# Formation of Nanoparticles of a Hydrophilic Drug using Supercritical $CO_2$ and Microencapsulation for Sustained Release

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## ABSTRACT

The objective of this work was to develop sustained release formulation for hydrophilic drugs with minimal burst effects. For this purpose, nanoparticles of a hydrophilic drug were produced using supercritical $CO_2$, which were then encapsulated into polymer microparticles using an anhydrous method, followed by studying their sustained *in-vitro* drug release. A hydrophilic drug, dexamethasone phosphate, was dissolved in methanol and injected in supercritical $CO_2$ with ultrasonic field for enhanced molecular mixing (SAS-EM technique). Supercritical $CO_2$ rapidly extracts methanol leading to instantaneous precipitation of drug nanoparticles of 150 nm. These nanoparticles, on encapsulation in poly(lactide-co-glycolide) polymer using anhydrous s/o/o/o technique, resulted in the well-dispersed encapsulation of drug nanoparticles in polymer microspheres of ~70 μm. Their *in-vitro* drug release showed sustained release of dexamethasone phosphate over a period of 700 hours with almost no initial burst release.

*Keywords*: sustained/controlled drug release, PLGA, SAS-EM, dexamethasone phosphate, s/o/o/o, supercritical $CO_2$, nanoparticle.

## 1  INTRODUCTION

Sustained release formulations of active pharmaceutical ingredients (API) using biodegradable polymer microencapsulation have been developed for delivery via oral [1,2], transdermal [3,4], subcutaneous depot [5] and other routes. Various microencapsulation procedures have been developed such as o/w solvent evaporation [6], w/o/w double emulsion [7], hydrous w/o/o [8], w/o/o/o [9], s/o/w [10], anhydrous s/o/o/o [11] and some novel phase separation techniques [9,12]. However, various problems with drug encapsulation and release, protein instabilities, aggregation, and denaturation still persist [13]. Some advances have been made in resolving these problems and stabilizing these API [14]. However, the problem with hydrophilic drugs still persists in that the drug particles are not very small as compared to the encapsulating polymer microparticles or are encapsulated in the form of small clusters within the polymer matrix, resulting in a high initial release.

Hence, the objective of this work is to produce sustained release formulations made of PLGA microparticles, with fine nanoparticles of a hydrophilic drug, dexamethasone phosphate encapsulated in them. Formulation is achieved in two steps. The first step is reducing the particle size of the drug particles by the use of supercritical antisolvent technique with enhanced mass transfer (SAS-EM®) [15]. The second step is microencapsulation of drug nanoparticles using s/o/o/o phase separation/coacervation technique [16]. Both these steps are completely anhydrous and hence are easily extendible to formulations of hydrophilic drugs including rigid proteins [11,17].

Two different issues have been addressed in this work. The problem of 'initial burst release' is solved by reducing the particle size by using the SAS-EM technique such that the relative size of the drug is very small as compared to the size of the polymer microparticles allowing the drug to be well dispersed in the polymer matrix. The other problem of poor percentage encapsulation of the drug in the polymer microparticles was resolved by using the anhydrous s/o/o/o technique that gives maximum encapsulation efficiencies for hydrophilic drugs and proteins [10].

## 2  MATERIALS

Poly(lactide-co-glycolide) (PLGA 50:50; inherent viscosity = 0.39 dL/g in HFIP @ $30^oC$) was obtained from Birmingham Polymers. Sodium salt of dexamethasone-21-phosphate was obtained from Sigma Chemicals. Dichloromethane (99.9% ACS grade), silicon oil (dimethicone, 350 cSt) were purchased from Sigma Aldrich, and methanol (HPLC grade), phosphate buffer saline (PBS, Labchem Inc., pH=7.4), and hexane (HPLC grade) were obtained from Fisher Scientific. All the materials were used as received.

## 3  APPARATUS AND PROCEDURE

### 3.1  Formation of Drug Nanoparticles

SAS-EM process for forming drug nanoparticles is explained in depth by Gupta and Chattopadhyay [18]. A schematic of the SAS-EM apparatus is shown in Figure 1. The main component of the apparatus is a high-pressure precipitation cell (*R*) with an approximate total volume of 80 $cm^3$. A titanium horn (*H*, Sonics and Materials, Inc.) with a tip of 1.25 cm in diameter is attached to the precipitation cell to provide the ultrasonic field generated by a 600 W (max. power), 20 kHz ultrasonic processor (*U*, Sonics and Materials, Inc). The experiment was carried out at constant temperature of $40^oC$ and constant pressure of 1500 psi to maintain supercritical conditions of $CO_2$. The

