

## Application Note XRD 607

## Small Angle X-ray Scattering in Modern Drug Development Self-Emulsifying Drug Delivery Systems (SEDDS)

## Introduction

More than 60 \% of the new chemical substances identified as promising active pharmaceutical ingredients (API) are poorly soluble in aqueous media and hence require special formulation strategies for drug delivery and bioavailability (Lipinski, 2000; Singh, et al., 2015). In this context, precise knowledge of the nano-structural properties of heterogeneous formulations - emulsion particles, liquid crystalline phases - is essential. Modern laboratory instrument based X-ray scattering techniques are uniquely suited to provide such information easily and rapidly.

In the focus of current strategic developments are self-emulsifying drug delivery systems, SEDDS (Gursoy \& Benita, 2004; Mercuri, et al., 2011). They consist of an isotropic mixture of drug, oil, surfactant and co-surfactant which spontaneously form a micro- or nano-emulsions upon contact with the aqueous phase e.g. of gastrointestinal fluids. The spontaneous emulsification only occurs when defined excipient compositions are mixed, which limit the interfacial tension with the lipid to almost zero and enable the formation of micro emulsion (Gursoy \& Benita, 2004; Mercuri, et al., 2011). Extensive excipient screening based on the builtup of phase diagrams from optical observations is usually
required to identify possible formulations. By doing so, only macroscopic manifestations of molecular interactions can be perceived, limiting the understanding of such systems and lengthening the formulation development. The mechanism of self-emulsification itself results from the succession of states found along the path connecting the pure aqueous phase and the pure lipid on the phase diagram. It has been shown that the presence of lamellar liquid crystals gives rise to "eruptions" during which small droplets of lipids are expelled into the continuous aqueous phase (Mercuri, et al., 2011; Wakerly, et al., 1986). On the contrary, for systems where no lamellar liquid crystals are observed, self-emulsification can be driven by diffusion and phase inversion (Wakerly, et al., 1986). Several independent experiments such as the determination of Critical Micellar Concentration (CMC) or the measurement of the droplet size distribution in the resulting micro emulsion are essential to characterize the macroscopic properties of these systems, but remain insufficient to correlate them with molecular interactions.

In this note, we describe how the use of small angle X-ray scattering (SAXS) can identify the supra-molecular assemblies formed in SEDDS at various points of the complex phase diagram.


Figure 1: Schematic representation (1. to 3.) of the self-emulsification mechanism via the erosion process and observation of the phenomenon under polarised light microscopy for the system soybean oil/tween 80/span 80 in water. 1. Water infiltrates into the oil/surfactant mixture forming reversed micelles; 2. As a consequence of further water penetration, a dispersed lamellar liquid crystalline mesophase starts to be formed; 3. The interface is disrupted by the rapid penetration of water and the gentle agitation which causes droplets ejection, with consequently emulsion formation. O indicates the oil; S the surfactant; W the water. Adapted from (Mercuri, 2009; Wakerly, et al., 1986).

## Investigating the hidden nano-structure of SEDDS through SAXS analysis.

The most common approach used to develop SEDDS formulation is the study of phase diagrams by varying the relative concentrations of the lipidic and aqueous components, and finding the composition in which the API of interest is best soluble. Such phase diagrams are initially based on visual tests by which formulations are classified depending on their ability to spontaneously form an emulsion. After having identified the critical surfactant ratio for self-emulsification, a phase diagram can be established by varying the surfactants, lipidic and aqueous phase concentrations. A typical example is shown in Fig. 1. The description of each phase is classically based on a combination of macroscopic observations (viscosity, optical transparency, macro-phase separation) and polarized-light microscopy to identify isotropic or certain liquid crystalline phases, such as lamellar, hexagonal or cubic ones.

