Organic Process Research & Development **2009,** *13,* 1284–1290

Crystallisation from Water-in-Oil Emulsions As a Route to Spherical Particulates: Glycine and the Hydrochloride Salts of Glutamic Acid and Ephedrine

K. Chadwick, R. J. Davey,* and R. Mughal

Molecular Materials Centre, School of Chemical Engineering and Analytical Sciences, University of Manchester, Manchester M60 1QD, U.K.

I. Marziano

Pfizer Research and Development, Pfizer Limited, Sandwich, Kent CT13 9NJ, U.K.

Abstract:

Emulsion crystallization has been reported as an approach to controlling particle properties, and is part of a wider set of techniques that can be used to form spherical particles. In the pharmaceutical industry, spherical particles are known to present distinctive advantages in terms of flow and compression properties, for example over needles or laths. This study sought to define a possible working space for the development of emulsion crystallisation of materials that could mimic pharmaceutically active compounds. Crystallisation of three water-soluble materials, glycine, L-glutamic acid hydrochloride, and ephedrine hydrochloride from water-in-oil emulsions is explored. In particular, work on these compounds shows that the combined importance of stirring and surfactant and templating additive choice is evident in developing a practical route to utilising this technology. It is also evident that the relative solubility of the solute in the two liquid phases may totally preclude the use of the drops as crystallisation environments and lead to the unwanted growth of large crystals in the continuous phase.

Introduction

The use of solution crystallisation technologies as means of simultaneous purification and particle preparation of active molecules is well-known.[1] While purification is considered an inherent and ever-present aspect of crystallisation processes, the use of crystallisation to control particle properties such as crystal size, shape, structure, powder flowability, bulk density, etc. often demonstrates a dependency upon process variables such as reactor configuration, cooling profiles, and solvent choice.[2] Thus, the use of large-scale crystallisation as a means of defining and controlling crystal size, for example, while possible, is somewhat limited, particularly for sizes below 50 μm. The ubiquitous use of large batch tanks for crystallisation leads almost inevitably to the production of crystalline materials having polydispersed sizes ranging from a few tens to a few hundred micrometers.[1] Subsequent milling procedures are often utilised to achieve sizes desirable for product formulation. One possible means of enabling more effective size control in the small size range whilst still utilising batch equipment might be to apply emulsion technology to the problem and create a system in which the solution to be crystallised is constrained within small supersaturated drops which each then behave as a microscopic and individual crystalliser. If nucleation and growth can be controlled to provide one crystal or particle per drop then the drop size of the emulsion may be used to control both the particle size of crystals of the active molecule and their particle shape. Initial work following this idea has been reported by Sjøstrom et al.[3] who used evaporative crystallisation of oil-in-water emulsions for the successful preparation of poorly soluble steroids.

The idea of crystallising both inorganic and molecular crystals from emulsified melts and solutions is, in fact, well-known both as a means of studying homogeneous nucleation[4] and also in the context of purification.[5] Much of this previous work has led to the realisation that the probability of a heteronucleus being present in a drop decreases with drop size so that smaller drops are effectively stabilised against heterogeneous nucleation, and the study of homogeneous nucleation becomes possible. This effect was first demonstrated by Ostwald in 1897 for the solidification of molten salol droplets[6] and later used by Turnbull in his work on metals,[7] but it was not until 1963 that Skoda and van den Tempel[8] recognised that nucleation could be *induced* in aqueous emulsions of triglycerides by use of emulsifiers whose molecular structure resembled that of the crystallising triglyceride. Similar effects were reported by Codiez et al.[9] for stearic acid emulsions and by McClements et al.[10] for aqueous emulsions of *n*-hexadecane. More recently Davey et al.[11,12] demonstrated the enhanced purification achievable in the crystallisation of aqueous emulsions of organic melts.

* Author for correspondence. E-mail: roger.davey@manchester.ac.uk.

