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Simultaneous *in situ* monitoring of free drug concentration and nanoparticles during dissolution testing of nanocrystalline and amorphous formulations

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PURPOSE

It has been demonstrated that the presence of nanoscale colloidal particles in suspensions could increase flux through biological membranes for poorly soluble compounds [1] and as a result improve their oral absorption. Hence, there is a need for in vitro tests that aid in the understanding of nanosuspension behavior. This is challenging as nanosized particles are difficult to separate by filtration or cetrifugation, they could absorb light obscuring *in situ* UV measurements often leading to erroneous concentration measurements. The goal of this study was to develop a method of de-convoluting the UV-Vis spectra measured *in situ* to not only obtain the concentration of free drug in the presence of light absorbing nanoparticles but also to quantify the concentration of nanoparticles present in suspensions.

METHOD(S)

The **µDISS Profiler™** instrument (Figure 1) controlled by **AuPRO™** software (Pion Inc.) was used to collect and analyze UV-VIS spectra. Nanosuspensions of Naproxen (NPX) and Griseofulvin (GSF) were obtained from Novartis AG and shown on Figure 2.

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

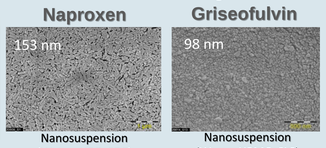
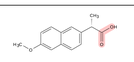
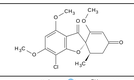
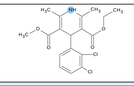


Figure 2. Electron microscopy pictures of nanosuspensions used in this work [2].

Structures and physicochemical properties of studied molecules shown in Table 1.

Table 1. Structures and measured physicochemical properties of compounds used in this study.

Compound	Structure	MW	pK_a	log P
Naproxen (NPX)		230	4.2(A)	3.3
Griseofulvin (GSF)		353	NA	2.2
Felodipine (FLD)		384.3	NA	5.6

RESULT(S)

Analytical Steps

1) Zero Intercept Method **(ZIM)** of 2nd derivative spectroscopy [2] was used to determine concentration of free drug in equilibrium with nano-crystalline particles (NPX, GSF) or amorphous solubility of FLD above which the 2nd liquid phase was formed [3].
2) Identifying **spectral characteristics of nanoparticles** present in the suspension or emulsion.
3) Use **multi-component regression** analysis to determine the concentration of free drug and nanoparticles ***in situ*** as a function of time.

(1) Zero Intercept Method (ZIM)

ZIM points, i.e. wavelengths where 2nd derivative of API spectra crosses the zero absorbance line, were determined using AuPRO™ software and shown in Figure 3.

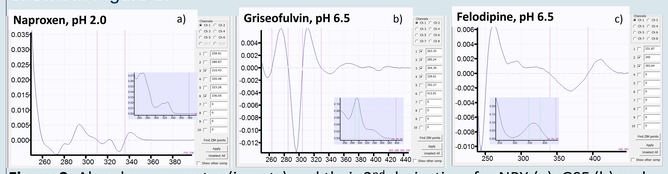


Figure 3. Absorbance spectra (inserts) and their 2nd derivatives for NPX (a), GSF (b) and FLD (c) in aqueous media. Selected ZIM points are indicated by dashed vertical lines.

Aliquots of nanosuspension (NPX and GSF) or concentrated methanol solution of FLD were added to the corresponding media and the sum of absolute values of 2nd derivative absorbance at selected ZIM points were monitored (Figure 4).

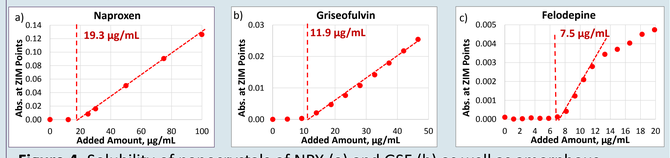


Figure 4. Solubility of nanocrystals of NPX (a) and GSF (b) as well as amorphous solubility of FLD (c) determined by ZIM method. Below the vertical dashed line only free API is present in the solution while above it there is at least one additional absorbing phase (e.g. nanoparticles for NPX and GSF and API-rich liquid phase for FLD.

