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Predicting the Relative *In Vivo* performance of Nanoformed and Crystalline Bulk Piroxicam Solid Suspensions from *In Vitro* Flux Measurements

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PURPOSE

In recent years, the formulation of APIs as solid nanosuspensions has become a popular strategy to address poor solubility and dissolution properties, to increase drug bioavailability.

In this study, using the example of nanocrystalline piroxicam (PRX) prepared using CESS® technology, we demonstrated the correlation of *in vitro* flux results to the outcomes of a pre-clinical rat study. We also predicted the *in vivo* absorption and pharmacokinetic (PK) behaviour in humans from *in vitro* dissolution/permeability data, employing an absorption model based on the gastrointestinal unified theoretical (GUT) framework[1].

METHODS

Formulation Preparation – Controlled Expansion of Supercritical Solution (CESS®)

Two PRX solid suspensions, a bulk material, prepared with untreated piroxicam, and nanoformed™ material produced by CESS® (Figure 1) were prepared for dissolution/permeability testing each with identical API loading of 100 mg/mL and excipient content (1% HPMC and 1% Tween). These suspensions were also used for an *in vivo* pharmacokinetic study in rats with an oral administration of 20mg/kg API.

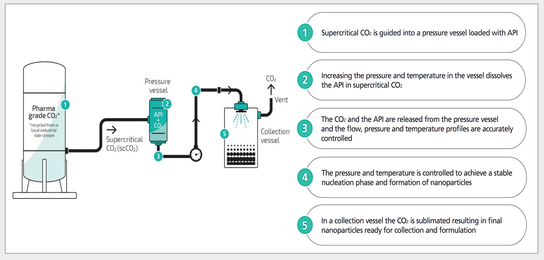

Figure 1. A schematic of the CESS® methodology with description of the process steps.

MicroFLUX™ Dissolution/Permeability Assays

Flux assays were carried out in quadruplicate using the MicroFLUX™ two-compartment dissolution/permeability testing apparatus (Pion Inc., Billerica, MA, USA). Quantitation of the API was performed by a Rainbow R6 FO spectrometer.

In silico Modelling – Human *In Vivo* Predictions

Predictions of human *in vivo* mass absorbed were generated using the fraction absorbed calculation in the Pion Predictor™ (v1.0) software package. *In vivo* C_{max} and T_{max} were estimated from mass absorbed *vs.* time plots exported from Predictor and published pharmacokinetic data[2] (Vd, K_{el}).

RESULTS

Characterization of PRX Solid Suspensions

CESS® technology is a particle production method based on supercritical carbon dioxide ($scCO_2$) expansion[3]. The piroxicam (PRX) nanoparticles were successfully prepared by carefully controlling the API saturation and $scCO_2$ depressurization. Unlike other nanoparticle manufacturing methods, the CESS® process does not require the use of excipients or surfactants, thus producing pure PRX nanoformed particles.

The bulk and the nanoformed materials were characterized (Figure 2) by scanning electron microscopy (SEM) and X-ray powder diffraction (XRPD). The PRX particles produced using Nanoform's CESS® technology resulted in nanocrystalline material with particle size distribution (PSD, d50) of 236 nm determined based on SEM images with the help of an in-house AI application, whereas the PRX bulk particles showed a d50 of ~2 µm.

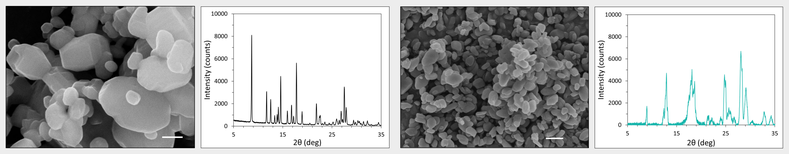

Figure 2. SEM image and XRPD pattern of the "bulk" and "nanoformed" PRX. Scale bar 1 µm.

In Vitro Flux vs *In Vivo* Rat Study

The appearance rate of PRX in the acceptor vessel of the MicroFLUX™ apparatus was 1.86 times greater for the nanosuspension as opposed to the bulk, whereas the AUC ratio between the appearance profiles exhibited a 2.16-fold improvement for the nanoformed™ product. Replicates showed consistent results (Figure 3).

The results of the *in vivo* preclinical rat study (Figure 4) demonstrated that a 20 mg/kg oral dose of nanoformed™ piroxicam possessed superior pharmacokinetic properties compared with piroxicam bulk, with 6 times faster T_{max}, 1.55 times higher C_{max} and 1.86 times larger AUC within the first 8 hours. By comparison, *in vitro* flux assays exhibited a 2.16-fold improvement in the acceptor vessel AUC, showing reasonable correlation in the relative increase in drug exposure between the formulations.