constant pressure was maintained by using a Teledyne ISCO syringe pump (Model 500D), and constant temperature by heating coil, temperature sensor and RTD controller device. Supercritical $CO_2$ was fed inside the precipitation cell through the inlet port located at the bottom of the vessel and purged from the exit located at the top of the vessel through an inline membrane filter to prevent the loss of any drug particles. At steady temperature and pressure conditions and known ultrasonic intensity, 10 mg/ml dexamethasone phosphate solution in methanol was injected into the precipitation cell, at the rate of 2 ml/min using a pump (Lab Alliance, Series 1500, dual head piston pump with pulse dampener). During the injection process, $CO_2$ was continuously being purged to allow extraction of methanol out of the system. The solution was injected through a horizontally placed 1/16th in. PEEK nozzle of internal diameter 100 μm, touching the center of the horn, so that the liquid jet experiences maximum ultrasonic intensity. These ultrasonic vibrations break the liquid jet into very fine droplets, their diameter being controlled by the ultrasonic intensity, which in turn is controlled by changing the amplitude of the ultrasonic vibration. 90 W power was used to drive the ultrasonic horn [14].
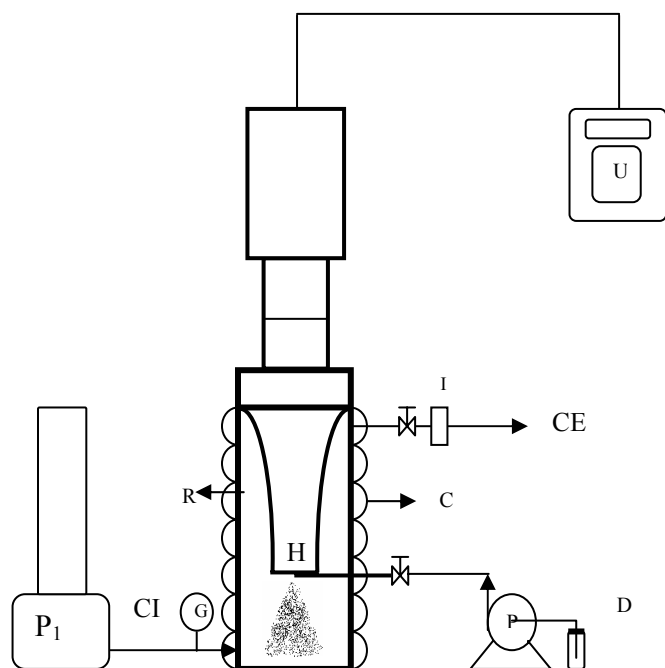


**Figure 1.** Schematic of SAS-EM apparatus. R: Precipitation Cell, H: Titanium Horn, U: Ultrasonic Processor, D: Drug Solution, CI: $CO_2$ Inlet, CE: $CO_2$ Exit, P: High Pressure Liquid Pump with Pulse Dampener, $P_1$: ISCO Syringe Pump for supercritical $CO_2$, I: Inline Filter, G: Pressure Gauge, C: Heating Coil with Temperature Sensor & RTD Controller.

After injecting the solution, the ultrasound was turned off and the system is allowed to purge with fresh $CO_2$ almost 5 times the volume of the precipitation cell to completely remove methanol from the cell. The cell was then depressurized to collect the drug nanoparticles.

## 3.2   Encapsulation of drug nanoparticles

Drug nanoparticles obtained from SAS-EM were encapsulated in PLGA microspheres by using the non-aqueous s/o/o/o solid dispersion technique [19,20]. 400 mg PLGA was first dissolved in 5 ml dichloromethane. 20 mg dexamethasone phosphate nanoparticles were then finely dispersed into 5 ml PLGA/ dichloromethane by sonication. A fine suspension of drug nanoparticles with no sedimentation was obtained. Under continuous mixing at around 1000 rpm, five ml of 350 cSt silicon oil was added to this suspension resulting in the coacervation of the organic phase. This caused PLGA to precipitate in the form of microspheres encapsulating drug nanoparticles. This suspension was added to 75 ml hexane, pre-cooled to 5°C, to extract out the dichloromethane and silicon oil. The microparticles were collected by vacuum filtration through a Whatman no. 1 filter paper. The microparticles were washed with cold hexane, to remove residual dichloromethane, and silicon oil. These microparticles were then air dried under mild vacuum to remove residual hexane.

Similar procedure was repeated for the encapsulation of dexamethasone phosphate particles as provided by the supplier without any further particle modification (termed as "unprocessed drug particles" henceforth) for comparative analysis.

