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Solubilization of Oleanolic Acid and Ursolic Acid by Cosolvency

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Solubilities of oleanolic acid and ursolic acid in aqueous surfactant solutions, liquid polyethylene glycols (PEG), and solvents of various polarity were measured. The results showed that the solutes were slightly or moderately solubilized in the surfactant solutions and the liquid PEGs. It was also revealed that the solutes were slightly soluble in the solvent of either extreme polarity or nonpolarity, but moderately soluble in solvents of intermediate polarity of which solubility parameters are around 10. The solubility parameters of these solutes were calculated from the group contribution to be 10.2 for both of them. Of the solvents tested, tetramethylurea was exceptionally effective in solubilizing the solutes. The solutes were also moderately soluble in the aqueous cosolvents containing tetramethylurea. This suggests that the mixed systems of tetramethylurea could be employed for the solubilization in the formulation of these compounds as an aqueous system.

Key words : Solubility, Oleanolic acid, Ursolic acid, Solubility parameter, Tetramethylurea

INTRODUCTION

Oleanolic acid (OA) and ursolic acid (UA) are triterpene acids widely distributed in plant kingdom, and have been recently attracting much concerns because of their various interesting biological activities (Lee and Kim, 1996, Sohn *et al.*, 1993, Lee *et al.*, 1994). OA and UA are very hydrophobic in structure due to their pentacyclic hydrocarbon skeleton as shown in Fig. 1 and there have been difficulties in handling these compounds in biological experiments because of their extremely low solubilities especially in aqueous systems.

It is well known that drug efficacy can be severely limited by poor aqueous solubility. It is also known that side effects of some drugs are the result of their poor solubility. Moreover, solubility problems are frequently encountered in the preparations of parmaceutical dosage forms (Yalkowsky, 1981, Banker and Rhodes, 1979). From the pharmaceutical stand point, the solubility of nonpolar drugs in aqueous system is the most important, and a variety of techniques have been developed to increase the aqueous solubility of a nonelectrolyte. A lot of attempts have been made to derive quantitative relations to predict the solubility of solids in liquids. However, the solubilization of solids in liquids can not be predicted in a wholly satisfactory manner as yet, because of the complicating factors involved. Superficially, solubilization is a very simple phenomenon. In reality, however, it is thermodynamically an extremely complicated one.

There are several techniques to promote the solu-

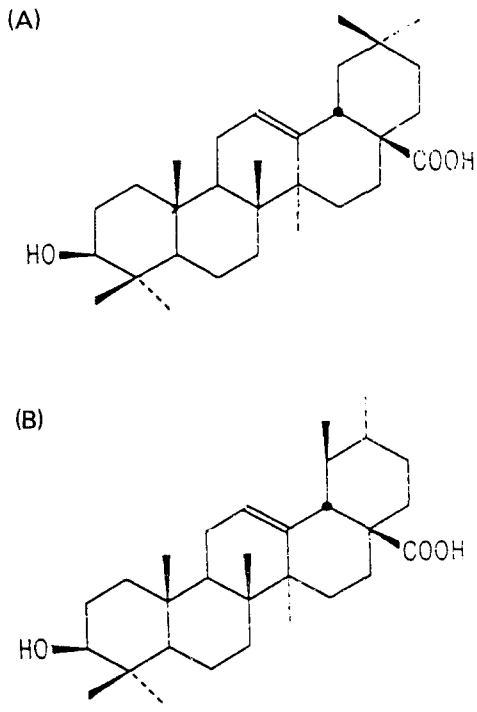

Fig. 1. Structures of oleanolic acid [A] and ursolic acid [B].

