Bioperformance Improvement: Small Particles and Optimal Polymorphs

Rolf Hilfiker[a]*, Markus von Raumer[a], André Geoffroy[a], Fritz Blatter[a], and Hans-Walter Haesslin[b]

Abstract: The average molar mass of active substances in crop protection as well as in pharmaceuticals has grown almost tenfold in the last century. In general, large molar masses are a drawback when looking at bioavailability. There are several strategies to overcome the problem of low bioavailability. Two of those strategies will be discussed in this paper: (i) Increasing the dissolution rate of the solids by increasing the specific surface and (ii) increasing the solubility by choosing an optimal polymorph or an amorphous substance. It will be shown what physicochemical measurements are useful to predict which excipients will stabilize suspensions of particles as small as 500 nm. In relation to optimal polymorphs, the importance of the optimal choice will be highlighted and examples of reliable stabilization of the amorphous form will be given.

Keywords: Adsorption isotherm · Amorphous · Bioavailability · Formulation · Ostwald ripening · Polymorphism

1. Introduction

Finding a new biologically active molecule is one thing, developing a good formulation for it is another. There is the famous Lipinski Rule of Five [1] which states that a molecule is hard to formulate and may have an insufficient bioavailability if several of the following conditions are fulfilled: MW > 500 g/mol, log P > 5, > 5 hydrogen bonds, solubility < 10^{-5} M. The average molar mass of active substances has grown roughly tenfold in the last century [2]. A larger molar mass means obviously a higher probability that any of the Lipinski criteria are fulfilled. It is therefore no surprise that finding a good formulation has become a harder task and that bioavailability can become a bottleneck. This applies to any biological system, *i.e.* to pharmaceutics, animal health and crop protection products.

The transfer of an active substance which is applied in an extravascular compartment (*e.g.* orally) occurs in at least two steps. First, the active substance has to be dissolved and then it has to be resorbed, *i.e.* pass a membrane. A biopharmaceutics classification system (BCS) has been suggested by the FDA which takes solubility and permeability into account (Table 1).

Solubility is the bottleneck for classes 2 and 4, and an increase of solubility or dissolution rate is therefore expected to lead to an increase of bioavailability. Examples where dissolution is the rate-limiting step are, for example, digoxin, warfarin, phenytoin and tetracyclins [3]. The dissolution rate of a substance can be described by the Noyes-Whitney equation [4] (Eqn. 1):

$$\frac{dC}{dt} = \frac{A \cdot D \cdot (C_{sat} - C)}{h} \qquad \text{Eqn. 1}$$

Where dC/dt is the dissolution rate, A the surface area, D the diffusion coefficient, h the thickness of the diffusion layer, C_{sat} the saturation concentration and C the actual concentration. Accordingly, the solubility and dissolution rate can be influenced by the following parameters:

- particle size
- chemical modifications (soluble prodrugs, salts)
- polymorphic form (polymorphs, solvates, amorphous form)

Correspondence: Dr. R. Hilfiker
[a]Solvias AG
Klybeckstrasse 191
Postfach
CH–4002 Basel
Tel.: + 41 61 686 60 21
Fax: + 41 61 686 65 65
E-Mail: rolf.hilfiker@solvias.com
[b]Syngenta Crop Protection Muenchwilen AG
Im Breitenloh 180
CH–4333 Münchwilen

Table 1. Biopharmaceutics classification

Class 1	Class 2
good solubility	bad solubility
good permeability	good permeability
(*e.g.* paracetamol)	(*e.g.* danazol)
Class 3	**Class 4**
good solubility	bad solubility
bad permeability	bad permeability
(*e.g.* cimetidin)	(*e.g.* hydrochlorothiazide)

- dispersions (solid solutions, melt extrusion, eutectics)
- complexation/solubilization (surfactants, cyclodextrins)

In the following, we will discuss two of these possibilities, *i.e.* particle size and polymorphic form. Many examples where micronization [5] and change of polymorphic form [6] can lead to an increase of bioavailability can be found in the literature.

