

# UV-vis Investigation of $\beta$ -Carotene in Presence of AOT/n-Heptane, Cyclohexane, Tetrahydrofuran/Water System

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**Abstract:** The precise measurements of  $\beta$ -carotene absorbance in presence of AOT/solvent/water systems at 25°C by UV-vis technique are reported. The solvents selected were: n-heptane, cyclohexane, and tetrahydrofuran. The concentrations of AOT [Aerosol-OT (sodium bis(2-ethylhexyl) sulfosuccinate)] were varied from 0.01 to 0.2mol/kg.  $\beta$ -carotene concentration in quvette during UV-vis spectrum registration was not the same in each solvents because of different absorption intensity depending on the solvent. Water concentration in the studied systems was defined by R parameter according to relation:  $R=[H_2O]/[AOT]$  and was equal 0; 5; 10 both in n-heptane and cyclohexane, and 0; 10 and 20 in tetrahydrofuran.

Obtained results showed a noticeable decreasing of  $\beta$ -carotene absorbance as a function of AOT concentration. There is evidenced red shift of  $\beta$ -carotene absorbance depending on the solvent in the following order: tetrahydrofuran >cyclohexane>n-heptane. Presented results show that  $\beta$ -carotene absorbance decreased with water concentration in the system. It had been happened because the water molecules located in the core of micelles make its curvature bigger and the hydrocarbon chains forming palisade layer in AOT reversed micelles get more space. As a consequence  $\beta$ -carotene molecules penetrated this layer dipper comparing to systems without water.

**Keywords:**  $\beta$ -carotene, AOT, n-heptane, cyclohexane, tetrahydrofuran, UV-vis.

## 1. INTRODUCTION

In the broad area of membrane chemistry reversed micelles have been used in the investigations of proteins solubilization in apolar solvents [1]. A long time ago inverted micellar structures were found in natural membranes [2]. Recently, "synthetic" reversed micelles have been used for mimicking some simple aspects of domain structure of bio-membranes. Amphiphilic molecules (surfactants) dissolving in organic solvents are capable of self-organization to form spheroidal aggregates. From the structural point of view amphiphilic molecules consist of the polar or charged head-groups (hydrophilic part) and hydrocarbon chains (hydrophobic part). Depending on the solvent polarity they form normal or reversed micelles. Among many surfactants that are suitable to form the reversed micelles in non-polar solvents AOT is one of the widely studied. There is a many papers regarding its properties and structure. Some of them belong to very often cited [3,4]. The ability of AOT molecules for aggregating results from the combined action of spatially separated hydrophilic and hydrophobic moieties of this surfactant. Such aggregates are built as inner cores occupied by the hydrophilic head-groups coated by the hydrocarbon tails which are extended into the bulk of non-polar solvent. It has been shown

that the peculiar capacity of AOT reversed micelles for a large amount of water depends on the surrounding non-polar medium (solute) and temperature [5]. Water is readily soluble in the polar core of such micelles. The amount of water enclosed in the micelles interior is characterized by R parameter defined as:  $R= [H_2O]/[AOT]$  [6]. The low R values ( $R<10$ ) promotes structures in which water molecules are strongly bounded to the AOT head groups. Contrary, at higher  $[H_2O]/[AOT]$  ratios ( $R>20$ ) free water in the micelles interior is predominant. The last structures are characteristic for swollen reversed micelles solutions [7,8]. The ionic nature of interior of AOT micelle easy dissolves water which, as it was mentioned previously can exist as water bounded to the surfactant polar groups or so-called "water pools" [5]. It is known [9] that micelles as well as reversed micelles and two-layer membrane cells in the vital organisms are distinguished. From the structural point of view systems containing AOT reversed micelles can be considered as a very simple model of the domains in natural biological membranes.

Antioxidants play a very important role in course of chemical reactions. In fact they defense organisms against damaging oxidizing reactions effects. The most popular natural antioxidant is  $\beta$ -carotene naturally occurring in many vegetables and fruits.  $\beta$ -carotene molecule has 11 conjugate double bonds in the hydrocarbon chain. From the structural point of view,  $\beta$ -carotene molecule consists of two retinal molecules. As

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a reagent  $\beta$ -carotene can interact with other dietary antioxidants like vitamin E and C [10].

The UV-vis spectra of  $\beta$ -carotene were found to be solvent-dependent and the absorbance of  $\beta$ -carotene in n-hexane is larger than in methanol, and increased linearly with concentration increasing. Contrary to n-hexane, in methanol  $\beta$ -carotene showed a non-linear concentration effect [11]. As a chemical compound,  $\beta$ -carotene is sensitive to light, heat and oxygen, making it necessary to store stock solution in screw-top amber vials, under nitrogen, and on ice. Tan and Soderstrom [12] found the absorbance of  $\beta$ -carotene standard solution with the maxima 448 nm. The relative solubility, stability, and absorptivity of  $\beta$ -carotene were already determined in 18 organic solvents [13].

