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Distribution Coefficient of β-Carotene between an Organic Solvent and Water

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The distribution coefficient was measured at 25 °C for β-carotene in binary aqueous immiscible systems of organic solvents: toluene, cyclohexane, hexane, methylcyclohexane, and $CHCl_3$. The values of the distribution coefficients of the solvents examined rise in the following order: hexane < cyclohexane < methylcyclohexane < toluene < chloroform. Chloroform was found unsuitable as a β-carotene solvent, as it forms a complex or most likely reacts with the latter.

Introduction

β-Carotene is a major product used as a provitamin A. It belongs to the polyene class of compounds, which accounts for its high reactivity as a free-radical inhibitor and antioxidant. β-Carotene readily undergoes isomerization, which is induced in particular by oxygen, on exposure to light and above 35 °C. Therefore, the solvent extraction technique is highly recommended as a nondestructive method that is feasible at room temperature.

The extractive approach for the isolation of β-carotene is the subject of interest of numerous papers (cf. Craft, 1992; Leo et al., 1971) and patents (cf. Rose, 1995 (U.S. patent); Kitoaka Motomitsu, 1995 (European patent)). No data were found, however, on the distribution of carotenoids.

β-Carotene is commonly isolated by chromatographic separation techniques and, for a larger scale, by solvent extraction. Low-boiling solvents are usually used for the extractive technique, as they easily removable from the extract under low pressure and are readily recoverable. A good extractant should feature a value of selectivity coefficient above unity. The higher the selectivity, the better the extractant and the lower the solvent consumption in the extraction process. The extractant selectivity is directly proportional to the distribution coefficient.

In connection with the isolation of β-carotene from carrots by the extraction technique, initially its distribution coefficient values were studied for water + organic solvent systems using organic solvents such as toluene, cyclohexane, hexane, methylcyclohexane, and chloroform. Moreover, the aim of the work was to select the most effective low-toxicity solvent for extraction.

Experimental Section

Materials. β-Carotene used in the study of a purity of 95% was supplied by Sigma. Solvents: toluene, analytical reagent grade (99%), from the Płock Refinery Industry R&D Centre; methylcyclohexane, pure (98%), supplied by Loba Chemie, Wien Fischamend, Austria; hexane, standard (99.95%), from Chemipan, Warsaw, Poland; cyclohexane, analytical reagent grade (99%), from Ubichem Ltd.; chloroform, analytical reagent grade (99%), ethanol-stabilized, supplied by the PPH POCh Gliwice (Poland); all were used without additional purification. The water used in the measurements was twice distilled. Refractive index values measured at 25 °C coincide with the literature values (cf. Table 1).

Table 1. Physicochemical Properties of Solvents: Molecular Weight $M^{(i)}$, Normal Boiling Point t_b, and Refractive Indices n_D at 25 °C, Measured in the Study and from the Literature

i	solvent name	$M^{(i)}$,[a] g/mol	$t_{b,i}$,[a] °C	d_i,[a] g/cm^3	n_D exptl	n_D lit.[a]
1	H_2O	18.015	100.0	0.998 07	1.3330	1.332 87
2	c-C_6H_{12}	84.162	85.5	0.666 6	1.4236	1.423 54
3	n-C_6H_{14}	86.178	68.7	0.661 1	1.3722	1.372 26
4	$C_6H_5CH_3$	92.141	110.6	0.669 9	1.4941	1.494 13
5	c-$C_6H_{11}CH_3$	98.189	100.9	0.665 5	1.4206	1.420 58
6	$CHCl_3$	119.4	61.2	1.444 4	1.4460	1.446 10

[a] Reference: Riddick and Bunger (1970).

Experimental Procedure. The β-carotene coefficient of distribution between an organic solvent and water was measured for at least three different β-carotene concentrations using the following procedure. An organic solvent containing β-carotene in various concentrations (yet always less the saturation concentration of the hydrated solvent) was agitated with water for ca. half an hour, and then the transmission of the organic phase was measured with a Specol, GDR 1975, instrument. The apparatus allowed transmission of light of a specific wavelength through the solution under investigation to be measured as percentage with reference to the sample not containing β-carotene. The β-carotene concentration in the organic phase was determined from the reference curve. The wavelength of the light for transmission measurement was chosen so as to make the transmission vs concentration curve continuous and monotonic over the entire concentration range studied. To determine the reference curves, a minimum of 20 measurements of transmission ϑ were made for β-carotene solutions of a known composition. To describe the reference curves corresponding to the β-carotene mole fraction, w_β, as a function of transmission ϑ, various correlation equations were used as gathered in Table 2 along with the wavelength value λ used in transmission measurements

