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Electrospray technique for solid lipid-based particle production

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Abstract

Background: Different preparation methods for the production of lipid micro- and nanoparticles as controlled release formulations have been widely developed. Novel techniques are attracting increasing attention for their preparation. *Method*: The objective of the present investigation was to produce solid lipid-based micro-nanospheres using the electrohydrodynamic atomization (electrospraying) and to evaluate whether it is a suitable method to prepare drug-loaded particles. *Results*: Narrowly dispersed spherical particles lower than 1 μm, easily internalized in cells, were obtained using stearic acid and ethylcellulose in a 4.5:0.5 (w/w) ratio. Tamoxifen, as model drug, was encapsulated with good entrapment efficiency. The in vitro release, after an initial burst effect, showed a prolonged drug release. *Conclusion*: The electrospraying method might be proposed to prepare in a single-step monodisperse lipid-based micro- and nanoparticles in powder form for drug delivery.

Key words: *Drug delivery; electrohydrodynamic atomization; electrospray; solid lipid-based particles; tamoxifen*

Introduction

Biodegradable polymeric micro- and nanoparticles are widely used as drug delivery systems. These particles, in which the drug is encapsulated into a biodegradable matrix, improve drug delivery and provide a relatively slow release rate, prolonged release time, and high local drug concentration compared with conventional dosage forms.

Micro-nanoparticles made of solid lipids are now an alternative to polymeric particles. These lipidic particles were designed as carriers for the parenteral administration of hydrophobic drugs[1]. However, as the potential of colloidal systems for transmucosal drug delivery has become evident, the utility of lipid micro-nanoparticles for improving the oral absorption of drugs has also been tested[2].

Recently, much work has focused on the production by electrical means of fibers with diameters as small as 10 nm[3]. This process has received considerable attention, probably because of the interest in nanotechnology, as ultrafine fibers or fibrous structures of various polymers with diameters down to submicrons to nanometers can easily be fabricated with this process.

The basic setup for electrostatic atomization comprises a nozzle connected to a high-voltage power supply and supplied with a liquid to be atomized[4]. In a typical process, a polymer solution is contained in a syringe, with a metal capillary connected to a high-voltage power supply as one electrode. A metal foil collector is placed opposite the capillary as counter electrode. Depending on the properties of the liquid, its flow rate, and the voltage applied, different modes of atomization can occur. The mode of interest to prepare nanoparticles is the cone-jet mode, in which liquid emerging at the nozzle forms hemispherical drops because of surface tension (electrospray). By increasing the electrical field, the hemispherical drops can be changed to a conical shape, which breaks up into highly charged droplets. By selecting suitable conditions, droplets can be generated with a narrow size distribution and nano-micrometer size range. Solid particles can be formed by evaporating solvent from the droplets produced traveling through the electrical field.

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The electrospray process for particle production has shown promising potential[5]. Thus far, more than 30 polymers have been successfully electrospun, but to the authors' knowledge no articles have yet been published on the electrospray of lipid-based nanoparticles[6].

This study examines the possibility of using the electrospray technique to produce lipid-based micro-nanoparticles in powder form as delivery system using tamoxifen as model drug. The morphology, mean size, polydispersity, zeta potential, and thermal behavior of the lipid particles were first examined. The encapsulation efficiency (EE) and the in vitro release behavior of tamoxifen were then determined to evaluate whether electrospraying is a suitable method to prepare monodisperse drug-loaded lipid-based particles.

Materials and methods

Materials

Hydrogenated phosphatidylcholine (Epikuron H) was a gift from Degussa. Stearic acid (SA), palmitic acid, tristearine, ethanol, *n*-propanol, *n*-butanol, *n*-pentanol, *n*-hexanol, and tamoxifen were purchased from Sigma (St. Louis, MO, USA). Ethylcellulose (EC, 64,000 Da, 7 mPa.s 5% toluene-ethanol 80–20, 25°C) was from Fluka (Milan, Italy). All chemicals were used without further purification or other processing. Double-distilled water was prepared freshly whenever required.