Knowledge on the antioxidant solubility in reversed micelles and their interactions can help to get to know and understand the analogous processes in biological membranes in a very simple way.  $\beta$ -carotene is not soluble in water but is soluble in non-polar solvents and fatty acids. Taking in account this property one may expect that  $\beta$ -carotene will be solubilized in the palisade layer of AOT reversed micelles formed with non-polar hydrocarbon chains. This hypothesis has been evidenced using calorimetric technique [14]. Wilczura et al., [14] explained the influence of hydrocarbon chain length of n-alkanes used as a solvents in the systems  $\beta$ -carotene/n-alkane/AOT/water as well. Still open question is "What is the solvent structure influence on  $\beta$ -carotene interactions with AOT reversed micelles"?  $\beta$ -carotene molecule, because of conjugated double bonds show a well documented UV-vis absorption bands [12,13]. As it was mentioned Zang *et al.*, have studied absorbance changes of  $\beta$ -carotene in n-hexane and methanol [11].

As far as we know there is not many works in the literature dealing of  $\beta$ -carotene with the surfactants [14-16] and generally there is lack of information about the  $\beta$ -carotene/solvent/AOT/water systems. In this paper

we focus on investigation of the solvent effect on  $\beta$ -carotene UV-vis spectrum at 25°C in solvents differing with structure: n-heptane, cyclohexane, and tetrahydrofuran. We also tried to answer the question how is the influence of AOT reversed micelles on  $\beta$ -carotene UV-vis spectrum in selected solvents.

The aim of the present work is to analyze the influence of concentrations and amount of water to AOT by UV-vis method at 25°C with  $\beta$ -carotene in n-heptane, cyclohexane, and tetrahydrofuran.

## 2. EXPERIMENTAL

Sodium bis(2-ethylhexyl)sulfosuccinate (AOT 99%) was from Sigma-Aldrich Steinheim, Germany, and was dried under vacuum at 25°C,  $\beta$ -carotene (95%) was from Sigma – Aldrich, Steinheim, Germany and used without further purification and solvents n-heptane, cyclohexane and tetrahydrofuran were purchased from POCH, Poland for spectroscopy quality. Additionally they were checked by UV-vis measurements before using. Water was of bidistilled quality.

The investigated solutions were prepared gravimetrically at 25°C and molalities were converted to molarities using solvents densities at 25°C. AOT solutions with concentrations 0.2 mol/kg were used as stock solutions. They were diluted with n-heptane, cyclohexane, and tetrahydrofuran suitable to obtain a series of concentrations of the surfactant in a given solvent – all above the CMC (critical micelle concentration). Before all investigations CMC for AOT reversed micelles formation in selected solvents was determined according to procedure described earlier [17] and compared with literature (Table 1). Each solution before UV-vis measurement was stirred 24 hours on magnetic stirrer. In a case of  $\beta$ -carotene solutions special procedure was employed. Solutions were prepared in presence of argon and because of its difficult solubility in selected solvents were sonicated 30 min in ultrasonic bath. After sonication  $\beta$ -carotene

**Table 1: CMC for AOT Reversed Micelles Formation in n-Heptane, Cyclohexane and Tetrahydrofuran, Measured Data and Literature**

solvent	measured value [mM]	literature data [mM]
n-heptane	0.42 (UV-vis)	0.54 <sup>a</sup> (UV-vis)
cyclohexane	0.31 (refraction)	0.95-1.1 <sup>b</sup> (UV-vis)
tetrahydrofuran	9.33(UV-vis) 12.1 (refraction)	-----

<sup>a</sup>18; <sup>b</sup>19.

solutions were filtered through Millex-LCR, 0.5 micrometer filter to obtain transparent liquid. Finally solutions were protected against light and air very carefully.

For all studied systems  $\beta$ -carotene absorption spectra were recorded in the  $\lambda$  range 300-600nm at 25°C with the lights turn off using Varian UV-vis Spectrophotometer (Model Cary 50 Conc.) single beam equipped with 1 cm length quartz cuvette. UV measurements were realized with the following procedure. Before each measurement base line was registered for suitable AOT/solvent system (with or without water) and afterwards a  $\beta$ -carotene solution in the same solvent was added. After adding  $\beta$ -carotene solution to the AOT once the whole liquid volume was stirred very carefully. The absorption band was registered as a function of AOT concentration. For the systems containing water the relevant amount of water added to the surfactant solution was calculated for a given R parameter value which was between 0 and 20. Parameter R was defined as water to AOT molarities ratio. The final  $\beta$ -carotene concentration in a quartz cuvette was  $1.36 \times 10^{-3}$  M in cyclohexane,  $5.40 \times 10^{-4}$  M in n-heptane and  $6.01 \times 10^{-3}$  M in tetrahydrofuran. Such concentrations involved obtaining a well-marked  $\beta$ -carotene absorption band in selected solvents. We also investigated the solvents effects on  $\beta$ -carotene absorption band. In these experiments  $\beta$ -carotene concentration was equal  $5.40 \times 10^{-4}$  M and was the same in all three solvents.

