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Liquid−Liquid Phase Transition of Mixtures Comprising Squalene, Olive Oil, and Ethyl Lactate: Application to Recover Squalene from Oil Deodorizer Distillates

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ABSTRACT: Experimental and modeling studies of the liquid−liquid equilibria at ambient pressure of three model systems—squalene + ethyl lactate, olive oil + ethyl lactate, and squalene + olive oil + ethyl lactate—were performed with the view to explore the potential application of an agrochemical solvent, namely, ethyl lactate, to recover squalene from pretreated olive oil deodorizer distillate. The favorable separation factors obtained reveal the strength and potential of ethyl lactate as a green solvent for the recovery of squalene from squalene + triglyceride mixtures.

INTRODUCTION

Squalene (2,6,10,15,19,23-hexamethyl tetracosaheaxaene) is an important component in cosmetic production. Further, it has been recently investigated for several therapeutic uses.[1,2] The main natural source of squalene is shark liver oil, but it can also be found in minor amounts in olive oil. Olive oil deodorizer distillates (OODDs) are mainly comprised of (30 to 40) % in mass fraction of free fatty acids, (20 to 30) % in mass fraction of fatty acid esters and hydrocarbons, mainly, squalene—(10 to 30) %.

Different processes to purify squalene from these sources have been reported in the literature. The most innovative employ countercurrent supercritical CO_2 extraction.[3−8] For example, Bondioli et al.[7] described a process in which first the free fatty acids and fatty acid esters were converted to their corresponding triglycerides, followed by an extraction of squalene from the squalene + triglyceride product using supercritical CO_2.

Ethyl lactate (ethyl 2-hydroxypropanoate) has recently received increased attention due to its many attractive characteristics:[9,10] it is fully biodegradable, noncorrosive, noncarcinogenic, and nonozone depleting and it has low volatility, broad liquid temperature range, and low viscosity. Due to its low toxicity, ethyl lactate is approved by the U.S. Food and Drug Administration (FDA) as a pharmaceutical and food additive. Recent works have reported the potential application of ethyl lactate to extract carotenoids from different sources (tomatoes, carrots, and corn)[11] and sclareol from salvia extracts.[12]

The goal of our research is to study the application of ethyl lactate as an extractive solvent for recovering squalene from OODDs. First, a series of experimental and modeling studies of the liquid−liquid phase transition of three model systems, namely, (squalene + ethyl lactate), (olive oil + ethyl lactate), and (squalene + olive oil + ethyl lactate), were performed. Then, bearing in mind the immiscibility gaps observed, the equilibrium liquid−liquid phase composition of a ternary mixture (squalene + olive oil + ethyl lactate) with a chosen initial composition was investigated. The partition coefficients and the squalene/triglyceride separation factors obtained as a result indicate the potential use of ethyl lactate to selectively recover squalene from triglycerides, which, to our knowledge, has not been studied and reported previously.

Additionally, the UNIQUAC model was applied to correlate the liquid−liquid phase equilibria behavior of the systems studied. The binary interaction parameters between squalene and ethyl lactate and olive oil (chosen here to be represented by triolein) and ethyl lactate were obtained by fitting the corresponding experimental transition temperatures. These parameters were satisfactorily applied to predict the liquid−liquid phase equilibria of the squalene + olive oil + ethyl lactate pseudoternary mixture.

MATERIALS AND METHODS

Chemicals. Squalene (98 % mass purity) and ethyl lactate (98 % mass purity) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Extra virgin olive oil was purchased from a food store. Reagents for chemical analysis, trimethyl-2,2,4 pentane, methyl-*t*-butyl-ether, propan-2-ol, and chloroform, were of HPLC grade (Lab Scan, Dublin).

Experimental Procedure. *Transition Temperature Determination.* Transition temperatures define the liquid−liquid region of a system and are usually determined by the cloud-point method, observing visually the onset of the phase transition.

