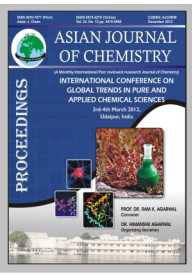
Asian Journal of Chemistry; Vol. 24, No. 12 (2012), 5457-5459

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in




# Effect of Sodium Dodecyl Sulfate on the Supramolecular Complexation Between Chitosan and β-Carotene†

S. KONGSRI[1], S. TECHAWONGSTEIN[2] and S. CHANTHAI[1,*]

[1]Department of Chemistry and Center for Innovation in Chemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand
[2]Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand

*Corresponding author: Fax +66 4320 2373; Tel. +66 43009700 ext. 12371; E-mail: sakcha2@kku.ac.th

AJC-11666

The chemical interactions between β-carotene and chitosan were investigated by using resonance light scattering intensity and Fourier transform infrared (FT-IR) spectroscopic methods. The optimum conditions for its aggregation and followed by flocculation were obtained. An ethanolic solution of β-carotene (14 μM) was added into an acidic solution of chitosan (4 mM) in acetate buffer solution pH 5 and heated at 40 ºC for 0.5 h. Under the weak acidic conditions, β-carotene was induced to interact with chitosan to form a stable aggregated complex. The effect of sodium dodecyl sulfate (2 mM) in association with other experimental parameters significantly affected their interactions. However, the soluble aggregated complexes were inductively become larger aggregates causing phase separation. It was found that the soluble aggregated complex became to be a labile supramolecular complex flocculating under the critical conditions. Regarding to FT-IR spectrum, it exhibited their characteristics of the supramolecular aggregated complex.

**Key Words: Chitosan, β-Carotene, Aggregation, Flocculation, Resonance light scattering, Infrared spectroscopy.**

## INTRODUCTION

Carotenoids are a group of important plant pigments particularly found in large amounts in tomatoes or other reddish orange fruits. They are powerful antioxidant compounds and essential nutrients in human diet because they can prevent cardiovascular diseases, regulate the immune system and are considered as anticarcinogenic agents and precursors of vitamin A[1,2]. Its characteristics of β-carotene produce the main problems of processing at high temperature and storage, which are easily damaged by oxygen, heat and light. Lose in antioxidant activity is avoided and further limits its applications in food and nutraceutical and pharmaceutical products[1,3,4]. Due to the important role of β-carotene for humans, it is necessary to be protected in order to maintain its structure and antioxidant property[5].

Chitosan is generally known as a cationic polysaccharide obtained from deacetylation of chitin, which is extracted from crustaceans and some insects. It is a copolymer of units of 2-deoxy-*N*-acetyl-D-glucosamine and 2-deoxy-D-glucosamine joined by β-1,4-glycosidic bonds[6,7]. The chitosan natural polymer is nontoxic, biodegradable and biocompatible[8]. It is soluble in weak acidic solution such as acetic acid, citric acid, lactic acid, formic acid and is chemically more versatile than chitin[9,10]. Due to their chemical configuration, this property is useful for preparation of film, gel and sphere[6]. Among many other uses, it has been used for an encapsulating agent. This agent provides the product that will be protected from odor, colour, flavour degradation during storage, which is widely used in many industry such as biomedicals, cosmetics, food industries and increasingly also in pharmaceutical sciences[5-10]. However, there are few studies to reveal its hydrophobic interactions of β-carotene aggregates, especially the effect of biopolymer such as chitosan on the aggregation behaviour of β-carotene. Thus, knowledge of their interactions is an important point of views in controlling the chemical structure of the β-carotene-chitosan complex for increasing their bioavailability and stability toward irradiation, reactive oxygen and other radical species.

Concerning on resonance light scattering, it is one of highly sensitive light scattering techniques which is widely used to study of an aggregation formation of biological macromolecules and nanoparticles in liquid sample and β-cyclodextrin inclusion with high sensitivity, rapidity and simplicity[11-13]. Numerous studies on the interactions of chitosan with synthetic dyes have also been reported[11-18]. However, a few articles on the interactions of natural dye with chitosan have been outlined.