## 3.3   Particle size analysis

The unprocessed drug particles, drug nanoparticles obtained from the SAS-EM process as well as the encapsulated drug/polymer microparticles obtained from the s/o/o/o technique were analyzed for their sizes and surface morphology using a scanning electron microscope (SEM: Zeiss, model DSM 940). The particles were initially spread on a carbon tape glued to an aluminum stub and coated with Au using a sputter coater (Electron Microscopy Systems, model EMS 550) under a vacuum of 0.2 mbar. The Au layer of ~10 nm thick is coated to make the particle surface conductive to electrons in the SEM. The amount of Au coated is insubstantial as compared to the size of the particles so as not to alter the particle morphology or surface texture. The particles were then observed under SEM and micrographs were recorded. Multiple images of different areas of the SEM stub were recorded for particles size analysis.

## 3.4   Extent of encapsulation

To determine the percentage of drug nanoparticles that were not encapsulated into the polymer (termed as surface drug), 5 mg of the above microparticles were suspended in

2 ml of PBS buffer. This aqueous buffer was then filtered through a 5 μm Alltech® syringe filter and tested for the drug content using HPLC (Model 600, Waters Corp.) for dexamethasone phosphate. The HPLC is equipped with a 7725i Rheodyne® six-port injection valve, model 600 HPLC pump controller with dual head pump, model 2410 refractometer and model 2487 Dual λ UV spectrometer. To determine the total amount of dexamethasone phosphate encapsulated, 5 mg of the microparticles were dissolved in 1 ml of dichloromethane and equilibrated overnight with 2 ml of PBS buffer. The aqueous phase was then tested for dexamethasone phosphate content using HPLC. In HPLC, the mobile phase was kept acidic to maintain the sodium salt of dexamethasone phosphate in un-dissociated form. Hence, the mobile phase was modified to 0.05 M PBS buffer : acetonitrile : acetic acid in the ratio of 70:28:2 flowing at 1 ml/min through a 3.9 mm x 150 mm Novapak C-18 column. The UV detector was set to 244 nm.

## 3.5  *In-vitro* release rate study

The release rate of the drug from the polymer microparticles was studied over a period of time. About 50 mg of PLGA microparticles, containing drug nanoparticles encapsulated in them, were suspended in 100 ml of 0.05 M phosphate buffer saline (PBS, pH=7.4) at 37°C in an incubator, and stirred at 50 rpm. Simultaneously, 50 mg of PLGA microparticles, containing unprocessed drug particles encapsulated in them, were also suspended in 100 ml PBS at similar conditions. Samples, after being filtered using a 5 μm filter, were taken approximately once or twice a day and analyzed for dexamethasone phosphate using HPLC as described above.

## 4  RESULTS AND DISCUSSION

The process of supercritical antisolvent precipitation with enhanced mass transfer is well explained earlier [21]. In this technique, the drug is dissolved in a solvent and injected in supercritical $CO_2$ in the form of a fine spray. The ultrasonic vibrations enhance mass transfer between supercritical $CO_2$ and the organic medium. $CO_2$ acts as an antisolvent and removes the organic solvent from the fine droplets resulting in the formation of drug nanoparticles.

The process of coacervation has been well established [22]. In this case, the ratio of drug to polymer was maintained to 1:20 in order to achieve a good dispersion of the drug in the polymer matrix. The polymer was about 6 wt. % of the polymer solvent i.e. dichloromethane.

## 4.1  Particle size and morphology

The unprocessed dexamethasone phosphate particles as provided by the supplier were ~50-100 μm irregular shaped crystalline particles (Figure 2a). By the SAS-EM technique, dexamethasone phosphate particles were converted into nanoparticles of 150-200 nm size (Figure 2b).
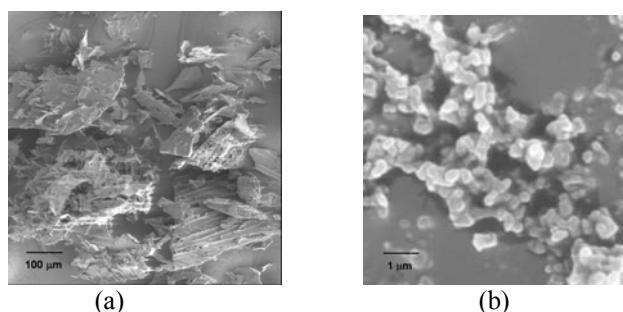


**Figure 2.** SEM micrographs of (a) dexamethasone phosphate microparticles as provided by the supplier and (b) dexamethasone phosphate nanoparticles obtained by SAS-EM.

