J. Chem. Eng. Data **1999,** *44,* 553−556

Distribution Coefficient of β-Carotene in Systems Composed of Cyclohexane, Water, and 2-Propanone and Methylcyclohexane, Water, and 2-Propanone

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The distribution coefficient of β-carotene between water and a mixed organic solvent was measured at $T = 298.15$ K. Binary solvent systems formed by 2-propanone with either cyclohexane or methylcyclohexane were used as the mixed solvent. The liquid−liquid equilibria, along with the tie lines, were determined for two ternary aqueous systems, viz., cyclohexane + water + 2-propanone and methylcyclohexane + water + 2-propanone. The systems exhibit a parabolic type miscibility gap. The coexistence curves were measured by the titration method, and results were smoothed by means of either three- or four-parameter equations.

1. Introduction

This work is an extension of the previous studies on the distribution coefficient of β-carotene between an organic solvent and water (Treszczanowicz et al., 1998). The need for use of low-boiling extractants resulted in an attempt to use mixed solvents exhibiting positive deviations from Raoult's law, which have a lower boiling point, compared with pure solvents. As a result, the energy consumption can be reduced, and if an inexpensive solvent, such as 2-propanone, is used, the hydrocarbon consumption can be clearly reduced. Data on the distribution coefficient of carotenoids are lacking in the literature despite the fact that β-carotene isolation is the subject of numerous patents and papers (cf. Treszczanowicz et al. (1998)). In this study, the distribution coefficient was investigated in systems formed by mixed solvents, water, and β-carotene. 2-Propanone with cyclohexane and methylcyclohexane were taken as the mixed organic solvent. Here results of investigations at $T = 298.15$ K are reported for two ternary aqueous systems: cyclohexane (1) + water (2) + 2-propanone (3) and methylcyclohexane (1) + water (2) + 2-propanone (3).

2. Experimental Section

Materials. *trans*-β-Carotene, of 95% purity, was supplied by Sigma. Organic solvents, cyclohexane (99%) standard, by Ubichem Ltd., and methylcyclohexane (98%) pure reagent grade, by Loba Chemie Wieden Fischamend, were used without additional purification. The water used in the measurements was twice distilled. Refractive index and specific gravity values of the reagents studied, measured at $T = 298.15$ K, agree well with the literature values (cf. Table 1).

Table 1. Physicochemical Properties of the Solvents Studied: *M*, Molecular Weight,[a] t_b, Boiling Point at Standard Pressure,[a] *d*, Density;[a] and n_D, Refractive Index at 298.15 K

		M_i/	d_i/	n_D		
i	solvent	(g mol^{-1})	(g cm^{-3})	expt	lit.[a]	t_b/°C
1	H_2O	18.015	0.99705	1.3330	1.33287	100.0
2	CH_3CO	58.080	0.78508	1.3561	1.35609	56.0
3	c-C_6H_{12}	84.161	0.77389	1.4236	1.42354	80.7
4	c-$C_6H_{11}CH_3$	98.188	0.76506	1.4206	1.42058	100.9

[a] Riddick et al. (1986).

Measuring Procedure. The liquid−liquid coexistence curve was determined at $T = 298.15$ K in the apparatus described by Treszczanowicz and Cieslak (1993) by means of the titration technique adapted with the Letcher et al. (1986) approach. One of the components was added to a known amount of the other component or mixture so that a two-phase or homogeneous mixture should be formed. From the quantities of components added as the determined mass, the composition of the equilibrium mixture was determined. The temperature was kept within ±0.02 K by pumping thermostated water through the jacket of the measuring cell. During the measurement the mixture was vigorously mixed. This procedure is applied to both β-carotene free subsystems and systems containing β-carotene.

