Synthesis, Purification, and Micronization of Pharmaceuticals Using the Gas Antisolvent Technique

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The synthesis, purification, and micronization of the nonsteroidal antiinflammatory [Cu_2-(indomethacin)$_4L_2$] [Cu−Indo]; indomethacin = 1−4-(chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indole-3-acetic acid, L = *N*,*N*-dimethylformamide (DMF)] has been investigated using DMF as the solvent and CO_2 as the antisolvent. The phase behavior of the binary system DMF + CO_2 and the ternary system DMF + CO_2 + Cu−Indo at 25, 30, and 40 °C and pressures up to 7.6 MPa was examined. The phase behavior of the ternary system DMF + CO_2 containing copper-(II) acetate monohydrate (Cu−Acetate), indomethacin, or acetic acid and the quaternary system DMF + CO_2 containing Cu−Indo and either Cu−Acetate, indomethacin, or acetic acid at 25 °C and pressures up to 5.8 MPa was also examined to determine optimum synthesis conditions. The effect of variables such as reactant concentration, CO_2 wash volume, and rate of expansion on the purity and characteristics of the Cu−Indo produced in the synthesis was investigated. The recrystallization of Cu−Indo from DMF was investigated and the effect of the rate of expansion on the size of the particles produced was determined at 25 °C. It was found that Cu−Indo solubility in a DMF expanded solution decreased with increasing pressure and decreasing temperature. The solubility of Cu−Acetate in a DMF expanded solution was slightly increased as the pressure increased to 2.7 MPa and decreased rapidly at higher pressures. Upon addition of CO_2 to DMF + indomethacin saturated solutions, a second liquid phase formed in the system and precipitation only occurred at pressures above 5.5 MPa. Acetic acid was found to remain soluble in the DMF expanded solution at the range of pressures and temperatures examined. The addition of a second solute to the DMF + CO_2 + Cu−Indo solutions was found to significantly influence the phase behavior of the system. The solubility of Cu−Indo increased in the presence of acetic acid and Cu−Acetate and decreased in the presence of indomethacin. The product, Cu−Indo, with greater than 95% purity was produced in a single step at 25 °C. The presence of a slight excess of either reactant did not alter the purity of the Cu−Indo produced. The rate of expansion substantially varied the size and morphology of the particles produced. Rapid expansion resulted in bipyramidal crystalline particles that were less than 10 μm in size. Slow expansion resulted in rhombic crystals with an average size of between 20 and 10 μm.

Introduction

The use of dense gases as antisolvents has become an area of much interest since the first reported study.[1] The two general methods of using dense gases as antisolvents are the gas antisolvent (GAS) and the aerosol solvent extraction system (ASES). The former method is a batch process, in which the gas antisolvent is introduced into the solvent to precipitate dissolved solids, and the latter is a semicontinuous process, in which the solution is sprayed into a vessel previously pressurized with the gas antisolvent. The major emphasis of these two processes has been to develop fine particles.[2,3] There has also been some work published on the use of these techniques to purify chemicals by selectively precipitating impurities and removing the substance of interest or by precipitating the desired substance and leaving the impurities in solution.[4−13]

A new application of GAS or ASES is in the area of chemical reactions. In most chemical reactions, it is desirable to obtain product of the highest possible purity in the least number of steps and using as little solvent as possible. This becomes increasingly important in the pharmaceutical industry, where the presence of impurities in the final product is most undesirable. Often removing these impurities is more complex than the initial reaction and is very costly in terms of equipment needed and time taken to carry out the purification steps. These impurities come from a number of sources, namely, unreacted reactants, side reactions, unwanted isomers, and residual solvents. The use of dense gases as antisolvents can provide a convenient way of removing these impurities.

A further advantage of using dense gases in chemical reactions is the ability to control solvent parameters such as density, viscosity, and diffusivity. Changes in these parameters have been well documented for reactions undertaken in supercritical fluids.[14,15] The ability of the solvent to dissolve the product can be altered by

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10.1021/ie000189n CCC: $19.00
Published on Web 11/15/2000

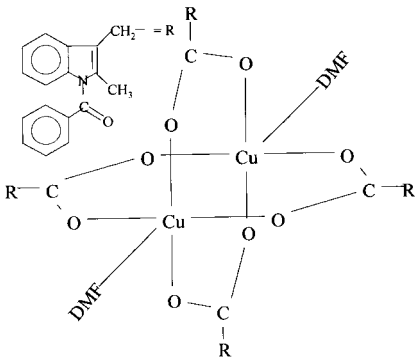

Figure 1. Molecular structure of $[Cu_2(indomethacin)_4(DMF)_2]$.

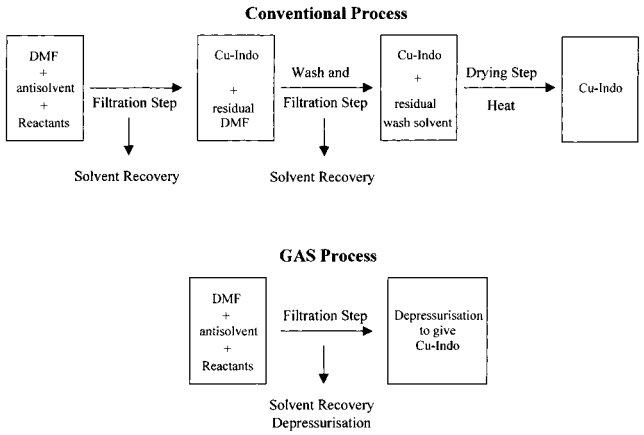

Figure 2. Comparison between the conventional process and the GAS process for the synthesis of Cu−Indo.

varying pressure and temperature. Reactions that are equilibrium controlled can then be controlled by selectively removing product from the reaction mixture through precipitation. An example is the reaction of isoprene with maleic anhydride in supercritical CO_2.[15] Undesirable side reactions can be avoided by removing the product from the reaction as it forms.

