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STABILISATION OF CURCUMIN WITH γ-CYCLODEXTRIN: PHASE SOLUBILITY STUDY AND ITS CHARACTERISATION

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Abstract*—Curcumin ($C_{21}H_{20}O_6$) is a yellow polyphenol molecule extracted from the rhizome of turmeric (*Curcuma longa*). However, curcumin are not stable during storage and have very low water solubility which limits its application to food system. Gamma cyclodextrin has been investigated to increase the solubility and stability of curcumin. Increase in solubility was noticed with the addition of gamma cyclodextrin until it reached its solubility limit at 7mM gamma cyclodextrin. The phase solubility diagram obtained was characterised as B_S. Based on phase solubility study, the molar ratio obtained was 1:3 (gamma cyclodextrin:curcumin) for complexation. Inclusion complex of curcumin was prepared using co-precipitation (CP) and kneading method (KM) and compared with physical mixture (PM). The complexes formed were characterized by using Fourier transform infrared spectrometry (FTIR), differential scanning calorimeter (DSC) and field emission scanning electron microscopy (FESEM). A strong C-O band at wavelength of 1027 cm^{-1} in co-precipitation and kneading methods in FTIR spectrum indicates that this bond has been strengthen and used for complexation. The DSC thermogram showed that the melting point of curcumin was at 176.52°C. The intensity of the peak has been reduced in co-precipitation and kneading methods. The results obtained from FTIR and DSC indicates that kneading and co-precipitation methods able to produce inclusion complex. However, FESEM images revealed that inclusion complex obtained using co-precipitation has smaller in crystal sizes and appeared as different morphology compared to pure compound. Therefore, co-precipitation was the chosen method for formation of inclusion complex between curcumin and γ-cyclodextrin.

Keywords: Curcumin, γ-cyclodextrin, phase solubility and inclusion complex.

I. INTRODUCTION

Curcumin is also known as diferuloylmethane is a yellow polyphenol extracted from the rhizome of turmeric (*Curcuma longa*). Curcumin is a main constituent of the Indian spice turmeric, is of growing interest due to its wide ranging pharmaceutical properties [1]. Curcumin compound has a great pharmaceutical activity, has an effective antioxidant as well as has free radical scavenger properties. This compound has potency against many diseases such as cough, diabetes, anorexia, Alzheimer disease, rheumatism, and hepatic disorders [2]. Besides it is extensively used in food industries. Raw materials itself will have variation in composition in term of colour, flavour and aroma compounds due to different harvesting time, size and growing site of the product. Curcumin has poor bioavailabity, low aqueous solubility and chemically unstable under acid and alkaline condition as well as light sensitivity[3]. The extract obtained from plant materials may contain microbiological contamination as well as undesirable alteration of the compounds might occur during storage. So, the colour compound might decrease during storages due to its sensitivity toward light. Besides, the storage of natural sources of certain colour has serious storage problems. This compound can be performing by steam hydrodistillation, solvent extraction and Pressurized Liquid Extraction (PLE) from extraction of plants [4] .

Cyclodextrin (CD) has a crown-like structure which is cyclic (α-1,4) linked oligosaccharide. It produces from starch by enzymatic conversion. CD is an unstable compound, so, it usually combined with other chemicals to form a stable aqueous compound. Typical cyclodextrins are constituted by 6-8 glucopyranoside units, it represents with the larger and the smaller openings exposing to the solvent secondary and primary hydroxyl groups respectively. Because of this arrangement, the interior of the CD is not hydrophobic, but considerably less hydrophilic than the aqueous environment and thus able to host other hydrophobic molecules. In contrast, the exterior is sufficiently hydrophilic to impart cyclodextrins (or their complexes) water solubility.

Usually, β-CD been used as an encapsulation agent. Not many studies been carried out to study the effect of γ-CD as encapsulation agent. γ-CD have larger diameter and has higher solubility compared to β-CD. Several researchers had encapsulated complex materials like oleoresin, essential oil (*Salvia sclarea L.* essential oil, *Lippia sidoides oil* and lemon oil) and fatty acid compounds (lineoleic acid and cholesterol) with cyclodextrin [5]. However, the interpretation of data was a bit difficult for complex materials. Therefore, pure compound, curcumin will be used as marker compound to investigate the properties of the inclusion complex formed. It may results in easier interpretation of data due to results obtained are unaffected by other compounds.

The results obtained from this study can be applied to other plant compounds as well as can be used in any field including food, fragrance, pharmaceutical and medical areas. Besides, this study can be used as a model study for future

research on inclusion complex of any turmeric extracts that contain curcumin.

