Simultaneous UV Imaging and Raman Spectroscopy for the Measurement of Solvent-Mediated Phase Transformations During Dissolution Testing

JESPER ØSTERGAARD, JIAN X. WU, KAISA NAELAPÄÄ, JOHAN P. BOETKER, HENRIK JENSEN, JUKKA RANTANEN

Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, Copenhagen DK-2100, Denmark

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ABSTRACT: The current work reports the simultaneous use of UV imaging and Raman spectroscopy for detailed characterization of drug dissolution behavior including solid-state phase transformations during dissolution. The dissolution of drug substances from compacts of sodium naproxen in 0.1 HCl as well as theophylline anhydrate and monohydrate in water was studied utilizing a flow-through setup. The decreases in dissolution rates with time observed by UV imaging were associated with concomitant solid form changes detected by Raman spectroscopy. Sodium naproxen and theophylline anhydrate were observed to convert to the more stable forms (naproxen, and theophylline monohydrate) within approximately 5 min. Interestingly, the new approach revealed that three intermediate forms are involved in the dissolution process prior to the appearance of the neutral naproxen during dissolution in an acidic medium. The combination of UV imaging and Raman spectroscopy offers a detailed characterization of drug dissolution behavior in a time-effective and sample-sparing manner. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:1149–1156, 2014

Keywords: dissolution; dissolution rate; hydrates/solvates; imaging methods; Raman spectroscopy; solid form transformation; UV/Vis spectroscopy; UV imaging

INTRODUCTION

In the case of poorly water-soluble drugs, dissolution is frequently the rate-limiting step in drug absorption.[1–4] The dissolution behavior of an active pharmaceutical ingredient (API) is closely related to the solid form (amorphous/crystalline, polymorphism, solvate/hydrate, and salt/free form) of the compound and solid form transformations occurring as part of the dissolution process may significantly affect the performance of the drug. Increased dissolution rates may be attained through the use of high-energy solid forms or supersaturating drug delivery systems, which have attracted a lot of interest because of the increasing number of poorly soluble development candidates.[5–8] However, solvent-mediated phase transformations from the high-energy forms to a more stable form with lower solubility may occur upon exposure to the dissolution medium.[9–12]

In simple cases, solvent-mediated solid form transformations have been inferred from *in vitro* dissolution experiments. However, generally, elucidation of solid form transformations may be challenging as several events/phenomena may occur simultaneously: nucleation and growth of the stable form, simultaneous dissolution of coexisting solid forms, and a continuously changing surface area available for dissolution.[11,12] Thus, verification and deeper characterization of phase changes and solid forms upon dissolution testing should be achieved using, for example, X-ray powder diffractometry[13] or Raman spectroscopy in order to obtain fast real-time characterization of the solid-state form. For this purpose, flow-through dissolution apparatuses with *in situ* Raman spectroscopy allowing real-time information on phase transformation kinetics have been found highly useful.[14–17] Synchrotron-based X-ray diffraction experiments have recently been suggested to be a promising approach for investigating fast transformation kinetics.[18] Recent advances in dissolution testing have also been achieved by the introduction of imaging techniques, including magnetic resonance imaging,[19,20] Fourier transformed infrared (FTIR)[20,21] and UV imaging[22–28] providing real-time data. UV imaging facilitates spatially (2D) and temporally resolved quantification of drug substance in solution immediately adjacent to the solid material.[22,24,29] Utilizing a flow-through cell, the UV imaging technique was first applied for monitoring the dissolution behavior of amlodipine besylate and the free base of amlodipine that may be subject to solvent-mediated phase transformations during dissolution.[22] Recently, this miniaturized flow-through dissolution approach has been applied to investigate dissolution of indomethacin, theophylline and ibuprofen,[25] carbamazepine and carbamazepine-nicotinamide cocrystals,[26] furosemide,[27] and paracetamol.[28] In these studies, efforts have also been made to investigate the propensity of the drug substances to undergo solid form changes during dissolution in parallel, but separate, experiments applying more or less similar conditions (e.g., flow conditions) to those applied in the UV imaging-based flow-through setup.

The objective of the present work was to develop and investigate the feasibility of a real-time UV imaging-based dissolution method coupled with *in situ* Raman spectroscopic measurements as a means for simultaneous detailed characterization of drug dissolution behavior using small amounts of

Correspondence to: Jesper Østergaard (Telephone: +45-35336138; Fax: +45-35336001; E-mail: jesper.ostergaard@sund.ku.dk)

Kaisa Naelapää's present address is Oral Formulation and Process Development, Novo Nordisk A/S, Denmark.

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API. As model systems, dissolution of theophylline anhydrate, theophylline monohydrate, and sodium naproxen was investigated representing different scenarios for solid form changes.

MATERIALS AND METHODS

Materials

Naproxen was obtained from Marsing & Company A/S (Copenhagen, Denmark). Sodium naproxen was obtained from Divi's Laboratories Limited (Hyderabad, India). Theophylline monohydrate was obtained from BASF (Ludwigshafen, Germany) and theophylline anhydrate was prepared by dehydration at 100°C overnight. The dissolution media were purified water from a Milli-Q deionization unit (Millipore, Bedford, Massachusetts) or 0.1 M HCl.