In this study the feasibility of using CO_2 as an antisolvent in the synthesis and processing of $[Cu_2(indomethacin)_4(dimethylformamide)_2]$ (Cu−Indo) is examined. Cu−Indo may be synthesized by the following reaction:

$$4(\text{indomethacin}) + 2(\text{Cu−Acetate}) \leftrightarrow \text{Cu−Indo} + 4(\text{acetic acid}) \quad (1)$$

In this reaction copper(II) acetate monohydrate (Cu−Acetate) is reacted with indomethacin in an organic solvent such as *N*,*N*-dimethylformamide (DMF) to yield Cu−Indo. A representation of the Cu−Indo complex is shown in Figure 1. Cu−Indo is a novel nonsteroidal antiinflammatory drug (NSAID) which has a number of advantages over traditional NSAIDs. Generally, traditional NSAIDs such as aspirin are ulcerogenic to the gastrointestinal tract, whereas Cu−Indo has reduced ulcerogenic activity, while simultaneously increasing the antiinflammatory properties.[16−19]

The conventional process for the synthesis of Cu−Indo involves multiple stages, as shown in Figure 2. Organic compounds, such as DMF as the solvent and ethanol as the antisolvent, are used in both the synthesis and purification of Cu−Indo, which results in the generation of solvent waste as well as solvent residues in the drug. The crystallization step, which commences once the ethanol is added to the synthesis mixture, can take as long as 24 h. The organic solvents are removed by filtration, which is a slow process mainly because of the blockage of filters. The larger the batch size, the more complex the problem becomes. A single filtration step is not sufficient to purify Cu−Indo, and a washing step is needed to remove impurities, requiring further filtration and product loss. Cu−Indo is then dried to remove the wash solvent. The drug is unstable at elevated temperatures, which means that lower temperatures need to be used for drying, thus requiring long drying times. Utilization of the GAS process in the Cu−Indo synthesis enables the process to be performed in a single step. Once the reaction is complete, the expanded solution is filtered. The reduced viscosity and increased diffusivity of the expanded solution results in a solution that is easier to filter. The antisolvent is then removed from Cu−Indo by a simple depressurization.

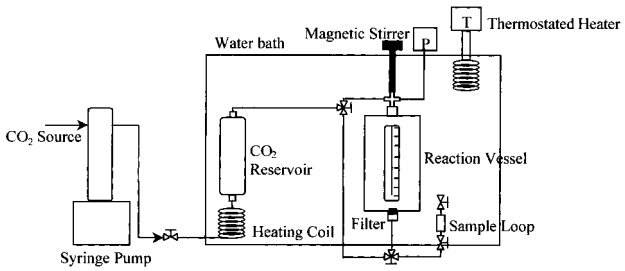

Figure 3. Experimental apparatus.

Experimental Section

Materials. Cu−Indo (90% purity) was donated by Biochemical Veterinary Research. Cu−Acetate (98% purity) was supplied by Sigma−Aldrich. Indomethacin (99% purity) was supplied by Shanghai Shon Long Pharmaceutical Factory. DMF (99.9% purity) was supplied by Burdick and Jackson. Glacial acetic acid (99.7% purity) was supplied by Ajax Chemicals. Carbon dioxide (food grade) was supplied by BOC Gases. All reagents were used as supplied.

Apparatus. The experimental apparatus is depicted in Figure 3. The apparatus consists of a high-pressure vessel (Jerguson sight gauge series no. 32) with an internal volume of 60 mL. The reaction vessel enabled observation of the phase behavior throughout each experiment. A magnetic stirrer was designed for the high-pressure vessel to ensure the solution was well mixed before and during sampling. A 0.5 μm stainless steel filter frit was situated at the bottom of the reaction vessel to sparge the CO_2 in the liquid phase during pressurization and to trap any precipitated solid in the washing step. A 500 mL stainless steel surge tank was placed before the reaction vessel to maintain a constant pressure while a sample was taken. An ISCO LC-500 syringe pump was used to generate the required pressure. Pressure monitoring was made possible by the use of a Druck pressure transducer (model DPI, 260 ± 0.007 MPa). The temperature was controlled to within 0.1 °C by submerging the apparatus in a water bath heated with a Thermoline Unistat 130 water heater. The flow of CO_2 through the reaction vessel was from the bottom during the pressurization step or from the top during the washing step and was controlled by two three-way ball valves (Whitey SS-41XS2). Two two-way ball valves (Whitey SS-41S2) separated by a filter housing (Swagelok ANUPROA filter) were used as a sample loop. The

sample loop had an internal volume of 1.85 mL (calibrated with water).

Phase Behavior. The phase behavior study consisted of loading the high-pressure vessel with approximately 20 mL of solvent or a saturated solution. In the systems that involve solid, an excess of solid was added to ensure that the solution was saturated throughout the experiment. The vessel was then placed in a controlled-temperature water bath in order to reach the desired temperature. The CO_2 was passed through a heating coil to maintain the CO_2 at operating temperature and then sparged through the solution via a frit located at the bottom of the Jerguson. The system was allowed to equilibrate with stirring. After equilibration a sample was taken at constant temperature and pressure. During sampling, the CO_2 was passed through the vessel from the top of the high-pressure vessel and a sample was taken by pushing the solution through the filter and into a sample loop. To ensure that there were no bubbles in the sample loop, the pressurized liquid was slowly let into the sample loop from the bottom by allowing the vapor to purge from the top of the sample loop. Once liquid started escaping from the top of the valve, sampling was ceased and the sample valve disconnected. Each experimental point was repeated until consistent results within 5% relative standard deviation were obtained.

Synthesis. Synthesis experiments were conducted by mixing Cu−Acetate and indomethacin in a 1:2 molar ratio in DMF. The reaction vessel was then charged with a 5−10 mL volume of the mixture and brought to the desired pressure by passing CO_2 through the filter. Once the reaction was complete, the liquid was removed at constant pressure by passing CO_2 from the top of the vessel and pushing the liquid through the filter. Cu−Indo that precipitated was washed and dried by removing the solvent-rich liquid phase at a constant pressure between 5.7 and 5.9 MPa and a CO_2 flow rate of 2−4 mL/min. In each batch 200−400 mL of CO_2 was used to minimize the residual solvent. The system was then depressurized, and a sample was taken for analysis.