(1) Davey, R. J.; Garside, J. *From Molecules to Crystallizers. An Introduction to Crystallization*; Oxford University Press: Oxford, 2000.
(2) Mullin J. W. *Crystallization*, 3rd ed.; Butterworth-Heinemann: Oxford, 1993.
(3) Sjostrom, B.; Kronberg, B.; Carlfors, J. *J. Pharm. Sci.* **1993**, *82*, 579–583.
(4) Keller, D. M.; Massey, R. E.; Hileman, O. E., Jr. *Can. J. Chem.* **1978**, *56*, 831–840.
(5) Davey, R. J.; Garside, J.; Hilton, A. M.; McEwan, D.; Morrison, J. W. *Nature* **1995**, *375*, 664–666.
(6) Ostwald, W. *Z. Phys. Chem., Liepzig* **1897**, *22*, 289–330.
(7) Turnbull, D.; Cech, R. E. *J. Appl. Phys.* **1950**, *21*, 804–810.
(8) Skoda, W.; van den Tempel, M. *J. Colloid Sci.* **1963**, *18*, 568–584.
(9) Cordiez, J. P.; Grange, G.; Mutaftschiev, B. *J. Colloid Interface Sci.* **1982**, *84*, 431–441.
(10) McClements, D. J.; Dickenson, E.; Dungan, R.; Kinsella, J. E.; Ma, J. G.; Povey, J. M. *J. Colloid Interface Sci.* **1993**, *160*, 293–297.
(11) Davey, R. J.; Garside, J.; Hilton, A. M.; McEwan, D.; Morrison, J. W. *J. Cryst. Growth* **1996**, *166*, 971–975.
(12) Davey, R. J.; Hilton, A. M.; Garside, J. *Trans. IChemE* **1997**, *75*, 245–251.

10.1021/op900123n CCC: $40.75 © 2009 American Chemical Society
Published on Web 08/11/2009

This work led to the realisation that the locus of crystallisation in such systems is not necessarily within the dispersed drops but may also be in the continuous phase, depending on the combination of surface-active additives used.[13] Overall this leads to a view that control over the nucleation site in such a system is essential if it is to be used for particle size control. Following recent studies on crystallisation at the air/water interface it is apparent that such control may be possible using close packed monolayers of tailored amphiphilic molecules to induce oriented crystallisation of both organic and inorganic substrates.[14,15]

In this current study crystallisation of three water-soluble materials, glycine, L-glutamic acid hydrochloride, and ephedrine hydrochloride from water-in-oil emulsions is explored. These solutes were chosen so as to encompass the pharmaceutically interesting phenomena of polymorphism (glycine) and molecular salt formation (L-glutamic acid and ephedrine hydrochlorides).

Experimental Section

Glycine, L-glutamic acid hydrochloride (GluHCl), and (1*R*,2*S*)-ephedrine hydrochloride (EphHCl) were obtained from Aldrich, with purities 99, 98, and 99%, respectively, and used without further purification. In this study dodecane was chosen as an appropriate oil phase, being readily available at high (99%) purity from Aldrich. Emulsions were prepared at 80 °C by dissolution of the desired surfactants into the oil phase and adding the aqueous phase with overhead stirring (stainless steel paddle, 300−1400 rpm). For all experiments the ratio of oil to aqueous phase used was 90:10 by volume. The aqueous concentrations (g per 100 g water) of model compounds used in every experiment were as follows; glycine −30.78, GluHCl −37.5, and EphHCl −40.08. The solubilities of both glycine and glutamic acid hydrochloride in dodecane were less than 10^{-3} g/100 g decane while ephedrine had a higher value of 1.4 g/100 g dodecane. The surfactant mixture chosen to stabilize the water-in-oil emulsions was a combination (a ratio 70:30 by weight respectively) of sorbitan monolaurate (Span 20) and sorbitan monooleate (Span 80), both obtained from Aldrich. Span 20 and Span 80 are nonionic surfactants consisting of a hydrophobic alkyl chain and a polar headgroup that is a derivative of the sugar alcohol sorbitol. Surfactant levels from 2.5−10 wt %, based on the total aqueous phase, were used to prepare the emulsions in order to determine the minimum level needed for emulsion stability.

Crystallisation of the model compounds within these emulsions was achieved by one of two methods in which the emulsion drops were supersaturated whilst maintaining stirring at speeds in the range 300−1400 rpm In the first, supersaturation was induced by evaporation of water from the emulsion drops at 80 °C under ambient pressures, while in the second emulsions were cooled to 25 °C to supersaturate the drops. In order to follow the progress of the crystallisation processes and obtain information on the size and morphology of the emulsion drops and resulting solids, emulsion samples were taken at specific time intervals after their initial preparation and placed onto glass microscope slides, and optical micrographs were recorded using a Zeiss Axioplan 2 polarising microscope and Linksys image capture software. The solid phases obtained at the completion of a crystallisation were characterised by X-ray powder diffraction using either a Bruker D8 or Rigaku Miniflex+ Desktop powder diffractometer. Electron microscope images of the crystalline particles were recorded using an FEI Quanta 200 ESEM, operating in low vacuum mode.