Experimental electrospray set-up

To obtain particles of a narrow size distribution, voltage and liquid flow were set such that the system operated in a cone-jet mode. The experimental setup consisted of a 5-mL syringe connected to an infusion pump (KDS 100; Biological Instruments, Varese, Italy). A Teflon pipe connected the syringe to the tip of a metal capillary (ID 0.6 mm). A strong electric field (Gamma high-voltage research, Ormond Beach, FL, USA) was applied between the tip and a collector plate consisting of a circular 150-mm diameter aluminum dish linked by a wire to a neutral ground. The shape of the dish was selected to maximize the symmetry of the electrospray system and improve uniformity of electric charge[7]. The distance from metal tip to collecting plate was 150 mm. Different parameters were used to obtain a cone-jet mode. A solution flow of 15 µL/min and a voltage of 25 kV for ethanol were used, whereas 15 µL/min/30 kV for *n*-propanol and *n*-butanol, and 10 µL/min/30 kV for *n*-pentanol and *n*-hexanol were used.

The same parameters were used to prepare the SA-EC particles. During free flight, the organic solvent evaporated and solid particles collected on the aluminum dish.

Preparation of the solutions for electrospray

Lipid solutions (Epikuron H, tristearine, PA, and SA) and SA-EC (4.5:0.5, w/w) at 5% (w/v) were prepared. Ethanol, *n*-propanol, *n*-butanol, *n*-pentanol, and *n*-hexanol were used as solvents.

Fluorescent lipid-based nanoparticles were obtained by adding a 6-coumarin solution (0.1%, w/w) to the SA : EC solution in *n*-pentanol. Tamoxifen-loaded particles were prepared by dissolving the drug together with SA and EC in the chosen solvent at a 10/1 (w/w) ratio between matrix and tamoxifen. All solutions were prepared under magnetic stirring at room temperature to obtain homogeneous systems.

Characterization of the solutions for electrospray

The surface tension of the alcoholic solutions was determined using a digital tensiometer (Kruss GmbH, Hamburg, Germany), the conductivity using a conductivity meter (Orion, Boston, MA, USA), and the viscosity using an Ubbelohde viscometer (Schott Gerate, Postfach, Germany). The determinations were carried out at 25°C.

Scanning electron microscopy and atomic force microscopy

The morphology of the particles was examined by scanning electron microscopy (SEM) (Leica Stereoscan 410, Wetzlar, Germany) A thin layer of particles was mounted on a copper stud, which was then sputter coated with gold (SCD 050, Lewica, Wetzlar, Germany) for 60 seconds under vacuum at a current intensity of 60 mA. The gold-coated particle layer was scanned using the accelerating voltage scanning of 20 kV.

Atomic force microscopy micrographs were obtained using DME Dual Scope C-21 Scanning probe (Danish Micro Engineering, Copenhagen, Denmark). The samples were all scanned in noncontact mode with three piezo electrodes and processesd with SPM software.

Particle size and zeta potential

The average particle size and polydispersity index of the lipid-based particles were determined in duplicate by photon correlation spectroscopy (90 Plus, Brookhaven Instrument, Holtsville, NY, USA). Measurements were carried out at an angle of 90° at 25°C. Samples were obtained by dispersing SLN in filtered water using ultrasound for size determination. For zeta potential measurements, the samples were prepared by dispersing the SLN in 0.001 N KCl to ensure that the light-scattering intensity and conductivity were within the instrument's sensitivity range (90 Plus, Brookhaven Instrument).

Evaluation of cellular uptake by confocal laser-scanning microscopy

Exponentially growing Vero cells were plated and cultured overnight in 24-well plates on glass coverslips. The cell monolayers were then incubated with 1 μg/mL of fluorescent labeled nanoparticles for the times indicated and extensively washed with phosphate-buffered saline to observe the living cells. Confocal sections were taken on an inverted Zeiss LSM510 fluorescence microscope.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) measurements (DSC7; Perkin Elmer, Nortwalk, CT, USA) were used to determine the melting peak of tamoxifen, and tamoxifen-loaded particles. In each experiment, 2–3 mg of sample was sealed in the sample pan and scanned, with a similar empty pan as reference, at a fixed heating rate (5°C/min) from 40°C to 120°C.