Micronization. Micronization experiments were conducted by charging 5 mL of a 90% saturated solution of Cu−Indo in DMF into the reaction vessel, and the system was pressurized by passing CO_2 into the vessel through the filter. Once pressurization was complete, the liquid was removed from the vessel at constant pressure (5.9 MPa) by passing CO_2 from the top through the reaction vessel. The precipitate was dried, after removal of the liquid, by passing 200−400 mL of liquid CO_2 through the vessel at constant pressure (6.2 MPa). The solid particles were gold-coated and analyzed by a scanning electron microscope (Hitachi S4500).

Analyses. The mole fraction of each component was determined by a mass balance. The sample loop was weighed and the total mass of the liquid recorded. The masses of CO_2 and DMF were determined by gravimetric means. The sample loop was weighed, then CO_2 was allowed to evaporate from the liquid by slowly releasing the pressure from the sample loop, and the liquid from the loop was collected in a cold trap. The collected liquid was sonicated for a few minutes, to ensure that all of the CO_2 was purged, and then weighed.

The mass of Cu−Indo and indomethacin was determined by a high-performance liquid chromatograph (HPLC). The HPLC used consisted of a Waters 600 pump, a 996 photodiode array detector, and a 717Plus autoinjector. The samples were separated using a Symmetry C_{18} column with a mobile phase made up of 70% acetonitrile and 30% 0.01 M tetramethylammonium chloride in 0.1% acetic acid. A flow rate of 1 mL/min was used, and the analytes were detected at a wavelength of 320 nm.

The mass of Cu−Acetate was determined by atomic absorption (AAS) using a Perkin-Elmer Analyst 300.

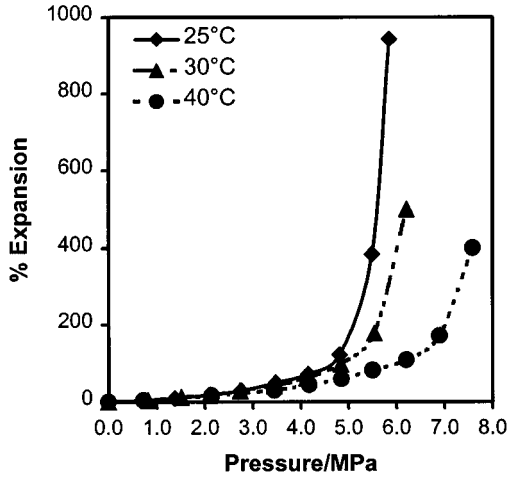

Figure 4. Volumetric expansion of DMF as a function of pressure.

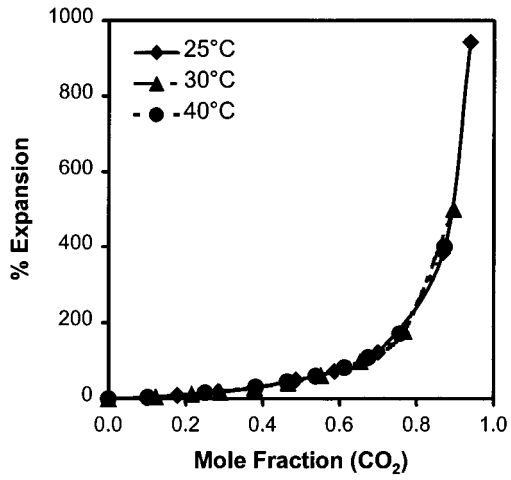

Figure 5. Volumetric expansion of DMF as a function of CO_2 mole fraction.

Results and Discussion

Phase Behavior of the CO_2−DMF System. The phase behavior of the DMF−CO_2 system at temperature ranges between 25 and 40 °C was examined. The solubility of CO_2 in the liquid phase was measured and is illustrated in Figures 4 and 5. The volume of the liquid phase was increased by increasing the pressure of the system. At a given pressure, as the temperature increased, there was a reduction in the volumetric expansion of the solution, as shown in Figure 4. The CO_2−DMF system has given similar results in previous studies.[1,20,21] Volumetric expansion, which is a result of an increasing mole fraction of CO_2 in the liquid phase, is expected to cause the solute that is dissolved in the organic solvent to precipitate by decreasing the cohesive energy of the organic solvent. The data in Figure 5 reveal an interesting observation that for all isotherms, for a given mole fraction of CO_2, the volumetric expansion of the liquid phase was the same. Kordikowski et

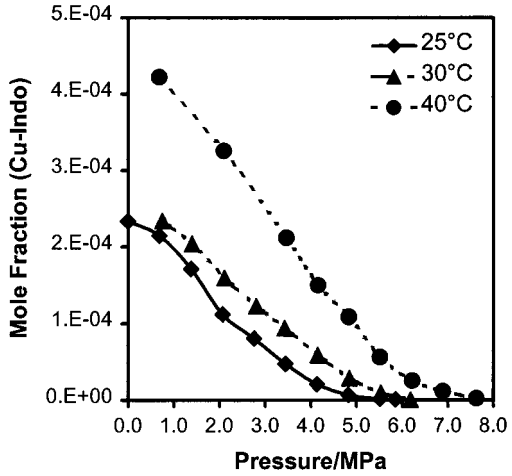

Figure 6. Mole fraction of Cu−Indo dissolved in the liquid phase.

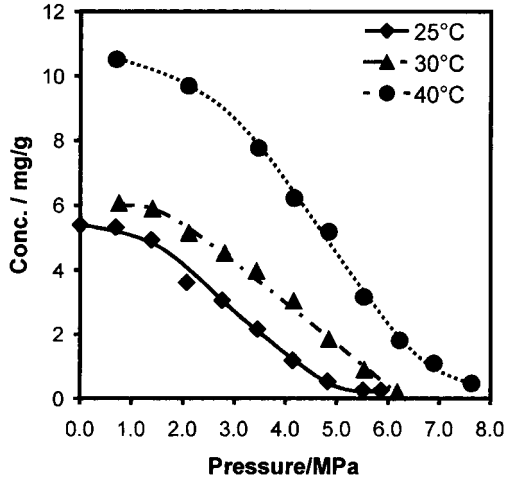

Figure 7. Concentration of Cu−Indo in DMF (CO_2-free basis).

al.[21] observed a similar trend for a number of different solvents pressurized with CO_2. It was suggested that the solute−solvent interaction is more important in the GAS process than the solute−CO_2 interaction.

