ANTI SOLVENT CO-PRECIPITATION OF BIODEGRADABLE POLYMERS AND DRUGS

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Controlled – delivery products receive considerable attention, because of the drug's therapeutic effect prolongation, that keeps the drug concentration in the body between a lower limit (therapeutic limit) and an upper limit (toxicity limit).
These devices are traditionally obtained by encapsulation of a drug in a polymer matrix using an organic solvent with the following steps:

- Solubilization of the active principle in an organic solvent;
- Partitioning of the active principle between the solvent and the polymer matrix;
- Residual solvent removal.

Problems arise for the elimination of residual solvent to acceptable limits.
Antisolvent precipitation can be an alternative method for the encapsulation of a drug in a polymer matrix.
In this study the feasibility of the technique known as Supercritical Anti Solvent recrystallization (SAS) will be investigated in order to obtain the co-precipitation of a drug and a biodegradable polymer from an appropriate organic solvent.
A biodegradable polymer, poly(L-lactide) (L-PLA) is considered, with a non steroidal anti-inflammatory drug (NSAID), nimesulide, as active principle.

INTRODUCTION

The encapsulation of pharmaceuticals, including proteins, into polymers has been attracting a significant amount of attention for controlled release applications. For drug delivery is often desirable to produce globular polymer particles on the order of 10 μm containing hydrophilic pharmaceuticals [1].
The main reason for the micronisation of pharmaceuticals are to allow the use of a more appropriate and/or convenient administration route, such as subcutaneous, intramuscular, topical and intestinal modes of administration, to decrease required dosage and to increase drug bioavailability by increasing the surface area to volume ratio [2].
Various microencapsulation techniques exist: conventional processes including solvent evaporation, spray drying or organic phase separation techniques have several disadvantages such as considerable amounts of residual organic solvent in the microparticles and low encapsulation efficiencies [3].
The development of a microencapsulation method with environmentally benign solvents still remains a challenge. Solvent impurities are often toxic and also may degrade pharmaceuticals within a polymer matrix [1].

In recent years, the use of supercritical fluids as media for the formation of microparticles has shown tremendous success. Supercritical fluids offer significant advantages over conventional routes of microparticles formation, namely, mild operating temperatures, high purity of products and production of solvent-free dry particles [4].
Carbon dioxide (CO_2) is one of the most commonly used supercritical fluid because of its relatively low critical parameters (T_C = 31.1°C, P_C = 73.8 bar). The low critical temperature of CO_2 makes it attractive for processing heat-sensitive flavours, pharmaceuticals and labile lipids. In addition, CO_2 is non-toxic, inexpensive and has a relatively high dissolving power [5].
In the antisolvent recrystallization process a SCF (antisolvent) is added to a solution of the solute of interest in an organic solvent. The antisolvent is miscible with the solvent but immiscible with the solute. Dissolution of antisolvent in the liquid phase expands the saturated liquid, reduces its solvent power, and eventually causes solute precipitation [6].
Different acronyms were used to denote the different antisolvent micronisation processes: aerosol solvent extraction system (ASES), precipitation with a compressed fluid antisolvent (PCA), gas antisolvent (GAS), solution enhanced dispersion by supercritical fluids (SEDS) and supercritical antisolvent (SAS) [7].
Biodegradable polymers can be used as controlled drug delivery systems of bioactive agents and drugs. They can be used for this purpose in form of films, sponges, microspheres and nanoparticles [8].

