Formation of valine microcrystals through rapid antisolvent precipitation

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ABSTRACT

In this work we have experimentally studied concentration effects on antisolvent precipitation of valine (an amino acid) from aqueous isopropanol solutions. Our experiments showed that the valine precipitation is very sensitive to both the supersaturation and to the water content in the final solution. Results from spectrophotometric measurements and supersaturation analysis showed that the crystal formation kinetics is strongly dependent on both mixing and concentration profiles in the early stages of the process, even though no visible change in the systems occurs immediately upon mixing with the antisolvent or subsequent dilution. Results from small angle static light scattering measurements showed that the first visible crystals are of micron size and they grow only little over time, while their number increases gradually. Taken together, these experiments point to intermediate phase separation of (possible amorphous) precursors, being either very small nanoparticles or droplets with their refractive index closely matching that of the continuous phase, which subsequently assemble into micron size valine crystals.

INTRODUCTION

We have reported previously (1-3) on the use of rapid antisolvent coprecipitation as a method for self-assembling common pharmaceutical ingredients such as proteins, nucleic acids and nanoparticles onto the surface of crystals. The microparticles obtained are typically monodisperse and form free-flowing dry powders that have attractive properties for a range of drug-delivery applications, such as excellent levels of protein bioactivity retained, low levels of aggregation under temperature and humidity stress conditions, ability to tailor the active loading on the crystal surface, while their production is well scaleable with low-capital costs compared to freeze-drying and spray-drying technologies. Here we report studies aimed at better determining the mechanism of formation of these microcrystals.

From earlier studies (3) it was clear that the morphology of the crystals produced by precipitation were often similar in the presence and absence of the coprecipitant although in the latter case the dimensions are generally larger. These observations point towards a common formation mechanism. In this study we have therefore examined crystal formation starting with a pure solution of crystal forming material as a means of simplifying the analysis of the system. The material chosen was DL-valine because the kinetics of precipitation of this material were found to be sufficiently slow to allow the process to be monitored in real-time (4). The microcrystals precipitated using DL-valine are generally plate-like with a very high aspect ratio- typically 5-10 microns diameter with a thickness of about 0.2 microns. They are therefore very different from the spherical microparticles formed by other types of precipitation processes.

The basic process involves addition of a concentrated aqueous solution of valine to a large excess of a water miscible solvent such as isopropanol with rapid admixing. This may be carried out as either a batch process using a dynamic mixer or as continuous process using a static or dynamic mixer to produce products of similar morphology. Since the amino-acid, DL-valine, is significantly less soluble in the solvent than in an aqueous phase, widely varying levels of supersaturation can be rapidly reached and particle formation can be observed on a time-scale from seconds to days depending on solution compositions and mixing procedures.

The mechanism of the process might be expected *a priori* to involve simple nucleation and growth, where small crystal nuclei are formed first when supersaturation values are at their highest and these nuclei then grow in size by incorporating the remaining solute from the solution (5). On the other hand, there has been recently growing body of evidence that various apparently straightforward crystallisation processes follow non-classical scenarios (6-9), such as formation of nanoparticle intermediates, possibly via liquid-liquid demixing or spinodal decomposition processes, which then go on to assemble or transform into resulting crystals.

In order to better design, scale-up and control microcrystal formation processes, a better understanding of underlying physico-chemical mechanisms is needed. In this paper we report experimental studies of concentration effects in the precipitation of valine microcrystals from aqueous isopropanol solutions.

EXPERIMENTAL

Materials used in experiments were of laboratory reagent grade: 2-propanol (isopropanol), DL-valine, and deionised water from in–house Millipore Water System were used without further purification. Laboratory instruments used for measurements were DU 800 Spectrophotometer (Beckmann Coulter) for turbidimetry and Malvern Mastersizer MS 2000 (Malvern Instruments) for small angle static light scattering measurements.

Typical sample preparation included injecting small volume of valine solution in valine saturated isopropanol and mixing for 20 s with a magnetically driven stirring bar. Isopropanol solution volume ranged from 10 to 20 ml, volume of valine aqueous solution was between 30 and 100 µl. For one set of experimental conditions, at least five measurements were performed. In dilution experiments, a 1–2 ml sample was diluted at dilution ratios varying from 1:1 to 1:4 after 20 s of mixing. Spectrophotometric measurement were performed at a light wavelength of 600 nm. The experiment consisted of taking a blank measurement, sample preparation, injecting a small volume of prepared sample in cuvette and measuring the sample for a sufficiently long time to achieve a plateau in absorbance, or for at least 600 s.

