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Counter-ion complexes for enhanced drug loading in nanocarriers: Proof-of-concept and beyond

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1,2-Dipalmitoyl-sn-glycero-3-phosphatidic acid, sodium salt (PubChem CID: 643979)
Gentamicin (PubChem CID: 3467)
Pluronic® F68 (PubChem CID: 24751)
Poly(DL-lactide-*co*-glycolide) (PubChem CID: 71391)
sodium dodecyl sulfate (PubChem CID: 3423265)
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ABSTRACT

Enhanced drug loading is an important prerequisite of nanomedicines, to reach administration dose while reducing the amount of excipient. Considering biocompatible and biodegradable polymers such as PLGA, pH dependent solubility characteristics along with limited organic solvent solubility of the drug hampers nanoparticle (NP) preparation. To improve loading of such molecules, a method based on using counter ions for complex formation is proposed. Formed complex alters the intrinsic solubility of active substance via electrostatic interaction without chemical modification. A proof-of-concept study was conducted with sodium dodecyl sulfate as counter-ion to fluoroquinolone antibiotic ciprofloxacin. Complex formation resulted in suppressed pH dependent solubility over pH 1.2–9.0 and an additional −80 fold increase in organic solubility was achieved. In consequence, NPs prepared by microjet reactor technology have shown enhanced drug loading efficiencies (−78%) and drug loading of 14%. Moreover, the counter-ion concept was also demonstrated with another class of antibiotics, water soluble aminoglycosides gentamycin and tobramycin. In addition, the counter ion was substituted by degradable excipients such as phosphatidic acid derivatives. Successful implementation has proven the counter-ion concept to be a platform concept that can be successfully implemented for a variety of active substances and counter-ions to enhance drug loading in nanocarriers.

1. Introduction

Biocompatible and biodegradable polymers are popular carriers for nanoparticulate drug delivery purposes, since they offer degradation into non-toxic components and elimination via metabolic pathway. Poly(lactic-*co*-glycolic) acid (PLGA) applications have attracted attention by first FDA approved products, such as Sandostatin® LAR from Novartis, being successfully used since decades for drug delivery (Barichello et al., 1999; Danhier et al., 2012; Govender et al., 1999; Makadia and Siegel, 2011; Sah et al., 2013). In addition to its low toxicity and biocompatible character, it also offers predictable biodegradation kinetics (Makadia and Siegel, 2011). The ratio of lactide to glycolide ratio or even molecular weight can successfully be employed for adjusting the release profile (Park, 1995). Furthermore, it is well known that the physicochemical properties of the encapsulated drug substance and the drug load also contribute to the release rate (Siegel et al., 2006). Many different nanoparticle (NP) preparation techniques (Danhier et al., 2012; Sah et al., 2013) have been employed for encapsulation of drug substances in PLGA for different administration routes (Dinarvand et al., 2011; Martin-Banderas et al., 2013; Tosi et al., 2013; Ungaro et al., 2012). Typically

Abbreviations: ACN, Acetonitrile; DMSO, Dimethyl sulfoxide; DPPA, 1,2-Dipalmitoyl-sn-glycero-3-phosphatidic acid, sodium salt; DSC, Differential scanning calorimetry; EE, Encapsulation efficiency; FTIR, Fourier transform infrared spectroscopy; HPLC, High pressure liquid chromatography; MJR, Microjet reactor; NP, Nanoparticle; PDI, Polydispersity index; PLGA, Poly(lactic-*co*-glycolic) acid; SDS, sodium dodecyl sulfate; XRD, X-Ray diffractometry.

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solvent evaporation and double emulsion techniques are used for PLGA nanoparticle preparation; however success of both techniques is dictated by water solubility of the drug substances. Solvent evaporation technique is suitable for hydrophobic drug encapsulations, whereas w/o/w double emulsion method serves for encapsulation of hydrophilic drugs (Cohen-Sela et al., 2009) suffering from low drug loading efficiencies. Nanoprecipitation, or the so-called solvent displacement method, was shown to be mainly efficient for hydrophobic drug applications (Barichello et al., 1999). Despite the variety of techniques that are applicable for PLGA NP preparations, all methods come along with some drawbacks which include but not limited to the use of unfavorable solvents, the necessity of high cost equipment, lack of scale up possibility and most important of all low encapsulation/drug loading efficiencies.

Encapsulation of active pharmaceutical ingredients showing pH dependent solubility characteristics along with limited organic solvent solubility is a challenge for all preparation techniques. Fluoroquinolone antibiotics, such as ciprofloxacin (Sweetman, 2009), can be listed among such drug substances. The drug can neither be dissolved in a common organic solvent due to low solubility nor in pH-adjusted aqueous solvents due to the *in vitro* degradation of the polymeric carrier by acidic and alkaline environment. Especially degradation of PLGA is well-known to be accelerated under such conditions (Shive and Anderson, 1997; Zolnik and Burgess, 2007). As a consequence, the reported drug loading of ciprofloxacin nanoparticles does not exceed 5% (Dillen et al., 2004; Isa et al., 2016; Jeong et al., 1998; Nardecchia et al., 2012).

