Supercritical Fluid Processing of Materials from Aqueous Solutions: The Application of SEDS to Lactose as a Model Substance

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Purpose. The objective of the work was to study the factors influencing the crystallisation of α-lactose monohydrate from aqueous solution using the Solution Enhanced Dispersion by Supercritical Fluids (SEDS) technique.

Methods. An aqueous solution of α-lactose monohydrate is dispersed with a homogeneous mixture of carbon dioxide-ethanol/methanol using a co-axial nozzle. Crystallised lactose particles were analysed for water content by Karl-Fisher analysis, anomeric composition by Differential Scanning Calorimetry (DSC) and characterised for crystallinity by powder X-ray diffraction and morphology by scanning electron microscopy.

Results. Water content in the lactose recrystallised with ethanol was higher compared to the product obtained with methanol as cosolvent. Rate of crystallisation could be altered by varying the CO_2 flow thereby modifying the water content in the lactose. At low flow rates of CO_2, the crystallisation occurred in a cosolvent rich antisolvent phase causing rapid crystallisation whereas high flow rates of CO_2 favoured a much slower crystallisation mechanism in the water rich phase. As a consequence, the morphology changed from thin long bands to large agglomerated chunks with mean particle size between 5 and 31 microns.

Conclusions. The SEDS process is an efficient method for forming micron sized particles of water-soluble compounds with controlled physico-chemical properties.

KEY WORDS: particle formation; lactose; supercritical CO_2; powders, nozzle.

INTRODUCTION

Supercritical fluid (SCF) technologies have advanced in recent years with ever increasing applications covering a wide variety of processes in the chemical, biochemical and the pharmaceutical industry. Pharmaceutical materials processing with SCF's is a relatively new and efficient approach to prepare high purity micron sized particles with defined morphological structures in a single step operation. In this context, production of high purity, chemically stable particles with controlled physico-chemical properties of drug compounds in a consistent, reliable and cost efficient process remains a challenge to the process industry. Supercritical fluids, due to their flexible physical properties for example density, and transport properties such as viscosity and diffusivity, and the relative ease by which these properties can be manipulated with temperature and pressure, promise an efficient means of solving some of these problems. Because of their liquid like densities, SCF's are capable of dissolving a number of the high molecular weight low vapour pressure pharmaceutical compounds, and thus a proportion of the impurities present in the drug material could be selectively removed. Several SCF processes for particle formation reported in the literature include (a) precipitation from supercritical solutions—*Rapid Expansion of Supercritical Solution (RESS)* (1) and (b) precipitation from liquid solutions using supercritical fluid antisolvent—Gas Antisolvent (GAS) process (1), Aerosol Solvent Extraction System (ASES) (2), Precipitation by Compressed fluid Antisolvent (PCA) (3), Supercritical fluid Antisolvent process (SAS) (4) and Solution Enhanced Dispersion by Supercritical fluid (SEDS) (5).

However, these processes are not suitable for aqueous systems due to poor solubility of supercritical carbon dioxide in water and vice versa. The purpose of the present paper is to demonstrate the ability of the modified SEDS process to crystallise water soluble compounds from an essentially aqueous environment using lactose as a model substance. Future work will then consider the preparation of labile macromolecules like proteins in this benign process as compared to conventional methods of freeze drying and spray drying.

Lactose, a widely used excipient in the pharmaceutical industry, is highly soluble in water, sparingly soluble in methanol and virtually insoluble in ethanol. It is therefore an ideal model compound to carry out a systematic investigation for crystallising water soluble compounds using the SEDS process. In earlier work using SEDS (6), it has been shown that lactose can be recrystallised from aqueous solutions with different solid state properties compared to the starting material. An objective of the present work is to evaluate the effect of various process parameters on the crystallisation process and their influence on the physico-chemical properties of the crystallised lactose.

Phase Equilibria of Water-methanol-CO_2 System

Methanol and ethanol have been chosen as the organic antisolvents in the present study and hence the phase behaviour of water-methanol-CO_2 and water-ethanol-CO_2 systems is of interest at two representative processing temperatures (i) 50°C and (ii) 90°C. References for the binary and ternary equilibrium data reported in the literature are given in Table 1. Whilst limited data are available in the literature in the relevant ranges of temperatures and pressures for this study, the phase behaviour of the ternary H_2O- MeOH-CO_2 system has been predicted using the Peng-Robinson equation of state (16) and is shown in Figure 1 at 50°C and 90°C. The binary interaction constants used in the quadratic mixing rules for the calculations are given in Table 2. Methanol is completely miscible with supercritical carbon dioxide at 50°C while water and carbon dioxide are not miscible at either temperatures. The solubility of carbon dioxide in water is low, less than 3.0 mole % at 50°C and that of water in carbon dioxide is less than 0.5 mole % (11). Owing to poor representation of the phase equilibrium by the equation of state in the critical region, these diagrams have been used in a qualitative manner to understand the fundamental concepts of particle formation with supercritical fluids. The phase behaviour of the

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Table 1. Literature Sources of Binary and Ternary Systems Relevant for the Present Study

System	Temperature, °C	Pressure, bar	Reference
MeOH-CO_2	50, 75, 100, 125	5–185	(7)
MeOH-H_2O	35, 50, 65	2–53	(8)
EtOH-CO_2	35, 50, 75	10–110	(9)
EtOH-H_2O	30, 50, 70, 90	0.04–1.7	(10)
H_2O-CO_2	40–350	25–1500	(11,12)
EtOH-H_2O-CO_2	35, 40, 50, 60, 70	80–190	(13,14)
MeOH-H_2O-CO_2	25	2–54	(15)

EtOH-H_2O-CO_2 system has not been computed and, whilst available in the literature for 50°C at 120 bar pressure (14), is presumed to be similar to that of the MeOH-H_2O-CO_2 system at 90°C.