II. MATERIAL AND METHODS

Curcumin (Aldrich, USA),γ-cyclodextrin (Wacker-Chemie GmbH, Munchen, Germany),Ethanol (ACS, Reag. PhEur. MERCK, Germany),KBr powder (BDH, UK) for FTIR analysis.

III. METHODOLOGY

A. Phase solubility

An excess amount of curcumin (20 mg) was added to screw-capped vials containing γ-CD in 5.0 ml of ethanol: water (25:75 v/v) solution at various concentrations, ranging from 0 to 9 mM for γ-CD. The vials then were shaken at 30°C for 48 hours in a water bath (Memmert, Germany) until reached equilibrium. The samples were centrifuged at 3000 rpm for 10 minutes. After attainment of equilibrium, the contents of the tube were filtered through Whatman filter paper (type 42). The extract solutions were determined from the absorbance at 549 nm using UV visible spectrophotometer model Perkin Elmer Lambda 35. The wavelength of absorbance of curcumin in ethanol solution was reported by Colin *et al.* (2008). The duplicate absorbances were made for each assay. To nullify the absorbance due to the presence of cyclodextrin, the apparatus was calibrated with ethanol as blank.

The appearance stability constant, K_c of curcumin and γ-CD inclusion complex was calculated from the slope and intercept of the linear segment of phase solubility line according to the following equation:

$$K_c = \frac{k}{S_o (1-k)} \quad (1)$$

S_o = intrinsic solubility of curcumin in ethanol: water solution (25:75)

k = slope of the straight line

B. Inclusion complex

The inclusion complex of curcumin: γ-CD was prepared by using co-precipitation and kneading methods and kneading and physical mixture as control were prepared following the method reported by [6]. The method of co-precipitation method was following the method used by [7].

C. Co-precipitation

Curcumin was added to screw capped vials containing γ-CD in ethanol: water (25:75 v/v) mixture of 5 ml. The vials were shaked at 30°C until equilibrium reached; this is done in water bath for 48 hours (Memmert, Germany). The samples were centrifuged at 3000 rpm for 10 minutes. The supernatant was decanted to form the complex as microcrystalline precipitate. The product obtained will be dried in oven at 40°C for 48 hours. The dried mass was sieved through 150 µm mesh (Endecotts Ltd., England).

D. Kneading method

γ-CD and curcumin with molar ratio 1:3 was added in mortar and kneaded for 45 minutes. During the kneading, 40% of ethanol: water (25:75 v/v) mixture was added to the mixture to maintain proper consistency. The products were dried in oven at 40°C for 48 hours. The dried mass was sieved through 150 µm mesh (Endecotts Ltd., England).

E. Physical mixture

For a control, physical mixture of the same weight ratio of curcumin: γ-CD were prepared by dry-pestling in a mortar and was kneaded for 5 minutes to obtain homogenous blend.

F. Fourier Transform Infrared Spectroscopy (FTIR)

In this procedure, the pellets were prepared by mixing the samples and KBr a pestle and on agate mortar and compacted with a hydraulic press. Fourier Transform Infrared Spectroscopy (FTIR) spectra of the samples were obtained in the range of 450-4000 cm^{-1} using a Perkin Elmer Model Spectrum One FTIR spectrophotometer. The resolution was 1.0 cm^{-1} and the spectra were results in averaging 4 scans.

G. Differential Scanning Calorimeter (DSC)

Samples (1-5 mg) were weighed and placed in aluminum pans with pinhole lid and it followed by heating at rate of 10°C/min in temperature range of 140°C to 250°C. The measurements were carried out under dry nitrogen at the flow rate of 50 ml/min. DSC curves of pure materials and all system was recorded on a Mettler Toledo differential scanning calorimeter (model DSC 1 STAR System). An empty pan of aluminium pan was used as reference.

H. Field Emission Scanning Electron Microscopy (FESEM)

Particles size and structure of spray dried microcapsules were evaluated by Scanning Electron Microscopy, Model SUPRATM 40VP (GEMINI). The microcapsules were attached to FESEM substances using a double-sided adhesive tape and then examined at 2 kV.

IV. RESULTS AND DISCUSSIONS

A. Phase solubility

The phase solubility diagram of curcumin was obtained by plotting the dissolved curcumin as a function of γ-CD concentrations. Although the solvent addition of used (ethanol) can affect the solubility constants, mixtures of ethanol and water were used to increase the solubility of lipophilic curcumin in water. A phase solubility study of resveratrol with CD using similar method was reported by [8]. The phase solubility diagrams for the complex formation between curcumin and γ-CD are presented in Figure 1, it can be classified as Bs type as described by [9]. Bs denotes complexes with limited solubility. Solubility curve been plotted for curcumin from 0 to 9 mM γ-CD. This plot shows that there was an increment in the solubility of curcumin up to 7 mM γ-CD. However, solubility limit reached at 7 mM and further addition does not increase in solubility. Addition of γ-CD above 7 mM resulted in formation of precipitate. At

this stage, two distinct types of precipitate were obtained, which is the less soluble inclusion complex (yellow precipitates) and excess CDs (white precipitates).

