

## Production of curcumin-betacyclodextrin nanocomplex from turmeric roots plant

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### Abstract

The idea of this study is to synthesize the curcumin-betacyclodextrin nanocomplex in sustainable green methods through extraction that depends on grinding the roots of turmeric plant and then dissolving it in ethyl alcohol and exposing it to ultrasound, which is considered the whole process to achieve sustainable development standards, This is done by linking curcumin to beta-cyclodextrin to increase its solubility and stability, thus increasing its bioavailability and then utilization by the living cell. We find that the results of the tests obtained first using the PHPLC preparatory high-performance liquid chromatography device showed that the ethyl extract of the roots of the turmeric plant contained the active compound curcumin. The best concentration of curcumin from which the nanocomposite was prepared was 10% of the four studied concentrations of curcumin, which carried a fixed percentage of betacyclodextrin. As for the stability rate of curcumin in betacyclodextrin and the time of its release, the value was about 80% at 72 hours and the pH reached 7.4. As for the nano-testes of the compound by scanning electron microscope of the compound of curcumin - betacyclodextrin, it was in the form of small, compact, regular crystals, measuring within nanoscale ranges of up to 120 nanometers, which was within the specifications of ideal nanoparticles in terms of not agglomerating with each other due to electrostatic forces at room temperature.

**Keywords:** turmeric plant, curcumin, betacyclodextrin, zeta effort, PHPLC

### Introduction :

Recently, unprocessed natural and biologically active plant extracts have received great attention, due to their curative properties known to most people of the world. One of the natural plant sources that were previously addressed is turmeric (*Curcum Longa*) [17]. The turmeric plant has been used for centuries in Indian medicine, where this herb was considered a miracle that made its methods to its important and because it contains a wide range of medicinal benefits[1]. A previous study found that the roots of the turmeric plant contain three main phenolic compounds, namely curcumin,

which constitutes 75%, Dimethoxycurcumin, which constitutes (10-25)%, and Bis-demethoxycurcumin, which constitutes 5%, [11]. Curcumin extracted from turmeric is a functional compound that possesses healthy and protective properties [10]. and has antioxidant, anti-inflammatory, antimicrobial and anti-mutagenic properties [13]. Curcumin is a molecular compound, also known as diferuloyl methane, with a chemical formula of  $C_{21}H_{20}O_6$ , a molecular weight of 368.38, and a hydrophobic distribution coefficient of Log P 3.0. This makes it highly insoluble in water and soluble in polar solvents such as methanol, ethanol, dimethyl sulfoxide, chloroform, ethyl

acetate, etc. It is slightly soluble in aliphatic hydrocarbon solvents, such as hexane benzene and kerosene [14]. As for the methods of extracting the active compound, ultrasound is a method followed recently and promising to extract biologically active compounds from their raw sources. Ultrasound waves cause swelling and expansion of the pores of the outer wall of the active substance, which facilitates the access of the extraction solvent to the inside of the active substance. It increases the size of the minute gaps in the wall of the active substance, which leads to its destruction and causes a shock that eventually leads to a disturbance in the wall of the active substance, and this process facilitates the exit of the components from the inside to the outside of the solvent [11]. on the other hand, other studies have indicated the possibility of preserving the active substance and not losing it through the gradual release of it by means of a coated substance loaded inside which improves its effectiveness and bioavailability, moreover, it acts as a protective coating against oxidizing factors (light, heat and oxygen). It can also mask the undesirable flavors and odors that the active ingredient may contain [2,3,4,7,8,9]. The research aim is to collect the extract of curcumin from the turmeric plant and then combine it with the polymer beta cyclodextrin nanosynthetic and study some of the nano properties of the new nanocomposite after the preparation process.

### Materials and Methods:

Curcumin used as a standard sample was purchased from (sigma-aldrich) company, while beta-cyclodextrin was purchased from sigma-aldrich company). The dried roots of the turmeric plant (*Curcum longa*) were obtained from the local markets of the city of Hilla - Babylon province and tested by the Ministry of Agriculture - the General Authority for Seed Examination and Certification, and a weight of approximately 250 g was selected. The turmeric roots were washed with pure water and dried in the oven at a temperature of 40 degrees Celsius

for a whole day, after which the roots were ground in a special mill for grinding grains for the purpose of obtaining turmeric powder.