Above the critical mixture composition, higher concentrations of carbon dioxide increases the vapour fraction (SCF phase in Figure 1) and lower concentrations result in a liquid phase rich in methanol with CO_2 dissolved in it. Thus at high concentrations of CO_2, for an aqueous phase in contact with a vapour phase rich in carbon dioxide with a cosolvent methanol dissolved in it, the distribution of the individual components between the two phases is kinetic dependent and a function of the diffusion coefficients and other transport properties. Such a process path intersects several tie lines with the mutual transport of methanol and CO_2 from the vapour rich phase to the aqueous rich phase and water from the aqueous phase into the vapour phase until the conditions of equilibrium are reached.

In the present study, the flow rates of co-solvent and aqueous solutions have been kept constant and the flow rate of carbon dioxide was varied. A liquid carbon dioxide flow of 18.0 ml min^{-1} provides the condition of a mixture consisting of high vapour fraction and a flow of 5.0 ml min^{-1} gives a mixture with properties similar to the phase rich in methanol and carbon dioxide. Accordingly, the rate of crystallisation in the miscibility region is fast due the high proportion of antisolvent (co-solvent + CO_2) and slower at the conditions of 'time dependent mass transfer'. It is therefore hypothesised that these two environments would initiate the particle nucleation and growth differently and thus help unfold some of the details of the particle formation process mechanisms. The experimental process flow conditions have been translated into mole fraction units per unit time and are shown on the ternary plots in Figure 1. Apart from the situation at 90°C and 150 bar, the different flow conditions fall in the vapour phase region. Since the conditions during a SEDS run are far from equilibrium, it is believed that the process path of a two phase flow (aqueous and CO_2 + co-solvent streams) reaching a homogeneous phase would be along the tie line particularly in case of a kinetic regime.

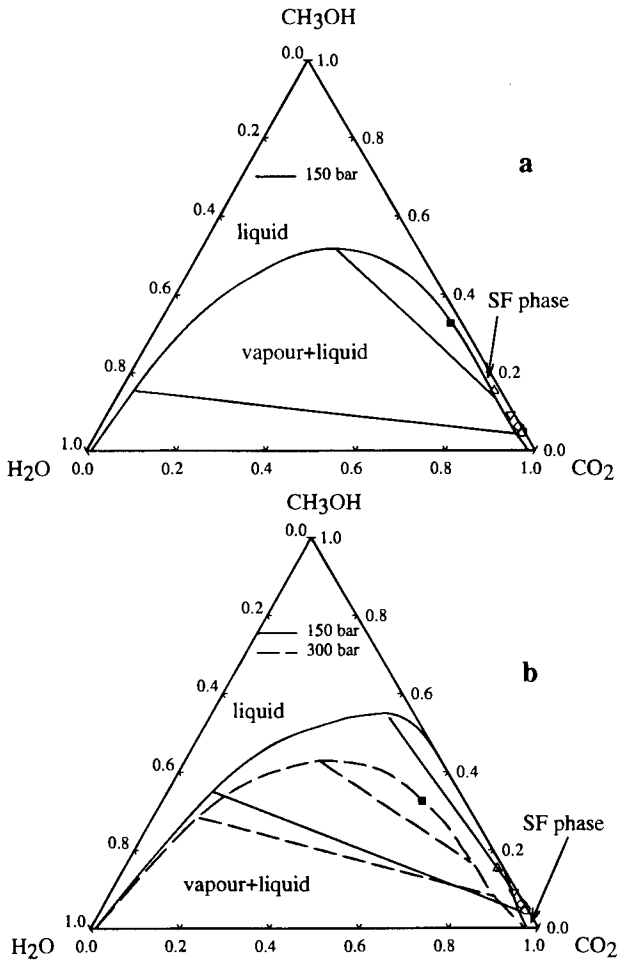

Fig. 1. Estimated phase behaviour of H_2O-CH_3OH-CO_2 system at (a) 50°C and (b) 90°C. ■ - Critical point. Process flow conditions: Aqueous lactose solution = 0.035 ml/min.; methanol solution = 0.665 ml/min.; liquid carbon dioxide: △ = 5.0 ml/min; ▽ = 9.0 ml/min.; ◇ = 14.0 ml/min.; ○ = 19.0 ml/min.