Drug recovery and encapsulation efficiency

Drug recovery (D_r) was determined by dissolving 5-mg sample of particle powder in 1 mL methanol, then 1 mL water was added and mixed. After centrifugation the solution was injected for high-performance liquid chromatography (HPLC) measurements. The D_r was then calculated by the following equation:

$$D_r = \frac{M_{pp}}{M_0} \times 100\%$$

where M_{pp} is the mass of drug detected in the particle powder and M_0 is the mass of drug used for SLN preparation.

The EE of the particles was measured in triplicate using HPLC after washing 5-mg sample three times with an ethanol:water solution (20:80, v/v). The residue was solubilized in 1 mL methanol, and 1 mL water was added and mixed. After centrifugation, the solution was injected for HPLC measurements. The EE was then calculated by the following equation:

$$EE = \frac{M_{SLN}}{M_0} \times 100\%$$

where M_{SLN} is the mass of drug detected in the SLN after washing and M_0 is the mass of drug used for SLN preparation.

In vitro tamoxifen release

Experiments of in vitro tamoxifen release from lipid particles were done in triplicate in 20-mL screw-capped tubes placed in an orbital shaker bath, which was maintained at 37°C and shaken at 120 rpm. To avoid rinsing particles away, about 10 mg of SLN powder were dispersed in 1 mL of 0.1 M phosphate buffer at pH 7.4 and immediately enclosed in a 2-cm long piece of dialysis membrane, 10 mm external diameter, and cutoff 100 kDa. The sealed membrane was immersed in the tube containing 10 mL of 0.1 M phosphate buffer at pH 7.4/methanol solution (5/1, v/v) to ensure sink condition. Aliquots (1 mL) were collected at regular time intervals for drug determination, and the receptor medium was refilled with 1 mL of fresh phosphate solution. A dilution-correction procedure was applied to calculate drug release. To verify whether the diffusion of the drug through the dialysis membrane was not the limiting step in the process, an aqueous solution of tamoxifen was used at the same concentration as in SLN and the experiment was carried out in the same conditions.

Tamoxifen content determination

Tamoxifen content was determined using an HPLC system consisting of Shimadzu liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with an SPD 10A variable wavelength ultraviolet detector and a CR6A integrator. A Lichrosphere C-18, 5 μm (Merck, Darmstadt, Germany) 25 cm × 4.6 mm ID reversed-phase column was used. The column was eluted with a solvent system containing methanol/water/triethylamine (89/11/1, v/v). The eluent was run at the rate of 1 mL/min and monitored at 265 nm following injected volumes of 20 μL of tamoxifen standard solutions and samples. The calibration curve was found to be linear in the range 0.05–20 μg/mL. The minimum concentration detected was 20 ng/mL of tamoxifen. Each sample was analyzed in triplicate.

Results and discussion

This research showed the feasibility of producing (in a single step) lipid-based micro-nanoparticles using the electrospray technique. The primary requirement to obtain particles using electrospray with properties suitable in the perspective of drug delivery is to use solvents with relatively high solubilization capacity for lipids. Moreover, this solvent must be removed easily from the particles to avoid toxicity problems arising from solvent residues.

Preliminary experiments were carried out to tune the process parameters using different fatty acids, triglycerides, and phospholipids dissolved in short- or medium-chain alcohols. The results showed that electrospray is a cost-effective method to produce solid lipid particles in

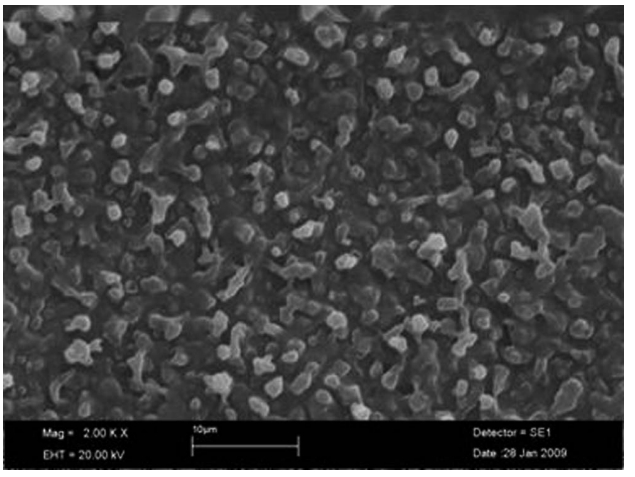

Figure 1. SEM image of hydrogenated lecithin particles obtained from a *n*-propanol solution.