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bility of water-insoluble drugs in aqueous system. Among them, the most common method is to employ micellar systems of surfactants (Altwood and Florence, 1983). Surfactant molecules form aggregates at the concentration above the critical micelle conecntration, and the hydrophobic core of the aggregates uptake water-insoluble hydrophobic solute molecules, and solubilize them. The nature of the aggregates and the consequent properties of the solution are dependent on the kind and the concentration of the surfactant in the system and exert great effects on their solubilization of hydrophobic compounds. Sometimes, macromolecular systems are employed to solubilize nonpolar solute in aqueous systems. Macromolecules such as polyethylene glycols form a hydrophilic coil in an aqueous system, which can accomodate nonpolar compounds, and eventually the aqueous solutions of the macromolecules have the capability to solubilize them (Martin *et al.*, 1993, Bailey and Koleske, 1967). Mixed solvents have a high degree of utility in the design of many types of liquid formulations, especially parenteral dosage forms. Because of the irritating effects of most surfactants, the low toxicity of many cosolvents, and the relatively greater ability of mixed solvents to solubilize nonpolar drugs, cosolvent systems have become the most useful means of solublizing drugs for both intravenous and intramuscular administration (Yalkowsky, 1981).

In this research, the solubilities of OA and UA in the mixed cosolvent systems were investigated in search of a better solubilizing system for these compounds.

MATERIALS AND METHODS

Oleanolic acid, ursolic acid, polyoxyethylene (n=10) oleyl ether (Brij 96), and tetramethylurea (TMU) were purchased from Sigma Chemical Co. (U.S.A.). Cetyltrimethylammonium bromide (CETAB) was purchased from Aldrich Chemical Company, Inc. (U.S.A.). Sodium lauryl sulfate (SLS), 1,4-dioxane, dimethyl formamide, butyl acetate, benzene, chloroform, and 1-octanol were purchased from Junsei Chemical Co. (Japan). Solvents for HPLC were purchased from J.T. Baker (U. S.A.). All other solvents were of reagent grades.

Shaker (Eyela. Unithermo Shaker NTS-1200 and Cooling Thermo Pump CTP-100) was employed for maintaining temperature and shaking till the equilibrium solubilization was reached. Centrifuge (Hansin Medical Co., LTD. HC-16) was used for taking the supernatant of the equilibrated solutions. High performance liquid chromatography (HPLC, Gilson, U.S.A. metering pump 306, pump heads and kits, manometric module 805, analytica mixer 118C, injection valve 7125, gradient assembly package, system interface module 506C and programable, variable wavelength UV/Visible 118 detector) was used for analysis of the concentration of the solute in the solution.

Solubility determination

The solubilities of OA and UA were determined by placing an excess amount of the solute in 5 ml screw-capped vial with 3 ml solvent and agitating for 48 hr in a shaker bath maintained at 25°C. Preliminary studies showed that this period of time was sufficient to ensure saturation at 25°C. After equilibrium was attained, the solutions were centrifuged at 553 g for 20 mim and the supernatant was filtered with 0.22 μm Millipore filter (Millex-GV_{13}). Then the filtrates were diluted with appropriate solvents and the diluted sample solutions were chromatographed on a C_{18} reversed-column (μ-Bondapak, 3.9× 300 mm, Waters) with eluting mobile phase of acetonitrile : water (90 : 10 volumn ratio). The flow rate of the mobile phase was 1 ml/min and the injection volume was 15 μl. The detector was set at 220 nm, and the retention times of OA and UA were 7.8 min, respectively. The calibration was made with cholesterol employed as an internal standard. Its rentention time was 2.7 min and it was well seperated from the peak of OA or UA.

RESULTS AND DISCUSSION

Solubilization of OA and UA in aqueous surfactant solutions

The results of the measurements of solubilization of OA and UA in aqueous solutions of various concentrations of SLS, CETAB or Brij 96 were shown in Fig. 2. They show that the surfactant systems have moderate solubilizing capacities for OA and UA in proportion with the concentration of the surfactant up to 10%, and OA was more solubilized than UA. The results also show that ionic surfactants had slightly greater capacity for solubilization of OA and UA than the nonionic surfactant. This difference is ascribed to the inherent difference of the micellar sizes and shapes of the ionic and nonionic surfactants. However, there was no significant difference in the solubilizing capacities between the anionic and cationic surfactants. This means that the interaction between the positive charge of the micellar surface of the cationic micelle and the carboxylic group of the solutes is insignificant. In this experiment, it was extremely difficult to get clear filtrate for analysis of the solubility, and the possibility of including dispersed particles in the solution can not be excluded. These results suggest that the surfactant system are not adequate for solubilizing OA or UA.