Solid Solubility in the Expanded Solution. A knowledge of the solubility of the reactants and products in a reaction medium is an important parameter to be verified before a synthesis is carried out. In an expanded solution, these data become increasingly important because the high-pressure nature of the process makes it difficult to modify the variable during the process. In a conventional synthesis reaction, variables such as the volume of the organic solvent or the concentration of one reactant can be easily varied. However, in the case of an expanded solution, which is under pressure, and in many cases with no means of visual observation, altering these variables would be difficult.

In the synthesis of Cu−Indo, there are two reactants, Cu−Acetate and indomethacin, and two possible products, Cu−Indo and acetic acid. All components may be present during the synthesis. The solubility of each component in a DMF expanded solution at 25 °C was determined. The presence of other solutes may influence the solubility of reactants and products.[5,12] Therefore, the solubility of a binary mixture of these compounds in the DMF expanded solution was also investigated.

The solubility of Cu−Indo in DMF expanded with CO_2 at 25, 30, and 40 °C is illustrated in Figures 6 and 7. The mole fraction of Cu−Indo decreased with increasing pressure and increased with temperature. As the temperature increased, the pressure required to precipitate the Cu−Indo was increased because of the reduction in the solubility of CO_2 in the DMF at higher temperature. At each temperature the solubility of Cu−Indo approached a minimum with increasing pressure. This minimum is expected to coincide with the solubility of the solute in CO_2.[22] A decrease in the mole fraction of Cu−Indo was due to the increased mass capacity of the solution (the dilution effect) as CO_2 dissolved in the liquid phase and was not necessarily indicative of precipitation. The concentration of Cu−Indo, where the concentration of Cu−Indo is expressed in terms of a CO_2-free basis, is shown in Figure 7 and indicates the pressure at which precipitation occurred in the system. The solubility of Cu−Indo decreased rapidly as the pressure was increased even at low pressures. Precipitation of the solute occurred even at low pressures. Because precipitation could only occur when supersaturation was reached, or exceeded by some value, CO_2 is an effective antisolvent for precipitating Cu−Indo. At 25 °C and 5.9 MPa the amount of Cu−Indo that precipitated from solution was greater than 95% of the total solute dissolved initially in the solution.

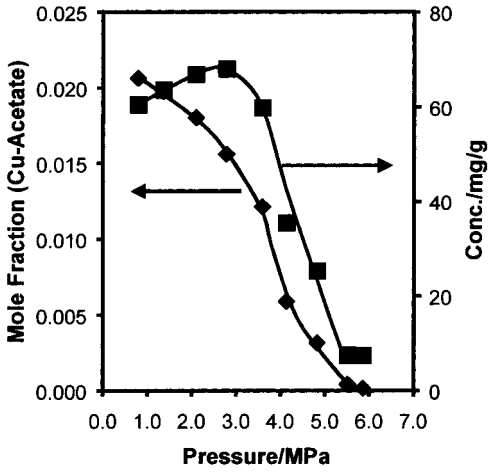

Figure 8. Solubility of Cu−Acetate in expanded DMF at 25 °C.

The solubility of Cu−Acetate in DMF expanded with CO_2 at 25 °C is illustrated in Figure 8. The mole fraction of Cu−Acetate decreased with increasing mole fraction of CO_2 in the liquid phase. The concentration of Cu−Acetate (CO_2-free basis) increased slightly as the pressure was increased up to 3 MPa and then decreased when the pressure was increased further. The initial increase in solubility may be due to the density increase of the liquid phase that occurs at low pressures.[21] This density increase results in CO_2 acting as a cosolvent at pressures below 3 MPa. The increased solubility of Cu−Acetate in the CO_2−DMF−Cu−Acetate system may be due to the high solubility of Cu−Acetate in DMF at atmospheric conditions. Cu−Acetate was 2 orders of magnitude more soluble in DMF than Cu−Indo at atmospheric conditions. The solute−solvent interaction between DMF and Cu−Acetate was greater than that between DMF and Cu−Indo. The initial density increase in the liquid phase outweighed the antisolvent effect of CO_2, and this led to a solubility increase for Cu−Acetate. The solubility increase continued until the concentration of CO_2 dissolved in the liquid phase reached a value where the antisolvent effect dominated the solute−solvent interaction. In the case of Cu−Indo, which was far less soluble in the organic solvent, at low pressures

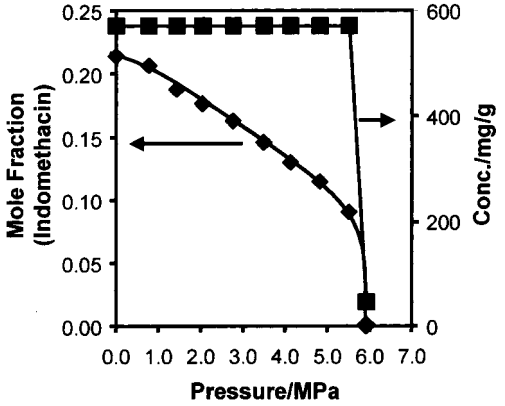

Figure 9. Solubility of indomethacin in expanded DMF at 25 °C.

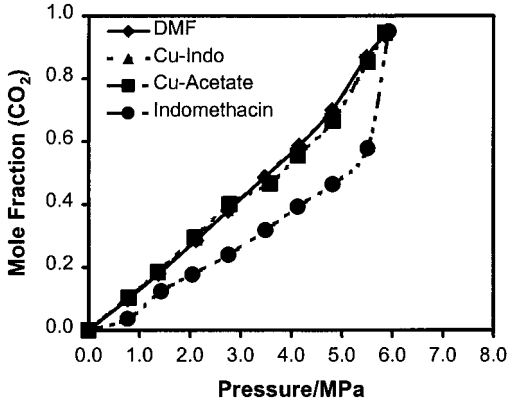

Figure 10. Mole fraction of CO_2 as a function of the pressure at 25 °C in the presence of a solute.

this initial density increase did not result in greater solubility because the solute−solvent interaction was dominated by the antisolvent effect of CO_2.

