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Characterisation and solubility study of γ-cyclodextrin and β-carotene complex

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Abstract: β-carotene, a precursor of vitamin A can be destroyed easily during high temperature processing. Thus, γ-cyclodextrin (CD) was investigated to increase thermal protection and solubility of β-carotene in water by formation of inclusion complex by using kneading, co-precipitation and physical mixture techniques. Characterisation of inclusion complex was performed using Fourier Transform Infrared Spectroscopy (FTIR) and Field Emmision Scanning Electron Microscopy (FESEM). The molar ratio needed for encapsulation was 1:1 (CD: β-carotene). Result obtained from FTIR revealed that the shift of C-H aliphatic band (2928cm^{-1}) and C=0 band (1718 cm^{-1}) to higher wavenumber for co-precipitation, indicates C-H and C=0 been used for the formation of inclusion complex. The same band also noticed in kneading and physical mixture but lower in intensity. FESEM photographs revealed that co-precipitation method produced compounds that have completely different morphology than β-carotene and γ-cyclodextrin. Co-precipitation was found to be best method that able to increase solubility of β-carotene.

Keywords: β-carotene, γ-cyclodextrin, inclusion complex, phase solubility, characterisation

Introduction

β-carotene is one of a group of natural chemicals known as carotenes or carotenoids which are responsible for the orange color of many fruits and vegetables such as carrots, pumpkins, and sweet potatoes. It is a precursor of vitamin A and plays an important role in metabolism and human health maintenance. The recognition of protective effects of β-carotene against serious disorders such as cancer, heart disease, and degenerative eye disease has stimulated intensive research on their role as antioxidants and regulators of the immune system. It is also the most effective scavengers of singlet oxygen and free radicals by protecting cells and tissues from the damaging effects of these free radicals and singlet oxygen (Di Mascio *et al*., 1989). However, β-carotene can be destroyed in food processing method at high temperature. From the result of Poznanskaja *et al*. (1994), it was found that the inclusion complex of β-carotene with β-cyclodextrin (CD) could protect β-carotene from heat and made β-carotene be easily dissolved in water.

Cyclodextrins are produced from starch by means of enzymatic conversion (Hedges *et al*., 1995). It is consisting of six α-cyclodextrin, seven β-cyclodextrin, eight γ-cyclodextrin or more glucopyranose units linked by α-(1,4) bonds. They act as flavour carriers which also can protect against oxidation, light-induced decompositions and heat-induced changes. Moreover, cyclodextrins improve shelf life of food products and mask or reduce undesired taste (Szente and Szejtli, 2004). Over the last few years they have found a wide range of applications in food, pharmaceutical and chemical industries as well as agriculture and environmental engineering. For examples, flavour-cyclodextrin complexes are used in the preparation of confectionery to control the release of the flavours and of chewing gum to retain its flavours for longer duration (Reineccius and Peppard, 2003).

Based on the stability constant, γ-cyclodextrin was at least twice more effective than β-cyclodextrin for the inclusion of turmeric rhizome oleoresin (Zaibunnisa *et al*., 2008). The stability of inclusion complexes is influenced by the sizes and configurations of the guests. Average particle size of γ-cyclodextrin obtained from SEM photographs is 86.86 ± 31.97 μm as reported by Zaibunnisa *et al*. (2009). For pure β-carotene, the mean particle size varied from 3.8 to 246.8 μm (Franceschi *et al*., 2008). Several researchers had study the possibility of using γ-cyclodextrin to encapsulate various guest molecules. However, encapsulation of β-carotene with γ-cyclodextrin has not been carried out before.

The intention of this research is to encapsulate β-carotene (guest) with γ-cyclodextrin and to characterise the inclusion complex formed. The specific objectives of the project include determining appropriate molar ratio needed for encapsulation to form most stable γ-cyclodextrin and β-carotene solid stage inclusion complex, to determine the efficiency of encapsulation method for β-carotene

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with γ-cyclodextrin by using kneading and co-precipitation methods. The inclusion complexes formed were characterised by using FESEM and FTIR. The stable encapsulated product can be applied as food antioxidant, natural colouring agent and also as nutrient. This encapsulated product also will be protected from flavour, aroma and color degradation during storage.