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Table 2. Comparison of Calibration Curves for β-Carotene Mass Fraction w_β, in the Solvents Studied as a Function of Transmission ϑ, and the Values of the Calibration Equations Parameters and the Mean Square Deviation σ_s, at 25 °C

solvent	w_β	λ, nm	a_1	a_2	a_3	σ_s
C_6H_{14}	$(100 - \vartheta)(a_1\vartheta^{-a_2} + a_3)$	520	−4.3285	0.000 10	4.3284	0.002
C_6H_{12}	$a_1\vartheta^{-a_2} + a_3$	535	511.88	0.004 748	−500.27	0.017
$C_6H_5CH_3$	$1/(a_1 + a_2\sqrt{\vartheta}) + a_3$	570	0.20365	0.009 40	−3.2001	0.024
$C_6H_{11}CH_3$	$a_1\vartheta^{-a_2} + a_3$	530	1.8538	0.363 84	−0.2928	0.002
$CHCl_3$	$a_1\vartheta^{-2} + a_2\vartheta + a_3$	576	0.0089	−0.181 11	9.7646	0.10

Table 3. Results of the Measurements of Transmission ϑ of Solutions and Mass Fractions of β-Carotene in the Solvent Phase before $w_\beta^{(0)}$ and after $w_\beta^{(2)}$ Extraction, and in the Aqueous Phase, Masses in the Aqueous m_w and Solvent m_r Phases, and the Values of Distribution Coefficients Evaluated, k_w, at $t = 25$ °C

ϑ^0, %	ϑ^e, %	$w_\beta^{(0)}$, 10^3	$w_\beta^{(2)}$, 10^3	m_r, g	m_w, g	$w_\beta^{(1)}$, 10^6	k_w
Hexane							
80.05	88.9	0.019 34	0.009 36	52.4193	1020	0.513	18.2
85.02	88.9	0.019 37	0.009 36	52.4193	1020	0.514	18.2
75.2	84.8	0.025 82	0.013 7	52.3041	1020	0.621	22.0
80.05	87.0	0.019 4	0.011 31	49.7406	1020	0.395	28.7
87.8	92.0	0.010 48	0.006 41	49.3427	1030	0.195	32.9
87.8	92.5	0.010 48	0.005 96	49.3427	1030	0.217	27.5
Cyclohexane							
3.5	4.1	0.857 42	0.819 20	19.4372	42.	17.69	46.3
7.2	14.1	0.683 44	0.521 87	70.0	1070	10.57	49.4
34.0	50.0	0.311 05	0.218 96	40.2001	1055	3.500	37.5
		0.222 60	0.142 7	38.3838	1050	2.921	48.9[a]
Toluene							
68.5	70.0	0.352 92	0.342 26	58.5752	1020	0.612	559.1
68.0	69.5	0.356 52	0.345 79	58.5752	1020	0.616	561.2
63.0	64.2	0.393 64	0.384 53	69.1127	1010	0.623	616.8
65.0	66.5	0.378 53	0.367 43	54.4872	1010	0.599	613.6
Methylcyclohexane							
57.2	59.3	0.132 47	0.126 93	55.5421	1020	0.302	420.6
57.2	59.7	0.132 47	0.125 90	55.5421	1020	0.270	466.5
57.2	59.9	0.132 47	0.125 39	55.5421	1020	0.300	423.0
57.5	59.15	0.131 66	0.127 31	55.5421	1020	0.237	537.9
57.5	59.7	0.131 66	0.125 90	55.5421	1020	0.314	401.5
51.2	53.0	0.149 96	0.144 43	52.1084	1030	0.280	516.1

[a] Obtained by evaporation method.

Table 4. Concentrations c_β of β-Carotene in the Chloroform (2) and Aqueous (1) Phases and the Calculated Values of the k_w Coefficients at $t = 25$ °C

$c_\beta^{(2)}$, mg/mL	$c_\beta^{(1)}$, mg/mL	k_s	k_w
0.8771	0.001 16	756.	511.
0.4432	0.000 31	1430.	966.
0.7285	0.000 65	1121.	757.
1.0720	0.001 03	1041.	703.

and the parameters of these equations and the standard deviation. For the evaluation of parameters of these equations use was made of the Levenberg and Marquardt least-squares method for the nonlinear functions (Press et al., 1988).

