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Short communication

Lyophilic matrix method for dissolution and release studies of nanoscale particles

Jenni Pessi[a,*,1], Sami Svanbäck[a,1], Ilkka Lassila[b], Edward Hæggström[b], Jouko Yliruusi[a]

[a] Division of Pharmaceutical Chemistry and Technology, University of Helsinki, Viikinkaari 5e, 00790, Helsinki, Finland
[b] Division of Material Physics, Department of Physics, University of Helsinki, Gustaf Hällströmin katu 2a, 00560, Helsinki, Finland

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ABSTRACT

We introduce a system with a lyophilic matrix to aid dissolution studies of powders and particulate systems. This lyophilic matrix method (LM method) is based on the ability to discriminate between non-dissolved particles and the dissolved species. In the LM method the test substance is embedded in a thin lyophilic core-shell matrix. This permits rapid contact with the dissolution medium while minimizing dispersion of non-dissolved particles without presenting a substantial diffusion barrier. The method produces realistic dissolution and release results for particulate systems, especially those featuring nanoscale particles. By minimizing method-induced effects on the dissolution profile of nanopowders, the LM method overcomes shortcomings associated with current dissolution tests.

1. Introduction

The dissolution rate of a drug is a physico-chemical property to be determined and modified during drug discovery and development [1,2]. For example, reducing the particle size to the nanoscale increases the dissolution rate and thus the bioavailability of poorly water-soluble drugs in classes II and IV of the Biopharmaceutics Classification System [3–5]. The dissolution rate of nanoscale particles correlates with the performance and quality of a formulation featuring nanoparticles [3]. Hence to assess the impact of nanonizing a poorly water-soluble drug, one needs reliable dissolution rate data of nanoparticulate systems. Such data could allow one to predict realistic *in vitro – in vivo* (IVIV)-correlation and facilitate determination of dose in animal experiments [6–8].

Current methods for investigating dissolution rates of nanoscale particles include the United States Pharmacopoeia (USP) I (basket), II (paddle), and IV (flow-through) methods, as well as modifications thereof, membrane diffusion methods (such as the dialysis methods), and sample and separate methods (such as centrifugal ultrafiltration) [6,7,9–14]. Additionally, dissolution rates of nanoparticles have been determined from tablets and admixtures using gel matrices [15,16]. Often, the measured values reflect features of the dissolution test device, equipment or method, rather than the nanoparticle properties.

The main issues with the current methods include: dispersion of non-dissolved particles, hydrodynamics-induced variability, membrane effects caused by diffusion barriers (e.g. gelatin, filters, or dialysis membranes), clogging and breaking of filters, sensitivity to flow and location in the dissolution vessel, as well as migration of nanoparticles to interfaces (e.g. wetting issues, floating, or adhesion) [17–24]. The UPS methods were not designed for dissolution studies of nanoscale particles and thus produce unrealistic results [13,17]. Dispersion and the consequent overestimation of nanoparticle dissolution rates in the USP I and II methods occur when the location of the particles is not fixed. Dispersion occurs in the USP IV method when a too large filter pore size is used [6]. On the other hand, constraining diffusion of the dissolved species by membranes or encapsulation, leads to measurement of the quality of the diffusion barrier rather than that of the nanoparticle dissolution, and often to underestimating the dissolution rate [17,19,25,26]. Using tablets or admixtures may alter the physical form of the drug during the tableting or mixing, and particles may detach from the tablet surface during the dissolution process, or induce a diffusion barrier [15,16]. Accordingly, there is a need for new methods and devices for determining dissolution rates of nanoparticles.

* Corresponding author.
E-mail address: jenni.pessi@helsinki.fi (J. Pessi).
[1] Authors contributed equally.

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2. Materials and methods

2.1. Chemicals

Indomethacin (Hawkins, USA) was used as poorly water-soluble model compound in the experiments and poloxamer 188 (BASF Co., Germany) was used as stabilizer. The chemicals used for preparing the media for the dissolution studies were monopotassium phosphate (Riedel-de Haën, Germany), sodium phosphate dibasic (Sigma-Aldrich, USA), and 5 M sodium hydroxide (VWR Chemicals BDH Prolabo, EC). All chemicals in the experiments were of analytical grade and were used as received.

2.2. Structure of the device

The experimental device comprised a lyophilic matrix, a cage, a vessel, and a mixing/heating plate (Fig. 1). The matrix has a core-shell structure comprising a core matrix that contained the particles of the test substance, and a surrounding shell matrix. The matrix material of both core and shell matrices is cotton (100% cotton, Curatex GmbH, Germany). The shell matrix consists of four layers of water jet-pressurized cotton with a dry specific surface weight of 5 ± 0.2 mg/cm^2. Cotton was selected as matrix material due to its unique properties; hollow cellulose fibers, high wet strength, inert nature, and substantial ability to absorb water-based media. The custom designed stainless steel cages (depth 3 mm × height 26 mm × width 16 mm) were 3D printed with selective laser sintering (Mlab Cusing, Concept Labs, Germany). The cage maintained the desired matrix geometry and provided a fixed diffusion distance.