Materials and Methods

Materials

γ-cyclodextrin was purchased from Munchen, Germany, *n*-hexane (ACS, Reag. Ph Eur. MERCK, Germany), ethanol (Merck EU Pharmacopier, Germany), β-carotene (Sigma-Aldrich GmGH, Germany), KBr powder (BDH, UK) and NaCl solution (Merck, Germany).

Phase solubility study

Phase solubility studies were carried out according to the method described by Higuchi and Connors (1965). An excess amount of β-carotene (20 mg) was added to screw capped vials containing gamma cyclodextrin in 5.0 ml ethanol: water (25:75 v/v) solution at various concentrations ranging from 0-4 mM for γ-cyclodextrin. The vials were shaken at 30°C until equilibrium reached, i.e. 48 hour, on a water bath (Memmert, Germany). The samples was centrifuged at 3000 rpm for 10 min. the resulting solutions was filter using cellulose filter and evaporated to remove solvent. *n*-hexane (5 ml) was added to samples and mixed for 20 min. Saturated NaCl solution (1ml) was added to facilitate the breakage of emulsion. The solvent phase containing β-carotene was separated by decantation and pipetting. Two successive extractions were carried out for each sample. The extract was pooled together and evaporated to 1 ml.

The stability constant, K_c, was calculated from the slope and intercept of the linear segment of the phase solubility line according to the following equation:

$$Kc = \frac{k}{So\ (1 - k)}$$

S_o = intrinsic solubility of β-carotene in ethanol: water solution (25:75)
k = slope of the straight line

Inclusion complex

The inclusion complex of β-carotene and γ-cyclodextrin was prepared using kneading and co-precipitation method. Kneading method and physical mixture as control were done following the method used by Zhang *et al*., (2005). Method for co-precipitation was according to Waleczek *et al*. (2003).

Co-precipitation method

β-carotene was added to screw capped vials containing γ-cyclodextrin in ethanol: water (25:75 v/v) mixture (5 ml). The molar ratio for β-carotene: γ-cyclodextrin was 1:1. The vials were shaken at 30ºC until equilibrium reached, i.e. for 48 hours, on shaking water bath (Memmert, Germany). Samples were kept at 5ºC for 1 hour. The samples was centrifuged using Clement (Sydney, Australia) at 3000 rpm for 10 min and the supernatant was decanted to provide the complex as microcrystalline precipitate. The product was dried at 40ºC for 48 hour. The dried mass was pulverized and sieved through 150 μm mesh (Endecotts, England).

Kneading method

β-carotene and γ-cyclodextrin with molar ratio 1:1 was added in a mortar and kneaded for 45 minutes. During the kneading, 40% ethanol: water (27:75 v/v) mixture was added to the mixture to maintain a proper consistency. The product then was dried at 40ºC for 24 hour and gently sieved through 150 μm mesh (Endecotts Ltd., England).

Physical mixture

As controls, physical mixtures of molar ratio 1:1 of β-carotene and γ-cyclodextrins was also prepared by dry-pestling in a mortar and kneaded for 5 minutes.

Characterisation of inclusion complex

Fourier transform infrared spectroscopy (FTIR)

The KBr disk method was used. In this procedure, the pellets were prepared by mixing the samples and KBr with a pestle on an agate mortar and compacted with a hydraulic press. FTIR spectra of the sample was obtained in the range 650 to 4000 cm^{-1} using a Perkin Elmer Model GX FTIR spectrophotometer. The resolution was 1.0 cm^{-1} and the spectrum was the result of averaging 10 scans.