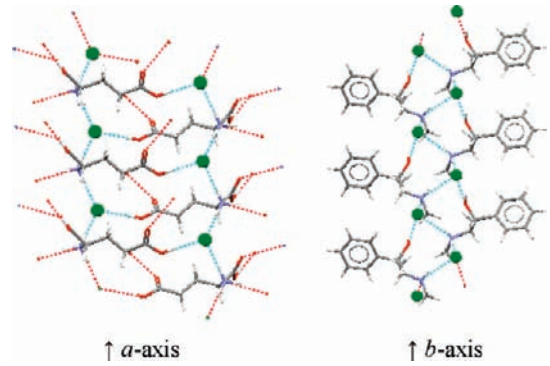

Figure 1. **Fast growth axes of, left, glutamic acid hydrochloride (*a*-axis) and, right, ephedrine hydrochloride (*b*-axis).**

The Use and Selection of Additives. Given previous reports[14,15] of using surfactant monolayers to 'template' crystal nucleation at interfaces, some potential additives were chosen as possible templates for the three solutes studied in this work. Such additives were chosen on the basis of both their molecular functionality and their hydrophobic/hydrophilic balance such that they would be simultaneously located at the oil−water interface and possess appropriate stereochemistry to template the fast-growing faces of the crystallising materials. In the case of glycine, previous work[16,17] was used as a guide, and hydrophobic amino acids (*R*,*S*)-leucine, (*R*,*S*)-norleucine, and 2-aminononanoic acid were chosen to induce crystallisation of different glycine polymorphs. For GluHCl and EphHCl no appropriate additives have previously been established and hence these were chosen on the basis of the crystal structure and morphology of the two salts.[18,19] Figure 1 shows projections of the fastest growth directions of the glutamic acid hydrochloride and ephedrine hydrochloride structures, which in both cases run in the direction of the strongest Coulombic interactions between chloride and protonated amino species. Additive molecules should be able to mimic the nature of this fast growth direction, hence a series of surface-active tetramethylammonium salts (hexadecyltrimethylammoniumbromide (CTAB), myristyltrimethylammoniumbromide (TTAB), hexadecyltrimethylammoniumchloride (CTAC), and trimethyletradecylammonium chloride (TTAC)) were selected since these possess protonated nitrogen groups capable of binding the chloride ion at the

(13) Davey, R. J.; Hilton, A. M.; Garside, J.; de la Fuente, M.; Edmondson, M.; Rainsford, P. *J. Chem. Soc., Faraday Trans.* **1996**, *92*, 1927–1933.
(14) Weissbuch I.; Popovitz-Biro R.; Lieserowitz, L.; Lahav, M. In *The Lock and Key Principle*; Behr, J. P., Ed. John Wiley and Sons: Chichester, 1994; Chapter 6.
(15) Mann, S.; Heywood, B. R.; Rajam, S.; Birchall, J. D. *Nature* **1988**, *334*, 692–694.
(16) Landau, E. M.; Popovitz-Biro, R.; Levanon, M.; Leiserowitz, L.; Lahav, M.; Sagiv, J. *J. Am. Chem. Soc.* **1989**, *111*, 1436–1440.
(17) Chen, B.-D.; Cilliers, J. J. L.; Davey, R. J.; Garside, J.; Woodburn, E. T. *J. Am. Chem. Soc.* **1998**, *120*, 1625–1626.
(18) Collier, E. A.; Davey, R. J.; Black, S. N.; Roberts, R. J. *Acta Crystallogr., Sect. B* **2006**, *62*, 498–504.
(19) Sequeira, A.; Rajagopal, H.; Chidambaram, R. *Acta Crystallogr., Sect. B* **1972**, *28*, 2514–2518.

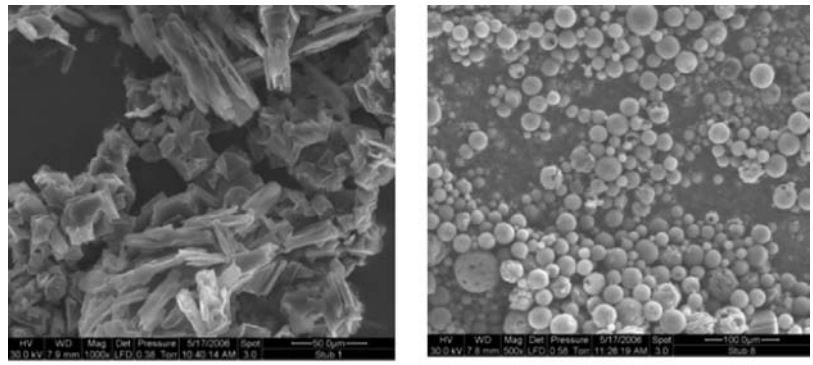

Figure 2. **SEM images of the glycine particles crystallised from water-in-oil emulsions by (a) cooling and (b) evaporation both at 700 rpm.**

