11.0. EXPERIMENT-7

Preparation of encapsulated curcumin chitosan nanoparticles (ECNPs) and its characterization.

11.1. Objective of the investigation

1. To study the synthesis of encapsulated curcumin chitosan nanoparticles (ECNPs)

2. The present experiment also intended to explore the physical and chemical characterization of ECNPs.

11.2. Results

11.2.1. Solubility of Nanoparticles

Figure 11.1 shows that free chitosan is insoluble in chitosan and free curcumin poorly insoluble in aqueous media. By contrast encapsulated curcumin chitosan nanoparticle (ECNPs) is fully clear and dispersible in aqueous media (Fig 11.1).

11.2.2. FT-IR Spectroscopic study

A spectral peak position at 3509 cm^{-1} is attributed the presence of the hydroxyl group (OH) in sample. The strong peak position at 1625 cm^{-1} is mainly shown to the (C=C) and (C=O) mode. An extensive band region at 1600 cm^{-1} is assigned to the symmetric aromatic ring which is important for the stretching vibrations mode of (C=C). The vibration band at 1505 cm^{-1} is attributed to the carbonyl (C=O) group whereas 1025 cm^{-1} is allocated to the enol (C-O) group. The stretching vibration modes at 960 cm^{-1} and 712 cm^{-1} are attributed to the benzoate trans–CH and CH vibration mode.

Figure 11.2 has shown the IR spectra of (a) only curcumin (b) chitosan and (c) ECNPs. In Fig (a) the spectra of curcumin peak position is not visible at 1640 cm^{-1}. However, a peak in the ECNPs spectrum is observed at 1640 cm^{-1} position and it corresponds to the amino deformation (Fig 11.2). Similarly a variation in the strong band position at 1089 cm^{-1} is resulted from the presence of keto group in the curcumin structure. The spectral band position

of ECNPs is also moved from 1640 cm^{-1} to 800 cm^{-1} (Fig 11.2). The FT-IR data helps to confirm that the nanoparticles were formed because of the interaction between amino groups of chitosan and phospho groups of TPP.

11.2.3. Sizes of nanoparticles

The prepared nanoparticles sizes were in the range between 8-40 nm. TEM image explored that synthesized nanoparticles were retained a spherical shape. It was found that most of the nanoparticles size was around 20 nm as evident from the particle size distribution plot in Figure 11.3.

11.2.4. XRD analysis

The XRD analysis revealed the modification of crystalline nature of samples and it is important for its powerful association with the drug inclusion as well as release rate (Fig 11.4). XRD analysis of the free curcumin established numerous peaks in the wide range (2 Theta) at 10–30° indicated their crystalline nature. Whereas, in ECNPs was not attributed the crystalline peaks (Fig 11.4).

Figure 11.1.

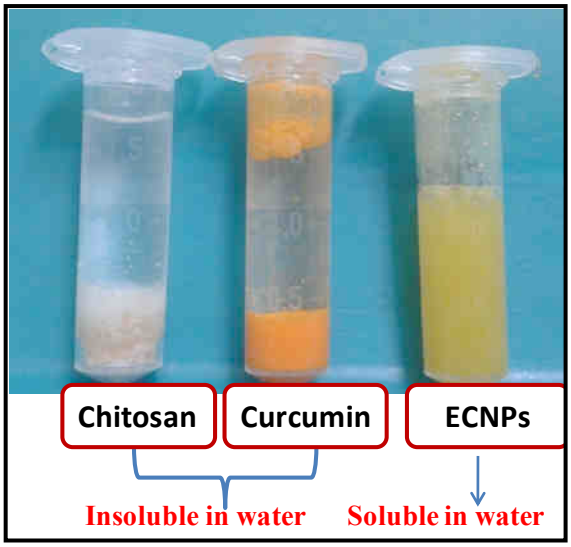

Fig 11.1. Represents the solubility of curcumin and encapsulated curcumin chitosan nanoparticles (ECNPs).

Figure 11.2.