Field Emmision Scanning electron microscopy (FESEM)

Particle size and structure of spray-dried microcapsules was evaluated by a FESEM, SUPRA™ 40VP FESEM. The microcapsulates was attached to FESEM stubs using 2-sided adhesive tape. The specimen was examined at 100-1000 kV.

Results and discussion

The phase solubility diagrams of cyclodextrin complexes with β-carotene were obtained by plotting the changes in guest solubility as a function of γ-cyclodextrin and shown in Figure 1. Based on the results obtained from the phase diagram, the inclusion complex of β-carotene and γ-cyclodextrin, is formed due to the smaller particle size of β-carotene than can be easily incorporated into the inner cavity of γ-cyclodextrin. The complex exhibit higher solubility than does the guest molecule. The rise in the solubility, which was not necessarily linear, was interpreted as the formation of a complex which dissolves in the ethanol: water (25:75 v/v) solution.

There was an initial increase in solubility of β-carotene and γ-cyclodextrin. The slopes obtained from the initial straight line from Figure 1, were 0.094, 0.119, 0.1218, 0.1222, 0.1333 and 1301, respectively. Assuming a 1:1 stoichiometry of host guest correlation with slope less than 1, stability constant K_C were calculated. A small K_C value indicates a weak interaction, while values of stability constant within the range of 100-1000 M^{-1} are considered ideal (Mukne and Nagarsenker, 2004).

$$Kc = \frac{k}{So\ (1 - k)}$$

S_o = intrinsic solubility of β-carotene in ethanol: water solution (25:75)
k = slope of the straight line

Thus, K_c = 43 M^{-1}

Fourier transform infrared spectroscopy (FTIR)

FTIR technique been used by researchers to detect the formation of inclusion complex in solid phase. This technique is also used to point out the implication of the difference functional groups of the guest and host molecules in the inclusion process. It can be done by studying the changes in the shape and position of the absorbance bands of guest, cyclodextrin, physical mixtures and inclusion complexes (Cannava *et al*., 2008). FTIR is a suitable method to characterise the inclusion complex (Crupi *et al*., 2008). Hence, the complexation between β-carotene and γ-cyclodextrin was investigated by using FTIR in this study. The IR spectrum of the β-carotene in Figure 2 was characterised by principal transmission peaks at 3420, 2928, 1718 and 963 cm^{-1}.

The peak at 2340 cm^{-1} due to the presence of conjugated phenol, can be observed in the β-carotene, γ-cyclodextrin, physical mixture and kneading method. However, these bands cannot be seen in co-precipitation method which indicates the formation of new solid phase.

C-H aliphatic band appears within the region 2928-2935 cm^{-1}, as can be observed in all spectra. Only for co-precipitation method, this band appeared to shift to higher wavenumber, at peak 2935 cm^{-1}, whiles others at peak 2928 cm^{-1}.

The major peak at 1718 cm^{-1} of the C=0 stretching of the carbonyl group, was the important characteristic of β-carotene. Carbonyl group of inclusion complexes of co-precipitation method presence at peak 1715 cm^{-1}, it indicates bond been strengthen, contribution of γ-cyclodextrin ring strain and distribution of strong hydrogen bonds to carbonyl in β-carotene replacement by a less intense association. There was also an overlapping effect in this region, as shown by the intense band located at 1650 cm^{-1} for physical mixture and kneading method, also for γ-cyclodextrin.

The spectrum within range 1160-1020 cm^{-1} can be attributed to the presence of the C-O stretching band, can be seen in γ-cyclodextrin spectrum. Intensity of these bands been reduced in kneading method and physical mixture. However, these bands can be seen in co-precipitate method which indicates formation of new solid phase.

Band at 1556-1560 cm^{-1} shows the presence of aromatic group which main characteristic of γ-cyclodextrin. However, these bands were disappeared in the spectrum complexes obtained by the co-precipitation method. The ring structure was changed to straight chain, as aliphatic group.