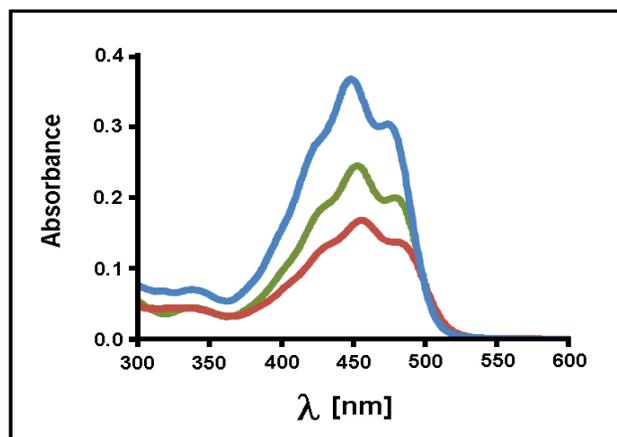
### 3. RESULTS AND DISCUSSIONS

#### 3.1. Solvents Effects on $\beta$ -Carotene

The concentrations of AOT solutions used in present study were above critical micelle concentration (CMC). Selected solvents were non-polar, as n-heptane, cyclohexane and tetrahydrofuran (with dielectric constants suitable: 2.00, 2.02 and 7.52) and because of that, surfactant formed reversed micelles in all studied systems [7].

The absorption spectra of  $\beta$ -carotene show an intense band between 360nm and 550nm [13]. In our

studies spectra of  $\beta$ -carotene were found to be solvent dependent (Figure 1). The highest absorbance was observed for  $\beta$ -carotene in n-heptane lower for cyclohexane and the lowest for tetrahydrofuran. Moreover, depending on the solvent a small shift of the maxima into a longer wave length (red shift) has been noticed in the following order: tetrahydrofuran >cyclohexane>n-heptane. The numerical values of peaks and valleys and suitable wave length are given in Table 2.



**Figure 1:** Absorption spectra for  $\beta$ -carotene in n-heptane – blue (top graph), cyclohexane – green (middle graph) and tetrahydrofuran - brown (bottom graph).

As is evidence in Figure 1,  $\beta$ -carotene absorption band show two maxima at 451nm (higher) and 477nm (lower). Depending on the solvent both peaks are sharp (as in a case of n-heptane) or not like in tetrahydrofuran. The shapes and intensities of  $\beta$ -carotene absorption bands obtained for studied solvents are presented in Figure 1.

#### 3.2. AOT Reversed Micelles Effects on $\beta$ -Carotene Absorbance

The process of AOT reversed micelles formation, as well as, structure of these micelles have been studied extensively, both experimentally and theoretically [3, 20, 21]. Kotlarchyk *et al.*, [20], Zulauf and Eike [22] and Assih *et al.*, [23] determined a spherical geometry of AOT reversed micelles in non-polar solvent. The size of

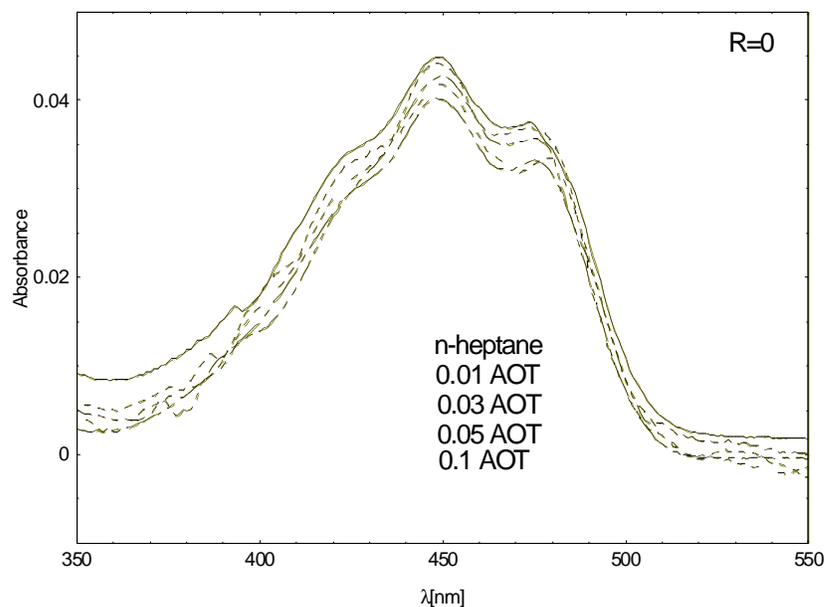
**Table 2:  $\beta$ -Carotene Spectral Features Depending on the Solvents**

Solvent	P <sub>1</sub> [nm]	Absorbance	V <sub>1</sub> [nm]	Absorbance	P <sub>2</sub> [nm]	Absorbance
n-heptane	448.0	0.3955	467.0	0.3278	474.0	0.3314
cyclohexane	452.0	0.2458	472.0	0.1967	478.0	0.2006
tetrahydrofuran	456.0	0.1686	477.0	0.1379	-	-