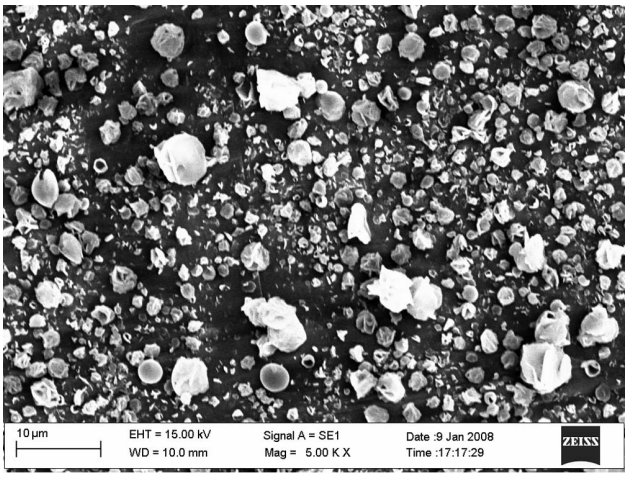

Figure 2. SEM images of SA particles obtained from a *n*-pentanol solution.

micrometer size range. A determined size and morphology could be obtained by adjusting the production parameters.

Figure 1 reports, as an example, the SEM photomicrographs of hydrogenated phosphotidylcholine particles obtained from a 5% *n*-propanol solution. Particles rather spherical with an average diameter lower than 1 µm are present besides some aggregates.

To investigate the effect of the solvent on the lipid particle formation SA and a series of alcohols ranging from ethanol to *n*-hexanol were selected. The solubility of SA at 25°C in the tested alcohols ranged from 5% for ethanol to about 7.5% (w/w) for *n*-hexanol, amounts suitable for the electrospray process.

The different SA alcoholic solutions were sprayed tuning the electrical fields and lipid solution flows to obtain the cone-jet mode[8]. Solid micro-nanoparticles obtained showed different sizes and morphology. The lipid particles obtained using ethanol, *n*-propanol, and *n*-butanol appeared similar, with some blade-like shaped particles besides the spherical ones, whereas *n*-pentanol formed spherical shape particles, but with different sizes. The same behavior occurred when the process parameter values (electric field and flow) were changed by ±20%.

Figure 2 shows the SEM images of SA particles prepared using *n*-pentanol as solvent.

The electrospray is a complex process, and an accurate estimation of particle size beforehand is very difficult. Many parameters can influence the transformation of solutions into fibers or particles through electroatomization as previously reported for polymer[9]. These parameters include the properties of the solution, such as viscosity, conductivity, and surface tension, and process variables such as hydrostatic pressure in the capillary tube, electric potential at the capillary tip, and the distance between the tip and the collector as well as environmental parameters such as the solution temperature, humidity, and air velocity in the electrospray chamber.

Different processes are possible after the droplets are formed, depending on the surface charge density and the droplet surface tension[10]. When the surface charge density is low, the Rayleigh limit, the maximum limit of surface charge density when the electrostatic forces exceed surface tension, is never reached. Another possibility is that the surface charge density of the droplets is high, so the Rayleigh limit is reached immediately or after solvent evaporation and droplets disintegrate (Coulomb fission), forming small charged droplets[8]. In the process the Coulomb fission should be avoided because droplets of uniform size are required.

In our experiments a series of 5% SA solutions with different alcohols were used to prepare the lipid particles. Table 1 lists the properties of the solutions employed, including the boiling points of the solvents, and the measured conductivities, surface tensions, and viscosities of SA solutions. All these properties can influence the sizes and morphology of nanoparticles. Probably the contribution of boiling point and viscosity plays the most important role in determining size of lipid particles.

A number of studies have investigated the influence of solution viscosity on polymeric particle formation; the results evidenced that viscosity below 100 mPs is necessary for droplet formation[11]. The effect of conductivity on particle formation has also been studied[12]: the increase in solution conductivity from µS/cm to mS/cm generally resulted in a marked reduction in the final particle size due to Coulomb fission. The surface

Table 1. Properties of solvents and SA (5%) and SA-EC (4.5/0.5%) alcoholic solutions.

