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(54) **Title**: AMPHIPHILIC PROTEINS AS MORPHOLOGY MODIFIERS

(57) **Abstract**: Disclosed is a process for modifying the morphology and/or polymorphism of an organic substance, which process

Amphiphilic proteins as morphology modifiers

The present invention relates to a process for modifying the morphology and/or polymorphism of solid organic compounds, such as crystal size, habit and modification, with amphiphilic proteins, a corresponding use of amphiphilic proteins and the modified solids obtainable by the present process.

Solidification and especially crystallization is a key separation and purification unit in most of the pharmaceutical, food and specialty chemical processes (for example pigments), with a significant impact on the efficiency and profitability of the overall process. The majority of pharmaceutical products contain active ingredients produced in crystalline form. Thus crystallisation is of fundamental importance to the industry. For efficient downstream operation (such as filtration, drying, compacting) and product effectiveness (e.g. bioavailability, tablet stability) the control of crystal purity, size distribution and shape can be critically important.
Typically pharmaceutical-grade crystalline products require a narrow particle size distribution, which implies that the primary production process must be well-designed and tightly controlled under optimal conditions. Control of crystal size and shape enables the optimization of the dissolution rate and this may maximize the benefit while minimizing the side effects. Many pharmaceuticals can form crystals that exhibit multiple morphological forms and habits that are of critical importance to the formulation and end use properties of the products.

Technical background

Figure 9 illustrates a typical crystallization process embracing the following steps:
(a) Organic molecules form randomly orientated molecular clusters by a diffusion process.
(b) These clusters can either break down to single molecules again or begin to form unstable lattice formations called embryos.
(c) Such embryos can break down into clusters again or grow sufficiently to form stable nuclei which precipitate out of solution (nucleation). Such critical size is dictated by the operating conditions (temperature, supersaturation, etc.).
(d) These nuclei then grow into larger crystallites which can continue to grow into single crystals or come together to form aggregates of crystallites.

(e) Such aggregates can range from soft aggregates that can be easily broken down to the original crystallites to hard aggregates that can only be broken down by an aggressive process such as milling.

Supersaturation is the driving force of the crystallization, hence the rate of nucleation and growth is driven by the existing supersaturation in the solution. Supersaturation is defined as concentration of the solute in excess of saturated concentration under given conditions of temperature. Once supersaturation is lost, the solid-liquid system reaches equilibrium and the crystallization process stops.

Nucleation and growth continue to occur simultaneously while the supersaturation exists.

Certain solvents, the presence of impurities or additives and compounds of similar chemical type to the compound undergoing the crystallization process can strongly influence its nucleation and crystal growth stages by changing the supersaturation properties of the crystallization process.

Depending upon the conditions, either nucleation or growth may be predominant over the other, and as a result, crystals with different sizes, different size distributions and habits (shapes) are obtained.

Crystal habits can be, for example, cubic, tetragonal, orthorhombic, hexagonal, monoclinic, triclinic, and trigonal.

Different polymorphs can also be produced by changes in the crystallization process.

Polymorphs are defined as crystalline phases that have different arrangements and/or conformations of molecules in the crystal lattice. These crystal forms differ in packing, thermodynamic, spectroscopic, kinetic, surface and mechanical properties.

Although polymorphs have the same elemental composition, polymorphs exhibit different physico-chemical and physicotechnical properties such as free energy, entropy, heat capacity, melting point, sublimation temperature, solubility, stability, dissolution rate, bioavailability, hardness, compatibility, flowability, tensile strength and compressibility, etc.

For this reason, polymorphism is of major importance in industrial manufacture of crystalline products

The nature of a crystallization process is governed by both thermodynamic and kinetic factors, which can make it highly variable and difficult to control. Factors such as impurity level, mixing regime, vessel design, and cooling profile can have a major impact on the size, number, and shape of crystals produced.

Poor control of crystal size and shape can also result in unacceptably long filtration or drying times, or in extra processing steps, such as recrystallization or milling, and can influence the purity of the product which is especially important in the food and pharmaceutical industries, in which the crystals are consumed.

It is known that the morphology and size of bio-active substances can be affected by the solvent used in the crystallization process. For example, monoclinic paracetamol is formed by crystallization from ethanol, but the less stable polymorph, orthorhombic paracetamol, is formed by slow crystallization from hot water only when multiple nucleation is prevented. However, the choice of crystallization solvents is severely limited on toxicity grounds.

A number of additives have already been used to influence either the growth or the nucleation phase, resulting in modification of either the polymorphic form or the crystal habit. In some cases, paracetamol serves here as a model substance.

Synthetic (co)polymers and surfactants have also been shown to modify the morphology of bio-active substances but this has limited commercial value again on toxicity grounds.

WO03/033462 proposes polymer libraries for initiating growth of crystal polymorphs and describes the use of certain polymers to modify the crystallization of paracetamol: The crystals are grown by cooling a solution of paracetamol in hot water. In the absence of polymers, these conditions would be expected to yield monoclinic paracetamol. There is a significant bias toward the production of orthorhombic paracetamol when crystallizations are carried out in the presence of Nylons or halogenated polymers. Rodríguez-Hornedo et al., J Pharm Sci. (2004) 93(2), 449-60, describe the use of surfactants sodium lauryl sulfate and sodium taurocholate

on the crystallization of dihydrate carbamazepine.

Garekani et al., Int. J. of Pharmaceutics 208 (2000) 87, and literature cited therein, report some methods for modifying the crystal habit of paracetamol by crystallization in the presence of additives.

WO 05/115344 claims that a rapidly dissolving form of paracetamol is obtained after re-crystallization in the presence of a crystallization modifier, which may be a polymer, or a protein such as albumin, papain, pepsin.

It has now been found that rapid nucleation of organic substances in solution may be induced, even at elevated temperatures, by an amphiphatic protein, especially a hydrophobin. It has also been found that such proteins alter the crystal growth behaviour of the organic substance during the crystallization process yielding unexpected crystal habits and crystal size distribution. Amphiphilic proteins like hydrophobins may be used as additives during or after crystallization, e.g. in order to control the morphology (stabilization of meta-stable polymorphs) and the size distribution of organic compounds such as bio-active substances, e.g. for cosmetical, biocidal, pharmaceutical or medical applications (such as cosmetical actives, active pharmaceutical ingredients [APIs], animal care products, agrochemicals, biocides, pigments, dyestuffs) or to stabilize certain polymorphs. The invention thus pertains to a process for modifying the morphology and/or polymorphism of an organic substance, which process comprises treating the solid substance, or a solution or dispersion thereof, with one or more amphiphilic proteins.

The process is advantageously carried out using a solution or dispersion of the organic substance and/or solution or dispersion of the protein. The solution usually is one in a polar solvent, often an aqueous solvent such as water, lower alcohol (such as methanol, ethanol, propanol, isopropanol, butanol, isobutanol) or mixtures of water and lower alcohol, especially water.

One of the most important aspects of this invention is the modification of crystal properties of bio-active substances by employing the use of amphiphilic proteins during the crystallization process.

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