Solubilities of OA and UA in PEGs

The liquid PEG 200, 400, and 600 exibited mode-

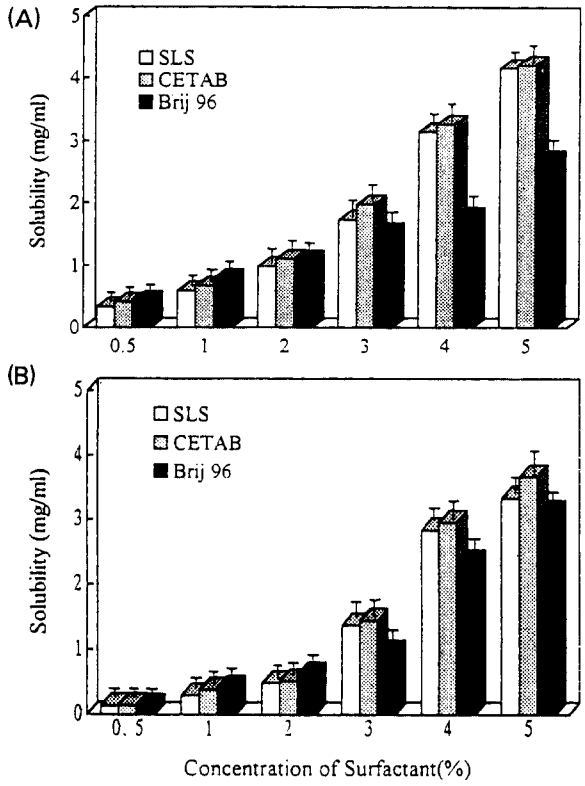

Fig. 2. Solubilization of OA(A) and UA(B) in various aqueous surfactant solutions (n=3).

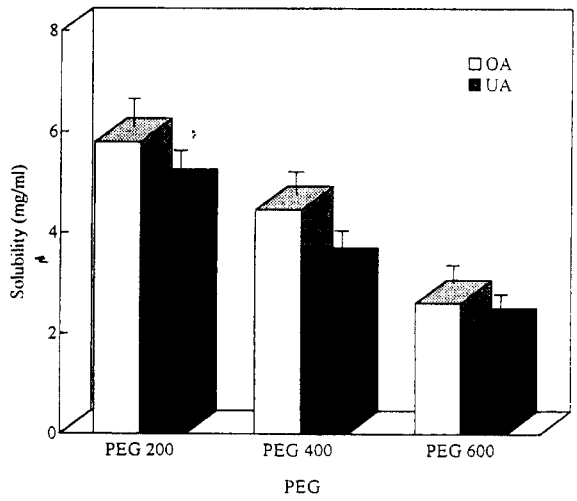

Fig. 3. Solubilities of OA and UA in various PEGs (n=3).

rate solubilizing capacities for OA and UA, respectively as shown in Fig. 3. It shows that PEG with lower degree of polymerization had greater solubilizing capacities. Addition of water to these PEGs exhibited abrupt decrease in the solubilizing powers. PEG 1000, 2000, 6000 and 10,000 are semisolids or solids at room temperature, and their aqueous solution didn't show significant solubilization for OA and UA. This suggests that PEGs have only limited solubilizing capacity for OA and UA. However, liquid PEG 200 might be employed for solubilization of OA or UA for addition of these compounds to samples in in-vitro experments.

