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CHARACTERIZATION OF β-CYCLODEXTRIN COMPLEXES WITH NATURAL DYE

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ABSTRACT

Natural dye was successfully solubilised in aqueous solution in forming inclusion complex with β-cyclodextrin (β-CD). To investigate molecular association, phase solubility studies were undertaken. In the presence of cyclodextrin, fluorescence absorbances were enhanced. Solid state inclusion complex was also prepared using co-precipitation method. The solid was characterized using Fourier Transform Infrared (FTIR) and X-ray Diffractometer (XRD). Using FTIR, the host-interaction have been evidenced by monitoring the changes in some guest molecule band relative to those observed in the spectra of the 1:1 physical mixtures and complex. XRD can give the order of structure in the solid inclusion. All the data from FTIR and X-Ray studies showed that it is possible to obtain an inclusion complex (1:1) in solid state.

Keywords: β-cyclodextrin, natural dye, inclusion complex, FTIR, XRD

INTRODUCTION

Nowadays, natural dye has been progressively applied in textile application. Natural dye is one of the product that have a commercial value instead of it is safe to the environment and consumer. Natural dyes are colorants obtained from biological matter through mechanical retention, covalent chemical bonds formation or complexes with salts or metals formation, physical absorption or by solutions. Plant is a major source that can produce natural dye. Natural colourant from plant sources are receiving growing interest from both food manufacturers and consumers in the continuing replacement of synthetic dyes. (Stinzing and Carle, 2004). Plenty of plants that can be sources of natural dyes such as henna leaves (Shaukat et al., 2009), Hibiscus mutabilis (Padma et al., 2009), Curcuma Longa (turmeric), Gardenia jasmoides Ellis (gardenia) and Carthamus tinctorius L (safflower) (Lee et al., 2008). Natural dyes have a big potential to be commercial because it have a vast economic significant because trade has a world market worth £ 2.5 billion/year. This paper proposes a natural dye from the dragon fruit peel that believed have fluorescence properties and suitable used for colorant either in textile or food industries. Hylocereus polyrhizus or more commonly known as pitaya or dragon fruit is a member of the Cactaceae family from the genus Hylocereus. The flesh of this fruit is red-purple in color when ripened and has gained a growing interest for cultivation in Malaysia (Hoa et al., 2006). Dragon fruit is one of the focuses for the next source of red dye because it is rich betalains which mainly used for food coloring. Cyclodextrins (CDs) are a family of natural or synthetically modified

cyclic molecules, consisting of typically six (α-CD), seven (β-CD) and eight (γ-CD) glucopyranose units (Duchene, 1987). The α, β, γ cyclodextrin (CD) are polysaccharides made up of six and eight D-glucopyranose residues, respectively linked by α-1,4 glycosidic bonds into a macromolecule (Mrozek et al., 2008). They have a toroidal shape with a hydrophobic central cavity and a hydrophilic outer surface, where the hydroxyl groups are located. (Cannava et al., 2008). These unique properties predispose them to form molecular microcapsules, namely inclusion complexes or host-guest complexes (Jiang, 2008). They can form host-guest inclusion complexes, both in solid and in solution phase. (Cannava et al., 2008). Among the natural CDs, β-CD is the most widely used because of its availability, low cost and dimensions of its cavity able to accommodate a number of molecules (Cannava et al., 2008). This paper reported the encapsulation of natural dye with β-CD preparation in order to improve the properties of fluorescence. Also, to characterize the inclusion mode and the stoichiometry that formed during the formation.

EXPERIMENTAL

Plant Material

The skin of pitaya fruits are collected from the local producer of organic food especially pitaya fruits and the materials stored at 4^{o}C. This is because to make sure that the skins of the fruit keep fresh and to avoid the lost of moisture contents.

Preparation of Natural Dye Sample

Natural dye was prepared by using water extraction. The purpose of using water extraction is to make sure that the colour is safe from the chemical and harmful substance. Then, the extraction was filtered and separated from the residue compound. Before extraction of natural dyes, the raw material was grind to small particles about 1mm. The natural dyes then extracted with boiling water by applying ratio 1:20 corresponding to the ratio of 1 g of raw material to the 20 mL of water. The duration of the extraction is fixed to 60minutes. The insoluble residue was separated by sedimentation and filtration through stainless steel filter fabric (0.3 mm mesh). (Bechtold et al., 2003). The resulting extract was used for the further experiment.