2.3. Characterization of the matrix

2.3.1. Matrix-medium interaction

The cotton matrix was examined prior to, during, and after medium exposure with light microscopy (Leica DMLB, Leica Microsystems Wetzlar, Germany) with a magnification of 200×, and prior to, and after medium exposure with scanning electron microscopy (SEM, Quanta^{TM} 250 FEG, FEI Inc., USA) with a magnification of 500×, voltage of 5.00 kV, spot size of 3.0, sputter coated with a 5-nm-thick platinum layer (Q150T Quomm, Beijing, China). The water intake properties of the matrix were investigated with a fast camera (1200 fps, Casio Exilim High-speed EX-FI1, Casio, Japan) and by weighing the matrix prior to and after exposure to the medium.

2.3.2. Drug-matrix interaction

The partitioning of the model compound between the matrix and medium was examined by partition coefficient and inverse partitioning coefficient studies. First, the retention of the model compound within the matrix was examined. This was done by partition coefficient tests, where the matrix containing 1 mg of bulk indomethacin was immersed in the medium, and collected after 22 h. The indomethacin retained in the matrix was determined by immersing the matrix into fresh medium for 22 h. This procedure was conducted with three parallel experiments in pH 5.5 and pH 7.4 phosphate buffer media [27] at 37.0 ± 0.5 °C with a stirring rate of 180 rpm (IKA RT 15 P, IKA Werke GmbH & CO. KG, Germany). The concentration of the medium was determined after the first and the second immersion at the 22 h time point. The concentration of the samples was analyzed with high performance liquid chromatography (HPLC Thermo System Products, Agilent 1200 Infinity Series, Agilent Technologies, Germany), using a Discovery C18 column (4.6 × 150 mm, 5 μm, Supelco, USA), 1.5 mL/min flow rate with a mobile phase consisting of 60:40 (V/V) acetonitrile (ACN) and 0.2% ortophosphoric acid (H_3PO_4) in water (MilliQ), operating at 30 °C with detection at 270 nm. The standard curve for indomethacin quantification was acquired from triplicate samples of indomethacin concentrations between 0.08 mg/L and 500 mg/L ($R^2 = 0.999$).

Second, the partitioning of the dissolved species into the matrix was examined. This was done by inverse partition coefficient tests, where an empty matrix was inserted into medium with saturated concentration of the model compound. The test was conducted in triplicate in phosphate buffer media with pH of 5.5 and 7.4. The empty matrices were inserted into the medium every 5 min and the test lasted 20 min. The concentration of the medium was monitored online using in-situ fiber-optic UV monitoring (Opt-Diss 410, Distek, Inc., USA) using probes with a path-length of 5 mm, exposure time of 44 ms (4 scans/data point) at an analytical wavelength of 320 nm.

2.4. Drug release studies

2.4.1. Preparation and characterization of the particles

A nanosized fraction, two sieved particle size fractions, and bulk indomethachin were tested with the LM method. Nanosuspension was prepared by milling with a Fritsch Pulverisette 7 Premium ball mill (Fritsch GmbH, Germany) to obtain particles for the experiments. Nanoparticles for the LM method were prepared of 2 g indomethacin suspended in solution containing 5.0 mL 0.24 g/mL poloxamer 188 solution (60 wt% relative to the drug amount) and 5.0 mL water (milliQ), and by grinding at 850 rpm in 5 cycles of 3 min using 60 g milling pearls (zirconium oxide, diameter 1 mm). The particle size distribution in the nanosuspension was determined with a Zetasizer Nano SZ (Malvern Instruments Ltd., UK).

The bulk indomethacin was divided into two fractions using a sieve with 125 μm eye size of (Fritsch GmbH, Germany). The particle size of the bulk powder and the two fractions were determined from SEM images (see Section 2.3.1.) ($n = 300$, ImageJ freeware, National Institutes of Health, USA). The bulk powder and the two fractions were each mixed with poloxamer 188 (60 wt% relative to the drug amount) to achieve physical mixtures with content identical to the nanosuspension.

2.4.2. Dissolution experiments

The test substances (corresponding to 0.5 mg of indomethacin) were distributed within the core matrix. The indomethacin suspension was introduced into the core matrix by wetting the core cotton evenly with the indomethacin suspension and then drying the core cotton. The dry powder was introduced into the core matrix by carefully mixing the particles with the core cotton in a mortal. The particles were collected into the core matrix as they adhere onto the core cotton fibers. Quantities corresponding to 3 mg of core cotton and 0.5 mg of indomethacin were weighed and the core cotton was placed between the shell matrices. The core cotton was distributed between the shell matrices as a square shaped even layer. The core matrix had a slightly smaller surface area than shell matrices to have the core matrix covered with the shell matrices from all sides. The matrix was then placed in the stainless steel cage acting as the matrix holder. Dissolution tests were conducted in triplicate for nanoparticles, bulk powder, and the two particle size fractions in pH 5.5 phosphate buffer medium [27] and for nanoparticles and bulk powder in pH 7.4 phosphate buffer medium [27]. All tests were performed in 100 mL of dissolution medium at 37.0 ± 0.5 °C using a stirring rate of 180 rpm (IKA RT 15 P, IKA Werke GmbH & CO. KG, Germany). The dissolution medium outside the matrix provided sink conditions. The stirring rate and the matrix geometry were optimized with preliminary experiments. Aliquots of 1 mL, subsequently replaced with the same volume of fresh medium, were taken at 12 time points: 30 s, 1 min, 2 min, 5 min, 10 min, 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, and 22 h. The samples were analyzed with HPLC as described in section 2.3.2. Cumulative release of indomethacin