I - BACKGROUND

Many attempts to perform the co-precipitation of a polymer and a drug are reported in literature.
Bleich *et al.* investigated the microencapsulation of a model drug, hyoscine buthylbromide, with poly (L-lactic acid) by precipitation of a solution of methanol and methylene chloride with the ASES technique. Other model drugs, as indomethycin, piroxicam and thymopentin, have also been coprecipitated with poly (L-lactic acid) from methylene chloride. The following results were obtained: a maximum drug loading (defined as the ratio between the mass fraction of encapsulated drug and the total mass of the sample) of 19.8 wt. % was obtained with hyoscine buthylbromide while a loading of 4.9 wt.% was achieved with thymopentin [9, 10].
Falk *et al.* studied the microencapsulation of ionic compounds, as gentamycin, naloxone, and naltrexone, with L-PLA using the PCA technique. Ionic compounds were dissolved in methylene chloride using hydrophobic ion pairing (HIP) to stoichiometrically replace polar counter ions with an anionic detergent, aerosol OT (AOT, sodium bis-2-ethylhexyl sulfosuccinate). The drug/polymer particles were spherical in shape and between 0.2 and 1.0 μm in diameter [11].
Bodmeier *et al.* adopted PCA method for the co-precipitation of L-PLA and two drugs, chloropheniramine maleate (water soluble) and indomethacin (water insoluble) from a methylene chloride solution. For both drugs the particle size was 1-5 μm while the drug loading was 3.73 % and 0.73% for chloropheniramine maleate and indomethacin respectively. The low drug loading value can be justified with the high solubility of the active principles in SC CO_2, aided by the methylene chloride cosolvent effect [12].
Microcapsules of hydrocortisone/poly(DL-lactide/glycolyde) (PLGA) were prepared with SEDS method by Ghaderi *et al.*. Hydrocortisone was successfully entrapped in the PLGA microparticles, with an entrapment efficiency (defined as the ratio between the entrapped hydrocortisone per batch microparticles and the initially added hydrocortisone per batch microparticles) of the 22% [5].

Sze Tu *et al.* investigated the feasibility of using dense CO_2 with the ASES technique employing a multiple nozzle to micronise and microencapsulate parahydroxybenzoic acid (p-HBA) from methanol solutions and lysozyme from DMSO solutions, with poly (L-lactic acid).
It was found that the high molecular weigh compounds, poly (L-lactic acid) and lysozyme, precipitates as micro and nanospheres, while the lighter weight compound, p-HBA, precipitated as crystalline particles resembling platelets of 3 μm in length. The maximum encapsulation efficiencies for p-HBA/ poly (L-lactic acid) particles obtained were 9.2%. Higher values (15.6%) were obtained with lysozyme [2].
In this study SAS method will be adopted in order to obtain microparticles of a drug encapsulated in a polymer matrix using CO_2 as antisolvent. SAS consists in the precipitation of a solution, of a solute in an organic solvent, by spraying it into the supercritical gas phase. A constant CO_2 stream will remove organic solvent over a period of time in the following drying process.
L-PLA was selected because it possesses the main requisites for pharmaceutical and biomedical applications: local biodegradability, biocompatibility, non-systemic toxicity. Furthermore, thanks to its semi-crystalline nature, it can be precipitated by compressed CO_2 without flocculation and plasticization typical of amorphous polymers, which were found to precipitate as micro-particles only under particular operative conditions [5].

II - MATERIALS AND PROCEDURE

L-PLA (M_w = 125000) was supplied by Fluka. Chloroform (99.8% purity) was supplied by Aldrich. CO_2 was obtained from SIAD with a purity of 99.98%.
The experimental apparatus for the precipitation measurements is presented in figure 1.
The precipitator (AISI-316 steel) with an internal volume of approximate 400 cm^3 has an inner diameter of 50 mm and a height of 200 mm and is equipped with a thermostatic water bath cover ensuring temperature to be kept within ± 0.5°C. The solution is kept at temperature like the precipitator by an electric heat plate.
The solution is sprayed into the precipitator through a nozzle (LECHLER 212.004.17.AC) with a diameter of 100 micron. A filter (0.5 micron) situated in the solution ensures that only dissolved drug enters the inlet tube, and the solution is pumped to the precipitator at constant flow rate by a high pressure pump (CONSTAMETRIC ® 3200 P/F). Liquid CO_2 is cooled down to approximate - 3°C and pumped to the precipitator from a volumetric pump (SIEMENS). Before the inlet of the precipitator the CO_2 is led through the thermostatic water bath to obtain the temperature of the reactor. Both the solution and CO_2 are added to the cell from the top, resulting in co-current flow. The outlet flow is filtered with a 0.5 micron filter to prevent precipitate to leave the precipitator. The regulation is done with a valve (WHITEY SS-21RS4), which is heated by an electric resistance heater in order to prevent freezing.
The precipitator is furthermore equipped with a safety valve (SWAGELOK SS-4R3A-EP, 100-155 bar) to prevent the pressure inside the cell to exceed 125 bar.
The temperature inside the reactor is controlled by a thermometer (Delta OHM HD 9214) and the pressure is measured with a pressure transducer (DRUCK DPI 280).