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Table 1. Transition Temperatures for the Binary Squalene (1) + Ethyl Lactate (3) and Olive Oil (2) + Ethyl Lactate (3) Mixtures at Atmospheric Pressure

squalene (1) + ethyl lactate (3)		olive oil (2) + ethyl lactate (3)	
w_3[a]	temperature/K	w_3[a]	temperature/K
0.92	283.1	0.96	292.0
0.88	294.7	0.95	297.9
0.84	301.4	0.92	305.4
0.80	305.4	0.89	311.6
0.76	308.1	0.86	314.7
0.72	309.3	0.83	315.7
0.68	310.4	0.81	316.7
0.64	310.8	0.79	317.2
0.60	311.0	0.76	317.7
0.56	311.5	0.72	317.7
0.52	311.7	0.70	317.9
0.48	311.9	0.66	317.9
0.44	312.2	0.63	317.6
0.40	311.8	0.60	317.2
0.36	311.2	0.57	316.9
0.32	310.2	0.54	316.1
0.29	307.7	0.49	314.3
0.24	304.3	0.43	310.9
0.20	301.2	0.39	307.3
0.16	297.0	0.37	305.9
0.12	291.7	0.35	303.9
		0.33	301.7
		0.30	299.2
		0.27	294.7
		0.22	284.7

[a] Mass fraction of ethyl lactate.

Furthermore, the influence of impurities of the substances is normally not taken into account.

In our experiments, in the case of the binary systems, a total of 3 g of liquid sample was prepared in a vial by weighting the corresponding amounts of substances (squalene and ethyl lactate or olive oil and ethyl lactate) on a precision analytical balance (Denver Instrument Apx-60) with 0.0001 g accuracy.

In the case of the ternary system, the study was carried out by adding different amounts of ethyl lactate to a squalene + olive oil mixture comprising 30 % mass of squalene. Also in this case a total amount of 3 g of liquid sample was prepared. The 30:70 squalene:oil mixture was selected to represent the squalene + triglyceride mixture obtained from OODDs after converting free fatty acids and alkyl esters to their corresponding triglycerides.

Once the sample was prepared, the vial was sealed. After stirring, it was introduced in a water bath, and heating was turned on (Bunsen, Magnetic Stirrer MC8). The sample was then heated to (323 to 325) K for about 15 min and was continuously homogenized using a magnetic stirrer. Heating was turned off, and the mixture was slowly cooled (typical cooling rate was 0.08 $\mathrm{K \cdot min^{-1}}$). The temperature decrease was continuously registered by coupling to the vial a mercury thermometer with an uncertainty of ± 0.1 K. The temperature at which turbidity started to appear in the mixture was recorded as the transition temperature, i.e., the temperature at which the homogeneous mixture splits into two liquid phases.

The repeatability of cloud-point determination was evaluated by duplicating each temperature measurement, starting from the one-phase region at different initial temperatures and followed by reducing the temperature until the cloud point was visually observed. The absolute deviation between both measurements was lower than ± 0.2 K for all experimental determinations. The temperatures reported in Table 1 correspond to the mean value of repeated experiments.

Liquid–Liquid Equilibria Measurements. The squalene + olive oil mixture (30 % mass squalene) and ethyl lactate were mixed in a decanter funnel and sealed. A total of 3 g of liquid sample was prepared by weighting the corresponding amounts of substances on a precision analytical balance (Denver Instrument Apx-60) with 0.0001 g accuracy. The decanter funnel was introduced in a water bath, and heating was turned on. Temperature was raised to (323 to 333) K until a homogeneous transparent phase was observed. Then, the decanter funnel was placed in a controlled water bath, and temperature was set to the desired value. The mixture was allowed to stand for 24 h. Samples from the top (light liquid phase) and from the bottom (heavy liquid phase) were withdrawn from the decanter funnel using its stopcock. The samples were analyzed by HPLC to determine their composition.

HPLC-ELSD Analysis. The samples collected were dissolved in chloroform prior to analysis. Chemical analysis was carried out on an HPLC system from Varian (Middelburg, The Netherlands) equipped with two pumps (model Pro Star 210), an injector (model 500), a gradient module (Pro Star 500), and a ELSD PL-ELS 2100 Ice (Polymer Laboratories, Varian, Middelburg, The Netherlands). The column, ACE 5 SIL (250 mm × 4.6 mm, 5 μm), was placed into an oven (Thar Instruments, Inc., Pittsburgh, PA) and maintained at 35 °C.

