CHARACTERISTICS AND DRUG RELEASE OF DRUG-LOADED MICROPARTICLES PREPARED WITH DIFFERENT SOLVENTS USING ELECTROSPRAYING

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Abstract:

Poly(lactic-co-glycolic acid) (PLGA) microparticles containing Celecoxib (CEL) were prepared with electrospraying using either acetone (ACE) or acetonitrile (ACN). The microparticles produced were characterized in terms of morphology, surface chemistry and physical state of the drug using scanning electron microscopy (SEM), X-ray powder diffraction (XRPD) and X-ray photoelectron spectroscopy (XPS) and drug release from the particle samples was studied.

Particles were around 3μm in diameter with porous surfaces and the particles prepared in ACE had a compact, raisin-like morphology while those prepared in ACN were collapsed, hollow spheres. The differences observed in morphology is explained by the higher solubility of PLGA in ACE and the higher evaporation rate of ACE resulting in better solute diffusion during particle formation.

XRPD diffractograms indicated that CEL was amorphous in a solid dispersion within the particles. Yet, XPS data representing particle surface element composition indicated 20% and 27% CEL concentration on the surface for particles prepared with ACE and ACN respectively, although all particles were prepared with 10% CEL content. The high surface drug content is explained by the migration of CEL towards the particle surface together with solvent during PLGA precipitation and solvent evaporation. The higher surface CEL concentration for particles prepared with ACN is explained by the slower solvent evaporation and faster precipitation of PLGA during particle formation.

The drug release study showed that the microparticles possessed diffusion driven release profiles with 70-80% cumulative release in 8 hours. The particles prepared with ACE showed a slower release than those prepared with ACN. This difference is explained by the larger surface area and higher surface drug concentration of particles prepared with ACN. The current study demonstrated the significant effects of solvent on the morphology as well as surface chemistry and drug release profile of the electrosprayed PLGA/CEL microparticles.

1. Introduction

Microparticles are being widely studied for pharmaceutical purposes and are seeing application in clinical practice [1]. In the development of such microparticle-based dosage forms it is often desirable to obtain particles with good control over properties including size, porosity, morphology and drug distribution

through rational particle design [2]. There are several techniques by which therapeutic microparticles can be produced, including emulsion-based techniques [3], spray drying [4] and electrospraying [5]. Electrospraying is an attractive technique with good control over particle properties such as size and morphology and can produce particle powders directly without subsequent drying or separation steps [6]. The technique is based on the atomization of liquid via strong electrostatic forces which break up the liquid into small charged droplets that subsequently result in a homogeneous population of particles [7].

Depending on the type of application of the microparticles there are certain particle attributes that are desirable to engineer. For instance for pulmonary drug delivery aplications it is desirable to have low density particles of a few micrometers with a rough surface, which can be accomplished by producing hollow particles with a porous or wrinkled surface [8]. In order to engineer such particles in a reproducable manner it is necessary to have a sound understanding of the particle formation process as well as the different parameters influencing particle characteristics and this is often not entirely the case [9]. The better performance of designed particles motivates further investigation in the underlying mechanisms of particle formation in order to achieve increasingly precise control of important particle features [10]. This is particularly relevant for a technique such as electrospraying for which good control of particle features has been observed but for which no commercial setup is currently available. Further understanding of the particle formation mechanisms with electrospraying is likely to aid the development of a commercial and automised device [11].

In this present study, microparticles were prepared from the biodegradable polymer, PLGA, which is FDA-approved and used for numerous applications including pulmonary delivery [12]. The PLGA microparticles were prepared loaded with the poorly water-soluble drug, CEL, which is often associated with low bioavailability due to its low solubility [13]. PLGA and CEL were atomized in a solution using either acetone (ACE) or acetonitrile (ACN) as their solvent component and with a solute concentration of 5% and a drug loading of 10%. The aim of the study was to examine the influence of the solvent on the particle characteristics including drug distribution as well as their drug release profile.

2. Materials & Methods

Materials

CEL powder was acquired from Dr. Reddy, Hyderabad, India (Mw=381.38 g/mol). Poly(D,L-lactide-co-glycolide (PLGA, acid terminated; 50:50 Resomer RG503H, Mw=24-38 kDa) was purchased from Sigma Aldrich (Poole, UK). Acetone (ACE, 99.9% HPLC grade) and acetonitrile (ACN, 99.9% HPLC grade) were purchased from Sigma Aldrich (Poole, UK). Phosphate Buffered Saline (PBS, 0.01M, pH 6.8) was made from sodium phosphate monobasic and sodium hydroxide purchased from Sigma Aldrich (Poole, UK), and Sodium Lauryl Sulphate (SLS) was purchased from Fagron (Waregem, Belgium).

