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Note

Characterization of thermal behavior of deep eutectic solvents and their potential as drug solubilization vehicles

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ABSTRACT

Deep eutectic solvent (DES) is a new class of solvents typically formed by mixing choline chloride with hydrogen bond donors such as amines, acids, and alcohols. Most DES's are non-reactive with water, biodegradable, and have acceptable toxicity profiles. Urea–choline chloride and malonic acid–choline chloride eutectic systems were characterized using differential scanning calorimetry (DSC) and thermal microscopy. A potential new 2:1 urea–choline chloride cocrystal with a melting point of 25 °C was characterized at the eutectic composition. The formation of this cocrystal suggests that DES should not be universally explained by simple eutectic melting, and may be useful in guiding the search for new DES systems. The lack of nucleation of the malonic acid–choline chloride system prohibited the construction of a phase diagram for this system using DSC. We also investigated possible uses of DES in solubilizing poorly soluble compounds for enhanced bioavailability in early drug development such as toxicology studies. For five poorly soluble model compounds, solubility in DES is 5 to 22,000 folds more than that in water. Thus, DES can be a promising vehicle for increasing exposure of poorly soluble compounds in preclinical studies.

1. Introduction

Mixtures of some high melting compounds can exhibit sub-ambient eutectic melting temperatures and can be used as solvents at ambient temperature. Eutectic mixtures have been used to generate liquid molten salts at ambient temperatures through interactions between quaternary ammonium salts and metal salts. (Welton, 1999; Wasserscheid and Keim, 2000; Abbott et al., 2001, 2003, 2004a,b; Wasserscheid and Welton, 2007) Ionic liquids containing aluminum chloride are highly reactive with water, while other metal containing salts such as $ZnCl_2$, $SnCl_2$ and $FeCl_3$ form ionic solutions that do not react with water. (Abbott et al., 2001; Wasserscheid and Welton, 2007) While useful in the fine chemical industry, applications of ionic liquids in the pharmaceutical industry have been very limited due to issues with toxicity, purity, and high costs.

Recently, it was shown that substituted quaternary ammonium salts with urea or carboxylic acids can also form eutectics that are liquid at ambient temperature and exhibit interesting solvent properties. (Abbott et al., 2003, 2004a,b) This class of solvents, termed deep eutectic solvent (DES), have increased solubilities for inorganic salts, aromatic acids and amino acids. DES's are advantageous because they can be easily prepared in high purity at low cost, and their components are biodegradable with low toxicity.

In this investigation, we characterized the thermal behavior of two known DES systems and then explored whether DES can be used to enhance solubility of poorly water soluble compounds.

2. Materials and methods

Choline chloride (Alfa Aesar, Ward Hill, MA) was dried and stored in a sealed chamber containing P_2O_5 (0–3% RH). Urea (J.T. Baker, Phillipsburg, NJ), malonic acid (Alfa Aesar, Ward Hill, MA), benzoic acid (J.T. Baker, Phillipsburg, NJ), danazol (MP Biomedicals Inc., Solon, OH), itraconazole (Sigma, St Louis, MO), and AMG517 free base (Amgen, Thousand Oaks, CA) were stored in sealed vials at room temperature, while griseofulvin (MP Biomedicals Inc., Solon, OH) was stored in a sealed vial in the freezer.

The eutectic mixtures were formed by heating and stirring two components between 100 and 150 °C until a colorless liquid was formed. A portion of each warm eutectic mixture liquid was loaded into a hermetically sealed aluminum pan. The sample was then cooled to −60 °C and then heated to 125 °C at 1 °C/min on a differential scanning calorimeter (DSC, Q1000, TA Instruments, New Castle,

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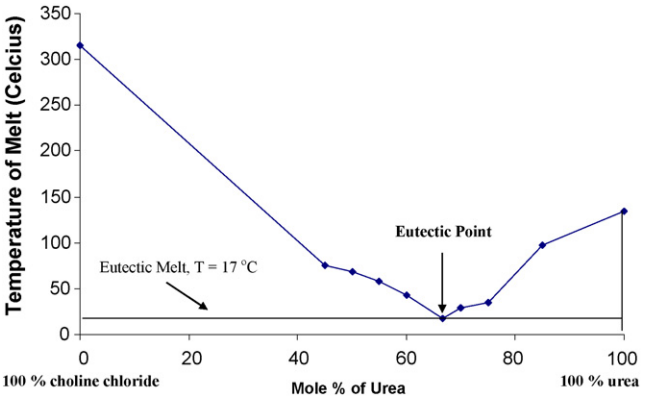

Fig. 1. Phase diagram urea–choline chloride mixtures.