Since the loading of these active substances into NPs, such as PLGA, turned out to be another hurdle due to low drug loading efficiencies achieved, this work aimed to improve drug loading efficiencies of NPs by utilizing the counter-ion method. This method relies on the modification of the solubility of the drug by complex formation based on a counter-charged ionic stabilizer. Thus, suppressing the pH-dependent solubility will enhance the loading. This novel approach is particularly applicable for ionizable drug substances with pH dependent solubility characteristics where use of organic solvents cannot be a choice for encapsulation purposes.

The proof-of-concept study for complex formation, characterization and its increased drug loading efficiency was conducted by using sodium dodecyl sulfate (SDS) as counter-ion for the fluoroquinolone antibiotic ciprofloxacin. Complex formation was proposed to occur as a result of the interaction between the secondary amine of piperazinyl group on ciprofloxacin C7 position (Fig. 1-A) and the sulfonyl head group of SDS shown in Fig. 1-C. The zwitterionic nature of ciprofloxacin shown in Fig. 1-B, is responsible for the solubility (Yu et al., 1994). A high complex yield should be achievable by using a positively charged ciprofloxacin molecule (piperazinyl group) increasing the interaction with the sulfonyl group. Proposed complex formation mechanism is provided in Fig. 1-D.

To have full control over the nanoparticle preparation MicroJet Reactor (MJR) technology was employed. MJR is based on a continuous mixing reaction due to impinging jets and the set-up enables control over the whole process parameters. Turbulent-like mixing in micron-volume chamber caused by impinging jets provides high NP quality, fine-tunable NP sizes and narrow particle size distribution (Wacker, 2013; Zhao et al., 2011).

2. Materials and methods

Ciprofloxacin base, tobramycin sulfate, Pluronic® F-68, sodium dodecyl sulfate (SDS) were purchased from Sigma Aldrich (Munich, Germany). Gentamicin sulfate was a generous gift from Dolder AG. PLGA (Expansorb ® 10P017, with a lactic to glycolic acid ratio of 50:50) was purchased from PCAS (Longjumeau Cedex, France). 1,2-Dipalmitoyl-*sn*-glycero-3-phosphatidic acid, sodium salt (DPPA) was obtained from NOF Corporation (Grobbendonk, Belgium). All solvents used were of analytical grade and were supplied by VWR, Darmstadt, Germany.

2.1. Ciprofloxacin-SDS complex preparation

Equimoles of ciprofloxacin and SDS were dissolved in 0.1 M HCl solutions of equal volumes. Ciprofloxacin solution was added to the SDS solution under continuous mixing. Precipitated complex is vacuum-filtered, washed twice with distilled water and dried at 30 °C under vacuum.

2.2. Ciprofloxacin-DPPA complex preparation

Equimoles of ciprofloxacin dissolved in 0.1 M HCl and DPPA dissolved in chloroform:methanol:pH 6.0 phosphate solution (1:1:0.1 v/v/v) were employed for complex preparation. Ciprofloxacin solution was added to the DPPA solution under continuous mixing. Precipitated complex is vacuum-filtered, washed twice with distilled water and dried at 30 °C under vacuum.

2.3. Nanoparticle preparation

For the NP preparations, 1% (w/v%) Ciprofloxacin-SDS complex dissolved in 0.5% (w/v%) PLGA in dimethyl sulfoxide (DMSO) solution (solvent system) and 0.25% Pluronic F68 in water solution (non-solvent system) were delivered to the MJR at 1:10 flow rate ratio with 180° angle by using Smartline S100 pumps (Knauer, Munich, Germany). Solvent delivering capillaries and the MJR were immersed into a water bath in order to control the system temperature. Experimental set-up is schematically depicted in Fig. 2. Purification of the nanoparticles was achieved with D-Tube™ Dialyzer Mega (VWR, Darmstadt, Germany) with a MWCO 6–8 kDa.

2.4. Characterization of complex and nanoparticles

2.4.1. Ciprofloxacin HPLC analysis

Chromatographic analyses were carried out using a Waters Symmetry C18 HPLC column (75 × 4.6 mm, 3.5 μm for assay analysis and 250 × 4.6 mm, 5 μm for impurity analysis) thermostated at 30 °C and isocratic elution mode using 25 mM phosphoric acid pH adjusted with triethylamine to 3.00 ± 0.05: acetonitrile (ACN) (87:13 v/v;) at a flow rate of 1.5 mL min^{-1}. Ciprofloxacin was detected at 278 nm.

2.4.2. Fourier-transform infrared (FTIR) spectroscopy

Infrared spectra were recorded using a FTIR 400 spectroscope from Perkin Elmer (Rodgau, Germany) between 4000 cm^{-1} and 550 cm^{-1}. Data analyses were performed with software PerkinElmer Spectrum Ver. 10.02.00.

2.4.3. Thermal analysis

Differential scanning calorimetry (DSC) was carried out with a Q100 DSC from TA Instruments (Eschborn, Germany) using hermetically sealed aluminum pans containing ca. 4 mg of sample. Analyses were performed over a temperature range of 20–300 °C following five minutes equilibration at 20 °C with a heating rate of 5 °C min^{-1}, under dry nitrogen purged at 50 mL min^{-1} and 100 mL min^{-1} through cooling unit. Data analyses were completed using Universal Analysis 2000 (Version 4.3A) software provided by TA Instruments.