**Synthesis and characterization of curcumin nanoformulation using solvent diffusion cum evaporation technique**

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**Abstract**

Turmeric can be used as aromatic, stimulant, preservative, flavouring and colouring agent in diet and it also has various medicinal properties. Turmeric mainly consists of curcuminoids, volatile oils and water soluble peptide. Curcumin is insoluble in water, photosensitive and unstable in alkaline pH and so curcumin has less bioavailability in the body due to inadequate absorption from the gastrointestinal track, higher metabolic rate and faster excretion. The study aims to develop encapsulated curcumin nano formulation with higher bioavailability and product stability. The prepared curcumin nanoformulation was basically a nanoemulsion which consists of aqueous and organic phase. The mean droplet size of formulation with higher bioavailability and product stability. The prepared curcumin nanoformulation was basically a nanoemulsion which consists of aqueous and organic phase. The mean droplet size of nanoemulsion was found to be 72.6nm, the polydispersity index was 0.478 and zeta potential was -50.2 mV after 1 hour ultrasonication with the surfactant concentration of 2.5 ml. The FTIR finger print confirms the presence of most of the absorption peaks pertaining to curcumin and beta cyclodextrin in the nanoformulation. The XRD pattern of curcumin nanoformulation showed that the crystallinity of the curcumin had decreased when curcumin was encapsulated within the carrier molecule thereby enhancing the aqueous solubility. Under thermo gravimetric analysis of nano formulation, the absence of peak at 185°C indicated that the curcumin was completely encapsulated into the carrier molecule.

**Keywords:** Curcumin nanoformulations, crystallinity, mean droplet size, polydispersity index, zeta potential and bioavailability

**1. Introduction**

Turmeric of commerce is the dried rhizome of the plant *Curcuma longa* L. Curcuma is a genus in the Zingiberaceae family, including 49 genera and 1400 species and the Indo-Malayan area is where the genus originated. About 40 species including *C. longa* are indigenous to India, implying that they are of Indian origin. In the diet, it has aromatic, stimulant, preservative, flavoring, and coloring effects (Ali et al., 2006) [2]. Curcuma spp contains turmerin (a water-soluble peptide), essential oils (monoterpenes and sesquiterpenes) and curcuminoids (curcumin, bisdemethoxycurcumin, demethoxycurcumin and cyclocurcumin) (Esatbeyoglu et al., 2012). Curcuminoids, which contain curcumin (77%), demethoxycurcumin (DMC 17%), and bisdemethoxycurcumin (BDMC 3%), are the primary active chemical constituents of turmeric roots (Ipa et al., 2019) [1]. Antioxidant, antibacterial, anti-inflammatory, anticancer, cardio protective, radio protective, anti-cytotoxicity, and hypoglycemic effects are all properties of curcuminoids (Jourghanian et al., 2016) [8] & (Gera et al., 2017) [9]. Antiproliferative, hypcholesterolemic, anti diabetic, antihepatotoxic, anti diarrheal, anticancer, and anti-inflammatory activities are all found in volatile oils Water-soluble peptides have antioxidant, DNA protectant and antimutagen properties. Curcumin has the potential to treat cardiovascular diseases (H. Li et al., 2019) and to cure aging-related diseases (Vaiserman. A et al., 2020) [22].

Curcumin has a number of limitations, including i) poor water solubility ii) physicochemical instability, iii) rapid metabolism, iv) limited bioactive absorption, v) poor pharmacokinetics and less bioavailability, vi) inadequate penetration and targeting efficacy. Curcumin administration through nanotechnology not only aimed to resolve less solubility, rapid drug metabolism, and drug stability challenges, but it should also disseminate or reach targeted tissues while reducing inadvertent effects to nearby normal cells/tissues. Nanoencapsulation techniques focus on providing sustained release, good stability, and increased solubility in water for bioactive components, they can also improve anti-microbial and antioxidant capacity.
of phenolic compounds like curcumin, leading to increased functionality and bioactivity without negatively impacting the quality or organoleptic properties.

2. Materials and Methods
2.1 Materials
Curcumin pure extract and sorbitan monolaurate were purchased from Sigma Aldrich Chemicals Pvt Ltd, β-cyclodextrin, polysorbate 80 and ethanol were obtained from Hi-media laboratories. All of the materials utilized in this formulation are analytical grade and also FDA approved compounds.