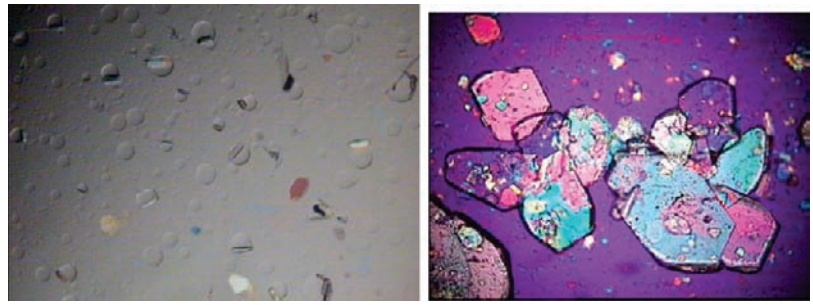

Figure 3. **Crystallisation of ephedrine hydrochloride in the oil phase, showing (left) nucleation at the drop surface and (right) the final crystals.**

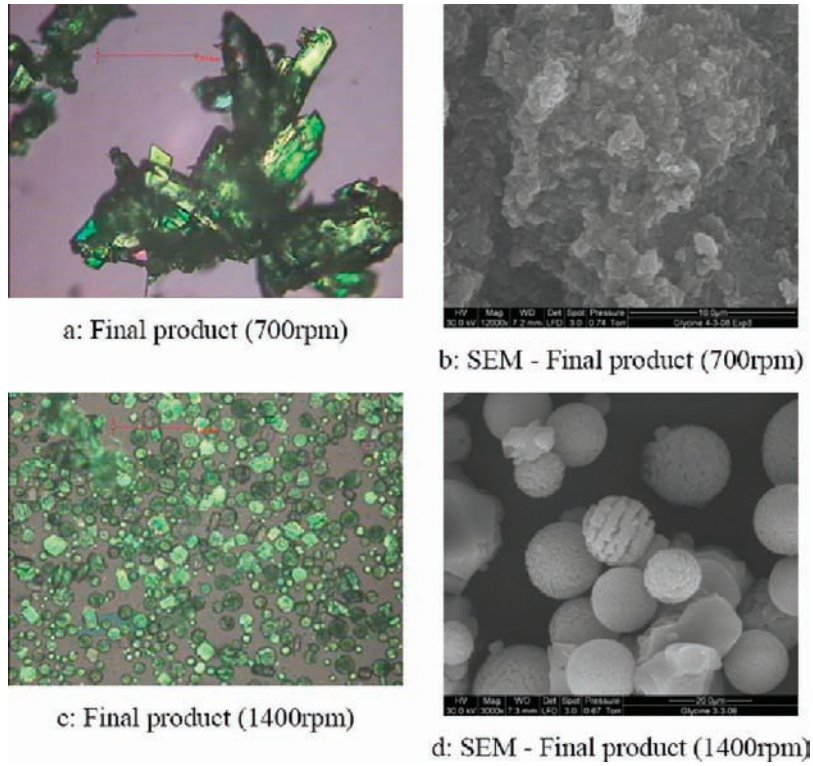

Figure 4. **Effect of stirring speed on the emulsion crystallisation of glycine showing both optical and SEM microscope images of products crystallised at 700 and 1400 rpm.**

oil−water interface and hence mimicking the fast growth direction of each salt. These 'template' additives were dissolved in the oil or aqueous phase (as required) prior to preparing the emulsion. The concentration of additive used ranged from 1−20% by the weight of the crystallising compound.

Results

Stable emulsions were created for all three systems, providing that the total surfactant levels were kept above 7.5%. This led to the choice of 10% loading in all experiments. The drop sizes depended strongly on the

