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General method for the quantification of drug loading and release kinetics of nanocarriers

Richard Schwarzl [a,*], Fang Du [b], Rainer Haag [b], Roland R. Netz [a]

[a] Freie Universität Berlin, Fachbereich Physik, 14195 Berlin, Germany
[b] Freie Universität Berlin, Institut für Chemie und Biochemie, 14195 Berlin, Germany

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ABSTRACT

Macromolecular nanostructures that are used as drug carriers are characterized by their loading and release kinetics. Release studies commonly employ the dialysis method, in which a cellulose membrane separates the solution of released drug from the nanocarrier solution. We demonstrate that it is necessary to take the effect of the dialysis membrane on the release kinetics into account. Using a two-step approach, consisting of a calibration experiment of drug diffusion through the dialysis membrane in the absence of nanocarriers, and an experiment in the presence of nanocarriers, we are able to determine all kinetic rates and in particular to disentangle kinetic dialysis membrane properties from kinetic nanocarrier properties. We apply our general approach to experimental dexamethasone release data from core-multishell nanocarriers and demonstrate that our method yields a consistent description of the nanocarrier release kinetics.

1. Introduction

Polymer micelles with fine-tuned core-shell structures gained attention in recent years for their function as drug delivery systems in tumor therapy and topical treatment [1–5]. They enable the transport of hydrophobic drugs in water by encapsulation in hydrophobic cores while the hydrophilic shells keep the system dispersed in water and protect the drug from the immune system [6–9]. In order to maximize therapeutic efficiency, the release kinetics of the drug from the nanocarriers has to be optimized and is an important factor when designing such nanocarriers [10]. Dendritic core-multishell (CMS) nanocarriers consist of a hyperbranched polar core, an inner shell and an outer shell. These nanocarriers are able to transport guest molecules in polar as well as nonpolar solvents and exhibit good tumor targeting as well as efficient topical drug delivery [11–13]. A recent study has shown that these CMS carriers can enhance penetration into the skin [12]. As a transport mechanism this may allow for drugs to be released into deeper skin layers [14,15]. In order for the drug transport via nanocarriers to be efficient, the release time of a specific drug from the carrier must be similar to the penetration time of the nanocarrier itself. Thus, reliable determination of drug-nanocarrier release kinetics is of great importance.

In this work we present a general approach to quantify the release kinetics of a drug from nanocarriers and apply our method to experimental data for dexamethasone released from CMS nanocarriers. Drug release from nanocarriers is in the standard setup studied by drug diffusion through a dialysis membrane, which poses the problem of disentangling the rate of drug release from the nanocarrier and the rate of drug diffusion through the dialysis membrane [16–19]. We solve this problem by a two-step procedure. In the first step, we perform a calibration experiment testing the release of dexamethasone from the aqueous solution inside of a dialysis bag to an outside phosphate-buffered saline solution in the absence of nanocarriers. Here we determine the drug diffusion rate through the dialysis membrane. In the second step, we measure the release kinetics from nanocarriers, and use in the analysis of the data the membrane diffusion rate from the calibration experiment. For the specific dialysis membrane and CMS nanocarrier used in our experiments, we find for the membrane release rate of dexamethasone $r_{MI} = 0.052\ \text{min}^{-1}$ and for the CMS nanocarrier release rate of dexamethasone $r_{NI} = 0.015\ \text{min}^{-1}$. We conclude that in our specific system, the

* Corresponding author.
E-mail address: rschwarz@zedat.fu-berlin.de (R. Schwarzl).

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release rate from nanocarriers is only roughly three times smaller than the membrane release rate and thus both rates are of the same order. In the following section we will elaborate on the structure of the CMS nanocarriers as well as on the experimental details and the theoretical model.

2. Materials and methods

2.1. CMS nanocarrier preparation

The CMS nanocarriers we used in our experiments consist of a polar core surrounded by a polycarprolactone (PCL) inner shell and a monomethoxypoly(ethylene glycol) (mPEG) outer shell. The molecular weight of a single nanocarrier is 163.8 kDa. Details about synthesis and structure of the nanocarriers have been published previously [20].

2.2. Dexamethasone loading and release studies

12 mg of dexamethasone film was incubated with 6 ml of CMS aqueous solution with a nanocarrier concentration of $c_{CMS} = 15$ mg/ml. After 30 s ultrasonication, 3 ml of solution was withdrawn and filtered immediately with a regenerated cellulose (RC) membrane filter (0.45 µm pore size). After 3 min of additional ultrasonication, the remaining 3 ml were filtered. At the start of the release experiment, 0.6 ml of each solution was placed in a dialysis bag with a molecular weight cut-off (MWCO) of 3.5 kDa. Since dexamethasone has a molecular weight of 392.46 Da [21], the dialysis membrane holds back the nanocarriers but lets the drug quite easily pass with a rate that we determine in the calibration experiment. The respective dialysis bag was then immersed in 30 ml of phosphate-buffered saline (PBS). Drug release studies were performed in a bioshaker with 100 rpm at 37 °C. Since the dexamethasone solution is thoroughly filtered before usage, we can safely assume that it corresponds to an equilibrium saturated solution and neglect the presence of aggregates in our analysis. Samples of 0.1 ml volume were periodically removed from the outside solution and the same volume of pure PBS was added. The amount of released dexamethasone was determined by high-performance liquid chromatography (HPLC). The HPLC measurements were carried out on a Knauer Smartline-HPLC system, equipped with a reversed-phase (RP) -C18 column (250 mm × 4 mm,5 µm particle size) and an UV/VIS detector at $\lambda = 245$ nm. The mobile phase was an acetonitril-water (40:60, v/v) mixture with a flow-rate of 1 ml/ min. For comparison, the release of free dexamethasone without CMS nanocarriers was conducted in the same condition. Each experiment was repeated three times. The release data are given by the mean of the three data sets. Errors have been calculated according to

