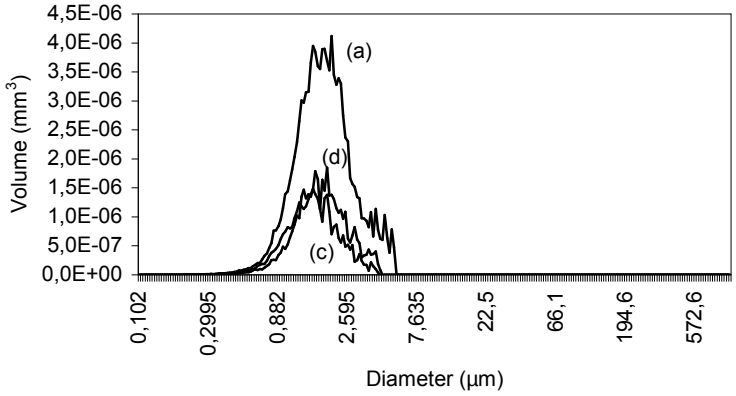


Figure 5 - SEM photo of particles obtained in experiment (d)

Particle size and size distribution



	D(4,3) (µm)	Specific surface area (m^2/g)
(a)	1,98	3,71
(c)	1,60	4,47
(d)	1,84	3,86

Figure 6 - Results of the particle size analysis for experiments (a), (c) and (d)

From the results presented above it is shown that Precirol forms microparticles much smaller than the nozzle used in the experiments and with porous a lamellar surface (figs.2,6). When trying to add an ascorbic acid solution to the lipid base, due to the difference in hydrophobicities of the compounds, a heterogeneous mixture was achieved (observed as darker spots in fig.3) and that is why no further analysis was performed in this sample. Regarding the anticaking agent effect, the structure formed seems to have sharper lamellas, almost like needles, and the average diameter has

decreased, increasing the specific surface area (figs.4,6). When adding the solution to this last system, a new hydrophilic anticaking agent also had to be added, since Aerosil R972 is hydrophobic, like Precirol, and now with water a hydrophilic anticaking agent was also needed (Aerosil 200). In this case already an homogeneous mixture was formed and still the particle sizes obtained were smaller than those achieved with the Precirol alone.
The reason for the sizes not matching in the SEM analysis and in figure 6 is probably due not only to the extreme porosity observed in the particles but also to the formation of aggregates: since the particle size analyser deagglomerates individual particles, its results give smaller values.

Differential scanning calorimetry

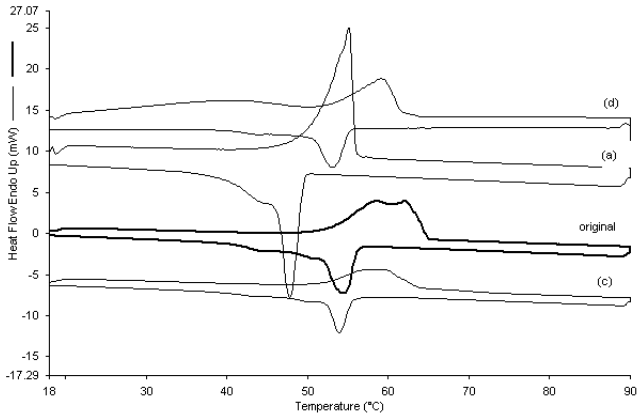


Figure 7 - DSC analysis results of experiments (a), (c), (d) and of Precirol supplied (original)

From the data obtained one can observe two peaks in the supplied Precirol that occur because it is in fact a mixture of diglycerides composed of C16 and C18 chains, and presents polymorphic forms (original). When processing the Precirol alone with CO_2, it seems that some kind of purification occurs leading to a sharper peak. Probably a more stable polymorph prevails (a). However, when anti-caking agents are present (c) it seems the original forms remains little altered, and even with water addition (d), from the ascorbic acid solution. Eventhough it is not exactly as the original form, it seems more similar to the original one than when processing Precirol alone.

Chemical Analysis

The HPLC analysis showed a content of 0,013g of ascorbic acid per gram of particle, which makes a yield of 53% compared to the initial input. Nevertheless the water content obtained by the Karl-Fisher titration showed only 25% of the initial water input was included in the particles.

CONCLUSION

The preparation of lipid particles containing ascorbic acid was successfully performed using a supercritical fluid process. Different experiments were carried out to check the effect of water and anticaking agents in the particles. Further development will involve the optimisation of the process in order to have better yields, the incorporation of other active substances, production of particles with other lipid matrices and also the incorporation of the particles produced in final products for the consumer.

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