Solubilities of OA and UA in solvents

Although the solubility of a solid in a liquid cannot be predicted perfectly, several theories have been proposed to predict and analyze the solubility of a solid in liquid. The equation derived from the fundamental thermodynamic considerations for an ideal solution for a solid in liquid is

$$-\log X_2^i = \frac{\Delta H_f}{2.303R}\left(\frac{T_m - T}{T_m T}\right) \quad (1)$$

in which X_2^i is the ideal solubility of the solute expressed in mole fraction, ΔH_f is the heat of fusion of the solute, R is the gas constant, T_m is the melting point of the solid solute in absolute degrees, and T is the absolute temperature of the solution. However, the solubility in real solution deviates far from the ideality, and the following Scatchard-Hildebrand equation has been proposed for the prediction of the solubility.

$$-\log X_2 = \frac{\Delta H_f}{2.303R}\left(\frac{T_m - T}{T_m T}\right) + \frac{V_2 \phi_1^{\,2}}{2.303RT}(\delta_1 - \delta_2)^{2)} \quad (2)$$

Here, δ is the solubility parameter and it expresses the cohesion between like molecules, and may be calculated from heats of vaporization, internal pressures, surface tensions, and other properties. Most commonly,

$$\delta = \left(\frac{\Delta H_v - RT}{V_1}\right)^{1/2} \quad (3)$$

Here, ΔHv is the heat of vaporization and V_1 is the molar volume of the compound at its liquid state. This equation suggests that the more alike are the δ values of the two components, the greater is the mutual solubility of the pair (Hildebrand *et al*, 1970).

In order to predict the solubility of a solute in solvent, it is necessary to know the solubility parameter of the solute. However, the heats of vaporization and the molar volumes of OA and UA are not reported yet. Alternatively, the solubility parameters of OA and UA were calculated from the group contributions of their chemical structures according to the Fedors method (Fedors, 1974). The results were included in Table I. It reveals that the solubility parameter values of OA and UA are 10.2, respectively. This suggests that solvents with solubility parameter around 10.2 might be best solvents for OA and UA.

The results of the experiments on the solubilities of OA and UA in solvents of various solubility parameters were listed in Table II, and illustrated in Fig. 4. They show that there are no significant differences or

Table I. Calculation of the heats of vaporization and molar volumes of OA and UA from the group contribution

Oleanolic acid				Ursolic acid			
group	No.	Δe_i (cal/ mole)	ΔV_i (cm^3/mole)	group	No.	Δei (cal/mole)	ΔV_i (cm^3/ole)
CH_3	7	1125	33.5	CH_3	7	1125	33.5
CH_2	10	1180	16.1	CH_2	9	1180	16.1
CH	4	820	-1.0	CH	6	820	-1.0
C	6	350	-19.2	C	5	350	-19.2
=CH	1	1030	13.5	=CH	1	1030	13.5
=C	1	1030	-5.5	=C	1	1030	-5.5
COOH	1	6600	28.5	COOH	1	6600	28.5
OH	1	7120	10	OH	1	7120	10
6-membered ring	5	250	16	6-membered ring	5	250	16
Σ		42085	402.8	Σ		42195	403.9
$\delta_{O.A.}$=10.2				$\delta_{U.A.}$=10.2			

$$\delta = \left(\frac{\Sigma \Delta ei}{\Sigma \Delta V_i}\right)^{1/2}$$

Table II. Solubilities of OA and UA in several solvents (n=3)

Solvent	Solubility parameter, δ_1	Solubility (mg/ml) Oleanolic acid	Ursolic acid
Ethyl ether	7.7	6.09±1.6	5.12±0.4
Cyclohexane	8.2	5.8 ±0.4	4.2 ±0.3
n-Butyl acetate	8.5	5.24±0.1	3.8 ±1.5
Carbon tetrachloride	8.7	6.0 ±0.4	4.38±0.4
Ethyl acetate	8.9	5.62±2.3	3.71±1.2
Benzene	9.1	5.3 ±1.3	4.9 ±3.0
Chloroform	9.3	8.04±0.7	7.8 ±6.7
Acetone	9.8	5.1 ±1.2	4.7 ±0.6
Dioxane	10.01	31.2 ±3.4	18.8 ±2.4
1-Butanol	11.3	16.91±1.6	16.33±1.6
1-Propanol	12	15.1 ±3.3	13 ±1.3
Dimethyl formamide	12.1	4.85±0.4	4.83±0.2
Ethanol	13	5.5 ±2.6	5.1 ±2.6
Methanol	14.5	6.2 ±2.1	7.6 ±4.2
Propylene glycol	14.8	2.8 ±0.3	3.14±0.6
1N-NaOH	-	$(4.0\pm3.5)\times10^{-3}$	$(3.1\pm2.9)\times10^{-3}$
Water	23.4	N.D.	N.D.
Tetramethyl urea	-	>100	>100

