



## Synthesis of Nanocurcumin-alginate Conjugate and Its Characterization by XRD, IR, UV-Vis and Raman Spectroscopy

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### ABSTRACT

The compounds which extracted from spices and herbs exhibit antiviral, anti-fungal and anti-cancerous effects having potential pharmacological uses. Curcumin, a component of turmeric, which has been used as a food additive and a coloring material in India and other Asian countries due to its potential medicinal properties. Earlier reports suggest that application of curcumin in foods is limited because of its low bioavailability and its high degradation in acid and alkaline medium. In this study the effect of stabilization of both free curcumin and nano curcumin–alginate conjugate in honey was studied by UV–Vis absorption, IR, vibrational spectroscopy (Raman) and XRD. Curcumin is degraded in acid and alkaline medium is highly stable with the nano formulation. From this work it was deduced that in presence of surfactant honey curcumin-alginate inhibits the formation of small sub-products. This work reveals the complexation of curcumin with alginate in presence of surfactant honey was demonstrated to protect this molecule from the degradation. UV–Vis, FTIR XRD and Raman spectroscopy were important to determine the nature of the structural modifications.

**Keywords:** Curcumin, UV-Vis, FTIR, XRD and Raman Spectroscopy.

### INTRODUCTION

Curcumin is a bright yellow chemical produced by plants of the ginger family (Zingiberaceae). It is the principal curcuminoids in herbal supplement, cosmetics ingredient, food flavoring, and food colouring<sup>1</sup>. Although curcumin has been used widely in *Ayurvedic* medicine, its potential medicinal

properties are till unproven and are an area of active investigating field. Chemically, curcumin is a diarylheptanoid with two methoxide and hydroxyl groups, belonging to the group of curcuminoids, which are natural phenols responsible for turmeric's yellow color. It is a tautomeric compound existing in enolic form in organic solvents and as a keto form in water (Figure. 2).<sup>2</sup>



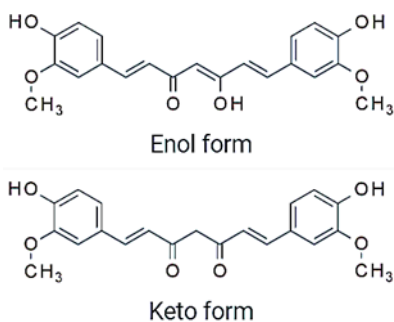


Fig. 1. Curcumin keto and enol form

Moreover, evidences from long-term use process and preclinical trials have demonstrated low toxicity of curcumin, even at relatively high doses.<sup>3</sup> However, it has been well known that the application of curcumin was limited due to its low water solubility, high degradation, and poor bioavailability.<sup>4-10</sup> For decades, several studies have been made to compensate for these disadvantages, with the development of improved drug delivery methods<sup>11-16</sup>. Recent researches witnessed the encouraging progress in the use of nanoscale drug delivery systems on curcumin such as loading curcumin into liposomes or nanoparticles, forming self-micro emulsifying drug delivery systems, cyclodextrin inclusions, and solid dispersions, as well as the latest reported technologies such as nano disks and nanotubes. However, good things never come easy. Applicational advancement of curcumin has been hindered by its water insolubility, degradation at alkaline pH, and photo degradation and thus extremely low bioavailability in both vascular and oral administration. Therefore, many inventions have been investigated, and an enhanced approach of using nanoscale drug delivery systems to overcome deficiencies. Among these methods, nanoscale drug delivery systems have become a source of concern for many researchers in the domain of traditional medicine.<sup>17</sup> Many successful examples of the combination of nanotechnology and traditional medicine have been reported. Alginate acid is a natural hydrophilic gel with special physical-chemical and biological properties, including non-toxicity, chelating metal ions, biodegradability, antibacterial and antifungal effects, immune system stimulation, etching metal ions, biodegradability, antibacterial and antifungal effects, immune system stimulation, etc.<sup>18,19</sup>. In this study curcumin- alginate conjugate in honey as surfactant nanoparticles were synthesized

by using the simple sonication green method and were characterized by SEM, TEM, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) AND XRD.

## MATERIALS AND METHODS

### Materials

All chemicals were of reagent grade and were used without further purification. Distilled water was obtained from a water purifying system; acetic acid and NaOH were obtained from laboratory supplies (Tvp) while curcumin powder and alginate acid were obtained from Merck.

