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Precipitation of Piperine from Hydrotropic Solutions: Study of Crystal Nucleation and Growth Kinetics from Batch Experiments

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ABSTRACT

Precipitation of piperine from aqueous hydrotropic solutions of sodium cumene sulfonate was investigated in a laboratory-scale agitated vessel by cooling aqueous sodium cumene sulfonate solution saturated with piperine from 353K to 303K. This temperature swing is a more economical approach than usual dilution by water for recovery of piperine from hydrotrope solutions. The time evolution of crystal size distribution was used to deduce simultaneously crystal nucleation and growth

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kinetics. The results indicate higher nucleation and slower crystal growth rates and thus formation of very fine crystals of piperine in the range of 1–400 μm.

Key Words: Piperine; Hydrotropy; Sodium cumene sulfonate; Nucleation kinetics; Crystal growth; *Piper nigrum*.

INTRODUCTION

Piperine ($C_{17}H_{19}NO_3$) (Fig. 1), a major alkaloid in black pepper, exhibits a strong chemoprotective effect against procarcinogens and also bacteria, fungal growth, and insects.[1] It also has been gaining increasing attention as a bioavailability enhancer in the formulation of several drugs.[2,3] The methylene dioxyphenyl group of piperine has an insecticidal activity. Piperine can also be administered to cancer patients before radiotherapy because of its protective effect against the radiation.[4] These applications suggest a need for pure piperine that is free from residual organic solvents to enable its direct use in medicinal formulations.

Extraction of piperine from black pepper by using hydrotropic solubilization seems to be a feasible alternative to extraction by using organic solvent.[5] This hydrotropic extraction gives high extraction efficiency and high selectivity toward piperine. The recovery of piperine from hydrotrope solutions is then done by diluting the hydrotrope solution by water. Although simple and elegant, the dilution results in large volumes of aqueous hydrotrope solutions, and reconcentration of these solutions for recycling makes the overall process somewhat expensive.

Hydrotropy is a phenomenon of increasing the solubility of water insoluble or sparingly soluble organic compounds in aqueous solutions in the presence of hydrotropes. The increase in the solubility is a strong function

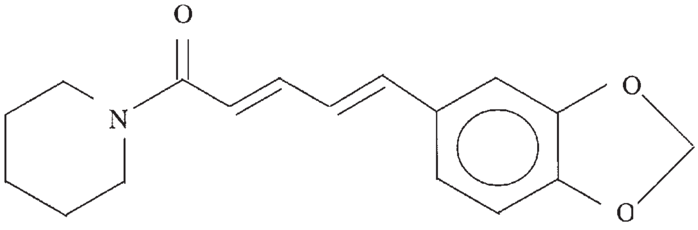


Figure 1. Piperine.

of hydrotrope concentration and varies with the nature of an organic solute. Hydrotropes are themselves highly water-soluble organic, but amphiphilic, salts.

Hydrotropic extraction is a very efficient process for rapid extraction of certain organic compounds from natural products.[5,6] To have a complete process, the recovery of the solute from the hydrotropic solutions also needs attention. The purpose of the present work is to study the precipitation of piperine from its hydrotropic aqueous solutions by exploiting the differential solubility behavior of piperine with temperature. The study is also aimed at developing crystal nucleation and growth rate expressions from the crystallization experiments by using synthetic solutions. Such an analysis should be useful to identify underlying principles and probably for better operation of the hydrotropic extraction of piperine and/or other similar products.

As there has been an increasing recognition of the importance of crystallization kinetics in design and performance assessment of the products, a few papers have studied the precipitation of a dissolved solute from hydrotrope solutions.[7−10] Recently, Dandekar and Gaikar[11] have reported the precipitation of curcuminoids from concentrated hydrotrope solutions by dilution with water. The time evolution of the crystal size distribution was used to deduce, simultaneously, the crystal nucleation and growth kinetics, and the kinetic data obtained from the experimental runs were correlated by a power law relative to the kinetic expression. The results indicated slow crystal growth and thus formation of very fine crystals of curcuminoids.

In this report, crystal nucleation and growth kinetics for precipitation of piperine from hydrotropic solutions of pure piperine is reported. However, instead of dilution with water, the variation in solubility with temperature is exploited for the recovery of piperine from the aqueous hydrotrope solutions. The method of *s*-plane analysis is followed to simultaneously deduce growth and nucleation kinetics expressions.[12]

MATERIALS AND EXPERIMENTAL METHODS

Standard piperine was obtained from Synthite Ltd., Cochin, Kerala (India). The raw pepper fruits were purchased locally. Sodium cumene sulfonate (NaCS) was purchased from M/s. Navdeep Chemicals, Mumbai, India, and was used as such. The solvents for analysis, dichloromethane (DCM), methanol, etc. [all high-performance liquid chromatography (HPLC) grade] were purchased locally and used as such.

Solubility Measurements

Solubility of piperine was determined by suspending pure piperine in aqueous NaCS solutions of different concentrations ranging from 0.05 to 1.0 mol/dm^3. The NaCS solution, in a 25-cm^3 stoppered conical flask, was equilibrated with excess solid piperine for several hours, at constant temperatures ranging from 303K to 353K by using a magnetic stirrer. The suspension was then filtered at the same temperature. The dissolved piperine from the hydrotrope solution was then extracted into DCM and was analyzed by HPLC. The HPLC analysis was performed with a 5-μm Eurosher-100, C-18 column. The column was mounted on a KNAUER HPLC chromatograph (Berlin, Germany) equipped with a 20-μL loop injector. The mobile phase was methanol : water (77 : 23) with a flow rate of 0.5 cm^3/min. The detection was done by an ultra violet detector attached to HPLC instrument with a detection wavelength of 343 nm.

Continuous Soxhlet extraction with methanol was separately conducted for 48 hr to determine the piperine content of the raw material. The amount of piperine present in the raw material was estimated to be 5% (w/w).

Extraction of Piperine into Hydrotrope Solutions

In earlier studies, while performing the extraction of piperine with hydrotrope solutions at room temperature, a decrease in the purity of extracted piperine from 98 to 89% was observed on reducing the particle size from 710 to 50 μm.[5] Pepper particles of 710 μm size, therefore, were used for the extraction studies. A 50-g sample of ground pepper was added to 500 cm^3 of 1.0 mol/dm^3 NaCS solution in a fully baffled, cylindrical, round-bottom, glass vessel (500 cm^3) equipped with a six-bladed pitch blade turbine impeller. This entire assembly was kept in a constant temperature bath at 353K during the experimentation. The suspension was agitated vigorously at 1000 rpm for a period of 2 hr. Samples ($\sim$2 cm^3) were taken out at predefined time intervals and were filtered immediately by using a syringe filter of pore size of 0.4 μm. After 2 hr, the remaining solution was allowed to settle for 40 min and was subsequently filtered at 353K. A clear, brown colored liquid was obtained as the filtrate. The filtrate was allowed to cool down, with constant agitation to 303K. A brown colored precipitate was separated from the hydrotrope solution by centrifugation and was dried and analyzed for purity.

