

THE VISIBLE SPECTRA OF SELF-ASSEMBLED AGGREGATES OF β -CAROTENE

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INTRODUCTION

Carotenoids are important group of multifunctional naturally occurring pigments used in several photosynthetic organisms where they act as molecules having important photophysical properties such as light harvesting and photoprotecting functions. They are also efficient quenchers of singlet oxygen and free radicals, where they interrupt the chain propagation reaction in oxidative degradation (Olsina *et al.*, 2012). The diverse functions of carotenoid molecules are directly related to their excited state properties. Carotenoids aggregation behavior is a common phenomenon in biological systems and it affects the functions of naturally occurring dyes. The formation of dimeric and polymeric aggregates of carotenoids can alter the photophysical properties of carotenoids such as absorption, reflection and emission of light.

The excited state dynamics and absorption spectra of monomeric carotenoids and their radical cations have been studied extensively by several researchers (Amarie *et al.*, 2010, Alwis *et al.*, 2015; Krawczyk, 1998). The absorption spectra show the characteristic, strongly allowed $S_0 \rightarrow S_2$ electronic transition with a vibronic structure corresponding to the 0–2, 0–1 and 0–0 transitions in the 400–500 nm regions. This strongly allowed transition is responsible for the bright red-orange colors to the carotenoid containing foods.

β -carotene is $C_{40}H_{56}$ hydrocarbon carotenoid consists of eleven conjugated double bonds and has β -ionone rings at the two ends of the structure (Figure 1).

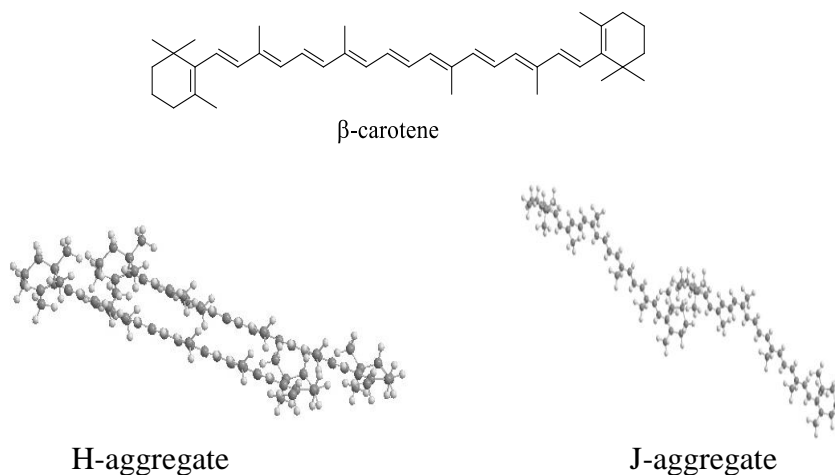


Figure 1. Molecular structures of β -carotene, J-, H-aggregates

The formation of aggregated structures in hydrated solvents is important both in natural and artificial systems. Typically carotenoids tend to form aggregates when present in lipid-bilayers and carotenoid-protein interacts in natural systems. In artificial systems carotenoids are usually form H-aggregates when deposited on conducting or semiconducting surfaces such as on TiO_2 nanoparticles (Olsina *et al.*, 2012). The aggregates formed in hydrated solvents, can be clearly distinguished by their absorption spectra. It is well known (Billsten *et al.*, 2005) that two types of aggregates are formed according to their assembling modes. The

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H-aggregate (card-pack) where the conjugated chains are closely packed parallel to each other. The other type of aggregate is J-aggregate, in which the conjugated chains are organized in a head-to-tail manner. The formation of either J- or H-aggregate depends on three factors such as (I) initial concentration of β -carotene (II) ethanol: water ratio and (III) pH of the solution.

In the present work we have investigated the molecular aggregates formed by nonpolar, hydrocarbon carotenoid, β -carotene. We studied the spectroscopic properties of self-assembled aggregates of β -carotene in ethanol/water mixtures at different pH values which have not been studied to our knowledge.

METHODOLOGY

β -carotene of a purity $\geq 93\%$ was purchased from Sigma Chemicals (PVT) Ltd. β -carotene was dissolved in absolute EtOH to achieve samples with initial concentration 50 and 100 μM . The UV-vis absorption spectra of the samples were measured with a Perkin-Elmer Lambda 35, double beam UV-Vis spectrophotometer. Samples were held in a quartz cuvette with 1 cm path length. Deionized water was used throughout this experiment.