The tie lines were determined for the starting solutions prepared by mass, whose concentrations remained within the middle of the miscibility gap. The mixtures prepared were vigorously mixed for approximately 1 h and subsequently were allowed to separate into two layers, while the temperature of 298.15 K was maintained within ±0.02 K. Subsequently, a sample of each of the layers was carefully withdrawn with a hypodermic injection syringe. The concentration of each of the layers was determined by a Hewlett-Packard model 6890 G gas−liquid chromatograph equipped with an electronic integrator. A good separation of the four components studied was obtained by means of the capillary column J-413 packed with phenylmethylsiloxane using argon as carrier gas. The relative accuracy of the measurement was estimated at ±0.5% for the hydro-

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10.1021/je980175c CCC: $18.00

Table 2. Coexistence Curve for Systems Formed by a Hydrocarbon (1) with Water (2) and 2-Propanone (3) at T = 298.15 K[a]

x_1	x_3	x_1	x_3	x_1	x_3
		Cyclohexane (1)			
0.000012	0.0000[b]	0.0489	0.5043	0.1830	0.6080
0.0015	0.1964	0.0623	0.5231	0.2281	0.6077
0.0049	0.2943	0.0710	0.5375	0.2365	0.6044
0.0109	0.3735	0.0944	0.5638	0.3481	0.5619
0.0168	0.3916	0.0967	0.5656	0.3802	0.5447
0.0244	0.4335	0.1196	0.5982	0.5296	0.4302
0.0323	0.4637	0.1419	0.5989	0.99942	0.0000[b]
		Methylcyclohexane (1)			
0.000003	0.0000[b]	0.0707	0.5781	0.2313	0.6225
0.0052	0.2999	0.0821	0.5896	0.2428	0.6212
0.0099	0.3966	0.1062	0.6052	0.2678	0.6112
0.0160	0.4408	0.1184	0.6116	0.3183	0.5880
0.0236	0.4733	0.1353	0.6212	0.3220	0.5850
0.0260	0.4876	0.1387	0.6200	0.4534	0.4943
0.0311	0.5098	0.1881	0.6291	0.5324	0.4345
0.0477	0.5426	0.2264	0.6226	0.99921	0.0000[b]

[a] Water molar fractions $x_2 = 1 - x_1 - x_3$. [b] Verified data by Sorensen and Arlt (1979).

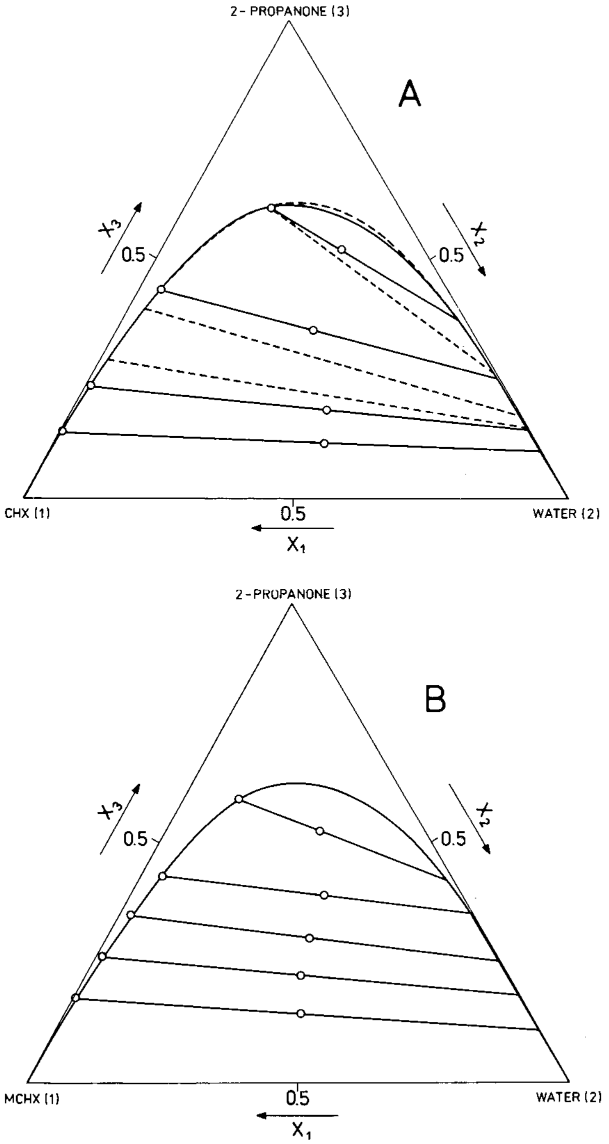

Figure 1. Liquid−liquid equilibrium in ternary systems at T = 298.15 K: (A) cyclohexane (1) + water (2) + 2-propanone (3) and (B) methylcyclohexane (1) + water (2) + 2-propanone (3). The continuous line corresponds to the experimental data smoothed with eq 1 and parameters given in Table 3; the straight continuous lines and points represent tie-line experimental data; the dashed line is the prediction by means of the UNIQUAC model and common parameters (Sorensen and Arlt, 1979).

