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Research paper

Online monitoring of dissolution tests using dedicated potentiometric sensors in biorelevant media

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ABSTRACT

The performance of the Ion-Selective Electrode (ISE) for *in vitro* dissolution testing using biorelevant media was evaluated in this study. *In vitro* dissolution was carried out using USP apparatus 2 (paddle method) with classical and with updated biorelevant media to simulate the pre- and postprandial states. The ISE was used as an analytical stand-alone system and in combination with a single-point HPLC–UV measurement. A modified method enabling the use of the ISE for very poorly soluble substances is also proposed.

In terms of f_2-factor, the results acquired using the ISE for the drug diphenhydramine-HCl were found to be very similar to the results obtained by manual sampling followed by HPLC–UV analysis. In Fed State Simulated Gastric Fluid (FeSSGF), a medium containing 50% milk, the ISE is more practical since the need to separate proteins from the analyte prior to HPLC–UV analysis is eliminated. Further work will be needed to establish ISE methodology for Fed State Simulated Intestinal Fluid (FeSSIF) media.

In summary, the ISE has promise as an analytical tool for research and development applications.

1. Introduction

Dissolution testing is an important analytical tool for the development of orally administered solid dosage forms. Originally introduced as a more discriminating, add-on test to the disintegration test, dissolution testing has become closely interwoven with the development of *in vitro–in vivo*-correlations [1]. Nowadays, dissolution testing is one of the most important tools in formulation development of new chemical entities.

According to the quality-by-design principle, it is essential to gather as much information about the dosage form as early as possible in the process of development. New dosage forms aiming at instant release of the drug tend to form a stable supersaturation (e.g. through complexation with cyclodextrins) or possess very rapid dissolution rates (e.g. melt extrudates and nanosized drugs) [2,3]. The latter formulations are often difficult to characterize accurately in dissolution testing. For instance, nanocrystals are often sufficiently small enough to pass through 0.45-μm filters, which are usually employed to separate the analyte from undissolved material [4]. In our laboratories, we have also observed that some lipid formulations and melt extrudates are hard to filter due to clogging of the filter. When just a limited number of samples are taken and the subsequent filtration proves to be difficult, the results are often incomplete and may even be misleading.

In other cases, it is not the dosage form itself that causes difficulties but the dissolution medium. Biorelevant media were introduced to investigate dissolution behavior and possible food effects of poorly soluble drugs [5,6]. In 2008, these media were updated to better resemble the human physiology and, among others, Fed State Simulated Gastric Fluid (FeSSGF) was introduced to simulate the fed state gastric fluids [7]. The use of milk as part of the dissolution medium in FeSSGF (50%) prohibits sample filtration through the standard pore size of 0.45 μm, since milk proteins clog the filter membrane. Therefore, a more labor-intensive sample preparation including filtration through larger pore size filters followed by protein precipitation, application of cosolvents and subsequent centrifugation has to be used [8]. This kind of sample preparation prohibits accurate characterization of the dissolution of nanosized drugs because the filter pore size used is 2.7 μm, which is by definition far larger than the average size of a nanosized API. Other authors have reported techniques as well to separate dissolved

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from solid drug in milky media, but none of them appears to be less labor-intensive or appropriate for very rapidly dissolving drug formulations [9,10].

For all these reasons, continuous *in situ* monitoring of the dissolution process would be highly desirable.

To monitor a dissolution process online, flow-through UV analysis and fiber optics can be employed, but both these methods are sensitive to light scattering effects caused by the medium or the formulation [11]. Since there is no filtration step in these methods, the baseline must be corrected for UV measurement [12].

In 2007, Bohets et al. introduced a potentiometric, Ion-Selective Electrode sensor system (ISE) to monitor the process of dissolution online. The ISE is sensitive for a given charged model drug (i.e. each ISE is conditioned for its target drug analyte), but insensitive to uncharged molecules and undissolved material [13]. Peeters et al. have shown that these potentiometric sensors are suitable for dissolution testing of various drugs (loperamide, cinnarizine and domperidone) in simple buffer media. Moreover, ISE can be used to obtain accurate results in turbid media [14]. It is not the first time an electrode has been used for monitoring the dissolution of a drug [15,16], but this is the first electrode which can be applied to any ionizable lipophilic drug since specificity is obtained by conditioning.

In this article, we utilized the ISE with two objectives:

(A) To test the suitability of the Ion-Selective Electrode as an analytical method which can be directly applied in biorelevant media, as an alternative to sampling with subsequent analysis by conventional methods such as HPLC–UV (Method A).
(B) To investigate the ISE as an analytical system to obtain a full dissolution profile in biorelevant media with the aid of one single draw which is analyzed by a conventional method, here HPLC–UV (Method B).

Dissolution profiles obtained by using ISE were compared to those acquired by HPLC–UV assay after conventional, manual sampling.

