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Biopharmaceutical Process Sciences
Preformulation and Non-Clinical Formulation,
Chemin du Foriest, B-1420 Braine-l'Alleud, Belgium.

Report: Technical evaluation	Type: Equipment evaluation
Substance ID: several NCEs	Program: /

Title:

Evaluation of Covaris Adaptative Focused Acoustics equipment for several Preformulation applications.

Keywords: Solubilization, particle size, homogenization, non-clinical formulation, preformulation

Distribution list: /

Summary:
Covaris Adapative Focused Acoustics technique seems to be well adapted to Preformulation and Non-clinical Formulation activities like:

- the determination of solubility
- the quick and easy preparation of non-clinical formulations
- the reduction of particle size of non-clinical formulations

The equipment is user-friendly requiring limited training. A limited number of parameters need to be understood and controlled for the various applications. A substantial saving of time is anticipated.
A positive recommendation is given for the purchase of the equipment.

Approval :	Date	Signature
Somville Pascal, Development Scientist, UCB Pharma s.a.	14 Dec 2011	
Schenkel Eric, Director, Preformulation & Non-Clinical Formulation, UCB Pharma s.a.	15-Dec-2011	

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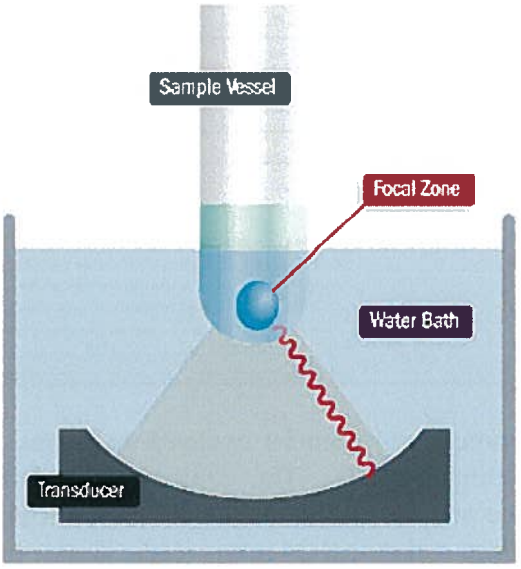
1. INTRODUCTION

Adaptative Focused Acoustics process works by transmitting focused acoustic energy wave packets from a dish-shaped transducer to the sample. The acoustic energy waves converge on the target sample in a small-localized area. As Acoustic energy transfers through the samples, this causes bubbles to form from the naturally dissolved gases. When the energy is then removed the bubbles collapse creating an intense, localized high velocity jet of solute. As the number of bubbles is high (around 100000 bubbles per sec) and the time interval is short (micro seconds), the mixing and/or disruption power capability of the process is significant.
When operated at low intensity levels, the computer controlled and focused waves, create a gentle mixing environment, suitable for accelerating any diffusion-dependent applications, such as compound dissolution, mass action binding events.
When operated at high intensity levels, the instrument can create a tunable shock wave environment with subsequent shear jet forces which has been demonstrated to be ideal for fragmentation applications.
Wavelength is typically 1 mm; frequencies are comprised between 500 to 1000 khz .



The objective of this study was, within 1 week, to evaluate the potential of the Covaris Adaptative Focused Acoustics equipment for further purchase. The potential of the equipment was assessed regarding 3 classical Preformulation activities:

- Reduction of solubilisation times of API and excipients
- Preparation of non-clinical formulations.
- Particle size reduction

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2. EXPERIMENTS

2.1 Reduction of solubilization time

Equilibrium solubilities of compound A obtained by the standard shake flask method at + 23°C for 24 hours and those obtained with AFA technique tuned at 1 min/100 cycles /1mL are summarized in table below. Both sets of results are very similar, indicating that the standard shake flask solubilization process of 24 hours could be replaced by a quick 1 min AFA solubilization cycle process.