### PHPLC Preparatory High Performance Liquid Chromatography:

The method [6]. was followed in the investigation and collection of the active compound (curcumin) extracted from what was mentioned, where a high-performance preparatory liquid chromatography device (Jasco, Gross-Umstadt, Germany) of the Ministry of Science and Technology - Department of Environment and water. The sample was prepared by dissolving 50 mg of the dried extract in 50 ml of ethanol in a 200 ml volumetric flask. The sample was placed in the ultrasonic device for 5 minutes for the purpose of complete homogeneity, after which 2 microliters were injected using the auto-injector. As for the separation column, it was of the type (250mm × C18) 5 μm; 4.6 Monochrom Diol Column, and the mobile phase was a mixture of 2% acetonitrile and acetic acid (50:50).

### Method of preparation of curcumin-β-cyclodextrin (the nanocombination process of curcumin and β-cyclodextrin)

The method [16]. was followed with some modifications in the method of work where 40 mg of β-cyclodextrin was dissolved in 8 ml of deionized water with stirring for 5 minutes in the magnetic stirrer for the purpose of obtaining complete homogeneity of the mixture and ensuring from the dissolution process. Concentrations (10%) -8 mg of curcumin were taken and dissolved separately in 500 μl in ethanol. The solutions were mixed in a closed dark glass container and then the container was placed in an ultrasonic device for 5 minutes, then the solution was mixed using a magnetic stirrer at 400 rpm for 5 hours without a lid to allow the ethanol to evaporate. The solution was then placed in a centrifuge at 3000 rpm for 15 min to separate the remaining particles. The solution containing curcumin and

betacyclodextrin nanoparticles was filtered by Buchner funnel and using 0.45 µm filter paper to remove the impurities and sediment formed in the solution, then the solution was dried to obtain the substance in powder form.

#### **Measurement of curcumin loading efficiency with betacyclodextrin:**

it followed the method described by [15]. with some modification, Where 1 mg of the nanocomplex (curcumin-betacyclodextrin) was dissolved in 50 ml of the organic compound dimethyl sulfoxide (DMSO) to liberate curcumin from betacyclodextrin and thus estimate the efficiency of the loading process. The sample is then shaken by a large capacity reciprocating shaker, Guohua, for approximately 12 hours at room temperature and in a dark place, then the sample is separated by centrifugation at a speed of 14,000 rpm to remove agglomerates of betacyclodextrin, then collect the pure supernatant of the solution (DMSO) containing curcumin for use in measuring the efficiency of encapsulation. The concentration of curcumin was estimated by means of an ultraviolet spectrophotometer at a wavelength of 430 nm. Note that the standard sample was composed of curcumin dissolved in DMSO solution.

#### **Measurement of curcumin-betacyclodextrin stability in laboratory:**

The stability of curcumin retention in the betacyclodextrin molecule was calculated through the method described by [19]. with some modification. The working method included a weight of 10 mg for the concentrations used for the curcumin-betacyclodextrin complex to be dissolved separately in 5 ml From 0.01 M physiological buffer solution (PBS) with pH 7.4 to be incubated in the vibrating incubator at a temperature of 37 °C for 72 hours using a spectrometer with the use of curcumin as a

standard sample. The following equation was calculated:

Curcumin retention percentage = (retained curcumin - liberated curcumin) / sequestered curcumin.

#### **Characterization of curcumin-betacyclodextrin by SEM scanning electron microscopy:**

The surface shape of the synthetic nano curcumin-betacyclodextrin extract was evaluated by scanning electron microscopy (SEM, Hitachi SU-8230, Japan) at an acceleration voltage of 15 kV. Then the dry samples were spread on double sided carbon tape.

