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Measurement of drug diffusivities in pharmaceutical solvents using Taylor dispersion analysis

Fengbin Ye, Henrik Jensen, Susan W. Larsen, Anan Yaghmur, Claus Larsen, Jesper Østergaard *

Department of Pharmaceutics and Analytical Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark

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ABSTRACT

Knowledge of drug diffusivity is of key importance in the understanding of a number of pharmaceutical and biological processes. However, experimentally determined diffusion coefficients and hydrodynamic radii are only reported for a limited number of drug substances. In this work, Taylor dispersion analysis conducted using capillary electrophoresis instrumentation coupled with a UV imaging detector, with two detection windows along the capillary, is introduced as a powerful method for the determination of drug diffusivities in nanoliter samples. Several potential advantages associated with applying two detection windows instead of one window as done in most previous studies were identified. Overall diffusion coefficient measurements performed using two detection windows are more robust and correction for changes in flow rate and sample volume is not required. The experimental conditions applied were suboptimal for performing single detection window measurements due to the relatively large sample volumes and may be optimized to alleviate the need for tedious correction procedures for this setup. The diffusivities of eleven aromatic compounds in water at 25 °C were determined, and showed a good agreement with the literature values. Furthermore, the diffusivities and hydrodynamic radii of four selected drug substances were determined in acetonitrile, methanol, isopropyl myristate, medium chain triglyceride, and propylene glycol in addition to water. The solvent viscosity was determined simultaneously along with the measurement of analyte diffusivity. Drug diffusivities decreased with increasing solvent viscosity. Taylor dispersion analysis is a robust, simple and automated method of quantification of diffusion coefficients even in media with a relatively higher viscosity than water.

1. Introduction

Diffusion is the transport of matter due to the random thermal motion of molecules and a spatial gradient in chemical potential. This mass transport process is of paramount importance to the pharmaceutical sciences [1,2]. The diffusion of drug substances through biological membranes is fundamental to drug absorption and elimination processes within the body. Diffusion plays an important role in various separation processes, e.g., dialysis, sedimentation and ultrafiltration. Equally important, the release from drug delivery systems often involves a diffusion component. Pertinent examples include the achievement of controlled drug release through rate-controlling membranes or polymer matrix-based systems [1,3].

The diffusivity (diffusion coefficient, D) of a drug substance in an isotropic solvent is related to the size (hydrodynamic radius, R_h) of the molecule and the properties of the diffusion medium as often expressed through the Stokes–Einstein equation:

$$D = \frac{k_B T}{6\pi\eta R_h} \quad (1)$$

where k_B is the Boltzmann constant, T is the absolute temperature, and η is the viscosity of solution, respectively. Despite the importance of the diffusion coefficient or effective size of a drug substance in various biological and pharmaceutical matrices, this physicochemical parameter is not commonly determined experimentally. This is, at least in part, related to the fact that measurement of diffusion coefficients is mostly cumbersome and time consuming. Diffusion coefficients of substances in solution may be determined using the diffusion cell method [4,5], optical methods (e.g. dynamic light scattering) [6,7], spectroscopic methods (e.g. NMR techniques) [8,9], or Taylor Dispersion Analysis (TDA) [7,10–19].

The TDA methodology is based on the studies by Taylor [20] and, subsequently, Aris [21] on dispersion of analytes subjected to Poiseuille laminar flow conditions in a tube. The parabolic flow profile causes the analyte molecules in the tube to move with different velocities according to their radial position in the tube or capillary which consequently lead to analyte dispersion. The degree of

Abbreviations: CE, Capillary electrophoresis; IPM, Isopropyl myristate; MCT, Medium chain triglyceride; PG, propylene glycol; TDA, Taylor dispersion analysis.

* Corresponding author. Tel.: +45 35336138; fax: +45 35336030.
E-mail address: joe@farma.ku.dk (J. Østergaard).

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dispersion of the analyte plug is dependent on the diffusivity of the analyte leading to redistribution over the tubular cross section (see Supplementary Data for details on TDA theory).

TDA is a simple, relatively rapid and absolute (calibration is not required) method for determination of diffusion coefficients [12]. TDA can be implemented using standard equipment such as High Performance Liquid Chromatography (HPLC), Flow Injection Analysis (FIA) and Capillary Electrophoresis (CE). In particular, commercially available CE instrumentation utilizing narrow bore fused silica capillaries and providing convenient autosampling and detection possibilities has been found well-suited for TDA studies and has contributed to the recent renewed interest in the technology. TDA has been used to assess the diffusivity of small molecules [11,17,22,23], macromolecules [11,12,15,16,18,24], as well as colloidal particles [13,25]. Also, TDA was successfully introduced to characterize complexation phenomena [14,26]. Recently, TDA studies utilizing two detection points along the capillary have been reported [7,16,18,25]. However, the potential advantages associated with having more than one detection window have hitherto not been investigated.