The formation of the new (absorbing) phase was indicated when 2nd derivative deviated from the zero absorbance line as indicated on Figure 4.

(2) 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 5 using NPX as an example. The procedure is automated in the AuPRO™ software version 6.0.

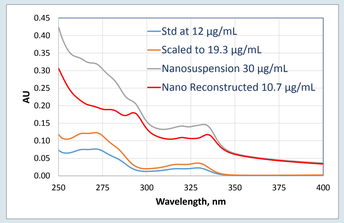


Figure 5. Reconstructing direct absorbance spectrum of nanoparticles: NPX spectrum at 12 µg/mL (blue) is scaled up to its saturated concentration of 19.3 µg/mL (orange) and subtracted from the nanosuspension spectrum at 30 µg/mL (grey) to obtain the spectrum of nanoparticles at 10.7 µg/mL (red).

The standard curves generated for nanoparticles of NPX, GSF and FLD are shown below on Figure 6.

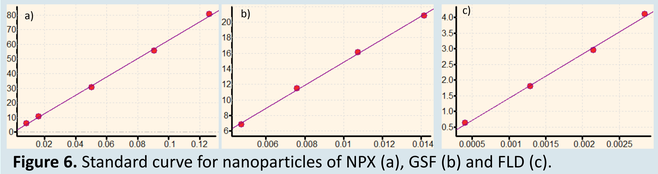


Figure 6. Standard curve for nanoparticles of NPX (a), GSF (b) and FLD (c).

(3) 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 procedure that shows the contribution of each standard component into their mixture.
The procedure has been implemented in the AuPRO version 6.0.

Validation, Naproxen

Validation of the method using NPX nanosuspension of 100 µg/mL load.

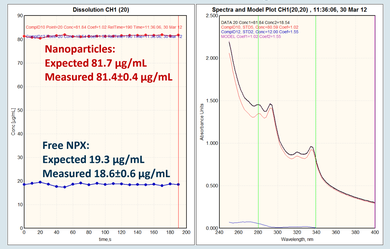


Figure 7. Multi-component regression toolbox in AuPRO™ software: concentration of components versus time on the left and their corresponding standard spectra on the right. Black spectrum is measured sample (superposition of nano-particles and free API) at a particular time point.

Liquid-Liquid Phase Separation of Felodipine

The spectrum of the newly formed liquid phase was characterized (red spectrum on the Figure 8, b) and then the spectrum of FLD emulsion was monitored over a period of 16 hours (Figure 8, a). Interestingly, precipitation of FLD to its crystalline solubility value (~1 µg/mL, blue dots on Figure 8, a) after 4 hours coincided with the disappearance of additional spectral influence from nanoparticles (red dots on Figure 8, a) indicating the formation of larger scale particles that only scatter light without absorbing it.

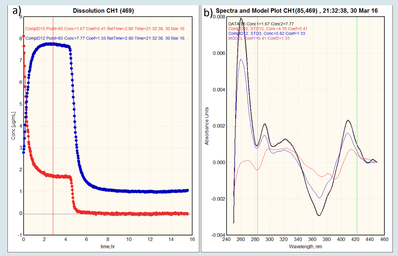


Figure 8. (a) Concentration of free FLD (blue dots) and nanodroplets [3] of API rich phase (red dots). (b) 2nd derivative spectra: FLD standard (blue curve), reconstructed nanoparticles standard (red curve), model (i.e. superposition of standards, dashed line) and sample measured at a selected time point.

CONCLUSIONS

A **novel analytical method** was developed that enabled *in situ* **simultaneous concentration monitoring of dissolved drug and nanosized** light absorbing **particles** that could be present during dissolution/precipitation processes especially in amorphous formulations.

The method added **new functionality** to fiber optic *in situ* concentration monitoring available with the **µDISS Profiler™** instrument and **AuPRO™ software**.

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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