carbon and ±1% for 2-propanone. For most of the mixtures the concentration measurement was limited to the organic layer as a results of the calculation procedure (see below).

Distribution Coefficient Measurement. For the measurement of β-carotene concentration in the equilibrium mixtures a HPLC chromatograph was employed using previously determined calibration curves. Analysis of the composition was carried out on a Waters model 501 apparatus using the Millenium 2010 controlling program. The mobile phase flow rate was 1 mL/min. The eluent used was a mixture composed of the following solvents: methanol, acetonitrile, and chloroform (50:40:10). The eluent was filtered through a Poly Cap A5 type filter. The measurement was carried out at a wavelength of 454 nm. The β-carotene concentrations, c_β/(g/L), in both layers at equilibrium are compared in Table 4. Definitions of distribution coefficients and their interrelations were defined in our previous paper (Treszczanowicz et al., 1998). The same notation is used here.

3. Results and Discussion

Results of the liquid−liquid equilibrium measurements determined at 298.15 K are reported in Table 2 and shown in Figure 1. The coexistence curve (binodal) coordinates, mole fractions of the 2-propanone x_3 and hydrocarbon x_1, were smoothed by means of a four-parameter equation

$$x_3 = (x_1 - x_1^{(2)})(x_1^{(1)} - x_1)\left(\frac{a_1}{1 + a_2x_1} + \frac{a_3}{1 + a_4x_1}\right) \qquad (1)$$

and by means of equations proposed by Letcher et al. (1992),

$$x_3 = a_1(1 - x_A)^{a_2}{x_A}^{a_3} \qquad (2)$$

$$x_3 = a_1(-\ln x_A)^{a_2}{x_A}^{a_3} \qquad (3)$$

where $x_A = (x_1 + 0.5x_3 - x_1^{(2)})/(x_1^{(1)} - x_1^{(2)})$ and $x_1^{(1)}$, $x_1^{(2)}$ are mole fractions of the hydrocarbon in phase 1 (nonaqueous, left in Figure 1) and phase 2 (aqueous, right in Figure 1) for the binary water + hydrocarbon systems. The parameters in eqs 1−3 were calculated by the Levenberg−Marquardt nonlinear least-squares method (Press et al., 1988) and are reported in Table 3 along with the standard deviation σ and maximum deviation values. The standard deviation is defined as

$$\sigma = [\sum_{i=1}^{n}(x_{3,i}^{(\text{calc})} - x_{3,j}^{(\text{expt})})^2/(n - m)]^{1/2} \qquad (4)$$

where n is the number of data points and m is the number of coefficients.

Studies on the coexistence curve and the tie lines of solutions with β-carotene show that the presence of β-carotene has no effect on the liquid−liquid equilibrium as compared with the experimental error in the composition determination. This allowed us to significantly simplify the method of the measurement of the distribution coefficient and of the method of determination of concentrations in the coexisting mixtures (the tie lines). The mole fraction ratio for the nonaqueous phase can be found from the concentration ratio of