Crystallization of Pure Piperine from Hydrotrope Solutions

Experiments were performed on precipitation of pure piperine from aqueous NaCS solutions. For the experiments, saturated solutions of piperine were prepared by adding solid piperine to 1.0 mol/dm^3 NaCS solution, and the suspension was stirred in a baffled agitated vessel at 353K till it became saturated and then was subsequently filtered at 353K. The amount of piperine in the solution was determined by HPLC as described earlier. The precipitation experiments were conducted in 100-cm^3 cylindrical glass vessel of 6.0-cm diameter, provided with a lid having three entry holes for impeller, thermometer, and sampling port. A pitch blade turbine impeller of 2-cm diameter was mounted at the central axis, with a clearance of about 2 cm from the bottom.

In a typical experiment 100 cm^3 of a saturated solution of piperine at 353K in 1.0 mol/dm^3 NaCS solutions was charged into the crystallizer. The temperature of crystallizer was allowed to come down naturally by air cooling over a period of 2–2.5 hr, with constant agitation. Samples (around 5 cm^3 each) were collected at predefined time intervals for analysis. The product crystal size distribution (CSD) was determined by using a Coulter counter (Model LS 230; Couter Corporation, Miami FL, USA) with a small volume module; measurements were made in the size range of 0.4–2000 μm. About 3 cm^3 of the sample was used to measure the CSD by using the Coulter counter; whereas, the rest of the sample was filtered immediately by using a syringe filter of pore size of 0.4 μm. The clear solution was analyzed for piperine content in the solution by using HPLC. In the first three runs (Runs 1–3), the stirring speed was kept constant at 1000 rpm. In the next two runs (Runs 4–5), the stirring speed was kept constant at 500 rpm.

RESULTS AND DISCUSSION

Solubility Measurements

The solubility of piperine in aqueous NaCS solutions of different concentrations and at different temperatures is depicted in Fig. 2. In water at 303K, the solubility of piperine is 0.01 mmol/dm^3. In a 1.0 mol/dm^3 NaCS solution at 303K, the piperine solubility increased by nearly four orders of magnitude to 63 mmol/dm^3. The increase in the solubility is significant only above the minimum hydrotrope concentration (MHC) of NaCS (0.1 mol/dm^3). The piperine solubility, in 1.0 mol/dm^3 NaCS solution, further increased from 63 to 282 mmo1/dm^3, i.e., 4.5 times as the temperature was increased

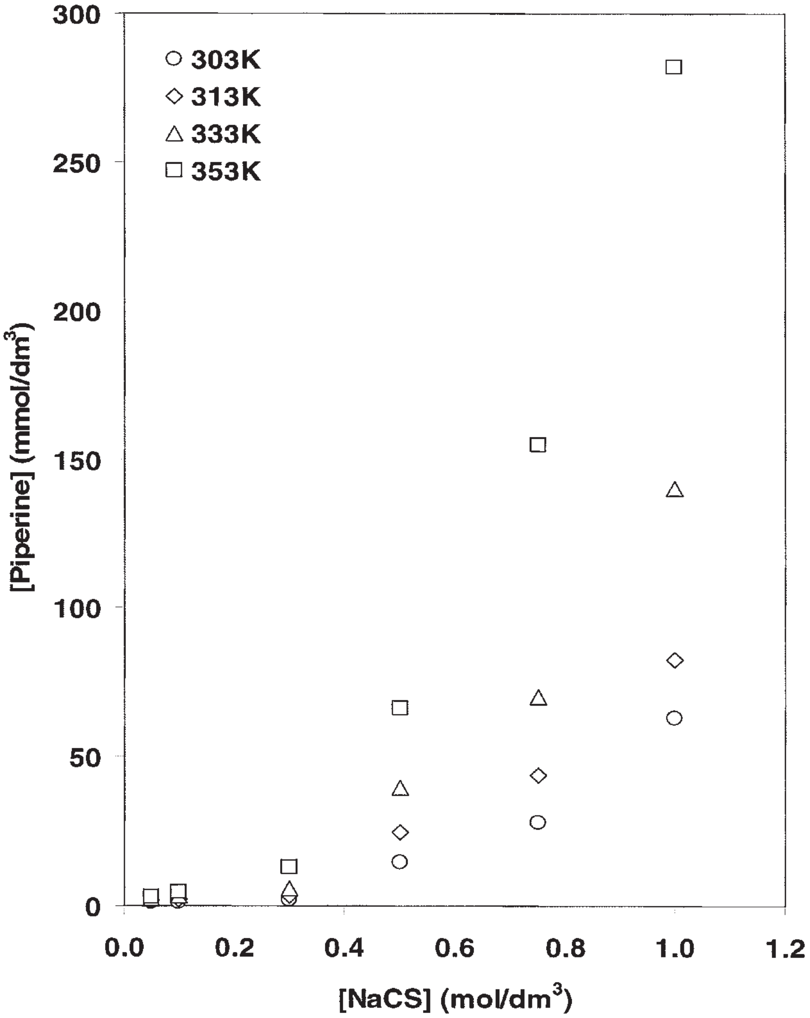


Figure 2. Solubility of piperine in aq. NaCS solutions.

from 303K to 353K. The solubility of piperine, therefore, is a strong function of the hydrotrope concentration, as well as of temperature.

These solubility data were adequately correlated, as represented by the solid curves in Fig. 3, into an exponential function as