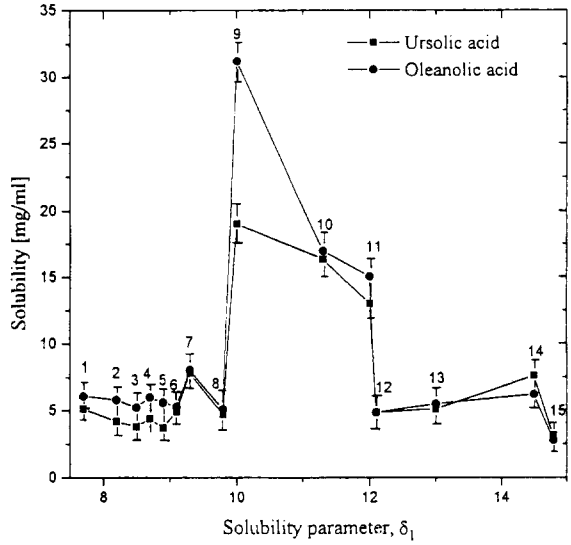

Fig. 4. Solubilities of OA and UA in soluvents* (n=3). [*1. ethylacetate (δ=7.7), 2. cyclohexane (δ=8.2), 3. n-butylacetate (δ=8.5), 4. carbontetrachloride (δ=8.7), 5. ethylacetate (δ=8.9), 6. benzene (δ=9.1), 7. chloroform (δ=9.3), 8. acetone (δ=9.8), 9. dioxane (δ=10.01), 10. 1-butanol (δ=11.3), 11. 1-propanol (δ=12), 12. dimethylformamide (δ=12.1), 13. ethanol (δ=13), 14. methanol (δ=14.5), 15. propylene glycol (δ=14.8)].

limited significance of differences between the solubilities of OA and UA except in dioxane, which dissolves significantly more OA than UA. OA and UA are extremely hydrophobic and didn't dissolve in water to any extent to be detected by the analytical method employed in this experiment. These compounds also showed extremely low solubilities even in 1.0N-NaOH aqueous solution. This means that they are very weak organic acids and do not dissociate significantly even in alkaline solution. The results also showed that OA and UA were moderately soluble in solvents having their solubility parameters around 10. This means that the calculation of the solubility parameter of OA and UA by the Fedors group contribution method is satisfactory. It is worthwhile to note that TMU was exceptionally effective in solubilizing OA and UA, although its solubility parameter is not reported yet. TMU is liquid at room temperature, and mixed well with water and most organic solvents in all proportion (Luthringhans and Dirksen, 1964). Moreover, it is known to be relatively nontoxic (LD_{50} i.v. in rats, 1.1g/kg) (Windholz). This suggests strongly that TMU can be employed itself or in mixed solvent systems to increase the solubility of water-insoluble drugs in aqueous systems.