Table 2. Binary Interaction Constants Used in the Calculation of Methanol-Water-CO_2 Phase Behaviour

System	k_{ij}	Remarks
	Temperature = 50°C	
CO_2-MeOH	0.079	regressed from experimental data (7)
MeOH-H_2O	−0.0966	extrapolated from the regressed values from experimental data at 150°C and 200°C (8)
CO_2-H_2O	−0.019	regressed from experimental data (11)
	Temperature = 90°C	
CO_2-MeOH	0.10	taken as the same regressed value at 100°C from experimental data (7)
MeOH-H_2O	−0.0926	extrapolated from the regressed values from experimental data at 150°C and 200°C (8)
CO_2-H_2O	−0.017	extrapolated from the regressed values from experimental data at 50°C and 75°C (11)

MATERIALS AND METHODS

Materials

Carbon dioxide (MG gases, UK) was 99.9% pure and α-lactose monohydrate (Sheffield Products, USA) consisted of 89% of α form and 11% β-lactose. Anhydrous lactose (Sheffield

Products, USA) was composed of 70% of the β form with the remainder in the α form. Methanol and ethanol (BDH Chemicals, UK) were of 99.9% purity.

Apparatus and Methodology

The laboratory scale SEDS experimental set-up has been described in detail elsewhere (6) and a schematic of the apparatus is shown in Figure 2. The apparatus consists of a pulse dampener, nozzle, and a particle formation vessel enclosed in an air oven. Liquid carbon dioxide was cooled by passing through a cooler (RTE-101, Neslab, UK) and pumped at the required flow rate by a high pressure reciprocating liquid chromatography pump (PU 986, Jasco, Japan). The liquid CO_2 passes through a 5 meter length of tube in the air oven (860-CO, Jasco, Japan, accuracy ± 0.1°C.) maintained at the required temperature which acts a heat exchanger changing the state of CO_2 from liquid to supercritical phase. A pulse dampener, consisting of a fixed bed of silica gel, smoothens the pulsed flow of carbon dioxide from the pump. In earlier experiments (6), a highly saturated lactose solution was prepared by adding the cosolvent, methanol, to the aqueous lactose solution in the ratio 95:5 by volume and was pumped through the nozzle along with carbon dioxide. With this arrangement, the effect of alternative cosolvents could not be studied as lactose is insoluble in other organic solvents such as ethanol. Hence the process has been modified by dissolving the cosolvent in supercritical carbon dioxide prior to entering the nozzle. A premixer has been added to the apparatus prior to the vessel to thoroughly mix the organic solvent and carbon dioxide into a homogeneous phase. The homogeneous CO_2-cosolvent mixture was then introduced into the particle formation vessel through the nozzle. Pressure in the system was maintained at ± 1 bar by the back pressure regulator (Model 880-81, Jasco, Japan).

An aqueous lactose solution was prepared by dissolving α-lactose monohydrate in distilled water and the solution was allowed to stand for one hour to reach the anomeric equilibrium composition of the α and β forms of lactose in water. At stable conditions of temperature and pressure, the aqueous lactose solution was pumped into the system through the nozzle along with carbon dioxide-cosolvent mixture. The two streams are thoroughly mixed and dispersed into the particle formation vessel by the nozzle. The particles formed were retained in the vessel by the 5 μm filter secured at the outlet end of the vessel. After a typical run time of one hour, aqueous solution and organic solvent flow were stopped and pure carbon dioxide pumped for a further 10–15 minutes to displace the CO_2-organic mixture in the vessel. Pressure in the system was then released very slowly in a controlled manner by means of the back pressure regulator. The particulate product in the vessel was collected and characterised for its physico-chemical properties.

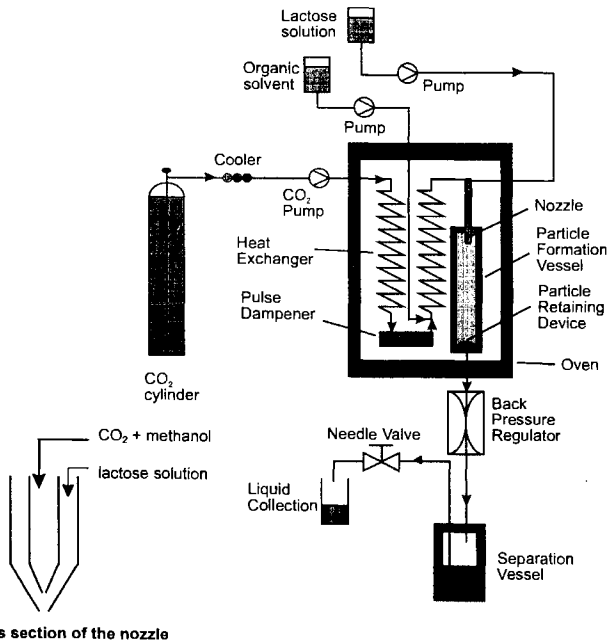

Fig. 2. Schematic diagram of the experimental set-up.

Particle Characterization

Water Content Analysis—Karl Fisher Titrometry

Moisture content of the lactose samples was determined semi automatically by the Karl Fisher method (701 KF Titrino, Metrohm Ltd., Switzerland). The titration for each sample was repeated three times and an average value taken.