2. Smaller Particle Size in Suspensions

While the concept of bioavailability improvement by particle size reduction is well established in the pharmaceutical industry, comparatively little has been done in the agrochemical sector. Concentrated suspensions are one of the favorite formulation types, however, for agricultural active substances (AS) like herbicides, fungicides or insecticides. Advantages of concentrated suspensions are that they have a very high AS concentration, are water-based, user friendly and relatively low cost. Prerequisites for a formulation of an active substance as a concentrated suspension are low solubility of the AS in water, a reasonably high melting point, and stability against hydrolysis.

The formulations must fulfill very stringent requirements, as they must be stable under rugged conditions. Extreme temperature variations and vigorous shaking are the factors which make the formulation chemist's life interesting. Moreover, the shelf life for commercial products should be in the order of several years.

Basically, three challenges have to be faced when preparing suspensions: (i) a reproducible, cost-effective way of preparing particles of the desired size has to be found, (ii) methods for reliable size measurement of the particles, ideally in the concentrated suspension, must be established and (iii) the particles have to be stabilized against Ostwald ripening (particle growth *via* a dissolution–crystallization process). All these challenges get much harder in general as the required particle size is decreased!

In the context of this article we will concentrate on the issue of stabilization. Normally, stabilization is achieved by means of surfactants, which adsorb on the solid-liquid interface. If the surfactant is ionic, colloidal particles are stabilized by electrostatic forces. The DLVO theory [7] provides a rational guidance to for-

mulating work with ionic surfactants. If the surfactant is non-ionic, steric stabilization [8] becomes more important. Independent of the surfactant class, several basic properties of the AS and of the surfactant must be known for successful stabilization. Assuming that the stability of the formulation is directly linked to the adsorption behavior of the surfactant (which certainly is only one aspect), then adsorption isotherms are of primary interest, since they directly reflect the adsorptive behavior of surfactants onto the AS surface.

Moreover, Ostwald ripening has proved to be one of the major obstacles in obtaining long-term stable suspensions. The rate of Ostwald ripening is governed by Eqn. 2 [9],

$$\frac{d}{dt}\langle r\rangle^3 \sim D\ \sigma\ C_{sat}\ V_m \qquad \text{Eqn. 2}$$

where σ is the interfacial tension between solid and liquid, r is the particle radius and V_m is the molar volume of the AS. σ is very much affected by surfactant adsorption and this reduction is, according to our experience, often the most important factor governing particle growth. The reduction of the interfacial tension through surfactant adsorption can be determined for ideal systems from the measured Langmuir isotherm by combining it with the Gibbs isotherm.

Basically two different concepts exist for the determination of adsorption isotherms. One concept is the quantification of the adsorbent concentration. The other concept is the monitoring of a property of the system, which is adsorbent concentration dependent. Both concepts have advantages and disadvantages. In the first case, the isotherm problem is reduced to the determination of polymer/surfactant concentrations, most often with indirect techniques. The formulation is centrifuged or filtered and the surfactant concentration in the supernatant is determined by means of an appropriate measurement. By comparison with the initial bulk surfactant concentration, the amount of adsorbed surfactant is obtained. For ideal systems (polymer or latex particles, minerals) normal spectroscopic techniques like UV-Vis spectroscopy are well suited for the surfactant quantification. If the adsorbent has no suitable chromophore, techniques like surface tension measurements [10] can be applied.

However, this apparently easy task is more difficult for non-ideal systems when (i) the solubility of the AS is affect-

ed by the adsorbent and is at the same time surface active, (ii) the AS is not pure and has by-products which are preferentially dissolved in the adsorbent, and (iii) the adsorbent is a complex mixture. Combination of all these hurdles makes it extremely difficult to obtain reliable adsorption isotherms. Unfortunately in the real world, the combination of these points is the normal case since both the AS and the surfactant are of technical quality. Given the manufacturing process of surfactants, they are almost always mixtures of chains with different lengths and other by-products. This represents an additional difficulty, especially for electrostatically stabilized systems. Minor changes of the surface charge through adsorbed impurities may have dramatic consequences for the stability of the formulation. The second concept, the monitoring of a surfactant concentration dependent system property has the advantage that the measured property levels out all heterogeneity of the AS and the adsorbent, and that an average isotherm is obtained. However detailed information is lost *e.g.* which fraction of adsorbent adsorbs preferentially. This approach needs a careful examination of the implicated process before extracting any data.