III - RESULTS

Nimesulide and L-PLA were co-precipitated from a single liquid solution with SAS technique using chloroform as solvent.

All the experiments were carried out at 40°C. The concentration of nimesulide or L-PLA in chloroform was 2.5% w/w. For the co-precipitation experiments the total concentration of drug + polymer was 2.5% w/w; different drug to polymer ratios were considered.

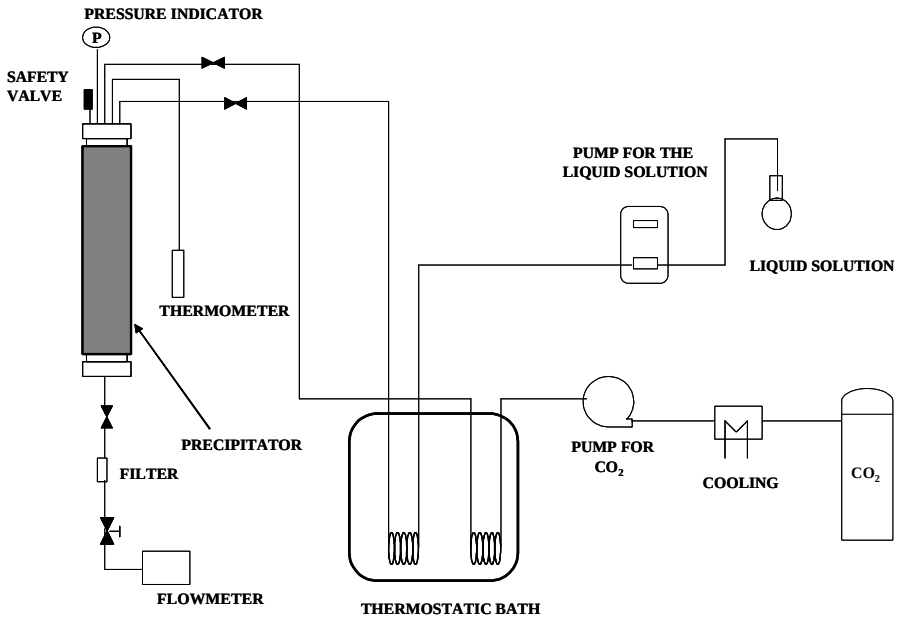


Figure 1: Experimental apparatus for the continuous SAS technique.

The organic solution was sprayed into the precipitator at rates ranging from 3.50 to 4.50 ml/min for 5 minutes while CO_2 flow was adjusted to 400-500 ml/min. All the experiments are synthesized in table 1.

Particles obtained were characterized by scanning electronic microscopy (SEM). Figure 2a shows the L-PLA precipitated from a 2.5% w/w chloroform solution at 40°C and 90 bar. The formation of a polymeric fibrous network with irregular size distribution, due to the high concentration of the organic solution, is evident.

Nimesulide precipitates from a 2.5% w/w chloroform solution at 40°C and 85 bar as needle crystals (see figure 2b). Agglomeration effects are observed, leading to an irregular particle size distribution.

Figure 3 shows the co-precipitation of nimesulide and L-PLA from a 2.5% w/w chloroform solution at 40°C and 103 bar with an initial drug to polymer ratio 1:1. The formation of nimesulide needles entrapped by the polymer fibers, consisting of a fibrous network of the drug and the polymer mixture, irregular in shape and in size distribution, is obtained. Similar results have been achieved for the co-precipitation experiments with an initial drug to polymer ratio 1:2 at 40°C and 103 bar.

