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Formulation and Evaluation of Curcumin in Inclusion Complex using Beta Cyclodextrin

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Turmeric (Curcuma longa L.) has a naturally occurring yellow substance called curcumin. However, in vitro tests have demonstrated a number of outstanding pharmacological properties, including anti-inflammatory, anti-tumor, anti-microbial, and antioxidant properties. Because beta-cyclodextrin (BCD) has advantages like improving drug release and/or permeation and stabilising drugs in the formulation or at the absorptive site, it is crucial to use BCD to form inclusion complexes that will increase curcumin's ability to penetrate the membrane upon transdermal administration. Curcumin and beta-CD inclusion complexes were made using a solvent evaporation technique with mole ratios of 1:1, 1:2, and 2:1. Using physical characterisation of X-ray diffraction, differential scanning calorimetry, and a scanning electron microscope, the solids of the curcumin inclusion complex that produced the beta-CD were compared to the curcumin single component, beta-CD, and a physical mixture of curcumin-beta-CD. Curcumin-beta-CD inclusion complex formation is indicated by a mole ratio of 1:2, according to physical characterisation data. Curcumin-beta-CD is utilised

in inclusion complexes to improve the stability and solubility of curcumin.
Keywords: Inclusion complexes, beta-cyclodextrin, curcumin, characterization.

1. Introduction

Turmeric (Curcuma longa L.) has a naturally occurring yellow substance called curcumin. Curcumin has not yet received approval for use as a drug, despite demonstrating numerous excellent pharmacological activities in in vitro experiments, including antioxidant, anti-inflammatory, anti-microbial, and anti-tumor activities [1–4]. This is partly because of its poor solubility (11 ng/mL in aqueous buffer, pH 5.5 [5]) and stability ($t_{1/2}$ of curcumin in PBS pH 7.2, < 10 min [6]). There have been numerous attempts to address these issues. Creating an inclusion complex with cyclodextrins (CDs) is one of the most widely used methods. Numerous CD types, including naturally occurring α-, β-, and γ-CDs as well as their derivatives like methyl and hydroxypropyl CDs, have been reported to be employed for inclusion complex formation with curcumin in order to improve the solubility or stability of curcumin. Theoretically, depending on the size of the molecule and the CD cavity, different molecules can fit in different CDs. Which kind of CD would fit the curcumin molecule the best is still up for debate. Prior studies have frequently generated inclusion complexes with curcumin using β- and γ-CD and their hydroxypropyl derivatives [7–10].

A drug molecule acting as a guest inside the host molecule's cavity creates an inclusion complex; the host molecule is typically a member of the cyclodextrin derivative class. Cyclodextrin's hollow structure contains a hydrophilic group on the outside and a lipophilic group inside the cavity. [11] Through host-guest interaction with the internal cavity that creates a hydrophobic environment, this structure enables the cyclodextr to form inclusion complexes with a variety of organic molecules1.The most common cyclodextrin group in formula development and drug delivery systems is beta-cyclodextrin (BCD).[12] By improving drug release and/or penetration, stabilising drugs in the formulation or at the absorptive site, improving the solubilisation of lipophilic drugs, reducing drug-induced local irritation, delivering sustained release of drugs from the vehicle, and modifying drug bioconversion in the viable skin, cyclodextrins may be crucial in optimising local and systemic dermal drug delivery, according to a number of studies. [13, 14]

Physical characterisation techniques like differential scanning calorimetry (DSC) to determine a solid's thermal profile, scanning electron microscopy (SEM) to determine the solid's morphological characteristics, and powder X-ray diffraction (PXRD) to determine a solid's diffraction pattern and degree of crystallinity could all be used to determine whether the formation of curcumin inclusion complexes in Beta-CD was successful. [15]

2. MATERIAL AND METHOD

Material: Curcumin (Sigma, Aldrich), Beta-cyclodextrin (Wayne, NJ, USA), methanol pro analysis and distilled water. α- and γ-Cyclodextrin were obtained from ISP Technologies, Inc. (Wayne, NJ, USA); β- and Hydroxypropyl-β-cyclodextrin were obtained from Wacker Chemie GmbH (Germany).