#### **Determination of the zeta potential of the nanocomposite (curcumin-betacyclodextrin):**

The average diameter of the encapsulated nano curcumin was determined by a Dynamic Light Scattering technique using Zetasizer nanoZS90 (Malvern Instruments Ltd., Malvern, UK) to determine the size of the encapsulated nanoparticles. The sample was prepared to measure the polydispersity and charge of nanoparticles after adding 5 µl of samples in a 2-ml Zeta device tube according to the instructions of the supplied company.

#### **Results and discussion:**

##### **PHPLC Preparatory High Performance Liquid Chromatography:**

After the process of extracting curcumin from the turmeric plant *Curcum longa* and purification using filtration under vacuum filtration using Pore size 0.45µm filter paper, the sample was injected into a Preparative HPLC high-performance liquid chromatography device in order to separate and diagnose curcumin extracted from turmeric, We notice from Figure (2-4) that its results converge with

the standard compound curcumin at a wavelength of 425 nm. Curcumin is extracted from the turmeric plant. The peak that appeared at the time (19.808) was considered to be the peak of the curcumin extract due to its appearance in the extract to be separated and its conformity with the time of its appearance in the standard sample. Its concentration in the active compound curcumin extracted from the turmeric plant was about 0.74 mg, And as shown in Figures (1-a,b), we find that the results agree with what [6], indicated about the diagnosis of

curcumin using the preparatory high-performance liquid chromatography technique. As for the two peaks that appeared in the standard compound, it may be due to some components of turmeric, such as Demethoxycurcumin and Bisdemethoxycurcumin. Finally, parts of the curcumin extract were collected by the Fraction Collector of the same preparatory high-performance liquid chromatography device and used in subsequent experiments.

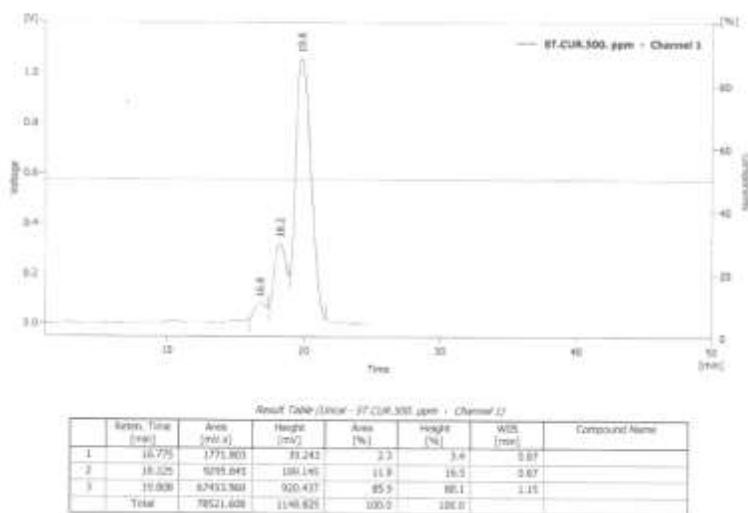


Figure 1-A: Peaks of standardized curcumin extract using PHPLC preparative high-performance liquid chromatography technique

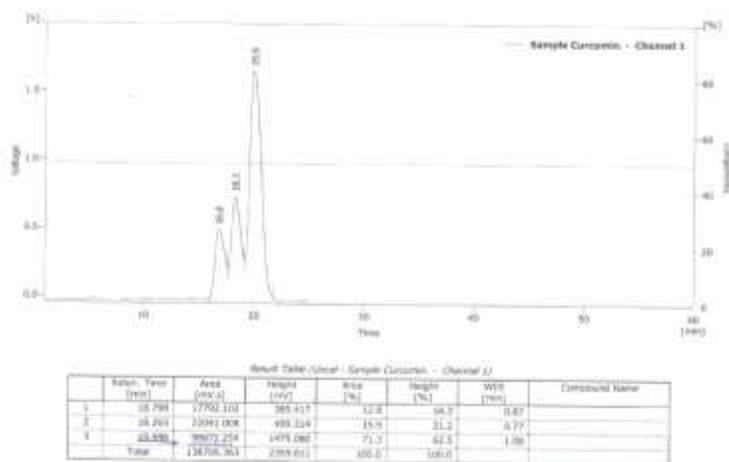


Figure 1-B: The peaks of curcumin extracted from the turmeric plant for the studied sample using PHPLC preparative high-performance liquid chromatography technique.