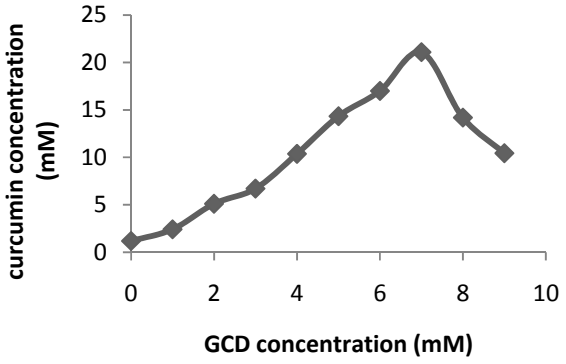


Figure 1. Solubility of curcumin as a function of γ-cyclodextrin in ethanol:water (25:75 v/v) solution at 30°C. Each data point is the mean of two measurements.

B. Inclusion complex

The inclusion complex was obtained using co-precipitation and kneading methods. Based on phase solubility diagram, it was interpreted that the formation of soluble 1:3 molar ratio γ-CD and curcumin inclusion complex which dissolved in ethanol:water (25:75 v/v) solution.

C. Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra for curcumin, γ-CD and the complexes were showed in Figure 2. The prominent spectrum of curcumin are as follows; 3512, 1627, 1508, 1429, 1281, 1206, 1153, 1026, 963, 856 and 814 cm-1. Generally, the stretching region of hydroxyl group, O-H was showed at the band range of 3200-3600 cm^{-1}. The band at 3510 cm^{-1} indicates the presence of hydroxyl group in the curcumin. The presence of water in γ-CD resulted in the presence of broad peak of OH which masks the presence of OH in curcumin.

The band for carbonyl group (C=O) peaks appeared at the band range of 1620-1650 cm^{-1}. The presence of OH also cause broad C=O band for γ-CD at 1620-1650 cm^{-1}, whereas sharp peak was noticed for carbonyl in curcumin. This sharp peak was noticed in kneading, co-precipitation method and physical mixture which indicates carbonyl of curcumin was not been used for complexation. Actually, the carbonyl presence in γ-CD was been used for complexation.

The band of alkanes (C-H) is shown at 1350-1512 cm^{-1}. The wavenumber for curcumin was noticed at 1508.57 cm^{-1}. However, the wavenumbers for KM, PM and CP were shifted to 1510.49, 1510.92 and 1510.90 cm^{-1}, respectively. The shift to higher wavenumber for C-H band in curcumin indicates C-H of curcumin been used and strengthen for complexation.

Stretching bands at 1000-1260 cm^{-1} indicated the presence of ether group (C-O). The spectrum at around 1026.25 cm^{-1} characterised the vibration of C-O stretching band for curcumin. The intensity of this peak appeared stronger for co-precipitation and kneading. However, physical mixture seems to have similar intensity when compared with pure curcumin. Therefore, C-O of curcumin been used for complexation. The results obtained from FTIR, revealed that co-percipitation and kneading can be used to form inclusion complex of curcumin with γ-CD.

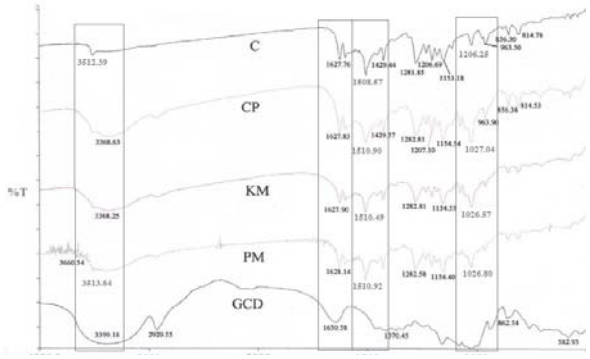
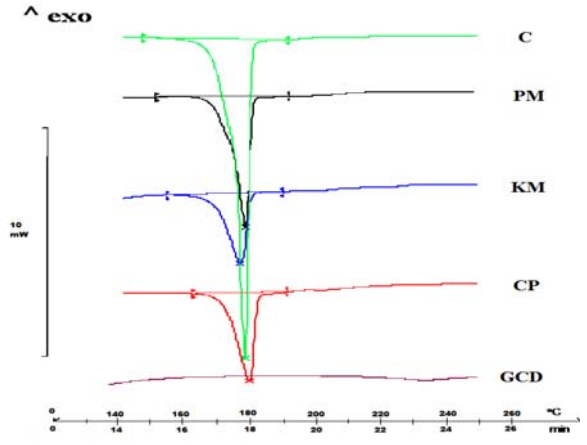