		Viscosity (mP.s)		Surface tension (mN/m)		Conductivity (μS/cm)	
Alcohol	Boiling point (°C)	SA	SA-EC	SA	SA-EC	SA	SA-EC
Ethanol	78.0	1.20	2.12	22.3	22.4	0.51	0.53
n-Propanol	97.2	2.36	3.67	23.1	23.9	0.28	0.26
n-Butanol	117.5	2.92	5.46	24.2	24.0	0.15	0.18
n-Pentanol	137.5	3.80	7.01	25.8	26.1	0.08	0.10
n-Hexanol	157.0	5.14	9.31	26.4	26.6	0.05	0.07

tension of the solution affects particle formation[3]: on decreasing the surface tension of the solution there is, in general, a slight decrease in average particle size, and a corresponding increase in standard deviation of the particle size.

From the data reported in Table 1 it appears that conductivity and surface tension of the series of alcoholic solutions did not change markedly and probably they have a little effect on the shape of the particles obtained. Moreover, the viscosities are below 100 mPa, value necessary for the droplet formation. Consequently, the main factor affecting particle shape seems to be the solvent boiling point, besides the solution flow.

Addition of a second component in the lipid solution has been investigated. Because few polymers are soluble in alcohol, EC was added to the lipid solutions. Different SA: EC ratios (w/w) were tested and the 4.5:0.5 (w/w) ratio was chosen, corresponding to a molar ratio of about 2000:1.

The SEM images of SA-EC (4.5:0.5, w/w ratio) particles prepared using different alcohols are reported in Figure 3. In the presence of EC, powders consisting of different shaped particles formed, including spherical particles with narrow size distribution, depending on the alcohol used. The different shapes of particles obtained, as in the case of SA solutions, may be due to the different alcohol boiling points and thus to the different solvent evaporation rates (Table 1).

It is supposed that the main mechanism in the presence of EC is presumably the solvent evaporation without droplet fission, as is shown by the uniformity of particle size.

The particle shape could be explained comparing the velocity of the shrinking front of the solution droplets and the diffusion of the polymer molecules in the solution droplets[10]. Because EC is a large molecule, its diffusion rate inside the droplets will be very slow. Loss of solvent through surface evaporation shrinks droplet size and increases EC concentration close to the surface of the droplet, leading to the formation of a shell of solid EC. Using low boiling point alcohols, and thus a high evaporation rate, makes the core of the particle structurally weak, so that the shell collapses and a blade-like shape particle is formed. With solvents having a low evaporation rate, the shell shrinks but does not collapse, because the core of the particle is structurally stronger because of the presence of more diffused EC. As it has been reported for electrosprayed polymer nanoparticles[7] the resistance of thinner jets depends on the viscoelastic force of the polymer solution. The most important variables that influence the viscoelastic force are the polymer molecular weight and the polymer concentration. In our case, because the polymer was the same for all experiments, the main factor influencing particle shape can be expected to be initial polymer solution concentration. During the process, the solvent evaporates and the solution concentration rises, thus the concentration in the thinner jet increases when the initial polymer concentration is increased, and consequently increases the viscoelastic force. Using higher EC concentrations fibers formed besides the spherical particles (data not shown).

The alcohol residues in the particles prepared at 4.5:0.5 SA:EC weight ratio were then determined using GC. The content in milligram of the different alcohols present per gram of particles ranged from 0.02 to 0.1 from ethanol to *n*-butanol and was 0.2 and 0.5 for *n*-pentanol and *n*-hexanol, respectively. As expected, the alcohol content decreased as the boiling point of the alcohol decreased. The relatively high alcohol content of the particles prepared using *n*-hexanol may contribute to the formation of the gel-like structure observed using SEM (Figure 3).

Fluorescent SA-EC nanoparticles were prepared to study their behavior after incubation in cell cultures. The fluorescent particles of about 500 nm were easily internalized in Vero cells as confocal microscopy analysis showed (Figure 4). Cellular uptake is fast and probably the surface hydrophobicity determines the interaction with cells before internalization.

