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Influence of salt type and ionic strength on self-assembly of dextran sulfate-ciprofloxacin nanoplexes

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ABSTRACT

We evaluated an analytical setup to identify optimal preparation conditions for nanoplex formation of small molecule drugs and polyelectrolytes using ciprofloxacin (CIP) and dextran sulfate (DS) as model compounds. The suitability of isothermal titration calorimetry (ITC) as a screening tool for rational formulation optimization was assessed. Besides ITC, static and dynamic light scattering, zeta potential measurements and scanning electron microscopy were applied to analyze the influence of different salt types and ionic strengths on CIP/DS nanoplex formation. The addition of low amounts of salt, especially 0.1 M NaCl, improved the formation of CIP/DS nanoplexes. The presence of low amounts of salt led to smaller and more numerous particles of higher uniformity but had no influence on the release of CIP from nanoplexes. Furthermore, the molar range, within which efficient complexation was achieved, was broader in the presence of 0.1 M NaCl than in the absence of salt with overall comparable complexation efficiency. Importantly, binding affinity correlated with particle shape and morphology, potentially enabling optimization of critical quality attributes based on ITC data. Altogether, ITC along with supplemental methods is a versatile screening tool for the evaluation of nanoplex formulation conditions regarding mixing ratio, salt type and ionic strength.

1. Introduction

Ciprofloxacin (CIP) is one of the few potent antibiotics against *Pseudomonas aeruginosa*, a bacterial pathogen common in severe respiratory diseases like chronic obstructive pulmonary disease (COPD) (Blondeau, 1999; Cheow and Hadinoto, 2012a; Zhanel et al., 2002). A major obstacle to efficient treatment of *P. aeruginosa* infections is its ability to form biofilms in which bacteria are protected from defensive attacks by the immune system as well as xenobiotics. Moreover, biofilm colonies are entrapped in the pulmonal mucus layer, which most free drugs cannot penetrate readily due to interactions with anionic and hydrophobic mucus components (Cheow and Hadinoto, 2012b; Ensign et al., 2012). Another major drawback in the treatment of lung infections is ciprofloxacin's poor solubility in aqueous media at physiological conditions (Breda et al., 2009; Cheow and Hadinoto, 2012c). Hence, the main therapeutic challenge is to reach sufficient CIP concentration at the site of action.

An approach to overcome these problems is encapsulation of the antibiotic into nanoparticles enabling higher local concentrations of CIP and circumventing solubility issues (Ramírez-Rigo et al., 2014). Further advantages of this drug delivery system could be improved mucus penetration to the site of action as well as slower and less effective clearance from the lung by phagocytosis through alveolar macrophages due to its small size (Cheow and Hadinoto, 2012b; Rogueda and Traini, 2007). Enhanced delivery to the site of action increases the therapeutic efficacy and is even considered to reduce occurrence of bacterial resistance against antibiotics (Cheow and Hadinoto, 2012b; Hadinoto et al., 2013). However, common nano-carriers often suffer from low drug loading, limiting their use for pulmonary delivery of antibiotics

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(Cheow and Hadinoto, 2012d). In contrast, nanoparticle complexes (nanoplexes) prepared by self-assembly of a drug and an oppositely charged polyelectrolyte allow higher drug loading (Cheow and Hadinoto, 2012d; Meng et al., 2012). Self-assembly of complexes has already been used in diverse pharmaceutical applications such as cancer therapy and to improve bioavailability of drugs with adverse biopharmaceutical properties (Al-Jamal et al., 2013; Ceschan et al., 2014; Meng et al., 2012). Polymer-drug complexes may also increase the solubility of poorly water-soluble drugs and stabilize the drug in the amorphous state (Baena et al., 2011; Cheow and Hadinoto, 2012c; Dai et al., 2007). Since CIP is well soluble at a pH of about 6 as a cation, it can be used herein for complex formation (Breda et al., 2009; Cheow and Hadinoto, 2012c). Dextran sulfate (DS), a natural biodegradable polyanion, readily forms complexes by ionic interactions when mixed with CIP at low pH (Liu et al., 2008). Additionally, hydrophobic interactions of the drug molecules presumably contribute to nanoplex formation and stabilization (Cheow and Hadinoto, 2012c). The presence of salt is thought to be a key parameter (Dautzenberg, 1997), as it may increase flexibility of the polyanion DS through reduction of intramolecular repulsion, in turn increasing complexation efficiency (Bertin, 2014; Cheow and Hadinoto, 2012c). Consequently, the type of cation, its valency and ionic strength were varied during formulation optimization in this study to analyze their influence on nanoplex formation.

The overall aim of this study was the evaluation of an analytical setup to identify optimal preparation conditions for nanoplexes using CIP and DS as model compounds. Isothermal titration calorimetry (ITC) is a convenient method for the investigation of interactions during complex formation (Priftis et al., 2012; Tian et al., 2007) and may serve as a tool for rational formulation optimization (Santos et al., 2007). Furthermore, complex stability in the presence of salt (Tian et al., 2007) and the origin of polyelectrolyte interactions may be analyzed by ITC (Feng et al., 2008). However, if calorimetric methods are applied to complex systems often complicated thermograms are obtained, which are difficult to interpret in isolation. Therefore, ITC data was supplemented by additional analytical methods such as static and dynamic light scattering (SLS and DLS), zeta potential measurements and scanning electron microscopy (SEM) helping to clarify the cause and the way of interaction of molecules (Bouchemal, 2008; Germershaus et al., 2014).