Thermogravimetric Analysis (TGA)

The analysis was performed on a thermogravimetric analyser (TGA7, Perkin Elmer Ltd., UK). The temperature axis was calibrated using the ferromagnetic standard. All samples were heated in open pans at 10°C min^{-1} from 50° to 300°C under a dry nitrogen purge.

Crystallinity

Powder X-Ray Diffraction

Samples, without further treatment, were filled into sample holders and scanned in an x-ray powder diffractometer (Siemens D 5000, Siemens, Germany) with two theta ranging between 1.5 and 60 degrees with a 0.05 degree step increase at a 3 second count interval using Cu K_a radiation.

Anomeric Composition

Differential Scanning Calorimetry (DSC)

About 2–5 mg of lactose sample was accurately weighed and scanned in a pierced, crimped aluminium pan by DSC (DSC 7, Perkin Elmer Ltd., UK). The heating range was between 50–300°C at 10°C min^{-1} under a dry nitrogen purge.

Size Measurement

Particle size was measured using an Aerosizer with Aerodisperser (API, Amherst, USA). In this technique a dry powder sample of 1–5 mg is fluidised by a partial air jet stream, dispersed by a nozzle, and is passed through the path of two parallel laser beams. The time of flight of the particles across the beams is detected by two photomultipliers and is translated into velocity units. From the true density of the particles, the geometric diameter of the particles is determined based on

differential volume. The analysis of lactose samples was repeated three times and an average value taken.

Particle Morphology

Images of lactose particles were obtained using a scanning electron microscope (Model S-520, Hitachi, Japan). Samples were coated with gold using a sputter coater (E5150, Polaron Equipment Ltd., UK) prior to analysis. An accelerating potential of 15 KV was used and the images were captured by computer using an *i-scan* software (ISS Group, UK).

RESULTS

Starting Materials

The XRPD patterns and DSC/TGA profiles of α-lactose monohydrate starting material and anhydrous β-lactose are shown in Figures 3 and 4 respectively. The DSC profile of the monohydrate form is characterised by the presence of a dehydration endotherm occurring at 140°C. The dehydration event is also shown in the TGA profile with a weight loss of 5% accounting for the water present in the sample. The fusion endotherm followed by decomposition occurs at 215°C for the monohydrate and at 230°C for the anhydrous form. The particle size and Karl-Fisher analysis values are presented in Table 3. The monohydrate form of lactose contains 5.27% of water as compared to 0.31% in the anhydrous form.

Effect of Methanol and Ethanol as Cosolvents

Methanol and ethanol as cosolvents have major effects on the crystallised product as can be seen from the experimental results at 50°C tabulated in sets 1 and 4 respectively in Table 3. An interesting feature is that the size of the particles with ethanol as a cosolvent is larger than those crystallised with methanol, with a trend of decreasing size with reduced flow rates of CO_2 in both the cases. Similar trends are also obtained for the water content of the particulate products, with Karl Fisher analysis values higher for product crystallised from ethanol than those from methanol. This observation is confirmed by the presence of a dehydration endotherm in the DSC profile shown in Figure 4 which is absent for lactose processed with methanol.

A further important observation is that the crystallised product obtained with either of the organic solvents, is a mixture of the two anomeric forms α and β lactose, as shown by the representative XRPD and DSC profiles in Figures 3 and 4 respectively. This finding is explained by the existence of an anomeric equilibrium between lactose monohydrate and anhydrous lactose formed by the partial conversion of lactose monohydrate in solution. Scanning electron micrographs of the particles crystallised with both methanol and ethanol are shown in Figure 5. At high flow rates of carbon dioxide, the particles appear as platelets fused together into large 'chunks'. The morphology changes from such 'chunks' to thin long bands with decreasing flow of carbon dioxide.

Effect of Temperature

The study was carried out at two temperatures, 50°C and 90°C, with methanol as a cosolvent. At 90°C and 150 bar