$$S = a \exp(b \times T) \tag{1}$$

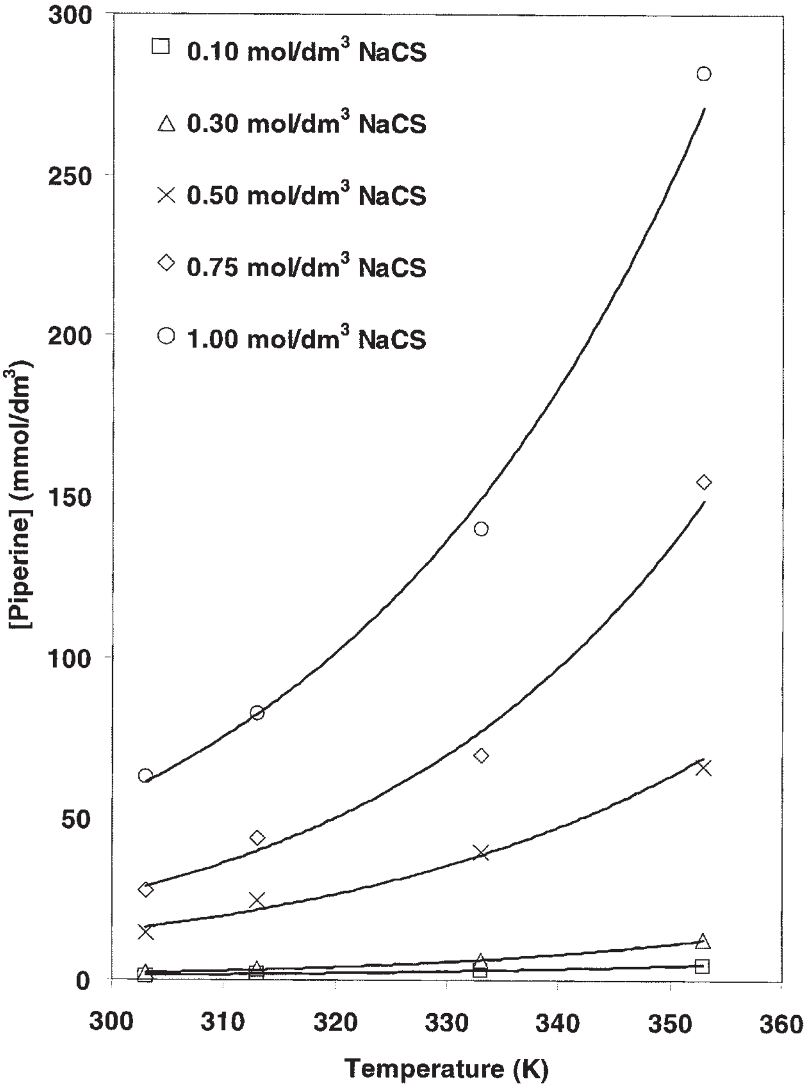


Figure 3. Solubility of piperine in aq. NaCS solutions at different temperatures.

where S is the solubility (mmol/dm^3) of piperine in aqueous NaCS solutions. The constants a and b, obtained from least square fitting of the experimental solubility data in aqueous NaCS solutions, are reported in Table 1. The data show that if the extraction of piperine is conducted with hydrotrope

Table 1. Solubility parameters for piperine in NaCS solutions [Eq. (1)].

NaCS, $\mathrm{mol/dm^3}$	a $\mathrm{mmol/dm^3}$	b $1/\mathrm{K}$
0.10	0.00070	0.0250
0.30	0.00007	0.0344
0.50	0.00250	0.0290
0.75	0.00140	0.0328
1.00	0.00750	0.0297

solutions at elevated temperatures, piperine can be recovered by simply cooling the hydrotrope solution. Compared with the dilution by water, the cooling procedure should be much more economical for the recovery of piperine.

Extraction of Piperine into Hydrotrope Solutions

Figure 4 shows that almost 80% of piperine is extracted within 30 min. When the extraction was conducted for longer times, the solution became very viscous. At higher temperatures, starch in the raw pepper also was solubilized, making the solution viscous. The solution, therefore, was very difficult to filter at 353K. The extraction, therefore, was restricted to 30 min at 353K. The particle size of 710 μm was useful for easy filtration of the hydrotrope solutions at these higher temperatures. The purity of recovered piperine was about 60%, unlike 90% reported by Raman and Gaikar.[5] At elevated temperatures, the extraction of undesirable oleoresins, and also solubilization of cellulose and/or starch, from the plant cells of the pepper resulted in reduced selectivity and, therefore, less purity of piperine compared with the extraction at lower temperatures, as reported earlier.[5]

A significant increase in the solubility of piperine in aqueous NaCS solutions (1.0 $\mathrm{mol/dm^3}$ concentration) and a decrease in the purity of recovered piperine were observed with the increase in temperature. The recovered piperine can be further purified by using hydrotropic solutions at room temperature to produce highly pure piperine, if necessary.

Almost 35% (w/w) extraction of piperine in 5 hr from raw black pepper by supercritical fluid extraction by using CO_2 was reported by Lajmi[13] between temperatures 308K and 323K, and at an operating pressure of 150 to 300 bar. The extracted piperine was yellowish in color, indicating the presence of impurities. Hydrotropic extraction of piperine, when compared with this supercritical fluid-extraction result, seems to be attractive, because of faster extraction, the ease of scale up, better purity, and much lower equipment cost.

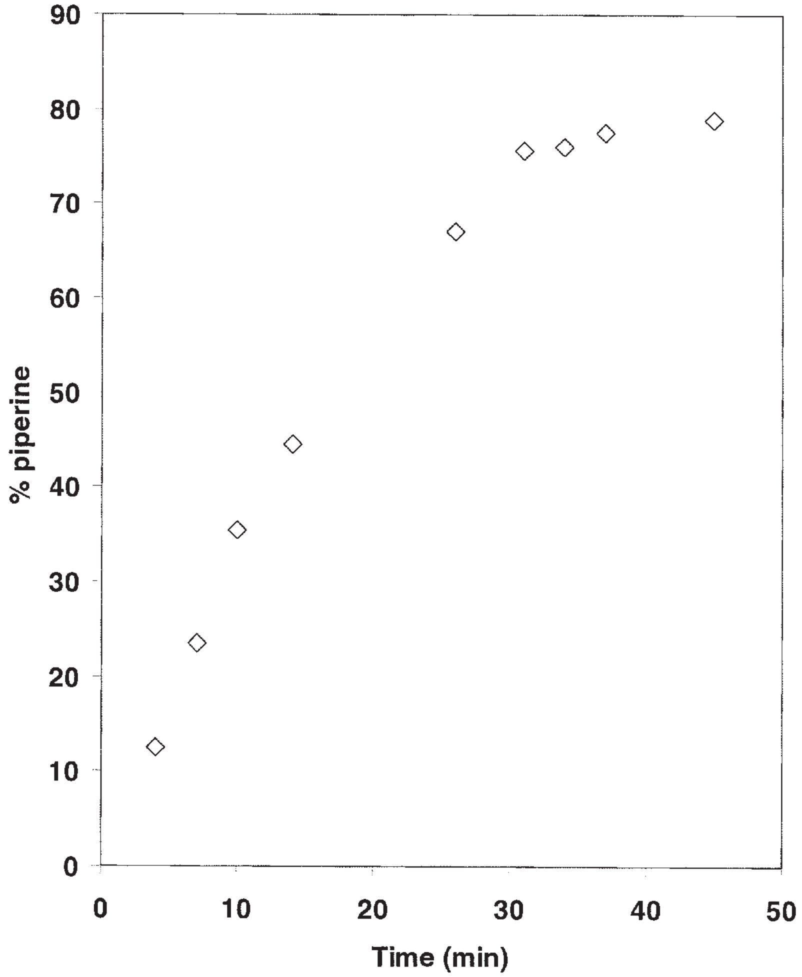


Figure 4. Percentage extraction of piperine with time in 1.0 mol/dm^3 NaCS solutions.

