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Relative Solubility, Stability, and Absorptivity of Lutein and β-Carotene in Organic Solvents

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The relative solubility, stability, and absorptivity of lutein and β-carotene were determined in 18 organic solvents. The solubility of both carotenoids was greatest in tetrahydrofuran, while hexane exhibited the least solubility for lutein; methanol and acetonitrile exhibited the least solubility for β-carotene. Stability was monitored for 10 days at room temperature by measuring absorbance changes at the wavelength maximum. In the majority of the solvents, initial absorbance decreased by less than 10% during the 10-day period. Degradation was greatest for both carotenoids in cyclohexanone. The relative absorptivities were determined by calculating the carotenoid concentration in a reference solvent using a reference absorptivity, and then Beer's Law was applied to the measured absorbance of the same carotenoid concentration in other organic solvents. Absorbance maxima and relative absorptivities were in good agreement with available literature values.

Interest in carotenoids has increased during the past decade. Carotenoids are not only natural pigments and vitamin A precursors but have been proposed as cancer prevention agents, ulcer inhibitors, life extenders, and heart attack inhibitors (Peto et al., 1981; Colditz et al., 1985; Mozsik et al., 1984; Cutler, 1984; Gaziano et al., 1990). Unfortunately, physical information about these compounds in organic solvents is limited. The wavelength maxima and absorptivity of carotenoids change with the nature of the solvent in which they are dissolved. For example, the visible spectrum of β-carotene in ethanol has little fine structure with absorbance maxima at 453 and 480 nm, while the visible spectrum in carbon disulfide has more fine structure and exhibits maxima at 484 and 512 nm (Davies, 1976). The molar absorptivities of β-carotene at λ_{max} in these two solvents are 140 700 and 107 800 L mol^{-1} cm^{-1}, respectively (Davies, 1976). In the past, absorbance maxima have been compiled for carotenoids in several solvents (Davies, 1976; De Ritter and Purcell, 1981), but such tables supply limited absorptivities, and frequently the maxima in a given solvent vary by several nanometers depending on the source of information. These tables also provide no information regarding the solubility and stability of the carotenoids in the solvents. The lack of information about carotenoid solubilities and molar absorptivities in a variety of organic solvents increases the difficulty associated with developing analytical methods for carotenoid research. Such practical information is important for the selection of solvents for use in sample preparation and liquid chromatography (LC) mobile phases and also for the identification and quantification of carotenoids in diverse LC mobile phases.

The two most prominent cyclized carotenoids in human serum and foods are lutein (β,ε-carotene-3,3′-diol) and β-carotene (β,β-carotene) (Bieri et al., 1985; Khachik et al., 1986). Not only are they the most prominent, but they also span a wide polarity range and are representative of the β,ε- and the β,β-carotenoids, respectively. Herein we describe the determination of the relative solubility, stability, and absorptivity of these two biologically important carotenoids in various organic solvents.

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MATERIALS AND METHODS

Reagents. Crystalline β-carotene (type I, Sigma Chemical Co., St. Louis, MO) and lutein (provided as a gift by Kemin Industries, Des Moines, IA) used throughout the study were assessed by spectrophotometric and liquid chromatographic techniques to be greater than 90% *trans*-β-carotene and 90% *trans*-lutein, respectively. The sources, descriptions, and lot numbers of the solvents used are listed in Table I.

Equipment. Spectral measurements were made using a photodiode array scanning spectrometer (H-P 8450, Hewlett-Packard, Palo Alto, CA). The spectrophotometer provided a 1-nm spectral band pass from 200 to 400 nm and a 2-nm spectral band pass from 400 to 800 nm. Wavelength accuracy was checked using a holmium oxide glass filter and found to be correct at the 279-, 361-, 460-, and 536-nm absorption maxima. Solutions were dispensed with calibrated pipets or gas-tight syringes. Carotenoid purity was determined as previously described (Craft et al., 1991).