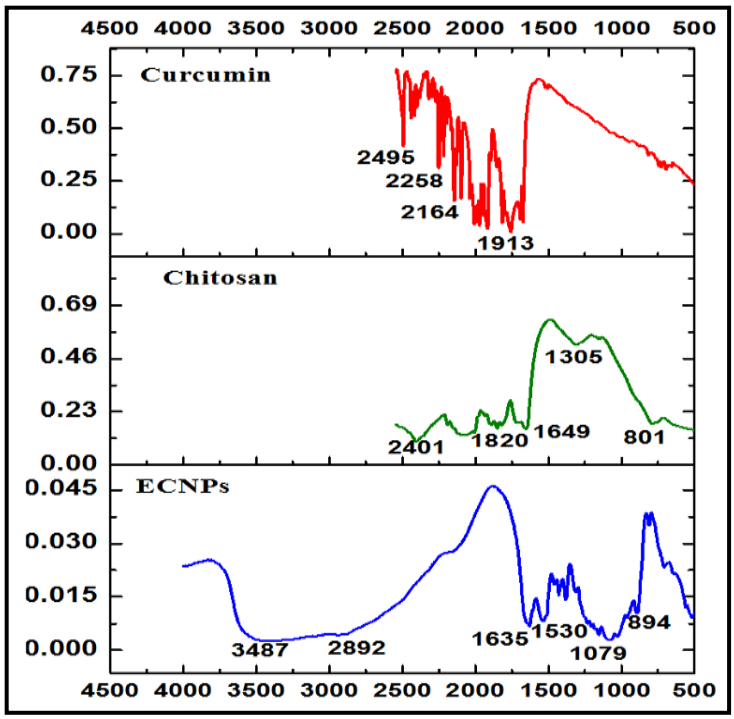

Fig 11.2. Represents the FTIR spectrum of (a) curcumin, (b) chitosan, and (c) ECNPs.

Figure 11.3.

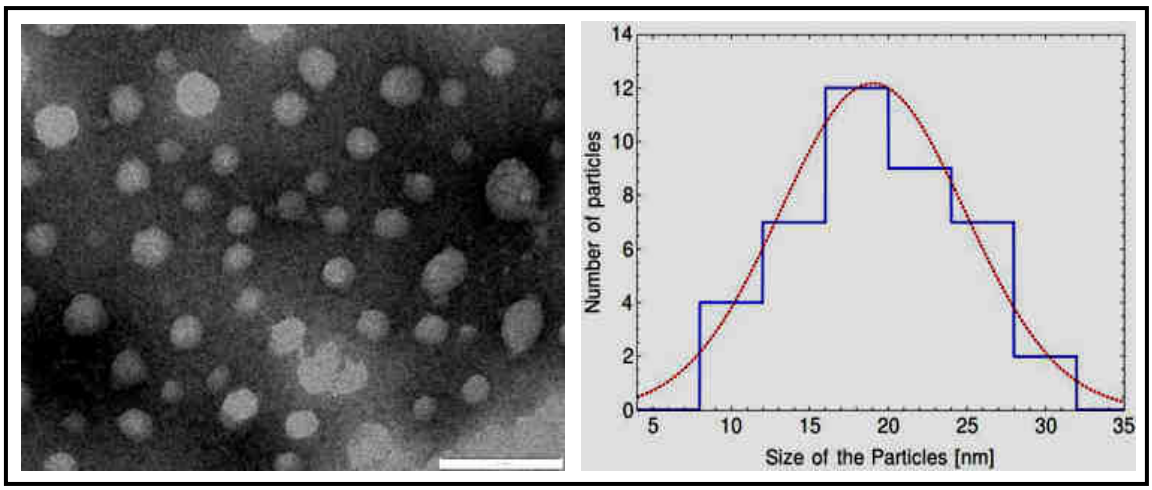

Fig 11.3. Represents the size characterization of the ECNPs using TEM studies.

Figure 11.4.

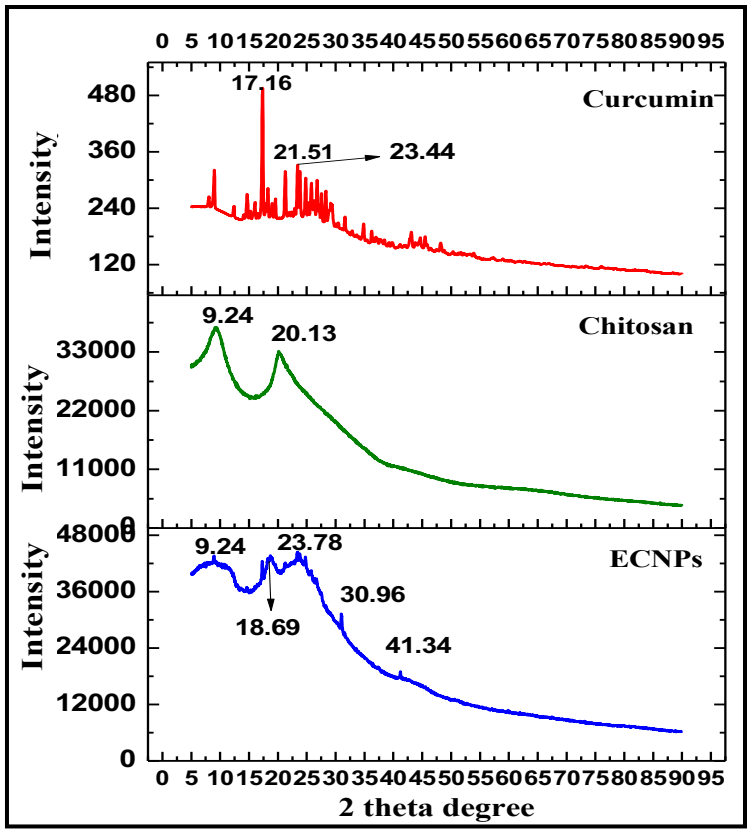

Fig 11.4. Analysis of (a) curcumin, (b) chitosan and (c) ECNPs using the help of X-ray diffraction spectroscopic.