2.2 Preparation of Curcumin Nanoformulation
Curcumin has been effectively encapsulated using solvent diffusion cum evaporation technique as modified protocol of (Lee et al., 2019). The aqueous phase mainly consists of water, an encapsulant (β-cyclodextrin), and a hydrophilic surfactant, whilst the organic phase is made up of 25mg of curcumin dissolved in 5ml of solvent and lipophilic surfactant were synthesized concurrently. The surfactant ratio was calculated using Hydrophilic Lipophilic Balance (HLB). To create a coarse emulsion, organic phase was introduced dropwise to aqueous phase and homogenized for 4 hours at 1200 rpm using a magnetic stirrer. Curcumin nanoformulation was prepared using 20 kHz ultrasonicator with 40% amplitude.

2.3 Characterization of Nanoformulation using Particle size analyzer: The mean droplet size, polydispersity index, and zeta potential were measured using a Horiba scientific Nano particle analyzer SZ-100. The particle size analyzer works on the basis of dynamic light scattering. Polydispersity index determines sample heterogeneity, and zeta potential determines sample stability.

2.4 Functional property - Fourier Transform Infrared Spectroscopy
The functional groups in the formulation were examined using the JASCO FT/IR-6800. FTIR identifies molecules through chemical bonding by creating an infrared absorption spectrum.

2.5 Structural property- X- ray Diffraction
The structural property was identified using Ultima IV X-RAY DIFFRACTOMETER works on the principle of Bragg’s law i.e. scattering of waves from the crystalline structure which helps to identify the arrangement of atoms in a molecules and also the crystallographic nature of sample.

2.6 Scanning Electron Microscope
Topography, morphology, composition and crystallographic information of the formulation were identified through QUANTA 250 Scanning Electron Microscope. SEM works on the principle when a primary beam of electrons hits the sample results in production of secondary electrons and these secondary electrons provide information about the sample.

2.7 Thermo Gravimetric Analysis
Thermal degradation rate of curcininnano formulation was studied using TG/DTA - EXSTAR/6300 (Thermo Gravimetric Analyzer) where empty alumina pan was taken as reference. The sample of 8.847 mg was subjected to the temperature range from 33˚C to 1000˚C with the gradual increase in temperature of 10˚C/ min.

3. Results and Discussion
3.1 Properties of Curcumin Nanoformulation
The prepared curcumin nanoemulsion formulation was basically a nanoemulsion which consists of two phases i.e. aqueous phase and organic phase (Figure 1). The aqueous phase consists of distilled water, tween 80 (hydrophilic surfactant) and carrier (β-cyclodextrin) meanwhile organic phase consists of curcumin along with solvent (ethanol) and span 20 (lipophilic surfactant). HLB of the mixture was calculated as 11.5 and the surfactants ratio was 1:1. Coarse emulsion was prepared by mixing these two phases under magnetic stirrer at 1200 rpm for 4 hours. Then the coarse emulsion was converted into nanoemulsion by ultrasonication with frequency of 20 KHz, amplitude of 40% and pulse on/ off of 10s/10s for 1 hour. (Lee et al., 2019) developed turmeric extract- loaded nanoemulsion with the HLB values in the range from 10.2 to 15. (Athira et al., 2018) [2] using wet grinding process developed octenyl succinylated cassava starch loaded nanocurcumin. (Behbahani et al., 2019) [4] using microemulsion method developed curcumin loaded nanostructured lipid carriers.

3.2 Particle Size Analyzer
The mean droplet size of nanoemulsion was found to be 31.3 nm (Figure 2), the polydispersity index was 1.89 and zeta potential was -47 mV (Figure 4) after 20 minutes of ultrasonication with the surfactant concentration of 2.5 ml. Under the same concentration of the surfactant but subjected to 1 hour ultrasonication, the mean droplet size was 72.6 nm (Figure 3), the polydispersity index was 0.478 and the zeta potential was -50.2 mV (Figure 5). (Jourghanian et al., 2016) [8] developed curcumin loaded solid lipid nanoparticles that had mean droplet size of 112 nm and polydispersity index of 0.114. (Darrandale and vavia, 2012) developed cycloexextrin based curcumin nanosponges which had the mean droplet size of 487.3 nm and polydispersity index of 0.476 and zeta potential of -27 mV. (Sun et al., 2010) [11] developed nanomedicine in the form of curcumin polybutylcyanoacrylate with mean droplet size and polydispersity index of 152 nm and 0.339. When compared to the earlier reports, the nanocurcumin synthesized in the current assignment has lowest particle size of 72.6 nm with zeta potential of -50.2 mV thus confirming better formulation efficiency and stability.