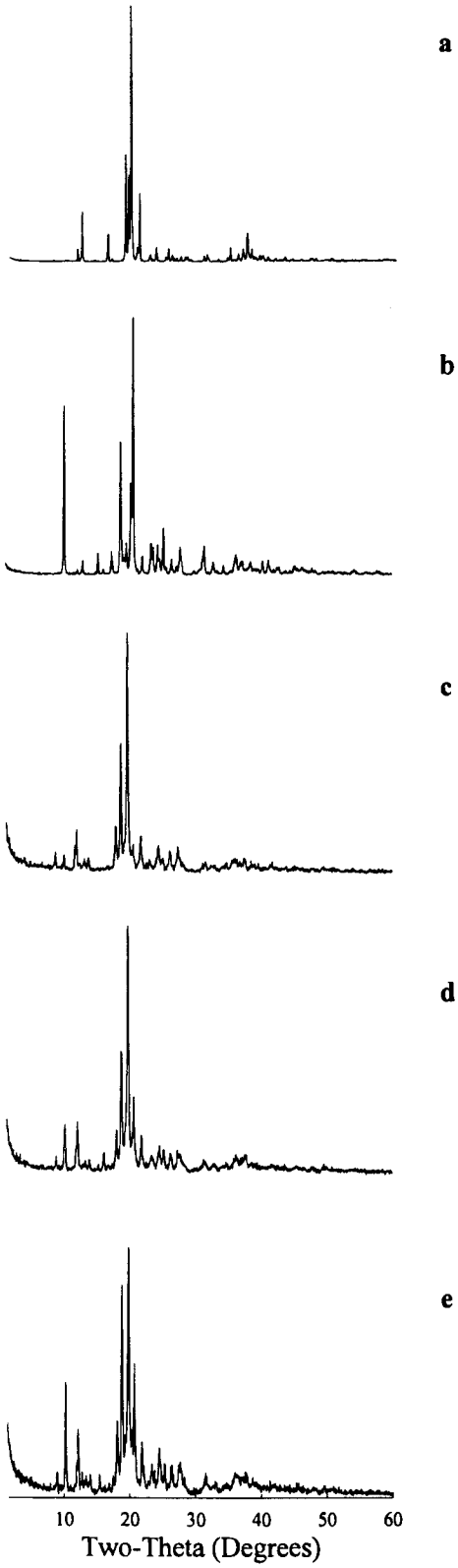

Fig. 3. XRPD patterns of unprocessed and SEDS processed lactose. (a) α-lactose monohydrate (b) anhydrous β-lactose. SEDS processing conditions: lactose concentration in water = 10% w/v; flow rate of consolvent = 0.665 ml/min; flow rate of liquid CO_2 = 19.0 ml/min (c) methanol – temperature = 50°C, pressure = 150 bar (d) ethanol - temperature = 50°C, pressure = 150 bar (e) methanol – temperature = 90°C, pressure = 300 bar.

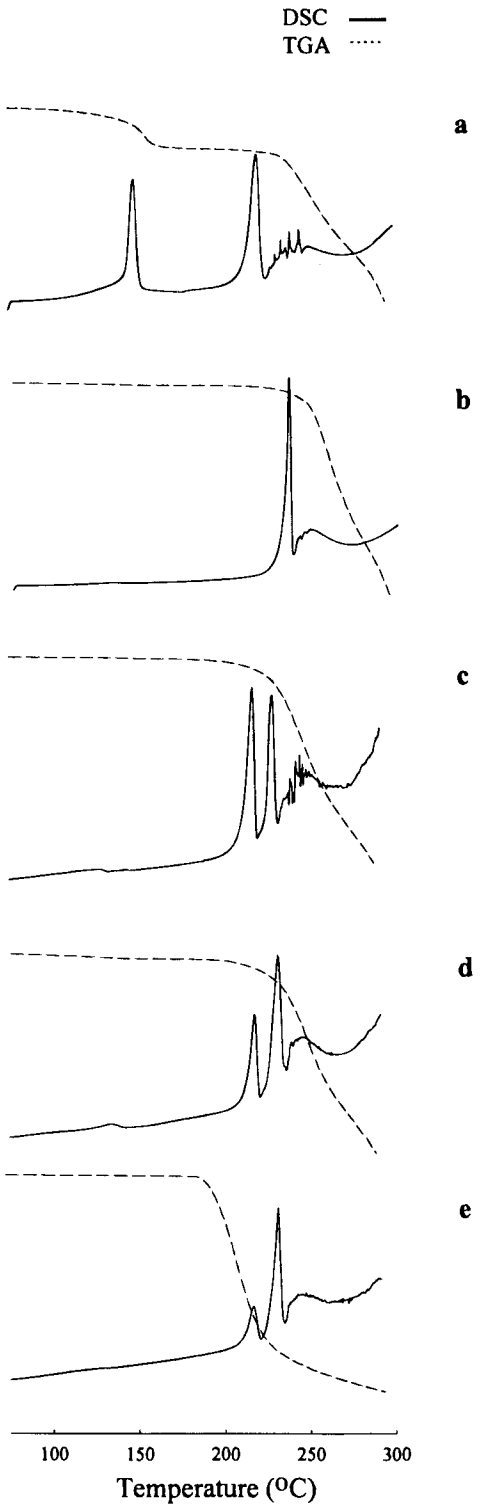

Fig. 4. DSC and TGA profiles of unprocessed and SEDS processed lactose. (a) α- lactose monohydrate (b) anhydrous β-lactose. SEDS processing conditions: lactose concentration in water = 10% w/v; flow rate of lactose solution = 0.035 ml/min flow rate of cosolvent = 0.665 ml/min; flow rate of liquid CO_2 = 19.0 ml/min (c) methanol – temperature = 50°C, pressure = 150 bar (d) ethanol – temperature = 50°C, pressure = 150 bar (e) methanol – temperature = 90°C, pressure = 300 bar.