The HPLC-ELSD method applied in our study is based on the one reported by Torres et al.[13] with some modifications. The solvent flow rate was 2 $\mathrm{mL \cdot min^{-1}}$ of (A) isooctane and (B) MTBE:propan-2-ol (80:20). The gradient was as follows: 2 min, 100 % A; 3 min, 95 % A; 8 min, 90 % A; 13 min, 80 % A; 15 min, 80 % A, maintained for 2 min; 18 min, 100 % A, maintained for 2 min. The ELSD conditions were 1.6SLM nitrogen, 35 °C nebulizer, 35 °C vaporizer, and gain 3.

Thermodynamic Modeling Framework. The description of the liquid–liquid equilibria (LLE) of the squalene + ethyl lactate, olive oil + ethyl lactate, and squalene + olive oil + ethyl lactate systems, which are highly nonideal, requires a thermodynamic model and robust and efficient flash and numerical routines. For LLE, the general equilibrium relation is

$$a_i^{\mathrm{I}} = a_i^{\mathrm{II}} \tag{1}$$

where a_i is the activity of component i and the superscripts I and II denote, respectively, each liquid phase.

To calculate activity, the UNIQUAC model of Abrams and Prausnitz[14] was applied. This model is based on a semitheoretical approach to the mixture problem that includes a local composition model and comprises two contributions—a configurational term, due to the differences in size and shape, and a residual term, due to the energetic interactions between the molecules.

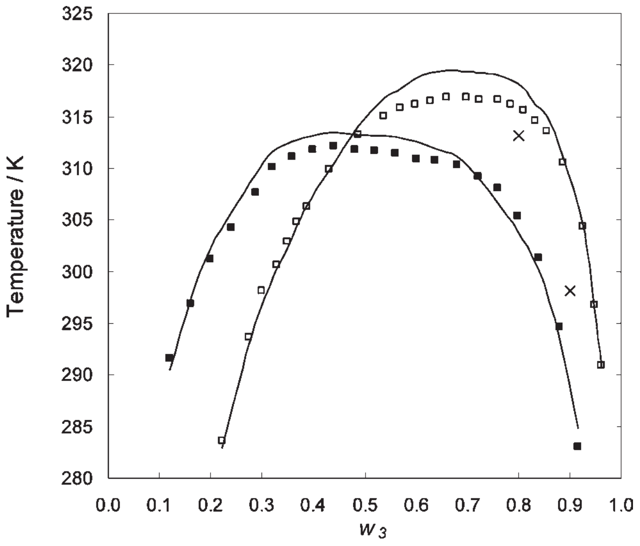

Figure 1. Transition temperature vs ethyl lactate mass fraction (w_3) for the two binary mixtures at atmospheric pressure: ■, squalene (1) + ethyl lactate (3); □, olive oil (2) + ethyl lactate (3) mixtures at atmospheric pressure; —, UNIQUAC model; ×, mixtures considered in the composition analysis of the coexisting liquid equilibrium phases.

Thus, the logarithm of the activity for each component in a mixture of n components is given by

$$\ln a_i = \ln \Phi_i^* + \frac{z}{2} M_i q_i^* \ln\left(\frac{\theta_i^*}{\Phi_i^*}\right) + M_i l_i^* - \frac{M_i \theta_i^*}{w_i} \sum_{j=1}^{n} w_j l_j^* + M_i \Phi_i^* \left[1 - \ln\left(\sum_{k=1}^{n} \Phi_k^* \tau_{ki}\right) - \sum_{j=1}^{n} \frac{\theta_j^* \tau_{ij}}{\sum_{k=1}^{n} \theta_k^* \tau_{kj}}\right] \qquad (2)$$

where

$$\Phi_i^* = \frac{w_i r_i^*}{\sum_{j=1}^{n} w_j r_j^*} \qquad (3)$$

$$\theta_i^* = \frac{w_i q_i^*}{\sum_{j=1}^{n} w_j q_j^*} \qquad (4)$$

$$l_i^* = \frac{z}{2}(r_i^* - q_i^*) - \left(r_i^* - \frac{1}{M_i}\right) \qquad (5)$$

and $z = 10$. Here, r_i^* and q_i^* are the volume and surface area parameters of component i per unit mass, respectively; w_i is the mass fraction; and M_i is the molar mass of component i.

