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Spectroscopic Study of β-Carotene

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Abstract

The spectroscopic study of bita carotene has been performed experimentally. A simple, rapid and cost efficient method has been utilized to extract required chemical (bita carotene) from natural carrot. Ethanol was used as organic solvent, water for the phase seperation and petroleum ether were used for purification purpose. This paper has aimed to get information related to electronic transition in some part of ultraviolet to visible region of electromagnetic spectrum. For observing absorption spectra, 400 nm to 500 nm excitatation wavelength were used in PC based double beam UV- Visible spectrometer. In analysis part of naturally extracted bita carotene from fresh carrot, we observe that conjugated chain in carotenoids absorbs visible (blue/green) part of electromagnetic spectrum and that is why Beta-carotene appears red-yellow as yellow colour get reflected back to us. And hence it has also been obtained that this symmetric molecule is a fluorescent chromophoric biomolecule. People have studied Raman spectra of bita carotene where in spectrum great enhancement has been observed in Raman signal in electronic transition region due to S_0 to S_2 transition which strongly support our result that bita carotene is a fluorescent chromophoric biomolecule having maximum electronic transition in visible (blue/green) region so appears yellowish red.

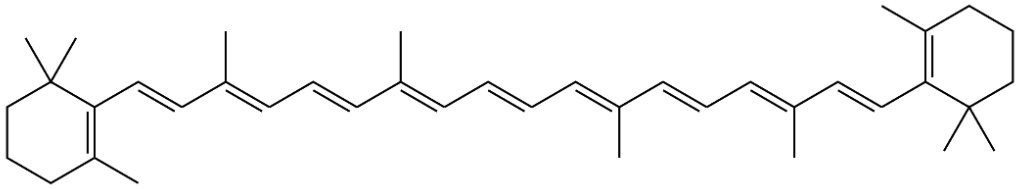
Keywords: UV-visible spectroscopy, Bita-carotene, Carrot, Electronic transition, Raman spectroscopy

1. Introduction

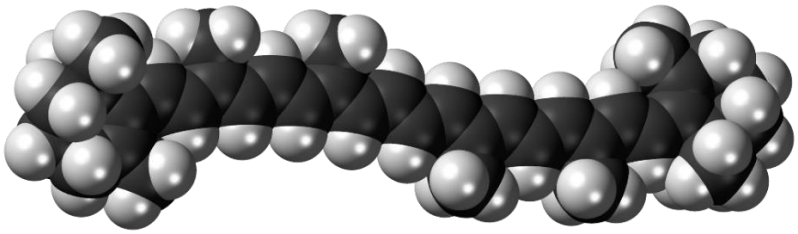
Beta carotene is one of the most important carotenoids and provitamins A precursor (retinol) so being used to prevent vitamin A deficiency. It cleaves to form two molecules of retinal, one of which is further metabolized to form retinoic acid and retinol. Beta carotene has three isomers alpha, bita and gamma out of which beta isomer is more active. It found mainly in carrot, spinach, vegetables, fruits, leaf and has antioxidant properties. Bita carotene has different structure and function from other carotenoids. it has been observed in in vitro study that bita carotene can also be act as a lipid radical scavenger and a singlet oxygen quencher [1].

Structure of bita carotene

Its chemical formula is $C_{40}H_{56}$, it consists of 2β-ionone rings at endpoints substituted with 40 carbon atoms in core structure of conjugated double bonds. All trans-β-carotene is the most important and suitable precursor of vitamin A [2].



(a)



(b)

Figure 1 (a) structure of Beta-carotene and 1(b) Molecular model of Beta-carotene.