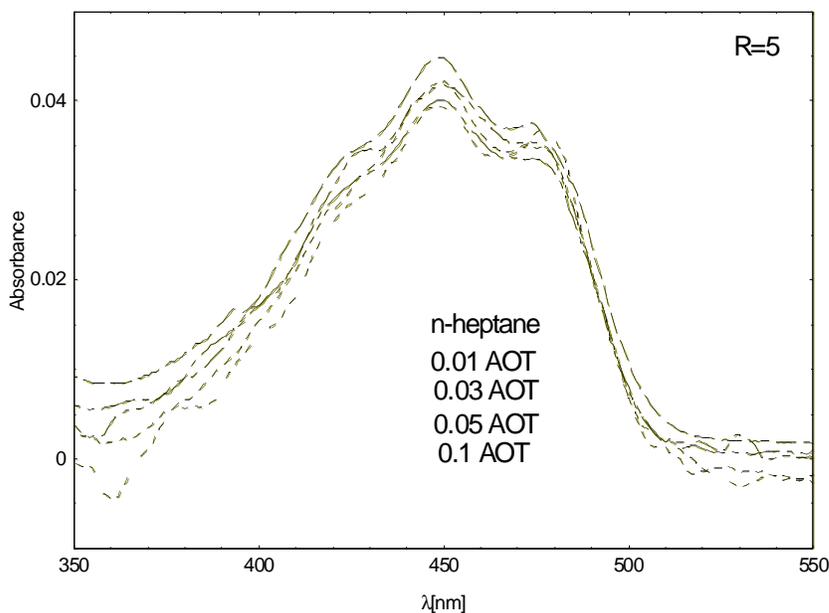
such micelles strongly depends on water contents defined by R parameter as a ratio of water to surfactant concentrations. When R is lower or equal 15 in solutions are present single AOT micelles. When R is higher than 15, the surfactant molecules form aggregates termed microemulsion and these dispersions contain both droplets and random bicontinuous structures [24]. As it was mentioned AOT, reversed micelles solutions are thermodynamically stable and transparent and therefore are widely used

as a systems mimicking in a simple way a properties of domains in natural membranes.

Moreover, what is very important, such systems because of their transparency can be easily used in UV-vis studies. In our experiments AOT concentrations were above CMC and R parameter values were 0; 5 and 10 in n-heptane and cyclohexane, and suitable 0; 10 and 20 in tetrahydrofuran. It means that only in one system was dispersion containing droplets and



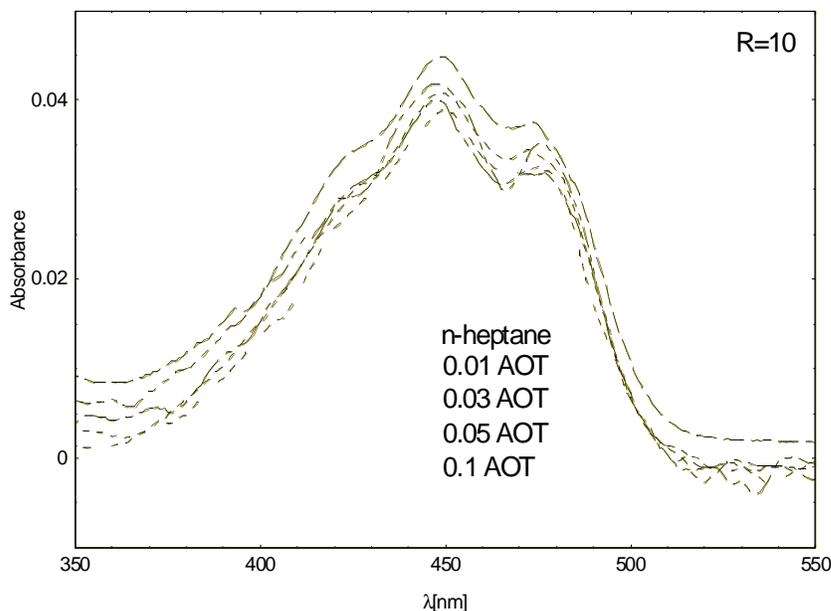
**Figure 2:** Absorption spectra for  $\beta$ -carotene with wavelength in n-heptane and different concentrations of AOT solution in n-heptane (R=0).



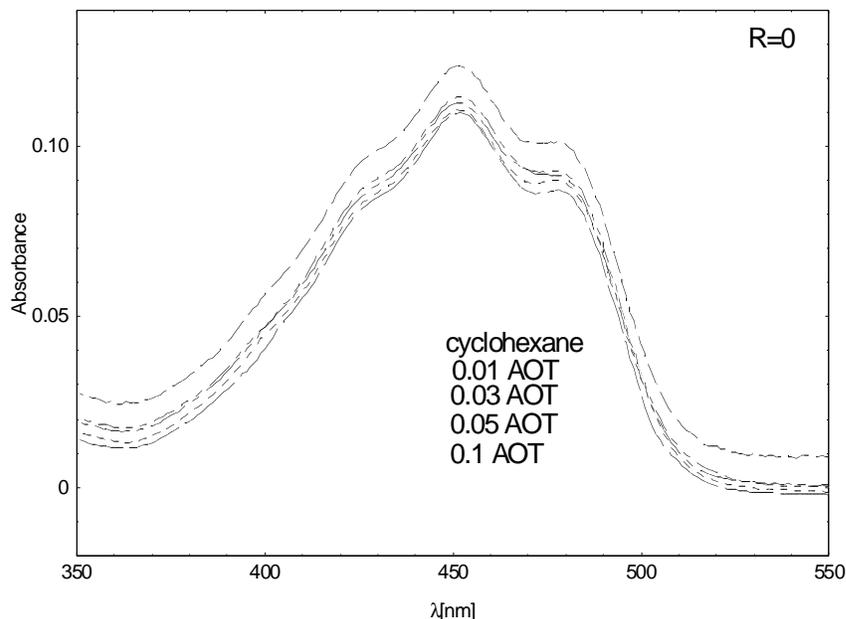
**Figure 3:** Absorption spectra for  $\beta$ -carotene with wavelength in n-heptane and different concentrations of AOT solution in n-heptane (R=5).

bicontinuous structure in all other was solutions containing reversed micelles [24]. In each experiment base line was recorded for AOT solution, and after that  $\beta$ -carotene solution was added. Before UV spectrum registration the mixture was stirred on magnetic stirrer 5 minutes. During this time  $\beta$ -carotene molecules interacted with AOT reversed micelles and finally distributed between organic bulk solvent and reversed micelles.