The binary interaction parameters between species i and j are given by

$$\tau_{ij} = \exp\left(-\frac{u_{ij}}{RT}\right) \qquad (6)$$

The application of the UNIQUAC model requires knowledge of r_i^* and q_i^* for each pure component and of the binary interaction parameters τ_{ij}.

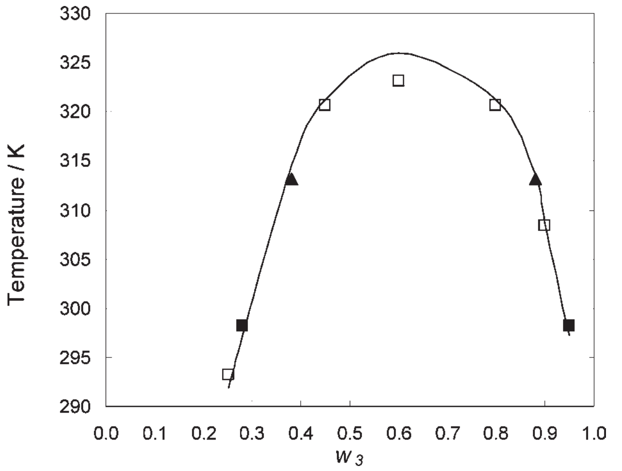

Figure 2. Transition temperature vs ethyl lactate content (w_3) of the pseudobinary squalene (1) + olive oil (2) (30 % mass squalene) and ethyl lactate (3) mixture: □, data obtained according to the experimental procedure described; ■, ▲, data obtained by HPLC analysis of L_1 and L_2 liquid samples; —, UNIQUAC model.

RESULTS AND DISCUSSION

Experimental Results. Table 1 reports the transition temperature of (squalene + ethyl lactate) and (olive oil + ethyl lactate) binary systems as a function of ethyl lactate concentration (mass fraction). Both systems exhibit UCST (upper critical solution temperature), with critical points (312.2 and 317.9) K, for the (squalene + ethyl lactate) and (olive oil + ethyl lactate) system, respectively.

Figure 1 compares the temperature−composition liquid−liquid phase diagrams of these two binary mixtures as a function of ethyl lactate concentration (in mass fraction). The two transition point curves intersect at 46 % in mass fraction of ethyl lactate. Since the main constituents of olive oil are triglycerides, (96 to 98) % in mass fraction, mixtures with (0 to 46) % in mass fraction of ethyl lactate will dissolve preferably triglycerides rather than squalene. For example, 30 % in mass fraction of ethyl lactate will completely dissolve triglycerides at 298 K, while higher temperatures are required to completely dissolve squalene. The opposite holds for mixtures with higher than 46 % in mass fraction of ethyl lactate (right side of Figure 1). Thus, it can be presumed that, for example, at 313 K, (50 to 90) % in mass fraction of ethyl lactate added to a triglyceride + squalene mixture will dissolve squalene better than triglycerides. Therefore, a selective liquid−liquid squalene extraction from the squalene + triglyceride mixture can be successfully carried out using ethyl lactate as extractive solvent.

The transition temperatures of a (squalene + triglyceride + ethyl lactate) mixture were also determined. As mentioned before, OODDs can contain up to 30 % in mass fraction of squalene. Thus, first a (squalene + triglyceride) mixture containing 30 % in mass fraction of squalene was prepared by mixing squalene and olive oil in the corresponding amounts; then, a set of samples were prepared by adding to the initial mixture different amounts of ethyl lactate, and the liquid−liquid phase behavior of the resulting pseudoternary systems was determined.

Figure 2 depicts the data obtained for the transition temperature of the squalene:triglyceride (30 % in mass fraction of squalene) + ethyl lactate mixture vs ethyl lactate composition.