Phase solubility study: To investigate the phase solubility, six distinct amounts of each CD were weighed, dissolved in one millilitre of distilled water, and then placed in a five millilitre glass vial to create six different concentrations ranging from zero to almost reaching its solubility (the solubilities of β-CD 178.87). For β-CD, the produced CD concentrations were 0, 2, 4, 6, 10, and 15 mM. Each CD solution received an excess of curcumin (~ 50 mg) and was agitated for 24 hours at 25 ± 1 °C until it achieved equilibrium. A 0.45 μm filter (Millipore, MA, USA) was used to filter the solution before a UV-visible spectroscopy at 430 nm was used to measure the amount of dissolved curcumin. The research was conducted in triplicate. The complexation constants were computed using the Higuchi and Connors method, and the associations between CD concentrations (x-axis) and curcumin concentrations (y-axis) were displayed. [16, 17]

Method of preparation: The solvent evaporation approach was used to create curcumin-based beta-CD. The mole ratios of 1:1, 1:2, and 2:1 were used to measure each curcumin and beta-CD component. After that, beta-CD was dissolved in distilled water and curcumin in methanol. For 24 hours, both were combined while being constantly stirred at 500 rpm. To create a precipitate, this combination was let to stand at room temperature until the solvent evaporated. Following drying, a 20 mesh screen was used to filter the precipitate of the inclusion complex compound, which was subsequently kept in a desiccator. [18-20]

Characterization: PXRD, FTIR, DSC, and SEM were used to analyse the physical properties of the curcumin and beta-CD single compounds. In order to determine the ratio that creates the compound of inclusion complexes of curcumin with beta-CD, the inclusion complex compound of curcumin-beta-CD was first prepared using the solvent evaporation method. It was then physically characterised using FTIR, PXRD, DSC, and SEM. [21-25]

Powder X-Ray Diffraction (PXRD): The X-ray diffractometer's sample chamber contained 100–200 mg of sample in the sample holder. The study was carried out using CuKα (Kα1 = 1.54060 nm; Kα2 = 1.54439 nm) at 40kV and 35mA in the range of 2θ 5-65° diffraction angles. [26]

Differential Scanning Calorimetry (DSC): Five to twenty milligrammes of sample were added to the DSC instrument's alumina crucible. The temperature range for thermal analysis was 30-300 °C, with a heating rate of 10 °C per minute. [27]

Fourier-Transform Infrared (FTIR) Spectroscopy: FTIR spectra of curcumin, curcumin-β-cyclodextrin complex, and equivalent physical mixes were performed using the Fourier transform infrared spectroscopy model, IRAffinity-1, Shimadzu, Japan, with a resolution value of 4 cm^{-1} in the 400–4000 cm^{-1} range. The powder was combined with KBr and pounded finely with a mortar and pestle prior to measurement. [28]

Scanning Electron Microscope (SEM): A few samples were put on the sample holder and auto fine-coated with gold-palladium. After that, samples are put on the SEM specimen chamber and examined on a computer so they can be photographed at the proper magnification. [29, 30]

3. RESULT AND DISCUSSION

Phase solubility: Figure 1 illustrates the correlations between curcumin concentrations and the CD. As the concentration of CD rose, so did the curcumin concentration. According to Higuchi and Connors' notions, the connections were linear, and the plots' slopes ranged from 0 to 1, suggesting an AL-type of phase solubility. These findings show that the curcumin-CD combination formed in the study at a 1:1 ratio.

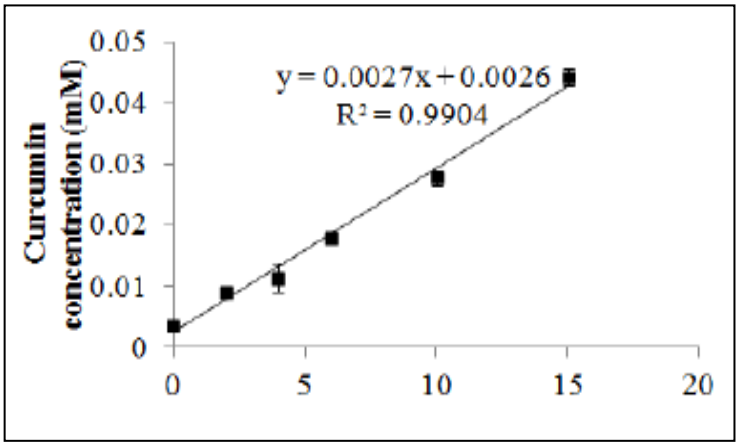


Figure 1: Relationships between curcumin and cyclodextrin concentrations

Preparation of Inclusion Complexes of curcumin-beta-CD: According to the solubility data, beta-CD was soluble in distilled water, however curcumin was soluble in methanol. Based on Table 1, the beta-CD inclusion complexes were created using mole ratios of 1:1, 1:2, and 2:1.