The present studies were undertaken to characterize further the feasibility of TDA for the measurement of diffusion coefficients and estimation of hydrodynamic radii of drug substances in selected pharmaceutical media. A UV imaging detector allowing two detection windows along the capillary connected to a commercially available CE instrument was used for the investigations. The effects of varying the experimental parameters, capillary dimensions, flow rates, and sample volumes, were studied highlighting the need for suitable corrections due to the ramp in fluid velocity and the finite width of the sample zone. In this context, several advantages of having two detection windows were identified. Using optimized experimental conditions, the diffusion coefficients of 11 low-molecular-weight analytes (mostly drug substances) in water were determined and compared to literature data. Most studies using TDA for the determination of molecular diffusion coefficients have been conducted in aqueous solutions. Consequently, it was found of interest to evaluate the feasibility of quantifying drug substance diffusivities in additional 5 pharmaceutically relevant media, including the more viscous isopropyl myristate (IPM), medium chain triglyceride (MCT), and propylene glycol (PG). In addition to the diffusion coefficients, measurements of vehicle viscosity were simultaneously attained by the TDA method.

2. Experimental

2.1. Chemicals and sample preparation

Phenol, *p*-nitrophenol, (R/S)-ibuprofen, (R/S)-warfarin, and salicylic acid were obtained from Sigma–Aldrich (St. Louis, MO, USA). Benzoic acid, histidine, isopropyl myristate (IPM), and propylene glycol (PG) were purchased from Fluka–Chemie (Buchs, Switzerland). L-Phenylalanine and L-tryptophan were obtained from Calbiochem–Novabiochem (Läufelfingen, Switzerland). Bupivacaine hydrochloride and lidocaine were obtained from Unikem (Copenhagen, Denmark). Benzene was obtained from J. T. Baker (Deventer, Holland). Toluene was obtained from Applichem (Darmstadt, Germany). Medium chain triglyceride (MCT, Myritol 318 PH) was kindly donated by Broste A/S (Lyngby, Denmark). Sodium dihydrogen phosphate monohydrate was obtained from Merck (Darmstadt, Germany). HPLC-grade methanol (MeOH) and acetonitrile (CH_3CN) were obtained from BDH (Poole, England). The free base of bupivacaine used was obtained from the corresponding hydrochloride as previously described [27]. All other chemicals were used as received. Throughout the experiments, purified water from a Milli-Q deionization unit (Millipore, Bedford, MA, USA) was used. All solutions were prepared by dissolving the analyte in the appropriate volume of water, organic or pharmaceutical solvent at ambient temperature. Ibuprofen was poorly soluble in water (0.12 mg/mL at 25 °C) [28]. Therefore, the saturated aqueous solution was prepared and filtered prior to TDA measurement.

2.2. Instrumentation

All TDA and solvent viscosity measurements were conducted on an Agilent ^{3D}CE instrument (Agilent Technologies, Waldbronn, Germany) with an external pressure source attached. An Actipix D100 UV area imaging detector (Paraytec Ltd., York, UK) was used for detection at two windows along the capillary. The capillaries were taken out of the Agilent CE cassette and looped into Actipix detector as detailed elsewhere [16]. The temperature of the capillary cartridge was set at 25 °C. The UV absorbance signals from each of the detection windows were recorded with Actipix control software (version 1.2). UV detection was performed at wavelengths of 200, 214 or 254 nm. The detection windows were 1.5 cm in width and produced by burning off the polyimide coating. Fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) were cut to a total length of 90.2 cm and prepared with lengths of 30.7 and 50.9 cm to the centre of the first and the second detection windows, respectively.

2.3. TDA measurements

New capillaries were conditioned with 1 M NaOH and water for 30 min each. Between two injections, the capillary was flushed with dispersion media for 2 min (water, MeOH, and CH_3CN) or 10 min (IPM, MCT, and PG). In case of the more viscous and greasy solvents (IPM, MCT, and PG), the capillary was cleaned by flushing with 96% (v/v) ethanol after measurements. Sample injection was hydrodynamically performed from the inlet side of the capillary by applying a pressure of 50 mbar (from 1 to 20 s) or 1000 mbar (from 9 to 24 s) for the more viscous media. Mobilization pressures of 50, 100, 150, 200, 250, 300, and 1000 mbar were applied with a dispersion medium filled vial in the inlet position to force the sample plug through the capillary. Each sample was analyzed 6 times unless otherwise noted. The experimental temperature next to the capillary loop was measured using a Fluke 1522 temperature probe (Fluke Corporation, American Fork, UT, USA).

2.4. Determination of the capillary radius

Capillary radii, R_c, were measured using the modified Hagen–Poiseuille method [13,17]:

$$u = \frac{R_c^2\ \Delta P}{8\eta L_t} \quad (2)$$

where u is the flow rate, η is the viscosity of water, ΔP is the pressure difference between the inlet and outlet of the capillary, and L_t is the total length of capillary. The capillary was first filled with water (η = 0.933 mPa s at 23 °C [29]) by flushing the capillary for 10 min. Then a small plug of phenol sample (0.2 mg/mL in water) was hydrodynamically injected (50 mbar, 7 s) and replaced by forcing water through the capillary at ambient (known) temperature and a pressure drop of 50 mbar. The flow rate was determined by measuring the capillary length and the residence time of the phenol sample between the two detection windows. The measurements were repeated 6 times, yielding a capillary radius that varied less than 1%. The average of these measurements was taken as the capillary radius.