### METHODS

About  $2.5 \times 10^{-4}$ M of curcumin was dissolved in 15mL of acetic acid solution containing t, followed by the addition of 5mL of 2% alginate acid and the mixture was stirred continuously for 1 hour. After 1 h, 1mL of 1;1 surfactant solution (1% w/v) was added drop-wise to the mixture and stirred for an addition of 1 hour. Curcumin was loaded nano particles formed during the stirring. The curcumin loaded nanoparticles were collected by centrifugation and the samples were washed several times with ethanol to remove any excess of curcumin that adhered at the surface of the nanoparticles. This experiment is also repeated with NaOH solution.

### Morphological studies of Materials

Powder X-ray diffraction patterns were recorded on an X-ray diffraction (XRD; D8 Discover, Bruker AXS GmbH ( $\lambda = 1.5406$  nm). X-ray Diffractometer (Bruker D8 Advance): X-ray diffractometer (Bruker D8), possess innovative design namely DAVICNI, which combines operating safety, ease of use and user safety. It can be operated in both Powder X-ray Diffraction (PXRD) and Grazing Incidence X-ray Diffraction (GIXRD) modes. Morphological analysis was examined on a Scanning Electron Microscope (SEM; JSM7600F, JEOL), Nanocurcumin solution prepared is evaporated to dryness and is analysed SEM by coating on the surface of carbon and sputtering with gold. The images of TEM were taken by a JEM-1010 (Jeol, Japan) operated at an accelerating voltage of 200 kV. Infrared data were examined on KBr pellets by

using a Shimadzu IR Prestige-21 spectrometer (Japan). UV-Vis and UV-Vis-diffuse reflectance spectra were collected on a DR-Jasco V630 or a UV-Vis DRS-Jasco V670 spectrophotometer that was equipped with a diffuse reflectance attachment in which BaSO<sub>4</sub> was the reference. Micro Raman Spectrometer (with excitation wavelength of 514nm and 785nm available), Micro Raman Spectrometer (with excitation wavelength of 514nm and 785nm available). FT Raman Spectrometer is a multi-RAM stand alone model. The spectral range is 4000-50 cm<sup>-1</sup>. About 50mg solid sample in liquid form is introduced in BRUKER RFS 27 stand-alone FT-Raman Spectrometer.

## RESULTS AND DISCUSSION

### Morphology of nanoparticle curcumin by SEM and TEM

An SEM image of Fig. 2(a), (b), (c) of NPs loaded with curcumin shows that particles are almost spherical in shape and not uniform. The particle size is in the range of 30-100nm. The SEM image of freshly prepared curcumin-micelles indicates that the self-assembled micelles are well dispersed as individual particles with spherical shape. This micrograph proves the incorporation of alginate inside the NPs matrix. Our results are consistent with those of other research. Morphological analysis was studied with electron microscopic images. In the SEM images of curcumin alginate nanoparticles, it can be seen clearly that the particles are aggregated, with approximate spherical shape having size 80–25 nm range (Figure 1).

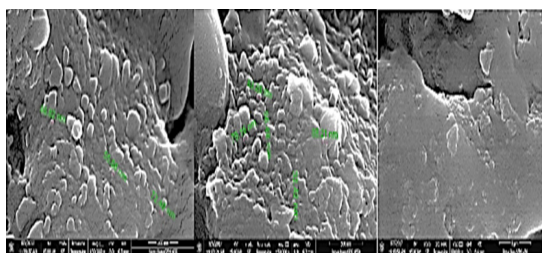


Fig. 2(a),(b) and (c) SEM images of curcumin loaded alginate nanoparticles

The TEM images in Fig. 3 curcumin-alginate nanoparticles reveal that the nanoparticles are spherical in shape, and the average size was 20-50nm. Thus, the immobilized polymer on nanoparticles did not lead to the aggregation

between the particles. This shows the appearance of the bonds on surface of nanoparticles