We determined adsorption isotherms using a large variety of the methods described above and found that in concentrated suspensions of agrochemicals and surfactants/polymers of technical grade quality, chromatographic, calorimetric and electrokinetic methods were best suited to cope with the difficulties mentioned [11]. Electrokinetic sonic amplitude (ESA) is particularly suitable for probing the surface charge of particles in suspension [12] but has not been used widely to study adsorption isotherms so far. Fig. 1 shows the viscosity compensated ESA signal of an AS formulated with varying Soprophor (sop) and Pluronic (Plur) concentrations. As expected, the ESA signal, which is directly proportional to the surface charge, changes with addition of anionic Soprophor and barely changes with addition of the neutral Pluronic. From these curves, together with known interactions between Soprophor and Pluronic, adsorption isotherms can be deduced. Moreover, this method is suitable to measure adsorption kinetics, a property which plays a very important role in the milling process.

Using and interpreting results such as these, relationships between stability and measured physicochemical properties could be established and used to speed up formulation development.

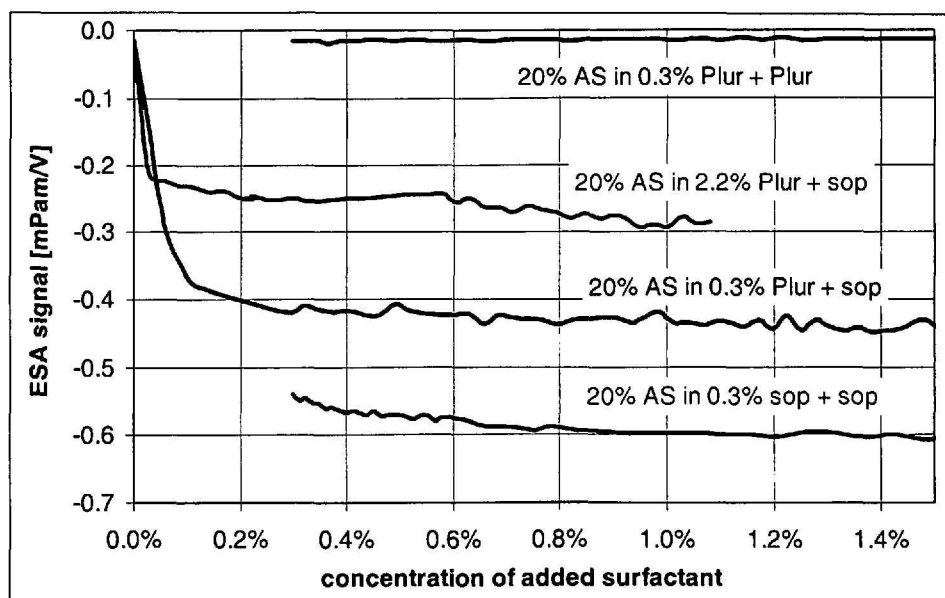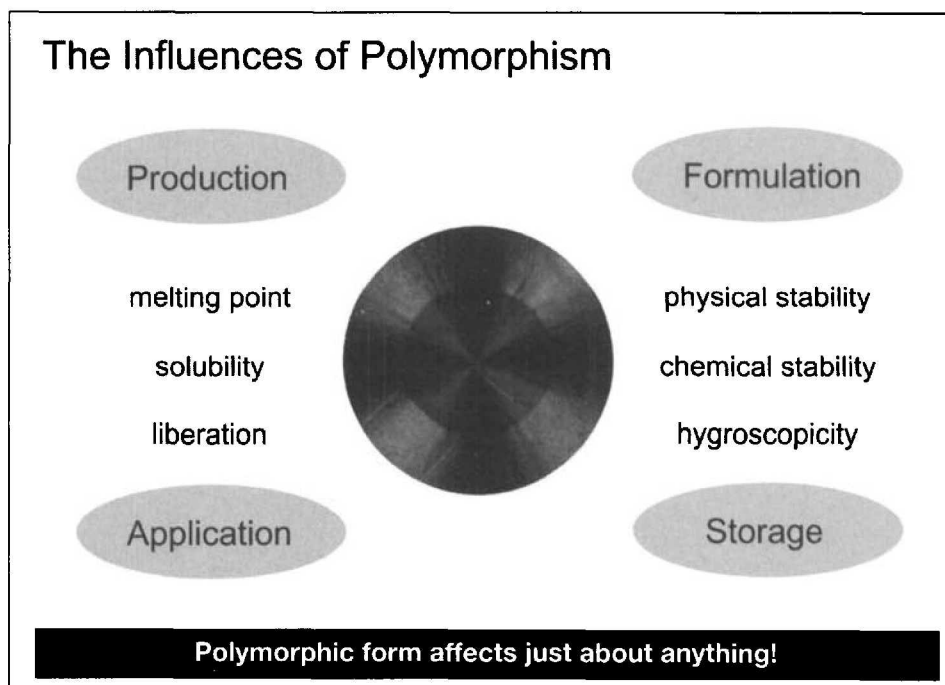Fig. 1. Viscosity compensated ESA signal of various AS formulations