To verify the possibility of using this process to prepare a suitable drug delivery system, only the particles obtained with *n*-pentanol (4:0.5 SA:EC ratio) were used. Tamoxifen (SA-EC:drug 10:1, w/w) was used to prepare drug-loaded particles. Samples of drug-loaded SA-EC particles are similar in shapes to the drug-free particles. The AMF image (Figure 5) revealed that the particle size was about 1 μm and that the particles had nearly spherical morphology and smooth surfaces. The size of the water-suspended drug-loaded particles, their polydispersity determined

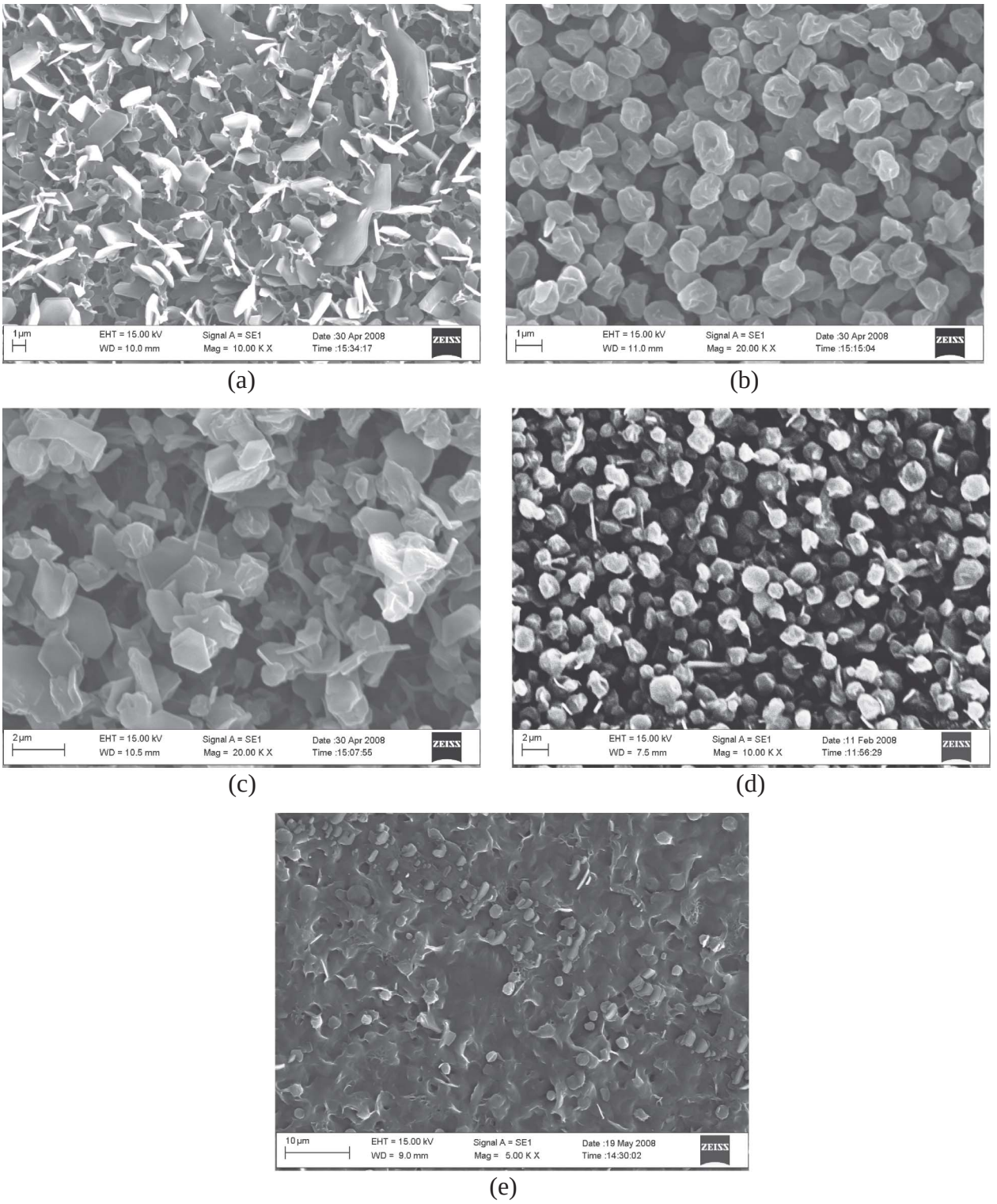

Figure 3. Effect of alcohol (SEM images) on the morphology of 4.5:0.5 SA-EC particles: (a) ethanol, (b) *n*-propanol, (c) *n*-butanol, (d) *n*-pentanol, and (e) *n*-hexanol.

using LLS, and their zeta potential were 922 ± 52 nm, 0.11 ± 0.02, and −29.1 ± 1.3 mV, respectively. The high negative zeta potential value indicates that suspensions formed from these particles do not easily aggregate.