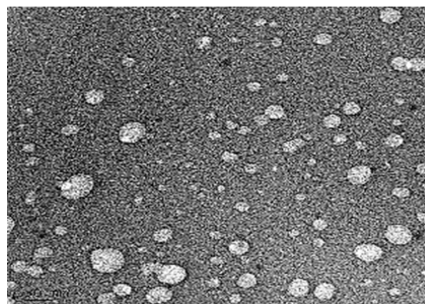
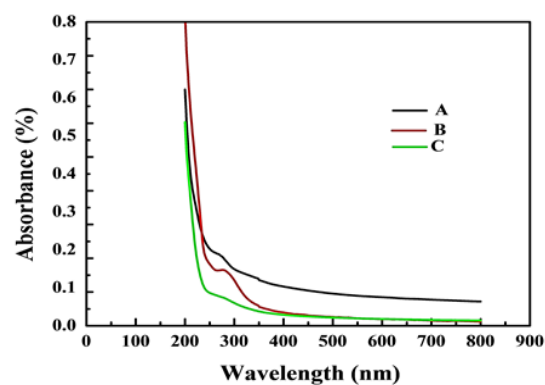
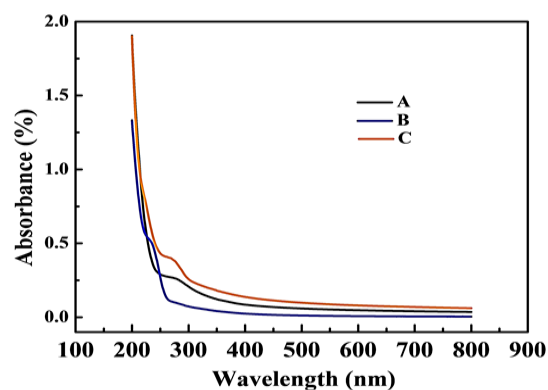


Fig. 3. Transmission Electron Microscopy of range 20 nm

### Ultraviolet-visible spectroscopy analysis



(a)



(b)

Fig. 4. (a) and (b) a-cur+acetic acid /HAC b-cur+acetic acid/ HAC+alginate acid+honey c-cur+acetic/HAC acid+alginate acid

UV-Vis spectra (Fig. 4.1a) shows a broad absorption band at 285 nm in comparison with the two others such as Fig. 6.1(a) and (c). This may be due to the formation of nanocurcumin. The medium for this nanocurcumin formation is HAC. In HAC

the nano curcumin formed is more stable than the nanocurcumin in NaOH. In NaOH the peak intensity is also less in comparison with the medium NaOH. In spectra (Fig. 4.1b) the peak intensity as well as broadening are greater for curcumin in HAC, but for curcumin alginate in surfactant has a less intense and much sharper peak. Formation of nanocurcumin shows the deviation of spectra. Fig. 4.1 also shows the same variation in spectra.

### Raman Spectroscopy for Analysis

Raman spectra (Fig. 5-2(a) and 2-(b)) reveals the nanocurcumin alginate in two different media in NaOH and in  $\text{CH}_3\text{COOH}$ . In NaOH medium the curcumin under goes some degradation hence intensity reduces. Nano particles formed are found to be less compared to the nanoparticles formed from acetic acid. curcumin normal, a functional group vibration in the region of  $1500\text{-}1650\text{ cm}^{-1}$  is shown in the figure which is due to  $\text{C}=\text{O}$  vibration, but in the case of nano curcumin the peak at this region is very intense and sharp. An other major peak is at  $1060\text{ - }1150\text{ cm}^{-1}$ . Raman spectra of nanocurcumin is too much intensive than normal curcumin.

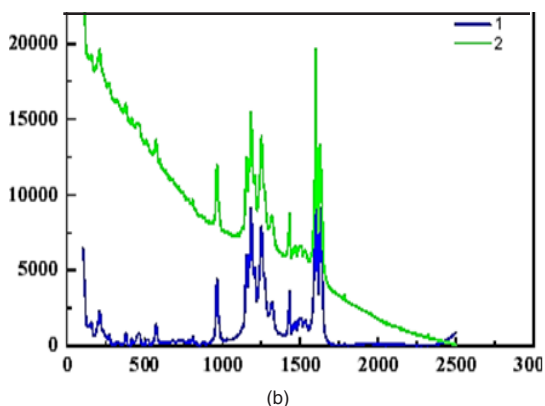
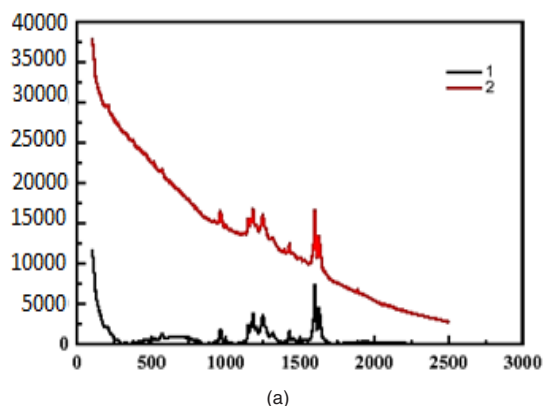


