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Research paper

# Encapsulating darunavir nanocrystals within Eudragit L100 using coaxial electrospraying

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ABSTRACT

Electrospraying is renowned for its simplicity and versatility, and which can effectively produce particles with well-controlled size, size distribution, particle shape, morphology and microstructure at the nano/microscale. In this study, coaxial electrospraying was used to investigate its feasibility for preparing nanoparticles made up of nanocrystals encapsulated within a polymer shell. Firstly, aqueous nanosuspensions of darunavir were prepared by wet media milling. Then the nanosuspension and solutions of an enteric polymer, Eudragit L100, were used as the inner/core liquid and outer/shell liquid in a coaxial electrospraying setup, respectively. As long as a sufficiently high voltage was applied, a stable Taylor cone-jet mode was obtained to produce very fine core-shell structure nanoparticles with high darunavir encapsulation efficiency of approximately 90%. The influence of the starting nanosuspension and the flow rates on the characteristics of the final electrosprayed particles was also evaluated. Using an optimized nanosuspension with reasonable size, size distribution and flow rates, the enteric coating layer reduced the percentage of DRV release in acidic medium in the in vitro dissolution test to ca. 20%. This study indicates that coaxial electrospraying is a potential and unique technique for encapsulating drug nanocrystals within a polymeric shell.



## 1. Introduction

Due to advanced technologies in high throughput screening, combinatorial chemistry and computer-aided drug design, a great number of new chemical entities with promising efficacy are being generated. However, a large proportion of these potential active pharmaceutical ingredients (APIs) are poorly water soluble [1] and such drugs are expected to have an erratic absorption profile and low and/or highly variable oral bioavailability as a result of too low solubility and dissolution velocity. Among the different formulation strategies for enhancing the solubility, dissolution rate and ultimately the bioavailability, nanosuspensions (sub-micron colloidal dispersions of pure crystalline drug particles which are stabilized by polymers/surfactants [2]) have been considered as a universal formulation approach. Compared to other commonly used strategies such as amorphous solid dispersions, inclusion complexation with cyclodextrins and derivatives or lipid-based approaches [3–7], which require the drug to have process-specific properties [8], nanosuspensions show to be applicable to all drugs, including drugs that are poorly soluble in water, organic solvents and oils [9].

However, nanosuspensions have their own drawback regarding long-term physical stability. The nanoscale size is responsible for a higher surface energy of the system. Spontaneous agglomeration and Ostwald ripening are characteristic for such nanosystems, hence adequate surface stabilization is required and can be accomplished by using formulation ingredients that favor electrostatic repulsion between charged surfaces or induce steric hindrance [10]. In addition, the extremely fast dissolution rate of nanocrystals can cause rapid or burst release of the drug which in turn can lead to toxicity and severe side effects once a nanosuspension is administered. Moreover, nanosuspensions are commonly obtained by particle size reduction of larger crystals (top-down approach), using high-pressure homogenization, microfluidization and media milling [11], and such processes often introduce mechanical or thermal stress to the crystal lattice [12]. As a consequence, the particle surface can become mechanically activated (i.e. disordering of the crystal surfaces), or local amorphous regions in the bulk of the materials can be formed [13–15], which may result in additional physical or chemical instability of the end product.

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A possible solution to overcome the problems posed by the large and highly energetic surface area of the nanoparticles is an effective encapsulation. This is also in particular interesting for drinkable solid dosage forms of nanosuspensions that are far more preferable, especially for children and elderly patients compared to common parenteral dosage forms which are intended for intravenous, subcutaneous or intramuscular administration. The dried encapsulated nanocrystals can be reconstituted into a drinkable nanosuspension once they are exposed to drinks like fruit juice, water or even to yoghurt. Depending on the properties of the encapsulating shell, nanocrystals will not dissolve in the drink, but in the stomach or at a specific site in the gastrointestinal tract for optimized drug absorption and consequently, better bioavailability.

A possible encapsulation route is the use of electrospraying that is renowned for its versatility and easy build-up/installation with low investment, which can effectively produce particles with well-controlled size, size distribution, particle shape, morphology and microstructure at the nano/microscale [16]. Many different types of drug particles, from simple solid dispersions of an amorphous drug in a polymeric matrix using a single nozzle, to more complicated core-shell microstructured particles using dual- or tri-capillary nozzles were successfully prepared by electrospraying. In this study, we investigate for the first time the feasibility of coaxial electrospraying for the preparation of nanoparticles made up of nanocrystals encapsulated within a polymer shell.

The poorly soluble drug model used in this study was darunavir (DRV), a second-generation protease inhibitor (PI) for treatment of infections with the human immunodeficiency virus (HIV). DRV nanocrystals are first prepared by wet media milling and subsequently encapsulated within Eudragit L100, an enteric polymer, using a coaxial electrospraying setup. The encapsulated DRV nanocrystal particles are one part of an 'advanced' fixed dose combination with ritonavir (RTV). The rationale to develop an 'advanced' fixed dose combination is to overcome concurrent release of both drugs from the same dosage form since both compounds influence each other's solubility and supersaturation behavior [17]. Also, more recent studies reported that in many cases where drugs are formulated together in the same dosage form, the amorphous solubility and maximum achievable supersaturation of each drug can be reduced as a result of their mutual influence, especially when they are miscible in the amorphous state [18–20]. Therefore, a system with the ability to release RTV and DRV consecutively should be developed. More specifically, such system should allow RTV to be released earlier in the stomach and be absorbed before the release of DRV from the encapsulated nanocrystals in the small intestine.

