Bioperformance Improvement: Small Particles and Optimal Polymorphs

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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 reabsorbed, 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):

\[
\frac{dC}{dt} = \frac{A \cdot D \cdot (C_{\text{sat}} - C)}{h}
\]

Eqn. 1

Where \(dC/dt\) is the dissolution rate, \(A\) the surface area, \(D\) the diffusion coefficient, \(h\) the thickness of the diffusion layer, \(C_{\text{sat}}\) 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)

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 in-
crease 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 in-
dustry, comparatively little has been done
in the agrochemical sector. Concent-
trated suspensions are one of the favorite
formulation types, however, for agricul-
tural active substances (AS) like herbici-
des, fungicides or insecticides. Advan-
tages of concentrated suspensions are
that they have a very high AS concentra-
tion, are water-based, user friendly and
relatively low cost. Prerequisites for a
formulation of an active substance as a
concentrated suspension are low solubili-
ty of the AS in water, a reasonably high
melting point, and stability against hy-
drolysis.

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 pre-
paring 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 es-
tablished and (iii) the particles have to be
stabilized against Ostwald ripening (par-
ticle growth via a dissolution-crystalliza-
tion 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 stabili-
ization [8] becomes more important. Inde-
pendent of the surfactant class, several
basic properties of the AS and of the sur-
factant must be known for successful sta-
bilization. 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 in-
terest, since they directly reflect the ad-
sorptive 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],

\[
\frac{d \sigma}{dr} \sim D \sigma \frac{C_m V_n}{\mu}
\]

where \(\sigma\) is the interfacial tension be-
\text{tween solid and liquid,} \(r\) is the par-
ticle radius and \(V_n\) is the molar volu-
me of the AS. \(\sigma\) is very much affected by
surfactant adsorption and this reduction is,
given our experience, often the most
important factor governing particle growth.
The reduction of the interfacial tension
through surfactant adsorption can be de-
termined for ideal systems from the
measured Langmuir isotherm by combin-
ing it with the Gibbs isotherm.

Basically two different concepts exist for the determination of adsorption iso-
therms. One concept is the quantification
of the adsorbent concentration. The other
concept is the monitoring of a property of
the system, which is adsorbent concen-
tration 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 centri-
fuged or filtered and the surfactant con-
centration in the supernatant is deter-
mined by means of an appropriate mea-
surement. 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 tech-
niques like UV-Vis spectroscopy are well
suited for the surfactant quantification. If
the adsorbent has no suitable chromo-
phore, 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 preferen-
tially dissolved in the adsorbent, and (iii)
the adsorbent is a complex mixture.
Combination of all these hurdles makes it
extremely difficult to obtain reliable ad-
sorption 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 elec-
trostatically stabilized systems. Minor
changes of the surface charge through
adsorbed impurities may have dramatic
consequences for the stability of the for-
mulation. The second concept, the mon-
toring of a surfactant concentration de-
pendent system property has the advan-
tage that the measured property levels out
all heterogeneity of the AS and the ad-
sorbent, 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 impli-
cated process before extracting any data.

We determined adsorption isotherms
using a large variety of the methods de-
scribed above and found that in concen-
trated suspensions of agrochemicals and
surfactants/polymers of technical grade
quality, chromatographic, calorimetric
and electrokinetic methods were best
suited to cope with the difficulties men-
tioned [11]. Electrokinetic sonic ampi-
tude (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 compen-
sated ESA signal of an AS formulated
with varying Soprophor (sop) and Plur-
onic (Plur) concentrations. As expected,
the ESA signal, which is directly propor-
tional 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 Sopro-
phor 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.
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 (different 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 ther-mally 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 characterization 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 crystallizing 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 (\( T_{g2} \)) of the mixture can be predicted. Several formulas exist, one of them is the Nielsen equation (Eqn. 3) [16].

\[
\frac{1}{T_{g,\text{mix}}} = \frac{w_1}{T_{g,1}} + \frac{1-w_1}{T_{g,2}}
\]

\( w_1 \) is the weight fraction of the plasticizer and \( T_{g,\text{mix}}, T_{g,1} \) 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 | AS  4 | AS  5 | AS  6 | AS  7 | AS  8 | AS  9 |
|----|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| MP (°C) | 76    | 113   | 79    | 111   | 63    | 144   | 179   | 131   | 143   |
| T_σ (AS) (°C) | -18   | 15    | -7    | 15    | -20   | -1    | 75    | recr  | 20    |
| T_g (calc) (50:50) demix | -2    | 48    | 34    | 48    | 25    | 38    | 82    | -     | 51    |
| T_g (meas) (50:50) demix | 42    | 32    | 65*   | 16    | 50    | 59    | 46    | 33    |

*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.

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