Figure 2. FTIR spectrum of Curcumin (C), γ-cyclodextrin-curcumin co-precipitation method (CP), γ-cyclodextrin-curcumin kneading method (KM), γ-cyclodextrin-curcumin physical mixture (PM) and γ-cyclodextrin (GCD).

D. Differential Scanning Calorimeter (DSC)

DSC is a very useful tool in the investigation of thermal properties of CD complexes [10]. It can provide both quantitative and qualitative information about the physiochemical state of guest inside the CD complexes. The complexation is result in the absence of exothermic peak or shifting to the other temperature which indicate changes in crystal lattice, melting, boiling or sublimation points. The thermogram of pure curcumin, pure γ-CD and kneading, physical mixture and co precipitation are represented in Figure 3 below. DSC thermogram of curcumin shows the exothermic peak at 176.52 ± 0.170°C indicating the melting point of curcumin.

The DSC thermograms for the curcumin: γ-CD systems show the persistence exothermic peak of curcumin in all products. However, the intensity of the peak is differing to each method used to form inclusion complexes. The melting peak for curcumin reduced in intensity in co-precipitation and kneading sample, whereas the intensity of melting peak for physical mixture remains the same. These results indicated there are major interaction between curcumin and γ-CD in the inclusion complex. Thus, the data obtained from DSC indicated that inclusion complex been formed using co-precipitation and kneading methods. However, physical mixture was unable to form inclusion complex.



F
curcumin (C), pure γ- CD (GCD), physical mixture (PM), kneading (KM); and precipitation (CP).

E. Field Emission Scanning Electron Microscopy (FESEM)

Figure 4 illusrates the FESEM images of pure curcumin, γ-CD and complex from co-precipitation, kneading methods and physical mixtures at magnification of

X 100. The curcumin was observed to be an irregular and flake shapes, while pure γ-CD appeared as pallelogram shapes. In physical mixture and kneading method, the images of complexes appeared as agglomerate, clumping to each other with similar particle sizes. However, the complex obtained by co-precipitation method appeared as crystals with a smaller particles size compared to kneading and physical mixture. The original structure of the raw materials disappeared and it was not possible to identify the original pure compounds. The drastic change in particle sizes indicates there is a new solid phase form in co-precipitation sample.

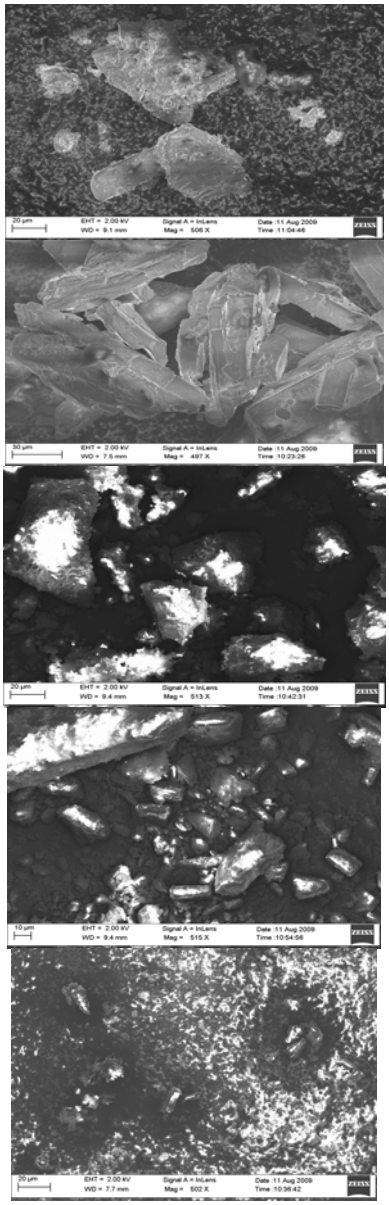


Figure 4. FESEM illustration of a) Curcumin, b) γ-cyclodextrin (GCD), c) Curcumin: GCD physical mixture (PM), d) Curcumin: GCD kneading method (KM), e) Curcumin: GCD co-precipitation method (CP) at magnification X 100.

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