# Dissolution, free drug concentration and permeability of crystalline nanoparticle formulations: study using *in situ* fiber optic and potentiometric techniques.

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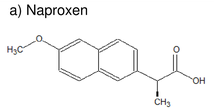
## INTRODUCTION

Recent studies have demonstrated that compound specific potentiometric sensors can be used for real time concentration monitoring in challenging opaque media often replacing time consuming HPLC methods[1]. The purpose of this study was to determine applicability and limitations of two *in situ* methods (spectroscopic UV-vis and potentiometric) in their ability to measure free drug concentration in the presence of nanoparticles.

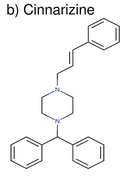
## MATERIALS AND METHODS

a) Naproxen



Acid: $pK_a$ 4.32; logP 3.24

b) Cinnarizine



Base: $pK_a$ 7.69, 2.55; logP 5.71

**Figure 1.** Chemical structure and phys.-chem. properties of NPX and CNZ

**Figure 2.** Free Drug Sensor and Reference electrode in the solution.

Nanoparticles of naproxen (NPX, Figure 1 a) and cinnarizine (CNZ, Figure 1 b) were prepared as 10 wt% suspensions in DI water with small amount (< 3 wt%) of nanoparticles stabilizing excipients. Free Drug Sensors, FDS (Figure 2, Pion Inc.) were conditioned in the solutions of NPX and CNZ at the pH values where compounds were fully ionized. After conditioning electrodes became specific to the drugs they had been conditioned with and could be used to measure concentration of these compounds *in situ* through monitoring of the change in mV response of the sensors in different concentrations of corresponding solutions of CNZ and NPX. µFLUX Profiler™ fitted with µFLUX apparatus (Figure 3, Pion Inc.) was used to measure simultaneously dissolution characteristics in donor compartment and flux of API through membranes in the receiver chamber.

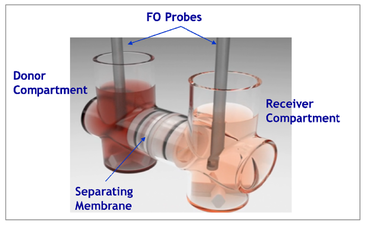



**Figure 3.** A schematic of the µFLUX apparatus showing a pair of the donor and receiver chambers. FO probes attached to the µDISS Profiler monitor concentrations in the donor (left) and receiver (right) compartments. The chambers can be separated by artificial, cell-based size exclusion, or other types of membranes mounted in the Membrane Holder.

Each pair (Figure 3) of a donor and an receiver compartment were separated by a filter-supported artificial membrane (Double-Sink™ PAMPA[3]) with 1.5 $cm^2$ surface area. The donor compartment was filled with 20 mL of the media of interest while the receiver compartment contained the same volume of Acceptor Sink Buffer at pH 7.4 (ASB-7.4, Pion Inc). The integrated fiber-optic UV probes were positioned in the donor and receiver compartments allowing real time concentration monitoring in all chambers. Zero Intercept Method (ZIM)[2] analysis was performed using Au PRO™ software version 5.1 (Pion).

## RESULTS AND DISCUSSION

### *Particle Size, Morphology and Crystallinity*

**Table 1.** Particle size distribution by laser diffraction for micronized and untreated CNZ and NPX.

| Powder | $x_{10}$ µm | $x_{50}$ µm | $x_{90}$ µm |
|---|---|---|---|
| CNZ-Untreated | 41 | 133 | 461 |
| CNZ-Micronized | 0.7 | 2.5 | 5.3 |
| NPX-Untreated | 3.2 | 12 | 40 |
| NPX-Micronized | 0.9 | 2.6 | 6.1 |

**Table 2.** Mean particle size and polydispersity index (PI) by dynamic light scattering for CNZ and NPX nanosuspensions.

| Nanosuspension | Mean Size, nm | PI |
|---|---|---|
| CNZ | 232 | 0.20 |
| NPX | 153 | 0.13 |

XRPD analysis confirmed the same crystalline structure for micronized and nano-sized NPX and CNZ.