Preparation of Solid Inclusion Complex

The inclusion complex of natural dyes and β-CD were prepared by co-precipitation method. Natural dyes and β-CD were accurately weighed 1:1 molar ratio. The β-CD (10wt %) was dissolved in 10% of ethanol solution at room temperature. Then the natural dye was slowly added into the ethanol solution. The solution was stirred continuously for 6h and then kept at 4^{o}C in a refrigerator for 12h. The solution was filtered using filter paper. The precipitate was air-dried at 50^{o}C and the final dry powders were stored in a desiccator at room temperature. (Jiang, 2008)

Preparations of Physical Mixtures

The 1:1 natural dye and β-CD were obtained by mixing the single components in an agate mortar until the mixture homogeneous. (Jiang, 2008)

Phase solubility diagram

Phase solubility studies were carried out in aqueous medium according to Higuchi and Connor. A 1mL of stock solution of natural dye was transferred into 10mL volumetric flask and then appropriate β-CD was added with increasing concentration of β-CD. The concentration of CD ranged is from 0 to 0.007 mol/L. The mixtures were shaking thoroughly for 48h at room temperature protected from light. After this period the suspensions were centrifuged at 12000 rpm for 5 minutes. The upper liquid was analyzed using UV-Vis spectrophotometer with the absorbance read at 1099nm (Jiang, 2008)

Characterization of inclusion complex

The samples collected in solid state were characterized as follows. FTIR spectra were obtained in KBr discs using Thermo Nicolet instrument with $4cm^{-1}$ spectral resolution. While, powder X-ray diffractometer using Cu κα (λ=1.506Å) with 40mA, 40kV and scanning rate $3^{o}C$/min (Marcia et al., 2007)

RESULTS AND DISCUSSION

Phase Solubility studies

The stoichiometry ratios and stability constant describing the extent of formation of the complexes were obtained by measuring the changes UV-Vis absorbance of the substrates. As shown in Figure 1, the adsorption intensities were increased by increasing the concentration of β-CD. Also, displays a linear increase of fluorescence absorbance with concentration of β-CD, the slope changing when all the natural dye concentration is fully complexed suggesting that the apparent solubility of fluorescence is enhanced by a binding process with macrocycles In the region where linear increase was observed, a linear regression analysis was performed and the equations turned out to be as follows:

$$y= 0.01x + 0.0054 \quad (1)$$

In the above expressions, x is the concentration of cyclodextrin in solution and y is the absorbance of natural dye. Since the slope of the diagram is less than 1, the stoichiometry of the complexes was assumed 1:1 (Cannava et al., 2008).

The fluorescence absorbance in different pH is shown in Figure 2. As the figure shown, the maximum wavelength of excitation is 974nm. Comparing to the curves, it is clear that the fluorescence intensity of natural dye was enhanced in the presence of β-CD.