Fig. 2. Influence of polymorphism on the product life cycle

3. Optimal Polymorphs

Polymorphism is the ability of a compound to crystallize in more than one distinct crystal structure. The probability that a particular drug substance can exist in different solid forms (polymorphs, solvates, hydrates and amorphous form) is very high. These crystal modifications or polymorphs have different lattice energies and hence different chemical potentials, which means that most physical and chemical properties will differ. This in turn will influence the whole life cycle (Fig. 2) of a product from production (reliable way to manufacture desired form), *via* formulation (some forms are easier to formulate than others) and storage (dif-

ferent chemical and physical stability) to application (bioavailability *via* solubility). Obviously, in the context of this article, solubility is the most important varying property.

The first step to defining which polymorph (or amorphous form) of an AS is most suitable is to find all relevant forms and to characterize them in terms of thermodynamic and kinetic stability as a function of temperature and other environmental variables. That knowledge is also important to make sure that no undesired changes occur during the production process or during the product lifetime. Moreover, it has been demonstrated several times that a sound polymorphic characterization is a powerful means of

extending the lifetime of one's own patent or, under favorable conditions, of getting patent protection on generic drugs.

Solvias has long experience in this area and has a clearly defined and structured strategy for polymorphic studies (Fig. 3). This strategy includes the search for new solid forms *via* thermoanalytical techniques as well as *via* different crystallization techniques from selected solvents. Any new relevant solid form is characterized spectroscopically and thermally and the hygroscopic behavior is analyzed. Finally, the thermodynamic relationship between the forms is established and an interrelation scheme is drawn. The extent of the study is adapted to the development stage of an AS, *i.e.*

Solvias Polymorphism Screens

clearly defined strategy, subdivided into "study-stages"

Study Stage 1   Characterization and
thermoanalytical search

Study Stage 2   Search for hydrates

Study Stage 3   Crystallization from
solvents (various techniques)

Study Stage 4   Physicochemical character-
ization of any new relevant
solid form

• possibility of starting at any of these stages

• variable program size (according to stage of product)
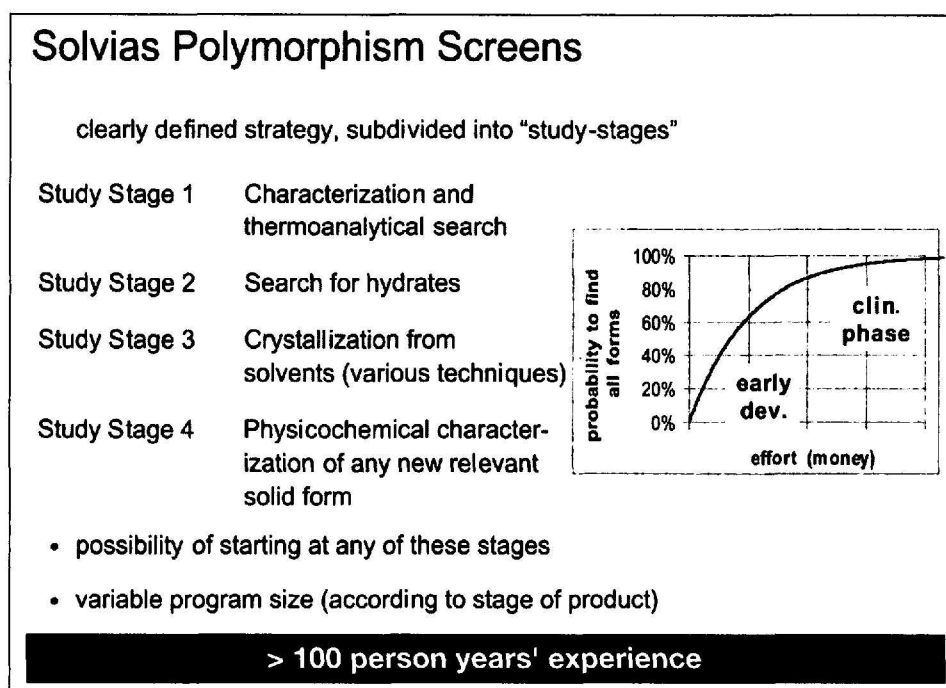
> 100 person years' experience