Table 3. Experimental Conditions and Particle Analysis Results of Lactose Prepared Using the SEDS Process[a]

	Lactose[d]		Liquid carbon dioxide flow rate, ml min^{-1}			
	α[e]	β[f]	5.0	9.0	14.0	19.0
Size[b]	31.17	16.99				
Water[c]	5.27	0.31				
Co-solvent - Methanol						
Set 1: Pressure = 150 bar; Temperature = 50°C						
Size[b]			4.20	7.58	10.52	7.16
Water[c]			1.59	1.33	1.24	1.47
Set 2: Pressure = 150 bar; Temperature = 90°C						
Size[b]			10.68	9.38	10.37	5.78
Water[c]			1.23	2.32	1.13	1.16
Set 3: Pressure = 300 bar; Temperature = 90°C						
Size[b]			5.89	17.92	18.79	9.13
Water[c]			1.03	1.39	1.49	2.59
Co-solvent - Ethanol						
Set 4: Pressure = 150 bar; Temperature = 50°C						
Size[b]			12.69	15.41	12.00	11.12
Water[c]			2.53	3.40	4.74	3.06

[a] Concentration of α-lactose monohydrate in H_2O = 10% w/v; flow rate of aqueous lactose solution = 0.035 ml min^{-1}; flow rate of Methanol = 0.665 ml min^{-1}.
[b] Average value of the geometric diameter measured from volume distribution (microns).
[c] Karl Fisher analysis (%w/w).
[d] Unprocessed lactose (Sheffield products).
[e] α-lactose monohydrate.
[f] anhydrous β lactose.

and at flow conditions of liquid CO_2 –5.0 ml/min, lactose solution—0.035 ml/min and methanol—0.665 ml/min, the mixture exists as vapour and liquid phases as shown in Figure 1. The results listed in sets 1 and 2 in Table 3, indicate that temperature does not affect significantly the particle size and water content. Shape of the particles observed at 50°C and 90°C, shown in Figures 5 and 6 at the respective flow rates at each of the temperatures, is similar.

Effect of Density of Supercritical Carbon Dioxide

The density of pure carbon dioxide at 50°C and 150 bar and 90°C and 300 bar is 0.73 g/cc. The phase behaviour at these two sets of conditions is similar as can be seen from Figure 1. The effect of density can be studied by comparing the results presented in sets 1 and 3 in Table 3 with methanol as cosolvent. The product is crystalline and a mixture of both the anomeric alpha and beta forms as illustrated by the representative XRPD and DSC/TGA patterns shown in Figures 3 and 4. It is interesting to note that the water content of the product prepared at the two conditions is comparable. At 90°C and 300 bar, water content decreases by two fold with reducing flow of carbon dioxide in contrast to 50°C and 150 bar where it is unaffected. Particles are micron sized and morphology of the particles prepared at these two sets of conditions is similar as shown in Figures

