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Study on the Oiling-out and Crystallization for the Purification of Idebenone

Jie Lu,*[,†] Yi-Ping Li,[†] Jing Wang,[†] Zhen Li,[†] Sohrab Rohani,[‡] and Chi-Bun Ching[§]

[†]National Engineering Laboratory for Cereal Fermentation Technology, School of Chemical and Material Engineering, Jiangnan University, Wuxi 214122, China

[‡]Department of Chemical and Biochemical Engineering, The University of Western Ontario, London, Ontario N6A 5B9, Canada

[§]School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore 637459, Singapore

ABSTRACT: Oiling-out, also termed as demixing or liquid−liquid phase separation (LLPS), frequently occurs in the crystallization process of pharmaceuticals and normally results in slow crystal growth, uncontrollable crystal morphology, and low purity of products. In this work, the oiling-out and cooling crystallization of idebenone were investigated. The cooling crystallization and the purity of its products were found to depend upon whether an oiling-out occurred in the course of cooling. At low initial concentrations (e.g., 12 mg/mL), oiling-out did not take place, and the crystallization products possessed higher purity. At moderate initial concentrations (e.g., 25 mg/mL) small quantities of oil droplets formed and then coalesced into larger droplets, which subsequently crystallized into products with lower purity. When high initial concentrations (e.g., 31 mg/mL) were employed, large quantities of oil droplets formed a stable oil phase, and thus no products could be harvested. In addition to initial concentration, other parameters such as solvent, cooling rate, and seeding also affected the occurrence of oiling-out. On the basis of the above results, the approach to purifying idebenone through cooling crystallization will be found.

1. INTRODUCTION

More than 80% of the substances used in pharmaceuticals, fine chemicals, agrochemicals, food, and cosmetics are isolated or formulated in their solid form, and crystallization is, in general, used as the last chemical purification step in their production.[1,2] However, when components of different polarities are involved, oiling-out may occur in the course of crystallization.[3−5] Oiling-out, also termed as demixing or liquid−liquid phase separation (LLPS), refers to the appearance of a second liquid phase during the crystallization of a compound from solution.[6,7] In practice, oiling-out is undesirable because the oil phase is often a good solvent for impurities which will lower the purity of final products, and it may stick to reactor walls and thus impede the whole crystallization process.[8] In tune with the Ostwald rule of stages, oiling-out was found to slow down the crystallization rate, as the first formed metastable liquid phase hinders primary and secondary nucleation.[9]

To date, there were only a few references in the literature with regard to the oiling-out of organic small molecules, e.g. bisphenol A,[10] 4,4′-dihydroxydiphenylsulfone (DHDPS),[11] $C_{35}H_{41}Cl_2N_3O_2$,[12,13] methyl(E)-2-[2-(6-trifluoromethylpyridine-2-yloxymethyl)-phenyl]-3-methoxyacrylate,[14] vanillin,[15] etc.[16−18] Serajudin and Pudipeddi[19] reported that there is an increase in the number of less hydrophilic and less polar drug candidates during the discovery of potent and improved target-specific drug molecules. Derdour[20] has attributed the oiling-out of less hydrophilic molecules to their inherent low polarity resulting in a lack of anchoring sites required for the growth in an organized manner when crystallizing from solvents and has categorized oiling-out into two types. One is liquid−liquid separation occuring where each phase contains reasonable amounts of solute. Veesler et al.[21] have found that this type of oiling-out usually occurs when a mixture of solvents is used, and in this case, the solute is generally not evenly distributed between the two phases. The large difference in affinity of target compound for each solvent can be the driving force for LLPS. In this type of LLPS, the crystallization of the drug is possible by adjusting the crystallization condition. In particular, seeding has been shown to be a good method to avoid oiling-out.[22] The other type is liquid−liquid separation occuring where one phase contains the solvent(s) and the other phase is mainly formed by the solute in the form of a very heavy, viscous, oily phase. This type of LLPS takes place usually for high solute concentrations at high temperatures which are close to the melting points of solutes.[14] In this case, lowering the concentration of the solution and crystallization temperature may prevent oiling-out.

