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Solvents Design on the Basis of Molecular-Microscopic Properties of Binary Mixtures for Lycopene Extraction

Hadi Salari[1], Hanie Rohani[2], Mohammad Reza Elahifard[1], Mohammad Hosseini[2], Mohammad Reza Gholami[1,*]

[1]Department of Chemistry, Sharif University of Technology, Azadi Ave., Tehran, P.O.Box 11365-9516, Iran
[2]Department of Chemical Engineering, Amirkabir University of Technology, Tehran, Iran

Abstract Lycopene is a natural compound with high-value of nutritional and medical properties. Nowadays, the extraction of Lycopene with mixtures of solvents to obtain more efficiency and less toxicity is interested. In this study, the Lycopene extraction in three binary mixtures of solvents (Ethyl-acetate/Ethanol, Hexane/Isopropanol and Benzene/Isopropanol) was measured and then the effects of solvatochromic parameters (π^*, dipolarity/polarizability; β, hydrogen-bond acceptor basicity; α, hydrogen-bond donor acidity) and solvophobicity (Sp) parameters on the extraction from tomato tissues have been considered. Among the all parameters, Sp shows the biggest contribution in the extraction that is well justified, knowing that Lycopenes are lypophobic. Our calculation results show that in addition to Sp, another parameter is needed to get the reasonable correlation between the parameters and the extraction. For Ethanol/Ethyle-acetate mixture, β, for Isopropanol/Benzene mixture, π^* and for Isopropanol/Hexane mixture, α, participated in the correlation equation in addition to Sp.

Keywords Lycopene, Extraction, Solvatochromic Parameters, Solvophobicity

1. Introduction

Carotenoids, the most important natural pigments found in becteria, plants, fungi and animals[1-3] cannot be synthesized by human; hence, they must be obtained from dietary sources. Lycopene as a carotenoid with antioxidant properties is accumulated in photo-synthesis pigment-pigment complex of plants that can be most easily seen in a ripe tomato, water melon, pink grape fruit, guava and papaya, giving them a characteristic red color[1-5]. Tomato and tomato products are the major source for Lycopene production.

The molecular structure of Lycopene consists of a long chain of conjugated carbon-carbon (11 conjugated double bonds) having various geometrical isomer; the all trans-isomer are of the dominate percentage in most raw material but the cis-isomers are more compatible in our body and with even stronger bioactivities[6,7]. In-vitro and in-vivo studies showed that Lycopene was promising bioactive components on lowering own the risk of some chronic diseases including certain cancer (e.g. prostate cancer) and coronary heart diseases[6].

The supplying of this product relies on the extraction of Lycopene from plants and zymotie liquid via chemical extraction with conventional solvent (that may assist with supersonic or microwave instruments), and super critical fluid (CO_2) extraction (SCFE)[4,8]. SCFE represents non-organic solvent residing as an advantage but the equipment and the energy consumption is very high.

Due to the hydrophobic nature and limited solubility of carotenoids in water, the selection of appropriate solvents has been recently subjected of large studies[9,10]. There are two important factors to select the best solvent, i.e. 1) extraction efficiency and 2) complete removing and less adverse effects on human health. Unfortunately, most solvents with high extraction efficiencies are considered to be toxic. This represents the mixture of solvents to obtain higher extraction with lower toxicity.

Although some solvents mixtures were used in carotenoids extraction[1,2], the investigation of solvent-solvent interactions and their impress on the extraction of various caroteniods have not been considered. Solute–solvent interactions developed in solvation shell of the solutes, cause physical and chemical effects in many processes. These effects of the medium are originated from changes in its polarity, solvophibicity and hydrogen bonding[11-14].

Interactions in mixed solvents are much more complex than in net solvents because a solute can be surrounded preferentially by the mixture components or formed complex [11]. The behaviour of solvents in chemical processes can be understood with an investigation of the solution interactions with a solute. Solvatochromic parameters which studies solute-solvent interactions can show specific and non specific interactions in mixed solvents[11,12].

In this study we applied Ethyl-acetate/Ethanol, Hexane/

* Corresponding author:
gholami@sharif.edu (Mohammad Reza Gholami)
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Isopropanol and Benzene/Isopropanol mixtures to lycopen extraction. On the basis of proper probes, Solvophibicity (Sp) and three solvatochromic parameters (i.e. π*, dipolarity/polarizability; β, hydrogen-bond acceptor basicity; α, hydrogen-bond donor acidity) are followed in net and mixed solvents versus extraction and then, the best correlation between them has been calculated and discussed.

2. Experimental

Lycopene with 97% purity was supplied from Sigma Aldrich, USA. All solvents, i.e. Hexane, Ethanol, Ethyl-acetate and Isopropanol were purchased from Merck. Red tomatoes were obtained from local market and they were floated in the boiling water for 2min, and then they were cooled so that their tissues could be cut more easily. All samples must be kept in the cold and unlighted condition.