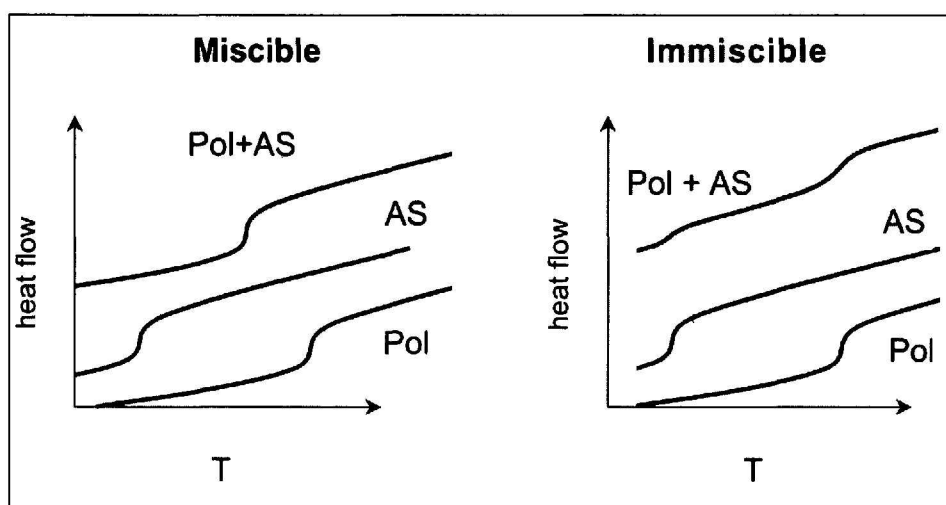Fig. 3. Solvias strategy and stages for polymorphism studies

Fig. 4. DSC traces for miscible and immiscible AS

for a substance in early development a smaller study makes economical sense, while in a later stage a full-scale study is necessary to optimize the product's potential and to exclude unexpected problems during the product's lifetime. After elucidating the polymorphic behavior of the AS, the physical and chemical stability in a given formulation and possible interactions with excipients are established. An example of the problems for patients, as well as the huge commercial losses for the manufacturer, which can occur in the case of such unexpected issues is Ritonavir [13].

The amorphous state plays a special role in the context of polymorphism. While in general it offers the highest solubility and bioavailability of all forms, it is metastable and therefore prone to crys-

tallizing during storage. This would of course be a disaster, as its solubility and effectiveness would then greatly decrease. One way to avoid this is to embed the AS in a polymer while fulfilling two requirements: (i) The AS/polymer mixture must be in the glassy state so that translational diffusion is excluded and (ii) the AS must be miscible with, *i.e.* molecularly dispersed in the polymer. If AS 'islands' were present in the polymer, these islands could still crystallize. For system optimization, a method to determine these two quantities reliably is therefore required.

DSC (differential scanning calorimetry) can provide all the necessary information. If AS and polymer are molecularly dispersed, then the AS will act as a plasticizer for the polymer [14][15]

(Fig. 4, left side) and the glass transition temperature (T_g) of the mixture can be predicted. Several formulas exist, one of them is the Nielsen equation (Eqn. 3) [16].

$$\frac{1}{T_{g,mix}} = \frac{w_1}{T_{g,1}} + \frac{1 - w_1}{T_{g,2}} \qquad \text{Eqn. 3}$$

w_1 is the weight fraction of the plasticizer and $T_{g,mix}$, $T_{g,1}$ and $T_{g,2}$ are the glass transition temperatures of mixture, plasticizer and polymer, respectively. Table 2 displays results of mixtures of several AS with a polymer ($w_1 = 0.5$). It shows that this polymer is suitable to stabilize 1:1 mixtures of AS 2, 6, 7, and 8 up to room temperature. For AS 4, one can immediately calculate that while a 1:1 mixture is

Table 2. Melting points and glass transition temperatures for several AS and 50/50 mixtures with a polymer.