Table 2. Compositions (Ethyl Lactate Free Basis) of Coexisting Phases in Equilibrium (L_1 and L_2) of Squalene (1) + Triglyceride (2) Mixtures (30 % Mass Squalene) at Different Temperatures (Ethyl Lactate Free Basis)

		L_1 (mass fraction) (triglyceride rich phase)		L_2 (mass fraction) (ethyl lactate rich phase)	
T/K	ethyl lactate/squalene−triglyceride ratio	w_1	w_2	w_1	w_2
experimental					
298.2	9	0.174	0.826	0.420	0.580
313.2	4	0.206	0.794	0.342	0.658
calculated					
298.2	9	0.130	0.870	0.413	0.587
313.2	4	0.163	0.837	0.370	0.630

Table 3. Liquid−Liquid Equilibria for the Squalene (1) + Triglyceride (2) Mixtures (30 % Mass Squalene) and Ethyl Lactate (3): Partition Coefficients k_1^{eq} and k_2^{eq} (Ethyl Lactate Free Basis) and Separation Factor $\alpha = k_1^{\mathrm{eq}}/k_2^{\mathrm{eq}}$

T/K	k_1^{eq}	k_2^{eq}	$\alpha = k_1^{\mathrm{eq}}/k_2^{\mathrm{eq}}$
298.15	2.419	0.702	3.447
313.15	1.615	0.840	1.922

Table 4. Pure Component Parameters (r_i^* and q_i^*) and Binary Interaction Parameters (τ_{ij} and τ_{ji}) for Systems Comprising Squalene (1), Triolein (2), and Ethyl Lactate (3)

component i	component j	r_i^*	q_i^*	τ_{ij}	τ_{ji}
1		0.04425	0.03588		
2		0.05607	0.04705		
3		0.04695	0.03954		
1	2			416.84	169.98
1	3			−909.29	2115.9
2	3			137.13	1445.17

As can be observed, the temperature of the three-component model system at which it attains complete miscibility, regardless of its composition, is higher (323.15 K) than the corresponding temperatures for squalene + ethyl lactate (312.15 K) and olive oil + ethyl lactate (317.15 K) mixtures, respectively.

To ascertain if a selective recovery of squalene from squalene: triglyceride (30 % in mass fraction of squalene) mixtures using ethyl lactate is possible, the liquid−liquid equilibria of two systems, namely, a system with 90 % in mass fraction of ethyl lactate and a system with 80 % mass ethyl lactate, were examined at two temperatures—298.15 K (ambient temperature) and 313.15 K, respectively. Both temperature−composition conditions are indicated in Figure 1. The conditions were selected in such a way as to favor the dissolution of squalene in the ethyl lactate rich phase (Figure 1). Additionally, it should be noted that both temperatures are sufficiently low, and hence gentle heating only is required. The compositions of the corresponding equilibrium liquid phases (L_1 and L_2) were determined by HPLC-ELSD analysis.

Table 2 shows the results of the chemical analysis. In both cases a selective extraction of squalene was achieved in the ethyl lactate rich phase. At 298.15 K a liquid phase (L_2) containing 42.0 % mass of squalene (solvent free basis) was obtained. This means that a 1.4-fold increase of squalene with respect to the original squalene:triglyceride (30 % in mass fraction of squalene) mixture was achieved. At 313.15 K, the L_2 phase contains 33.3 % in mass fraction of squalene (solvent-free basis), and a 1.2-fold increase was obtained.

Table 3 shows the partition coefficients k_i^{eq} (% mass in L_2/% mass in L_1) for the squalene (SQ) and triglycerides (TG) compounds, respectively, calculated on ethyl lactate free basis, at the two temperatures investigated. Also given in the table are the corresponding separation factors ($\alpha = k_{\mathrm{SQ}}^{\mathrm{eq}}/k_{\mathrm{TG}}^{\mathrm{eq}}$). As can be observed from the values obtained, the selectivity of the process toward the extraction of squalene is higher at 298.15 K. This means that a selective recovery, with sufficiently high separation factor ($\alpha > 3$), of squalene from squalene + triglyceride mixtures using ethyl lactate can be achieved at ambient temperature, thus saving operating costs related with heating requirements.

Thermodynamic Modeling. *Determination of Model Parameters.* The most reliable procedure for the determination of the UNIQUAC model parameters involves a fit to available experimental data over a wide range of liquid compositions. In view of this, for the ethyl lactate + squalene and ethyl lactate + triolein binaries, the corresponding interaction parameters τ_{ij} were determined by fitting the UNIQUAC calculated to the experimentally determined transition temperatures.