Preparation of CEL-PLGA microparticles using electrospraying

CEL-PLGA microparticles were prepared with a single nozzle electrospraying setup as shown on Figure 1. The setup was comprised of a power source (Glassman Europe Ltd, Tadley, UK) with a high voltage output, a syringe pump (PHD 4400, Harvard Apparatus, Edenbridge, UK) with a highly precise, adjustable flow rate, and a cylindrical stainless steel nozzle with outer and inner diameters of 2.34mm and 1.77mm respectively. The spray solutions containing PLGA and CEL were prepared and electrosprayed at a flow rate of 30µl/min and an electrical potential of 10-11 kV. The particles produced were collected 70mm from the nozzle onto a sheet of aluminum foil and the samples were stored in a desiccator. A video camera with an in-built magnifying lens (Leica S6D JVC-color) was used to monitor the nozzle tip and jet at all times during preparation of the microparticles to ensure a stable cone-jet.

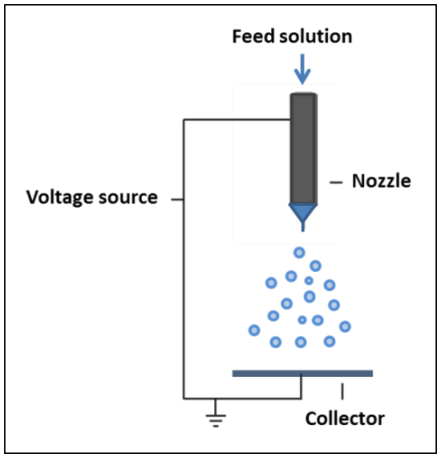

Figure 1. Electrospraying setup

To study the solution properties the kinematic viscosity and intrinsic viscosity of ACE and ACN solution containing CEL and PLGA were determined using an Ubbelohde viscometer (Cannon instruments) at 25 ºC in a water bath. The evaporation of the solutions were examined using thermogravimetric analysis (TGA) by measuring the % loss in weight over time. A measure for evaporation rate was determined as the average % loss in weight per minute during the linear part of the evaporation curve.

Characterization of CEL-PLGA microparticles

The size and shape of the microparticles were examined using a scanning electron microscope (SEM) (Quanta 200 ESEM FEG). Samples, consisting of a monolayer of particles, were sputter-coated with gold, and viewed at an accelerating voltage of 3 kV. The solid state of the drug in the microparticles was analysed using X-Ray Powder Diffraction, which was done on a PANalytical X'Pert PRO MPD (PW3040/60, Philips, Netherlands) using a copper anode for radiation with λ=1.542Å, 45 kV and 40mA. The samples were placed on an aluminum sample holder and measured from 5° - 40° 2θ with a step size of 0.053° and 0.025° s^{-1}.

X-ray photoelectron spectroscopy (XPS)

The surface chemistry of particles samples was examined by XPS using a K-Alpha (Thermo Scientific) equipped with a monochromated AlKα X-ray source. Wide energy survey scans (0-1350 eV binding energy) were acquired with pass energy 200 eV and step size 1.0 eV. An angle of 90° was used between sample surface and analyzer (take-off angle). The surface drug content (in weight %) of the microparticles was determined by ratioing the detected amount of fluorine in the sample to the amount of fluorine in pure CEL.

Drug release study

In brief, the microparticle samples were weighed and placed in 500 mL dissolution media of PBS (0.01M, pH=6.8) + 1.5% sodium lauryl sulfate (SLS) using a Sotax AT7 dissolution station (Sotax, Switzerland) equipped with a USP 2 (paddle) apparatus and 1000 mL glass vessels. The study was performed at a paddle rotation of 50 rpm, with a constant temperature bath at 37 ºC. Samples of 5ml were extracted at 9 time points and the drug content in the extracted samples was analyzed using a HPLC unit with Pump P680 and ASI 100 sample injector and UVD340U (Dionex, Germany) equipped with Kromasil 126 column (Kromasil, Sweden). A mobile phase of acetonitrile:water (60:40 v/v) was used at a flow rate of 0.5 ml min-1 and the injection volume of 10µl was detected at a wavelength of 230 nm. A standard curve with good linear correlation was achieved in the range of 0.5µg/ml and 50µg/ml.

3. Results & Discussions

3.1 Solution characteristics

The solvents ACE and ACN were selected for this study based on the criteria that the solvents should be able to easily dissolve both PLGA and CEL at the concentrations used and further have a high enough evaporation rate to result in dry particles upon collection without use of any post drying processes. The solution characteristics presented in Table 1 indicates that the ACE solution is slightly more viscous than the ACN solution with the intrinsic viscosity also being slightly higher for the ACE solution. The intrinsic viscosity refers to the ability of a specific polymer to increase the viscosity of the solvent in which it is dissolved and thereby gives an indication of the solvent power of the solvent [14]. The higher intrinsic viscosity of the ACE solution thus indicates a higher solvent power of ACE and a less compact comformation of the polymer in ACE.