Idebenone (6-(10-hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone, I_1 in Scheme 1) is an organic compound of the quinone family which has been used for the treatment of various cerebral diseases.[23] It has been found that there are mainly two analogous impurities (I_2 and I_3 shown in Scheme 1) in the product from chemical synthesis.

In this work the purification of raw idebenone has been attempted by use of crystallization from hexane/methylene chloride mixtures, in which the effects of such parameters as cooling rate, solvent, initial concentration, and seeding on the occurrence of oiling-out and the purity of products were experimentally investigated. The optical microscope was used to photograph the oil droplets. High performance liquid chromatography (HPLC) was used to analyze the purity of samples.

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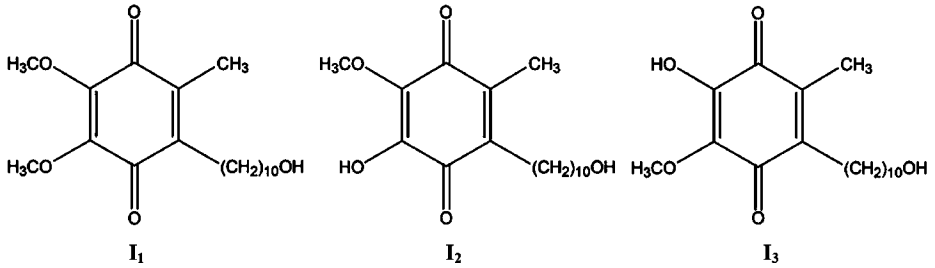
Scheme 1. Structures of idebenone (I_1) and analogous impurities I_2 and I_3



I_1 I_2 I_3

2. EXPERIMENTAL SECTION

Materials. Raw idebenone with a purity of 96.2% (HPLC) was supplied by Suzhou Lixin Pharmaceutical Company. Hexane, methanol, and methylene chloride (99.5%) at an analytical grade were purchased from Sinopharm Chemical Reagent Company. Ultrapure water was obtained from Millipore filter system.

Polarity Calculation. When polarities were calculated, the structures of target molecules were optimized by the *Gaussian 03* program package.[24] The structures are in ideal state which gives minimum energy, not in real state.

Solubility Measurements. The solubility of recrystallized idebenone was measured in hexane, methylene chloride, and their mixtures at various temperatures. Excess powders of idebenone were first added to the solvents in a 100-mL jacketed glass crystallizer. The Teflon-coated magnetic stirring bar ensured proper mixing in the crystallizer. The temperature of the crystallizer was controlled by an RTE-740 Digital Plus refrigerated bath (Thermo Neslab, Newington, NH). After 24 h when the sample had reached equilibrium, the agitation was stopped, and the solution was allowed to settle. The supernatant in equilibrium with a macroscopically observable solid was then filtered through Millex-VV 0.1 μm filters (Millipore, Billerica, MA). The concentration of filtered supernatant was determined by use of the dry mass method. About 10 g of the filtered supernatant was withdrawn with a pipet and placed in a sample bottle preweighed by use of a Sartorius CP225D analytical balance (Sartorius, Goettingen, Germany) with a resolution of $\pm$0.01 mg. The sample was placed in a Vacucenter VC 20 oven (Salvis-Lab, Rotkreuz, Switzerland) and vacuum-evaporated to dryness at 278.15 K until the mass was constant. The low evaporation temperature was chosen to eliminate decomposition. The solid residue masses were determined, and the concentration was then calculated. All experiments were replicated three times. The data reported in this work are the average of the replicates.

Cooling Crystallization Experiments. The experiments were performed in a 200-mL jacketed glass crystallizer of which the temperature was controlled by a Julabo FP 50 programmable circulator (Julabo Labortechnik, Seelbach, Germany). A suspension of the desired amount of raw idebenone was first heated to 3 °C above the equilibrium temperature to dissolve all powders. It was then filtered and added to the crystallizer in which temperature was kept at the desired value. When the solution was cooled down to 0 °C, the product was harvested. The cooling rates conducted were 0.05, 0.1, 0.2, and 0.5 °C/min. The ratios of hexane to methylene chloride used were 7, 8, 9, and 10 (w/w). Initial solution concentrations employed were 40, 31, 25, 20, and 12 mg/mL. In some cases, seeding was employed for the purpose of comparison. For all cooling crystallization experiments, the stirring was employed by a Corning PC-353 magnetic stirrer (Corning, NY).