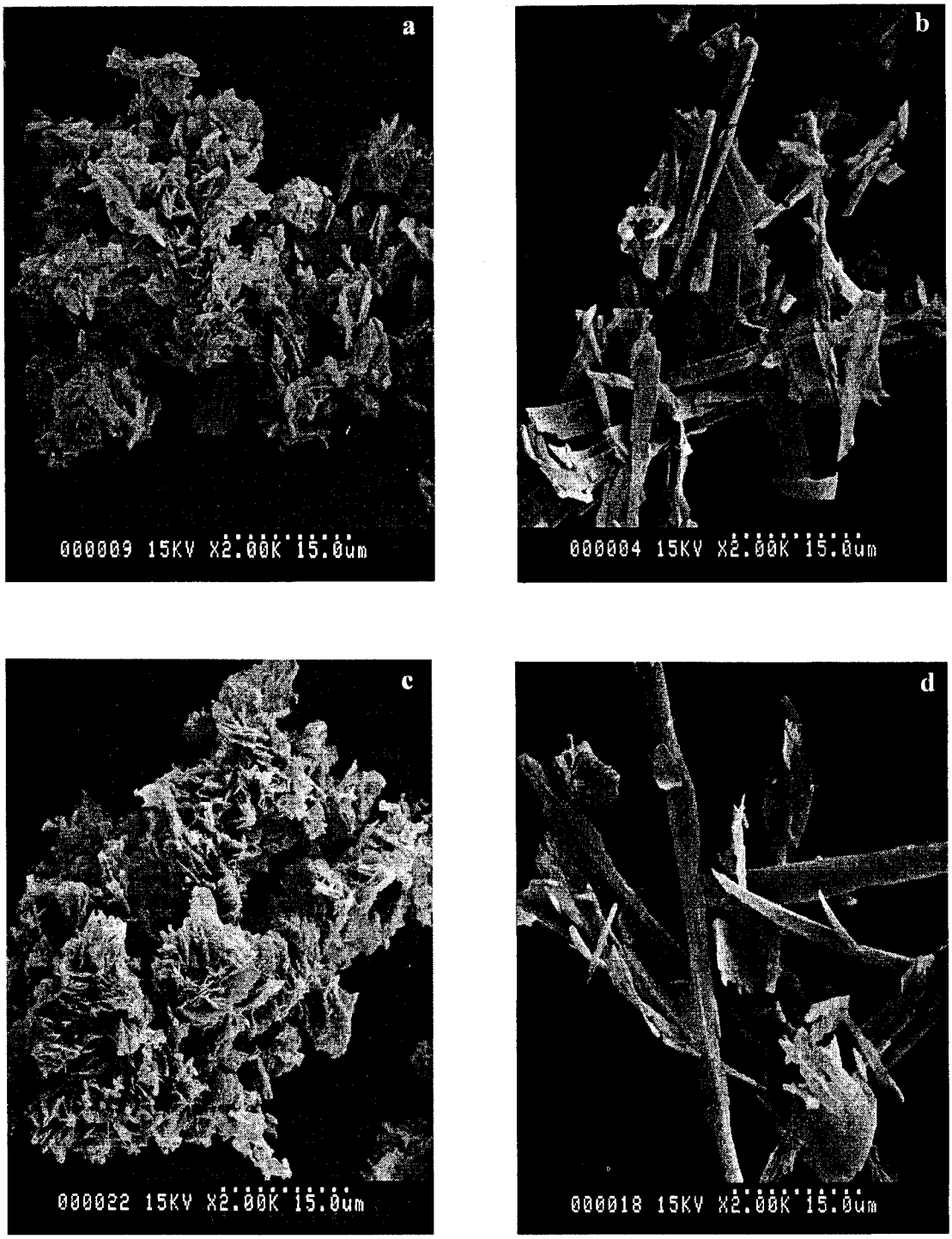

Fig. 5. Scanning electron micrographs of the SEDS processed lactose samples. Process conditions: temperature = 50°C, pressure = 150 bar; lactose concentration − 10% w/v in water; flow rate of solution = 0.035 ml/min; flow rate of cosolvent = 0.665 ml/min; (a) methanol, flow rate of liquid CO_2 = 19.0 ml/min; (b) methanol, flow rate of liquid CO_2 = 5.0 ml/min (c) ethanol, flow rate of liquid CO_2 = 19. ml/min (d) ethanol, flow rate of liquid CO_2 = 5.0 ml/min.

5 and 6 at the respective flow rates at each temperature. These observations confirm that the process is controlled neither by temperature nor pressure thereby attractively reducing the number of process variables.

DISCUSSION

Though the flow conditions of the individual streams are chosen in such a way that the mixture exists in a single homoge-