DE). X-ray powder diffraction (XRPD) patterns were collected using a Phillips X-ray automated powder diffractometer (X'Pert, Phillips Analytical, Netherlands) equipped with a sub-ambient chamber accessory. Diffraction patterns were collected between 3–40° 2θ at a scan rate of 0.5° 2θ per minute with 45 kV voltage and 40 mA current. Microphotographs were taken using a microscope (Nikon Eclipse E600 POL, Melville, NY) with a hot-stage accessory (Linkam LTS 350, United Kingdom). For hot-stage microscopy, the urea–choline chloride eutectic was heated from −100 °C to 30 °C at a rate of 5 °C/min, while the malonic acid–choline chloride eutectic was heated from −150 °C to −50 °C at a rate of 5 °C/min.

Solubility was determined by saturating a given solvent with a test compound at room temperature for 24 h while being agitated. The suspension was then filtered using a syringe membrane filter (0.25 μm). Concentration in each saturated solution was determined using a HPLC (HP1100, Agilent, Santa Clara, CA) equipped with a Luna 3u C18(2) 100A column (30 × 4.60 mm, 3 μm). Analyses were run with a gradient method using mixtures of 98% (wt%) water + 2% acetonitrile and 98% acetonitrile (wt%) + 2% water as the mobile phases. Phase identity of equilibrium solid was analyzed by XRPD.

3. Results and discussion

Fig. 1 shows the phase diagram for urea and choline chloride. Similar to a previous report, the eutectic composition is 67.7% (mol%) urea and 32.3% choline chloride. (Abbott et al., 2003) Upon heating the eutectic mixture from −60 °C, an exotherm with an onset temperature of −27 °C was observed (Fig. 2). This was followed by an endotherm with an onset temperature of 17 °C. This type of thermal behavior is consistent with crystallization of a glass that is followed by melting. On sub-ambient hot-stage, the liquid solidified into a glass, which did not exhibit birefringence, upon cooling to −90 °C (Fig. 3). Upon heating, crystallization of the amorphous glass commenced at −40 °C and completed at −24 °C. Melting of the crystalline phase began at approximately 17 °C and completed at 26 °C. The unique XRPD pattern of the eutectic mixture from −40 °C to −20 °C confirmed the presence of a new crystalline phase with a composition of two urea for each choline chloride, hereafter called 2:1 cocrystal (Fig. 4). Cocrystals are crystals that contain two different molecules that do not rely on ionization of the API and the counter-ion to make a solid. They have recently been recognized as a way to enhance solubility/dissolution,

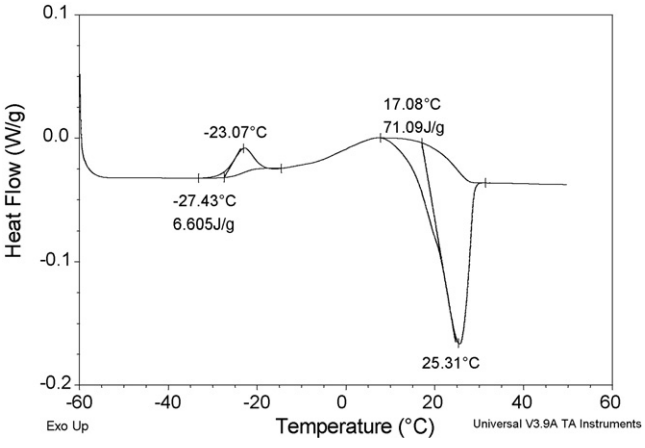

Fig. 2. DSC curve for the urea–choline chloride eutectic.