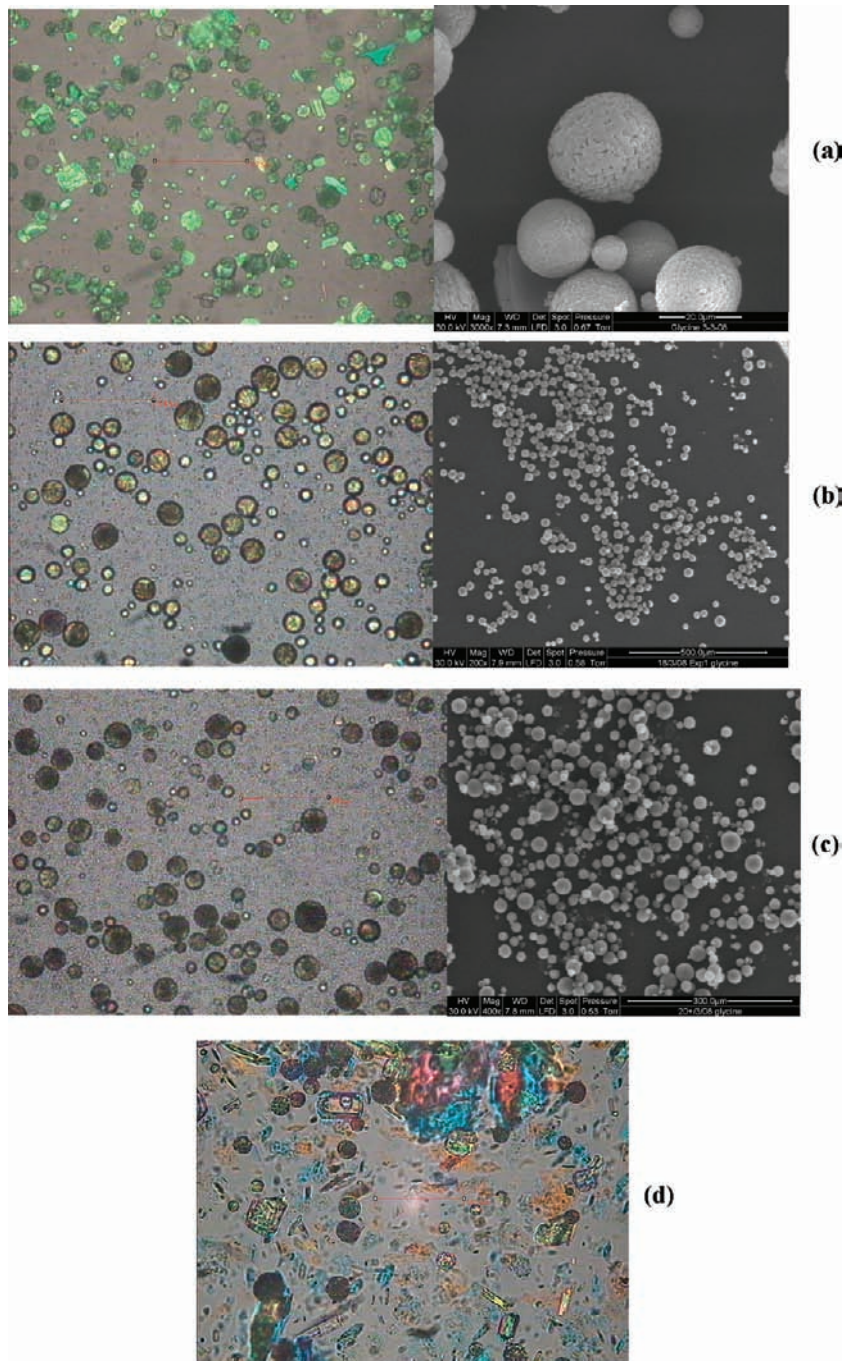

Figure 5. **Glycine emulsions crystallised in the presence of templating additives. (a) No additive, 1400 rpm, (b) 2% TTAB, 700 rpm, (c) 2% CTAB, 700 rpm, (d) 2% leucine, 700 rpm.**

stirring rate. At 300 rpm sizes were in the range 500 to 20 μm; at 700 rpm from 200 to 20 μm; at 1400 rpm all droplets were 60 μm or smaller (below 30 μm with templates TTAB and CTAB). Crystallization of the emulsions by cooling proved an ineffective method for inducing droplet crystallisation regardless of the stirring speed, surfactant concentration, or use of additives. Crystallization by evaporation, on the other hand, yielded significant droplet crystallization when using the correct experimental conditions. For glycine, Figure 2 shows a typical example of the difference in particle size and shape observed when using cooling and evaporative methods at constant stirring and equivalent additive and surfactant additions. The SEM images clearly show that when cooling the emulsions from 40 to 25 °C needle-like crystals, orders of magnitude larger than the emulsion drops, form in the oil phase, while when evaporation at 80 °C is used crystallisation of the droplets occurs and spherical glycine particles are obtained. This difference is, presumably related to the lower supersaturations and temperatures experienced in the cooling experiment which increase the chances of crystals growing in the continuous phase. As a consequence of this outcome, all subsequent experiments utilised the evaporative technique. In the case of ephedrine hydrochloride no conditions tested in this work enabled crystallisation in the droplets. As seen in

