Colloids and Surfaces B: Biointerfaces 166 (2018) 24–28

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Release kinetics and cell viability of ibuprofen nanocrystals produced by melt-emulsification

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ARTICLE INFO

Article history:
Received 25 July 2017
Received in revised form 27 February 2018
Accepted 2 March 2018
Available online 5 March 2018

Chemical compound studied in this article:
Ibuprofen (PubChem CID: 3672)

Keywords:
Nanocrystals
Poorly water soluble drugs
Release kinetics
Caco-2 cells
AlamarBlue®
Cell viability

ABSTRACT

The clinical use of poorly water-soluble drugs has become a big challenge in pharmaceutical development due to the compromised bioavailability of the drugs *in vivo*. Nanocrystals have been proposed as a formulation strategy to improve the dissolution properties of these drugs. The benefits of using nanocrystals in drug delivery, when compared to other nanoparticles, are related to their production facilities, simple structure, and suitability for a variety of administration routes. High pressure homogenization (HPH) is the most promising production process, which can be employed at low or high temperatures. Ibuprofen nanocrystals with a mean size below 175 nm, and polydispersity below 0.18, have been produced by melt-emulsification, followed by HPH. Two nanocrystal formulations, differing on the surfactant composition, have been produced, their *in vitro* ibuprofen release tested in Franz diffusion cells and adjusted to several kinetic models (zero order, first order, Higuchi, Hixson-Crowell, Korsmeyer-Peppas, Baker-Lonsdale and Weibull model). Cell viability was assessed at 3, 6 and 24 h of incubation on human epithelial colorectal cells (Caco-2) by AlamarBlue® colorimetric assay. For both formulations, Caco-2 cells viability was dependent on the drug concentration and time of exposure.

1. Introduction

Pharmaceutical nanocrystals are innovative drug carriers, produced by down-sizing technology from the microscale to nanoscale, aiming to dramatically change the physicochemical properties of compounds. Nanocrystals are drug nanosuspensions of solid particles, surface-stabilized with a surfactant layer, featuring a size in the nanometer range (i.e. mean diameter below 1 μm) [1]. In the beginning of the 90s, Elan Nanosystems (San Francisco, CA, USA) preferentially encouraged the use of nanocrystals for oral bioavailability enhancement instead of the use of microcrystals, which were suspended in water [2]. Nanosuspensions consist of the dispersion of drug nanocrystals in liquid media [3]. The dispersed solid particles are usually stabilized by surfactants or polymeric stabilizers [4]. Water, aqueous or non-aqueous solutions can be used as dispersion media [5]. Comparing to conventional dosage forms, the bioavailability of drug nanocrystals increases significantly, both by the increased surface area and by the fact that particles are made of 100% of drug [6]. This property contributes for the achievement of a high therapeutic concentration at the site of action, thus exhibiting an improved pharmacological effect [7]. In addition, due to their high loading capacity, nanocrystals are very effective in transporting drugs [5]. Nanocrystals are usually stabilized by electrostatic and/or steric stabilization by surfactants such as lecithin, alone or in combination with sodium cholate or non-ionic surfactants, e.g. Tween 80, poloxamer 188 and polyvinylpyrrolidone. These surfactants are accepted for intravenous injection, while using binary or ternary mixtures of electrostatic and steric surfactants was found to be effective for long-term stability [8,9]. For other administration routes, e.g. oral administration, several other surfactants can be also used [10].

Several methods have been described to solubilize poorly water soluble drugs, however the selection depends on the chemical

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https://doi.org/10.1016/j.colsurfb.2018.03.005
0927-7765/

properties of the drug, such as its solubility in organic media, conformation and/or molecular size [11]. Although it is possible the use of surfactants or co-solvents, these options can lead to adverse side effects and toxicity [12,13].

Ibuprofen is an acidic poorly soluble compound (pKa = 4.91), with a non-steroidal analgesic, anti-inflammatory activities [14]. Ibuprofen (IUPAC name IUPAC name: (RS)-2-[4-(2-methylpropyl) phenyl] propanoic acid) shows higher solubility in a neutral-alkaline environment [15]. **Ibuprofen has been frequently used as a model drug for the purpose of sustained/controlled release** [16]**, as it is a compound of low solubility (intrinsic solubility of approximately 21 mg/L at 25 °C)** [17], **high permeability and is considered as a class II compounds according to The Biopharmaceutical Classification System (BCS)** [18]**. As its solubility in water is very low, it shows limited oral absorption and dissolution rate**; ibuprofen has been therefore selected as model drug for BCS class II for the development and characterization of poorly-water soluble drug nanocrystals. In the present work, ibuprofen nanocrystals stabilized with hydrophilic surfactants (Tween 80 or Polyvinyl alcohol in combination with Span 80) were produced by down-sizing approach based on melt-emulsification process, and analysed in terms of drug release and *in vitro* cytotoxicity in Caco-2 cell lines.