The solubility of indomethacin in DMF expanded with CO_2 at 25 °C is depicted in Figure 9. The mole fraction of indomethacin decreased gradually, and at 5.5 MPa it dramatically decreased. The concentration of indomethacin (CO_2-free basis) remained constant at pressures below 5.5 MPa and then decreased to 46 mg/g of DMF at 6 MPa. A second liquid phase appeared between 5.5 and 6 MPa. The second liquid phase converged with the first liquid phase once precipitation of the indomethacin occurred.

The mole fractions of CO_2 dissolved in the liquid phase for the DMF, Cu−Indo + DMF, Cu−Actetate + DMF, and indomethacin + DMF systems at 25 °C are compared in Figure 10. At pressures below 5.5 MPa the mole fraction of CO_2 in the liquid phase was lower for DMF solutions containing indomethacin than for the other systems. The CO_2−DMF expansion behavior (the amount of CO_2 dissolved in DMF) did not change when solutes of low solubility such as Cu−Indo and Cu−Acetate were present in the system. The solubilities of Cu−Indo and Cu−Acetate in DMF at atmospheric pressure and 25 °C were 5.4 and 60 mg/g, respectively. A similar observation was made for the expansion profile of the dimethyl sulfoxide−CO_2 system when protein with low solubility was added to the system.[23] However, the data in Figure 10 show that highly soluble compounds such as indomethacin, which has a solubility of 571 mg/g in DMF at atmospheric pressure and 25 °C, dramatically influence the phase behavior of the system. The lower solubility of CO_2 in DMF in the presence of indomethacin may be due to intermolecular

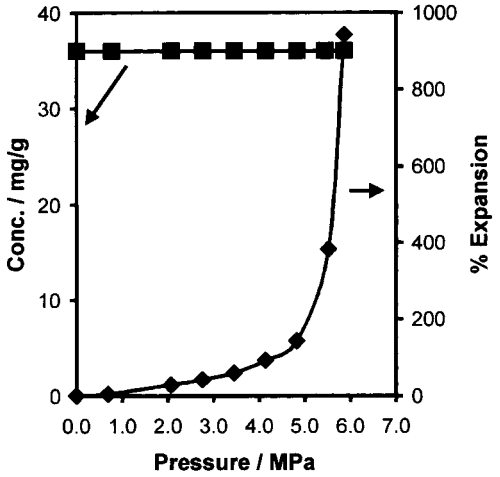

Figure 11. Phase behavior of DMF containing acetic acid at 25 °C.

forces between the solute and solvent. An analogous observation has been made for the citric acid−ethanol system.[24] A high solubility suggests that there are strong cohesive forces between the solute and the solvent and the system no longer behaves as pure DMF. The strong cohesive forces, which leave little solvent available to interact with CO_2, resulted in a second liquid phase forming at higher pressures. A splitting of the liquid phase occurred in the CO_2−naphthalene−toluene system in which one liquid phase was rich in CO_2 and the other was rich in the solute.[25] As the pressure increased, the amount of CO_2 increased, which results in an increased competing force for DMF between CO_2 and indomethacin. Eventually, at pressures greater than 5.5 MPa, the DMF−indomethacin and DMF−CO_2 phases split. A further pressure increase caused the indomethacin to precipitate out of solution. Once precipitation occurred and the indomethacin concentration in solution decreased, the two liquid phases merged and the phase behavior of the system approached that of the CO_2−DMF system.

Acetic acid and DMF are totally miscible in all proportions. The phase behavior of the DMF−CO_2−acetic acid (36 mg/g) system at 25 °C is shown in Figure 11. The presence of acetic acid did not influence the expansion profile of the DMF−CO_2 system over the pressure range tested.

The data from the ternary systems containing a solute indicate that Cu−Indo could be separated from Cu−Acetate and indomethacin at 25 °C by pressure manipulation. The solubility of Cu−Indo in DMF and DMF expanded solutions at all pressures examined was lower than those of Cu−Acetate and indomethacin. The saturation concentrations of Cu−Acetate and indomethacin at various pressures can be obtained using the data in Figures 8 and 9. The synthesis process would be performed at conditions where the concentrations of the reactants, Cu−Acetate and indomethacin, are far from saturation. If the initial concentration of a solute in solution is less than saturation, precipitation will not occur until the solid−liquid equilibrium line is crossed.[25] The phase behavior studies show that at each pressure the solubility of Cu−Indo was lower than those of Cu−Acetate and indomethacin; hence, it crosses the solid−liquid equilibrium line before the others.

The solubility of a compound in an expanded solution may be influenced by the presence of another solute. The solubilities of Cu−Indo at 25 °C in DMF expanded

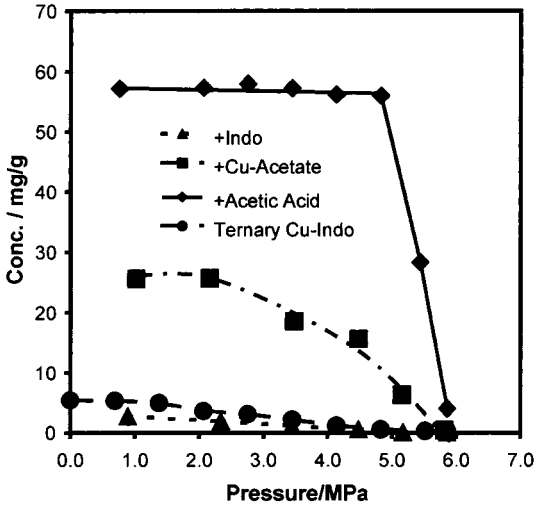

Figure 12. Solubility of Cu−Indo in quaternary systems at 25 °C.

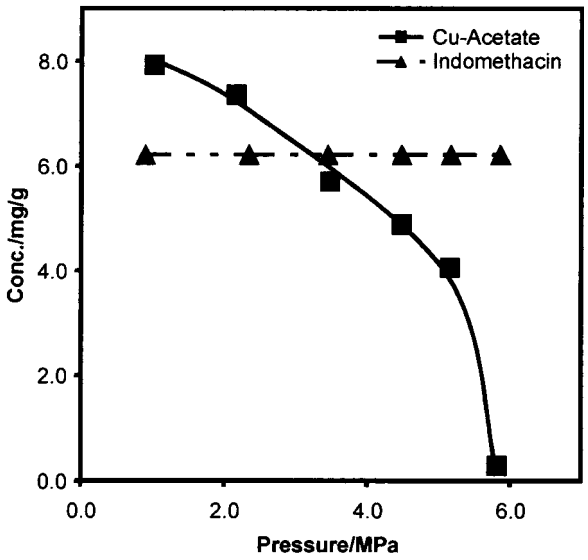

Figure 13. Quaternary solubility of the second solute at 25 °C.

solutions containing either acetic acid, Cu−Acetate, or indomethacin are depicted in Figure 12. The initial concentrations of acetic acid, Cu−Acetate, and indomethacin in DMF were 60, 8, and 6.2 mg/g, respectively. These concentrations were chosen as they are close to the conditions that may be used during the Cu−Indo synthesis.