	AS 1	AS 2	AS 3	AS4	AS5	AS6	AS7	AS8	AS9
MP (AS) (°C)	76	113	79	111	63	144	179	131	143
T_g (AS) (°C)	-18	15	-7	15	-20	-1	75	recr	20
T_g (calc) (50:50)	-2	48	34	48	25	38	82	-	51
T_g (meas) (50:50)	demix	42	32	65[a]	16	50	59	46	33

[a] partial demix

not suitable, a mixture containing 20% AS is. With information from Table 2 and knowledge about relationship between *e.g.* molar mass and glass transition temperature, it is now also rather easy to find conditions where the remaining AS can be stabilized.

4. Biological Tests

Biological tests showed that both strategies worked with certain AS but not with all AS tested.

This is the expected result, since solubility is not always the limiting factor as discussed above.

5. Conclusion

In conclusion, we have shown that bioperformance can be increased by reducing particle size in suspensions or by choosing optimal polymorphs. We are convinced that nano-sized particles as well as optimal solid forms are important for future formulation technologies.

Sound physicochemical knowledge coupled with the appropriate experiments greatly speed up development processes and help to find the critical parameters to optimize a system, which in turn significantly reduces the all-important time to market for a product.

[1] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, *Advanced Drug Delivery Reviews* **1997**, *23(1-3)*, 3–25.

[2] Adrian Friedmann, Syngenta AG, priv. comm.

[3] C.D. Melia, S.S. Davis, *Aliment. Pharmacol. Therap.* **1989**, *3*, 223–232.

[4] V.G. Levich, 'Physicochemical Hydrodynamics', Prentice-Hall, Englewood Cliffs, NJ, **1962**.

[5] S. Pfeifer, P. Pflegel and H.-H. Borchert, 'Grundlagen der Biopharmazie: Pharmakokinetik, Bioverfügbarkeit, Biotransformation', Verlag Chemie, Weinheim, **1984**.

[6] C.D. Melia, S.S. Davis, *Aliment. Pharmacol. Therap.* **1989**, *3*, 513–525

[7] see references in special issue of: *Colloids Surfaces B: Biointerfaces* **1999**, *14* .

[8] G.J. Fleer, M.A. Cohen Stuart, J.M.H.M. Scheutjens, T. Cosgrove, B. Vincent, 'Polymers at Interfaces', Chapman & Hall, London, **1993**.

[9] A.S. Kabalnov, E.D. Shchukin, *Adv. Coll. Interface Sci.*, **1992**, *38*, 69–97.

[10] E. Hecht, H. Hoffmann, *Tenside Surf. Det.* **1998**, *35(3)*, 185.

[11] M. von Raumer, H.-W. Haesslin, R. Hilfiker, *Colloids Surfaces A: Physicochem. Eng. Aspects*, **2001**, *183-185*, 795–804.

[12] R.W. O'Brian, D.W. Cannon, W.N. Rowlands, *J. Colloid Interface Sci.* **1995**, *173*, 406, and references cited therein.

[13] S.R. Chemburkar, J. Bauer, K. Deming, H. Spiwek, K. Patel, J. Morris, R. Henry, S. Spanton, W. Dziki, W. Porter, J. Quick, P. Bauer, J. Donaubauer, B.A. Narayanan, M. Soldani, D. Riledy, K. McFarland, *Org. Proc. Res. and Dev.* **2000**, *4*, 413–417.

[14] M. del Pilar Buera, G. Levi, M. Karel, *Biotechnol. Prog.* **1992**, *8*, 144–148.

[15] R. Vukovic, V. Kuresevic, G. Bogdanic, D. Fles, *Thermochimica Acta* **1992**, *195*, 351–364.

[16] L. E. Nielsen, in 'Mechanical properties of polymer', Reinhold, New York, **1962**, p. 21.