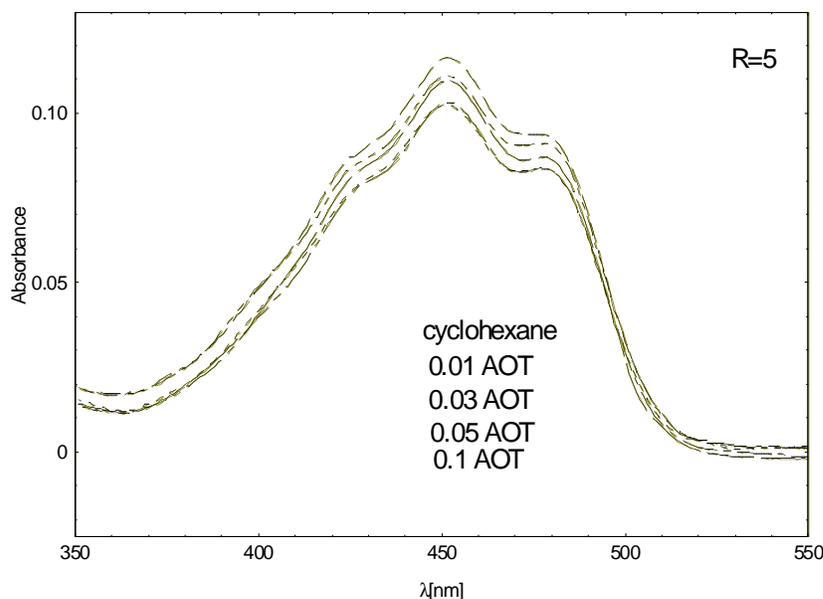
In Figures 2-10 are presented the dependence of absorbance with wavelength of  $\beta$ -carotene in n-heptane, cyclohexane and tetrahydrofuran on AOT concentration with different R parameter respectively. In Figure 2 for R=0, the absorption band of  $\beta$ -carotene in n-heptane is sharp for peak and valley where as decreases with AOT concentrations till  $0.03 \text{ mol.kg}^{-1}$  and then slightly increases and then monotonously decreases. In Figure 3 for R= 5, the absorption band are lower than for R=0 (Figure 2) but higher than for



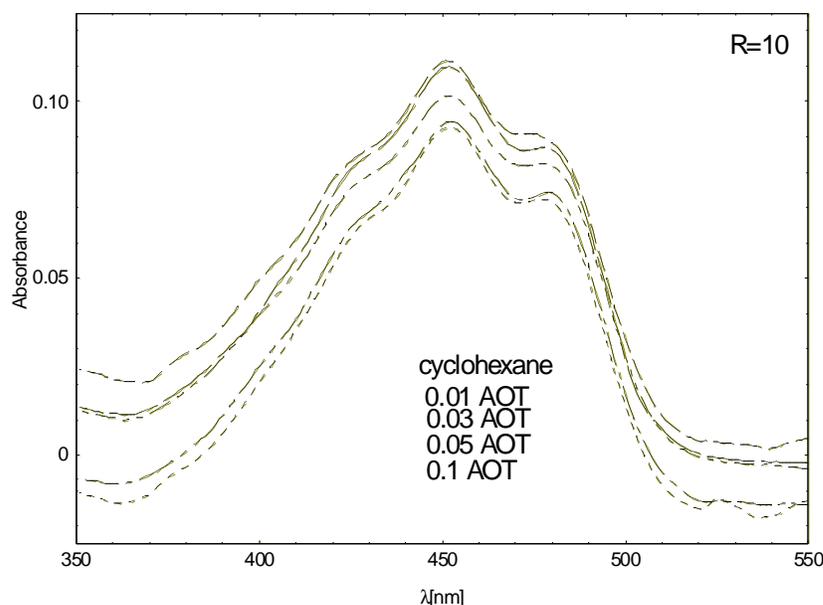
**Figure 4:** Absorption spectra for  $\beta$ -carotene with wavelength in n-heptane and different concentrations of AOT solution in n-heptane (R=10).



**Figure 5:** Absorption spectra for  $\beta$ -carotene with wavelength in cyclohexane and different concentrations of AOT solution in n-heptane (R=0).



**Figure 6:** Absorption spectra for  $\beta$ -carotene with wavelength in cyclohexane and different concentrations of AOT solution in n-heptane ( $R=5$ ).