The presence of acetic acid resulted in a 10-fold increase in the solubility of Cu−Indo. Precipitation only occurred at a pressure above 5 MPa corresponding to a CO_2 mole fraction of 0.7. Above these pressures, pure Cu−Indo precipitated from solution. Cu−Indo is dissociated in the presence of acetic acid to indomethacin and Cu−Acetate as described in eq 1. A driving force is needed to push the equilibrium toward the formation of Cu−Indo. Because carbon dioxide is able to induce precipitation of Cu−Indo at pressures above 5 MPa, at these conditions CO_2 is able to drive the equilibrium toward the formation of Cu−Indo by its antisolvent action.

The presence of Cu−Acetate resulted in a 4-fold increase in the initial concentration of Cu−Indo. In this case, however, the precipitation of Cu−Indo followed a trend similar to that of the ternary CO_2−DMF−Cu−Indo system, where precipitation occurred throughout the pressurization process. In the ternary CO_2−DMF−Cu−Acetate system, an initial Cu−Acetate concentration of 8 mg/g would result in precipitation occurring at pressures above 5 MPa; however, in the presence of Cu−Indo, the precipitation of Cu−Acetate began at 2 MPa (Figure 13). It is suggested that Cu−Indo lowered the solubility of Cu−Acetate in the DMF expanded solution.

The presence of indomethacin resulted in a 2-fold decrease in the initial concentration of Cu−Indo. Precipitation of Cu−Indo occurred throughout the pressurization process shown by the solubility decrease in Figure 12. The concentration of indomethacin in the expanded solution is illustrated in Figure 13. The concentration of indomethacin remained constant at all pressures, which implies that Cu−Indo could be separated from indomethacin simply by increasing the pressure of the system. It is interesting to note that, at a concentration of 6.2 mg/g of indomethacin in DMF, the second liquid phase did not appear in the system.

The phase behavior studies of the binary, ternary, and quaternary systems demonstrate that the solubility of a compound such as Cu−Indo in an expanded solution depends not only on the temperature and pressure but also on the concentration and presence of other compounds dissolved in the system. The effect of pressure and temperature can be related to CO_2 density and is predictable. However, the phase behavior and hence the solubility of Cu−Indo is a complex function of the solvent and other solutes.

Table 1. Yield and Purity of the Cu−Indo Syntheses in a DMF Expanded Solution at 25 °C

max pressure/ MPa	wash vol/ mL	time to max expansion/ min	initial concn of Cu−Indo/ (mg/g)	% yield	% purity
5.8	200	120	5	85	
5.8	200	120	10	91	
5.9	200	10	20	98	96.3
5.9	200	10	20	98	93.9
5.9	200	120	50	98	93.5
5.9	400	120	200	96	93.5
5.9	200	120	200	96	91.0
5.9	400	10	200	96	100
5.9	400	120	200	96	100

Synthesis and Purification in the Expanded Solution. One of the objectives in the synthesis of Cu−Indo is to maximize the yield and at the same time reduce solvent usage. Both reactants need to be soluble to enable the reaction to take place; therefore, the limiting reactant in the Cu−Indo synthesis is Cu−Acetate with a solubility of approximately 60 mg/g (0.3 mmol/g) in DMF at 25 °C. According to the stoichiometry of the reaction, the concentration of indomethacin would need to be 215 mg/g (0.6 mmol/g), which results in a Cu−Indo concentration of 255.5 mg/g (0.15 mmol/g) and an acetic acid concentration of 72 mg/g (0.12 mmol/g).

The results of the synthesis experiments are given in Table 1. The target Cu−Indo concentration was varied from 5 mg/g, below the ternary saturation concentration to 200 mg/g, which is close to the maximum possible synthesis concentration.

The behavior of the system during the synthesis was dependent on the concentration of the solute. In the dilute solutions (e.g., 5, 10, and 20 mg/g), Cu−Indo precipitated from solution at 5.2 MPa. The higher concentration solutions (e.g., 50, 100, and 200 mg/g) resulted in a second liquid phase, which appeared as

liquid droplets. This second liquid phase dried into solid Cu−Indo upon further pressurization. The extent of formation of the second liquid phase increased with increasing concentration of the reactant and, hence, Cu−Indo. The second liquid phase was hardly noticeable when 50 mg/g of Cu−Indo was produced, while at 200 mg/g it was significant. The nature of the precipitates was a function of the Cu−Indo concentration produced during the synthesis. Free-flowing precipitates made up of individual particles were collected from dilute solutions, while agglomerated particles were formed from solutions of higher concentration.

The yield of each synthesis experiment was calculated by comparing the initial concentration of Cu−Indo to the concentration in the liquid removed once precipitation had ceased. The yield was greater than 90%, except at lower concentrations, where the yield was below 90%. The amount of Cu−Indo that remained soluble in the expanded DMF was a function of the acetic acid concentration. Quaternary data for the CO_2−DMF−Cu−Indo−acetic acid system indicate that the solubility of Cu−Indo increased in the presence of acetic acid. During the synthesis, 4 mol of acetic acid are formed for each 1 mol of Cu−Indo formed. Therefore, the yield decreases because of the increased concentration of acetic acid. However, a yield of 90% is quite reasonable.