2. Materials and methods

2.1. Chemicals

Diphenhydramine-HCl (DPH) was chosen as the model drug. The dissolution tests were carried out with Nustasium© tablets (lot # 07H30) containing 50 mg diphenhydramine-HCl from Labima, Belgium. Diphenhydramine-HCl drug substance, NaCl, NaOH, glacial acetic acid, NaH_2PO_4, H_3PO_4 KH_2PO_4 and acetonitrile were purchased by Sigma–Aldrich, Germany. Egg phosphatidylcholine (Lipoid E PC®, 97.9% pure, lot # 108015-1-/042) was kindly donated from Lipoid GmbH, Ludwigshafen, Germany. Glycerylmonooleate (GMO, Rylo MG19 Pharma®, 99.5% monoglyceride, lot # 173403-2202/107) was provided by Danisco Specialities, Brabrand, Denmark. Hydrochloric acid (31–33%) was obtained from Hedinger, Stuttgart, Germany. Ortho-phosphoric acid (85%) and pepsin (Ph. Eur., 0.51 U/mg, lot # 1241256) were purchased from Fluka Chemie AG, Buchs, Switzerland. Sodiumoleate (82.7% pure, lot # 51110) was obtained from Riedel-de Haën, Seelze, Germany. Sodium taurocholate (NaTC, >97% pure, lot # 2007100274) was purchased from Prodotti Chimici e Alimentari SpA, Basaluzzo, Italy. Long-life whole milk was obtained from Milfina, Germany and from Lidl, Germany.

2.2. Filter adsorption studies

Filter adsorption of diphenhydramine-HCl onto regenerated cellulose filters (Minisart© RC 25, 0.2 μm, lot # 17764, Sartorius, Germany) was investigated by filtration of each medium at a concentration of about 100 μg/ml diphenhydramine-HCl. The samples were analyzed by HPLC–UV and compared to the unfiltered medium ($n = 3$) [17].

2.3. Calibration of the Ion-Selective Electrodes

Ion-Selective Electrodes were provided by Janssen Pharmaceutica and have been described previously by Bohets et al. [13]. Prior to dissolution testing, the electrodes were conditioned at 37 °C in the dissolution medium at a DPH concentration of about 110 μg/ml for at least 48 h. In preliminary experiments, it had been shown that this time-frame was sufficient to condition the ISE to DPH in simple buffer media (no data shown). The DPH concentration is equivalent to 110% dissolution of Nustasium© tablets. This procedure resulted in low drift and fast response of the ISE to DPH. Calibration curves were constructed by stepwise addition of a standard solution into a vessel containing 500 ml of the test medium. Two milliliters of aliquots were added in five steps, with each 2 ml containing approximately 11 mg of DPH. As in the conditioning step, the final concentration was approximately 110% of the concentration expected at the end of the dissolution of Nustasium© tablets. Dissolution tests were initiated when the correlation coefficient (R^2) of the linear fit of the calibration data exceeded 0.9995. A secondary criterion was that the mean slope of this calibration curve did not exceed the value of 63 mV. Furthermore, the sensor response obtained from the stepwise addition of the standards had to be fast and stable; $t_{90} < 60$ s and drift less than 0.3 mV for the 2nd to 5th addition.

2.4. Dissolution testing

Dissolution tests were carried out at 37 °C with a USP type II (paddle) dissolution tester Erweka R6©, (Erweka, Heusenstamm, Germany). In some experiments, 2 or 3 ISE were placed in a single dissolution vessel to evaluate reproducibility among electrodes. In other experiments, the ISEs were compared to manual sample removal and subsequent HPLC analysis. Manual samples were taken at 5, 10, 15, 20, 25, 35 and 45 min without volume replacement. The filtered samples were diluted appropriately with mobile phase.

2.5. Quantitative analysis

2.5.1. Ion-Selective Electrodes (ISE)

The conversion of the measured potential to percentage dissolution was carried out according to the procedure described in Bohets et al. [13], using an in-house Potential-to-Concentration software (LabView©, Version 6.1, National Instruments). The system is able to measure the electrochemical potential of the solution every four seconds. An endpoint calibration to correct the drift of the ISE system was conducted in two different ways:

Method A: With this method, the ISE was placed into a solution containing a known concentration of DPH, typically the calibration solution, after completion of the dissolution run.
Method B: With this method, a manual sample was drawn at one time-point (corresponding approximately to the completion of drug release, e.g. t_{45} for Nustasium© tablets) and the concentration of DPH was determined by HPLC–UV. The dissolution profile was calculated on the basis of this value.

2.5.2. HPLC–UV

Samples from dissolution test were analyzed by HPLC–UV. The HPLC–UV-System consisted of a LaChrom© L-7100 pump, a LaChrom© L-4250 UV–Vis-Detector, a LaChrom© L-200 autosampler