β-Carotene concentration in the aqueous phase was found from the formula

$$w_\beta^{(1)} = m_r(w_\beta^{(0)} - w_\beta^{(2)})/m_w \tag{1}$$

where m_r and m_w are the masses in the solvent and aqueous phases, respectively, $w_\beta^{(0)}$ and $w_\beta^{(2)}$ are the mass fractions of β-carotene in the organic phase before and after the extraction, respectively. The β-carotene concentration in the organic phase was also verified by gravimetric method by evaporating the solvent from a withdrawn solution sample. Results of transmission determination, masses of the phases, and the mass fractions of β-carotene are collected in Table 3.

During the study of the coefficient of distribution of β-carotene between chloroform and water, a lowered transmission was found for the β-carotene solution after the extraction process. In this case, therefore, use was made of another analytical procedure. Concentration of the components in the organic and aqueous phases was determined by HPLC using a Waters instrument type 501 with a Waters type 486 detector and the Millennium 2010 software for its control. The mobile phase flowed at a rate of 1 mL/min. The eluent used was a mixture of the following solvents: methanol + acetonitrile + chloroform (50:40:10 by volume). The eluent was filtered through a Polycap AS filter. The measurements were made at a wavelength of 454 nm. Samples were analyzed directly after the equilibrium measurement. Prior to their introduction into the column, all the samples were filtered through a syringe filter type Millex-LC of Waters. Prior to proper determination, a calibration curve was plotted.

A synthetic β-*trans*-carotene, of a purity of 95.% and supplied by Sigma, was used as reference.

3. Results and Discussion

The distribution coefficient k_x of a substance β is defined as a ratio of mole fractions $x_\beta^{(k)}$ of the solute between two immiscible liquid phases: the aqueous phase (1) and the organic solvent phase (2):

$$k_x = x_\beta^{(2)}/x_\beta^{(1)} \tag{2}$$

In general use the coefficients are either determined by the ratio of concentrations (in moles per liter of solvent) c_β

$$k_c = c_\beta^{(2)}/c_\beta^{(1)} \tag{3}$$

or determined as mass fraction ratios w_β listed in Table 3

$$k_w = w_\beta^{(2)}/w_\beta^{(1)} \tag{4}$$

For conversion of the k_x and k_c or k_w, the following formulas are used

$$k_x = k_w(M^{(2)}/M^{(1)}) \quad \text{and} \quad k_c = k_m(d^{(2)}/d^{(1)}) \tag{5}$$

where $M^{(1)}$, $M^{(2)}$ and $d^{(1)}$, $d^{(2)}$ are mean molecular masses and densities, respectively, of the aqueous and solvent phases. In the case when the water and the organic solvent exhibit limited mutual solubility and the solute (β-carotene) is present in both phases in a low concentration, then $M^{(1)}$, $M^{(2)}$ and $d^{(1)}$, $d^{(2)}$ are the molecular weights and densities of the water and solvent, respectively.

The evaluated distribution coefficients of β-carotene between water and the solvent are gathered in Table 5. The distribution coefficients of β-carotene between water and the organic solvent are independent of concentration. The values calculated for distribution coefficients of β-carotene for different initial concentrations differed within the measurement error. As the value of distribution coefficient,

Table 5. Average Values of Distribution Coefficients of the β-Carotene k_w, Measured at $t = 25$ °C[a]

solvent	k_w
n-C_6H_{14}	24.6 (6.0)
c-C_6H_{12}	45.5 (5.5)
$C_6H_5\,CH_3$	588. (28.)
c-$C_6H_{11}\,CH_3$	461. (44.)
$CHCl_3$	735. (188.)

[a] Mean square deviation is given in parentheses.

an average of all the measurements for β-carotene and a given solvent was assumed.

The declined transmission of the wave of light observed in the solution following extraction with chloroform, thus a rise in color intensity of the solution, may be due to isomerization of β-carotene (Britton, 1985; O'Neil and Schwartz, 1992) or polymerization (deeper color, Muller (1957)) induced by trace amounts of HCl or free Cl dissolved in chloroform or alternatively by the formation of EDA or H···p complexes with chloroform (Woycicki, 1980). A preliminary NMR study carried out in this work appears to indicate only the appearance of specific interaction or formation of a chloroform complex. These doubts lead to the need for additional study. The study confirms the point that chloroform cannot be used as carotenoid extractant.

The values of the distribution coefficients of the solvents examined rise in the following order

$$\text{hexane} < \text{cyclohexane} < \text{methylcyclohexane} < \text{toluene} < \text{chloroform}$$

which is associated with the sequence of the rising solubility of β-carotene and corresponds to the sequence of selectivity coefficients, except chloroform (Craft, 1992). The distribution coefficients for all the solvents examined exhibit high values. However, for commercial process hexane or cyclohexane seems to be the best, as their relatively high vapor pressure at room temperature makes the solvent evaporation process more cost-effective.

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