The purity of the precipitates collected was determined by HPLC and was found to vary between 91 and 100%. The purity of Cu−Indo depends on a number of factors, namely, residual solvent, coprecipitants, and molecules of solvation. A thorough characterization of Cu−Indo indicated that it is not unusual for molecules such as water and DMF to be included in the Cu−Indo crystals.[26] The water most likely comes from Cu−Acetate, which has a water molecule included in its crystal. These are molecules that do not form part of the Cu−Indo structure (Figure 1), but they are included in the crystal as molecules of solvation. These molecules of solvation could contribute up to 6% of the Cu−Indo precipitate and are difficult to remove because they are quite strongly bound in the crystals. A purity of greater than 94% is therefore considered acceptable. Purities greater than 94% will occur when these molecules are not included in the crystal.

The volume of CO_2 used for washing was an important factor in removing residual solvent. Cu−Indo washed with 400 mL of CO_2 possessed less residual solvent than that washed with 200 mL of CO_2. The rate of expansion has little effect on the purity of Cu−Indo, with similar purity results obtained for both rapid and slow expansion experiments. At the conditions under which 100% pure Cu−Indo was produced, all included solvent molecules, which do not form part of the Cu−Indo structure, had been removed from the crystal.

Two Cu−Indo synthesis experiments were conducted at 25 °C, one with excess Cu−Acetate and the other with excess indomethacin, to determine what effect this would have on the Cu−Indo precipitation. The results are given in Table 2. A 5% excess of Cu−Acetate or indomethacin was added in each case.

In the presence of excess Cu−Acetate, the yield of Cu−Indo was 96% and the purity of the precipitate collected was 94%. An excess of Cu−Acetate does not have any effect on the purity of the Cu−Indo produced. This is contrary to the data for the CO_2−DMF−Cu−Indo−Cu−Acetate system, which indicated that Cu−Acetate would precipitate with Cu−Indo. This result demonstrates the complex interactions that occur in multicomponent mixtures. In the presence of excess Cu−Acetate, there are five components in this mixture, namely, CO_2, DMF, Cu−Indo, Cu−Acetate, and acetic acid. The presence of the acetic acid on the mixture has resulted in an increased solubility of Cu−Acetate in expanded DMF.

Table 2. Yield and Purity of the Cu−Indo Syntheses in a DMF Expanded Solution at 25 °C in the Presence of Excess Reactant

max pressure/ MPa	wash vol/ mL	time to max expansion/ min	reactant in excess	% yield	% purity
5.9	400	120	indomethacin	99	92.9
5.9	400	120	Cu−Acetate	96	94.8

Table 3. Comparison between CO_2 and Ethanol as Antisolvents for the Cu−Indo−DMF System at 25 °C

antisolvent	pressure/ MPa	antisolvent mole fraction	% yield
ethanol	0.1	0.5	90
	0.1	0.8	96
	0.1	0.9	96
	0.1	0.95	96
CO_2	3.5	0.5	60
	4.8	0.7	90
	5.5	0.9	95
	5.8	0.95	95

Table 4. Comparison between GAS and the Conventional Process for the Synthesis of Cu−Indo at 25 °C

antisolvent	pressure/ MPa	Cu−Indo concn/ (mg/g)	% yield
ethanol	0.1	5	40
CO_2	5.8		85
ethanol	0.1	50	75
CO_2	5.8		98
ethanol	0.1	200	91
CO_2	5.8		96

The presence of excess indomethacin resulted in a Cu−Indo yield of 99% and a Cu−Indo purity of 93%. In this case the yield has improved which can be explained from the quaternary data for the CO_2−DMF−Cu−Indo−indomethacin system. The solubility of Cu−Indo was reduced in the presence of indomethacin, which resulted in more Cu−Indo precipitating and an increase in the yield of the synthesis. The purity remained the same, implying that the excess indomethacin has remained in solution.

Comparison between CO_2 and Ethanol as Antisolvents. The conventional synthesis for Cu−Indo involves the use of ethanol as an antisolvent. The results of CO_2 and ethanol as antisolvents for the DMF−Cu−Indo system in terms of the yield of precipitate relative to the mole fraction of the antisolvent at 25 °C are given in Table 3. The yield of Cu−Indo precipitated was greater than 90% for mole fractions of ethanol from 0.5 to 0.95. In the CO_2 system the yield of Cu−Indo was greater than 90% at mole fractions of CO_2 greater than 0.8. Ethanol is a better antisolvent in terms of the yield at low mole fractions and is as effective as CO_2 at higher mole fractions.

Carbon dioxide and ethanol as antisolvents, in the synthesis of Cu−Indo, are compared in Table 4. Sufficient ethanol was added to each solution to give a mole fraction of antisolvent 0.95. The synthesis solutions contained target Cu−Indo concentrations of 5, 50, and 200 mg/g. Using ethanol as an antisolvent only resulted in 40 and 75% yields for the 5 and 50 mg/g solutions,