Analogously, the regression procedure for the ternary model system was performed, but just the squalene vs triolein τ_{ij} energy interaction parameters were treated as unknowns and determined. For the ethyl lactate vs squalene and the ethyl lactate vs triolein, τ_{ij} parameters were used, the values already estimated by the fitting procedures for the respective binary systems. The binary interaction parameters obtained are shown in Table 4.

It should be noted that it is not uncommon for the objective function in such nonlinear parameter estimation problems to be nonconvex and thus to have several local minima. Because the methods typically used to solve such problems, like the one used in the present study, are local methods that are strongly dependent on the initial estimates applied, they provide no guarantee that the global optimum has been found. Thus, the sets of the binary interaction parameters obtained probably represent just one of the possible sets of parameters.

In the thermodynamic modeling, as mentioned previously, olive oil was considered to be represented by a single component, namely, triolein. This simplification is based on the fact that (96 to 98) % in mass fraction of olive oil are triglycerides. Furthermore, a typical composition of this triglyceride fraction (fatty acid basis) is 75.5 % oleic acid (C18:1), 11.5 % palmitic acid (C16:0), and 7.5 % linolenic acid (C18:2). Thus, triolein is the more abundant triglyceride of the neutral fraction, and that is why it was selected to represent olive oil.

The volume and surface area parameters of each pure component (ethyl lactate, squalene, and triolein) were calculated applying the UNIFAC (Dortmund) parameter matrix.[15] The corresponding parameter values/per unit mass for each of the pure components are shown in Table 4.

Liquid—Liquid Equilibria Calculations. The calculation of either the transition temperatures or the liquid—liquid equilibrium compositions requires reliable algorithms and robust, efficient, and effective numerical routines. Conventional LLE algorithms can be unreliable and difficult to converge with either a strong attraction to the trivial solution or failing to converge to a physically feasible solution at all. In the present study, we apply the algorithms and initialization strategy advocated in ref 16, as these are targeted particularly at the solution of LLE and LLLE problems.

Figure 1 shows a comparison between the experimentally measured transition temperatures and their values calculated using the UNIQUAC thermodynamic model. As can be observed from the figure, good representation of the transition temperatures is achieved in both cases. Nevertheless, deviations are somewhat greater in the case of the olive oil + ethyl lactate system, which can be attributed, to a certain extent, to the fact that olive oil was (simply) represented as triolein.

With respect to the ternary squalene + olive oil + ethyl lactate, again a good representation was obtained applying the UNIQUAC model (Figure 2). Still, for this particular case, it is of greater interest to compare the experimental and calculated liquid phase compositions. Table 2 shows the experimental and calculated L_1 and L_2 compositions (mass fractions) of squalene:triglyceride (30 % mass squalene) mixtures at different temperatures and ethyl lactate content. The mean absolute average deviation (AAD = $(1/N)\Sigma|(z_i^{\text{exp}} - z_i^{\text{cal}})/z_i^{\text{exp}}|$) is 12.7 %, while the largest deviations (i.e., for the smaller mass fractions) are around 25 %. Thus, it can be concluded that satisfactory representation of both L_1 and L_2 compositions was achieved using the UNIQUAC model.

CONCLUSIONS

Transition temperatures for the squalene + ethyl lactate and the squalene + olive oil + ethyl lactate mixtures were measured in this work, delimiting the liquid partial miscibility region in a temperature vs ethyl lactate composition phase diagram. The UNIQUAC model was employed to represent the phase behavior of the systems studied, and its capabilities to satisfactorily reproduce the transition temperatures and the phase equilibrium compositions were demonstrated.

On the basis of this phase equilibria analysis, it was shown that a selective recovery of squalene from squalene + triglyceride mixtures using ethyl lactate is feasible. At 298.15 K (ambient temperature) and providing an ethyl lactate/squalene:triglyceride (30:70) ratio lower than 10, a 1.4-fold increase of squalene in the ethyl lactate rich phase was achieved. Thus, the selectivity of squalene extraction is almost 3.5 higher than that of the triglycerides.

The results obtained in our study demonstrate the viability of a liquid—liquid countercurrent extraction process to purify squalene from pretreated olive oil deodorizer distillates and can serve as a basis for its design.

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