2. Materials and methods

2.1. Materials

Polysorbate 80 (Tween 80®) was purchased from Uniqema (Everberg, Belgium). Ibuprofen was kindly donated from Medinfar (Amadora, Portugal). Polyvinylpyrrolidone (PVP) K30 was acquired from Fluka (**Buch**, Switzerland). Phosphate buffered saline (pH 7.40), sorbitan monooleate (Span 80®), and Trypsin EDTA 0.25% were purchased from Sigma-Aldrich (Steinheim, Germany). **Cellulose membrane Millipore® HA with an average pore size of 0.22 μm was purchased from Millipore (Madrid, Spain)**. RPMI 1640, BioWhittaker®, was obtained from Lonza (**Verviers**, Belgium), **Caco-2 ATCC® (HTB-37 TM) cell line from LGC Standards S.L.U. (Barcelona, Spain)**, and Fetal Bovine Serum from **Biowest (Nuaillé, France)**. Ultra-purified water was obtained from Milli® Q Plus system, home supplied.

2.2. Production of drug nanocrystals by melt emulsification

Ibuprofen nanocrystals composed of 0.25% (m/v) of drug in aqueous solution of surfactants were produced by melt-emulsification process as described by Fernandes et al. [19]. Briefly, ibuprofen was added to the aqueous solution of surfactants, Tween 80® (T80) and Polyvinylpyrrolidone (PVP) K30 or T80 and Span 80® (S80). The obtained drug suspension was heated up to 80 °C to melt the drug, and then processed by high shear homogenization (Ultra-Turrax®, T25, IKA) for 10 min, to obtain a coarse emulsion. This hot emulsion was transferred to a high pressure homogenizer (EmulsiFlex®-C3, Avestin) and homogenized at 1000 bar for 20 min in the continuous mode, operated at 80 °C. The hot emulsion was then cooled down, by placing it in an ice-bath.

2.3. Mean particle size and polydispersity

The mean particle size and polydispersity index (PI) of freshly prepared ibuprofen nanocrystals were determined by dynamic light scattering (DLS) in a DelsaNano C Submicron (Beckman Coulter Delsa, Krefeld, Germany). To avoid multiple scattering, nanocrystals were diluted in Milli Q water to an appropriate concentration prior to analysis. The reported results are the mean of triplicate runs per sample.

2.4. In vitro *ibuprofen release*

In vitro release studies of ibuprofen from nanocrystals were performed using Franz glass diffusion cells of 5 mL (PermeGear, USA), using a pre-hydrated cellulose membrane Millipore® mounted between the matched donor and receptor compartments. Ibuprofen nanocrystals were placed onto the membrane surface of the donor compartment. At determined time-intervals, samples were collected and the same volume was replaced with buffer. The ibuprofen content in collected samples was analysed spectrophotometrically at 264 nm. Phosphate-buffered saline (PBS), at pH 7.40 and maintained at 37 °C, was used as receptor medium.

2.5. In vitro *cell viability evaluation*

The human intestinal cell line (Caco-2) was purchased from ATCC® (HTB-37 TM). The human intestinal cells were cultured in Dulbecco's Modified Eagle Medium (high glucose) supplemented with penicillin 100 U/mL, streptomycin 100 μg/mL, 10% (v/v) inactivated fetal bovine serum, at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. Cell viability was assessed on human epithelial colorectal cells (Caco-2) pre-exposed to the final formulations (T80, S80, T80 + S80, PVP K30, T80 + PVP K30) and compared with non-exposed cells (control). The method for cell viability evaluation was the colorimetric assay using AlamarBlue®, in which resazurin (a blue, non-fluorescent compound), the active ingredient, enters the cells and is reduced to resorufin (a red compound that is and highly fluorescent) in metabolically active cells. Viable cells continuously convert resazurin to resorufin, increasing the overall fluorescence (or red color) of the media surrounding cells. Briefly, Caco-2 cells were seeded at the density of 0.1×10^6 cells/well, in 96-well microplates with a final volume of 200 μL. After 24 h, the medium was removed and the different formulations were added at a final volume of 200 μL. After 3, 6 and 24 h, the medium was completely removed and new medium containing 10% resazurin was added per well. Cells were further incubated during 3.5 h at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. After this incubation time, the absorbance was read using an ELISA microplate reader at 570 nm and 600 nm. The results were expressed as percentage of resazurin reduction relatively to control cells, from three independent experiments, as reported in previous works [20–23].

2.6. Statistical analysis

All the experiments were performed in triplicate, being the results expressed as **mean ± standard error of mean (SEM)** of three independent experiments. Statistical analyses were performed using two-way ANOVA, with a Dunnett's multiple comparisons test. The differences between the means were considered significant for values of $p < 0.05$. The statistical tests were applied using GraphPad Prism, version 6.00 (GraphPad Software, San Diego, CA, USA).

3. Results and discussion

Nanosuspensions of 0.25% (m/V) of ibuprofen, stabilized by a combination of 0.2% Tween 80 and 1.2% PVP K30 (Formulation F1) or 0.2% Tween 80 and 1.2% Span 80 (Formulation F2), have been produced by melt-emulsification process, according to Fernandes et al. [19]. **As described by Fernandes et al., when the percentage of Tween 80 is higher the average size is decreased, however with the increase of the PI. This result is attributed to the rearrangements of polysorbate chains on the surface of**