Crystallization of Piperine from Hydrotrope Solutions

In all the crystallization experiments, concentration of dissolved piperine in the solution was determined at various time intervals by HPLC. By comparing this data with the solubility values estimated when using Eq. (1) and Table 1, supersaturations were estimated and supersaturation curves were plotted for each run (Fig. 5). The hydrotrope solution gets saturated and

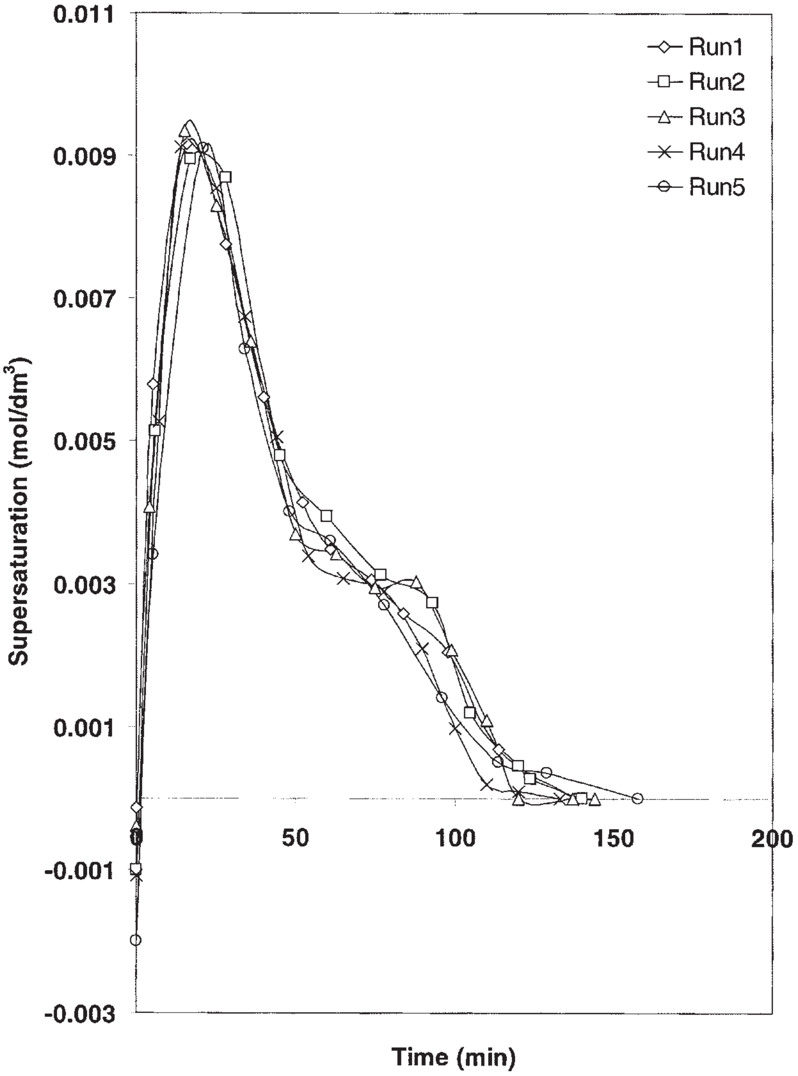


Figure 5. Supersaturation of piperine in aq. NaCS solutions.

then supersaturated on reducing the temperature of the hydrotrope solution. Both particle formation and its subsequent growth deplete the level of supersaturation. The effect of stirring speed on the supersaturation curve was not prominent when in the 500–1000 rpm range. The supersaturation was the main driving force for nucleation and growth of precipitation; it was, further, kinetically correlated with growth and nucleation rates, along with stirring speed (N) and suspension density (M_T).

Some results of size-distribution data for Run 5, obtained from the Coulter counter measurements are shown in Fig. 6, and indicate a distribution containing more fine size crystals than large size crystals. A small but not significant hump in the population density curve at a higher particle size

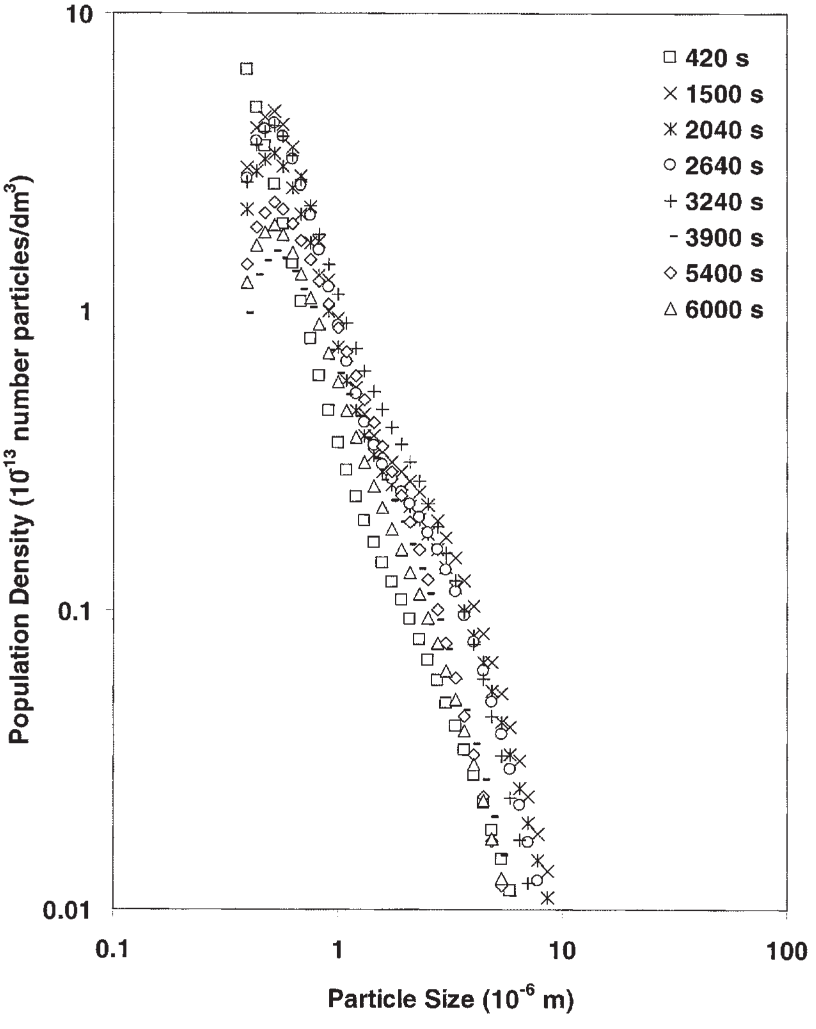


Figure 6. Population density of crystals at different times (Run 5500 rpm).

was observed, either due to agglomeration or to crystal growth. Although the crystals showed growth with time, continuous reduction in temperature resulted in formation of newer nuclei as the density of the smaller crystals increased with time.

Figures 7 and 8 show the CSD data obtained from the Coulter counter as volume percentage vs. particle diameter for Run 1 and Run 5, respectively. In Run 1, there was no substantial growth of the particles during the experiment, which can clearly be observed for Run 5 (Fig. 8). The influence of agitation on the nucleation process is complex. It is generally agreed that mechanical disturbances can enhance nucleation, but it has been shown by Mullin and Raven[14] that an increase in the intensity of agitation does not always lead to an increase in nucleation.