Table 3. Coefficients a_i in Equations 1−3 for Systems Formed by a Hydrocarbon (1) + Water (2) + 2-Propanone (3) at T = 298.15 K, Standard Deviation (σ), and Maximum Deviation (mx) with Respect to Mole Fraction x_1

quantity	eq 1	eq 2	eq 3
	Cyclohexane (1)		
a_1	252.50	2.9279	2.5680
a_2	681.79	1.1462	1.0724
a_3	4.3873	1.1224	1.5161
a_4	6.0642		
σ	0.0056	0.0044	0.0055
mx	0.0092	0.0090	0.0124
x_1	0.0049	0.5296	0.5296
	Methylcyclohexane (1)		
a_1	137.93	3.2716	2.9082
a_2	276.40	1.2130	1.1497
a_3	2.7200	1.1662	1.6030
a_4	4.2701		
σ	0.0040	0.0031	0.0034
mx	0.0096	0.0054	0.0063
x_1	0.0099	0.4534	0.4534

$$x_3^{(1)}/x_1^{(1)} = (c_3^{(1)}M_1/c_1^{(1)}M_3) \qquad (5)$$

where $c_3^{(1)}$ and $c_1^{(1)}$ are concentrations (g/L) of components 3 and 1 in the organic phase (1) determined by the GLC technique and M_1 and M_3 are their mole masses. The x_3 vs x_1 function for the coexistence curve defined by eq 1 can be used to evaluate mole fractions $x_3^{(1)}$ and $x_1^{(1)}$ for various values of their ratio. The tie-line coordinates for the aqueous layer, $x_3^{(2)}$ and $x_1^{(2)}$, were obtained as a result of the intersection of a straight line drawn through point ($x_1^{(1)}$, $x_3^{(1)}$) and point ($x_1^{(0)}$, $x_3^{(0)}$) corresponding to the composition of the starting mixture with this part of the coexistence curve described by eq 1 which relates to the aqueous layer. Results of calculations of composition of the mixture at equilibrium, $x_1^{(1)}$, $x_3^{(1)}$ and $x_1^{(2)}$, $x_3^{(2)}$ together with the composition of the starting mixtures $x_1^{(0)}$, $x_3^{(0)}$ are collected in Table 4. Results of measurements of the β-carotene concentrations in the organic and aqueous layers, $c_\beta^{(1)}$ and $c_\beta^{(2)}$, and calculations of the distribution coefficient values, $k_c = c_\beta^{(1)}/c_\beta^{(2)}$, are reported also in Table 4.

The solubility of β-carotene in 2-propanone is larger than in either of the hydrocarbons studied. Hence, as shown in Figure 2 A,B, the distribution coefficient for the mixed solvent of cyclohexane + 2-propanone as well as methylcyclohexane + 2-propanone initially increases with 2-propanone concentration at a low water content in the nonaqueous phase. However, with increasing water concentration in the nonaqueous layer, the distribution coefficient value k_c decreases due to both the diminishing solubility of β-carotene in the nonaqueous phase and its concomitant higher solubility in the aqueous phase (cf. the

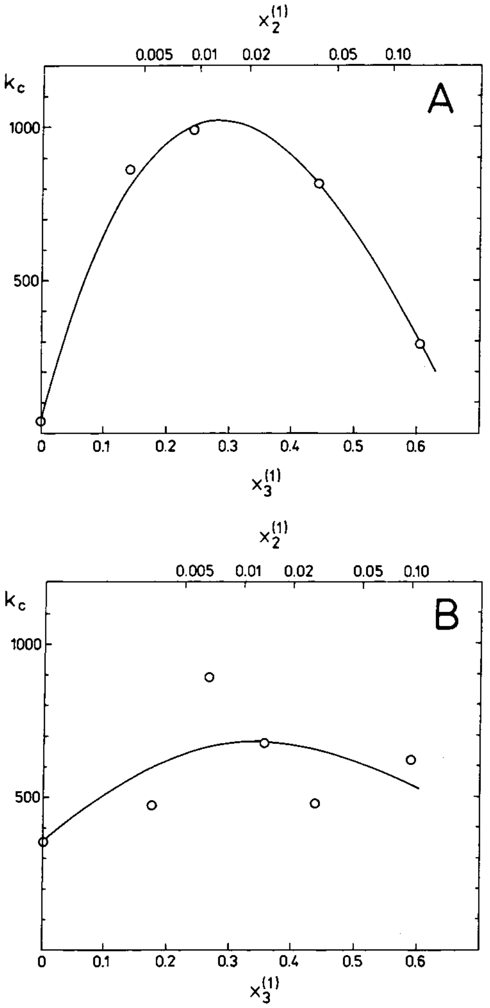

Figure 2. Distribution coefficient k_c of β-carotene at T = 298.15 K as a function of the mole fraction of 2-propanone $x_3^{(1)}$ and of water $x_2^{(1)}$ in the nonaqueous (organic) phase: (A) cyclohexane (1) + water (2) + 2-propanone (3) and (B) methylcyclohexane (1) + water (2) + 2-propanone (3). The distribution coefficient values for $x_3^{(1)}$ = 0 were taken from the work by Treszczanowicz et al. (1998).