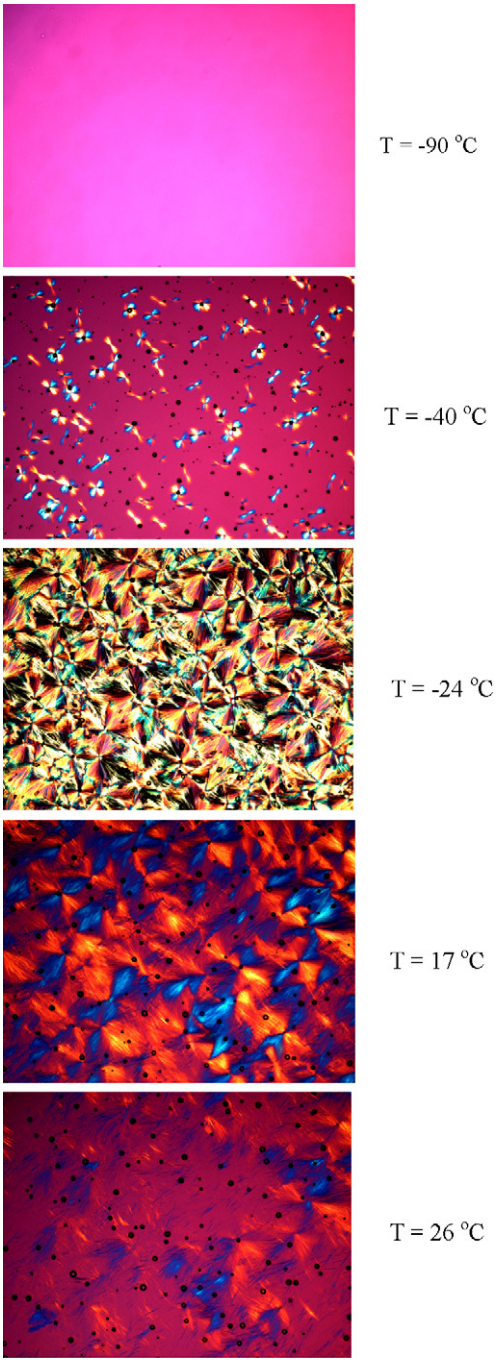

Fig. 3. Hot-stage microscope images for the urea–choline chloride eutectic.

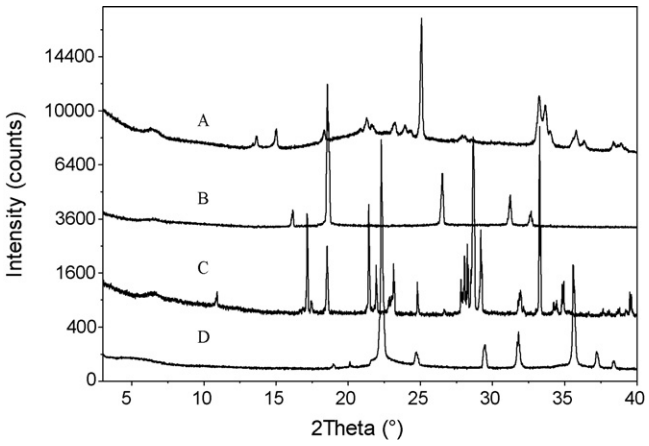

Fig. 4. XRPD patterns for the (A) urea–choline chloride eutectic point mixture (B) choline chloride form B (C) choline chloride form A and (D) urea.

hygroscopicity, stability and the IP position with respect to the development of active pharmaceutical ingredients. This 2:1 cocrystal suggests that the formation of a new compound with a very low melting point is responsible for the observed deep eutectic behavior of the urea–choline chloride system. The formation mechanism of the DES therefore should not be restricted to simple eutectic melting. We expect the 2:1 cocrystal to form eutectic systems with urea and choline choride. However, we did not detect distinctive eutectic melting temperatures in mixtures containing either 60 mol% or 70 mol% urea (Fig. 1). This can happen if the eutectic composition between the cocrystal and either urea or choline chloride is very close to that of the cocrystal. Heating mixtures of compositions different from the 2:1 cocrystal showed XRPD patterns of excess component, urea or choline chloride, but not that of the 2:1 cocrystal. This suggests that a small excess of urea or choline chloride can substantially slow down crystallization of the 2:1 cocrystal.