## 2. Materials and methods

### 2.1. Materials

Darunavir, in the form of darunavir ethanolate was obtained from Cilag AG (Zug, Switzerland). Poloxamer 338 and poloxamer 188 were generous gifts from Janssen Pharmaceutica (Beerse, Belgium). Tween 20 and Tween 80 were purchased from Fagron (Waregem, Belgium). Eudragit L100 was obtained from Degussa Rohm GmbH (Darmstadt, Germany). Hydroxypropyl methylcellulose 2910 5 mPa s (referred as HPMC afterwards) was provided by Colorcon (Dartford, UK). D-α-tocopheryl polyethylene glycol 1000 or 400 succinate (vitamin E TPGS 1000 or 400) were obtained from Isochem (Gennevilliers, France). Sodium lauryl sulfate (SLS) was obtained from Sigma-Aldrich (Missouri, USA). Isopropanol (IPA) and acetonitrile (HPLC grade) were obtained from VWR (Leuven, Belgium) and Acros Organic (Geel, Belgium), respectively. Other chemical reagents were of analytical grade. In all the experiments deionized water (>18 MΩ) (Maxima Ultra Pure Water, Elga Ltd, Wycombe, England) was used.

### 2.2. Methods

#### 2.2.1. Preparation of nanosuspensions by media milling

An aqueous nanosuspension of DRV was prepared by wet media milling. Several types of stabilizers such as Tween 20, Tween 80, HPMC, poloxamer 338 or 188, vitamin E TPGS 1000 or 400, and SLS were chosen for screening. The preparation of nanosuspensions involved two steps. Firstly, stabilizer solutions were prepared by dissolving the required amount of stabilizer in purified water to obtain the desired concentration (2.5% w/v). An exact amount of 1 g DRV was then dispersed in 10 ml of the stabilizer solutions to obtain a constant ratio of drug to stabilizer (4/1 w/w). After addition of a fixed amount of yttrium-stabilized zirconium oxide beads of 1 mm in diameter (Tosoh Corp., Tokyo, Japan), nanogrinding was performed at room temperature using an in-house build roller mill with ACS310 control (ABB, Helsinki, Finland). The milling process parameters were set as follows: rotation speed of 80 Hz, and milling time of 24, 48 or 72 h.

#### 2.2.2. Preparation of micro/nanoparticles of DRV nanocrystals encapsulated within an enteric polymer by coaxial electrospraying

The core-shell nano/microparticles of DRV nanocrystals were prepared by electrospraying using a two-concentric stainless steel nozzle (COAX_2DISP, Linari Engineering, Italy). The coaxial electrospray setup is illustrated in Fig. 1. Darunavir nanosuspensions were used as the inner/core liquid, whereas a solution of Eudragit L in IPA (3% w/v) was used to form the particle shell. The core and shell liquids were delivered to the nozzles using two syringe pumps (PHD 4400, Harvard Apparatus, Massachusetts, US) at different flow rates. During electrospraying, a stable cone-jet mode was obtained by applying a sufficiently high voltage (from 12 to 15 kV depending on the flow rates). Electrospraying was performed at 30 °C and 40% relative humidity in a closed climate-controlled electrospinning chamber (Electrospinning Apparatus EC-CLI, IME Technologies, the Netherlands). Each formulation (different starting nanosuspension or different ratio of core/shell flow rates) was prepared in duplicate. The distance between the tip of the nozzle and the collector was fixed at 6 cm during the experiment. Electrosprayed particles were collected in 10 ml of 0.1% (w/v) SLS solution in water (pH 4.5) using a homemade conductive container consisting of an aluminum plate at the bottom and a wall made of Geberit high density polyethylene. Air was pumped through two symmetrically arranged needles onto the surface of the SLS solution to facilitate dispersion of the electrosprayed particles. At the end of the experiment, a milky suspension was obtained and the micro/nanoparticles of DRV were collected after water removal by freeze-drying at −50 °C and 0.04 mBar for 24 h (Christ Alpha 1-2 LD plus freeze-dryer, SciQuip LTD, UK). In the end, between 80 to 100 mg of electrosprayed particles were collected and stored in room condition for characterization later.

#### 2.2.3. Encapsulation efficiency determination

The encapsulation efficiency (EE), the ratio of the mass of DRV effectively entrapped in the core of the particles to the total mass of drug available, was determined as follows: 1 ml of the final suspension collected after electrospraying was centrifuged at 14,000 rpm and 21 °C for 20 min (centrifuge 5804R, Eppendorf, Belgium). The sediment, which represented the electrosprayed core-shell DRV particles, was rinsed twice with 1 ml of 0.1 M HCl solution each time and then dissolved in 100 ml of phosphate buffer pH 6.8, whereas the supernatant and the above washing solution were mixed and then filtered through a PTFE membrane