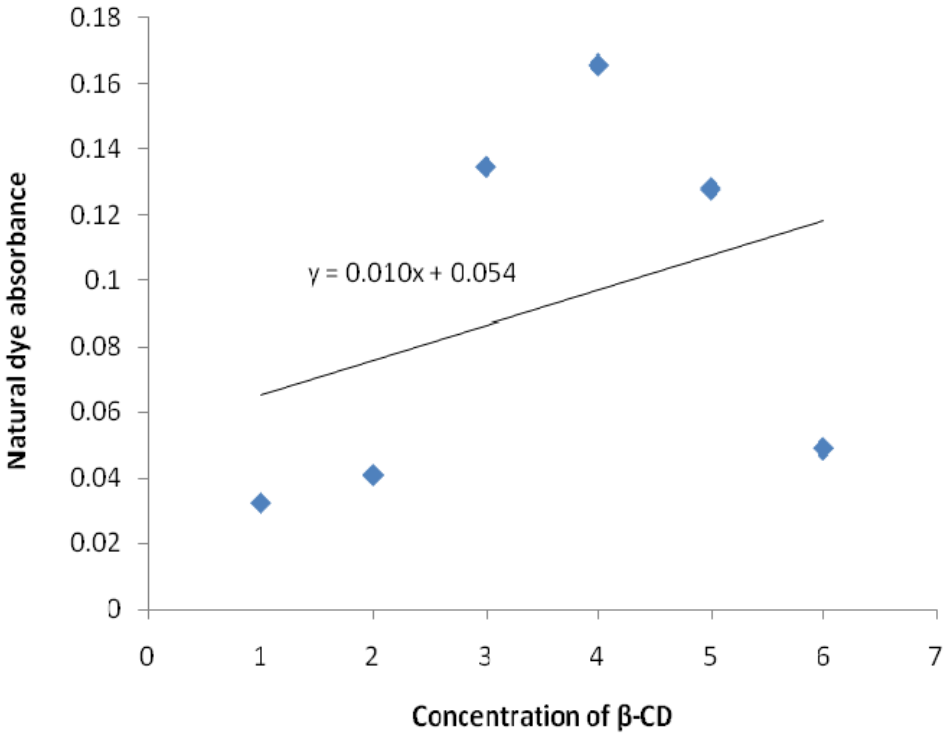

Figure 1: Phase solubility diagram of natural dye in solution with β-CD in pure water at 25°C. Each data point is the mean of three measurements.

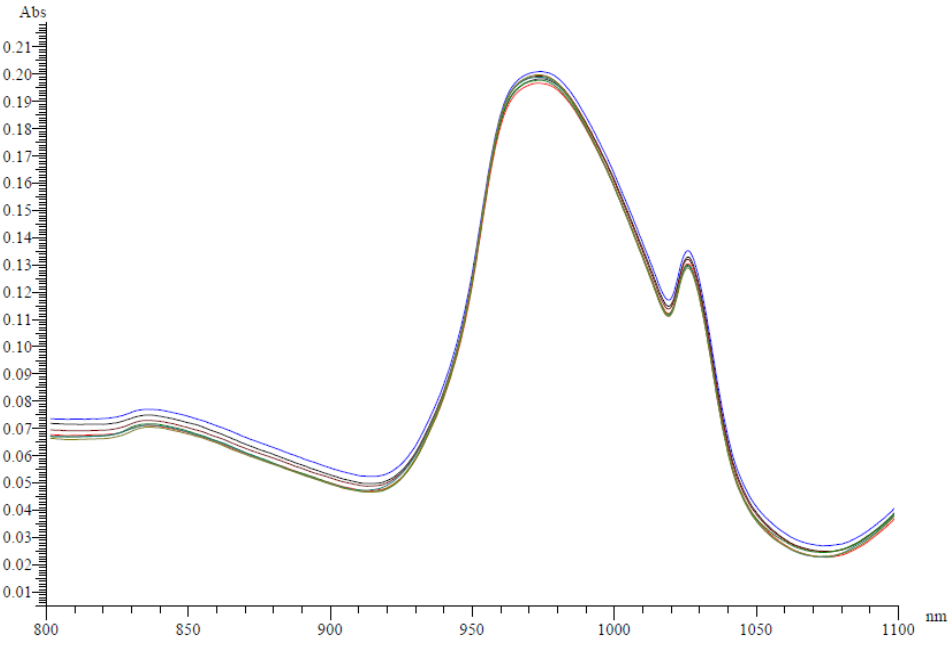

Figure 2: Absorption spectrum of complex natural dye and β-CD in solution by using UV-Visible Spectrophotometer

Figure 3 shows the effect of pH on the fluorescence absorbance in the natural dye encapsulated with β-CD. It was found that the fluorescence enhancement in acidic medium was stronger that alkaline medium. According to the previous studies, betalains that may content in the natural dye favor a pH range 3 to 6 in the presence of oxygen and also anaerobic conditions. (Herbach et al., 2006). The relatively high intensities were obtained at pH 3, so it is determine that pH3 is the best condition to get the best flourescence.