Solubilities of OA and UA in mixed solvents

The solubilities of OA and UA in EtOH-water mix-

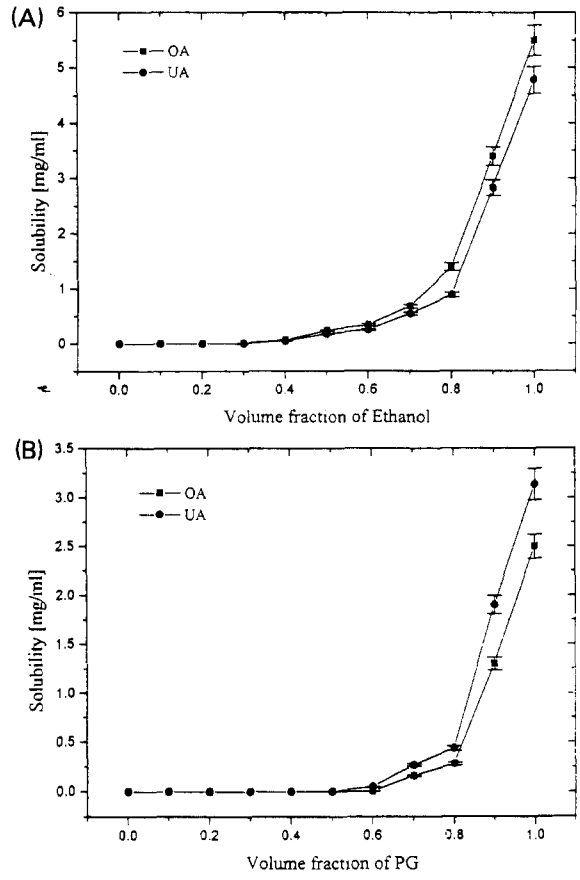

Fig. 5. Solubilities of OA and UA in the mixtures of EtOH-water (A) and PG-water (B).

tures and PG-water mixtures were measured, and the results were shown in Fig. 5. The results show that OA and UA began to dissolve where the volume fraction of the cosolvent is over 0.5~0.6, but the extent of solubilization is not great, especially in case of PG-water mixtures. As the differences between the solubility parameters of EtOH/PG and the solute are quite large and this trend was as expected. This suggests that the mixed solvent systems of water and PG or EtOH are not adequate for solubilizing OA and UA. TMU itself is an excellent solvent for OA and UA. However, when TMU was mixed with small amount of water, the solubilizing capacity of TMU decreased abruptly. This suggests that the binary mixture of water and TMU is not adequate for solubilizing these solutes. Fig. 7 and 8 show the results of the solubilities of OA and UA in ternary mixed aqueous solutions of EtOH-TMU-water and PG-TMU-water, respectively. They show that the ternary solvent systems are effective for solubilizing OA and UA, and the EtOH-TMU-water mixtures were far more effective in solubilizing OA and UA than the PG-TMU-water mixtures. The solubilities of OA and UA in these system increased in proportion to the content of TMU, and

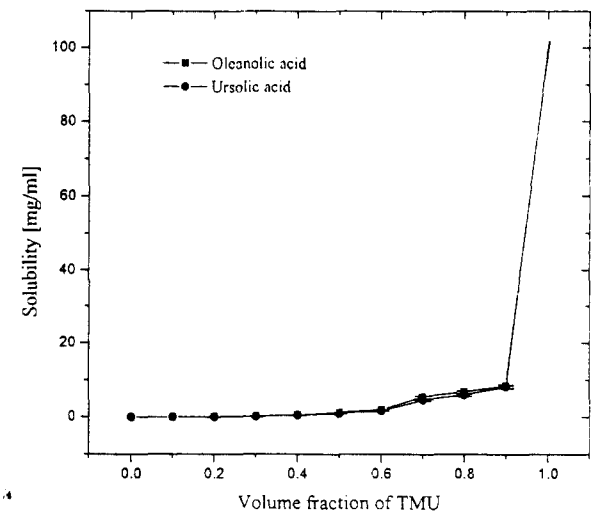

Fig. 6. Solubility of oleanolic acid and ursolic acid in TMU-water mixtures.