### *Nanoparticles Solubility by Zero Intercept Method (ZIM™)*

Nanoparticles in solution absorb light[2,4] effectively acting as additional component to the dissolved API. To determine concentration of dissolved API in the presence of nanoparticles, a special analysis of the second derivative spectrum (*Zero Intercept Method*, ZIM™) was developed[2]. In ZIM, the 2nd derivative absorbance values at wavelengths where the corresponding spectra of fully dissolved API, $AU''_{API}(\lambda_{ZIM})$ equal to 0 (cross the wavelength axis, Figure 4) is plotted versus amount of nanoparticles added. At these special wavelengths only nanoparticles contribute to the second derivative spectrum, i.e.: $AU''_{Total}(\lambda_{ZIM}) = AU''_{Nano}(\lambda_{ZIM})$. The plot of $\sum|AU''_{Total}(\lambda_{ZIM})|$ versus load of API from nanosuspension (µg/mL) will consist of horizontal portion where only dissolved API is present (first 2 points on Figure 5) and a sloping line when nanoparticles remain un-dissolved in the media (dashed line on Figure 5).

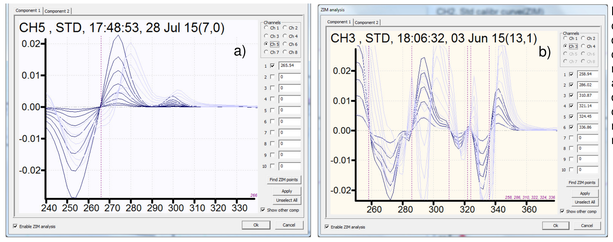
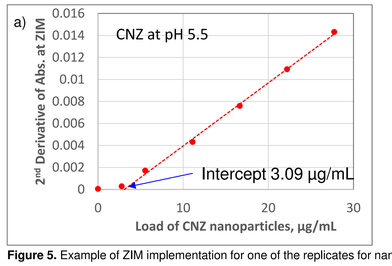



**Figure 4.** Zero intercept points of CNZ (a) and NPX (b) used to determine solubility of these compounds from corresponding nanosuspension. Black lines are 2nd derivative spectra of dissolved API at various concentrations while grey lines represent different additions of nanosuspensions.



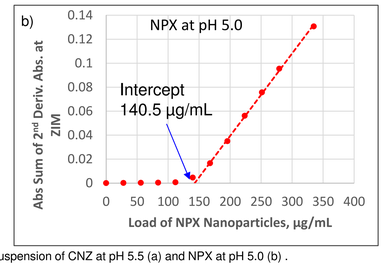




**Figure 5.** Example of ZIM implementation for one of the replicates for nanosuspension of CNZ at pH 5.5 (a) and NPX at pH 5.0 (b) .

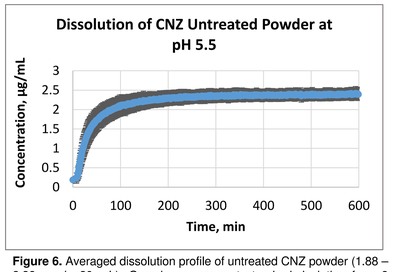



**Figure 6.** Averaged dissolution profile of untreated CNZ powder (1.88 – 2.38 mg in 20 mL). Grey bars represent standard deviation from 8 measurements.

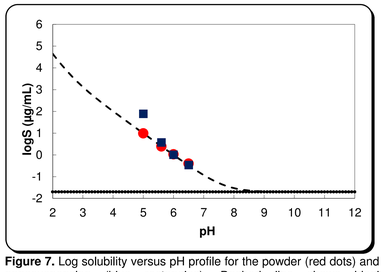



**Figure 7.** Log solubility versus pH profile for the powder (red dots) and nanosuspension (blue rectangles). Dashed line shows ideal Henderson-Hasselbalch dependency while dotted line represents extrapolated intrinsic solubility of CNZ (20 ng/mL).