The mechanism of self-emulsification proceeding through an erosion process can be changed when a co-solvent such as glycerol is added. SAXS was used in this study to investigate the self-emulsification mechanism change, as the understanding of this is an essential prerequisite for planning successful formulations. This procedure can be repeated and

Table 1 Some examples of SEDDS pharmaceutical formulations (modified from (Kohli, et al., 2010)).

| API | Trade name | Dosage forms |
| :---: | :---: | :---: |
| Tretinoin | Vesanoid (Roche) | Soft gelatin capsule, 10 mg |
| Tretinoin | Tretinoin Capsules (Teva) | Soft gelatin capsule, 10 mg |
| Isotretinoin | Accutane (Roche) | Soft gelatin capsule, 10,20 and 40 mg |
| Cyclosporine | Panimum bioral (Panacea Biotec) | Capsule, 50 and 100 |
| Cyclosporin A | Gengraf (Abbott) | Hard gelatin capsule, 25 and 100 mg |
| Cyclosporin A | Sandimmune Neoral (Novartis) | Soft gelatin capsule, <br> 25,50 and 100 mg |
| Cyclosporin A | Ciqorin (Teva) | Soft gelatin capsule, $10,25,50$ and 100 mg |
| Lopinavir and Ritonavir | Kaletra (Abbott) | Soft gelatin capsule, Lopinavir 133.33 mg and Ritonavir 33.3 mg |
| Sanquinavir | Fortovase (Roche) | Soft gelatin capsule, 200 mg |
| Tipranavir | Aptivus (Boehringer Ingelheim) | Soft gelatin capsule, 250 mg |
| Amprenavir | Agenerase (GSK) | Soft gelatin capsule <br> 50 and 150 mg |



Figure 2: Phase diagram of the system Span 80 : Tween $80(1: 1) /$ soybean oil/water. L2 indicates a w/o microemulsion, LC indicates an area in which lamellar liquid crystals can be observed, $G$ is a gel-like viscous phase and $E$ an o/w emulsion. Modified from (Mercuri, et al., 2011).


Span ${ }^{\circledR} 80$

$n=6$ or 8

Captex 300


Tween ${ }^{\circledR} 80$
Figure 3: Chemical structures of Span 80; Tween 80 (Mercuri, 2009) and Captex 300.


Figure 4: Composition of samples studied by SAXS: A) SEDDS with $0 \%$ glycerol in presence of $5,7,10,15,20$ and $28 \%$ of pure water and B) SEDDS in presence of $5,7,10,15,20$ and $28 \%$ of glycerol solution at $58.5 \%$.
refined in a restricted part of the phase diagram. Typically, once a formulation has been established it is possible to study the effects of aqueous phase parameters such as concentration, pH , temperature, and presence of cosolvent(s) on the self-emulsification and on the resulting micro/nano emulsion. In this case, only a line joining the pure lipidic/ surfactant(s) phase and the pure aqueous phase has to be screened. Apart from being very time and material consuming, despite its relative simplicity, this approach remains incomplete due to the lack of detailed nano-structural information.

## Liquid crystalline mesophases

Liquid crystals can be divided in two categories: those that change phase according to temperature variations and those that change phase when a solvent is added and/or temperature is changed. The former are known as thermotropic liquid crystals and the latter as lyotropic liquid crystals. The lyotropic liquid crystals mesophases are the lamellar, cubic and hexagonal phases (Hyde, 2001). Common substances that form lyotropic liquid crystals are amphiphile molecules (also known as surfactants): chemicals which have hydrophobic
and hydrophilic parts within the same structure. The arrangements of the formed mesophase is determined by the packing parameter $(P)$, which is the ratio between the volume of the hydrophobic hydrocarbon chain $\left(v_{0}\right)$ and the product between the length of the surfactant $\left(I_{0}\right)$ and the hydrophilic polar group cross sectional area ( $a_{0}$ ) (Myers, 1999; Myers, 2006).

Each mesophase can exist in its inverse structure, in accordance with the characteristics of the amphiphile three dimensional structure, as described by the packing parameter, Figure 5. The lamellar phase is characterized by low viscosity and birefringence when observed at the microscope under polarized light. Also the hexagonal phase is birefringent and has a higher viscosity than the lamellar phase. The cubic phase is non birefringent and is highly viscous (Hyde, 2001). Any factor influencing the polar head or the length and volume of the hydrophobic tail, will alter the packing parameter and therefore the mesophases formed by the surfactants.