$$\Delta\Phi_O = \sqrt{\frac{1}{n-1}\sum_{j=1}^{n}\left(\Phi_{Oj} - \frac{1}{n}\sum_{i=1}^{n}\Phi_{Oi}\right)^2}. \tag{1}$$

2.3. First order rate equations

We describe the release of dexamethasone using a four-state model schematically illustrated in Fig. 1. We denote the fractions of dexamethasone inside the nanocarriers, the inner solution volume, the dialysis membrane and the outer solution volume as Φ_N, Φ_I, Φ_M and Φ_O, respectively. The release kinetics of dexamethasone from the CMS nanocarriers can be described by a system of coupled rate equations:

$$\frac{d\Phi_N}{dt} \equiv \dot{\Phi}_N(t) = -r_{NI}\Phi_N(t) + r_{IN}\Phi_I(t), \tag{2}$$

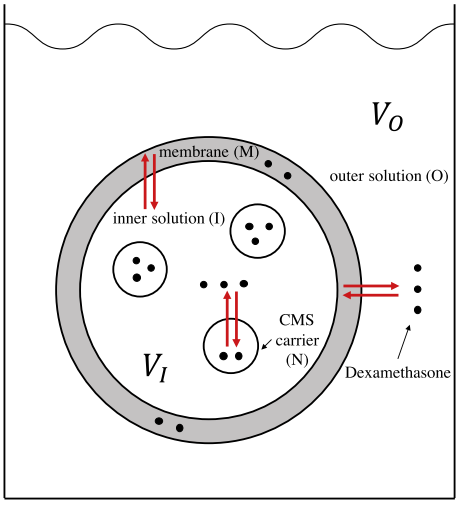

Fig. 1. Schematic illustration of the experimental setup: The dialysis bag is immersed in the outer solution with a volume V_O. The black dots schematically denote dexamethasone molecules, that are either inside the nanocarriers (N), the inner solution (I) with a volume V_I, the membrane (M) or the outer solution (O). During the experiment dexamethasone first diffuses from the nanoparticle interior to the inner solution, then into the membrane and finally into the outer solution. The dialysis membrane is impenetrable for the nanocarriers, leading to the desired separation of the released dexamethasone from the nanocarriers.

$$\frac{d\Phi_I}{dt} \equiv \dot{\Phi}_I(t) = -(r_{IN} + r_{IM})\Phi_I(t) + r_{NI}\Phi_N(t) + r_{MI}\Phi_M(t), \tag{3}$$

$$\frac{d\Phi_M}{dt} \equiv \dot{\Phi}_M(t) = -(r_{MI} + r_{MO})\Phi_M(t) + r_{IM}\Phi_I(t) + r_{OM}\Phi_O(t), \tag{4}$$

$$\frac{d\Phi_O}{dt} \equiv \dot{\Phi}_O(t) = -r_{OM}\Phi_O(t) + r_{MO}\Phi_M(t), \tag{5}$$

where r_{NI} is the transition rate of going from state N to state I and analogous for the other rates. Two properties are used to simplify the set of Eqs. (2)–(5). One is the conservation of the total amount of drug,

$$\Phi_N(t) + \Phi_I(t) + \Phi_M(t) + \Phi_O(t) = 1. \tag{6}$$

The other is the rate symmetry

$$r_{MI} = r_{MO}, \tag{7}$$

which reflects that the inner and outer membrane surfaces have identical properties and areas. Note that we neglect any diffusive process inside the solutions, the nanocarriers and the membrane. This means that we assume the membrane to be relatively thin and the solutions to be well-mixed. We also neglect the finite drug loading capacity of the nanocarriers, which would lead to nonlinear effects. This assumption is justified since the nanocarriers are only loaded with a few dexamethasone molecules at the beginning of the release experiments and thus should be far from their loading capacity, as we will demonstrate in the Discussion section at the end. Using Eqs. (6) and (7), the simplified set of equations is given by:

$$\dot{\Phi}_N(t) = -r_{NI}\Phi_N(t) + r_{IN}\Phi_I(t), \tag{8}$$

$$\dot{\Phi}_I(t) = -(r_{IM} + r_{IN})\Phi_I(t) + r_{NI}\Phi_N(t) + r_{MI}(1 - \Phi_I(t) - \Phi_O(t) - \Phi_N(t)), \tag{9}$$

$$\dot{\Phi}_O(t) = -r_{OM}\Phi_O(t) + r_{MI}(1 - \Phi_I(t) - \Phi_O(t) - \Phi_N(t)). \tag{10}$$