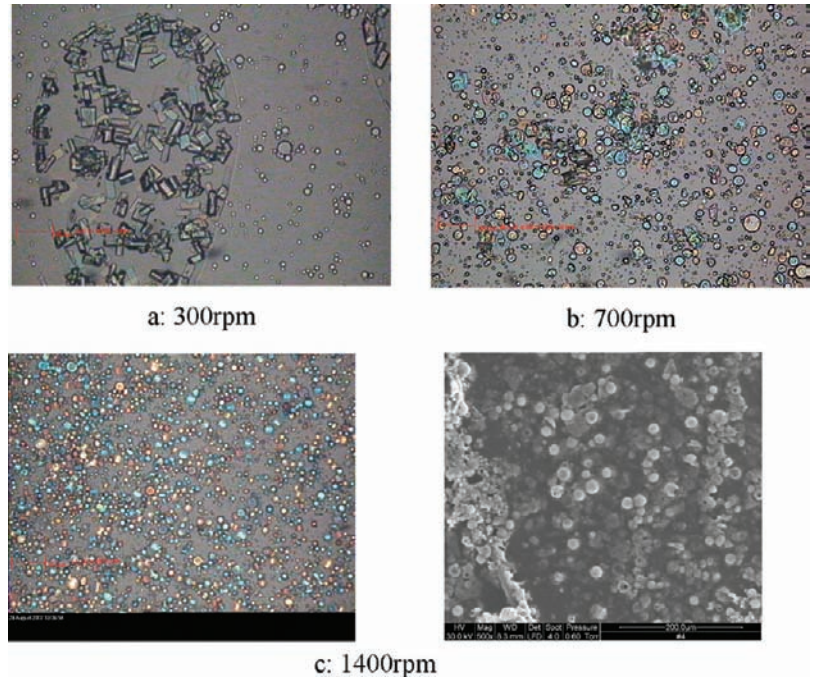

Figure 6. **Effect of stirring speed on the emulsion crystallisation of Glu HCl when using 1% TTAC as an additive (c shows both optical and SEM images of product material).**

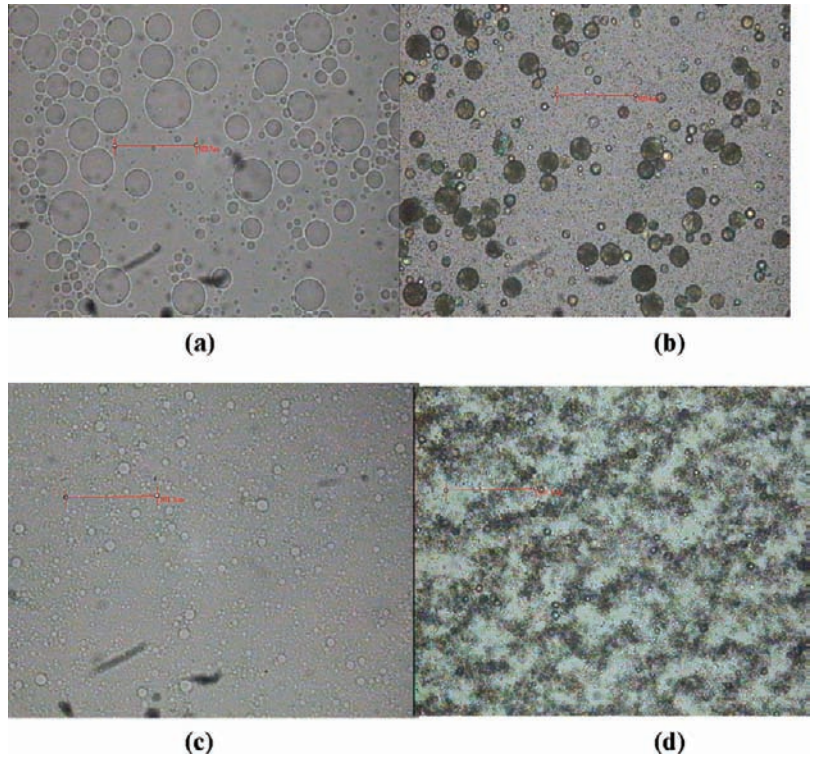

Figure 7. **Relationship between drop and particle sizes for glycine crystallisation: a and b with 2% CTAB at 700 rpm, c and d with 5% TTAB at 1400 rpm (scale bars ~100 μm in each case).**

Figure 3, nucleation appeared to occur at the aqueous/oil interface followed by subsequent crystallisation in the oil phase, leading to the growth of large platelet crystals. This point is discussed later.

In defining appropriate conditions to achieve droplet crystallisation, the effect of stirring speed was also found to be profound. Figure 4, for example, shows the results of the evaporative crystallisation of glycine at 80 °C at both 700 and 1400 rpm without the use of a template additive. At 700 rpm crystallisation occurs in the continuous phase with plate-like crystals observed, while increasing the stirring speed to 1400 rpm promoted droplet crystallisation.

The action of additives in templating nucleation within the drops is also apparent in the case of glycine. Thus, while Figure 4 shows how agitation can be utilised to induce drop nucleation, Figure 5 demonstrates the impact of added template molecules.