Take 1gr of tissues in to 250 ml flask; add 30 ml of the solvents. The mixtures were stirred magnetically with 1000 rpm at room temperature for 30 min. Lycopene content in the extract was determined according to the absorbance measured at ~440, ~470, and ~500 nm relating to the three isomers of Lycopene (Fig. 1) via UV-Vis spectrophotometer (GBC cintra40).

3. Results and Discussion

Tables 1-3 and Figures 2-4 show the Sp, solvatochromic parameters and Absorption variation in Hexane/ Isopropanol, Ethyl-acetate/Ethanol and Benzene/Isopropanol mixtures. Solvophibicity is the consequence of increased hydrophobic interactions caused by a solute molecule that enforces the water structure. The values of Sp for many binary mixtures give a fair linear correlation with the volume fraction of constituents[15,16]. Thus, we have estimated the Sp values for Ethyl-acetate/Ethanol, Isoropanol/Hexane and Isoropanol/Benzene mixtures by the following equation (Eq. (1)):

$$Sp= f_1\ Sp_1+ (1- f_1)\ Sp_2 \qquad (1)$$

Where f_1 is the volume fraction of solvent 1 and Sp_1 and Sp_2 are solvophibicity parameters in pure solvents 1 and 2, respectively. The Sp values of the corresponding volume fractions and values between those listed for binary mixtures were linearly interpolated and have been summarized in Tables 1-3.

Inverse to Sp, the solvatochromic parameters do not show a linear correlation with the volume fraction of constituents in the mixture, so they must be measured. All these parameters have been derived from previous studies[17-19] that are listed in Tables 1-3.

Maximum Absorption in different wave lengths in three binary mixtures and in Molecular solvents is summarized in Table 4. The results show that instead of Ethyl-acetate/ Ethanol mixture, two other mixtures have a maximum point in the extraction that referenced the stronger solute-solvent interaction in the mixture than net solvents for these cases.

The single and multi-parameter correlation of Absorption values versus solvent parameters have been calculated and the single and double-parameter data are shown in Table 5. All efforts to derive single-parameter regression for Lycopene extraction in the three binary mixtures did not respond well which can be summarized as fallow;

Sp; the correlation coefficient of Absorption versus Sp is negative and by increasing the solvophibicity, the Absorption decreases. According to the hydrophobic structure of caroteniods the Sp parameter plays a predominant role in all net and mixtures undoubtedly. Although, Sp has the biggest coefficient between single parameter (Table 5), it could not give a reasonable mathematical model to fitting data. There is no acceptable correlation between maximum extraction and every solvatochromic parameters in any of the mixtures, either. Therefore, Multi-parameter Linear Regression (MLR) analysis was carried out. The double-parameter regression showed a good mathematical model (Table 5).

Table 1. Solvatochromic and Solvophobicity(Sp) parameters in Ethanol/Ethyl-acetate mixture. V is the volume faction of Ethanol.

V	α	β	π*	Sp
0	0	0.45	0.45	0.06
0.2	0.67	0.57	0.61	0.076
0.4	0.76	0.6	0.63	0.092
0.6	0.8	0.63	0.65	0.108
0.8	0.82	0.65	0.7	0.124
1	0.86	0.75	0.54	0.14

Table 2. Solvatochromic and Solvophobicity(Sp) parameters in Isopropanol/Benzene mixture. V is the volume faction of Benzene.

V	α	β	π*	Sp
0	0.65	0.9	0.5	0.1
0.2	0.56	0.83	0.57	0.08
0.4	0.49	0.7	0.63	0.06
0.6	0.42	0.61	0.64	0.04
0.7	0.41	0.54	0.64	0.03
0.8	0.39	0.43	0.64	0.02
0.9	0.35	0.28	0.62	0.01
1	0.04	0.09	0.6	0

Table 3. Solvatochromic and Solvophobicity(Sp) parameters in Isopropanol/Hexane mixture. V is the volume faction of Hexane.

V	α	β	π*	Sp
0	0.67	0.9	0.49	0.1
0.2	0.66	0.89	0.45	0.0818
0.4	0.65	0.91	0.39	0.0636
0.6	0.66	0.91	0.3	0.0454
0.7	0.71	0.92	0.23	0.0363
0.8	0.75	0.83	0.15	0.0272
0.9	-	0.68	0.06	0.0181
1	0.1	0.03	0.06	0.0091

When small quantities of the Ethanol are added to Ethyl-acetate, the α parameter increases sharply and then is smoothly enhanced to the value of pure Ethanol. There are

increasing profiles for β and Sp parameters by increasing Ethanol content.