Throughout the experiments, domination of nucleation over the growth was observed. The particles of micron size were finally generated. The reason for small crystal size can be attributed to the precipitation as fine crystals from different regions of the microphases of hydrotrope aggregates, rather than growing by mass transfer from one region to another. The solute is divided into different regions of hydrotrope assemblies. Although, the hydrotrope's self-aggregation is a prerequisite for the enhanced solubility of piperine in aqueous solutions, the solute-driven hydrotrope aggregation cannot be ruled out. When piperine is located in such different regions, crystallization of smaller particles from these assemblies is more likely on reducing the solubility by cooling of the solution. The process needs to be understood in terms of the changes in the hydrotrope assemblies on reducing temperature, which is also likely to vary with different assemblies of different aggregation numbers, making the process inherently more random and subjected to variation.

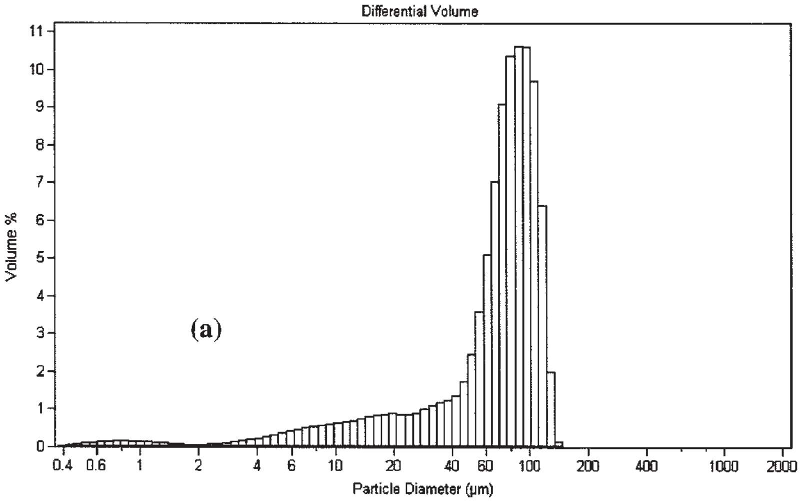
Kinetic Correlation

The CSD data obtained from the Coulter counter measurements were used to calculate the growth and nucleation rates by the method of *s*-plane analysis. Growth and nucleation rates obtained from all the experimental runs (20 observations from 5 runs), using the successive pairs of population density curves, were correlated by power law kinetic expressions in terms of observable parameters as

$$G = k_g N^{m'} \Delta c^g \tag{2}$$

and

$$B = k_r N^{m''} M_T^j G^i \tag{3}$$



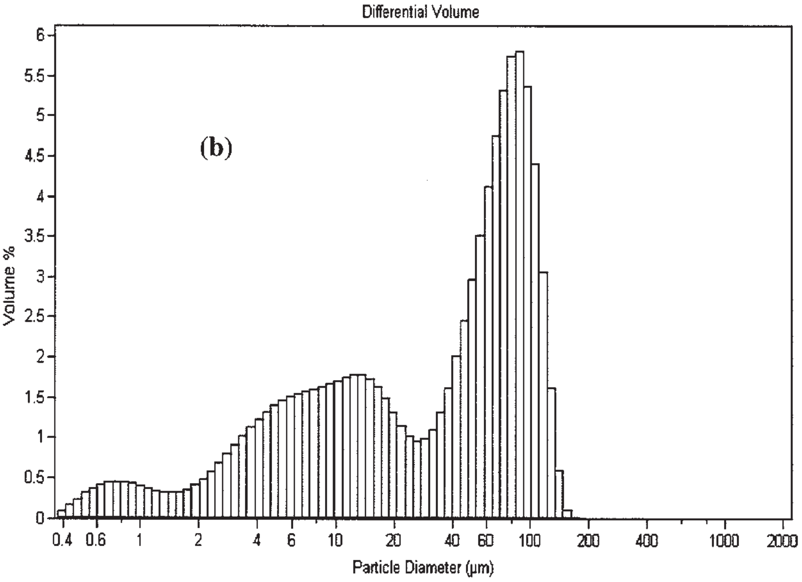
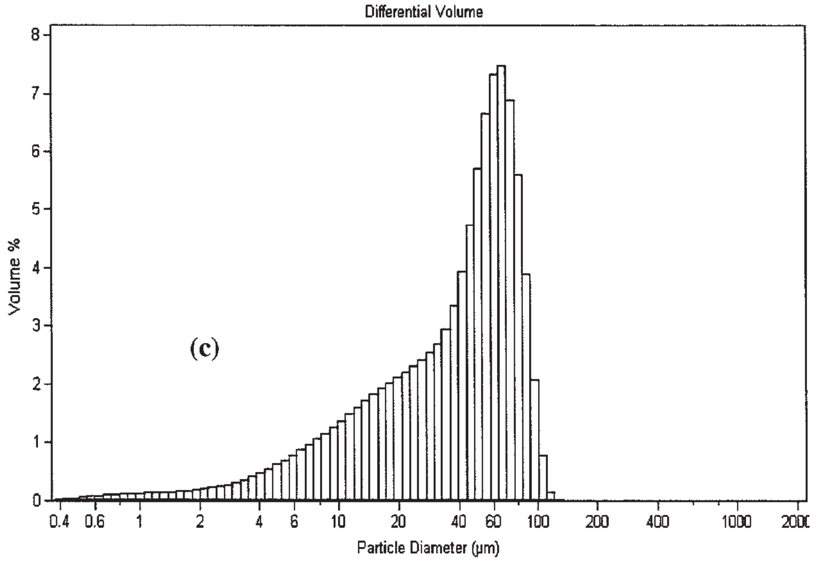
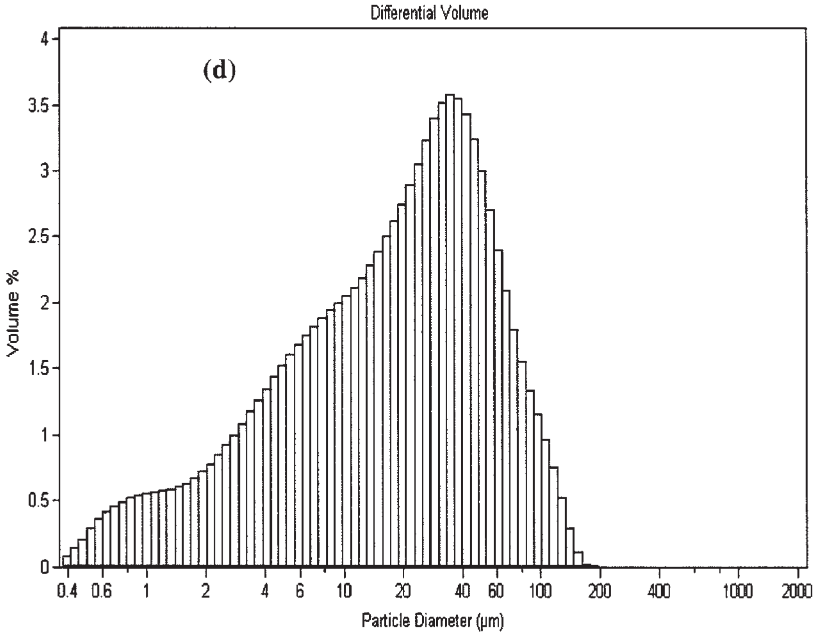


Figure 7. CSD variation of piperine with time (Run 1; rpm: 1000); (a) t = 5 min; (b) t = 28 min; (c) t = 52 min; (d) t = 74 min; (e) t = 98 min; (f) t = 114 min.