Optical Microscopy. The oil droplets and the crystallization products were observed by an optical microscope (C3230B, Shanghai Precision Instruments Company) and recorded with a camera (CX-1, Shanghai Precision Instruments Company). Along with the crystal shape and size, the microphotographs were useful for observing the crystal form and how the oil droplets changed.

HPLC. The HPLC system (Waters 600S, Waters Corporation, Milford, MA) was utilized to analyze the purity of samples. Column used was a Grace C18 with dimensions 4.6 mm × 250 mm and particle size 5 μm. Operation conditions were the following: 30 °C, elution with methanol–water (72:28 v/v), 1.0 mL/min, 20 μL injection, concentration for sample (degassing before injection) 0.2 mg/mL.

DSC. DSC was conducted by use of a Mettler-Toledo DSC-822^e differential scanning calorimeter (Mettler-Toledo, Columbus, OH). Indium was used for calibration. Accurately weighed samples (2–5 mg) were placed in hermetically sealed aluminum pans and scanned from 25 to 250 °C at 10 °C/min under nitrogen purge.

3. RESULTS AND DISCUSSION

Polarity Calculation. Table 1 lists the calculated polarity of solvents, I_1, I_2, and I_3. It is shown that the polarities of impurities, I_2 and I_3, are larger than that of product, suggesting that the increase in the polarity of the solvent will favor the removal of the impurities from the product.

Table 1. Calculated polarity of solvents, I_1, I_2 and I_3

chemical	I_1	I_2	I_3	hexane	methylene chloride
polarity	2.15	3.12	3.62	0.06	3.40

Solubility. Figure 1 presents the solubility of idebenone in different hexane/methylene chloride mixtures at different temperatures. The solubility of idebenone generally increases with the temperature and the polarity of the solvents.

Oiling-out. In most cases, as the temperature was lowered, oiling-out phenomenon could be observed distinctly. When oiling-out took place, the transparent solution became cloudy and oil droplets appeared on the wall of the crystallizer (Figure 2a). To ensure that the cloudiness of the solution was caused by liquid–liquid demixing and not by crystal formation, the stirrer was turned off, and after several seconds, two liquid phases separated, and the oil phase sank to the

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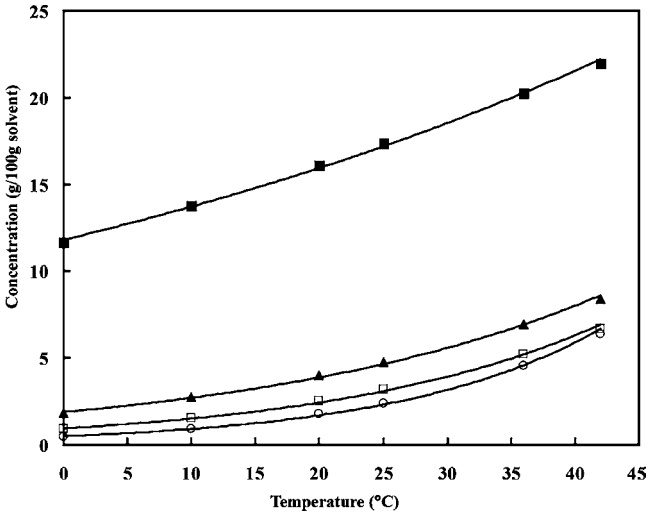

Figure 1. Solubility of idebenone in different hexane/methylene chloride mixtures (w/w): ■ 3:1; ▲ 5:1; □ 7:1; ○ 9:1.

