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Assessing the drug release from nanoparticles: Overcoming the shortcomings of dialysis by using novel optical techniques and a mathematical model

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ABSTRACT

The aim of the present investigation was to develop a reliable method which can be applied to the measurement of *in vitro* drug release from nanocarriers. Since the limited membrane transport is one major obstacle to the assessment of drug release with dialysis techniques, the determination of this parameter was our objective. Therefore, a novel drug release automatic monitoring system (*DREAMS*) was designed to conduct continuous measurements during the dialysis process. Moreover, a mathematical model was used for evaluation of the experimental data. This combination of mathematical and analytical tools enabled the quantification of the total amount of free drug in the system. Eudragit® RS 100 nanoparticles loaded with the model compound 5,10,15,20-tetrakis(m-hydroxypheny)chlorin (*m*THPC) were investigated and the drug release was continuously monitored by using a fluorescence spectrometer that is part of the setup. Free drug and drug-loaded nanoparticles were tested to discriminate between the two formulations. In addition, two types of membranes composed of different materials were evaluated and the kinetics of membrane transport was determined. The data obtained from the apparatus were further treated by a mathematical model, which yielded distinguishable release profiles between samples of different compositions. The method offers a promising option for release testing of nanoparticles.

1. Introduction

The past two decades have seen a rapid development of nanotechnology being adopted in pharmaceutical science and formulation development (Zhao et al., 2011). Nanocarriers hold great promise with regards to poorly soluble compounds (Dai et al., 2007) and drug targeting applications (Kumari and Yadav, 2010; Faraji, 2009). After encapsulation of active pharmaceutical ingredient (API) into nanocarriers, the drug release is controlled by the delivery system and determines bioaviability of the drug at the site of action (Barzegar-Jalali et al., 2008). Since a multitude of parameters affect this process, the mechanisms of drug release from polymeric carriers are still poorly understood. For microparticles, mathematical models have been set up, that calculate the drug release as a result of diffusion and erosion processes (Siepmann and Siepmann, 2008). For other polymers, the drug–polymer interactions or the swelling of the matrix material play a crucial role for drug release. Drug release testing is one important tool in quality control and pharmaceutical formulation development. On one hand, these techniques rapidly provide information about the performance of dosage forms and reproducibility of the production processes. On the other hand, physiology-based drug release models are utilised for the prediction of the *in vivo* performance and allow an optimised formulation design based on *in vitro* data (Jünemann and Dressman, 2012). For nanoencapsulated drugs, the separation procedures for dissolution tests are challenging due to the extraordinarily small size of the drug vehicles (Wacker, 2013).

A variety of methods have been used to assess the release rate of drug from nanoparticles (NPs), including the sample-and-separate method (Valo et al., 2013; Corrigan and Li, 2009; Matsumoto et al., 1999). Though it is a simple and direct method, it suffers from a number of drawbacks which cause erroneous results, e.g. a premature release caused by the centrifugation which was usually used to separate the NPs and free API may occur (Chidambaram and Burgess, 1999; Bhardwaj and Burgess, 2010) or the sample taken for measuring may still undergo release during the run time of the

Abbreviations: DREAMS, drug release automatic monitoring system; RC, regenerated cellulose; CE, cellulose easter.

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separation process (McCarron et al., 2000). Furthermore, depending on the properties of the drug, special release medium with surfactant addition may be required. Since this may be problematic for high pressure liquid chromatography (HPLC) or UV-visible spectrometer, sample pre-treatment such as solid-phase extraction is necessary in this case (Kamberi and Tran, 2012). Coupled with HPLC or mass spectrometry, solid-phase extraction assures sensitive analysis of the released drug (Monteiro et al., 2005), especially for samples that are obtained from dissolution media containing proteins or serum. After all, when the drug formulations endure a few days or even weeks of release, tedious and repetitive measurements during the long-lasting process are inevitable.

Alternatively, dialysis membranes are utilised to separate NPs from the released drug (Chidambaram and Burgess, 1999; Nastruzzi et al., 1994; Essa et al., 2011; Xu et al., 2012). A variety of techniques and setups are based on this concept, such as the rotating dialysis cell (Schultz et al., 1997; Larsen et al., 2008; Parshad et al., 2003), Franz diffusion cells (specially for skin permeability tests) (McCarron et al., 2000; Herrera et al., 2012; Sonavane et al., 2008), the dialysis bag method (Rawat and Burgess, 2011; Saarinen-Savolainen et al., 1997) and reverse dialysis (Chidambaram and Burgess, 1999; Xu et al., 2012). Recent developments in this area include modified versions of apparatus I and IV listed in the United States Pharmacopeia (USP). For microparticles and liposomes, a dissolution adapter for USP apparatus IV, the flow-through cell, was developed by Bhardwaj and co-workers in 2010 (Bhardwaj and Burgess, 2010) by mounting a small dialysis chamber into the dissolution tester. Similarly to the dialysis bag method, the hydrodynamic situation inside the chamber is limiting the dissolution properties.