Similar work was performed to construct the phase diagram for the malonic acid–choline chloride system. The eutectic composition was suggested to be 50 mol % malonic acid. (Abbott et al., 2003) When the mixture was heated on the DSC from −80 °C to 100 °C, no thermal events were observed except for the shift in baseline at above −50 °C (Fig. 5). This behavior is consistent with a glassy material that undergoes glass transition but does not crystallize upon heating. This interpretation is supported by sub-ambient microscopy. Upon cooling the liquid to −125 °C, the sample solidifies as a glass (Fig. 6). Upon heating, the glassy material liquefied between −84 and −60 °C. The liquid is very viscous when handled at room temperature and may be a reason why we do not observe

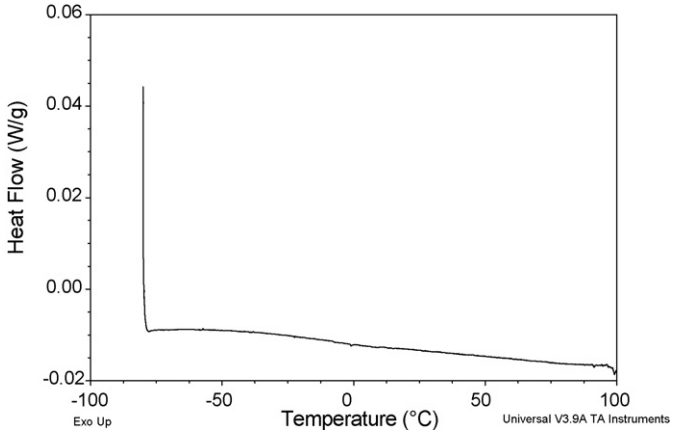

Fig. 5. DSC curve for the malonic acid–choline chloride eutectic.

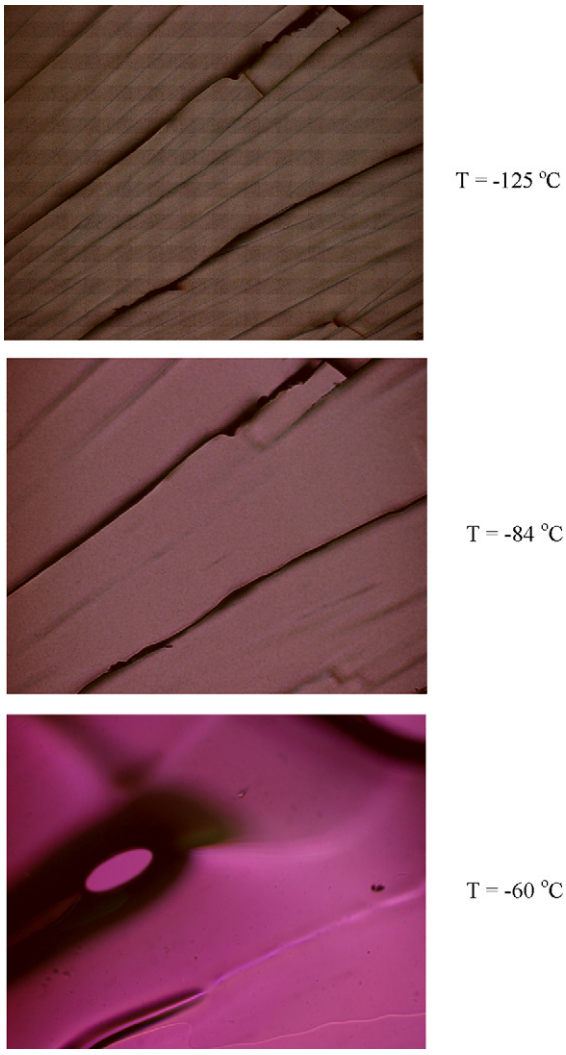

Fig. 6. Hot-stage microscope images for the malonic acid–choline chloride eutectic.

readily nucleation and crystal growth during heating. The lack of nucleation prohibited the derivation of a phase diagram for this system using DSC. The previous work on this system was based on freezing point measurement of the liquid upon cooling. (Abbott et al., 2004a,b) Our attempts to determine the phase diagram for the malonic acid–choline chloride system by preparing powder mixtures in a liquid nitrogen bath (instead of starting with the liquid eutectic) were unsuccessful because of the rapid eutectic melting upon mixing.