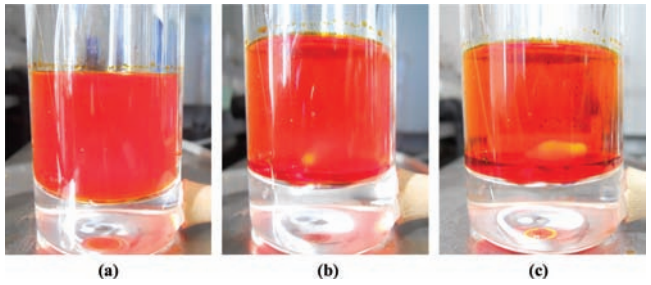

Figure 2. (a) The oil phase appeared, and the solution became cloudy during cooling; (b) After the magnetic stirrer was stopped, a visible phase separation interface can be observed; (c) After 30 min the upper solution became transparent and no crystals were detected visually.

bottom (Figure 2b). After 30 min, the opaque phase would become transparent, while the oil phase still existed, as shown in Figure 2c.

Figure 3a is a photomicrograph of oil phase which was obtained from the bottom of the liquid–liquid phase, observed under the optical microscope. With the temperature decreasing, the oil droplets solidified, the solidification product was showed in Figure 3b. Figure 3c is a photomicrograph of the needle-like crystallization product in the absence of oiling-out.

Figure 4a shows HPLC chromatogram of idebenone crystallized from hexane/methylene chloride mixture in the absence of oiling-out using UV detection with an ultraviolet absorption maximum at 278 nm, which indicates that the product is very pure with only a small, main, related organic impurity (retention time =17.97 min) by peak area ratio (99.55%). Figure 4b shows the chromatogram of the solidification product of the oil phase with a lower purity (99.21%). The DSC results (Figure 5) also demonstrate that the appearance of oiling-out will deteriorate the purity of crystallization products of idebenone. It is noted that, in general, the higher-purity sample must have not only the higher melting point but also the narrower melting range. In this work the wider melting range of the crystallization product may be attributed to the types and the amounts of particular impurities still included in the crystallization product.

The oiling-out was found to be significantly influenced by such operational parameters as the polarity of solvent (Table 2), cooling rate (Table 3), initial concentration (Table 4), and seeding (Table 5). In tables where abundant oil droplets occurred, normally no products can be harvested and thus no yield. As shown in Table 2, when the polarity of solvent mixtures was decreased (i.e., the difference in polarity between solvent and solute was increased), the oiling-out was promoted, which is in accordance with the experimental results of Kim et al.[16]

As shown in Table 3, fast cooling can also lead to the occurrence of oiling-out. Meanwhile, at low initial concentrations (e.g., 12 mg/mL, Table 4), oiling-out did not take place, and the obtained product possessed higher purity. At moderate initial concentrations (e.g., 25 mg/mL) small quantities of oil droplets formed and then coalesced into larger droplets, which subsequently crystallized into products with lower purity. When high initial concentrations (e.g., 31 mg/mL) were employed, large quantities of oil droplets formed, and no products could be harvested. In addition, seems to reduce the occurrence of oiling-out (Table 5).

Purification through Crystallization. According to Ostwald's Rule,[1] in the kinetic process of the system when leaving a given state and transforming to another thermodynamically unstable one, crystal nucleation and growth are hindered, and the solute crystallizes into an intermediate, undercooled liquid phase.[9] However, for the system of idebenone studied in this work, a stable liquid phase will form when idebenone solutions of high concentration are cooled. Following the works of Derdour[20] and Maeda et al.,[25] a typical phase diagram can be schematically outlined to depict oiling-out and crystallization of idebenone from solvents, as shown in Figure 6. When the solution with a high concentration (e.g., point A) is cooled down, a stable liquid–liquid phase separation (LLPS) will form, which leads to no crystal products. When starting from point B, a metastable LLPS will happen, followed by nucleation and crystal growth. In this case, more impurities will be included as part of oil droplets will directly solidify into the product. LLPS can be

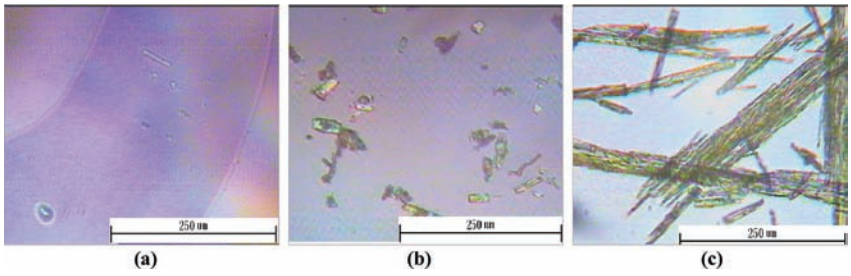

Figure 3. Optical microscope photos: (a) Oil droplets. (b) The solidification product of the oil droplets. (c) Classical crystallization product.