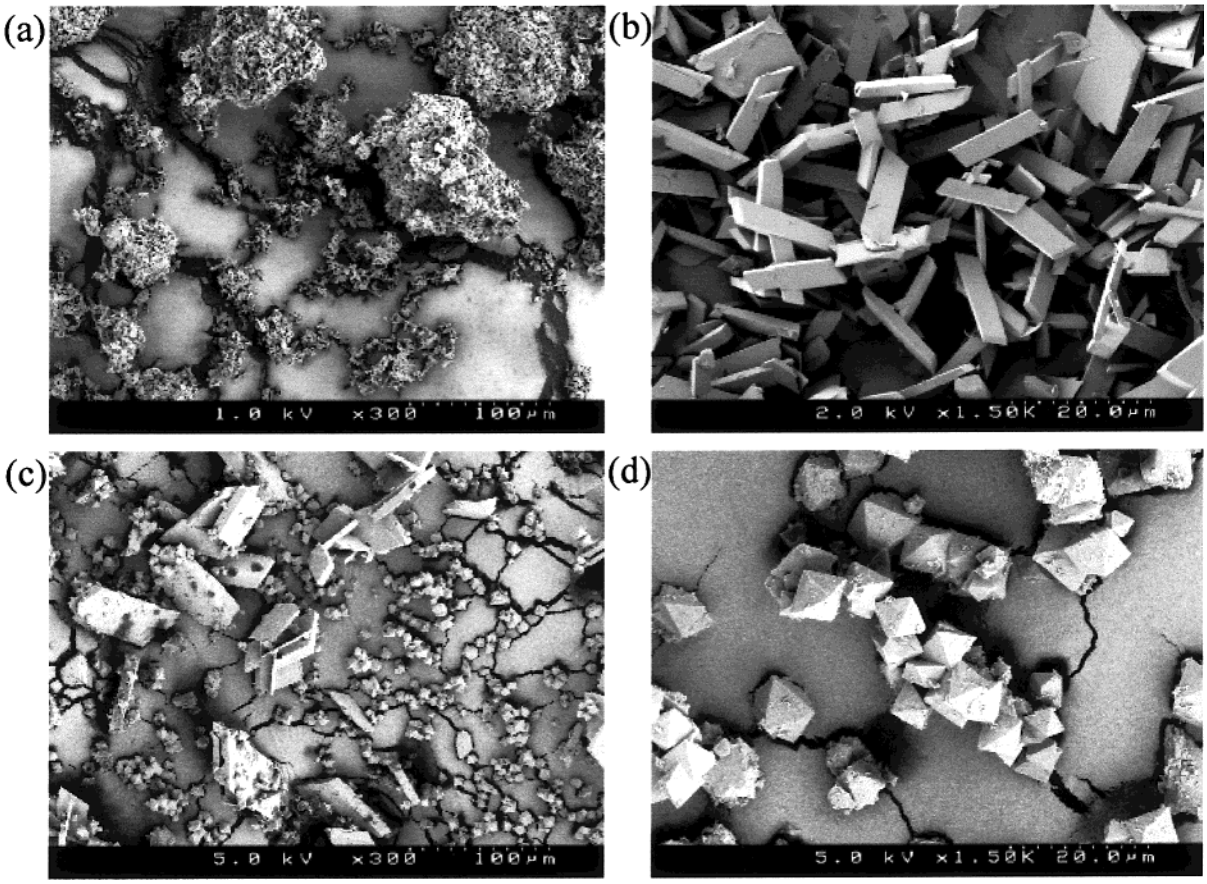

Figure 14. SEM images of the particles produced from the GAS and the conventional process: (a) conventional process; (b) slow expansion; (c and d) fast expansion.

Table 5. Micronization Results

time to max expansion/min	particle size/μm	morphology
120	10−20	rhombic crystals
10	few 100	rhombic and bipyramidal
	most less than 10	crystals

respectively. A 91% yield was obtained for the 200 mg/g solution. At the same mole fraction, CO_2 gave yields of 85% for the 5 mg/g solutions and greater than 95% for the 50 and 200 mg/g solutions. The data in Table 4 demonstrate that for the Cu−Indo synthesis CO_2 is more effective as an antisolvent, especially when using low concentrations of Cu−Indo.

The benefit of replacing ethanol with CO_2 is not only to give improved yields. Another advantage of CO_2 over ethanol as an antisolvent in the synthesis of Cu−Indo was in the time taken to crystallize Cu−Indo. Crystallization was complete within 2 h at 25 °C when CO_2 was used as the antisolvent, while at least 24 h was required when ethanol was used as an antisolvent. There is also the advantage of the ease of recycling CO_2. Purification of the CO_2, once it has been used as the antisolvent, is achieved by depressurizing in a cold trap. Purification of ethanol is achieved by distillation, which is expensive in terms of energy used and time taken.

Crystallization by GAS. All experiments were conducted in DMF at 25 °C. The results of the micronization study by GAS are given in Table 5, and examples of the particles produced are shown in Figure 14. The precipitate obtained from the conventional process formed aggregates (Figure 14a). The results in Figure 14b,c indicate that the GAS process placed far less stress on the particles formed. The particles from the GAS process retained their sharp edges and were more crystalline in appearance.

The rate of expansion has a marked effect on the morphology and size of the particles formed. The particles produced by slow expansion of the solution were crystalline, rhombic in shape, and around 15 μm in size (Figure 14b). The particles generated from rapid expansion were not uniform, and two different crystal morphologies were observed (Figure 14c). The first crystal form was similar in shape to those produced by slow expansion but larger, of the order of 100 μm. The second type was bipyramidal in shape with a size on the order of 10 μm (Figure 14d). The morphology of the second crystal form may be the result of a second liquid phase that formed with rapid expansion because of inefficient mixing. The two phases include a DMF−Cu−Indo-rich phase and a CO_2-rich phase. At the interface of these two liquid phases, a precipitate formed that diffused into the DMF-rich phase. Further expansion of the system resulted in the two liquid phases merging and further precipitation occurring from the single liquid phase. The smaller particles shown in Figure 14d are most likely the particles that formed in the second liquid phase. The larger particles, which have the same morphology as the particles produced from slow expansion of the system, most likely precipitated from the single liquid phase.

Conclusion

The GAS technique was successfully used for the synthesis and purification of Cu−Indo. Using this

technique, a high-purity product can be achieved in a single step. The nature of the particles produced from the synthesis is determined by the concentration of the solutes in the reaction solution. Higher concentration solutions gave agglomerated particles, while low concentration solutions gave crystalline free-flowing particles. The reactants and byproducts of the Cu−Indo synthesis had a marked effect on the solubility of Cu−Indo in DMF expanded with CO_2. A thorough study of the phase behavior of the system containing the various solutes is required to understand the synthesis of Cu−Indo in DMF expanded with CO_2. Replacing CO_2 for ethanol as an antisolvent was found to increase the yield of the process.

The micronization of Cu−Indo from DMF using CO_2 as the antisolvent gave two crystal morphologies depending on the rate of expansion. Slow expansion gave rhombic crystals, while fast expansion gave a mix of rhombic and bipyramidal crystals, with the majority having the bipyramidal morphology. An average size reduction was observed with increasing expansion rate.

Carbon dioxide provides an excellent alternative to ethanol as an antisolvent in the synthesis of Cu−Indo, giving the advantages of a single-step synthesis, faster crystallization rate, reduced solvent requirement, and controllable particle size.

Acknowledgment

The authors thank the Australian Government for their financial support through the auspices of the Australian Research Council SPIRT scheme (C89917624) and for the provision of an Australian Post Graduate Award (B.W.) and also Biochemical Veterinary Research P/L for financial support and the provision of analytical instrumentation.

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Received for review February 7, 2000
Revised manuscript received July 13, 2000
Accepted July 15, 2000

IE000189N