Characteristics of solutions			
Solution	**Kinematic viscosity**	**Intrinsic viscosity (dL/g)**	**Evaporation rate (%/min)**
5% PLGA in ACE	1.11	0.28	27
5% PLGA in ACN	1.08	0.22	7

Table 1. Properties of solutions used.

3.2 Particle morphology

Figure 2 shows representative SEM images from the two microparticle samples. The images indicate that the microparticles were generally around 3µm in diameter, they were near-monodisperse and had some degree of porosity on their surfaces. The microparticles prepared with ACE were generally more dense on the surface compared with those prepared in ACN whereas the particles prepared wih ACN were collapsed and indicated a hollow inner structure. The morphological differences in the samples are explained by the higher evaporation rate and solubility to PLGA of ACE compared with ACN. During particle formation the droplet shrinks while solvent evaporates at the droplet surface and at this point there is an important relation between solute diffusion and solvent evaporation rate [10]. If the solvent evaporates quicker than the solutes can diffuse inwards the polymer precipitates out at an early stage and generally results in hollow particles [15]. With the ACN based solution this seems to be the case while with the ACE based solution the more compact structure observed indicates that solute diffusion could keep up with the solvent evaporation at the surface for a longer time before precipitating.

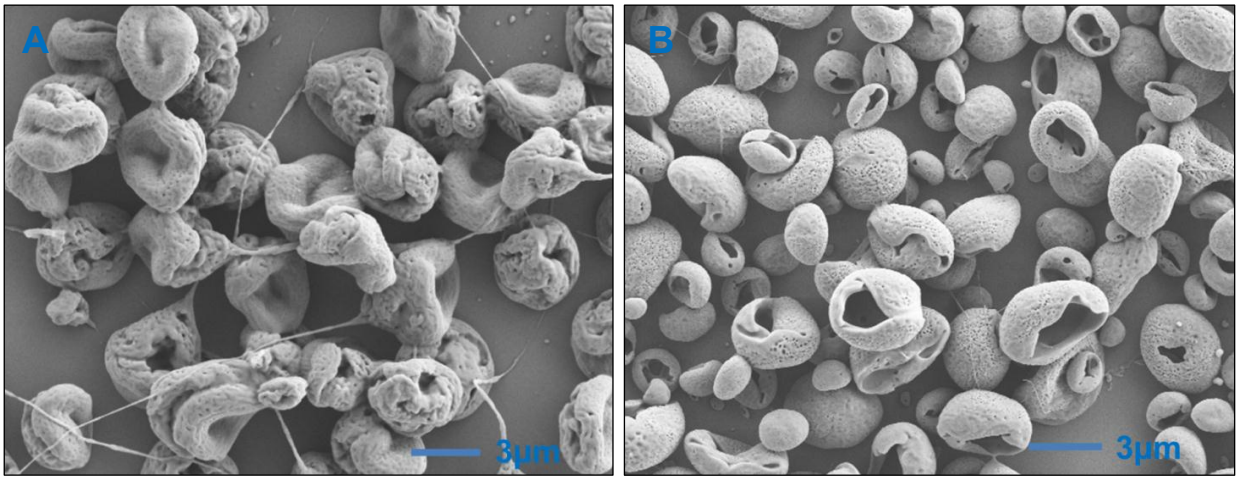

Figure 2. SEM images of CEL-PLGA microparticles.

3.3 Physical form

The XRPD diffractograms of the electrosprayed microparticles on Figure 3 show typical halo shapes for both microparticle samples. The absence of crystallinity in the particles suggest that the drug molecules were molecularly dispersed within the polymer matrix as a solid dispersion.

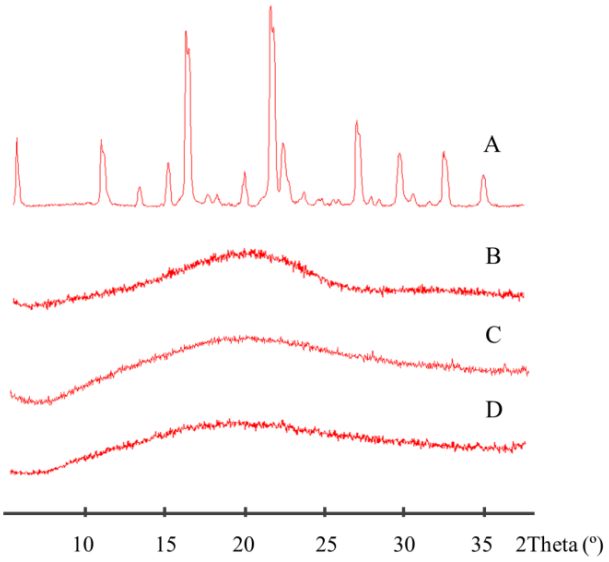

Figure 3. X-ray diffractograms of CEL crystalline powder (A), PLGA powder (B), particles prepared in ACE with 10% drug loading (C) and particles prepared in ACN with 10% drug loading (D).

