M1030-06-38

Novel Method for Monitoring of Free Drug Concentration in Presence of Nano-sized Formulations or Dynamically Formed Colloidal Nanoparticles

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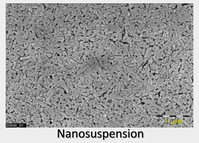
PURPOSE

The presence of **nanoscale particles in suspensions** could increase flux through biological membranes for poorly soluble compounds [1] and as a result improve their **oral absorption and bioavailability**. Hence, there is a need for *in vitro* method that aid in the understanding of dynamic nanosuspension behavior. This is challenging as nanoparticles both scatter and absorb light, obscuring *in situ* fiber optic UV measurements. That can lead to wrong interpretations of UV absorbance data and as a result erroneous concentration estimates. The goal of this study was to continue developing a method of de-convoluting the UV-Vis spectra measured *in situ* to obtain the **concentration of free drug in the presence of light absorbing nanoparticles.**

METHOD(S)

(1) Determining the **solubility nanoparticles** or **LLPS concentration** by **Zero Intercept Method** (ZIM [2]);
(2) Subtracting the absorbance spectrum of free drug at its solubility value from the combined spectrum of free drug+nanoparticles to obtain the **absorbance spectrum of nanoparticles;**
(3) Building a **standard curve for nanoparticles**.
(4) Performing **multicomponent regression analysis** to extract the concentration of **free drug** and quantify amount of **nanoparticles** present in the suspension.

Figure 1. µDISS Profiler used for *in situ* UV measurements.



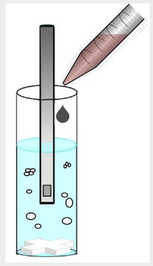
Nanosuspension (Water+HPC+SDS)

Figure 2. Electron microscopy pictures of 10% w/v naproxen nanosuspension used in this work [2].

The µDISS Profiler™ instrument run by AuPRO™ software version 6.0 (Pion Inc.) was used to monitor UV-VIS spectra *in situ*. ZIM implemented in the AuPRO™ software determined the concentration when the second absorbing component (i.e. nanoparticles) appeared along with dissolved API.
Nanosuspension of crystalline naproxen (NPX) was received from Novartis AG (Basel, Switzerland) and used to evaluate the method using aqueous pH 2.0 and pH 5.0 buffers. A solvent shift method was used to form amorphous precipitations of felodipine (FLD) in pH 6.5 buffer and albendazole (ABZ) in FaSSIF.

RESULT(S)

Amorphous or Nanocrystalline Solubility by ZIM



Aliquots of the concentrated solution in organic solvent (stock solution) or crystalline nanosuspension are added to the media of interest and 2nd derivative of the UV spectra are monitored.
Figures 3 – 4 demonstrate changes in the shape of the 2nd derivative of the absorbance spectra due to appearance of the extra absorbing component, e.g. nanocrystalline naproxen (Fig. 3) or drug-rich colloidal phase of felodipine (Fig. 4).

Figure 3. Schematic of the serial addition with simultaneous monitoring of the UV spectra using fiber optic UV probe in the solution

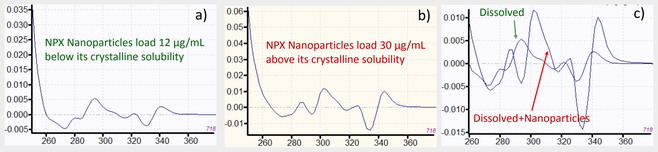


Figure 4. 2nd derivative of the absorbance spectra of fully dissolved naproxen (a), dissolved naproxen in the presence of nanoparticles (b) and their superposition illustrating spectral change (c).

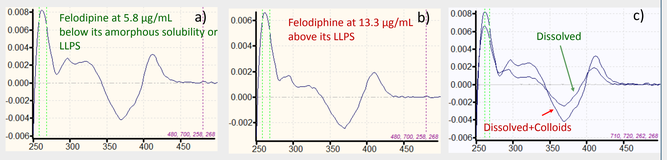


Figure 5. 2nd derivative of the absorbance spectra of fully dissolved felodipine (a), spectrum of felodipine at concentration above its LLPS (b) and their superposition illustrating spectral change (c).

ZIM points, i.e. wavelengths where 2nd derivative of fully dissolved API spectra crosses the zero absorbance line, were determined using AuPRO™ software. Concentration where signal begins to build up at these points determine the kinetic solubility of the studied API form.

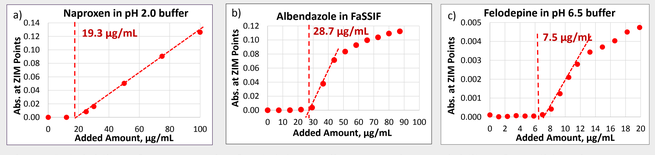


Figure 6. Solubility of nanocrystals of NPX (a) as well as amorphous solubility of ABZ (b) and FLD (c) determined by ZIM method.