Figure 5: Schematic representation of the mesophases formed by lyotropic liquid crystals as a function of water addition to an oil/surfactant mixture. The formation of mesophases is related to the packing parameter of the surfactant. Modified from (Jonsson, 1998).

## SAXS - Method and instrumentation

In contrast to conventional X-ray diffraction, XRD, SAXS is not limited to materials in their crystalline state. Technically, SAXS is the extension of powder diffraction, which is normally limited to small-molecular distances (less than a few nanometers), to the macro- and supra-molecular range (up to 100 nm ). Combining it with wide-angle scattering (SWAXS) allows to measure nano-particle or domain size and crystalline packing simultaneously, in the same experiment (Laggner \& Mio, 1992). The schematics of SAXS and SWAXS is shown in Figure 6.

The realization in a compact table-top instrument is shown in Figure 6. The challenge is to provide a high-brilliance monochromatic primary beam - flux $>10^{7} X$-ray photons/sec in a point beam cross section of $<0.3 \mathrm{~mm}$, with practically zero background (from air, optics, windows). This is realized in the Bruker MICRO SWAXS platform (Figure 5).

An essential feature is the sample environment: the active volume probed by the X-ray beam is as small as one drop (< $50 \mu \mathrm{~L}$ ), contained within a thermally controlled capillary cuvette, which can be rotated during exposure to minimize radiation damage and to avoid preferred orientation artifacts. Typical exposure times are in the order of 5-10 minutes.

In one special embodiment the SWAXS camera is combined with an integrated microcalorimeter (Bruker MICROcalix ${ }^{\top}$ ). This further allows the simultaneous measurement of DSC curves and SWAXS during temperature scans, thus providing thermodynamic and structural information in one and the same scan.


Figure 6: SWAXS - the combination of small- and wide-angle X-ray scattering covers the structure of liquids and solids from the atomic to the large nanometric range.


Figure 7: The Bruker MICRO SWAXS camera with high-brilliance micro-source (Incoatec, Germany), point-focusing optics and two linear position sensitive detectors.

## Information from SAXS

SAXS data provide information regarding the type of the liquid crystal under investigation (e.g. lamellar, hexagonal or cubic phase) and the size and shape of particle/ micelle structures (Tyler, et al., 2015; Dong \& Boyd, 2011; Laggner, 1999).

- SAXS allows to identify the type of liquid crystal by studying the relative positions of diffracted peaks. The specific geometry can be deduced from the corresponding peak positions in the SAXS spectrum.
- SAXS allows to determine particle sizes and shapes within microemulsion phases. It is possible to distinguish globules, cylinders, sheets and measure their rugosity (roughness).
- SAXS allows to identify the characteristic fingerprint of the hydrocarbon chain packing from the wide-angle spectrum.


Figure 8: Schematic view on the general types of information that can be obtained by SAXS on different kinds of samples.

A graphical representation of the general types of information that can be extracted from SAXS is shown in Figure 8.

## Practical example

Effects of glycerol on SEDDS formed by Captex 300, Tween 80 and Span 80

Glycerol/water in the very concentrated regime

Addition of glycerol induces a change in the phases. In particular, the isotropic area observed in the glycerol-free system is expanded as the glycerol amount is increased. At the same time the gel phase becomes reduced and finally disappears completely with higher glycerol concentration.


Figure 9: A) Schematic view of structures formed with increasing oil content. At the both ends of the sequence are micellar solutions, while at intermediate regions elongated micelles, lamellar LC phases, cubic (3D-mesh) phases occur.
B) Phase diagrams of SEDDS/Glycerol/water mixtures based on visual observations. L2 indicates a o/w microemulsion, G indicates a gel phase, E indicates an emulsion phase.

## Glycerol suppresses the lamellar liquid crystal formation

The SAXS data presented in Figure 10 show that admixture of water leads to the appearance of a lamellar liquid crystalline (LC) mesophase (Fig. 10). A lamellar repeat distance of $99 \AA$ has been found from the position of the first two orders of Bragg peaks, in good agreement with the values reported previously for a pure system of Tween80/ Span80, 7:3 (Dai \& Xiao-Yan, 1995).