The π* trend demonstrates a positive deviation with an increase in the maximum value in V=0.8. The π* values confirm high solvent-solvent interactions in this media. The prominence of the synergetic effect in the Ethanol mixture with Ethyl-acetate can by related to hydrogen bonding interaction between Ethanol and Ethyl-acetate to give a structural complex which is more polar than the two constituents of mixture. In Ethyl-acetate/Ethanol mixture, the double-parameter correlation of Absorption versus Sp and β gives a reasonable mathematical equation which is summarized in Eq. (2).

$$A= 5.545(\pm1.338) -5.367(\pm4.333)\beta -6.798(\pm1.4311)Sp \quad (n=11,\ R=0.959,\ s=0.278,\ F=17.09) \qquad (2)$$

Where, n, R, s, and F are the number of data, the regression error, the standard deviation, and the statistical factor, respectively. A is the maximum Absorption.

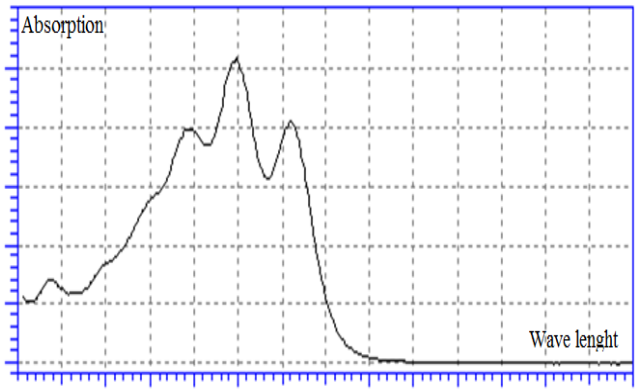

Figure 1. UV-Vis spectrum of Lycopene

Ethyl-acetate which consists of hydrogen bond acceptor group can form strong hydrogen bonding with Ethanol. With addition of Ethanol to Ethyl-acetate these inter molecular hydrogen interactions are drastically elevated, which leads to less interaction with Lycopene. An Absorption decreasing profile which is observed in Ethyl-acetate/Ethanol mixture originates from these interactions (Fig. 2).

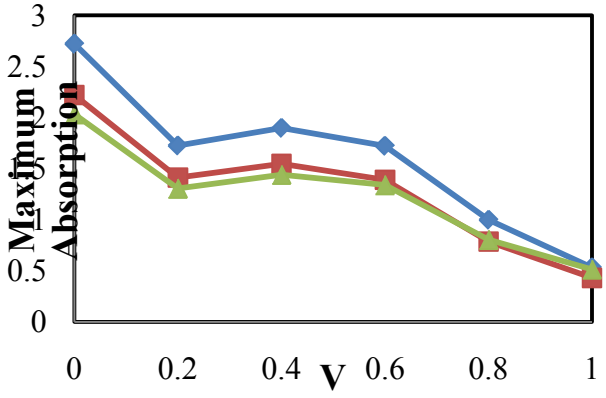

Figure 2. Maximum Absorption versus Volume fraction in Ethanol/Ethyl-acetate mixtures. (♦) in λ=473 nm, (■) in λ=504.5 nm and (▲) in λ=446 nm. V is volume fraction of Ethanol.

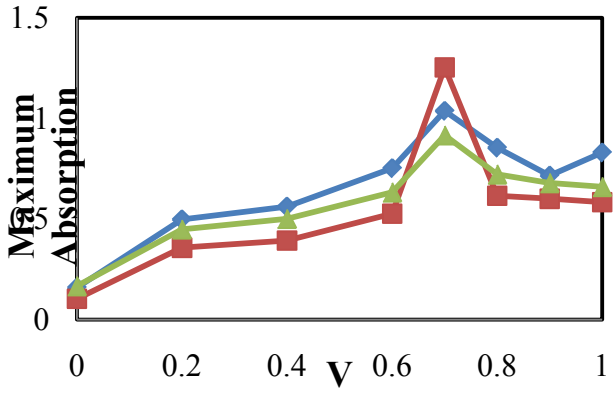

Figure 3. Maximum Absorption versus Volume fraction inIsopropanol/Benzene mixtures. (♦) in λ=470.6 nm, (■) in λ=500.4 nmand (▲) in λ=445.8 nm. V is volume fraction of Benzene.