Fig. 5(a) and (b) a-Raman 1 normal curcumin poly8 Smooth and - b Raman 2 nano form

### FTIR analysis

The study of Infrared spectra (Fig. 6) gives an idea about macro and nanoparticles of curcumin. Nanocurcumin has an intense and sharp peaks compared curcumin which have broad and less intense peaks. Curcumin nanoparticles have a stretching frequency near  $1690\text{ cm}^{-1}$  similar to the value of carbonyl frequency in curcumin and another stretching frequency value at  $3300$  region which is of broad and of H-bonded  $-\text{OH}$  group in curcumin. The nanoparticles a less intense peaks in the region  $950\text{-}750\text{ cm}^{-1}$  is due to aromatic  $\text{C}=\text{C}$  stretching. Next we have done a comparative study of nanocurcumin in different media in HAC and in NaOH. This also proves the stability and nanocurcumin formation of nanocurcumin. FTIR was studied to confirm the functional groups of the curcumin conjugate nanoparticles. The spectra of pure alginate, honey, nano curcumin alginate complex in honey-alginate conjugate( in NaOH and in HAC) were shown in Fig. 3. The presence of two strong absorption bands of all materials at around  $3100\text{-}3400\text{ cm}^{-1}$  and  $1650\text{-}1700\text{ cm}^{-1}$  shows the formation of nanoparticles. Moreover, the sharp intense peak band at  $1690\text{ cm}^{-1}$  was confirmed as the strong stretching vibration of the two  $\text{C}=\text{O}$  functional group structure. The absorption bands at  $1500\text{-}1400\text{ cm}^{-1}$ , attributed to  $\text{C}-\text{O}$  groups in ether. Intense broad peaks around  $3400\text{-}3100\text{ cm}^{-1}$  is due to the  $\text{O}-\text{H}$  stretching of the nanoparticles. A less intense peaks in the region  $950\text{-}750\text{ cm}^{-1}$  is due to aromatic  $\text{C}=\text{C}$  stretching. The very less intense and very broad peaks at  $1510\text{ cm}^{-1}$  and the band at  $1627\text{ cm}^{-1}$  is attributed to the  $\text{C}-\text{C}$  stretching vibration of the aromatic ring. On the other hand, the appearance of two strong bands at about  $13000$  and  $1040\text{ cm}^{-1}$  was confirmed as vibration of methyl phenylether group ( $\text{C}-\text{O}-\text{CH}_3$ ). This suggests that curcumin binds very strongly with alginate nanoparticles.

### XRD Analysis

Figure reveals the results of X-ray diffraction (XRD) analysis for curcumin conjugate nanoparticles synthesized by simple sonication method. The diffraction patterns have seven main peaks at  $2\theta$  values bet ween  $15.2\text{-}30$  corresponding to the reflection from different crystal planes. Positions and relative intensities of all the peaks are in similar to crystalline system of curcumin nanoparticles. The narrow shape peaks of the substance indicate that the nanoparticles have relatively very good

crystalline state corresponding to the diffraction peaks of (110), (104) at 2θ positions. Broadness of the diffraction peaks was related to particle sizes. Scherer's equation