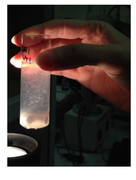


**Figure 8.** Appearance of the solution at pH 5.0 after addition of ~ 50 µg/mL load of nanosuspension.

Solubility of CNZ nanosuspension determined by ZIM method was compared to the solubility of micronized and untreated powder determined from powder dissolution experiment (Figures 6 and 7).
At pH 5.0 nanosuspension precipitated forming visible particles as depicted on Figure 8 while at other studied pH values solutions remained homogeneously opalescent what was characteristic for nanoparticles present in the solution. Formation of larger particles may be responsible for the overestimation of the CNZ nanosuspension solubility measured by ZIM method, i.e. nanoparticles got converted into bigger conglomerates thus no contributing any more to the 2nd derivative spectrum.

### *In Situ Concentration Measurements using Free Drug Sensors (FDS™)*

Part of the study was aimed to investigate the ability of FDS to measure dissolved (free) drug concentration in the presence of nanoparticles. Media at pH 5.5 was selected to prove the concept.

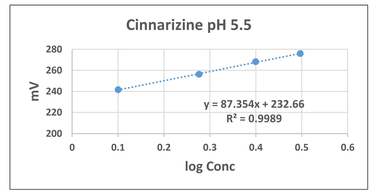



**Figure 9.** Standard curve (FDS mV response versus logarithm of concentration) from adding of pre-dissolved CNZ into pH 5.5 buffer. Concentration range 0.63 – 3.14 µg/mL.

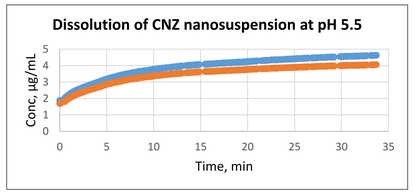



**Figure 10.** Dissolution profile of CNZ added from CNZ-nanosuspension (11.1 µg/mL load). Orange dots represent measurements corrected for the mV drift of FDS reading over time by exposing FDS to the standard solution of 3.14 µg/mL before and after the measurements.

Standard curve for CNZ was constructed by adding aliquots of pre-dissolved stock solution to pH 5.5 buffer while measuring mV response of FDS (Figure 9). After that FDS was placed in the media free of drug and 2 µL of nanosuspension (110 mg/mL CNZ nanoparticles load) was added to the media. Standard curve from Figure 9 was used to convert mV reading into concentration (Figure 10).

### *Flux of CNZ through Double Sink™ PAMPA Membrane*

The slope of concentration-time profiles in the receiver compartment can be used to calculate the flux of CNZ through artificial membrane at particular time point. As evident from Figure 11, flux of CNZ from nanosuspension and suspended micronized powder increased over time that could be explained by reduction in particle size as CNZ is partitioned to and penetrates through PAMPA membrane. It should be noted that change in the 2nd derivative spectra co-insided with increasing flux could indicate that suspended micronized powder began forming into nano-sized powder in the course of the flux experiment.

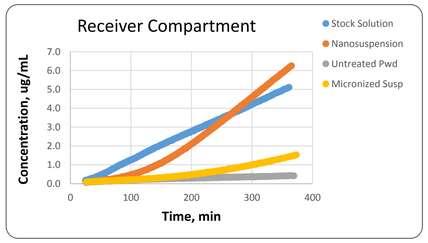



**Figure 11.** Concentration-time profile of CNZ in receiver compartment of µFLUX system when introduced as 50 µg/mL load from different forms .

## CONCLUSIONS

Using Zero Intercept Method it was possible to determine concentration of dissolved CNZ and NPX in equilibrium with nano-particles at pH 5.0 – 6.5.

Free Drug Sensors were able to measure concentration of free drug (CNZ) in the presence of nanoparticle at pH 5.5.

Flux from nanosuspension of CNZ was increasing over time surpassing flux from pre-dissolved CNZ.

Flux from suspended micronized powder of CNZ was increasing over time surpassing flux from untreated CNZ powder

## REFERENCES

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