(*continued*)





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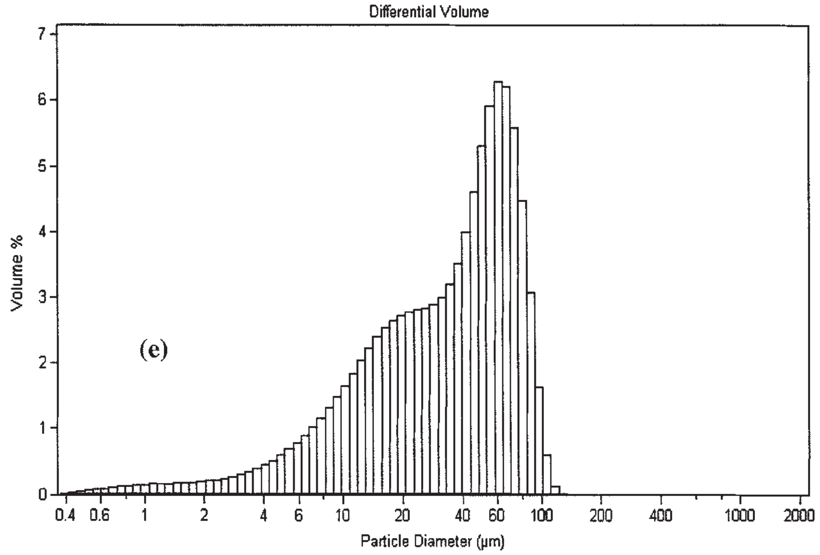

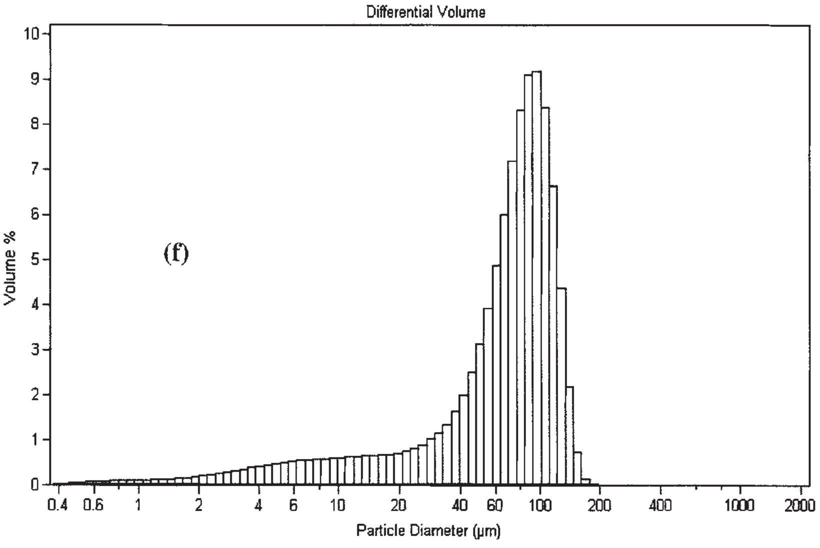

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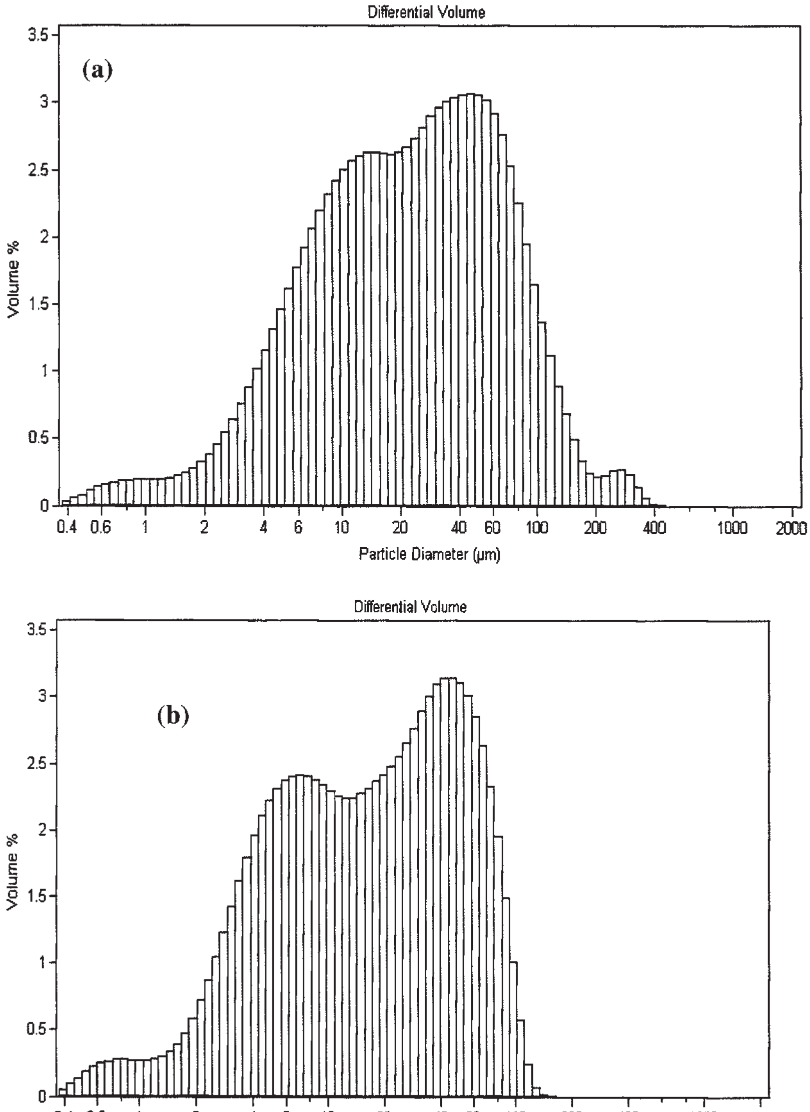

Figure 8. CSD variation of piperine with time (Run 5, rpm 500); (a) t = 7 min; (b) t = 25 min; (c) t = 54 min; (d) t = 78 min; (e) t = 110 min; (f) t = 133 min.