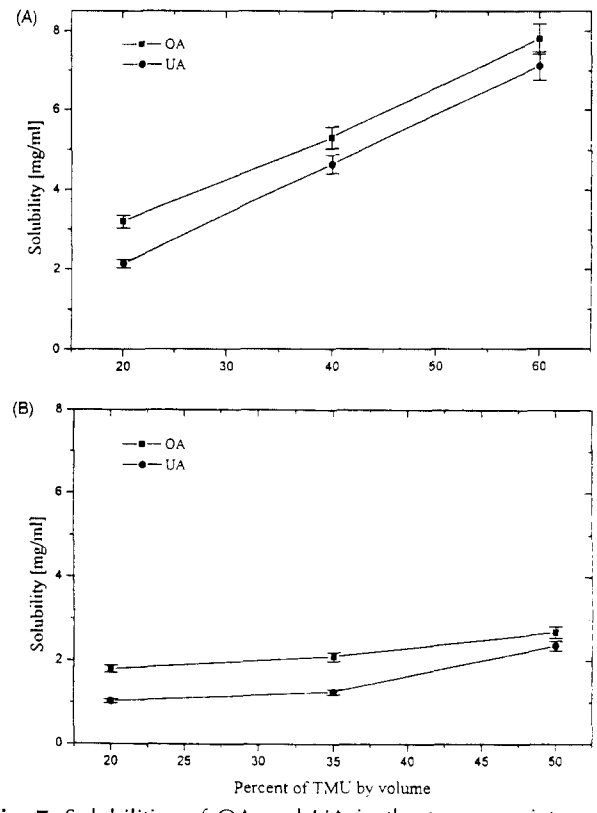

Fig. 7. Solubilities of OA and UA in the ternary mixtures of TMU, EtOH, and water (n=3). (A) water content 20%, (B) water content 30%.

the content of water in these ternary mixtures is critically important in solubilization of the solutes; the mixtures of 20% water is far more effective than the mixtures of 30% water. This suggests that the content of water in the mixed solvent should be as low as possible. OA was slightly more soluble in these systems than UA. These results also suggests that there

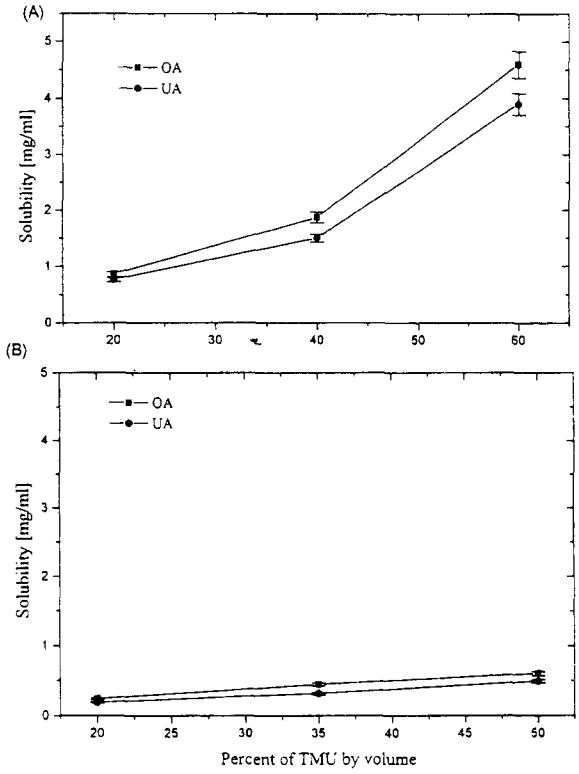

Fig. 8. Solubilities of OA and UA in the ternary mixtures of TMU, PG, and water (n=3). (A) water content 20%, (B) water content 30%.

exists a possibility of application of the mixed solvent system containing TMU in preparing parenteral aqueous solutions or any other solutions of OA, UA and other water-insoluble compounds.

CONCLUSION

From the results of the experimet, it was concluded that surfactant solutions and PEGs had only moderate solubilizing capacity for OA and UA. These solutes were extremely insoluble in water, and slightly or only moderately soluble in most organic solvents. However, they showed relatively high solubilities in the solvents with their solubility parameter around 10. The calculation of the solubility parameters of OA and UA form the group contribution method predicts that both of the values are 10.2, and the calculated vlaues were successful in predicting their solubility trends. Of the solvents tested, TMU was exceptionally effective in solubilizing OA and UA, and it was also effective in solubilizing OA and UA in aqueous mixed solvent systems. The results suggest that TMU has a potential to be employed as a component of aqueous mixed solvent system for enhancing the solubilities of OA, UA and possibly other water-insoluble drugs in liquid dosage formulation.

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