Following a dose of 5 mg/kg, suspension with nanoformed™ material demonstrated reduced AUC by 3.85 times showing dose-dependent pharmacokinetics compared with 4 times higher dose of 20 mg/kg nanoformed™ piroxicam.

In Vivo Pharmacokinetic Prediction

The Predictor software was used to convert the flux appearance profiles into plots of absolute mass absorbed (Figure 5), from which the human *in vivo* C_{max} and T_{max} values were estimated. The *in vivo* predictions of the nanoformed™ material provided close estimates of T_{max}, (1.43 hours) and C_{max} (2.01 µg/mL). In the human pharmacokinetic study, a dose of 20 mg administered as a tablet resulted in a T_{max} of 1.75 hours and a C_{max} of 2.23 µg/mL[4].

Formulation - Nanosuspension	*In Vitro* (Prediction)	*In Vivo*
Absolute Fraction Absorbed	99.99%	100%
Tmax (Hours)	1.43	1.75
Cmax (µg/mL)	2.01	2.23
AUC (0-1 Hour) (µg*h/mL)	1.21	1.15
Formulation - Bulk Suspension	***In Vitro* (Prediction)**	***In Vivo***
Absolute Fraction Absorbed	99.99%	100%
Tmax (Hours)	2.22	2.75
Cmax (µg/mL)	1.99	2.23
AUC (0-1 Hour) (µg*h/mL)	0.676	0.836

Table 1. Predictor v1.0 results from in vitro flux data compared to human in vivo data for the nano (upper) and bulk (lower) suspensions.

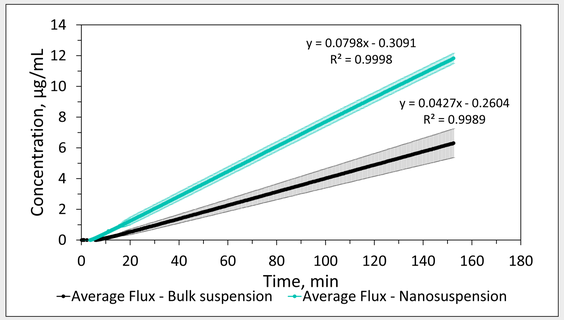

Figure 3. Average acceptor appearance profiles for PRX nanosuspension and PRX bulk suspension.

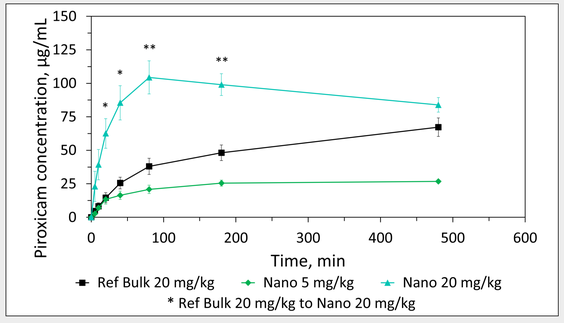

Figure 4. Piroxicam plasma levels in rats after 20 mg/kg and 5 mg/kg PRX administration.

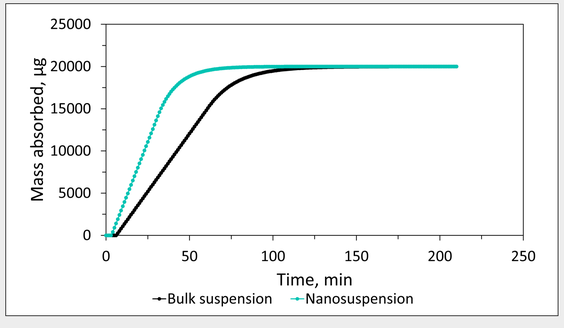

Figure 5. Calculated PRX absolute mass absorbed over a human intestinal residence time of 210 minutes using Predictor v1.0.

CONCLUSIONS

The data presented illustrates the applicability of *in vitro* flux assays in conjunction with predictive modelling software to predict the relative *in vivo* performance of formulations containing nanoformed Piroxicam.

The boost in the exposure of nanoformed™ Piroxicam as opposed to untreated bulk at equivalent dose is demonstrated clearly in rats. In this case, flux assay results alone directly correlated with the relative *in vivo* performance improvement in rats due to nanoforming.

Using the predictor software, the human *in vivo* predictions of the nanoformed™ material provided estimates of pharmacokinetic parameters close to the results of human clinical studies.

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