The mixtures of ethanol/water were prepared by adding water to a final content of 0 %, 25%, 35%, 45%, 60%, 70% and 80% (v/v) to a stock solution of β -carotene in EtOH having initial concentration of 100 μM . The UV-vis absorption spectra were obtained for all the above solutions at room temperature. The formation of aggregates were also analyzed by absorption spectra obtained for ethanol and water mixtures by the addition of water to a final content of 80% to a stock solution of β -carotene in EtOH having initial concentration of 50 μM .

The pH dependence of aggregates was investigated by varying the pH of the β -carotene solution having initial concentrations of 100 and 50 μM separately. The pH dependence was made by addition of water with pH 4, 7, 8 and 10 to samples of β -carotene in EtOH. The required pH values were obtained by adding strong HCl/NaOH.

RESULTS AND DISCUSSION

UV-VIS ABSORPTION SPECTROSCOPY

The formation of aggregates of β -carotene in EtOH/water mixtures was studied by UV-vis

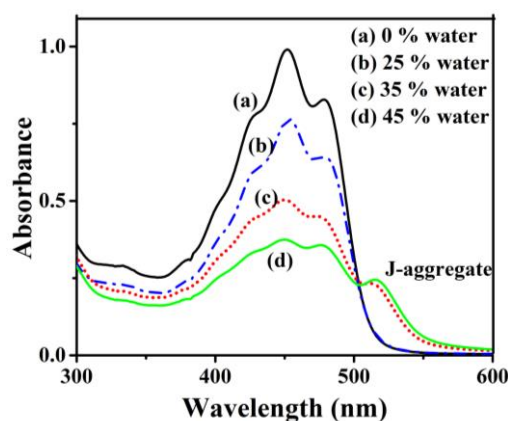


Fig. 2 UV-vis absorption spectra of J-aggregates of β -carotene formed with the addition of water. Initial concentration of β -carotene in EtOH 100 μM .

spectral shifts and the shapes of the spectra obtained at room temperature. It was observed that the strongly allowed $S_0 \rightarrow S_2$ electronic transition of β -carotene monomer with vibronic features corresponds to 0-2, 0-1 and 0-0 transitions occurred in the 400-500 nm region with maximum absorption at 452 nm. Analysis of UV-vis spectral data of β -carotene aggregates show that aggregation behavior of carotenoids in hydrated solvents depends on several factors. Effects of EtOH:water ratios, initial concentration of β -carotene and the pH of the solution on aggregation behavior are some of the factors determined during the present work.

The formation of J- or H-aggregates with varying ethanol:water ratios was determined by analyzing the UV-vis

spectra obtained at different water content in 100 μM β -carotene in EtOH. The results shown in (Figure 2.) indicate that addition of water up to 25 % has not observed any difference in the

absorption spectrum even its vibronic features were retained. The appearance of new red shifted peak around 510-520 nm region with decrease of resolution of the vibrational bands was appeared with increasing the water content upto 35-45 %. The similar type of spectral shift was also observed by (Olsina *et al.*, 2012) for the carotenoid astaxanthin in dimethylsulfoxide solutions.

This red shifted band observed at high initial concentration of β -carotene and at 35-45 % water content could be assigned to the J-aggregates. However, further increasing water content from > 45 %, observed the relative amplitude of red band increases slightly with disappearance of vibrational features of the monomer, clearly indicates the stabilization of the J-aggregates at this conditions (Billsten *et al.*, 2005).

The H-aggregates of β -carotene was distinguished from the monomer by their UV-vis absorption spectra (Figure 3). UV-vis spectra obtained at 50 μ M initial concentrations of β -carotene in EtOH and the addition of 80 % water to the mixture. A new absorption band was observed at \sim 437 nm which is blue shifted compared to the monomer absorption. This band was appearing with the addition of 80 %

water to the 50 μ M concentration of β -carotene in EtOH could be assigned to the H-aggregates (Olsina *et al.*, 2012). However the analysis of band positions suggest that this H-aggregate consists of the strongly coupled H-aggregate band (\sim 437 nm) and a subpopulation of weakly coupled band (as a weak shoulder around \sim 510 nm) as shown by the Fig.3 (b) (dotted line). Therefore it can be suggested that at low initial concentration of carotenoids and low ethanol:water ratios is the preferred conditions for the formation of H-aggregate of β -

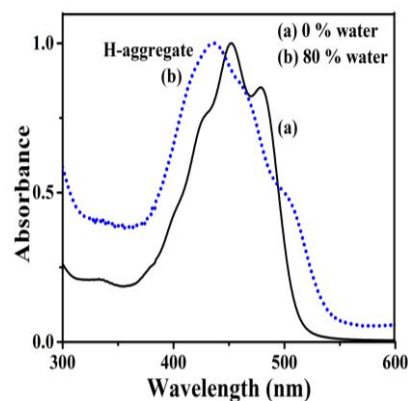


Fig. 3 UV-vis absorption spectra of H-aggregates of β -carotene formed with the addition of 20:80 (v/v) (EtOH/water). Initial concentration of β -carotene in EtOH 50 μ M.

carotene. In general, a high initial concentration of β -carotene and ethanol:water ratios are the key factors to produce J-aggregate of β -

carotene. Another important factor that determines the type of aggregate form in EtOH is the pH of the solution. The effect of pH on the determination of either J- and H-aggregate formation was investigated by changing the pH of water added to EtOH solution of β -carotene.