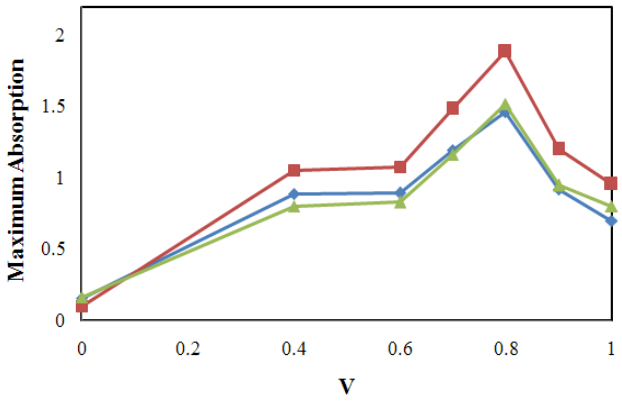

Figure 4. Maximum Absorption versus Volume fraction in Isopropanol/Hexane mixtures. (♦) in λ=470.6 nm, (■) in λ=500.4 nm and (▲) in λ=445.8 nm. V is volume fraction of Hexane.

In Isopropanol/Benzene system, π* demonstrates the increasing behaviour which is in agreement with extraction percent. By adding Benzene to Isopropanol, other solvatochromic parameters decrease. As a consequence, π* parameter has a positive effect in extraction, which is shown in Eq.(3).

$$A= -0.938(\pm0.904) +2.916\ (\pm1.392)\ \pi^* -3.530\ (\pm1.951)Sp \quad (n=8,\ R=0.916,\ s=0.127,\ F=13.114) \qquad (3)$$

π* parameter is related to specific solute-solvent interactions in solvents. Benzene with an unsaturated structure can interact with Lycopene through π*- π* interactions. Considering the results and the above discussion, in the region of high composition of Benzene these π*- π* interactions caused extraction with high efficiency.

In Isopropanol/Hexane mixture, absorption has the best agreement with Sp and α parameters (Eq. (4)).

$$A= 0.682(\pm0.145) +1.540(\pm0.262)\alpha -15.065\ (\pm2.023)Sp \quad (n=7,\ R=0.976,\ s=0.124,\ F=30.70) \qquad (4)$$

The decreasing behaviour for β, π* and Sp is shown in Table 3. There is a maximum Absorption in V=0.8 and α parameter exhibits a maximum in this volume fraction too. By adding Hexane to Isopropanol, α parameter increases rapidly and finally decreases to its corresponding value in pure Hexane through a maximum at V=0.8 and synergism

effect is observed for α parameter. Strong hydrogen interactions between Lycopene and the complex structure of solvent conclude more extractions in this medium.

Table 4. Maximum absorption in Binary mixtures in three λ. V is volume fraction of cosolvent.

	λ 1	*λ 2*	*λ 3*
V(Ethanol)		**Ethanol/Ethyl-acetate**	
0	2.721	2.221	2.034
0.2	1.726	1.413	1.305
0.4	1.899	1.547	1.442
0.6	1.723	1.39	1.339
0.8	0.999	0.787	0.804
1	0.531	0.431	0.513
V(Benzene)		**Isoropanol/Benzene**	
0	0.159	0.103	0.166
0.2	0.498	0.357	0.448
0.4	0.561	0.393	0.501
0.6	0.752	0.526	0.632
0.7	1.037	1.253	0.916
0.8	0.854	0.615	0.721
0.9	0.716	0.600	0.678
1	0.83	0.583	0.659
V(Hexane)		**Isoropanol/Hexane**	
0	0.159	0.103	0.166
0.4	0.889	1.054	0.805
0.6	0.894	1.079	0.83
0.7	1.195	1.483	1.164
0.8	1.465	1.885	1.519
0.9	0.921	1.2	0.947
1	0.698	0.957	0.802

Table 5. Regression coefficients in different mixtures

Sp	*α*	*β*	π*	R
		Ethanol/Ethyl-acetate		
-23.76				0.93
	-1.88			0.81
		-7.33		0.95
			-3.73	0.43
-6.79		-5.36		0.96
		Isoropanol/Benzen		
-6.42				0.83
	-0.97			0.65
		-0.66		0.67
			4.7	0.85
-3.53			2.91	0.92
		Isoropanol/Hexane		
-8.19				0.62
	0.55			0.30
		0.19		0.16
			-1.29	0.52
-15.06	1.54			0.98

4. Conclusions

Our results about the solvent-solvent and solute-solvent interaction in the Lycopene extraction process by three binary mixtures show:

1- In Ethanol/Ethyl-acetate mixture Sp and β show the best correlation. By increasing the Ethanol fraction the solvent-solvent interaction increase causes the Lycopene extraction to decrease.

2- Extraction in the Isopropanol/Hexane, Absorption had the best agreement with Sp and α parameters. The maximum extraction point and α parameter in the 80% of Hexane represented the more effect of the α parameter in extraction in this mixture.

3- In the Isopropanol/Benzene, Sp and π* with negative and positive coefficients showed the best correlation versus extraction. The maximum extraction and the π*parameter in the 70% of benzene show the new complex of Isopropanol with more π*- π* interactions between this complex and Lycopene.

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