The microencapsulation of dexamethasone phosphate nanoparticles into the PLGA microparticles yielded 70-80 μm large particles (Figure 3a-b). Since the size ratio of drug nanoparticle to polymer microparticle is about 1:400, the drug nanoparticles can be imagined to be well dispersed within the polymer microparticle matrix. Whereas, the encapsulation of unprocessed drug particles resulted in some smaller particles in the range of 40-50 μm and some extremely large particles of about 100-120 μm (Figure 3c-d). Hence, only part of the drug particles got encapsulated and some PLGA particles had no drug loading.
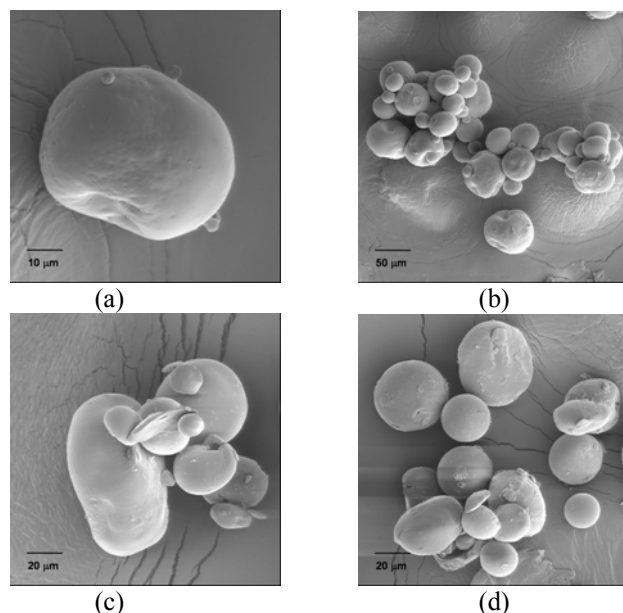


**Figure 3.** SEM micrographs of drug/ polymer microparticles containing (a-b) drug nanoparticles and (c-d) unprocessed drug particles. Each microparticle contains 10 wt.% dexamethasone phosphate nanoparticles, and 90 wt.% PLGA.

## 4.2  Percentage encapsulation

For the case where the unprocessed drug particles as provided by the supplier were used, out of the total quantity of drug taken, the net drug encapsulated was only 70%.

This shows that only smaller drug particles were encapsulated leaving behind the larger ones. However, for the case where drug nanoparticles were used for encapsulation, the surface drug was only about 1.5%. The net percentage of drug nanoparticles encapsulated was about 90% with minimal drug loss.

## 4.3 *In-vitro* release rate study

*In-vitro* drug release of dexamethasone phosphate nanoparticles encapsulated in PLGA microspheres was compared to that from the unprocessed drug particles also encapsulated in PLGA microspheres. The *in-vitro* drug release of the unprocessed drug particles (but microencapsulated) showed an initial burst release of about 5% at time zero and all the drug was released within 24 hours (Figure 4a). Whereas, the *in-vitro* drug release of the nanoparticles (also microencapsulated) obtained using SAS-EM showed a continuous release for 700 hours (Figure 4b).
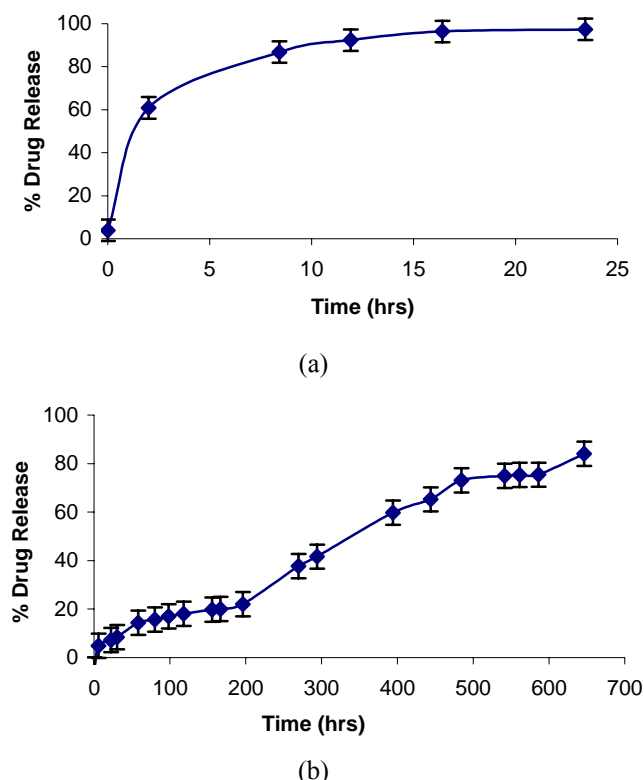


(a)



(b)

**Figure 4.** *In-vitro* release profile of dexamethasone phosphate from PLGA microencapsulated (a) drug microparticles as provided by the supplier (Figure 2a) and (b) drug nanoparticles obtained from SAS-EM (Figure 2b).

## 5 CONCLUSION

Nanoparticles of dexamethasone phosphate can be produced using SAS-EM® technique. When microencapsulated, these nanoparticles provide high encapsulation efficiencies and sustained drug release without initial burst. Since the complete process is anhydrous, it can be easily extended to produce sustained release formulation of other hydrophilic drugs, peptides and proteins.

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