In order to study the pH effect on J-aggregate formation, water having different pH values which was obtained by adding strong HCl/NaOH to β -carotene in EtOH solution with initial concentration of 100 μ M until the final concentration of water reaches to 40 % was analyzed. In this

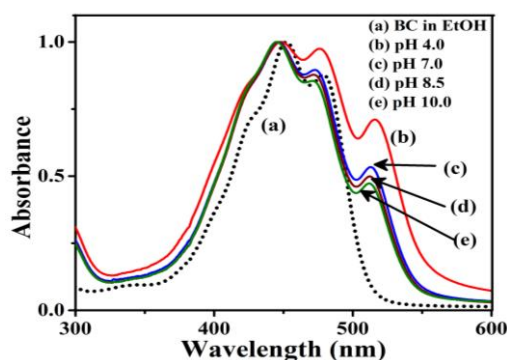


Fig. 4 UV-vis absorption spectra of β -carotene with the addition of 40% water at different pH values. Initial concentration of β -carotene in EtOH 100 μ M. (Normalized at 452 nm).

mixture addition of acidic pH (pH \sim 4) water, the formation of J-aggregate as the distinct peak at \sim 515 nm (Figure 4(b)) was observed. The appearance of a shoulder implies that a very low fraction of H-aggregates could also be formed. However the addition of water with pH \sim 7 and under more basic conditions (pH \sim 8 and 10) the magnitude of red band is decreased (Figure 4 (c,d,e)).

It was also observed that the variation of pH had little effect on the aggregation pattern beyond pH~7. The H-aggregation of β -carotene can be induced by adding water at different pH values to 50 μ M β -carotene in EtOH up to final concentration of 80%. When acidifying this mixture (pH~4) we could expect clearly to produce only the blue shifted band for H-aggregate as the dominant band. But significantly a high fraction of J-aggregate was also present at low pH (pH~4) value indicated by a peak observed (Figure 5(b)) ~ 515 nm.

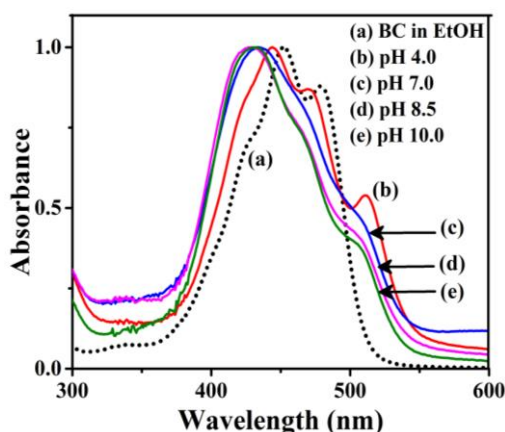


Fig. 5 UV-vis absorption spectra of β -carotene with the addition of 80% water at different pH values. Initial concentration of β -carotene in EtOH 50 μ M. (Normalized at 452 nm).

However at neutral and basic conditions the magnitude of the red band was disappeared and a distinct blue shifted peak at 437 nm was stabilized (Figure 5 (c,d,e)). Interestingly no significant change was observed in the spectra obtained at pH values beyond 7. The carotenoids such as β -carotene which

lack hydroxyl groups can be stabilized by other molecular forces such as π - π stacking or weak van der Waals interactions. In the case of β -carotene two carotenoid molecules are bound each other via weak hydrogen bond between the water molecules and π -electrons of the polyene chain (Wang *et al.*, 2012) and hence favorable for the formation of both aggregates at low pH.

CONCLUSION

Formation of both H-aggregates with blue shifted absorption, maximum around 437 nm and J-aggregates with red shifted absorption band peaking around 490 nm for β -carotene was observed. Formation of H-aggregate is possible under the low initial concentration of β -carotene and low ethanol: water ratios, whereas J-aggregates formed at higher initial concentration of β -carotene and higher ethanol: water ratios. Two β -carotene molecules are bound each other via weak hydrogen bond between the water molecules and π -electrons of the polyene chain and hence favorable for the formation of H-type aggregates. Therefore at low pH (pH ~ 4) both types of aggregates can generate irrespective of the concentration of β -carotene and ethanol: water ratio.

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