3.3 Fourier Transform Infrared Spectroscopy
The chemical structure of the nanoformulation was identified using Fourier Transform Infrared Spectroscopy (FTIR) and represented in figure 6. The absorption peak of 3737 cm⁻¹ indicates the O-H stretching of alcohol group, 3339 cm⁻¹ indicates the N-H stretching of aliphatic primary amine group, 2118 cm⁻¹ indicates C=O stretching of alkyne group, 1739 cm⁻¹ indicates the C=O stretching of esters group, 1638 cm⁻¹ indicates the C=O stretching of alkene group, 1454 cm⁻¹ indicates the C-H bending of alkane group, 1229 cm⁻¹ indicates the C=O stretching of aromatic ester group, 1126 cm⁻¹ indicates the C=O stretching of alkyl aryl ester group, 1086 cm⁻¹ indicates the C-O stretching of aliphatic ether group and 1045 cm⁻¹ indicates the S=O stretching of sulfoxide group. Importantly most of the absorption peaks pertaining to curcumin and beta cycloexdextrin were retained in the nanoformulation. The absorption peaks of curcumin at 3506
cm⁻¹ indicates the phenolic O-H stretching, 1427 cm⁻¹ indicates the olefinic C-H bending, 1273 cm⁻¹ indicates aromatic C-O stretching (Darandale and Vavia 2012) and the 1627 cm⁻¹ indicates enol carbonyl stretching (Mangalathillum et al., 2012) [10].

3.4 X-ray Diffraction
The nature of the samples can be identified using x-ray diffraction and when x-rays hit the sample, some of the atoms present in the sample which satisfies the Bragg’s law get diffracted and this diffracted x-rays can be identified using diffractometer. Crystalline phases and orientation of the sample, atomic arrangement of the sample, grain size and phase composition of the sample can be identified using XRD. The XRD pattern of raw curcumin showed that it was crystalline in nature. Meanwhile the XRD pattern of curcuminnanoformulation showed that the crystallinity of the curcumin had decreased when curcumin is encapsulated within the carrier molecule thereby enhancing the aqueous solubility and so adequate absorption from the gastrointestinal track thereby prevent the rapid metabolism and excretion which is the encouraging factor for bioavailability. XRD pattern for raw curcumin and nanoformulation was shown in figure 7.

3.5 Scanning Electron Microscope
When primary electrons interact with the sample, it will produce secondary electrons, backscattered electrons, x-rays, auger electrons and cathodery luminescence. This electron-sample interaction gives information about the sample. SEM images can give information of the sample like topography, morphology, composition and crystallographic nature. SEM images of Nano formulation was shown in figure 8 which shows the morphology of the formulation, homogeneous nature and also confirmed that the formulation was in nano range.

3.6 Thermo Gravimetric Analysis
TG, DTG and DTA curves are shown in figure 9. TG graph which indicates that 12%, 28% and 52% of mass were lost at 216˚C, 328˚C and 420˚C respectively. DTG curve shows that the thermal process consists of three steps as shown by three different peaks at around 269˚C, 338˚C and 403˚C. DTA is very similar to DSC and this graph indicates the melting point of curcumin nanoformulation that occurred at 252˚C, 339˚C and 401˚C. The absence of peak at 185˚C in curcuminnanoformulation indicated that the curcumin was completely encapsulated into the carrier molecule. (Ahmad et al., 2019) [1] and (Jourghanian et al., 2016) [8] reported that the melting point of curcumin was 185˚C which confirmed crystallinity of curcumin.

4. Figures and Graphs

Fig 1: Preparation of curcumin nanoformulation
Fig 2: Mean droplet size of CN at 20 mins ultrasonication

Fig 3: Mean droplet size of CN at 1 hr ultrasonication

Fig 4: Zeta potential of CN at 20 mins ultrasonication

Fig 5: Zeta potential of CN at 1 hr ultrasonication
Fig 6: FTIR pattern of curcumin, beta cyclodextrin and nanoformulation

Fig 7: XRD pattern of Curcumin and Curcumin Nanoformulation
5. Conclusion
The solvent diffusion cum evaporation technique resulted in curcumin nanoformulation with an mean droplet size 72.6 nm, polydispersity index of 0.478 and zeta potential of -50.2 mV. While encapsulating the curcumin nano particles in β-cyclodextrin, the crystallinity and the thermal degradation rate of curcumin were modified positively to provide better formulation efficiency and stability. This curcumin nanoformulation is readily aqueous soluble and less crystalline that are important prerequisites for good absorption with higher bioavailability.

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