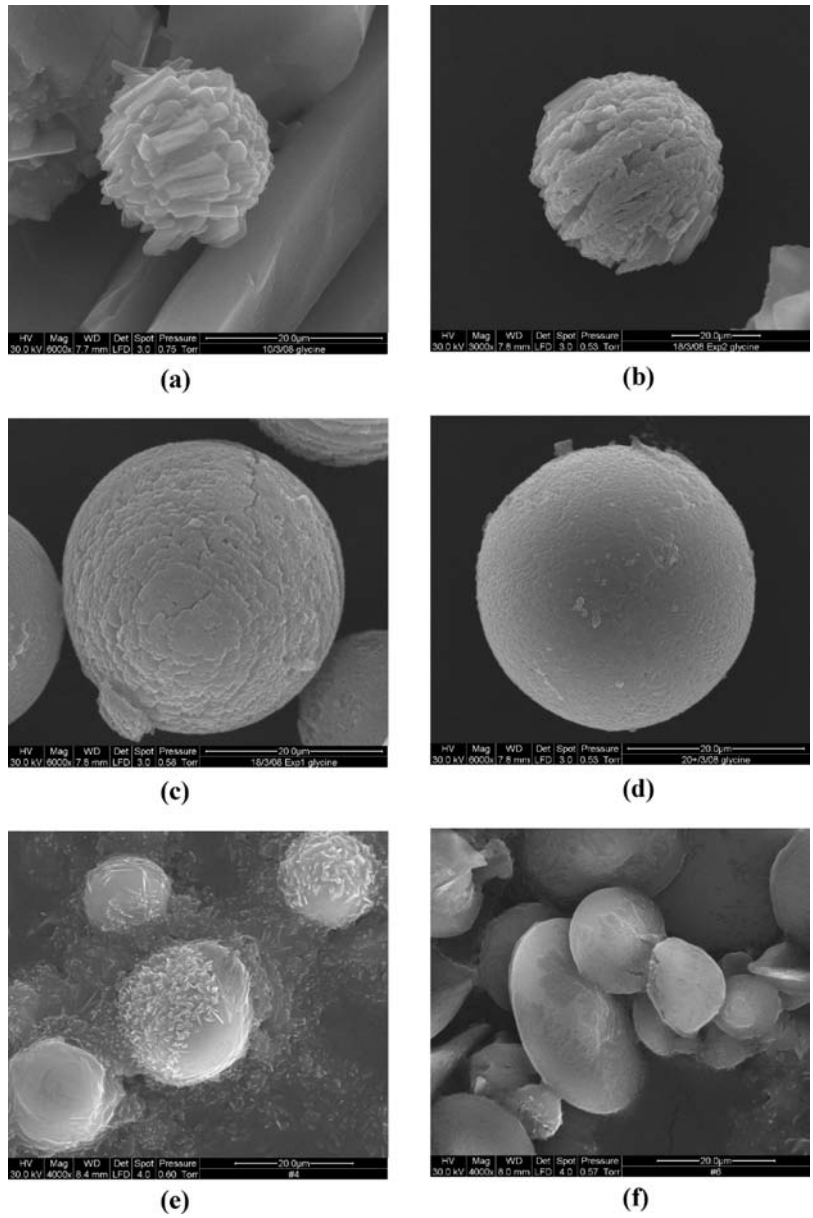

Figure 8. **The microstructure of emulsion-crystallised particles: a and b, *β*-glycine; c and d, α-glycine; e and f, GluHCl.**

Figure 5a shows again how, with no additive at 1400 rpm, a mixture of faceted crystals and crystallised drops formed. The additional use of 2% TTAB or CTAB (Figure 5b and c) induced almost total droplet crystallisation, even though the stirring speed was lowered to 700 rpm (cf. Figure 4a). *R,S*-leucine (2%) was less effective, as seen in Figure 5d, giving a mixture of spherical particles and crystals with ill-defined morphology that had, presumably, crystallised from the continuous phase. The choice of additive also affected the polymorphism of glycine obtained from the crystallisations with results that were broadly in agreement with the earlier work of Allen et al.[20] Pure solutions gave a mixture of α and β as did the presence of *R,S*-leucine and *R,S*-norleucine. CTAB and TTAB gave pure β, while γ glycine was never observed in these experiments.

Glutamic acid hydrochloride behaved in a similar way to glycine but with droplet crystallisation requiring the use of additives irrespective of the stirring rate. Figure 6 shows an example of an emulsion crystallisation of Glu HCl employing 1% TTAC as an additive. At 300 rpm crystallisation (Figure 6a) occurred in the oil phase with rod-like crystals observed. When the stirrer speed was increased to 700 rpm, significant droplet crystallisation was observed (Figure 6b). On increasing the stirrer speed further to 1400 rp, almost all the L-glutamic acid hydrochloride crystallised in the droplets (Figure 6c). Further emulsion crystallisations were carried out at (1400 rpm) for 1 and 5% concentrations of CTAB, TTAB, and TTAC. Droplet crystallisation was observed in all cases, suggesting that the templating process was not dependent on the counterion of the additive and that the additives were all successful at mimicking the functionality of the fastest growing axis as discussed above (Figure 1).