Relative Solubility of Lutein and β-Carotene in Organic Solvents. Approximately 10 mg of lutein or β-carotene was added to 3 mL of each of the solvents listed in Table I. Vials were ultrasonically agitated for 5 min. If a clear solution with no residual crystals resulted, additional carotenoid was added until crystalline material remained undissolved. Each solution was then filtered through a 0.2-μm membrane, and appropriate dilutions were made until the absorbance at the wavelength maximum was between 0.5 and 1.0 absorbance unit at ambient temperature. The background absorbance of each solution was subtracted using the appropriate solvent containing no carotenoid. Carotenoid concentration was calculated using Beer's law and the relative absorptivities determined below (Determination of Relative Absorptivity). Measurements were performed in triplicate and the calculation used is

$$(\text{absorbance}_s \text{ at } \lambda_{max})(\text{dilution factor})/\text{molar absorptivity}_s$$

where the subscript s is a given solvent. The measured values were rounded to one significant figure since this experiment was not designed to determine absolute solubility but rather to indicate solubility relative to other solvents.

Determination of Relative Absorptivity. Concentrated solutions (approximately 3 g/L) of lutein and β-carotene were prepared in tetrahydrofuran (THF) containing butylated hy-

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Table I. List of Solvents, Sources, and Lot Numbers

solvent	source, grade	lot	safety hazards[a]
acetone	Mallinckrodt, SpectAR	2438	1, 2, 5
acetonitrile	J. T. Baker, HPLC	C28108	2, 3, 5
benzene	J. T. Baker, Photrex	C14603	3, 5
chloroform	EM Science, Omnisolve	5102	3
cyclohexane	EM Science, Omnisolve	6041	1, 2, 5
cyclohexanone	Kasai, GR	FAV01	1, 2
dichloromethane	J. T. Baker, HPLC	D25082, D18131	2, 3
dimethylformamide (DMF)	Burdick and Jackson, HPLC	AK285	1, 2
dimethyl sulfoxide (DMSO)	Mallinckrodt, SpectAR	KMCD	1, 2, 4
ethanol, absolute	Warner-Graham		1, 5
ethyl acetate	EM Science, Omnisolve	7278	1, 2, 5
ethyl ether	EM Science, GR	9130	1, 2, 4, 5
hexane	J. T. Baker, HPLC	D33095	1, 2, 5
2-propanol	Mallinckrodt, AR	3037KDEV, 3035KCAY	1, 5
methanol	EM Science, Omnisolve	8352	3, 5
methyl *tert*-butyl ether (MTBE)	EM Science, reagent	11P23	2, 5
tetrahydrofuran (THF) + BHT	J. T. Baker, HPLC	C24654	2, 5
toluene	Burdick and Jackson, HPLC	AK80	1, 2, 5

[a] 1, harmful when entering the body; 2, irritant to skin, eyes, and respiratory organs; 3, toxic (harmful if inhaled, ingested, or absorbed through the skin); 4, explosive; 5, flammable.

Table II. Relative Solubility and Absorptivity of Lutein and β-Carotene in Organic Solvents

	lutein				β-carotene			
solvent	solubility, mg/L	λ_{max},[a] nm	absorptivity $E^{1\%}$, cm^{-1}	molar absorptivity,[a] L mol^{-1} cm^{-1}	solubility, mg/L	λ_{max},[a] nm	absorptivity $E^{1\%}$, cm^{-1}	molar absorptivity,[a] L mol^{-1} cm^{-1}
acetone	800	446	2540	144 500	200	452 (452)	2559	137 400
acetonitrile	100	446	2559	145 600	10	452	2540	136 400
benzene	600	456 (458)	2350	133 700 (127 200)	4000	462 (462)	2304	124 000 (125 500)
chloroform	6000	454 (458)	2369	134 800	2000	462 (461)	2330	125 100 (128 600)
cyclohexane	50	448	2520	143 400	2000	454 (457)	2508	134 700 (134 500)
cyclohexanone	4000	454	2359	134 200	2000	462	2359	126 700
dichloromethane	800	452	2320	132 000	6000	460	2369	127 200
DMF	1000	454	2390	136 000	200	460	2389	128 300
DMSO	1000	460	2369	134 800	30	466	2259	121 300
ethanol	300	444 (445)	2550	145 100[b]	30	450 (449)	2529	135 800 (140 700)
ethyl acetate	800	446	2529	143 900	500	452	2520	135 300
ethyl ether	2000	444	2629	149 600	1000	448	2659	142 800
hexane	20	444 (445)	2589	147 300	600	448 (453, 450)	2592	139 200[c]
2-propanol	400	444	2599	147 900	40	450	2508	134 700
methanol	200	442 (444)	2629	149 600	10	450	2540	136 400
MTBE	2000	444	2589	147 300	1000	450	2588	139 000
THF	8000	450	2469	140 500	10000	456	2399	128 800
toluene	500	456	2290	130 300	4000	462 (463)	2270	121 900

