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Optical microscopy as a comparative analytical technique for single-particle dissolution studies

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ABSTRACT

Novel, simple and cost effective methods are needed to replace advanced chemical analytical techniques, in small-scale dissolution studies. Optical microscopy of individual particles could provide such a method. The aim of the present work was to investigate and verify the applicability of optical microscopy as an analytical technique for drug dissolution studies. The evaluation was performed by comparing image and chemical analysis data of individual dissolving particles. It was shown that the data obtained by image analysis and UV-spectrophotometry produced practically identical dissolution curves, with average similarity and difference factors above 82 and below 4, respectively. The relative standard deviation for image analysis data, of the studied particle size range, varied between 1.9% and 3.8%. Consequently, it is proposed that image analysis can be used, on its own, as a viable analytical technique in single-particle dissolution studies. The possibility for significant reductions in sample preparation, operational cost, time and substance consumption gives optical detection a clear advantage over chemical analytical methods. Thus, image analysis could be an ideal and universal analytical technique for rapid small-scale dissolution studies.

1. Introduction

The trend in physicochemical screening, including dissolution studies, is towards smaller sample amounts with more rapid and efficient analysis (Kerns, 2001). While UV-spectrophotometry is the most widely available chemical analytical technique, the analysis of smaller and thus more dilute samples, requires more sophisticated methods for the determination of dissolved concentrations (Bernal et al., 1998). High-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) or tandem mass spectrometry (HPLC-MS/MS) are the most used techniques for high-throughput quantitative analysis of dilute liquid samples (Korfmacher, 2005; Tiller et al., 2003). While mass spectrometry coupled analysis is a valuable tool for quantitation of low concentrations and simultaneous determination of several analytes, *e.g.* drug compounds and metabolites, the apparatus is expensive and costly to operate (Saunders, 2004). There is thus a need for novel, simple and cost effective methods to replace advanced chemical analytical techniques, whenever the analytical integrity can be retained.

Apart from the high operational cost of advanced chemical analytical methods, other disadvantages also exist. One weakness of commonly used chemical analytical techniques is the high amount of sample preparation, which includes both sample and calibrant preparation, *e.g.* extraction, dilution and filtration (Alsenz and Kansy, 2007; Paakkunainen et al., 2009). As the total measurement error is determined by the inherent error in every step of the sampling chain, sample preparation should be kept to a minimum (Paakkunainen et al., 2009). This is especially true for miniaturized high-throughput experiments where the smaller sample sizes further increase the probability of measurement errors.

Image based physical analysis could thus provide a more accurate method, since it requires minimal sample preparation. While optical microscopy has been a widely used technique for morphological characterization of particles, recent advances in digital micrography, computing and image analysis software have enabled the use of optical microscopy for automated real-time analysis (Allen, 1990; Almeida-Prieto et al., 2006). Traditional optical microscopy has, however, two major limitations. While the theoretical minimum resolution is 0.2 μm, the realistic threshold for particle size analysis is considered to be 0.8 μm (Allen, 1990).

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The other limitation is the shallow depth of focus. Despite these limitations, the advantages of studying dissolution through image analysis, instead of chemical analysis, could be significant.

Due to the wide variability in structural properties of pharmaceutical compounds, different chemical analytical methods are required for different compounds and compound–solvent combinations, with the compound specific method development consuming a lot of time (Sacher et al., 2001; Tiller et al., 2003). For example, in an attempt to analyze 60 different pharmaceutical compounds with a minimum amount of analytical methods, whilst retaining analytical integrity, 6 different methods were needed (Sacher et al., 2001). Particle size analysis from micrographs, on the other hand, does not depend on the chemical properties of the studied substance or solvent, and can therefore be seen as a universal analytical method. The only required calibration is a size calibration to account for the different refractive indices of solvents. Significant gains in time and substance consumption could thus be achieved through optical detection.

Another way of reducing uncertainty of the analytical data is by minimizing the assumptions involved. While, according to Aristotle: "the whole is greater than the sum of its parts", it can nevertheless be argued that the whole is determined by its individual parts. This is also the case with powders and powder dissolution, where the primary particles composing the bulk are the main determinants of the overall bulk properties (de Villiers, 1996).

The general way of studying dissolution rates of small sample amounts has thus far been through the multiparticulate bulk approach (Marabi et al., 2008). However, multiparticulate systems are highly complex and therefore difficult to model accurately (Hulse et al., 2012). This is because important factors affecting the dissolution rate of solid substances such as particle size distributions, shape distributions and the degree of aggregation and agglomeration are difficult to measure accurately (Avdeef et al., 2009; Mosharraf and Nyström, 1995; Wang and Flanagan, 1999). The difficulty is further increased by the dynamic change of these factors during the dissolution process. Thus, the reliability of data extracted from multiparticulate systems depends on the accuracy of the underlying statistical calculations (Allen, 1990).