Small angle light scattering experiments were performed using the Small Volume Dispersion Unit attached to measurement cell, which first had to be wetted with isopropanol to ensure bubble removal from the cell window. Only after this procedure, the measurement itself could be started. Each measurement included a background scan. In batch experiments, samples were prepared identically to those measured by the spectrophotometer. A small sample was taken at regular intervals after the initial mixing for 20 s. It was immediately injected into a glass beaker filled with a large volume of a saturated quenching solution and well stirred for several seconds. Raw data from Mastersizer measurements were analyzed with a custom made data fitting procedure to obtain the mean radius of gyration. Radius of gyration is equal to the geometrical radius divided by 1.41 ($=2^{1/2}$) for thin disks, which correspond to crystal shape of DL-valine observed in these experiments.

RESULTS AND DISCUSSION

Figure 1 shows sensitivity of precipitation kinetics to the volume ratio of aqueous and isopropanol solutions. In terms of both initial absorbance increase as well as the final absorbance reached, it can be seen that even a small difference in the volume ratio has extensive effects.

Absorbances for two volume ratios of aqueous and isopropanol solutions are shown separately in Figure 2, together with the measured radii of gyration. In the case of classical nucleation and growth mechanism, many small nuclei would form in the first moments of precipitation and in later stages only their size increase would contribute to absorbance increase.

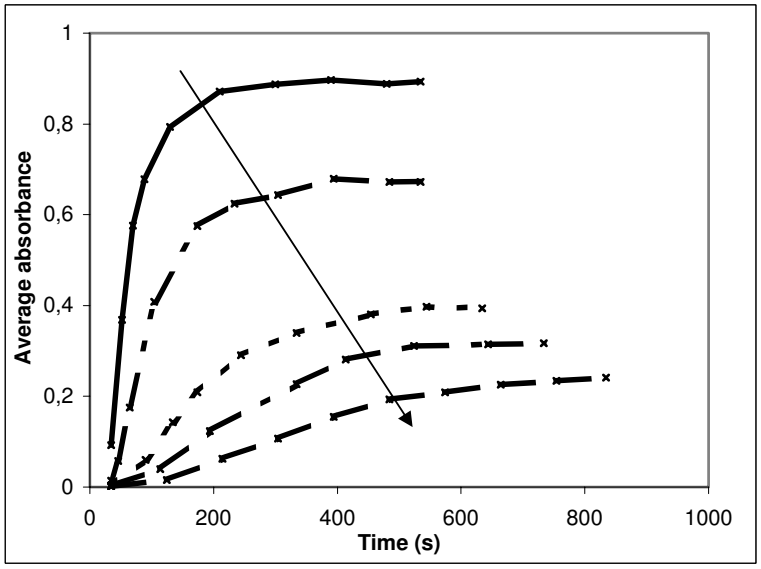

Figure 1. Spectrophotometric data on valine crystallisation kinetics. Samples were prepared by injecting 100 µl of 50 mg/ml aqueous valine solution into various volumes of valine saturated isopropanol ranging from 10 to 15 ml (arrow indicates increasing isopropanol volume).