The one-to-one relationship between emulsion drop size and final particle size is well demonstrated in the case of glycine and shown in Figure 7. Clearly the particles are smaller than the drops since water is evaporated during crystallisation; thus, at the larger end of the size distribution 50 μm drops become 25 μm particles, while at the lower end 10 μm drops become 7 μm particles.

The microstructure of the particles is evidenced in detail in the SEMs of Figure 8. Thus, typically β-glycine particles comprise oriented crystals (Figure 8a) often giving the particles a layer-like appearance (Figure 8b) as reported previously.[20]

α-Glycine spheres, however (Figure 8c and d), have smoother surfaces and appear to comprise randomly oriented microcrystals consistent with extinction under crossed polars, (Figure 5). The surfaces of GluHCl particles appear essentially smooth with random areas of texture (Figure 8e) and also a tendency to adopt a disk-like morphology (Figure 8f).

In the case of ephedrine hydrochloride, as mentioned above, crystallisation always takes place in the continuous oil phase, despite examining the effect of stirring, reducing the surfactant concentration to prevent possible micellar transport from aqueous to oil phases, reducing the supersaturation to slow down the nucleation process and despite changing the oil phase to tetradecane in which ephedrine hydrochloride is slightly less soluble (1 mg/mL).

A simplistic view of the overall process suggests three main kinetic processes, any one of which may be rate controlling: nucleation in the aqueous drops, nucleation in the oil, and molecular transport of solute from the aqueous to the oil phase. In the case of glycine and L-glutamic acidHCl it appears that nucleation in the aqueous phase is the fastest of these processes with molecular transport and nucleation in the oil phase limited by slow kinetics. On the other hand, for ephedrine hydrochloride the reverse seems true with nucleation in the aqueous phase the slowest step. It is tempting to conclude that this difference derives from relative solubility of the actives in the continuous (oil) phase which, being relatively high for ephedrineHCl, leads to facile nucleation in the oil phase and consequent supply of material from the solution drops to the growing crystals. When the emulsion is prepared, the solute will partition itself between disperse and continuous phases and upon solvent evaporation, providing equilibrium is achieved up to the point of nucleation, the supersaturation will be the same in each phase. According to the nucleation eq 1

$$J = K_J \exp(-B\gamma^3/T^3\sigma^2) \qquad (1)$$

the relative nucleation rates (J) in the aqueous and continuous phases at fixed temperature should therefore depend only on the absolute concentration of the solute in both phases incorporated into the pre-exponential factor, (K_J,) and the free energy barrier as reflected in the interfacial tension γ. The parameter B incorporates the shape of the nucleus and the molar volume and is the same in both phases. For a given supersaturation (σ) and temperature (T) therefore, nucleation will be maximised in that phase which offers the most favorable combination of solubility and interfacial tension. For ephedrine hydrochloride the hydrophobic nature of the phenyl ring leads to significant solubility in the oil phase, not seen in either gylcine or GluHCl, which increases K_J and decreases γ, giving rise to favorable conditions for nucleation in the continuous phase which no process modifications appear to obviate. Classical nucleation theory offers no insight into the combined effects of templates and strirring observed for glycine and Glu HCl. In the case of the templates the results confirm the thesis that appropriately functionalised interfaces will induce nucleation in the drops. The impact of stirring is more difficult to interpret but may be related to effect of the agitation in preventing drop coalescence and hence halting the growth of relatively large crystals which can then transfer from aqueous to oil phase.

Conclusions

This work has explored some practical issues surrounding the design of emulsion crystallisation processes for water-soluble solutes, including the pharmaceutically relevant class of molecular salts. The combined importance of stirring, surfactant, and templating additive choice is evident in developing a practical route to utilising this technology. It is also evident that the relative solubility of the solute in the two liquid phases may totally preclude the use of the drops as crystallisation environments and lead to the unwanted growth of large crystals in the continuous phase.

Acknowledgment

We thank Pfizer Inc for supporting this work through a postdoctoral research fellowship.

Received for review May 11, 2009.

OP900123N

(20) Allen, K.; Davey, R. J.; Ferrari, E. C.; Towler, C.; Tiddy, G. J.; Jones, M. O.; Pritchard, R. *Cryst. Growth Des.* **2002**, *2*, 523–527.