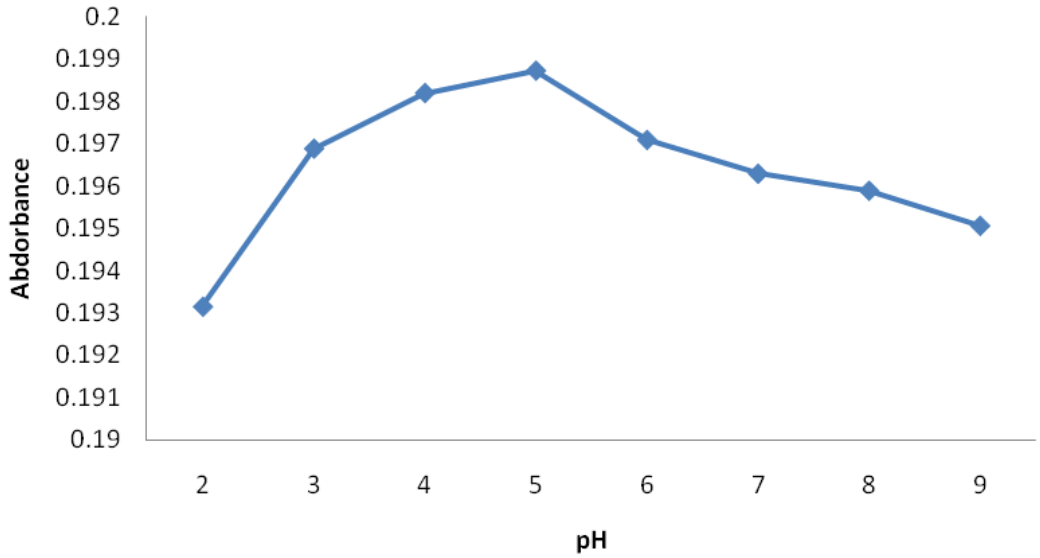

Figure 3: Dependence of fluorescence absorbance of natural dye at different pH values in β-CD media.

CHARACTERIZATION OF INCLUSION COMPLEX IN SOLID PHASE

Fourier-transform infrared spectroscopy (FTIR)

The use of FTIR technique allows the detection of complex formations in solid phase and to point out the implication of the different functional groups of guest and host molecules in the inclusion process by analyzing the significant changes in the shape and position of the absorbance bands of natural dye, β-CD, physical mixture and inclusion complexes. Figure 4 illustrates the IR peak of the solid natural dye, β-CD, physical mixture and inclusion complex. The β-CD exhibited significant FTIR peak at wave number of 942, 1094, 1166, 1337, 2929 and 3467 cm^{-1}. While, natural dye showed FTIR peaks at wave number of 1059, 1102, 1161, 3362, 3434 and 3464 cm^{-1}.

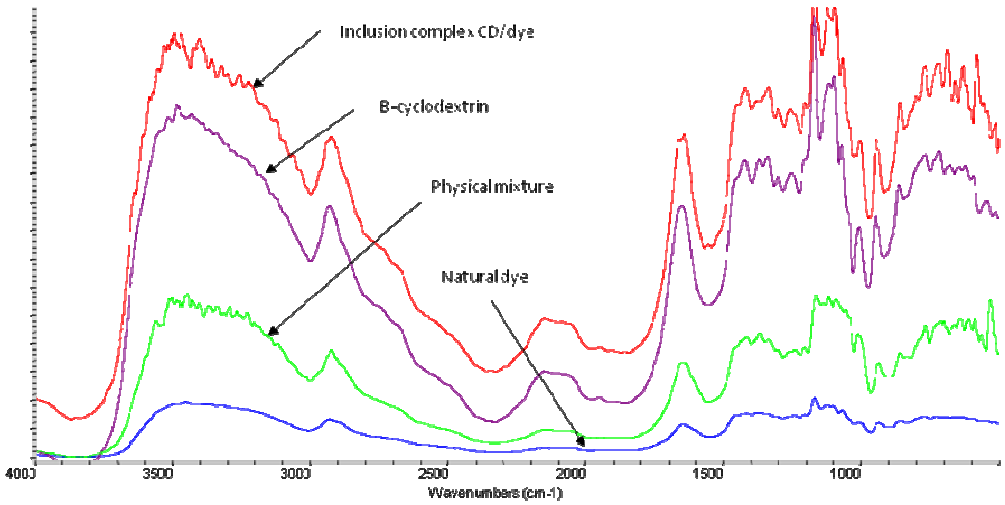

Figure 4: Experimental FTIR spectra collected in the high frequency region for the solid inclusion complex.

Table 1: Wavenumbers (cm^{-1}) and assignments for observed in the FTIR spectra of natural dye and β-CD

Infrared bands (cm^{-1}) and assignments	
Natural dye	**β-CD**
3464, 3434 and 3362: H-bonded O-H stretch	3467: ν(O-H)
2930 and 1420: Alkane**,** ν(C-H) and δ(C-H)	2926: ν(C-H)
1161: ν(C-O)	1420: δ(C-H) from CH_2 and CH_3
1102: ν(C-O)	1337: coupled δ(C-C-H), δ(C-O-H), δ(H-C-H)
1059: ν(C-O)	1166 and 1094: coupled ν(C-O),ν(C-C), ν(C-O-H)
	942: skeletal vibration involving α-1,4 linkage.