Table 4. Compositions of the Conjugate Solutions[a] of the Tie Lines: Experimental ($x_1^{(1)}$, $x_3^{(1)}$), Calculated ($x_1^{(2)}$, $x_3^{(2)}$), and Initial Mixture ($x_1^{(0)}$, $x_3^{(0)}$) and Experimental Concentrations of β-Carotene $c_\beta^{(1)}$, $c_\beta^{(2)}$ in Both Liquid Phases for the Quaternary System of a Hydrocarbon (1) + Water (2) + 2-Propanone (3) + β-Carotene (β) and Distribution Coefficient k_c at T = 298.15 K

organic phase			initial mixture		aqueous phase			
$x_1^{(1)}$	$x_3^{(1)}$	$c_\beta^{(1)}$/(g L^{-1})	$x_1^{(0)}$	$x_3^{(0)}$	$x_1^{(2)}$	$x_3^{(2)}$	$c_\beta^{(2)}$/(g L^{-1})	k_c
			Cyclohexane (1)					
0.234	0.607	4.103	0.151	0.518	0.0111	0.368	0.0141	291
0.527	0.435	6.074	0.286	0.350	0.0027	0.250	0.0075	810
0.753	0.237	6.780	0.347	0.184	0.0009	0.138	0.0069	983
0.855	0.141	8.342	0.386	0.117	0.0005	0.097	0.0097	860
			Methylcyclohexane (1)					
0.305	0.582	5.140	0.195	0.522	0.0136	0.423	0.0083	619
0.535	0.436	6.491	0.252	0.389	0.0072	0.348	0.0136	477
0.634	0.352	5.714	0.324	0.300	0.0033	0.246	0.0085	672
0.729	0.264	8.342	0.378	0.221	0.0019	0.174	0.0094	887
0.824	0.174	4.086	0.420	0.142	0.0010	0.110	0.0087	470

[a] Water mole fractions: $x_2 \approx 1 - x_1 - x_3$.

coexistence curve in Figure 1). A maximum occurs on the curve that depicts the 2-propanone concentration effect on the distribution coefficient k_c for β-carotene in the cyclohexane (1) + water (2) + 2-propanone (3) system. The maximum appears approximately at $x_3^{(1)} = 0.3$, which corresponds to $x_2^{(1)} \cong 0.005$. For the mixed solvent containing cyclohexane the increase in the distribution coefficient k_c with the 2-propanone concentration is greater, as compared with the solvent containing methylcyclohexane. For the cyclohexane system this increase is exceptionally significant (cf. Figure 2) compared with the extraction using the hydrocarbon only. Both the cyclohexane system and the methylcyclohexane system demonstrate significant positive deviations from Raoult's law. The system made up by methylcyclohexane with acetone is a nearly tangent zeotrope (Horsley, 1973 (cf. Swietoslawski (1963)), whereas in the cyclohexane + 2-propanone system there is an azeotrope with a boiling point by nearly 3 K lower than the boiling point of 2-propanone under normal pressure (Horsley, 1973). This could possibly justify the use of this mixed solvent instead of the hydrocarbon only.

The liquid−liquid equilibria for ternary system formed by cyclohexane were compared in Figure 1A with those predicted by the UNIQUAC model using common parameters given by Sorensen and Arlt (1979). The agreement observed between the values measured in this study and their corresponding literature data seems to be good for the coexistence curve and the tie-line slopes. For the ternary system formed by methylcyclohexane no any data are found in the literature.

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Received for review August 3, 1998. Accepted January 25, 1999.

JE980175C