Figure 6 shows the DSC profile of tamoxifen-loaded SA-EC particles, SA, and tamoxifen. The DSC curves of the loaded SA particles were similar to SA bulk material without the presence of a peak at the melting

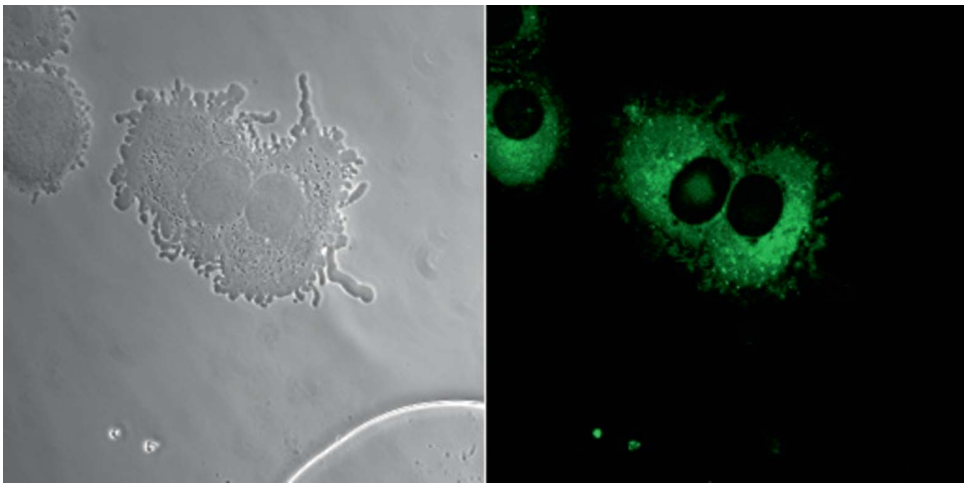

Figure 4. Cell uptake of fluorescent nanoparticles in Vero cells by confocal laser-scanning microscopy.

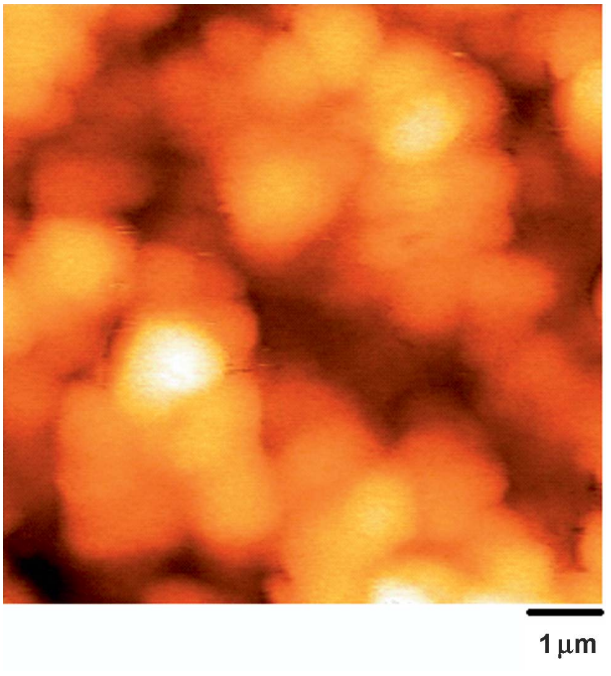

Figure 5. Atomic force microscopy photograph of drug-loaded particles (4.5:0.5 SA-EC).

temperature of tamoxifen (97°C). The disappearance of the drug peak in the DSC thermogram indicated that tamoxifen is dispersed in the lipid matrix and did not crystallize.

The D_r was about 96% whereas the entrapment efficiency, which corresponds to the percentage of tamoxifen encapsulated within the particles, was about 70%.