[a] Calculated molar absorptivities and λ_{max} in parentheses are taken from Davies (1976). [b] Reference absorptivity for lutein. [c] Reference absorptivity for β-carotene.

droxytoluene (BHT) as an antioxidant and filtered through 0.2-μm membranes. To 30 mL of each of the solvents listed in Table I was added 30 μL of concentrated lutein or β-carotene in THF. We were cautious to work well within the carotenoid solubility limits (determined above) of the solvents being examined to avoid precipitation of the carotenoid compounds. Sealed vials were ultrasonically agitated for 3 min to assure dissolution. Spectrophotometric scans were performed from 250 to 550 nm, and absorbance at the wavelength maximum was determined. The concentrations of the lutein and β-carotene solutions were determined using the most widely accepted molar absorptivity for lutein in ethanol and β-carotene in hexane (145 100 and 139 200 L mol^{-1} cm^{-1}, respectively) (Davies, 1976). Relative absorptivities in the different solvents were determined on the basis of the calculated concentrations of lutein and β-carotene determined in ethanol and hexane, respectively, and the absorbance of the carotenoid solutions at the wavelength maximum in a given solvent using Beer's law. Absorbance measurements were performed in triplicate, and the combined error (mean standard deviation of absorbance measurements and estimated limits of bias) associated with the measurements was approximately 1%.

Lutein and β-Carotene Stability. The solutions prepared under Determination of Relative Absorptivity were stored in amber glass vials with Teflon-lined screw caps at room temperature. The UV–vis absorbance spectrum from 250 to 550 nm was monitored over a 10-day period. Decreases in absorbance and shifts in wavelength maxima were indicators of carotenoid degradation. Degradation is reported as percent of initial absorbance at λ_{max}.

RESULTS AND DISCUSSION

Both carotenoids tested were most soluble in THF (Table II). β-Carotene was least soluble in methanol and acetonitrile, while lutein was least soluble in hexane. Many existing extraction techniques partition carotenoids into hexane or petroleum ether from aqueous alcohol or acetone (Bieri et al., 1985; De Ritter and Purcell, 1981; Simpson et al., 1985). Given the poor solubility of dihydroxy and more polar carotenoids in hexane, this may lead to losses. Diethyl ether has also been used to partition carotenoids from aqueous/polar organic mixtures (Britton, 1985; De Ritter and Purcell, 1981; Rodriquez-Amaya, 1989); on the basis of the solubilities listed in Table II, this may present a more effective approach. One possible disadvantage is the solubility of fatty acid soaps in ether which must be thoroughly removed with water (Britton, 1985). Although THF is subject to peroxide formation, it has found increased use (Bureau and Bushway, 1986; Khachik et al., 1986; Peng et al., 1987) for carotenoid extractions due to the high solubility of a wide polarity range of carotenoids. We are unaware of published absorptivities for carotenoids in THF. This lack of information may hamper its use as a solvent and result in the introduction of errors associated with evaporation and solvent-transfer steps due to the use of less appropriate solvents with published absorptivities. The information listed in Table II should prove

Table III. Relative Lutein and β-Carotene Degradation in Organic Solvents

	% of initial absorbance of lutein at λ_{max}						% of initial absorbance of β-carotene at λ_{max}					
	time, days				λ_{max}	cis	time, days				λ_{max}	cis
solvent	1	3	6	10	shift,[a] nm	peak[b]	1	3	6	10	shift,[a] nm	peak[b]
acetone	99	96	95	95	0	+	98	96	96	93	0	+
acetonitrile	98	97	94	94	−2	+	99	96	93	92	0	+
benzene	100	99	100	97	0	−	87	77	71	67	0	−
chloroform	97	96	93	90	−2	+	97	91	92	91	−2	+
cyclohexane	100	100	98	99	0	−	98	98	93	91	0	+
cyclohexanone	88	70	49	37	−2	+++	86	67	45	32	−4	+++
dichloromethane	95	91	88	83	0	−	77	59	47	34	−30	+
DMF	100	100	97	97	0	−	96	94	93	90	0	−
DMSO	100	99	99	97	0	−	94	90	86	80	0	−
ethanol	96	96	93	91	0	+	98	94	92	91	−2	+
ethyl acetate	98	97	95	96	0	−	99	97	96	95	0	−
ethyl ether	96	88	79	65	−2	++	94	78	70	69	−2	+
hexane	99	100	100	98	0	−	96	95	94	92	0	+
2-propanol	99	99	99	95	0	−	97	94	94	89	0	−
methanol	97	95	95	90	−2	++	97	92	89	88	−4	++
MTBE	97	90	82	76	0	++	96	89	82	74	−2	++
THF + BHT	100	100	100	99	0	−	99	98	99	97	0	−
toluene	99	98	100	97	0	−	90	80	76	71	0	−