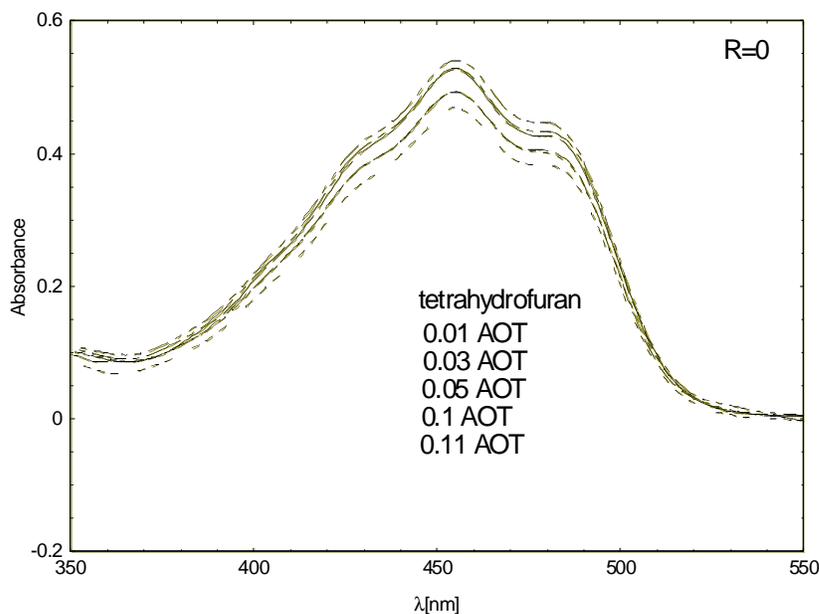
**Figure 7:** Absorption spectra for  $\beta$ -carotene with wavelength in cyclohexane and different concentrations of AOT solution in n-heptane ( $R=10$ ).

$R=10$  (Figure 4) and then somehow similar trends for absorption band like for  $R=0$  (Figure 2).

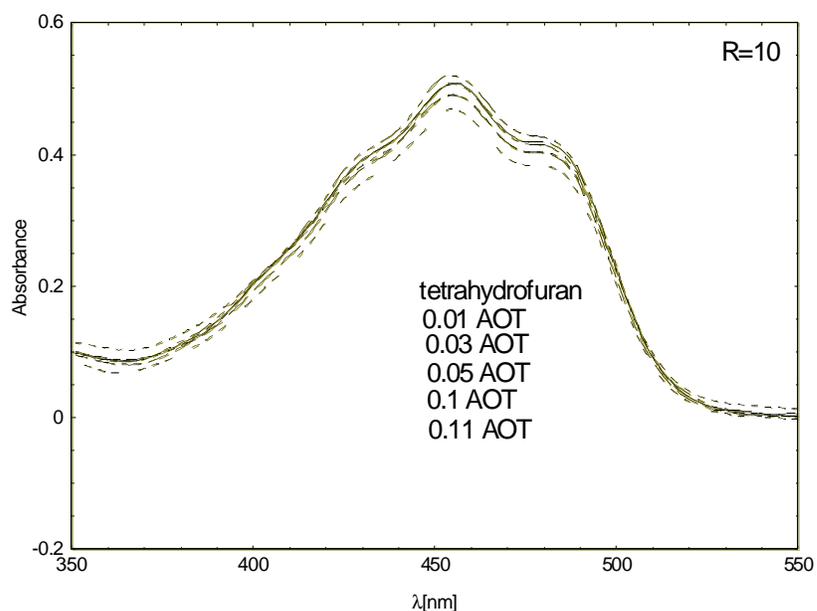
In Figure 5 for  $R=0$ , the absorption band of  $\beta$ -carotene in cyclohexane is sharp for peak and valley where as increases with AOT concentrations upto  $0.01 \text{ mol.kg}^{-1}$  and decreases till  $0.03 \text{ mol.kg}^{-1}$  and then slightly increases. In Figure 6 for  $R=5$ , the absorption band are lower than for  $R=0$  (Figure 5), but higher than for  $R=10$  (Figure 7) and then somehow similar trends for absorption band for  $R=0$  (Figure 5).

In Figure 8 for  $R=0$ , the absorption band of  $\beta$ -carotene in tetrahydrofuran is sharp for peak and valley where as increases with AOT concentrations up to  $0.1 \text{ mol.kg}^{-1}$  and then decreases. In Figure 9 for  $R=10$ , the absorption band are lower than  $R=0$  (Figure 8) but higher than for  $R=20$  (Figure 10) and then somehow similar trends for absorption band like for  $R=0$  (Figure 8).

In three solvents system, absorption band of  $\beta$ -carotene decrease in presence of water and this trend



**Figure 8:** Absorption spectra for  $\beta$ -carotene with wavelength in tetrahydrofuran and different concentrations of AOT solution in n-heptane ( $R=0$ ).