This issue can be avoided by assessing the dissolution rate from single particles. By studying single particles, the cohesive interactions between particles can be ignored and the particle shape and size may be exactly observed. The single-particle approach thus minimizes the assumptions regarding factors influencing the dissolution rate, and the data can therefore be regarded as more reliable. Thus far, dissolution studies using individual particles has been implemented only in a few cases, mainly in chemical engineering as compiled by Marabi et al. (2008), but lately also in pharmaceutical sciences (*e.g.* Prasad et al., 2002; Raghavan et al., 2002; Østergaard et al., 2011). It can be concluded based on literature, that in the few single-particle dissolution studies that have been made, optical microscopy has been the most prevalent analytical method (Marabi et al., 2008; Prasad et al., 2002; Raghavan et al., 2002).

In all previous single-particle studies where optical microscopy has been used, no evaluation of the analytical technique has been performed. The aim of the present study was therefore to investigate and verify the applicability of optical microscopy, as an analytical technique for drug dissolution experiments. The evaluation was performed by comparing image analysis data with chemical analysis data of individual dissolving pure drug substance particles. Since optical microscopy can be seen as a universal technique, its possible use would mean that dissolution rates of single particles of any drug substance could be assessed by a single analytical technique. This would give significant advantages over the current norm of studying multiparticulate samples by multiple chemical analytical techniques.

2. Materials and methods

2.1. Materials

Pure substance pellets of the initial mass range 0.20–0.85 mg were used in the single-particle dissolution studies. Pellets of three model drug compounds: acetylsalicylic acid (ASA) (acetylsalicylic acid, Orion Pharma, Espoo, Finland) and paracetamol (paracetamolum, Ph. Eur. 4th ed. 2002, Oriola, Espoo, Finland) as model acids, as well as anhydrous theophylline (theophylline anhydrous, BASF, Ludwigshafen, Germany) as model base, were used. The pellets were produced from micronized powders, using a miniaturized extrusion-spheronization method recently developed at the Division of Pharmaceutical Technology, Faculty of Pharmacy, University of Helsinki.

2.2. Experimental setup

The dissolution of single pellets was performed at ambient temperature ($23 \pm 0.5\,^{\circ}C$) in 24-well plate wells, using degassed distilled water as dissolution medium. One well could hold 3 ml of medium, which was sufficient to allow adequate dissolution times for the studied substances, ranging from one to a few hours. In order to even out the concentration in the well before collecting liquid samples, the well plate had to be shaken before every sampling time. Shaking was performed by turning the well plate upside down three times, with a frequency of 1 turn/s. This was done approximately 30 s before sample collection, which allowed a suitable time-frame for the particle to settle at the bottom, and for positioning of the well plate, to allow particle imaging.

The pellets were weighed using an analytical balance (DeltaRange AX105, Mettler-Toledo GmbH, Greifensee, Switzerland), with an accuracy of 0.01 mg. The accuracy of the balance was affirmed by weighing ten pellets three times each and one pellet ten times. Dissolution experiments of each substance were performed in triplicate, in order to enable comparison between individual and collective single-particle dissolution data. This would allow the assessment of the reproducibility and accuracy of the method. The experiments are referred to as ASA/PCM/TP1-3 for ASA, paracetamol and theophylline, respectively.

An experiment was initiated by adding 3 ml of dissolution medium to a well with a pellet. Depending on the initial weight of the particle, sampling time-points were at 2, 5 and 10 min, and after this every 5 or 10 min. When approaching the end of the experiments in which 10 min intervals were used, sample collecting was again shifted to every 5 min, increasing the frequency of data points. Between two sampling-points the well plate was kept undisturbed. The time was recorded for both image capturing and liquid sample collecting, and the process was continued until the particle size had decreased, so that a pellet no longer could be found when repositioning the well plate.

2.3. Optical microscopy and image analysis

The USB-microscope (DigiMicro 2.0 Scale, dnt Drahtlose Nachrichtentechnik Entwicklungs- und Vertriebs GmbH, Dietzenbach, Germany) was connected to a computer via the PC USB port and controlled with the accompanying software (MicroCapture V2.0, dnt Drahtlose Nachrichtentechnik Entwicklungs- und Vertriebs GmbH, Dietzenbach, Germany). By inverting the USB-microscope and imaging a settled particle through the transparent bottom of the 24-well plate, it was possible to use a fixed magnification and resolution of 0.8 μm/pixel for all experiments (Fig. 1). Before the first experiment, an image was taken of a micrometer graticule for subsequent calibration of the scaling, and the graticule was imaged again before every experiment in order to