[a] Indicates direction and amount of spectral shift at day 10. [b] Indicates presence/absence of cis peak. Number of +'s indicates intensity of cis peak.

valuable in the selection of solvents employed for carotenoid extractions and dissolutions.

The wavelength maximum for β-carotene in the various solvents ranged from 448 to 466 nm. The λ_{max} in hexane was 448 nm, which is 4–5 nm below the referenced value (Davies, 1976), although the same reference lists other citations reporting λ_{max} in hexane at 449 nm but without an absorptivity. The difference in the reference λ_{max} and the λ_{max} obtained in this work can be attributed to aromatic contaminants in some solvents, the presence of cis isomers in the β-carotene employed, and differences in the wavelength calibration or spectral band pass of the spectrophotometers used for the measurements. For the purposes of this work, the maximum absorbance was used to determine relative solubilities and absorptivities. The wavelength maximum for lutein ranged from 442 to 460 nm. The λ_{max} of lutein in ethanol was within 1 nm of the reported value of 445 nm (Davies, 1976). It may be that, in the lutein used, the presence of ~6% zeaxanthin, which has a higher λ_{max}, offset the wavelength lowering due to cis isomers or differences in wavelength calibration. The spectrophotometer used for this work was limited to a 2-nm spectral band pass in the visible region. These two observations may explain why the measured λ_{max} values were consistently 1–2 nm lower than reported values.

Because the absorptivity and λ_{max} of carotenoids vary in different solvents and these values are only published for a few solvents, relative absorptivities and λ_{max} were determined for lutein and β-carotene in the solvents listed in Table I. These values, given in Table II, were used to calculate the solubility of the carotenoids, also reported in Table II. Relative absorptivity values listed in Table II are in good agreement with previously published values (Davies, 1976; De Ritter and Purcell, 1981). The primary advantage of the determination of the spectral maxima and absorptivities is that carotenoid concentrations can be determined directly in a wide range of solvents. In addition, data obtained using diode array detectors in conjunction with LC can be better interpreted when the wavelength shifts that occur in different solvents are known.

Finally, the stability of these two carotenoids in the various solvents was monitored spectrophotometrically over a period of 10 days. Carotenoid degradation was accompanied by decreases in the absorbance and, in some cases, a downward shift in the λ_{max} (Table III). We are aware that some degradation products (e.g., geometric isomers and carotenals) contribute to the absorbance in the visible region; however, all degradation products exhibit lower absorptivity at the wavelength maximum of the parent compound. This also implies that changes in absorbance are not necessarily proportional to the concentration of lutein or β-carotene in the solution. The definitive measure of degradation would have been to monitor the trans isomer of both carotenoids by HPLC; however, while attempting to do this, we encountered technical difficulties. First, it was not possible to make all of the measurements by HPLC at the appointed times without staggering the experiments; second, few of the solvents could be injected directly into the HPLC system; and third, complete redissolution of carotenoids was questionable if a solvent evaporation was included. For these reasons we opted to record the UV–vis spectra to monitor major changes in the analytes. When the absorbance expressed as percent of initial absorbance at λ_{max} was plotted against time, the degradation function was similar in all solvents but proceeded at different rates (Figure 1). Stability was poorest for both lutein and β-carotene in cyclohexanone, retaining only 37% and 32%, respectively, of their initial absorbance by day 10. The degradation of β-carotene in cyclohexanone was followed closely by degradation in dichloromethane with ~34% absorbance remaining at day 10. In general, the rate of lutein degradation was slower than β-carotene degradation as illustrated by the curves shown in Figure 1 representing the average rate of degradation in all solvents. Even on day 10, the carotenoid absorbance in most solvents was clustered above 90% of the initial absorbance. The conditions incorporated were selected to exacerbate the degradation process so that stability/instability would be evident. In a laboratory setting, greater efforts would be made to stabilize carotenoid solutions, e.g., by the incorporation of antioxidants and use of lower storage temperatures.

