In Situ Method for Monitoring Free Drug Concentration Released from Nanoparticles

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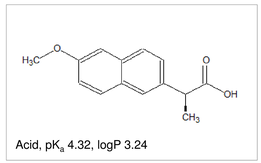
INTRODUCTION

Determining concentration of API released from nanoparticles presents a challenge as conventional solid separation techniques become unsuitable while nano-scale size of the particles influences the UV spectrum for UV-based techniques. A Zero Intercept Method (ZIM) presented in this work overcome these challenges allowing *in situ* concentration monitoring of free API as it is being released from nanoparticles.

MATERIALS AND METHODS



Acid, pK_a 4.32, logP 3.24

Figure 1. Chemical structure and physico-chemical parameters of naproxen.

Naproxen (API, Figure 1) was used as a model compound for the study. Spray-dried micronized (45.8% API load) and nano-sized (~ 150 nm, 39.4% API load) powder of naproxen was obtained from Novartis AG (Basel, Switzerland). Micro-suspension and nano-suspension[1] (10% w/w) in a mixture of HPC (type Klucel LF 2.5% w/w), SDS (0.05% w/w) and deionized water (87.45% w/w) were from the same source.

The dissolution behavior of naproxen powders and suspensions in pH 1.2 USP buffer and the suspension medium were studied using the µDISS Profiler (Pion Inc., Billerica MA, USA, Figure 2). The effect of micronization and suspension formulations on the flux of naproxen through a GIT-mimicking artificial membrane (Double-Sink™ PAMPA[2]) was investigated using the µFLUX add-on (Pion Inc.) to the µDISS Profiler. In the µFLUX apparatus three pairs of temperature controlled side-by-side permeability chambers separated by a PAMPA (or other) membrane (Figure 3) are mounted on top of the stirring platform and. Concentration in the donor and receiver compartments can be simultaneously monitored with integrated fiber-optic UV dip probes.

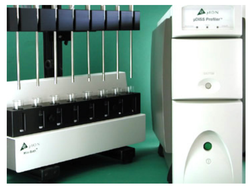


Figure 2. The µDISS Profiler from Pion Inc. monitors concentration in real time in 8 temperature controlled vessels using only 1 – 20 mL of dissolution media.

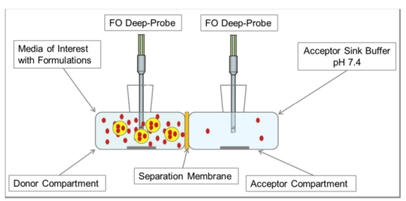


Figure 3. Schematic presentation of one donor-acceptor pair of the µFLUX apparatus. FO dip-probes attached to the µDISS Profiler (Figure 2) monitor concentrations in the donor (left) and receiver (right) compartments. The chambers are separated by PAMPA, cell-based (caco-2 or MDCK), dialysis, or other type of membrane.

Zero Intercept Method (ZIM)

In situ measurement of the concentration of free API as it is being released from a nanoparticle formulation is challenging due to the complex effect of simultaneous scattering and absorption of UV radiation by the nanomaterial[3].

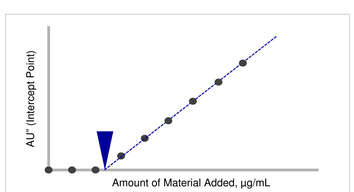


Figure 4. Dots show a hypothetical dependence of second derivative absorbance values at a ZIM point (Y-axis) as a function of amount of nanomaterial added (X-axis). The blue triangle indicates the intersection of the extrapolated linear fitting (dashed line) with the X-axis which indicates the solubility of the nanoparticles.

Recently, the Zero Intercept Method (ZIM) was introduced[1] to overcome this challenge. ZIM relies on the fact that for a particular API the derivative (first, second, etc.) of a UV-Vis spectrum may cross zero at one or more wavelengths. Figure 4 illustrates that at ZIM points the derivative absorbance from a mixture consisting of both dissolved API and nanoparticles would be influenced only by the nanoparticles and would become ~0 if the nanoparticles were fully dissolved.

RESULTS AND DISCUSSION

Solubility of Naproxen in Suspensions

Solubility (free fraction) of naproxen in nano-suspension and micro-suspension was determined by serial addition of a suspension (100 mg/mL load) to the corresponding medium (placebo). For the nano-suspension, four ZIM points of the 2nd derivative absorbance curve of the API (Figure 5) were used to extrapolate naproxen nano-particle solubility in the suspension (Figure 6).

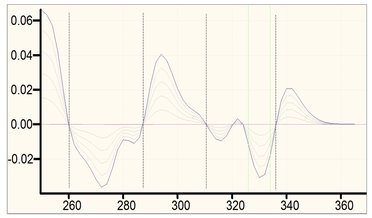


Figure 5. Overlapping 2nd derivative spectra profiles of naproxen at different concentrations with dark vertical dashed lines indicating ZIM points.

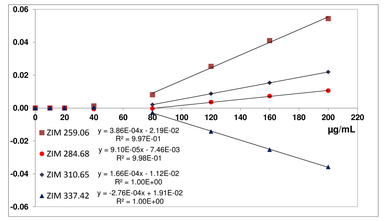


Figure 6. Example of processing data from one channel of the µDISS Profiler. Extrapolation of the linear fitting lines were used to determine the solubility of naproxen nano-powder in a suspension medium.