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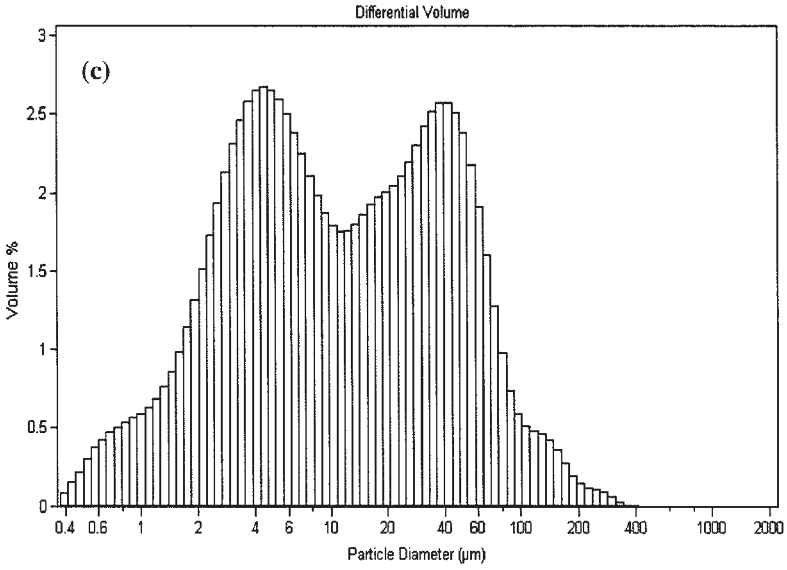

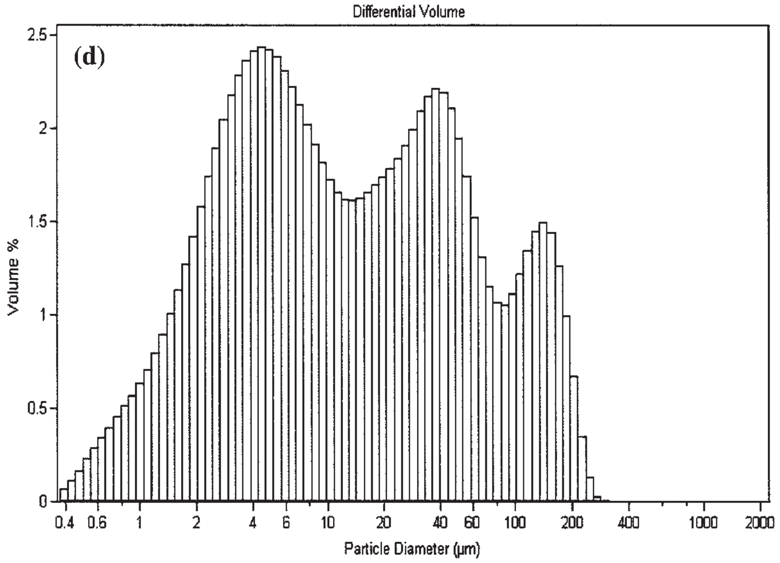

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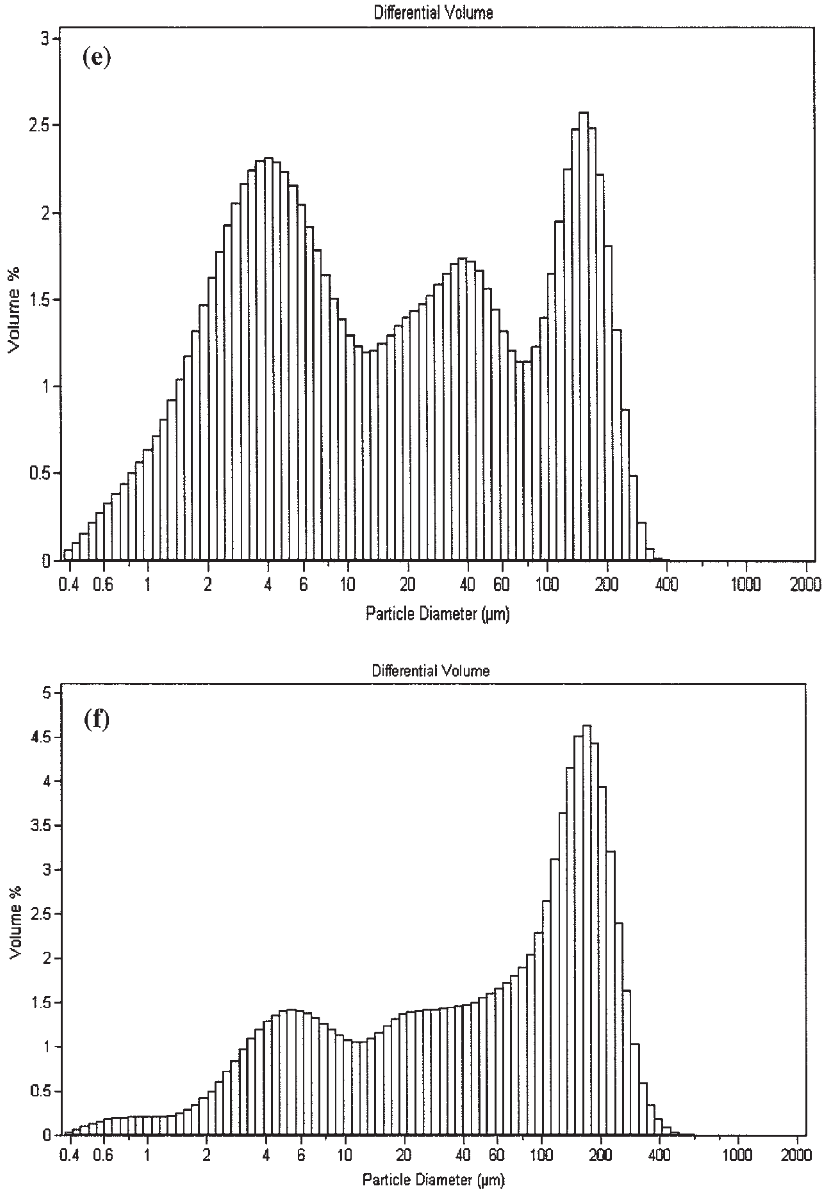

Figure 8. Continued.