2. Extraction of Bita-carotene from fresh Carrot (Sample preparation)

The extraction of beta carotene with organic solvent usually requires multiple extraction steps. We used ethanol as solvent. Sample were prepared in our Lab from naturally fresh Carrot purchased from local market of Prayagraj and properly cleaned in running fresh water. 150 gm fresh carrot were taken, cut into slices and crushed properly in a porcelain mortar. Crushed carrot had kept in a 1000 ml beaker. Then 450 ml ethanol were added in beaker slowly further 15 ml water were also added in beaker for the separation of phases. This mixture in beaker was kept in water bath at 65 to 70 ^{o}C for an hour and were shaken after each 15 minutes. 450 ml petroleum ether were taken in a separating funnel further water bathed carrot in ethanol mixture were added in same separating funnel. Within fraction of seconds two level produced and were separated as 50-50 by volume in separating funnel, upper level of liquid was collected which contained β-carotene solution in ethanol.

3. UV/Visible study

UV-Visible spectroscopy refers to absorption spectroscopy provides spectra of electronic absorption in Ultraviolet to visible region in electromagnetic spectrum. Samples having chromophore can be studied by UV-Visible spectroscopy.

In our study UV-Visible spectra of bita-carotene were recorded on PC based double beam spectrometer (Model 2202) with error of 0.5 in ethanol solution between absorption range 400 nm to 500 nm. In our more carefull observation we find biita carotene shows major part of absorption band in visible region (mainly green/blue) between 400-500 nm and shows much stronger absorption at about 450 nm in ethanol due to nine fully conjugated double bonds [3-4]. In UV-Visible spectra we also find two relatively weaker absorption bands at about 427 nm and 466 nm. The conjugated chain in carotenoids absorbs visible part that is blue and green colour of electromagnetic spectrum and so beta-carotene appears orang as red to yellow colour get reflected back to us.

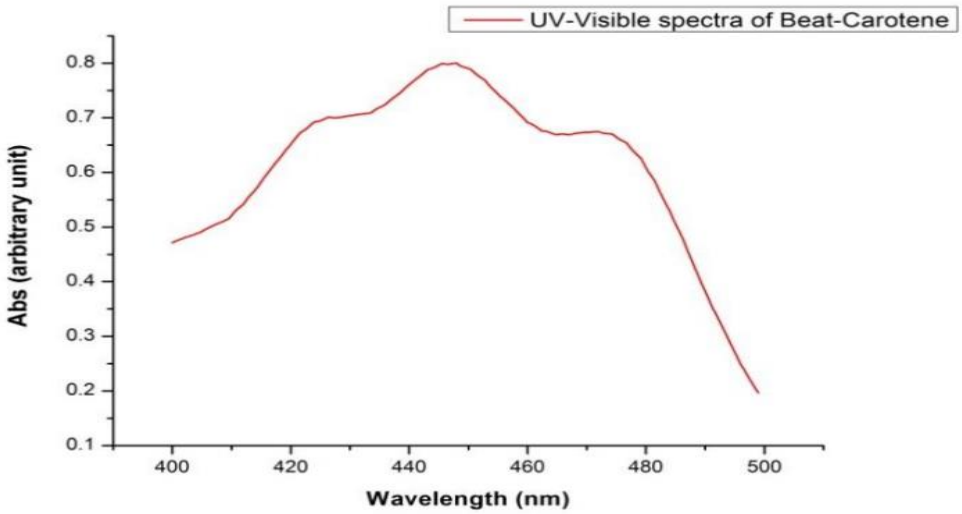


Figure 2 UV/Visible absorption spectra of Beta-carotene solution in Ethanol, wavelength (nm)

4. Raman study

Raman spectroscopy is one of the fastest and non-destructive analytical technique which provides an excellent method for identifying molecules, substances by unique fingerprint spectra [5]. In Raman spectroscopic technique frequency shift of inelastic scattered light from sample is measured when photon from incident light beam strikes onto molecule and produces scattered photon. If scattered photon has lower frequency than original incident photon in this case it is known as Stoke's Raman scattering and when scattered photon has frequency higher than incident photon then it is known as Anti stoke's Raman scattering [6].

