In Situ Solubility and Dissolution Measurements of Highly Concentrated Solutions

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INTRODUCTION

In situ UV concentration monitoring proved to be an indispensable tool in pre-formulation and final drug product testing. However, the method has its limitations when it comes to measuring high concentrations of the active pharmaceutical ingredients (API) due to saturation of the UV detector. The aim of this study was to evaluate applicability of Attenuated Total Reflection (ATR) UV probes to accurately measure highly concentrated solutions in real time with no need for dilution and solid separation.

MATERIALS AND METHODS

ATR probes use the principle that light crossing the interface between materials with high and low refractive index is mostly reflected, but it is also refracted (Figure 1). The ratio of refracted and reflected energies at a particular wavelength depends on the molar absorptivity and concentration of compound dissolved in the solution and this value could be used as a measurable characteristic of compound concentration in the solution. ATR probes were attached to the µDISS Profiler™ instrument (Figure 2, Pion) for concentration measurements in aqueous buffers and organic solvents (e.g., DMSO, 1-Propanol, Ethanol). In-Spec® Visible Standard (VIS-1.0) was used to simulate turbidity in the solution.

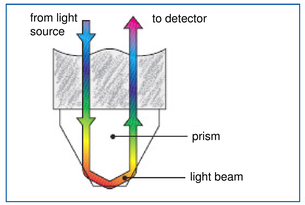

Figure 1. Simplified schematic of the ATR probe detection principle. Unlike in conventional dip-probe the light does not passes the solution.

Figure 2. The µDISS Profiler monitors concentration in real time with ability to dynamically change media in 8 temperature controlled vessels using only 1 – 20 mL volume.

RESULTS AND DISCUSSION

Solubility of Metoclopramide in DMSO

DMSO is often used as an organic solvent to store concentrated solutions (1 - 100 mM) of research compounds and use small aliquots of such "stock" solutions diluted in aqueous media to prepare analytical solutions for various measurements. However, if compound is not soluble in the DMSO then its concentration after dilution in the aqueous buffers become uncertain and all consequent tests may have misleading outcome. Thus, independent measurement of the drug concentration in the DMSO becomes an important task. ATR probes were applied to measure metoclopramide concentration in DMSO without *in situ* without any additional dilution step.

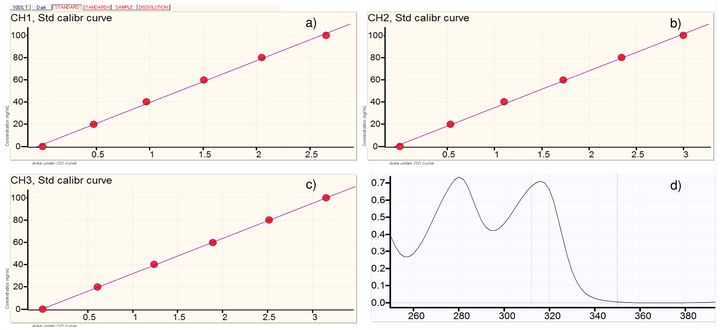

Figure 3. Standard curves shown in the reversed axis (concentration, mg/mL, versus absorbance) for Metoclopramide in DMSO a) – c) and spectrum of Metoclopramide at 100 mg/mL concentration d).

Figures 3 a) – c) show standard curves for Metoclopramide in the 0 – 100 mg/mL concentration range while Figure 3 d) shows an example of Metoclopramide spectrum in DMSO at concentration 100 mg/mL measured by ATR probes. It can be seen that absorbance level was below saturation (usually ~ 2.0 AU).

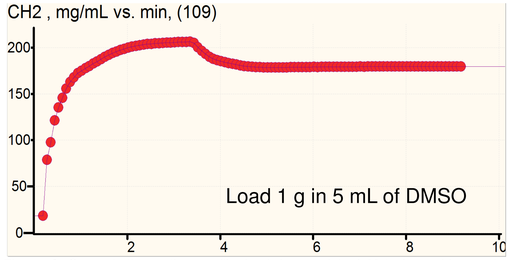

Figure 4. Concentration – time profile (mg/mL vs. min) of Metoclopramide powder dissolving in pure DMSO.

Figure 4 shows dissolution profile of Metoclopramide powder dissolving in pure DMSO. Data suggests that after a short supersaturation phase concentration equilibrates at ~ 180 mg/mL that was considered to be solubility of Metoclopramide in DMSO.