Little information of this type has been reported previously. In comprehensive reviews (Davies, 1976; De Ritter and Purcell, 1981), references to factors important to solvent selection are mentioned, but specific information about the influence of solvents on carotenoid stability is lacking. Information presented in Table III should be used for comparative purposes since stability is also dependent on solvent supplier and lot number. The solvents

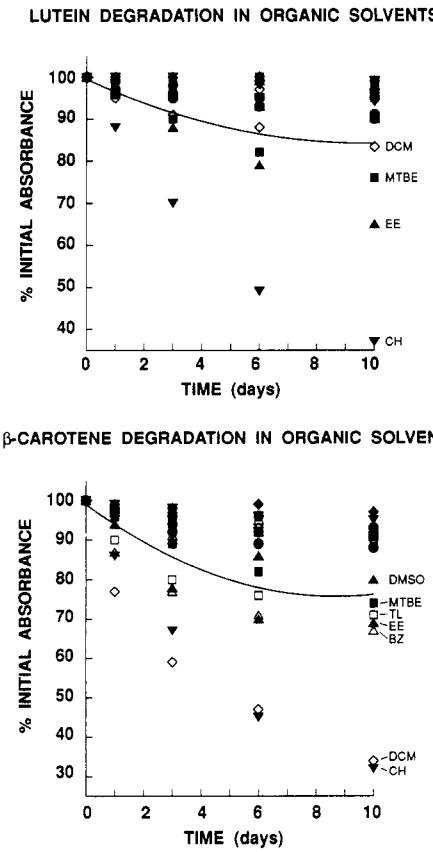

Figure 1. Percent initial absorbance at λ_{max} of lutein and β-carotene monitored in 18 organic solvents over a period of 10 days at ambient temperature. Solid line represents the average rate of degradation in all solvents. Actual values for a given solvent are listed in Table III. A specific symbol is used to illustrate the degradation in a given solvent; however, duplicates of most symbols were necessary to account for all solvents. As such, legends are not given for each symbol; however, solvents with less than 85% of initial absorbance at day 10 are indicated by solvent abbreviations. Abbreviations: BZ, benzene; CH, cyclohexanone; DCM, dichloromethane; EE, ethyl ether; MTBE, methyl *tert*-butyl ether; TL, toluene.

used may or may not be representative of current lots from a given supplier. No attempt was made to sample various sources of each solvent, and no additional antioxidants were added to the solvents used. However, our experience with 2-propanol and dichloromethane indicates that the source and lot of solvent used substantially influence the stability of carotenoids in solution.

While developing methods for the extraction and determination of carotenoids, we found that critical information was frequently missing from tabulated data and found it necessary to fill in some gaps. The information provided in this paper supplements published absorptivities and λ_{max} values for solvents for which information is currently unavailable. This information should also aid in the selection of solvents to be used for carotenoid research by giving an indication of stability and solubility of two carotenoids, which vary greatly in polarity. Finally, since the chromophore is not strongly influenced by the presence of hydroxyl groups outside the conjugated double bond system, the molar absorptivity values for lutein can be used for estimating concentration values of other β,ε-carotenoids such as α-cryptoxanthin and α-carotene; the molar absorptivity values for β,β-carotene can be used for β-cryptoxanthin and zeaxanthin.

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Received for review June 27, 1991. Accepted December 2, 1991. Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation of endorsement by NIST, nor does it imply that the equipment or materials identified are necessarily the best available for the purpose.

Registry No. Lutein, 127-40-2; β-carotene, 7235-40-7; acetone, 67-64-1; acetonitrile, 75-05-8; benzene, 71-43-2; chloroform, 67-66-3; cyclohexane, 110-82-7; cyclohexanone, 108-94-1; dichloromethane, 75-09-2; dimethylformamide, 68-12-2; dimethyl sulfoxide, 67-68-5; ethanol, 64-17-5; ethyl acetate, 141-78-6; ethyl ether, 60-29-7; hexane, 110-54-3; 2-propanol, 67-63-0; methanol, 67-56-1; methyl *tert*-butyl ether, 1634-04-4; tetrahydrofuran, 109-99-9; toluene, 108-88-3.