Field Emmision Scanning electron microscopy (FESEM)

New applications are emerging in nanotechnology, where fabrication techniques are so advanced that SEM technologies have been further developed to enable researchers to image the structures that they make (Apkarian, 1997). The FESEM photographs of β-carotene, γ-cyclodextrin, physical mixture, kneading and co-precipitate products are shown in Figure 3. The β-carotene appeared as agglomerates, while pure γ-cyclodextrin appeared as parallelogram shapes. Both products from simple physical mixing and kneading methods of β-carotene with γ-cyclodextrin appeared as oily agglomerates and clumping to each other. In the co-precipitation sample, the original morphology of raw material disappeared, and the shape is smaller than physical mixture and kneading products. It showed a new single phase was formed which differ from particle size of complexes of physical mixture and kneading method.

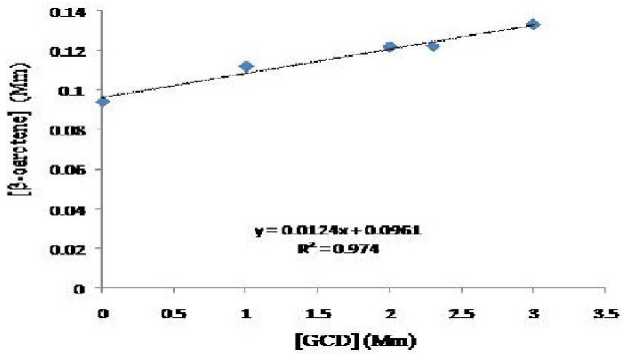

Figure 1. Solubility of β-carotene as a function of γ-cyclodextrin in ethanol: water (25:75 v/v) solution at 30°C

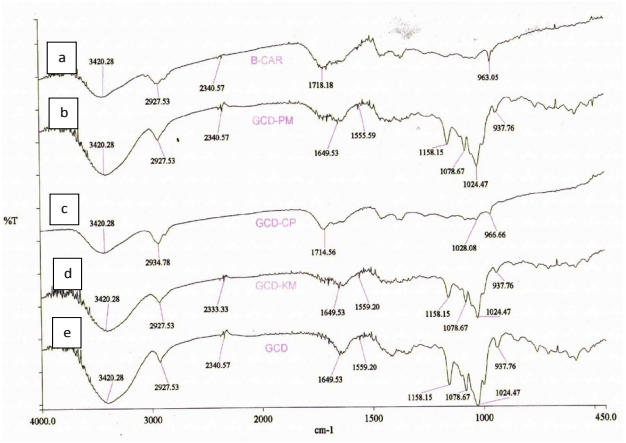

Figure 2. FTIR spectrum of β-carotene (a), physical mixture (b), co-precipitates complex (c), kneading complex (d), γ-cyclodextrin (e)

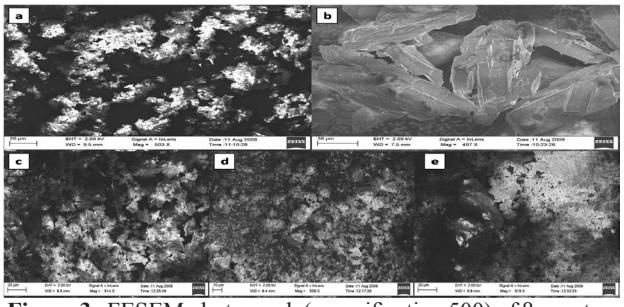

Figure 3. FESEM photograph (magnification 500) of β-carotene (a), γ-cyclodextrin (b), kneading complex (c), physical mixture (d), co-precipitate complex (e)

Conclusion

Inclusion complexes can lead to advantageous changes in the chemical, biological and physical properties of the β-carotene. β-carotene was found to form inclusion complex with γ-cyclodextrin with molar ratio of 1:1 (γ-cyclodextrin: β-carotene). The solubility of β-carotene was enhanced in the presence of γ-cyclodextrin. The result obtained by FESEM and FTIR show that co-precipitation was the best method for complexation.

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