Effective Path Length Equivalent of ATR Probes

According to the Beer-Lambert's law an absorbance $AU(\lambda)$ at a particular wave length λ is proportional to the concentration of dissolved compound c, path length l and molar absorptivity of the compound at this wave length $\varepsilon(\lambda)$:

$$AU(\lambda) = cl\varepsilon(\lambda) \qquad (1)$$

When measuring absorbance using ATR probes the light does not passes through the liquid phase, so the path length is not defined parameter in the conventional meaning of this term. However, by comparing absorbance measured with ATR probe to one measured by a conventional method (e.g. with a dip-probe equipped with a particular path length tip) one can estimate so-called "effective" path length, that is a path length of a conventional absorbance measurement that would measure the same absorbance as ATR probe if immersed in the same solution. For example, if $AU_{ATR}(\lambda)$ is absorbance at wave length λ measured with ATR probe in the solution with concentration c_{ATR} and $AU_{conv}(\lambda)$ is absorbance measured by a conventional method in the solution with concentration c_{conv} using path length l_{conv} then effective path length of ATR probe, l_{ATR}, can be calculated using equation (2):

$$l_{ATR} = \frac{AU_{ATR}(\lambda)\cdot c_{conv}\cdot l_{conv}}{AU_{conv}(\lambda)\cdot c_{ATR}} \qquad (2)$$

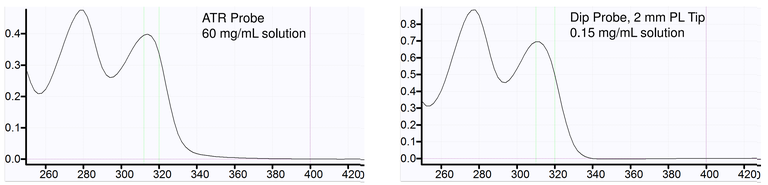

Figure 5. UV absorbance spectrum of 60 mg/mL solution of Metoclopramide in DMSO measured using ATR probe (left) and 0.15 mg/mL solution measured using 2 mm path length tips (right).

Figure 5 illustrates the principle of effective path length calculation using equation (2). For example, taking absorbance value at 312 nm and using Eq. (2) it is easy to estimate l_{ATR}:

$$l_{ATR} = \frac{0.4\cdot 0.15\cdot 2}{0.7\cdot 60} \approx 0.003\,mm \qquad (3)$$

Averaging calculations over several measurement channel gave the following estimation for the effective path length for the ATR probe:

$$l_{ATR} \approx 3.4 \pm 0.4\,\mu m$$

Measurements in Extremely Turbid Solutions

Ranitidine was used to study the effect of high turbidity on the accuracy and precision of measurements using ATR probes. Figure 6 shows standard curve of Ranitidine in the range 0.0 – 50.6 mg/mL collected in the clear buffer (USP pH 6.8 buffer). After that the highest standard concentration was diluted 2 fold with VIS-1.0 standard solution that has a broad Tyndall-like spectrum to simulate high turbidity in the solution.

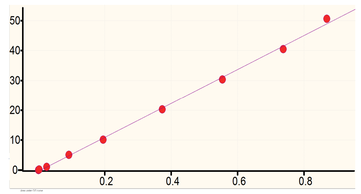

Figure 6. Standard curves shown in the reversed axis (concentration, mg/mL, versus absorbance) for Ranitidine in USP pH 6.8 buffer

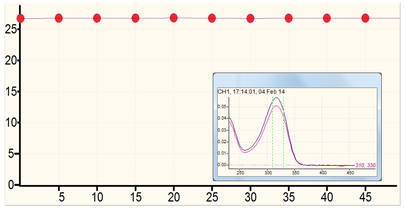

Figure 7. Concentration of Ranitidine measured in the very turbid solution using standard curve presented on Figure 6. Insert shows comparison of UV spectra in the turbid solution (pink) and in the clear solution (blue).

Figure 7 shows a series of scans of dissolved Ranitidine in the turbid solution containing 1/1 ratio of the buffer and VIS-1.0. Concentration was determined using the standard curve shown on Figure 6. An area under the direct spectrum (310 – 330 nm) was used in the concentration calculation. Reported concentration of 26.7 mg/mL was very close to expected concentration of 25.3 mg/mL. An insert in the Figure 7 demonstrates that direct UV spectrum of Ranitidine in the turbid solution (pink line) was not distorted comparing with spectrum of the standard measured in clear buffer (blue line).

CONCLUSIONS

Study suggested that ATR probes can become a useful addition to *in situ* concentration monitoring capabilities of µDISS Profiler for measuring solubility and dissolution of highly concentrated solutions (1 – 200 mg/mL) of drug compounds in various media.

The probes can handle high level of turbidity much better than conventional dip-probes.

This detection technique cannot provide enough sensitivity for solutions with concentration below about 1 mg/mL.

For concentrations below 2 mg/mL the conventional dip probes with short wave length tips provide better sensitivity than ATR probes.

More research is needed to establish the range of applications for ATR probes and better understand benefits and limits of their usage.