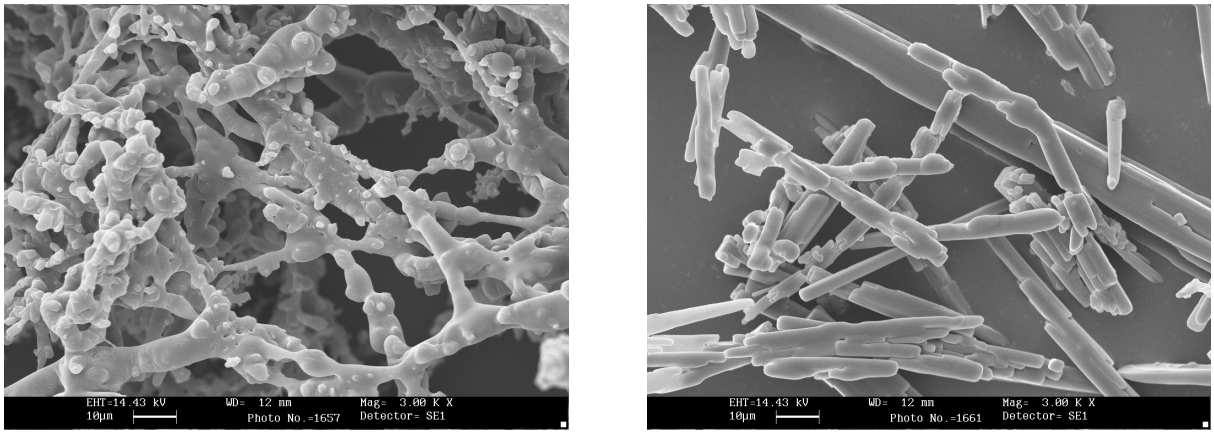
On the opposite side, starting from a solution with an initial drug to polymer ratio 2:1, only needle of nimesulide with aggregated microspheres of L-PLA have been obtained, demonstrating a very poor encapsulation efficiency.

The amount of nimesulide in nimesulide/L-PLA particles has been determined by dissolving a sample of the precipitate in 25 ml of ethanol in order to extract the drug. The resulting solution

was then analysed for nimesulide concentration with an UV spectrometer (UNICAM Helios α) at 297 nm. A drug loading of about 50 % was obtained for all the nimesulide/L-PLA systems (2:1, 1:1 and 1:2 w/w ratios).

Solute	**P (bar)**	**Particle description**
L-PLA	90	Fibers
Nimesulide/L-PLA (1:1 w/w)	103	Needles of nimesulide covered by aggregated fibers of L-PLA
Nimesulide/L-PLA (1:2 w/w)	103	Needles of nimesulide covered by aggregated fibers of L-PLA
Nimesulide/L-PLA (2:1 w/w)	93	Needle of nimesulide with aggregated microspheres of L-PLA
Nimesulide	85	Needle-shaped crystals

Table 1: Experimental operating conditions for the precipitation with SAS technique.



(a) (b)

Figure 2: Precipitation from 2.5% w/w chloroform solution of L-PLA particles at 40°C and 90 bar (a) and Nimesulide particles at 40°C and 85 bar (b).

CONCLUSIONS

This study investigated SAS method in order to obtain the co-precipitation of nimesulide and L-PLA to encapsulate the drug in the polymer matrix. Different weight ratios between the drug and the coating material have been considered. Nimesulide was successfully entrapped in L-PLA when precipitation occurs from chloroform solution with an initial ratio between the drug and the polymer of 1:1 wt or 1:2 wt. In the first case a higher drug loading (49.74%) was obtained.

A future development of the work will be to decrease of the drug and the polymer concentration in the organic solution in order to achieve smaller particle size.

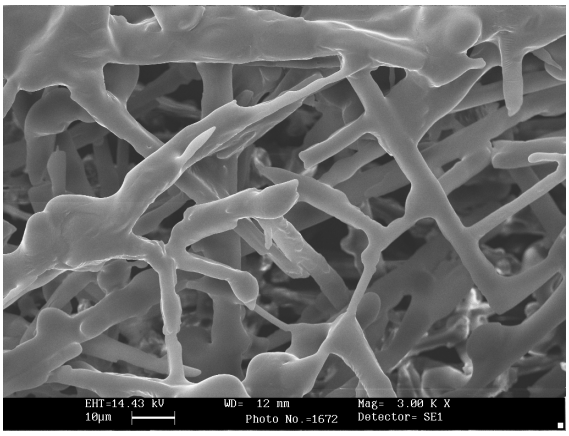


Figure 3: Nimesulide/L-PLA (1:1 wt ratio) particles precipitated from chloroform at 40°C and 103 bar.

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