**Measurement of curcumin loading efficiency with betacyclodextrin:**

The loading efficiency of betacyclodextrin for nano curcumin was appropriate for each of the concentration (10)% of curcumin added to the synthetic mixture. The percentage of free curcumin that is not bound to the biopolymer was less than it is compared with the high concentrations of curcumin, which amounted to

about (20, 30%), It showed the inability of betacyclodextrin to withstand the increase in the percentage of added curcumin, which is due to the inability of the inner cavity of the biopolymer to absorb, and this is somewhat different with what was mentioned [16], who showed that a concentration of 20% of curcumin may be suitable for the process Loading with betacyclodextrin.

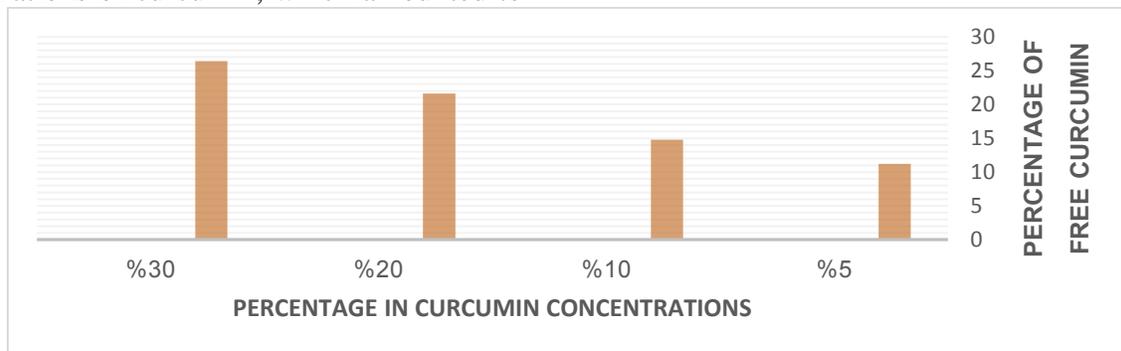


Figure 2: shows the efficiency of curcumin loading inside the cavity of betacyclodextrin at different concentrations (5, 10, 20, 30%)

**Measurement of the stability of curcumin-betacyclodextrin in laboratory:**

We note that the length of retention and stability period of the curcumin-betacyclodextrin

nanocomplex for all the studied ratios is equal, which ranged between 89-80% for up to 72 hours, which shows the efficiency of the curcumin loading process inside the vacuoles of the biopolymer.

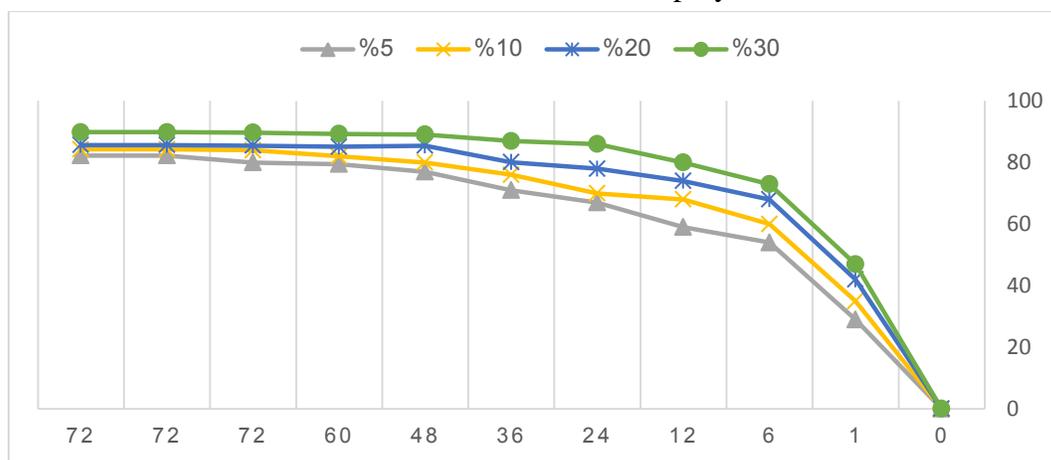


Figure 3: shows the ranges of curcumin release at different concentrations (5, 10, 20, 30) % of betacyclodextrin.