2. Materials and methods

2.1. Materials

Sodium dextran sulfate (DS) with an average molecular weight of 5 kDa was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Ciprofloxacin (CIP) (≥98% (HPLC)) was obtained from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). All other chemicals were at least of analytical grade. Purified water was used in all experiments (Milli-Q Synthesis Ultrapure Water Purification System, EMD Millipore Corporation, Billerica, USA).

2.2. Preparation of polyelectrolyte-drug nanoplexes

The nanoplexes were prepared as described before (Cheow and Hadinoto, 2012c) with slight modifications. CIP was dissolved in a 2% (V/V) aqueous acetic acid solution and subsequently diluted tenfold with water to a final concentration of 18.29 μM. The CIP solution was added to an aqueous DS solution of 0.1 μM at varying molar ratios of CIP to DS in absence or presence of salt. To identify optimal conditions for complex formation, the salt type and ionic strength were varied. Hence, nanoplexes were prepared in absence (0 M) and presence of NaCl, KCl or $CaCl_2$ each at an ionic strength of 0.1, 0.3 and 1 M. Immediately upon addition of CIP to DS, the solution turned cloudy and/or formed white precipitates depending on the amount of CIP used. Therefore, the prepared nanoplex suspensions were centrifuged at 13,000 × *g* and the pellet was resuspended in water three times to remove non-complexed CIP, DS and salt.

2.3. Characterization of polyelectrolyte-drug nanoplexes

2.3.1. Determination of CIP complexation efficiency (CE)

The optimal CIP/DS molar ratio for nanoplex preparation at the different ionic strengths was evaluated with regards to CIP complexation efficiency (CE) by addition of increasing volumes of CIP (18.29 μM) to 2 mL of aqueous DS solution (0.1 μM) in presence of NaCl at 0, 0.1, 0.3 and 1 M ionic strength. CE was also determined in the presence of different salt types and ionic strengths. CE was calculated based on the amount of non-complexed CIP after preparation of the CIP/DS nanoplexes and the amount of CIP initially added. The amount of non-complexed CIP was determined after the first centrifugation step by UV absorption measurement of the supernatant at λ = 277 nm (Genesys 10 s UV–vis spectrophotometer, Thermo Fisher Scientific Corporation, Waltham, USA). The amount of CIP complexed within the nanoplexes represented the absolute CE whereas the relative CE was calculated by dividing the amount of complexed CIP by the total amount of CIP initially used for preparation.

2.3.2. Static light scattering (SLS)

The time-averaged intensity of scattered light was used to analyze the extent of nanoplex formation depending on CIP/DS mixing ratio, salt type and ionic strength. Dependence of scattering intensity on mixing ratio during the actual nanoplex formation process was analyzed in 0.1 M NaCl prior to centrifugation. In addition, SLS intensity was determined at a fixed CIP/DS molar ratio of 31 depending on ionic strength and salt type after washing of the nanoplexes. Light scattering was measured using a LS 50B luminescence spectrometer (PerkinElmer, Waltham, USA) at a fixed angle of 90° for 60 s using a data interval of 1.2 s, an integration time of 0.1 s at λ = 638 nm and slit width of 2.5 nm.

2.3.3. Dynamic light scattering (DLS)

The particle size distributions of the CIP/DS nanoplexes were measured using a Delsa Nano HC Particle Analyzer (Beckman Coulter, Inc., Fullerton, CA, USA) at 25 °C. To prevent disturbance from larger agglomerates, the samples were left to settle down overnight before analyzing the supernatant. Each run consisted of three individual measurements comprising 70 accumulations each, and size distribution by intensity, average hydrodynamic diameter and polydispersity index (PdI) were determined.

2.3.4. Electrophoretic light scattering (ELS)

Zeta potential measurements were performed by ELS using a Delsa Nano HC Particle Analyzer (Beckman Coulter, Inc., Fullerton, CA, USA) using laser light at λ = 658 nm and a scattering angle of 15° at a temperature of 25 °C. Each measurement consisted of 10 accumulations and 3 repetitions. The applied voltage was set to 60 V. System performance was checked using polystyrene latex particles (Otsuka Electronics Co., Ltd., Osaka, Japan, P/N A50695).

2.3.5. Scanning electron microscopy (SEM)

For SEM, nanoplex suspensions prepared using different salt types and ionic strengths were lyophilized (primary drying: −10 °C, 0.16 mbar; secondary drying: 5 °C and later 30 °C, 0.06 mbar) in a laboratory freeze-dryer alpha 1-4 (Martin Christ GmbH, Osterode, Germany) without addition of additional excipients. Subsequently, samples were sputter-coated with