Table 1: The weight of Curcumin and beta-CD in each mole ratio

Molecule	Measured weight based on the mole ratio		
	1 : 1	1 : 2	2 : 1
Curcumin	0.701 g	0.701 g	1.402 g
Beta-CD	2.270 g	4.540 g	2.270 g

P-XRD Techniques: Curcumin's distinctive peaks (Figure 2) may be seen at 8.62◦, 11.9◦, and 14.2◦, 17.02◦ (2θ). There have been prior reports of similar peaks in the literature [88]. Additionally, these peaks can be linked to the crystalline curcumin polymorph. Curcumin characteristic peaks disappeared from the PXRD pattern of the curcumin complex with β-cyclodextrin (Figure 2), and the emergence of additional weak peaks in the complex's diffractogram suggests the production of an inclusion complex that exists as a new crystalline phase. Using the co-precipitation approach, similar results were previously reported for the complexation of curcumin with β-cyclodextrin polymer.

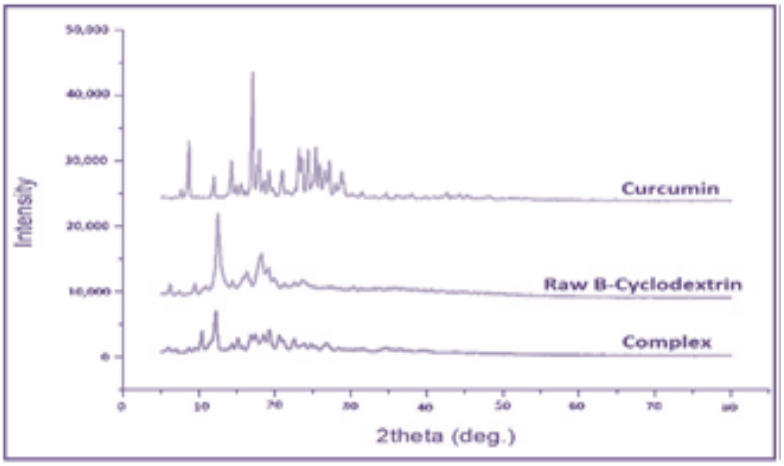


Figure 2: PXRD patterns of curcumin-β-cyclodextrin complex

DSC Technique: Curcumin's DSC thermogram (Figure 3) revealed an endothermic peak at 173.3 ◦C, which is the compound's melting point. Curcumin's melting point has been reported in the literature to be between 172.85 and 187 ◦C. Two endothermic peaks were seen in the β-cyclodextrin thermogram (Figure 3): the melting point of the β-cyclodextrin polymer is represented by the peak at 312.7 ◦C, while the broad peak at 115.3 ◦C indicates water evaporation. The curcumin endothermic peak vanished from the DSC thermogram of the curcumin combination with cyclodextrin. This demonstrates that curcumin is encapsulated in the cavity of the β-cyclodextrin polymer, which replaces water molecules, and validates the development of the inclusion complex; comparable results have been documented in the literature.

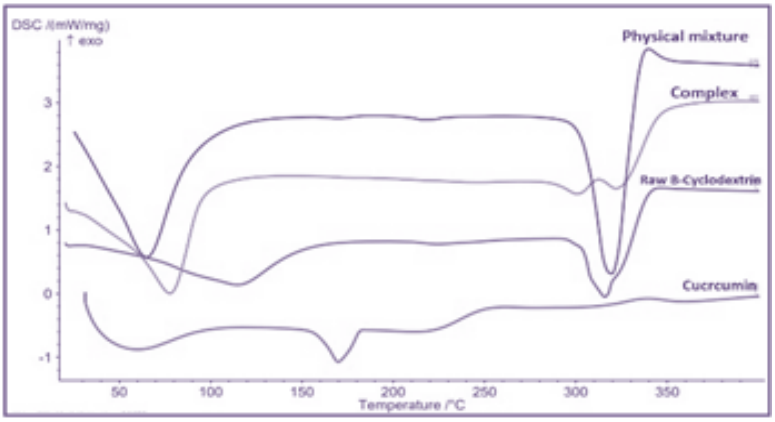


Figure 3: DSC thermograms of curcumin-β-cyclodextrin complex

Fourier-Transform Infrared Spectroscopy: The curcumin combination with β-cyclodextrin, raw curcumin, and the matching physical mixture peaks are shown in Figure 4. The O–H stretching vibration at 3516 cm−1 was identified as the representative peak of curcumin, followed by the benzene ring stretching vibration at 1624 cm−1, the presence of mixed (C–C)

and (C–O) vibrations, as well as (C=O) carbonyl bond vibrations and in-plane bending vibrations around aromatic (CC–H) of keto and enol configuration forms of curcumin at 1514 cm−1, the aromatic (C=C) stretching vibration at 1427 cm−1, and the (C–O) bending vibrations of the phenolic band at 1262 cm−1. Alongside the distinctive peaks of the cyclodextrin polymer at 2933 cm−1 corresponding to the (CH_2) group stretching vibration and at 1033 cm−1 reflecting the (C–O–C) stretching vibration, the physical mixture's FTIR spectrum showed the distinctive bands of curcumin. The typical peaks of curcumin were absent from the FTIR spectrum of the curcumin combination with β-cyclodextrin. On the other hand, distinctive β-cyclodextrin peaks are visible, providing a strong hint of inclusion complex development.