†Presented at International Conference on Global Trends in Pure and Applied Chemical Sciences, 3-4 March, 2012; Udaipur, India

At present, use of resonance light scattering technique to study of chitosan interaction with β-carotene has not been carried out yet. This study, therefore, attempts to discuss the interactions that occur during the aggregation formation of the β-carotene-chitosan complex by using resonance light scattering intensity and subsequently to characterize the inclusion complex formed due to its flocculation effect using FT-IR. The results obtained provide more detailed understanding of original characteristics of such interactions occurring between the hydrophobic structure of β-carotene and the hydrophilic part of chitosan, regarding of which useful information in the design of industrial products or process of foods, drugs and cosmetics.

## EXPERIMENTAL

Chitosan powder (average MW 10,253 g/mol, 85 % degree of deacetylation) derived from shrimp was purchased from Sigma-Aldrich (Japan). β-Carotene (≥ 97 %) was purchased from Fluka (USA). A stock solution of 487.66 mM chitosan was prepared by dissolving in 2 % (v/v) acetic acid. Stock solution of 37.25 mM β-carotene was prepared in ethanol. Sodium dodecyl sulfate was used as an anionic surfactant effect.

Spectrofluorophotometer (RF-5301 PC Shimadzu, Japan) was used for resonance light scattering intensity measurement. The resonance light scattering spectra were obtained by scanning simultaneously the excitation and emission monochromators of the instrument from 250 nm to 700 nm with $\Delta\lambda = 0$ nm. The slit widths of the excitation and emission were kept at 5.0 nm and the resonance light scattering intensity was measured at 485 nm. The FT-IR spectra of the samples were measured with FT-IR spectrometer (Perkin Elmer, USA) in the wave number range of 400-4000 $cm^{-1}$. Samples for FT-IR spectroscopic characterization were prepared by grinding the dry blend with an appropriate amount of KBr powder and compressing the mixture to form the KBr pellet.

**Measurement of resonance light scattering intensity of aggregation:** Both of β-carotene and chitosan solution was added into a 5 mL calibrated flask. Then acetate buffer solution (pH 5) was added in succession. The solution was mixed thoroughly and incubated on water bath at 40 ºC for 30 min. After incubation, the samples were cooled to room temperature. The resonance light scattering spectrum of the aggregated complex solution was recorded with synchronous scanning mode. For optimization conditions, various experimental parameters affecting the aggregation formation of the dye-chitosan binding complex were investigated. All measurements were performed at room temperature.

**Flocculation of β-carotene-chitosan complex:** The flocculation of β-carotene-chitosan complex occurred in the mixture solution of 5 mM chitosan and 16 mM β-carotene at pH 6. The solution test tube was shaken thoroughly and then incubated at 40 ºC on water bath for 30 min. After the reaction incubation, the solution was cooled down to room temperature. Precipitates of the aggregated complex were found at the bottom of the vessel after 1 h left standing. The sample was then centrifuged at 3,000 rpm for 15 min and the supernatant was decanted to get the gel-like precipitate. This gel product was dried at 40 ºC for 48 h.

## RESULTS AND DISCUSSION

The resonance light scattering spectra of β-carotene, chitosan and β-carotene-chitosan complex are shown in Fig. 1. Under the optimum conditions, each of β-carotene and chitosan solutions alone gave very weak signal of resonance light scattering intensity. When β-carotene-chitosan complex was formed, its resonance light scattering spectrum greatly enhanced with their maximum peak characteristics. The enhanced resonance light scattering intensity could be clearly observed with three peaks located at 453, 485 and 562 nm. Therefore, the peak at 485 nm was selected as the analytical wavelength of the aggregated complex. It is suggested that the aggregation of β-carotene-chitosan complex can be formed as large particles, resulting in strong resonance light scattering signal. However, the experimental parameters affecting the aggregation formation of the natural pigment complex with chitosan were insight investigated.

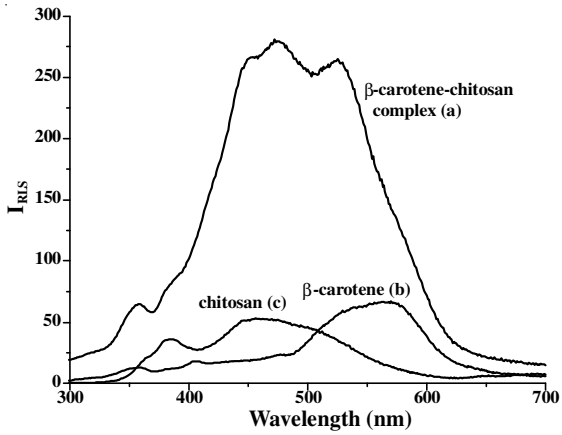



Fig. 1. Resonance light scattering spectral characteristics of the interactions between 14 μM β-carotene and 2 μM chitosan (a), 14 μM β-carotene (b) and 2 μM chitosan (c) in 60 mM NaCl and acetate buffer pH 5

**Effect of type and concentration of surfactant:** In this study, the effect of types of surfactant including anionic surfactant (sodium dodecyl sulfate, SDS), cationic surfactant (cetyltrimethylammonium bromide, CTAB) and nonionic surfactant (Tween 20) on the interactions between chitosan and β-carotene was investigated (Fig. 2).