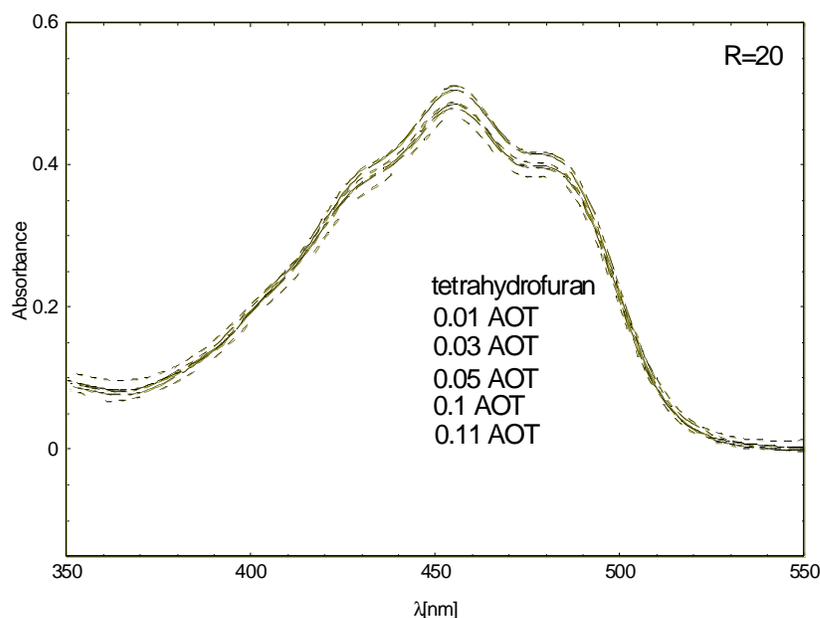
**Figure 9:** Absorption spectra for  $\beta$ -carotene with wavelength in tetrahydrofuran and different concentrations of AOT solution in n-heptane ( $R=10$ ).

is stronger with amount of water increasing in the system. The reason is that the size of AOT reversed micelle increases with increasing  $R$  [25], and because of that hydrocarbon chains forming palisade layer gets more space and  $\beta$ -carotene molecules easier penetrate and have a good opportunity for stronger interactions with the polar heads of AOT.

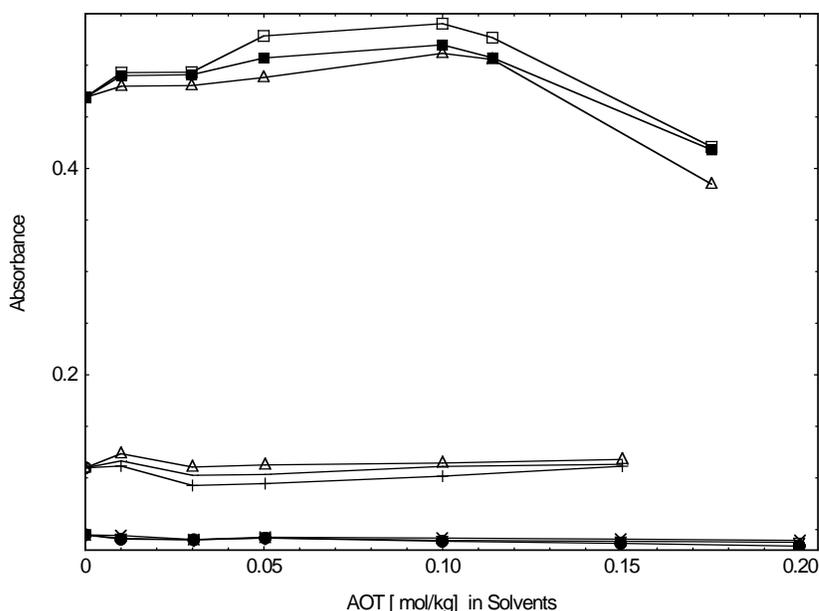
Figure 11 is the overall graph between the maximum absorbance value of  $\beta$ -carotene with AOT concentration in absence and presence of water

containing three selected solvents namely: tetrahydrofuran, cyclohexane and n-heptane. Among them the total absorbance of  $\beta$ -carotene in tetrahydrofuran systems in both cases in absence and presence of water have the highest values because of the highest  $\beta$ -carotene concentration in this solvent comparing to cyclohexane and n-heptane.

Generally, in all investigated systems has been observed a dependence of  $\beta$ -carotene absorbance on surfactant concentration as well as with water contents.