The values of the model parameters in Eqs. (2) and (3) were estimated by the least square multiple linear regression analysis. Supersaturation (Δc) and the stirring speed (N) were the significant variables influencing the precipitation process. Figures 9 and 10, however, show a large scatter attributed to uncertainties associated with estimation of the variables and, in particular, with supersaturation (Δc). The higher value of the relative rate coefficient for nucleation correlation ($k_r = 4.3 \times 10^7$) in comparison with the growth

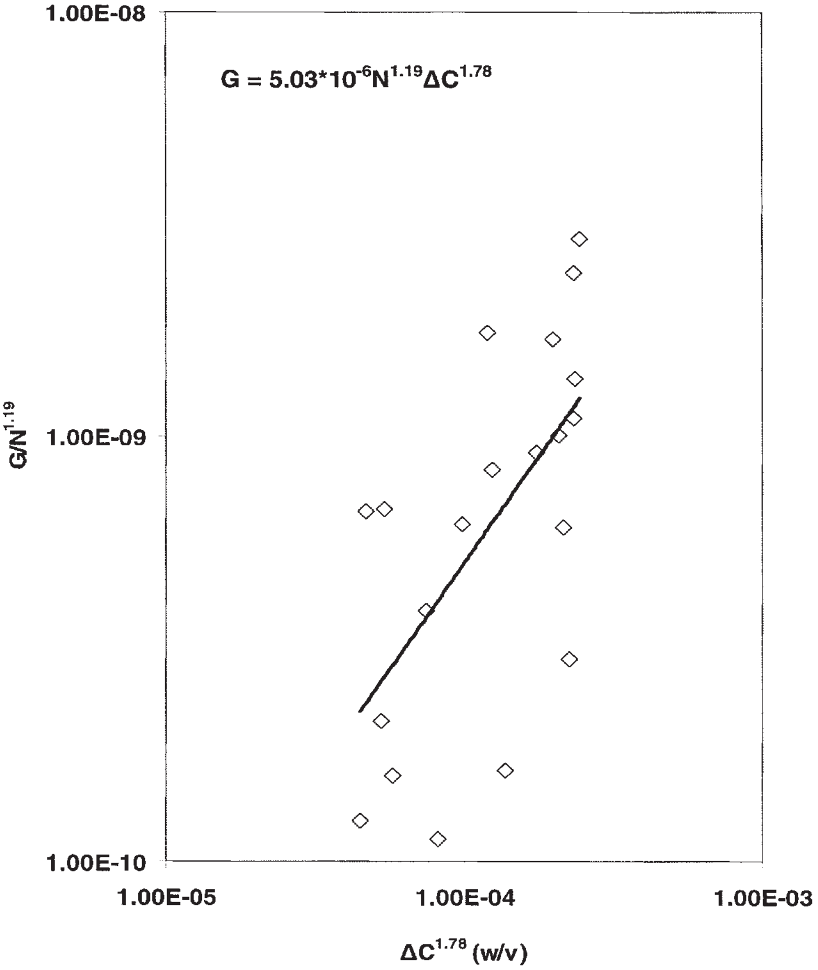

Figure 9. Relative kinetic correlation for crystal growth [Eq. (2)].

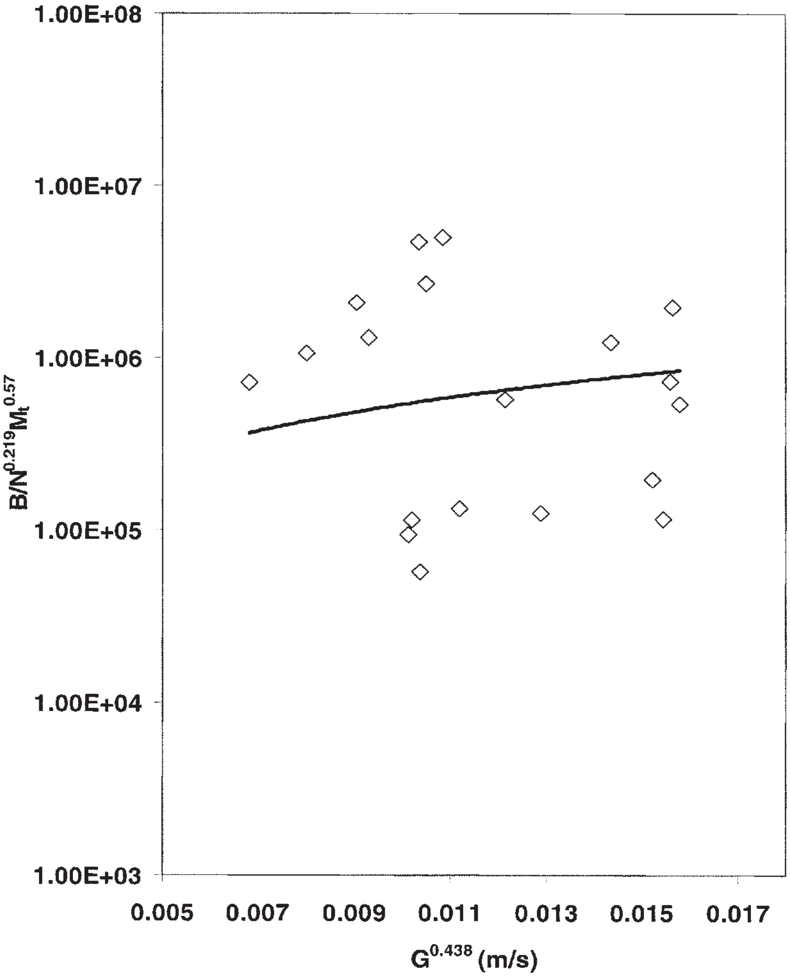

Figure 10. Relative kinetic correlation for nucleation [Eq. (3)].

rate coefficient ($k_g = 2 \times 10^4$), however, signifies the stronger influence of nucleation over growth of the crystals. A similar scatter and dominant nucleation over crystal growth was reported in our previous paper while studying precipitation of curcumin from hydrotrope solutions.[11] It would be worth investigating the characteristics of the crystals obtained from aqueous solutions of hydrotropes as the characteristic of respective hydrotrope solutions.

At the end of the experiment, the total recovery of piperine was $\sim$88%. The remaining piperine was in the dissolved form in the hydrotrope solution, which corresponded to the solubility of piperine in $1.0\,\mathrm{mol/dm^3}$ NaCS solution at a room temperature of 303K.

The nucleation and growth rates determined by this parameter estimation technique represent average values over an average time interval, and so, all other stated variables incorporated in the kinetic correlation should correspond to an average time. In a few cases, incorrect parameter estimates (e.g., negative rates) were predicted. Such predictions may be attributed to inaccuracies associated with taking representative samples and in measuring the population density curves. It is important to note that the method of *s*-plane is usually rather insensitive to the noise levels associated with both the experimental observations and the numerical techniques used for integral evaluations.

The lower solubility of piperine increases the relative uncertainties in the determination of supersaturation, which affects the further kinetic correlation of the data. During this study, it was observed that the precipitation rate of piperine from the pure piperine solution was not high compared with the mixing speed. Also, the CSD observed over time is not varying to a large extent and a constant pattern was observed for a population density curve, even over a sufficiently large time period.

CONCLUSION

The nucleation and growth rates of precipitation of piperine from hydrotrope solutions can be simultaneously estimated by using laboratory-scale batch crystallization. The method, based on the Laplace transform domain, was found to be suitable for this purpose. The precipitation of piperine from aqueous hydrotrope solutions was achieved by exploiting the change in solubility of piperine in a hydrotrope solution with temperature, which appears to be a better option compared with the precipitation by dilution with water. The precipitation from the aqueous hydrotrope solutions showed higher nucleation rates compared with the crystal growth rates, leading to finer crystals in the product.

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