However, when the formulation is mixed with an aqueous solution of glycerol, the Bragg peaks disappear (Fig. 10). It is known that glycerol, as well as other alcohols, tends to expand the interfacial film, while Span 80 condenses it and therefore is used to reduce the interfacial tension to the negative values required to form a microemulsion (Attwood, et al., 1974; Osipow, 1963). Furthermore, it has been shown that addition of glycerol to block copolymers induces swelling of the interlayer spacing between bilayers, and the turbid vesicle phase becomes completely transparent, as the glycerol refractive index matches that of the surfactant bilayers (Yan, et al., 2011).

## The lamellar LC phase is replaced by micelles

A closer inspection of the SAXS pattern at different fractions of the aqueous phase (Figure 11) reveals the following picture: The initially formed micelles increase in size, as seen by the decrease in width of the quasiGaussian curves and, in parallel, their rugosity (surface roughness) decreases. Above a certain fraction of aqueous phase, no further evolution is observed: the interglobule distance corresponds to close packing, macroscopic observations show a phase separation.

From Guinier plots and log-log plots of these SAXS curves (standard SAXS software, e.g. Bruker DIFFRAC.SAXS), the radius of gyration and the Porod slope can be obtained. These results are presented in the Figure 12. The Porod slope continuously increases with the water content starting with 3.2 at $5 \%$ of water


Figure 11: SAXS of SEDDS with glycerol $58.5 \%$ at increasing water/glycerol concentrations from 5 to $28 \%$. and reaching 3.9 for $20 \%$. A value of the Porod slope between 3 and 4 indicates that particles are $3-D$ objects. The variations of the Porod slope between 3 and 4 correspond to changes in surface rugosity. With a value of 3 , the particles are expected to have irregular interfaces while a value of 4 represents smooth surfaces. In parallel to this evolution, we observe an increase of the radius of gyration from $52 \AA$ at $5 \%$ of water to $86 \AA$ at $28 \%$ of water. These values correspond to globular micelle diameters of 134 and $221 \AA$, respectively. The fact that the micelles are indeed globular is underlined by the behaviour at increasing fractions of water/glycerol (Fig. 13), showing exponential swelling law with an exponent of 0.31 , which is close to 0.33 as theoretically expected for swelling micelles (Hyde, 2001).


Figure 12: Left: Radius of gyration (full diamonds) and Porod slope (hollow squares) obtained from SAXS analysis for the SEDDS with glycerol $58.5 \%$ at increasing water concentrations (figure 10). Right: Schematic illustration of variation of the Porod slope, P, with the rugosity of the micelle surface.


Figure 13: Swelling law. Log of the radius of gyration as a function of log of water content. The experimental slope of 0.31 is very close to the theoretical exponent of 0.33 for globular micelles (Hyde, 2001).

The decrease of surface rugosity and swelling are consistent with the notion that the added glycerol solution remains localized in globules.

## SUMMARY

SAXS is a highly informative analytical tool in the development of SEDDS formulations. It provides nanostructure information which is inaccessible by classical methods, and which is a key to rational development. Contrary to classical approaches (such as macroscopic observations, viscosity, optical transparency, macro-phase separation, and polarizedlight microscopy) which provide only macroscopic information about SEDDS formulations, SAXS enables to go a step further in their analysis. Indeed, by screening phase diagrams SAXS enables to identify liquid crystals and measure their lattice dimensions. The decisive role of co-solvent such as glycerol is clearly identified by SAXS. In the present formulation based on Captex oil, Tween $80^{\circledR}$ and Span $80^{\circledR}$, the presence of glycerol in the aqueous solution results in the disappearance of lamellar liquid crystals. In consequence, the erosion mechanism of self-emulsification is not functional anymore. Instead, spherical globules are formed whose size increases with aqueous volume. In parallel, the Porod exponent allows estimating the roughness of the globules formed in presence of glycerol. Its decrease with increasing aqueous volume fraction is in good agreement with the swelling of reverse micelles. These elements obtained by SAXS analysis offer detailed, quantitative information on supra-molecular interactions and assemblies for various formulations. SAXS appears as the key analytical technique for modern SEDDS formulations development.

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