Media	Shaking time reversal agitation/AFA	Measured pH	Solubility by reversal agitation (mg/mL)	Solubility with AFA process (mg/mL)	Solubility (according to the Eur Ph.)
100% KCl 50mM pH1.2	24 hours /1 min	1.5	2.910	2.700	Slightly soluble
20% labrasol in water	24 hours /1 min	2.9	0.788	0.685	Very slightly soluble
10% labrasol in water	24 hours /1 min	3.1	0.411	0.407	Very slightly soluble
5% labrasol in water	24 hours /1 min	3.3	0.191	0.204	Very slightly soluble
20% miglyol in water	24 hours /1 min	4.7	<0.001	<0.001	Practically insoluble
20% cremophor EL in water	24 hours /1 min	5.0	0.176	0.187	Very slightly soluble
20% cremophor RH in water	24 hours /1 min	5.6	0.148	0.154	Very slightly soluble
5% labrasol + 10% PEG 400 in water	24 hours /1 min	3.7	0.189	0.181	Very slightly soluble
5% labrasol + 10% propylene glycol in water	24 hours /1 min	3.3	0.312	0.322	Very slightly soluble

Descriptive term of solubility according to the Eur. Ph:		*Equivalence in concentration*
☐ *Very soluble :*	*Less than 1 volume of solvent (ml) per gram of solute*	*-> more than 1 g/mL*
☐ *Freely soluble :*	*From 1 to 10 volume of solvent (ml) per gram of solute*	*-> 100 mg to 1 g/mL*
☐ *Soluble :*	*From 10 to 30 volume of solvent (ml) per gram of solute*	*-> 33 mg to 100 mg/mL*
☐ *Sparingly soluble :*	*From 30 to 100 volume of solvent (ml) per gram of solute*	*-> 10 to 33 mg/mL*
☐ *Slightly soluble :*	*From 100 to 1000 volume of solvent (ml) per gram of solute*	*-> 1 to 10 mg/mL*
☐ *Very slightly soluble :*	*From 1000 to 10.000 volume of solvent (ml) per gram of solute*	*-> 0.1 to 1 mg/mL*
☐ *Practically insoluble :*	*More than 10.000 volume of solvent (ml) per gram of solute*	*-> less than 0.1 mg/mL*

A similar comparison (AFA tuned at 1 min/50 cycles/5 mL) has been made on a poorly aqueous soluble compound (compound B). The equilibrium solubility of compound B in water (12 µg/mL) is close to the one determined using AFA (9.4 µg/mL) indicating again that a short AFA process could replace a classical 24 hours shake flask process.

3 mL sample of a suspension of compound D in 10% HPBCD in 0.9 % NaCl was processed with various AFA processing times (5, 10, 15, 30, 60 and 120 sec). Solubility measurements done by HPLC are summarized in the table below. These results show that after only 5 seconds the equilibrium was reached. Based on this, a very short solubilisation processing time (1 min at maximum power) could be used as first approach for solubility determinations and replace the standard 24 hours shake flask agitation time.

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3 mL suspension at 1 mg/mL nominally AFA 100 cycles/min	Measured solubility (µg/mL)
5 sec	859
10 sec	897
15 sec	812
30 sec	775
60 sec	830
120 sec	910

2.2 Preparation of non-clinical formulation

Classical non-clinical suspension preparation procedure, typically composed of the five main following steps described hereunder

- Grinding step using pestle and mortar;
- weighing of the required amount of ground material;
- addition of vehicle (for instance: 1% methylcellulose + 0.1 % Antifoam + 0.1 % Tween 80 in an adapted buffer);
- homogenizing step using high-shear homogenizer device(Ultra-Turrax® at pre-defined speed);
- de-foaming step by slow magnetic agitation

has been compared to a simplified alternative procedure using AFA process, as described hereunder

- Weighing of the required amount of API;
- addition of vehicle (1% Methyl cellulose + 0.1 % Antifoam + 0.1 % Tween 80 in buffer);
- homogenizing step using Adaptative Focused Acoustics process at several intensity.

Experiments have shown that for several UCB compounds, well known for their difficulties to be formulated as solutions or suspensions, the Covaris AFA process has allowed to quickly and easily prepare homogenous formulations.

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7 UCB compounds, well known for their difficulty to be formulated were formulated using Covaris Adaptative Focused Acoustics process. 5 mL at 1 mg/mL API in 1 % methyl cellulose (400 cps) + 0.1 % Tween 80 + 0.1 % Antifoam in water were homogenized at ± 210 watt for 4 minutes. At the end of the process, the visual homogeneity of each formulation was checked. Microscopic examination revealed homogeneous suspensions composed of particles < 50 µm. It is to be noticed that for higher concentrations it should be suitable to split AFA process in 4 steps of 1 minute punctuated by 1 minute of vortex homogenization in order to fetch particles that could be stuck on the inner surface of the container.