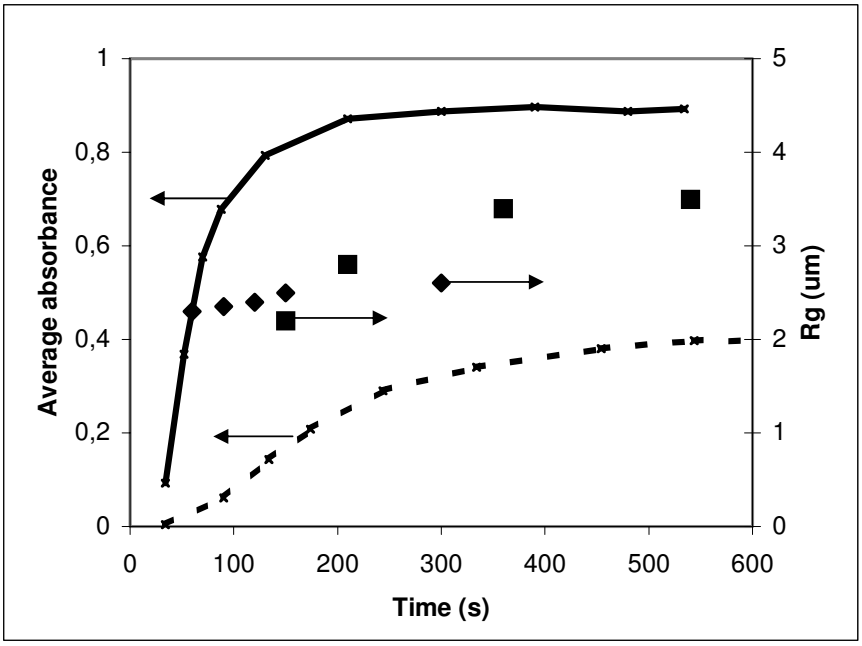

Figure 2. Comparison of spectrophotometric (left axis) and particle size (right axis) data for two different supersaturation values. Samples were prepared by injecting 100 µl of 50 mg/ml aqueous valine solution into valine saturated isopropanol: 15 ml (solid line, diamonds), 10 ml (dotted line, squares).

However, while absorbance doubles or even triples, particle size grows only by 15 % (diamonds) or by 60 % (squares). Furthermore, absorbance dependence on particle size is of order 1/3 - 2/3 (absorbance ~ $d_p^{1/3}$ to $d_p^{2/3}$) so that it is not the particle size growth, but particle number increase that is responsible for the observed absorbance increase. This confirms that a non-classical precipitation mechanism is present in this system.

Spectrophotometric data were subjected to supersaturation analysis based on DL – valine solubility data (4). In Figure 3 data from several experiments are shown. (S-1) is the supersaturation driving force for crystal growth, where $S=C/C_s$, C is the concentration of valine in supersaturated solution and C_s is its solubility in the corresponding solution. Data from dilution experiments are included as well. Overall, significant change in absorbance growth rate dependence on (S-1) value can be seen. Slopes of linear regression fits in this chart give exponents, x, in the power law dependence of the absorbance growth rate written as $(S-1)^x$. Values of the exponent x decrease notably upon dilution from 4 for original solutions (circles) to 2 for diluted solutions (squares). This observation is again inconsistent with simple nucleation and growth mechanism, where nucleation should cease or at least slow down significantly after dilution, thus leaving the already formed nuclei to grow.

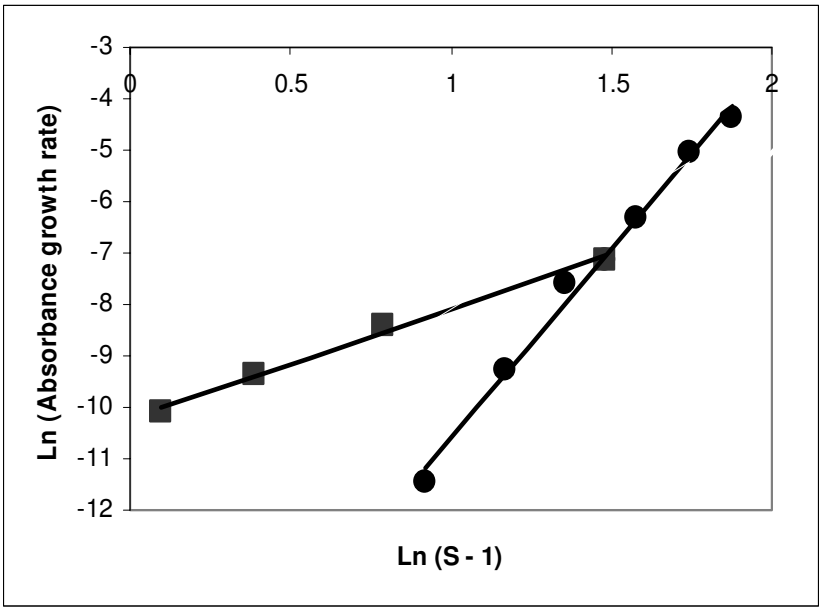

Figure 3. Dependence of absorbance growth rate on supersaturation. Samples were prepared by injecting 100 µl of 50 mg/ml aqueous valine solution into various volumes of valine saturated isopropanol (circles). Results for subsequently diluted samples are also shown (squares).