Spectra of Nanoparticles or Amorphous Liquid Phase

The steps of reconstructing the spectral characteristics of newly forming absorbing phase (e.g., nanoparticles) are illustrated on Figure 7 using NPX as an example. The procedure is automated in the AuPRO™ software version 6.0.

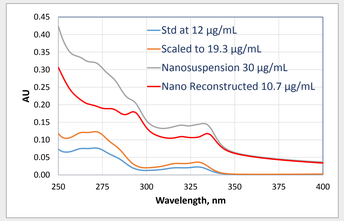


Figure 7. Reconstructing direct absorbance spectrum of nanoparticles: NPX spectrum at 12 µg/mL (blue) is scaled up to its saturated concentration of 19.3 µg/mL (orange) and subtracted from the nanosuspension spectrum at 30 µg/mL (grey) to obtain the spectrum of nanoparticles at 10.7 µg/mL (red).

Multi-component Regression

Theoretical Background

The method is based on modified classical least squares (CLS) technique to determine a contribution of known spectra (a.k.a. standard spectra) in the superposition of these spectra by minimizing the difference:

$$\chi^2 = \sum_{\lambda_1}^{\lambda_2}\left(A_{Measured}(\lambda) - \sum_{i=1}^{N} x_i A_{st,i}(\lambda)\right)^2$$

where $A_{Measured}(\lambda)$ is absorbance at wavelength λ of a sample containing N absorbing component, $A_{St,i}(\lambda)$ is absorbance of a standard for component i and x_i is a coefficient to be determined by the regression procedure that shows the contribution of each standard component into their mixture.
Implementation of the method in the AuPRO Advanced (Ver. 6.0) software was used in the study.

Evaluation - Naproxen Nanoparticles

Evaluation of the method was performed using NPX nanosuspension of 100 µg/mL load in the pH 2.0 buffer and it is shown on Figure 8.

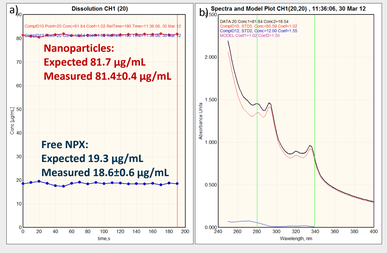


Figure 8. Multi-component regression toolbox in AuPRO™ software: concentration of components versus time (a) on their corresponding standard spectra (b). Black spectrum (b) is measured suspension sample (superposition of nanoparticles and free API) at a particular time point.

Monitoring of Dynamic Changes in Colloidal Nanoparticles after Liquid-Liquid Phase Transformation (LLPS)

Both FLD and ABZ formed LLPS at their amorphous solubility concentration. Example for the evolution of FLD colloidal particles are shown on Figure 9. The spectrum of the newly formed colloidal phase was characterized (red spectrum on the Figure 9, b) and then the spectrum of FLD emulsion was monitored over a period of 16 hours (Figure 9, a). Interestingly, precipitation of FLD to its crystalline solubility value (~1 µg/mL, blue dots on Figure 9, a) after 4 hours coincided with the disappearance of additional spectral influence from nanoparticles (red dots on Figure 9, a) indicating the formation of larger scale particles that only scatter light without absorbing it.

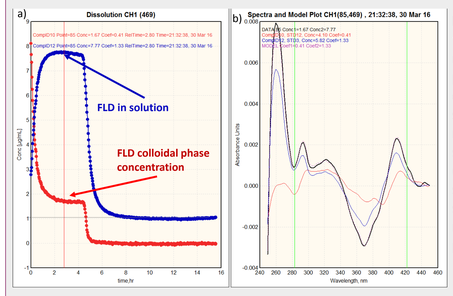


Figure 9. Concentration of free FLD (blue dots, a) and nanodroplets [3] of API rich phase (red dots, a). 2nd derivative spectra (b): FLD standard (blue curve), reconstructed nanoparticles standard (red curve), model (i.e. superposition of standards, dashed line) and sample (black curve) measured at a selected time point.

CONCLUSION(S)

A novel **method** enabled **simultaneous concentration monitoring** of dissolved **drug and nanoparticles** that could be present during dissolution/precipitation processes.

The method was implemented in the **AuPRO™ software** and expanded capabilities of in situ fiber optic technique.

REFERENCES

1. Stewart AM, et al. Impact of Drug-Rich Colloids of Itraconazole and HPMCAS on Membrane Flux in Vitro and Oral Bioavailability in Rats. Mol Pharm. 2017 Jul 3;14(7):2437–49.
2. Tsinman K, Tsinman O, Riebesehl B, Grandeury A and Juhnke M. In Situ Method for Monitoring Free Drug Concentration Released from Nanoparticles. Poster presented at AAPS Annual Meeting and Exposition. November 10 - 14, 2013, San Antonio, TX.
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