The solubilization potential of the urea–choline chloride and malonic acid–choline chloride eutectic systems were studied using several poorly soluble compounds including benzoic acid, griseofulvin, danazol, itraconazole and a recently reported experimental drug AMG517 (Stanton and Bak, 2008). Solubility was measured in the pure DES, mixtures of the DES and water (75:25 and 50:50 by weight), and pure water. The data is summarized in Tables 1 and 2. The results indicate that the solubility of the test compounds increased by 5 to 22,000 folds when compared with their solubility in water.

We also determined the solubility of the various compounds in aqueous solutions of the individual components of the eutectic mixtures (Table 3). Compositions for solvents were based on the molar ratios required to mimic the 75:25 DES–water mixtures with the absence of one component from the eutectic. Solubilization power of these solvents is significantly lower than that of DES

Table 1
Summary of solubility data in urea–choline chloride eutectic/water.

Solvent	Drug solubility (mg/mL)[a]				
	Benzoic acid	Danazol	Griseofulvin	AMG517	Itraconazole
Pure eutectic	229	0.048	0.034	0.010	<0.001
75:25 Eutectic:water	23 (4.4)	0.0061 (9.4)	0.016 (9.2)	0.00022 (9.4)	<0.001 (8.9)
50:50 Eutectic:water	14 (4.1)	0.002 (9.4)	0.015 (8.7)	<0.0001 (9.2)	<0.001 (9.0)
Pure water	3 (3.8)	<0.0005 (8.9)	0.007 (8.9)	<0.0001 (9.5)	<0.001 (8.5)

[a] pH in brackets

Table 2
Summary of solubility data in malonic acid–choline chloride eutectic/water.

Solvent	Drug solubility (mg/mL)[a]				
	Benzoic acid	Danazol	Griseofulvin	AMG517	Itraconazole
Pure eutectic	35	0.160	1.0	0.4727	22
75:25 Eutectic:water	18 (0.2)	0.0044 (0.3)	0.1007 (0.3)	0.014 (0.4)	6.6 (0.6)
50:50 Eutectic:water	11 (0.6)	0.002 (0.6)	0.043 (0.7)	0.002 (0.7)	1.2 (0.85)
Pure water	3 (3.8)	<0.0005 (8.9)	0.007 (8.9)	<0.0001 (9.5)	<0.001 (8.5)

[a] pH in brackets

Table 3
Summary of solubility data in the individual aqueous components.

Solvent[b]	Drug solubility (mg/mL)[a]				
	Benzoic acid	Danazol	Griseofulvin	AMG517	Itraconazole
Aqueous urea	11 (3.9)	0.01 (8.2)	0.053 (8.0)	<0.0001 (8.7)	<0.001 (5.2)
Aqueous choline chloride	7 (3.1)	<0.0005 (6.2)	0.005 (7.2)	<0.0001 (8.0)	<0.001 (5.8)
Aqueous malonic acid	9 (0.9)	<0.0005 (0.9)	0.084 (0.9)	0.005 (0.9)	12 (0.9)
Pure water	3 (3.8)	<0.0005 (8.9)	0.007 (8.9)	<0.0001 (9.5)	<0.001 (8.5)

[a] pH in brackets
[b] Compositions for solvents were based on the molar ratios required to mimic the 75:25 eutectic:water mixtures sans one component from the eutectic

for all drugs tested. Thus, the optimum solubilization requires the presence of both components in the DES and is not simply due to the complexation of drugs with either component in the DES.

4. Conclusions

The previously reported DES of urea and choline chloride system resulted from the formation of a urea–choline chloride (2:1) cocrystal with a low melting point, not from a simple eutectic system.

DES could improve solubility of poorly soluble compounds by 5 to 22,000 folds when compared with the solubility in water. The material safety data sheets indicate that the toxicity profile for rats (oral LD 50) for urea, choline chloride and malonic acid are 8471 mg/kg, 3400 mg/kg and 1310 mg/kg, respectively, while the toxicity profile for mice (oral LD 50) for urea, choline chloride and malonic acid are 11 g/kg, 3900 mg/kg and 4000 mg/kg, respectively. Because the components in DES are pharmaceutically acceptable, they could potentially be used as vehicles for oral dosing of rats during early development pharmacokinetic investigations where exposure is limited by solubility/dissolution and the typical ideal dosing volume is typically 2.5 mL for rats and 0.25 mL for mice.

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