11.3. Discussion

Nanoparticles are prepared from various biodegradable substances and their dimensions are normally less than 500 nm (Anitha, et al., 2011). The gelation method was used in this experiment to load curcumin in chitosan (Calvo et al., 1997; Anitha et al., 2011). Chitosan has poor solubility in aqueous media (Le Tien et al., 2003). Chitosan solution was made by dissolving in acetic acid (0.1%) solution. The solubility is small at pH 7 aqueous solution. However, because of solubility is rises its rigid crystalline form in organic and inorganic solvent (Muzzarelli, 1973). Solubility of chitosan not remain constant at pH 5.2 condition (Sadeghi et al., 2012). Decrease in aggregates was noticed due to increase of ionic strength of these molecules in increasing alkali side. Free amino groups was formed the intermolecular

hydrogen bonds to oxygen as a result increase in aggregates at the pH larger than 6.5. Although the acetyl groups are situated in chitosan chain, free amino groups trounce the associative forces which was formed because of intermolecular hydrogen bonding. The solubility was also increased at the same time in acidic solution below pH 6.5. Curcumin has poor absorption and low bioavailability. Curcumin has been applied for nanoparticles synthesis. Curcumin solution when added with Tween 80 in association with chitosan increases the solubility of curcumin (Anjana et al., 2012). The Tween 80 was used because it's increased the curcumin solubility and reflects the stability in the solution. Curcumin as nanoparticles loaded in chitosan developed transparent solution in soluble form. Here we noted that curcumin loaded in chitosan nanoparticles more soluble than the solubility of free curcumin (Fig 11.1). Chitosan bears a positive charge at low pH as it has an amine group (NH_2) which is protonated as NH^{3+}. The curcumin molecule have two hydroxyl group (-OH) and keto group (C=O) in the benzene ring. The presence of intermolecular hydrogen bond in it has the major interactive role. Hydroxyl group is the major binding position for the chitosan molecules. The negative condition of TPP has high charge affinity. It is mostly interacted with chitosan like positive charged polymer by cross link ionic interaction between chitosan amino group and P_3O_{105}-anions. Finally, it forms encapsulated nanoparticles (Yadav et al., 2012). TEM analysis was done to assess the morphological characteristics of the prepared nanoparticles. Fig 11.3 shows that the encapsulated curcumin-chitosan nano particle size distribution. The range of our developed nanoparticles are between 8-40 nm. Here we observed that TEM images of the prepared nanoparticles have a spherical shape with and it is uniform in nature (Fig 11.3). Other investigator developed curcumin loaded chitosan nanoparticles with 100 ± 20 nm size (Das et al., 2010). Akhtar et al 2012 also prepared curcumin loaded chitosan nanoparticles with the size of 200 nm but our nanoformulation produced nanoparticles of 8-40 nm size. XRD analysis the crystalline modification is

important because this is strongly associated with drug incorporation. The morphological characterеristic of the encapsulated curcumin in chitosan nanoparticles was performed using XRD analysis that suggests that particles are spherical in nature (Fig 11.4). Curcumin also exhibited a numerous crystalline peaks particular at 10–30° (2 Theta) region. However the numerous peaks was not visible in curcumin nanoformulation. From these results confirmed that, curcumin will remain entrapped into the polymer (Chaubey et al., 2014). We further verified the loading as well as in encapsulation of curcumin in chitosan nanoparticles by FTIR analysis. The spectrum analysis is demonstrated in Fig 11.2. The IR spectra of curcumin shows the absorption peak position at 1627.97 cm^{-1} and is attributed to the C=O stretching vibration mode. These typical bands of the prepared nanoparticles occur in between 1600-1500 cm^{-1} and at around 1400 cm^{-1}. These represent the stretching because of C=O and C-O bonds elongation of phenolic and alcoholic groups. These above spectrum peak position are situated in the physical mixture of curcumin entrapped chitosan nanoparticles though in the end position these are stretched and smoother.

Our study helps to indicate the chemical shifts arising due to bonding between the part of positive amino (-NH3+) of chitosan and part of negatively of TPP (P_3O_{105}). IR spectra hence confirmed the association of curcumin in the chitosan and also formulated the encapsulation curcumin chitosan nanoparticles. There was an interaction between keto and amine group of curcumin and chitosan respectively that helps into the drug loading. It is confirmed from this experiment that the encapsulation of curcumin within nanoparticles initiate a new avenue to improve and make the drug responsiveness for the treatment of several diseases.