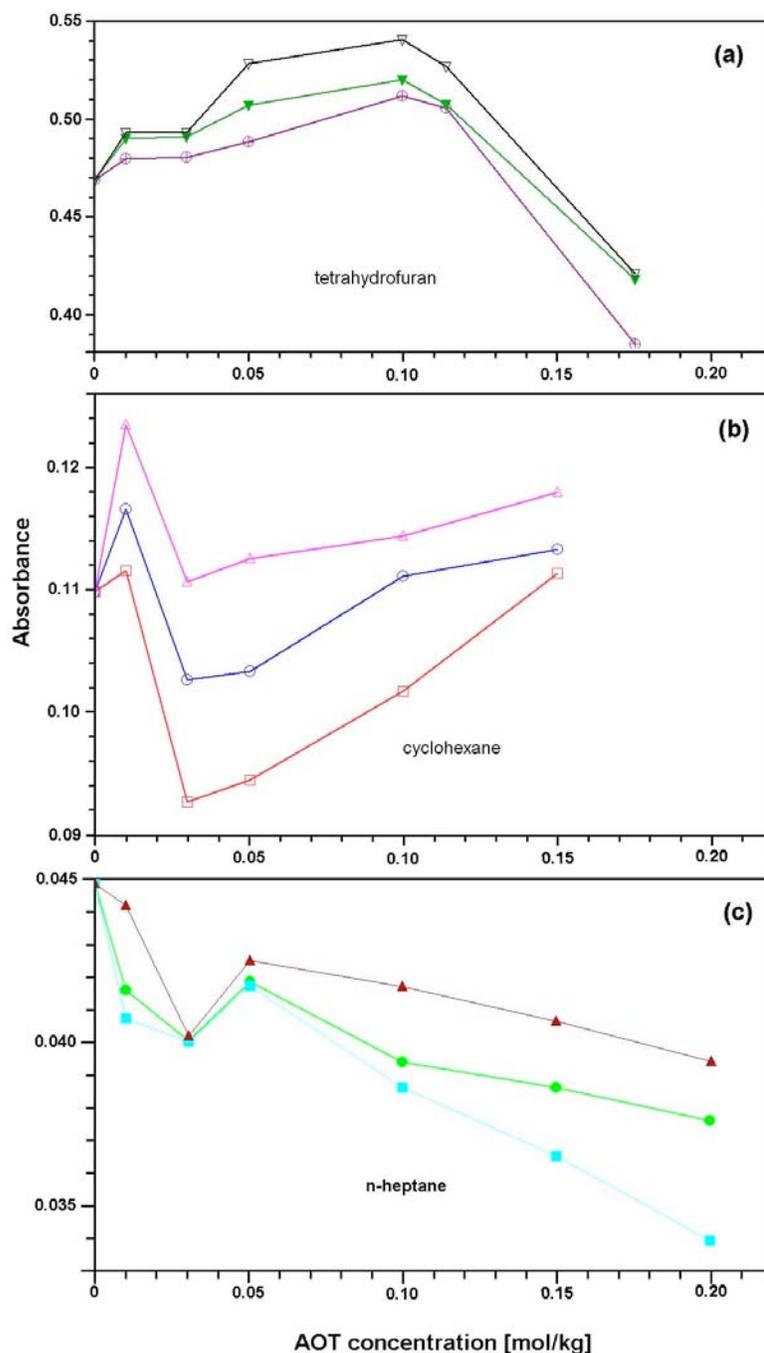
**Figure 10:** Absorption spectra for  $\beta$ -carotene with wavelength in tetrahydrofuran and different concentrations of AOT solution in n-heptane ( $R=20$ ).



**Figure 11:** Absorbance of maximum peak for  $\beta$ -carotene with AOT concentrations in tetrahydrofuran (top three graphs,  $R=0, 10, 20$ ), cyclohexane (middle three graphs,  $R=0, 5, 10$ ) and n-heptane (bottom three graphs,  $R=0, 5, 10$ ).

Keeping enough precise Y scale (Figure 12) is possible to see changes of  $\beta$ -carotene absorbance in studied solvents. For the system with tetrahydrofuran  $\beta$ -carotene absorbance increases for AOT concentration between 0 and 0.1 M, than decreases for AOT in the range 0.1 and 0.18 M (Figure 12a). For the system with cyclohexane  $\beta$ -carotene absorbance increases for AOT between 0 and 0.01 M, than decreases for AOT concentration between 0.01 and 0.03 M and finally increases for AOT concentration range 0.03 and 0.15

M (Figure 12b). For the system with n-heptane  $\beta$ -carotene absorbance decreases for AOT between 0 and 0.03 M, than increases for AOT between 0.03 and 0.05 M and again decreases for AOT concentration between 0.05 and 0.2 M (Figure 12c). The water concentration influence on  $\beta$ -carotene absorbance has been noticed with the same trends in all investigated systems, namely while R parameter increasing  $\beta$ -carotene absorbance decreasing. Obtained data show that in all investigated systems AOT inverted micelles



**Figures 12:** (a) Absorbance (peak) as AOT concentration for for  $\beta$ -carotene with AOT concentrations in tetrahydrofuran with R=0(opposite triangles), R= 10( closed opposite triangles), and R=20( crossed circles); (b) Absorbance (peak) as AOT concentration for  $\beta$ -carotene with AOT concentrations in cyclohexane with R=0(triangles), R=5 ( circles), and R=10( squares); (c) Absorbance (peak) as AOT concentration for for  $\beta$ -carotene with AOT concentrations in n-heptane with R=0(closed triangles), R=5 ( closed circles), and R=10( closed squares).

interact with  $\beta$ -carotene and as results have been registered changes in  $\beta$ -carotene absorption bands.

#### 4. CONCLUSION

Experimental results for the UV-vis measurement of  $\beta$ -carotene in presence of AOT/n-heptane, cyclohexane and tetrahydrofuran/water at 25°C have

been presented as a function of AOT concentration and wave lengths. It was found the solvent effect on  $\beta$ -carotene absorption band intensity. The highest values were noticed for n-heptane, the lowest for tetrahydrofuran. The highest absorbance was noted for  $\beta$ -carotene in tetrahydrofuran with AOT concentration in absence and presence of water in comparison to

cyclohexane and n-heptane. In all investigated systems the intensity of  $\beta$ -carotene absorption bands decreases with water concentration increasing. This behaviour suggests that  $\beta$ -carotene molecules dipper penetrate a palisade layer of AOT reversed micelles in presence of water comparing to systems without water.

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