*Wavenumbers values and assignments for β-CD and natural dye in β-CD/natural dye inclusion complex are due to the same vibrational modes. *ν, stretching vibration;δ, bending vibration.

X-ray Diffractometry

X-ray diffractometry (XRD) is an instrumental technique that is used to identify minerals as well as other crystalline materials. In order to investigate the characteristic of the inclusion complexes, the XRD is the suitable equipment that can provide the information of the degree of crystallinity of the materials presents, possible deviation of the minerals from their ideal compositions and others. The ordering degree in the structure of solid inclusion complex was compared to that the parents solids by XRD measurements showed in Figure 5. The X-ray diffractometry patterns or the physical mixtures of natural dye and

β-CD are approximately the superposition of the patterns of the raw materials. On the other hand, the co-precipitate products have completely different patterns in which it is no longer possible to distinguish the characteristic peaks of natural dye, thus confirming the existence of new compounds.

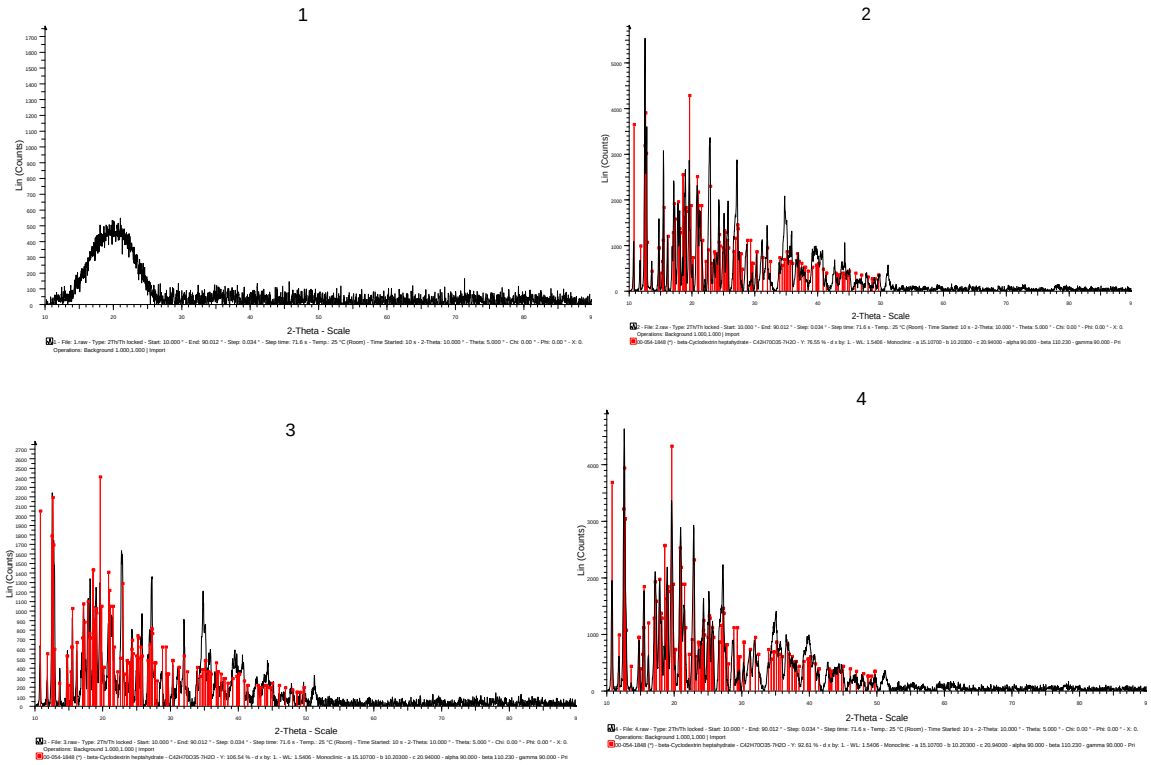

Figure 5: XRD patterns of solid of inclusion complex (1), physical mixture (2), β-cyclodextrin(3) and natural dye (4)

CONCLUSION

β-CD has an ability to form complex with variety of molecules include natural dye. This is useful in providing promising application in textile and batik industries. All the data from FTIR and X-Ray studies showed that it is possible to obtain an inclusion complex (1:1) in solid state.

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