Resonance Raman spectroscopy can be an efficient technique for analysing bita-carotene. In a recent study Raman spectra of bita-carotene were recorded using Lasers of different wavelengths 488 nm, 514 nm, 532 nm and 633 nm by L Lu et. al, when Laser energy approaches electronic transition from S_0 to S_2 state, there is enhancement in Raman signal and actual resonance Raman without self absorption has been observed. In the study Raman spectra of bita carotene appeared for all these wavelengths but maximum enhancement in Raman picks observed at 488 nm. Higher intensed Raman peaks at 1157 cm^{-1} and 1525 cm^{-1} corresponded to symmetric mode (C-C) and stretching mode (C=C) of bita-carotene respectively. Due to rocking motions of methyl groups in bita-carotene a smaller Raman peak observed at 1006 cm^{-1}. Some low intensed Raman peaks of bita-carotene observed at 2161 cm^{-1}, 2310 cm^{-1}, 2529 cm^{-1} and 2672 cm^{-1} at 488 nm and very less intensed at 514 nm but almost disappeared when Laser wavelength was greater than 532 nm. In this study bita-carotene were used to examine self absorption effect in resonance Raman spectroscopy. It is observed that for 532 nm excitation there appears less fluorescence and weaker self-absorpti on effect so 532 nm excitation Laser may be better for biological tissue study [7].

5. Applications/Benefits

The consumption of bita carotene rich food has been associated with numerous health benefit. Beta-carotene is being converted into vitamin A. vitamin A is essential for enhancing functions of body immune system, protects cornea (eye surface) improves night vision, promotes healing of wounds, illnesses [8]. Research havs shown that people having high blood levels of carotenoids may reduce the risk of macular degradation upto 35 percent [9]. Beta -carotene have significant antioxidant activity [10-11] as it neutrilize

the reactive oxygen species including free radicals. Due to antioxidant effect of beta-carotene, it reduces oxidative stress and oxydative damage to DNA, improves memory and cognitive function, reduces symptoms of Alzheimer's disease and age related cognitive decline, protects skin against UV radiation from the sun and maintain skin he3alth [9]. Recent research supports Beta-carotene has been identified to play roles in adipogenesis, lipolysis, insulin resistance, decreasing type 2 diabetes, mellitus, cardiovascular diseases, metabolic syndrome [12]. There is substantial improvement in biological activity of beta-carotene in aqueous media when it is present in form of nanoparticles [13].

The effect of beta carotene on cataract showed a small reduction in progression of age related cataract [14]. Lettuce is rich source of beta-carotene people who eat higher amount of lettuce have been found to have significantly lower mortality rates [l15]. Carrot, Spinach, Lettuce have enough amount of beta-carotene having lots of specific health benefits and boost human immune system. Consuming carrot may lower risk of some types of cancers such as lung cancer [16]. Recently It has been observed using spectroscopic studies that consumption of carrot leads to measurable increase in blood plasma concentration of beta-carotene [17] and spinach lowers blood pressure also decreases the risk of certain types of cancers [18].

6. Conclusion

Bita carotene is a non polar compound has unique structure and cleavage efficiency so it is the most efficient provitamin A. Due to antioxidant, it quenches singlet molecular oxygen and scavenges reactive oxygen species mainly paroxysm radicals. Bita carotene decomposes and can not be regenerated upon radical scavenging which suggest that the major function of bita carotene is that of a provitamin A in nutrition of human body. It is always better to consume bita carotene obtained from natural sources such as fruits, vegetables rather than bita carotene supplement, in some cases it has been observed that bita carotene supplement may have dangerous for human body health [16] for example bita carotene supplementation increases lung cancer risk in smoking people [19]. Uv/visible spectroscopy, Raman spectroscopy and many other spectroscopic, microspectroscopic techniques can be applied for the detailed study of bita carotene. Research is going on, people from different fields are growing their interest in bita carotene study for observing more possible applicatins and implementations in near future.

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