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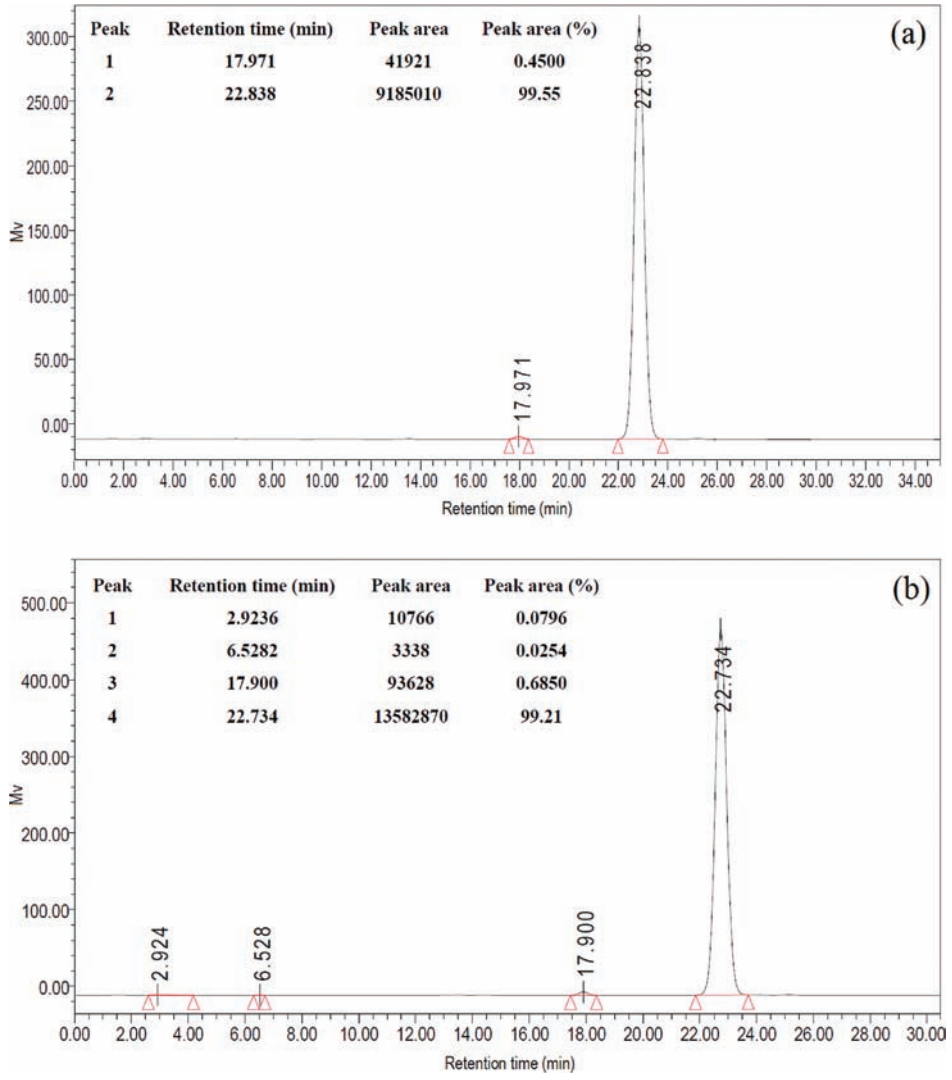

Figure 4. (a) Chromatogram of idebenone crystallized from hexane/methylene chloride mixture in the absence of oiling-out with a content of 99.55%. (b) Chromatogram of the solidification product of the oil phase with a content of 99.21%.