3.4 Surface chemistry

The surface chemical composition of the dry particle samples was analyzed using XPS to examine the surface drug content and thereby understand the drug distribution in the particles. The CEL concentration on the surface of the microparticles were measured to be 20% for the microparticles prepared with ACE and 27% for the microparticles prepared with ACN as shown in Table 2. Although the CEL only accounted for 10% of the total solute content in the spraying solution it comprised twice or more that concentration on the particle surface. This tendency of CEL to migrate towards the surface of the particles is explained by the small size of CEL (381 mol/g) compared with PLGA, which makes it much more mobile in the droplet during solvent evaporation. It is believed that CEL migrated out to the surface with the solvent as the solvent escaped from within the solidifying particle. The higher surface CEL concentration observed in particles formed with ACN can be explained by the significantly slower evaporation rate of ACN, which provide more time for the CEL molecules to migrate towards the particle surface.

Elements Analysis				
Sample	**N/F**	**S/F**	**F/C**	**Conc. of Cel**
5S10ACE	0.92	0.33	0.05	20±1%
5S10ACN	0.81	0.28	0.07	27±1%

Table 2. XPS surface chemistry analysis and surface CEL concentration of microparticles.

3.5 Drug release

From the release curves of CEL-loaded PLGA microparticles with 10% drug content (see Figure 4) it is observed that the drug was released during the entire measurement and that the cumulative release reached

between 70-80% of the total drug content in the 8h of measurement. It is further seen that the release was quicker for the particles prepared with ACN than for particles prepared with ACE. This is explained by two observations from the previous results sections, partly because of the larger surface area of particles prepared in ACN from their collapsed and hollow morphology and also partly because of their higher surface drug concentration as observed by XPS compared with the particles prepared with ACE.

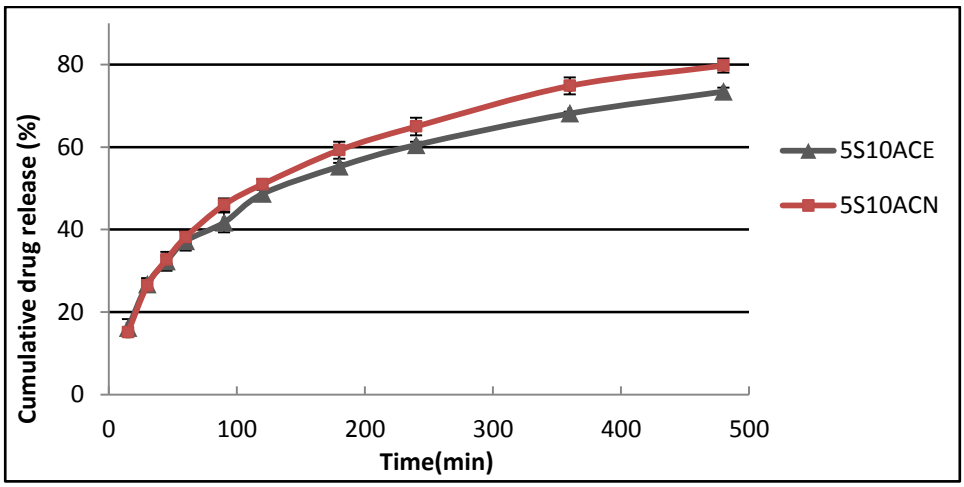

Figure 4. Drug release curves of microparticles prepared in ACE (grey/triangles) and ACN (red/squares).

4. Conclusion

Microparticles composed of Poly(lactic-co-glycolic acid) (PLGA) and Celecoxib (CEL) were prepared with acetone (ACE) and acetonitrile (ACN) using electrospraying. The morphology of the particles ranged from hollow, collapsed-spherical to raisin-shaped with the presence of pores on the surface. X-Ray powder diffraction indicated that the drug was molecularly dispersed in the PLGA matrix. The surface chemistry analysis measured with X-ray photoelectron spectroscopy showed surface enrichment of CEL, particularly for microparticles prepared with ACN, signifying the implications of the differences in morphology and particle formation process with the two solvents. The drug release study showed differences in the release profile of the two particle samples with particles prepared in ACN releasing their payload quicker. This further indicates the influence of the particle characteristics and demonstrates the interplay between solution properties, particle formation, the resulting particle features and the drug release profile. The results indicate that electrospraying is a useful technique for producing drug loaded microparticles that can be customized to fit the intended drug delivery application.

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