Compacts were made by filling portions of approximately 3 mg of substance into a stainless steel cylinder (inner diameter: 2 mm; height 2.4 mm) held in a manual press (Actipress; Paraytec, York, UK). A torque screwdriver was used to compress the samples at a constant torque (pressure) of 60 or 120 cNm for 30 s.

UV Imaging and Raman Setup

An Actipix SDI300 dissolution imaging system with Actipix flow-through type dissolution cartridge CADISS-3 (Paraytec) was used for UV imaging dissolution studies. The syringe pump was used for infusion of dissolution medium. The selected imaging area was 9×3 mm^2 and the pixels (7×7 μm^2) were binned horizontally in sets of 10 (10×1 pixel binning). UV imaging was performed at 297 and 214 nm (light path 4.0 mm) for dissolution studies involving theophylline and naproxen, respectively. Images were recorded with 2.8 frames per second and analyzed using Actipix D100 software version 1.6 (Paraytec). Pixel intensity was converted into absorbance using the Actipix software allowing the concentration of the drug substances to be determined as a function of position and time by the use of calibration curves.[29] A wide angle (Phat) probe (Kaiser Optical Systems, Inc., Ann Arbor, Michigan) with a spot size of 3 mm was mounted above the dissolution cartridge. A 785-nm Invictus TM NIR diode laser with a standard resolution 9 of 5 cm^{-1} and approximately 100 mW laser power (RamanRxn1; Kaiser Optical Systems) was used for *in situ* Raman monitoring. The spectra were collected continuously using 7 s exposure time and one accumulation to obtain each spectrum (a total of 15 s was used to obtain one spectrum). Simultaneous UV imaging and Raman spectroscopic measurements were performed using a modified CADISS-3 cell as shown in Supporting Information Figure S1.

Dissolution Studies

The UV imaging dissolution experiments with *in situ* Raman measurements were performed as follows. First, dark images (lamp turned off for 10 s) and reference images (10 s) were recorded with the flow cell filled with dissolution medium. After 60 s of data collection, data recording was paused, the cell disassembled, and the compact containing the drug substance inserted. Upon introducing the compact into the flow cell, UV imaging data collection was resumed while applying the dissolution medium to the cell at a flow rate of 1.0 mL/min for up to 30 min. At the same time, Raman spectroscopy was used to monitor the compact surface at 15 s on/off intervals. Dissolution rates were calculated using the Actipix D100 software (Paraytec) from the pixels in the quantification region (0.5×2.5 mm^2) downstream to the sample[22] and averaging data over 5 s intervals. The dissolution studies were conducted at room temperature (19°C–23°C). UV imaging calibration curves needed for the calculation of dissolution rates were constructed by flowing standard solutions through the dissolution cell at a flow rate of 1 mL/min in 4 min for each concentration level. The concentration ranges were $5.0{\cdot}10^{-5}$–$7.5{\cdot}10^{-4}$ and $1.0{\cdot}10^{-6}$–$5.0{\cdot}10^{-5}$ M for theophylline and naproxen, respectively.

X-Ray Powder Diffraction

X-ray powder diffraction measurements were conducted on the drug substances prior to and upon dissolution testing using a PANalytical X'Pert Pro θ/θ diffractometer with a PIXcel detector (PANalytical B.V., Almelo, The Netherlands). A continuous 2θ scan was performed in a range of 2°–40° using Cu Kα radiation ($\lambda = 1.5418$ Å) using a step size of 0.0390°2θ and a speed of 0.05°2θ/s. A nickel filter was used to eliminate K_β radiation. The voltage and current applied were 45 kV and 40 mA, respectively. Sample spinning was applied during the measurements to minimize the effects from preferred orientation. The data were collected using X'Pert data collector version 2.2 and analyzed with X'Pert high score plus version 2.2.4 (PANanalytical B.V.).

Multivariate Data Analysis

Raman spectra recorded during dissolution experiments of theophylline anhydrate and sodium naproxen were analyzed using multivariate curve resolution (MCR) to achieve estimation of the kinetic phase transformation profiles. The Raman data sets were arranged in time series and MCR[30–32] was employed using in-house developed Matlab (ver. 7.14; Mathworks, Natick, Massachusetts) routine together with MCR–alternating least squares (MCR-ALS), a Matlab-based toolbox developed by Jaumot et al.[33] The MCR technique allows decomposition of a complex spectrum into its pure chemical component spectra and the scores of their respective contribution. For each experiment, the Raman data were row-wise arranged in a matrix D. Followed by variable selection, the data matrix was standard normal variate corrected,[34] and a constant offset was subsequently added to D in order to correct for the negative elements. Prior to MCR modeling, the number (n) of components that the model should be based upon was estimated using evolving factor analysis (EFA).[35–37] For MCR model optimization on the theophylline dissolution experiment, the Raman spectra of theophylline anhydrate and monohydrate references were used as initial estimates. Likewise, Raman spectra of sodium naproxen and naproxen references were used as initial estimates for the MCR modeling. The basic MCR model structure is presented in Eq. 1 [32]:

$$D = CST + E \tag{1}$$

in which each preprocessed spectrum in D is decomposed into a n underlying pure chemical spectra arranged in S^T, and their respective relative concentration scores arranged in matrix C. E is the residual matrix. To minimize the ambiguity problem related to MCR, the constraints applied were non-negativity on both C and S matrices, and unimodality and closure constraint were applied on C matrix.[32]