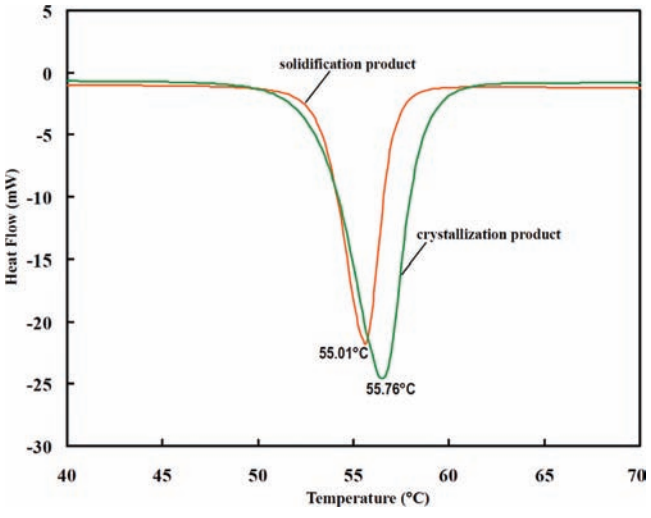

Figure 5. DSC curves of the products from crystallization and solidification of oil droplets.

avoided by lowering the solute concentration, i.e. starting from point C, which results in crystal products with higher purity.

Table 2. Effect of the ratio of hexane to methylene chloride on the oiling-out

hexane:methylene chloride	cooling rate (°C/min)	initial concentration (mg/mL)	purity (%)	yield (%)	oiling-out
7:1	0.1	25	99.55	78	less
8:1	0.1	25	99.54	67	little
9:1	0.1	25	99.21	–	much
10:1	0.1	25	–	–	more

Table 3. Effect of cooling rate on the oiling-out

cooling rate (°C/min)	hexane: methylene chloride	initial concentration (mg/mL)	purity (%)	yield (%)	oiling-out
0.05	7:1	25	99.56	78	nil
0.1	7:1	25	99.55	78	less
0.2	7:1	25	99.45	66	much
0.5	7:1	25	99.41	57	more

Furthermore, seeding could be used to induce the crystallization of idebenone before oiling-out,[5,12,16,20] as shown in Table 5.

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Table 4. Effect of initial concentration on the oiling-out

initial concentration (mg/mL)	hexane: methylene chloride	cooling rate (°C/min)	purity (%)	yield (%)	oiling-out
40	7:1	0.1	–	–	more
31	7:1	0.1	–	–	much
25	7:1	0.1	99.55	78	less
20	7:1	0.1	99.55	60	less
12	7:1	0.1	99.56	55	nil
12	7:1	0.05	99.56	50	nil

Table 5. Effect of seeding on the oiling-out

amount of seed (% w/w)	size of seed (μm)	hexane: methylene chloride	cooling rate (°C/min)	initial concentration (mg/mL)	purity (%)	yield (%)	oiling-out
1	113–150	7:1	0.1	25	99.56	68	nil
3	150–180	7:1	0.1	25	99.56	69	nil

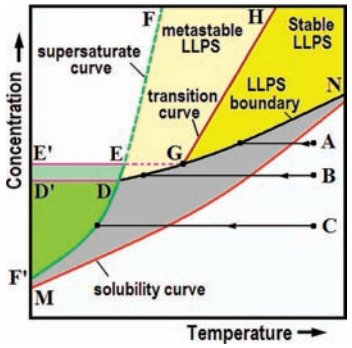

Figure 6. Schematic phase diagram for idebenone.

4. CONCLUSIONS

The experimental results indicate that the oiling-out has a great influence on the cooling crystallization of idebenone. In order to purify raw idebenone by the use of crystallization, the oiling-out should be avoided through adjusting the polarity of the solvent, initial concentration, cooling rate, and seeding strategy. Further investigation will include constructing accurate phase diagrams, looking into the effectiveness of seeding on preventing oiling-out, employing drowning-out in the purification of drugs that oil out, etc.

AUTHOR INFORMATION

Corresponding Author

*Telephone: +86-510-85917090. Fax: +86-510-85917763. E-mail: lujie6742@sina.com.

Notes

The authors declare no competing financial interest.

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