In order to explain the observed dependence of absorbance growth rate on system dilution, all nuclei present would have to grow to hundreds of microns in size, which would cause rapid sedimentation. However, this was not observed. One can therefore conclude that new crystals continued to be formed for an extended time after dilution. Moreover, the absorbance growth rate after dilution was always higher than the one for undiluted samples at the same overall supersaturation.

Figure 4 provides further evidence for non-trivial composition effects in the precipitation mechanism. It shows the time of clouding of supersaturated solutions prepared by mixing various amounts of aqueous valine solutions at concentrations of 60, 30 and 20 mg/ml with valine saturated isopropanol. It can be seen that clouding times at higher valine supersaturations depend very significantly on the overall water content in the resulting mixtures (different symbols in Figure 4 correspond to variously concentrated valine solutions used to achieve the same overall valine supersaturation

values). However, at smaller supersaturations (S around 3), the observed clouding times were essentially insensitive to the overall water content. This suggests that concentration profiles during the initial mixing play role in the subsequent crystallisation kinetics, although no changes in solutions immediately upon mixing were observed with the experimental techniques applied in this work.

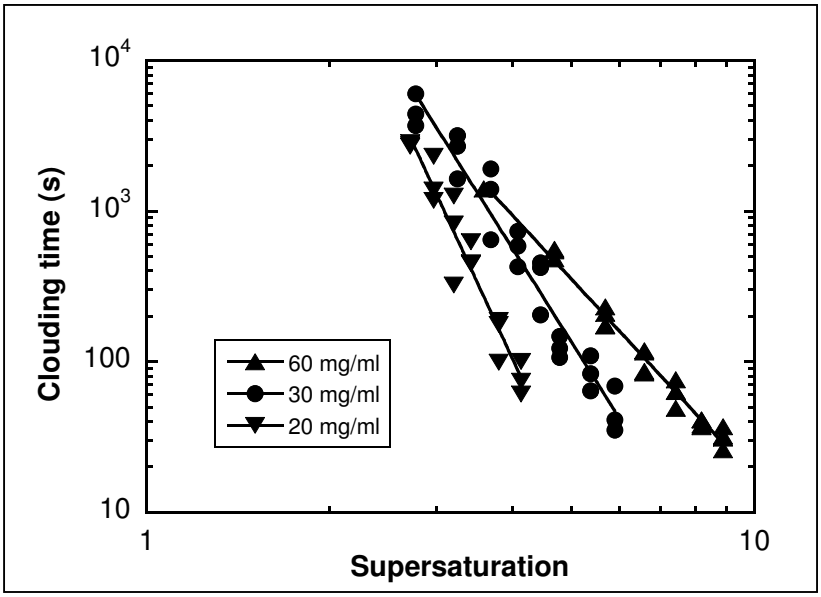

Figure 4. Clouding time dependence on the overall valine supersaturation. Samples were prepared by injecting small volumes of aqueous valine solutions with various concentrations (mg/ml) - 60 (upper triangles), 30 (circles), 20 (lower triangles) – into a large volume of valine saturated isopropanol.

We have seen that crystal formation kinetics in this system is strongly dependent on concentration profiles in the early stages of the process, even when no visible changes in the system occur immediately upon mixing with the antisolvent or subsequent dilution. Results from spectrophotometric and small angle static light scattering measurements taken together showed that the first visible crystals are of micron size and they grow only little over time, while their number increases substantially. Taken together, these experiments point to intermediate phase separation of (possibly amorphous) precursors, being either very small nanoparticles or droplets with their refractive index closely matching that of the continuous phase, which subsequently assemble into micron size valine crystals.

CONCLUSIONS

In this work we have experimentally studied effects of mixing and dilution on precipitation of DL-valine from aqueous isopropanol solutions. The spectrophotometric data showed that the precipitation process is very sensitive to both the supersaturation and water content in final solution. Seeding the solution with valine crystals can further speed up the precipitation kinetics. Dilution experiments show different absorbance vs.

time dependence, compared to standard undiluted samples with the same supersaturation. The results from spectrophotometric measurements and supersaturation analysis show that DL-valine precipitation is strongly dependent on concentration profiles in early stages of the process, even though there are no visible particles formed immediately upon mixing with antisolvent or subsequent dilution. Together with indications from small angle static light scattering, they point to intermediate phase separation of (possibly amorphous) precursors with negligible scattering, i.e., either very small nanoparticles or droplets with their refractive index closely matching that of the continuous phase.

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