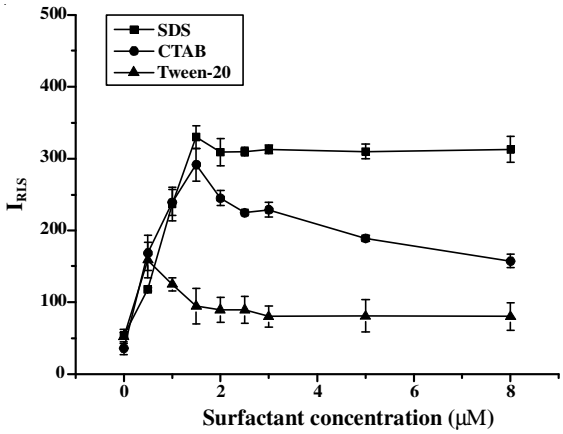



Fig. 2. Effect of type and surfactant concentration on resonance light scattering intensity of β-carotene-chitosan complex. Conditions: concentration of chitosan 2 mM and ß-carotene 14 mM, in 60 mM NaCl and acetate buffer pH 5

The results showed that the resonance light scattering intensity slightly increased with an increasing of sodium dodecyl sulfate and CTAB concentrations. But the resonance light scattering intensity of the aggregated complex in the presence of sodium dodecyl sulfate was much more pronounced than that of CTAB. It was evident that the resonance light scattering intensity of the aggregated complex was strongly enhanced by the concentration of sodium dodecyl sulfate. When the concentration of sodium dodecyl sulfate increased, the resonance light scattering intensity was sharply increasing with the sodium dodecyl sulfate concentration from 0.5-1.5 mM until a maximum value was reached, after that it remained constant. It is possibly suggested that the enhanced resonance light scattering signals is indicating the interactions of chitosan with both sodium dodecyl sulfate and β-carotene through an electrostatic attraction force and hydrophobic interactions to become the aggregated products. The aggregation of the β-carotene-chitosan complex may thus be occurred through the electrostatic interactions between both positively charged chitosan and negatively charged of sodium dodecyl sulfate (hydrophilic head) with hydrophobic interactions of β-carotene and the hydrophobic tail of sodium dodecyl sulfate[12]. Therefore, the anionic surfactant can bind strongly to chitosan *via* electrostatic interactions, which leads to the complexes formation in the presence of surfactant. Although CTAB molecule has a positively charged head, this may attribute to increase in charge repulsions between the CTAB itself and the positively charged groups of chitosan, resulted in an obstruction for the interaction binding of chitosan with β-carotene. However, the addition of Tween-20 did not has any more affecting on the interaction between chitosan and β-carotene probably concerning the functional groups of chitosan that can be disturbed by non-ionic surfactant.

**Characterization of the supramolecular aggregates of β-carotene-chitosan complex by FT-IR:** The flocculation between β-carotene and chitosan was investigated by using FT-IR. The FT-IR spectrum of chitosan shows characteristic absorption bands at 3443, 2923 and 2867 $cm^{-1}$, which represent the presence of OH group and $CH_2$ and $CH_3$ groups, respectively.

The amino group has a characteristic absorption band in the region of 3500- 3400 $cm^{-1}$, at which has been masked by the absorption bands of the OH group[19]. The C=O stretching (amide I) peak at 1659 $cm^{-1}$ is generally representing the structure of *N*-acetylglucosamine, as well as the $NH_2$ stretching (amide II) peak at 1567 $cm^{-1}$ is representing the glucosamine functional group of chitosan[20]. The IR spectrum of β-carotene shows the characteristic peaks at 3420, 2928, 1720 and 963 $cm^{-1}$, which represent the presence of an OH, C-H, C=O stretching and C-H bending bands. When compared among those of FT-IR data, it was found that the characteristic absorption bands of this chitosan at about 1590 $cm^{-1}$ (-$NH_2$) seems to disappear to give rise to two new bands located at 1625 and 1525 $cm^{-1}$ and with an increase of the peak intensity indicating the formation of the aggregated complex obtained. However, these bands were well agreed with the corresponding spectrum of the supramolecular aggregates of lycopene[21]. Its conditions were also adopted for the preparation of supramolecular aggregates of the β-carotene-chitosan complex in this study.