### Surface characterization of nanoparticles using scanning electron microscopy (SEM):

From the figure below, which shows the form of (curcumin - betacyclodextrin), (curcumin) and (betacyclodextrin) under different magnification strength, where it was observed from right to left that the nanocomposite in picture (C) may

be in the form of regular crystal clusters and stacked to each other, encapsulating the free curcumin compound that appears in picture (B) in the form of crystal rods, while in picture (A), we find that the biopolymer itacyclodextrin has Crystalline flakes appeared through a scanning electron microscope.

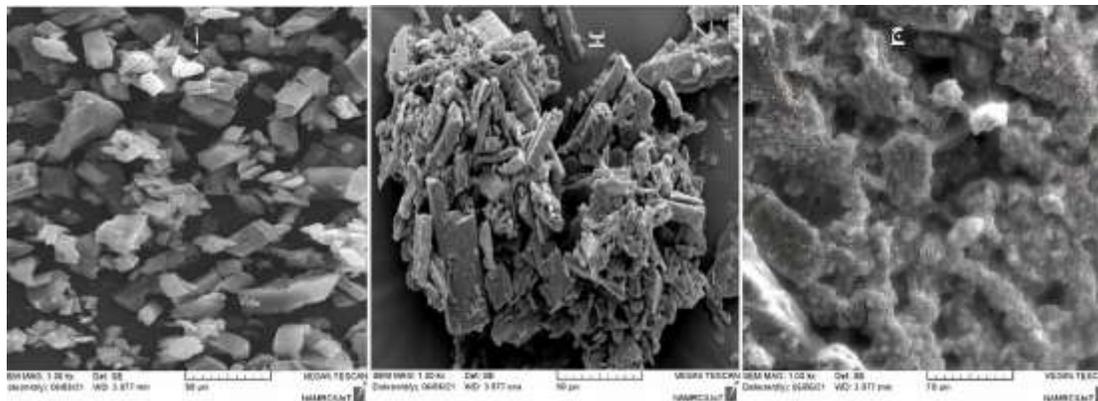


Figure 4: SEM image of (a) betacyclodextrin, (b) free curcumin, (c) curcumin-betacyclodextrin overlay.

### Estimation of the zeta potential of the nanocomposite (curcumin-betacyclodextrin):

Through the attached picture below, we find that the zeta potential measured by a Nano ZS-Malvern Instruments Ltd zetasizer device for the 10% curcumin-betacyclodextrin nanoparticle was -33.8 millivolts. This shows the efficiency of the process of loading the active compound (curcumin) with the biopolymer through the repulsion of the charges available on the surface of the nanoparticle and the charges of the dispersion medium immersed in it and thus its stability, which must exceed the value of the zeta potential of  $\pm 25$  millivolts, which indicates the extent of stability Synthesized curcumin-

betacyclodextrin particles and not agglomerate or combine with each other by electrostatic forces at a temperature of 30 °C. A relatively high polydispersion index (PdI) was obtained of 0.126, which is an indicator of sample heterogeneity, which should not exceed 0.2 for most particles that are within nanotechnology specifications, and this was shown by previous studies [18,12]. As for the particle size of curcumin-beta-cyclodextrin, it was about 120.8 nm at the highest concentration of the synthesized particles, and these results are consistent with the standard dimensions for the synthesis of highly effective nanoparticles that meet the bioavailability specification for the active compound [5].