Weighted average of the results from 3 different cannels of µDISS Profiler were used to determine the solubility of naproxen in the nano-suspension which resulted in a value of 65 ± 3 µg/mL. Because the micro-suspension does not alter the 2nd derivative profile of spectral data, the concentration of dissolved API can be monitored using the conventional derivative spectroscopy methods implemented in the µDISS Command Software.

Aliquots of a micro-suspension were added to the placebo medium until the concentration of API reached saturation indicating the solubility of naproxen in the micro-suspension. Figure 7 demonstrates parallel measurements in 4 channels of the µDISS Profiler. The averaged solubility of naproxen in microsuspension determined to be 76 ± 2 µg/mL.

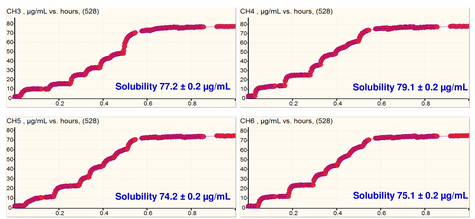


Figure 7. Solubility determination of naproxen in its suspension by adding aliquots of microsuspension to the placebo medium.

pH Effect

It should be noted that the suspension medium has no (or very limited) buffering capacity and that the solubility of an ionizable API like naproxen can be greatly effected by the load of material and environmental factors (initial pH, purity of the water, etc.)

Dissolution of Naproxen Formulations in pH 1.2 Buffer

Micronized powder exhibited a high dissolution rate quite comparable to the one measured on the micro-suspension[1]. Estimation of effective particle sizes that would generate the experimentally observed dissolution curves[4] of Figure 8 yields values of 3.2 µm and 2.2 µm for the higher and lower loads respectively.

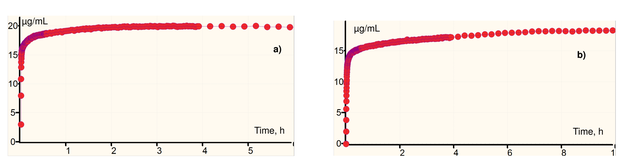


Figure 8. Dissolution profile for micronized powder 3.7 mg load (a) and 1.2 mg load (b) in 20 mL of pH 1.2 buffer.

Depending on the load, nano-powders behave differently. Analysis of the derivative spectra shows that with small loads (~1 mg in 20 mL) nano-powder in the solution behaves similarly to micro-powder. Figure 9 below shows the dissolution profile of nano-powder in pH 1.2 buffer. Higher loads of nano-powder distort the UV spectrum of naproxen. Unlike nano-suspension, nano-powder seems to be forming aggregates of different sizes when going into solution that makes application of the ZIM method more complex and requires some more development.

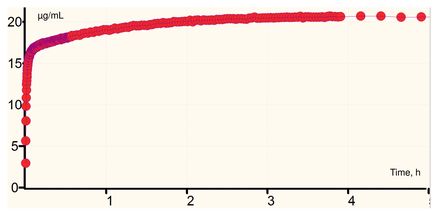


Figure 8. Dissolution profile for nano-sized powder 1.0 mg/ 20 mL load in pH 1.2 buffer.

µFLUX Method to Study Absorption Potential of Formulations

Flux of a drug through an *in vivo* GIT barrier can be limited by solubility, dissolution rate, or permeability of the drug. The µFLUX apparatus (Figure 3) allows investigation of these factors by comparing flux of the drugs through membranes (e.g., the Double-Sink™ PAMPA membrane[2]). Figure 9 shows concentration versus time profiles in the receiver compartment of a µFLUX system from different formulations of naproxen. It can be clearly seen that although all forms have the same solubility, flux from the nano-suspension was ~ 1.7 times higher than flux from the microsuspension (Table 1).

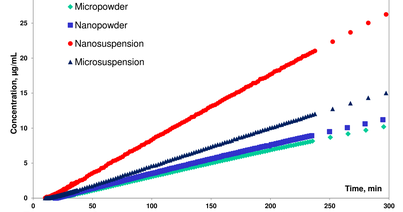


Figure 9. Concentration versus time profile in the receiver compartment of the µFLUX system (pH 7.4) with the donor compartment containing naproxen formulations in pH 1.2 buffer.

Flux from the nano-powder was slightly higher than from the micro-powder despite the lower load of the nano-powder (Table 1).

Table 1. Flux through a Double-Sink PAMPA membrane (0.79 cm^2) from different naproxen formulations.

Formulation	Load in Donor mg/mL	Flux µg/(min-cm^2)
Micro-powder	0.20	0.62
Nano-powder	0.16	0.69
Micro-suspension	0.40	0.90
Nano-suspension	0.40	1.56

CONCLUSIONS

The ZIM method allows *in situ* measurement of free API concentration in solution to be made in the presence of nano-particles.

The solubility of Naproxen formulated as a nano-powder was found comparable to the solubility of a micro-powder.

Nano-powders and nano-suspensions dissolve differently because naproxen stays in nano-form when delivered from nano-suspension while forming a mixture of nano-particles and bigger aggregates when added as nano-powder.

A µFLUX experiment can measure and differentiate factors limiting absorption thereby facilitating and providing valuable insight into formulation development.

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