Typically, when the dialysis method is applied for dissolution testing, the concentration profiles in the acceptor phase are directly employed to estimate the release kinetics of the nanospheres (Van Eerdenbrugh et al., 2011), considering that a linear gradient within the membrane under quasi-steady state adjusts instantaneously to the acceptor phase if the system is well stirred and a thin membrane is adopted (Pedersen et al., 2005). In fact, the rate-limiting properties of the membrane, especially for the membrane with a relatively small molecular weight cut-off (MWCO), are one major obstacle to the quantification of drug release by using dialysis methods (Moreno-Bautista and Tam, 2011). More often membrane permeability was limiting the drug release other than the dosage form (Zambito et al., 2012; Jünemann and Dressman, 2012; Heng et al., 2008; Washington, 1989). Despite of this, Fugit and Anderson (2015) demonstrated that a nearly identical release rate constant was observed in their study on drug release from liposomal formulations using dialysis method.

Furthermore, *in-situ* methods such as drug-selective electrodes (Moreno-Bautista and Tam, 2011) and fibre optic (Li et al., 2000; Zolnik et al., 2005; Lu et al., 2003) have been reported to be successful. However, a drug-selective electrode is conditioned for electroactive model drugs only (Elzoghby et al., 2012) and for dissolution testing of different APIs, a new set of sensors is required (Peeters et al., 2008). Considering the fibre optic analytics, though the sample separation is not required for it, the drug carrier debris accumulating on the probe mirrors often results in inaccuracy of the sensor (Johansson et al., 2002). Additionally, fibre optics is easily disturbed by light scattering effect when small particles such as NPs are present (Van Eerdenbrugh et al., 2011; Jünemann and Dressman, 2012; Peeters et al., 2008).

In the present study, the dialysis method was applied to separate the API released from the NPs. The obstacle of a membrane as the diffusion barrier has been overcome by utilising a mathematical model in combination with a continuous measurement of drug release by using fluorescence spectroscopy. By this, the repeated sampling and measuring procedures have been avoided. The continuous real-time data measured in the acceptor compartment can be processed by a mathematical model and consequently yield the results of the whole amount of free API in the system at each time point. Experiments on two types of membranes were carried out to validate the methods applied.

Polymeric carriers loaded with 5,10,15,20-tetrakis(m-hydroxypheny)chlorin (*m*THPC) as API were selected for dissolution tests. *m*THPC, also known as temoporfin, has been suggested to be one of the most promising drug molecules applied in photodynamic therapy (Oleinick and Evans, 1998). The nano formulations of *m*THPC have been suggested to reduce the side effect and to target the API to a specific site of action (Wacker et al., 2010). Besides, a successful formulation to prepare *m*THPC-loaded with Eudragit® RS 100 nanoparticles has been reported earlier in our work (Beyer et al., 2014). Eudragit® RS 100 is a co-polymer of poly(ethylacrylate, methyl-methacrylate and chlorotrimethylammonioethyl methacrylate) containing quaternary ammonium groups (Bodmeier and Chen, 1989). Being commonly used as tablet coating, this polymer exhibits sustained release properties and a pronounced swellability. In this context, numerous authors (Adibkia et al., 2011; Pignatello et al., 2002) aimed to generate nanoparticles with controlled release properties by using Eudragit® RS 100. However, the performed release experiments often had difficulties to discriminate between the API bound to the NP surface and the API being truly encapsulated into the nanocarriers. Intending to overcome this shortcoming, in the present study the often applied step of purification by multiple cycles of centrifugation and decantation was used for the evaluation of excipient-drug interaction by performing release experiments on various compositions and mixtures of those NP suspensions. The experimental data monitored by a fluorescence spectrometer of the setup were processed by a mathematical model to obtain the corrected release profiles. Furthermore, variable compositions of samples were tested to gain a deeper insight into the release kinetics of nanocarrier samples and the effect of the purification procedure.

2. Material and methods

2.1. Chemicals and reagents

Polysorbate 20 (lot 9005-65-6) was purchased from Fluka Chemika (Buchs, Germany). Eudragit® RS 100 (lot E100108020) was kindly provided by Evonik Röhm GmbH (Darmstadt, Germany). *m*THPC (lot 081112) was kindly provided by biolitec research GmbH (Jena, Germany). Trifluoroacetic acid (purity>99.8%, lot ZA 3765762) and lithium chloride (lot B0726979 202) were purchased from Merck KGaA (Darmstadt, Germany). Methyl-β-cyclodextrin (lot STBC7393V) was purchased from Sigma-Aldrich Chemie GmbH (Munich, Germany). A cellulose ester (CE) membrane (Spectra/Por® Biotech CE Tubing, MWCO: 50 kDa, thickness: 80 μm, release area: 6.1 cm^2) and a regenerated cellulose (RC) membrane (Spectra/Por® 6, MWCO: 50 kDa, thickness: 60 μm, release area: 8.0 cm^2) were purchased from Serva Electrophoresis GmbH (Heidelberg, Germany).

2.2. Preparation and characterisation of nanoparticles

Polymeric NPs loaded with *m*THPC were prepared according to the protocol published by Beyer et al. (2014). Nanoparticles were generated by dissolving 150 mg Eudragit® RS 100 and 7.5 mg temoporfin in 1 mL ethanol 96% [v/v]. Nanoprecipitation was induced by dropwise addition of polysorbate 20 in an aqueous solution with a concentration of 0.01% [w/v] and a speed of 12 mL min^{-1}.