One of these compounds, compound D, was formulated at 30 mg/mL under the same AFA conditions. Accuracy of the achieved concentration and homogeneity (6 samples taken at random in the formulation (2 top, 2 middle and 2 bottom)) were analytically checked. Satisfactory results, summarized in table below, were obtained.

Formulation analysis summary

Visual observation	Microscopic observation	Accuracy (HPLC meth ref. FA1 Ver1)	Homogeneity	Particle size Wet mode
Homogeneous suspension	Homogeneous suspension	97.8 % recovery (mean n=6)	RSD 2.4 % (n=6)	D_v0.5: 21.2 µm D_v0.9: 72.5 µm

2.3 Particle size reduction

Particle size distribution of non-clinical formulation of compound E (50mg/mL in 1% Methyl Cellulose 400 cps + 0.1% Tween 80 + 0.1% Antifoam in citrate buffer pH 5.0) was determined by optical measurement in wet mode (QicPic). The results summarized in table below show that alternative procedure using a 10 min AFA process at 500 cycles/min leads to particle size distribution results similar to those obtained with the classical formulation preparation procedure. The use of a higher number of cycles/min (up to 1000 for Covaris S) can reduce the processing time.

	Dv 0.5 (µm)	Dv 0.9 (µm)
Classical process	22	47
AFA 5 min 500 cycles/min	29	229
AFA 10 min 500 cycles/min	21	34
AFA 15 min 500 cycles/min	18	29
AFA 15 min 500 cycles/min + 5 min 1000 cycles/min	14	21

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2.4 Potential critical parameters of AFA technique

2.4.1 Volume of sample

As a large variety of containers with volumes ranging from 0.1 mL to 300 mL can be used with the equipment, the impact of the sample volume on the particular size distribution was quickly assessed. In the case of a suspension similar to those described in section 2.2, particles size distribution results summarized in table below demonstrate the impact of the volume on this parameter.

	Dv 0.5 (µm)	Dv 0.9 (µm)
1 mL suspension AFA 2 min 1000 cycles/min	24	38
5 mL suspension AFA 2 min 1000 cycles/min	37	76
20 mL suspension AFA 2 min 1000 cycles/min	95	324

2.4.2 Sample viscosity

Sample viscosity of formulation can considerably reduce homogenization efficiency of the process. Visually, particle homogenization is optimal in absence of viscosifying agent. But when 1 % methylcellulose (400 cps) is added to the vehicle, the vortex created by the high cavitation process in AFA is strongly reduced. This potential issue could be minimized by doing an interruption at mid AFA process followed by a manual homogenization of container (vortex). Nevertheless, results described in section 2.3 show the potential of the equipment to reduce particle size with standard viscosifying vehicle.

2.4.3 Chemical and Physical stability of API

Knowing that AFA process is highly energetic, overheating of samples could be an issue. The Covaris AFA can be connected to an external heater/cooler in order to maintain a selected temperature during process. The demo system was equipped with this option and no increase of sample temperature have been observed after long homogenizing process at the highest energy that Covaris S equipment can deliver. Solubility determination samples (section 2.1), were analyzed by means of a stability-indicating HPLC method and no increase of total related substance or additional peak have been observed after AFA process.

For the same reasons, physical transformation of the initial material form into another form is another potential issue. In order to investigate it, the crystalline form of 7 API's have been checked by XRPD analysis after 5 min of AFA process at the highest energy the Covaris S equipment can produce and compared to starting material. No difference with starting material were observed for all of them and more especially for 2 of them which are both known for their potential to transform into two different polymorphic forms in aqueous suspensions.
It has to be noticed that the AFA technique used for the preparation of a formulation of

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Compound G Form A, compared to other techniques used for the preparation of the same formulation, was the only one that did not produce any crystalline transformation of form A into form B after processing and upon a 24 hours storage.

3. CONCLUSIONS

Covaris Adapative Focused Acoustics technique seems to be well adapted to Preformulation and Non-clinical Formulation activities like:

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- the reduction of particle size of non-clinical formulations

The equipment is user-friendly requiring limited training. A limited number of parameters need to be understood and controlled for the various applications. A substantial saving of time is anticipated.

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