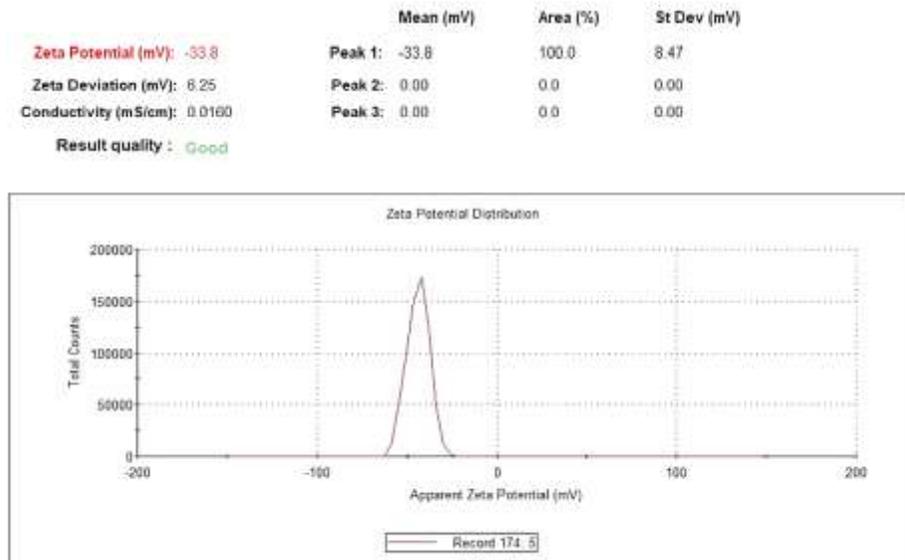


Figure 5: Zeta potential for nano curcumin-beta-cyclodextrin nanocomplex at 30 °C after thirty days of synthesis.

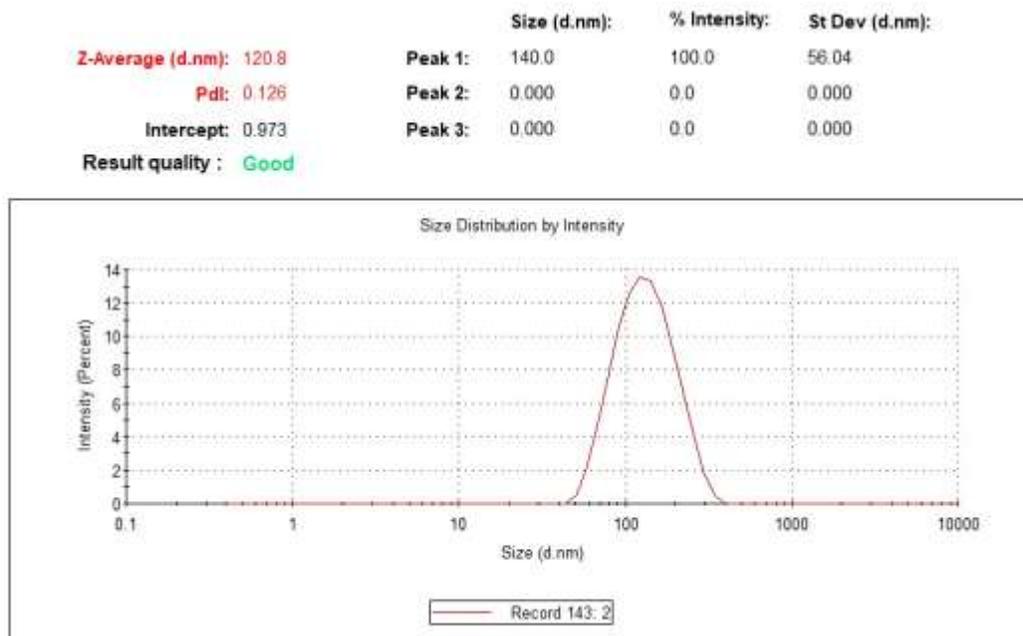


Figure 6: Nanoparticle size and dispersion coefficient measurement using a Zetasizer for the curcumin-beta-cyclodextrin complex at 30°C.

**Conclusion :**

The obtained results show the success of the curcumin loading process on the beta-cyclodextrin particle, However laboratory experiments must be conducted to confirm the

maintenance of the antioxidant and anti-microbiological properties after the loading process.

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