$$D = \frac{K\lambda}{\beta \cos\theta}$$

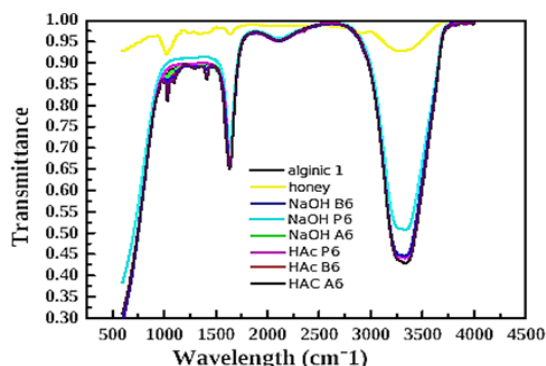


Fig. 6(a). FTIR Spectra

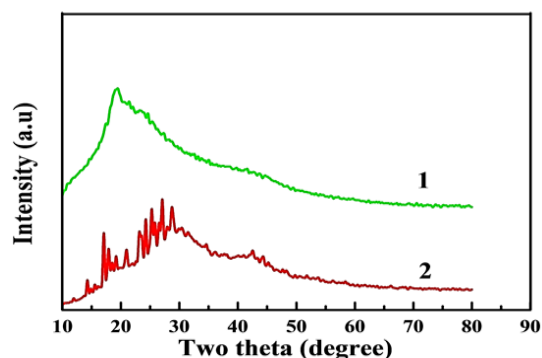


Fig. 6(b)1-Normal nano curcumin, 2-nanocurcumin-chitosan conjugate in honey

was used to calculate the average particle size D. In this equation θ is the angle of the peak, β is the full width at half maximum (FWHM) of the respective XRD peak, λ is the x-ray radiation wave-length in angstroms, and k is a constant. The broadening of Bragg's peaks indicates the formation of nanoparticles. The calculated mean crystallite size curcumin conjugate nanoparticles synthesized by the method 30-100nm range. Further studies are needed for detailed XRD analysis.

**In vitro cytotoxicity assay**

The cytotoxicity of free curcumin and curcumin-alginate conjugate nanoparticles evaluated against A549 cell lines with the concentrations ranging from 6.25 μg ml<sup>-1</sup> to 100 μg ml<sup>-1</sup> is presented

in Table 1. It was observed that cell inhibition was decreased at lower concentrations of nanoparticles. That is due to the lower concentrations of drug loaded into nanoparticles. The cancer cells treated with a lower concentration (10 μg ml<sup>-1</sup>) of the curcumin loaded alginate nanoparticles show a low percentage of inhibitions (4.98%) whereas curcumin loaded alginate nanoparticles with higher concentrations (100 μg ml<sup>-1</sup>) have a very high ability to inhibit cancer cells (78.55 μg ml<sup>-1</sup>). With this concentration, the free-curcumin inhibited cancer cells were 94.4%. Because of the slower release rates of curcumin from nanoparticles, it reduces the interaction of the drug with a cell from the obtained results, table Curve software was used to calculate the IC<sub>50</sub> values. IC<sub>50</sub> is the drug concentration at which the drug includes 50% inhibition of the growth of the cells. The results obtained from the two samples of free-curcumin with an IC<sub>50</sub> value of 73.03 and 11.37 μM ml<sup>-1</sup>, respectively. It suggests that curcumin n and curcumin loaded alginate nanoparticle shown the activity on cancer the A549 cell line n loaded alginate nanoparticles have no cytotoxicity wall.

**Table 1: Cytotoxicity assay of free-curcumin and curcumin loaded alginate nanoparticles**

A549 cells concentration μg/ml	Free curcumin (% of inhibition)	Curcumin-alginate nanoparticles
6.25	11.47	4.98
12.5	68.26	16.39
25	85.5	24.32
50	91.4	31.59
100	94.4	78.55
IC <sub>50</sub> 12	11.37	73.03

**CONCLUSION**

Curcumin-alginate nanoparticles are synthesized by simple sonication method. The synthesized nanoparticles (NPs) showed relatively small size and good responsivity. The alginate-curcumins prepared were characterized by XRD, FTIR, SEM, TEM, and UV-Vis techniques. TEM results showed that the c nanoparticles have almost spherical shaped morphology and the average size from 18 to 50 nm. The FTIR data indicate that the functional groups were successfully attached on surface of the nanoparticles. The outcomes of these results showed that these nanoparticles are stable and highly soluble. The *In vitro* studies of

curcumin-loaded alginate showed up to 70% of adsorbed drug released in the buffer solution after 2800 minute. The *In vitro* release profile suggests that curcumin-loaded alginate is promising in drug delivery carriers in cancer therapy.

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