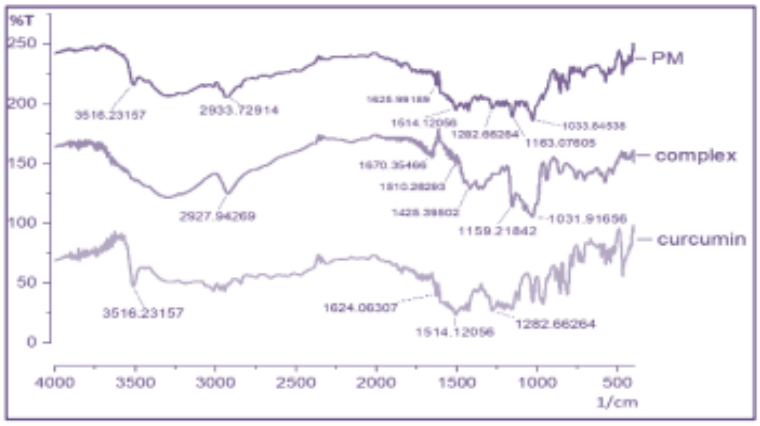
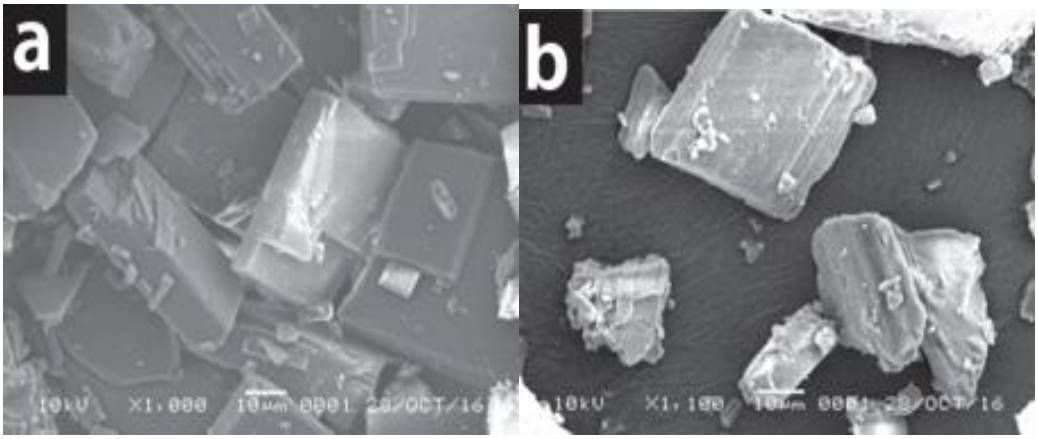


Figure 4: FTIR spectra of curcumin-β-cyclodextrin complex

Scanning Electron Microscope: Pure curcumin (Figure 5a) exhibits the flattened rod or needle shape in the SEM image. Although it is not readily visible, pure BCD (Figure 5b) is a tubular cylinder that is distinct from this. In contrast to each individual component of the curcumin and beta-CD habit that forms the irregular or amorphous habit, the solid from the outcomes of the curcumin-beta-CD inclusion complex exhibits distinct habits. This suggested that curcumin and beta-CD might interact to generate an inclusion complex molecule.



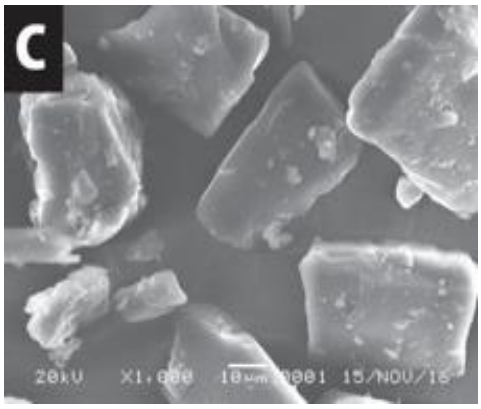


Figure 5: SEM micrograph. a; Curcumin, b; beta-CD, c; physical mixture of curcumin-beta-CD

4. CONCLUSION

The inclusion complex of Curcumin-beta-CD was generated at a mole ratio of 1:2, according to the three analyses or characterisations. The typical shift from crystalline to amorphous was shown by the PXRD study. The Curcumin melting point was invisible according to the DSC data, but the beta-CD thermal profile was visible. Curcumin-beta-CD inclusion complexes have an amorphous habit and a particle size of less than 10 μm, according to the morphological investigation by SEM.

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