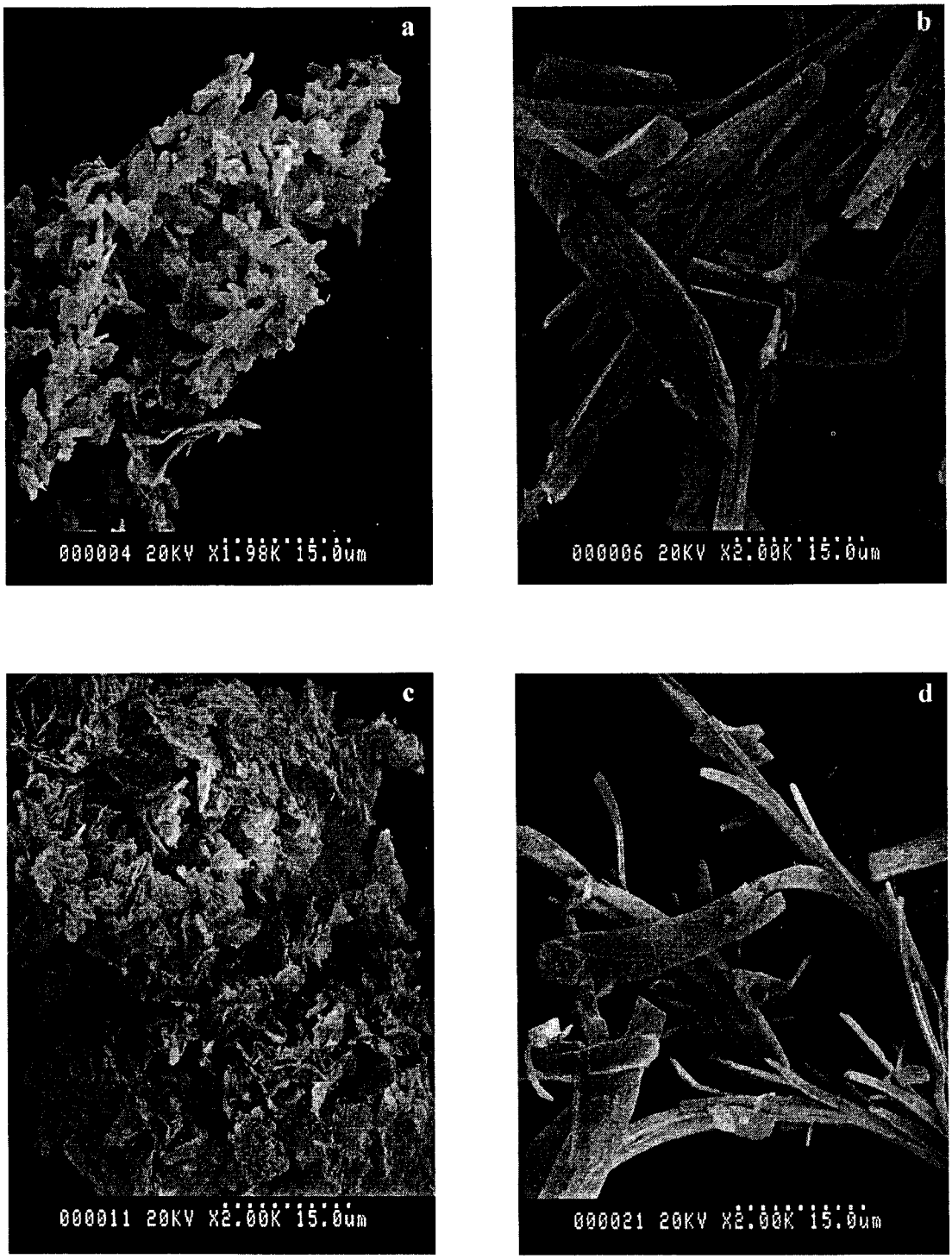

Fig. 6. Scanning electron micrographs of the SEDS processed lactose samples. Process conditions: temperature = 90°C, lactose concentration – 10% w/v in water; flow rate of solution = 0.035 ml/min; flow rate of methanol = 0.665 ml/min; (a) pressure = 150 bar, flow rate of liquid CO_2 = 19.0 ml/min; (b) pressure = 150 bar, flow rate of liquid CO_2 = 5.0 ml/min (c) pressure = 300 bar, flow rate of liquid CO_2 = 19.0 ml/min (d) pressure = 300 bar, flow rate of liquid CO_2 = 5.0 ml/min.

neous phase, the conditions in the vessel are not in equilibrium at the point where the streams are introduced. During such a transition from non equilibrium conditions of two phases to a single phase at equilibrium, the concentrations (solute free basis) in the respective phases could change along the tie line. The rate of mass transfer N_i (moles min^{-1}), of a component i, between the two phases is given by (17)

$$N_i = k_{L,G}a(C_{i,e} - C) \quad (1)$$

where, a is the mass transfer area, $C_{i,e}$ is the equilibrium solubility in mole fraction units of the component i in the respective phase, C is the concentration in mole fraction units at a particular instant of time. $k_{L,G}$ (moles cm^{-2} min^{-1} mole $fraction^{-1}$) is the overall mass transfer coefficient either in the liquid (L) or gas (G) phase which is a function of the system hydrodynamics given by the following correlation for flows in circular pipes (17) between the Sherwood number (Sh), Reynolds number (Re) and Schmidt number (Sc):

$$Sh_{L,G} = c\, Re_{L,G}^{d} Sc_{L,G}^{e} \quad (2)$$

where c, d and e are constants. During such a stage of bulk mass transport at high CO_2 flow rates/concentrations, the selectivity of the organic solvent towards the CO_2 rich phase is high and the liquid phase is essentially rich in water with a ratio of water: (methanol + CO_2) less than 8:1 (along the tie line in Figure 1) at 50°C and 150 bar. The flow of carbon dioxide within the nozzle is highly turbulent with Reynolds numbers greater than 20,000 and hence the limiting factor to the mass transfer is the diffusion coefficient of water in CO_2. For a H_2O-CO_2 system at 303 K and pressures between 5–60 bar, it is reported (18) that the mass transfer coefficient of CO_2 into an aqueous phase is independent of pressure while that of water into the CO_2 phase is inversely proportional to the total pressure in the system. The rate of achievement of the thermodynamic equilibrium state is therefore a function of time which is greater than the nucleation times.

In such a regime, as lactose is only slightly soluble in methanol, the level of supersaturation attained in the aqueous rich phase is low. As a result, the particles grow after nucleation resulting in fused chunks as shown in Figures 5 and 6 at both 50 and 90°C. Yields were also low, as expected, typically between 60–70%. This mechanism also helps to account for the higher water content in the product crystallised at high CO_2 flow rates. On the other hand, at low flow rates of CO_2 where the aqueous phase and the cosolvent + CO_2 phase are miscible, the time scales of water extraction and particle nucleation events are similar. Further, the mole ratio between the organic solvent and water is high and thus, lactose crystallises rapidly as thin long bands due to an antisolvent effect of cosolvent + CO_2. Such change in the crystal growth habit of lactose at high rates of crystallisation has been reported previously (19). This change in particle morphology was not observed in our earlier study (6) when the organic and aqueous solutions were mixed together before contacting with supercritical carbon dioxide since the product was formed rapidly from a highly saturated methanol rich liquid phase.

CONCLUSIONS

The underlying mechanism controlling the SEDS process or any crystallisation processes involving supercritical fluids, organic solvents and aqueous solutions, is defined by the phase behaviour of the resulting ternary system. α-lactose monohydrate was recrystallised as micron sized particles with two alternative morphologies when working at different non equilibrium solubility regions of the aqueous and CO_2 + cosolvent phases. Though crystallisation with ethanol and methanol gave similar morphological structures, the water content of the resultant lactose was lower with methanol compared to ethanol. When crystallisation was initiated in a water rich liquid phase, lactose exhibited a higher water content compared to those samples crystallised in the cosolvent-carbon dioxide rich phase. Such flexibility in altering the physico-chemical properties of the crystallised material is attractive and exemplifies the fact that the SEDS technique is an efficient and reliable means of comminuting water soluble compounds in a simple and environmentally benign process.

ACKNOWLEDGMENTS

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