The in vitro tamoxifen release from drug-loaded particles showed an initial burst of about 30% and a continuous slow release over time of the incorporated drug (Figure 7). The burst value was quite similar to the difference between recovery and EE values, indicating that this drug amount is located on the surface of the particles and it is immediately released into the receiving medium. Following this step, the release of tamoxifen slowed down and was at a relatively constant rate reaching about 50% after 24 hours. This release rate is similar to that reported for tamoxifen loaded in chitosan–SLN microparticles[13].

This research is in progress to verify the influence of different solvents and the presence of other excipients like surfactants on particle production. Moreover, formulations and process parameters will be developed to decrease the particle sizes.

Conclusions

Lipid-based micro-nanoparticles in powder form with sizes lower than 1 μm and with narrow size distribution, spherical structure, and smooth morphology could be successfully obtained in a single step with the electrospray method developed. Tamoxifen was encapsulated in lipid-based particles with good entrapment efficiency showing an in vitro prolonged release profile.

In conclusion, the electrospray method could be proposed as suitable and easy technique to obtain dry monodispersed solid lipid-based micro-nanoparticles for drug delivery.

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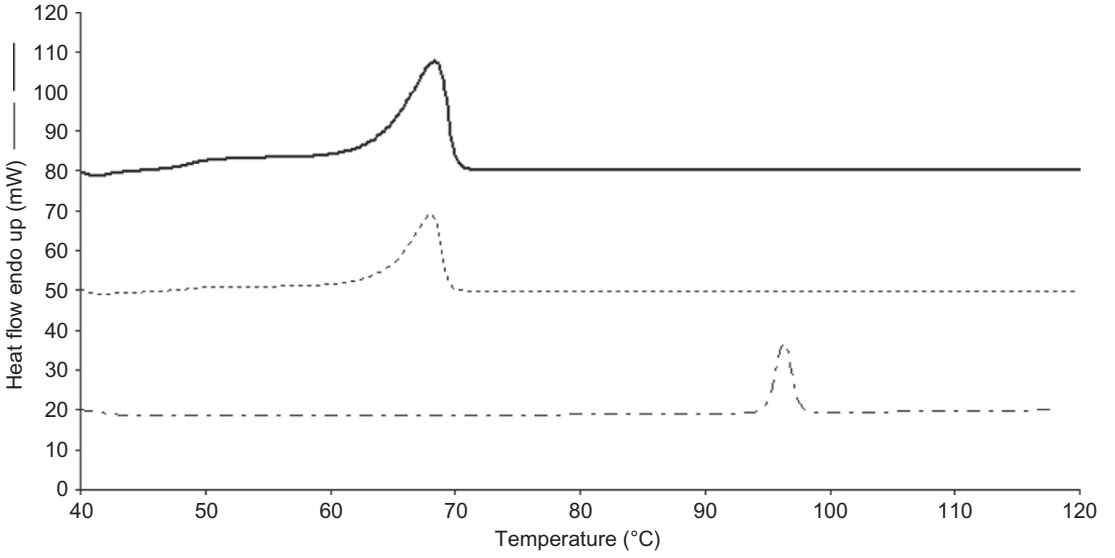

Figure 6. DSC thermograms: (——) SA, (- - -) tamoxifen-loaded particles, and (— - —) tamoxifen.

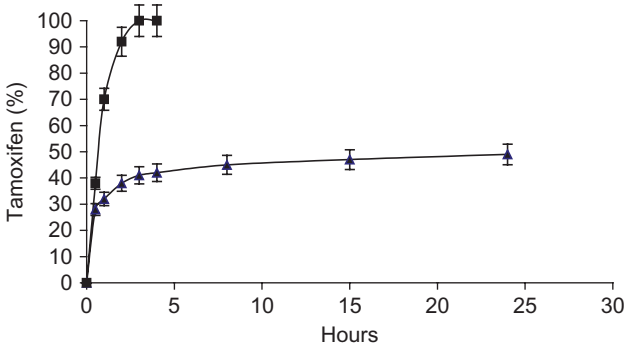

Figure 7. In vitro release profile of tamoxifen from drug-loaded particles (■) in confront to the solution (▲) through dialysis membrane.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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