## Conclusion

Both resonance light scattering spectrometry and Fourier transform infrared spectroscopy measurements have been well proved to be the analytical methods for monitoring the occurrence of such interactions between chitosan and β-carotene. The sodium dodecyl sulfate was strongly pronounced on the enhanced resonance light scattering intensity of the supramolecular complex. It is, therefore, demonstrated that anionic head of sodium dodecyl sulfate would drive an electrostatic interaction direct to the cationic surface of the chitosan substrate, while its hydrophobic tail may form hydrophobic interaction with the main chain of β-carotene. Then the induced β-carotene-chitosan complex can be formed under their optimum conditions of aggregation and flocculation effects.

## ACKNOWLEDGEMENTS

Financial supports from National Research University (NRU) and the Center for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education are gratefully acknowledged.

## REFERENCES

1. M. Careri, L. Furlattini, A. Mangia, M. Musci, E. Anklam, A. Theobald and C. von Holst, *J. Chromatogr. A*, **912**, 61 (2001).
2. F. Xu, Q.P. Yuan and H.R. Dong, *J. Chromatogr. B*, **838**, 44 (2006).
3. A.I. Barba, M.C. Hurtado, M.C. Mata, V.F. Ruiz and M.L. Tejada, *Food Chem.*, **95**, 328 (2006).
4. R. Charoensiri, R. Kongkachuichai, S. Suknicom and P. Sungpuag, *Food Chem.*, **113**, 202 (2009).
5. A.H. Zaibunnisa, M.N.A. Marhanna and M.A. Atirah, *Inter. Food Res. J.*, **18**, 1017 (2011).
6. G. Crini and P.M. Badot, *Prog. Polym. Sci.*, **33**, 399 (2008).
7. N. Mahae, C. Chalat and P. Muhamud, *Internat. Food Res. J.*, **18**, 1543 (2011).
8. T.P. Alexandre, I.S. Julliana, C.G. Juliana and J. Nozaki, *Carbohydr. Polym.*, **64**, 98 (2006).
9. R. Kumar, *React. Funct. Polym.*, **46**, 1 (2000).
10. M. Rinaudo, *Prog. Polym. Sci.*, **31**, 603 (2006).
11. Q. Wang, Z.F. Zhong, L. Kong and S.P. Liu, *Chin. J. Anal. Chem.*, **35**, 365 (2007).
12. Y.W. Wang, N.B. Li and H.Q. Luo, *Luminescence,* **23**, 126 (2008).
13. A.K. Anil, T. Astrid, F. John and S. Harjinder, *Colloids Surf. B*, **64**, 104 (2008).
14. Z. Chen, W. Ding, F. Ren, J. Liu and Y. Han, *Can. J. Anal. Sci. Spectr.*, **50**, 54 (2005).
15. H. Yong, F. Xiong, X.D. Chen, J. Yang, L.S. Yang and M.Q. Zhang, *Polym. Bull.*, **62**, 549 (2009).
16. J. Peng, S. Liu, L. Wang, Z. Liu and Y. He, *J. Colloid. Interf. Sci.*, **338**, 578 (2011).
17. Y. Zeng, L. Cai, H. Wang, L. Li, W. You, L. Guo and G. Chen, *Luminescence*, **25**, 30 (2010).
18. M.R. Kasimova, C. Adri and M.N. Hanne, *Biomacromolecules*, **12**, 2534 (2011).
19. R.M. Silverstein and F.X. Webster, Infrared Spectroscopy, In: Spectrometry Identification of Organic Compounds. John Wiley & Sons, New York, USA, edn. 6, (1998).
20. J. Nunthanid, A. Laungtana, P. Sriamomsak, S. Limmatvapirat, S. Puttipipatkhachom, L.Y. Lim and E. Khor, *J. Control. Rel.*, **99**, 55 (2004).
21. L.X. Wang, Z.L. Du, R.X. Li and D.C. Wu, *Dyes Pigments*, **65**, 15 (2005).