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RESEARCH ARTICLE

Enhanced Water Dispersibility of Curcumin Encapsulated in Alginate-polysorbate 80 Nano Particles and Bioavailability in Healthy Human Volunteers

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Abstract: ***Background*:** The turmeric (Curcuma longa) plant, a perennial herb of the ginger family, is an agronomic crop in the south and southeast tropical Asia. Turmeric an Indian yellow gold and universal spice is described in Ayurveda, an ancient treatise on longevity and quality life for the treatment of various inflammatory disorders. The oral bioavailability of curcumin is low due to poor aqueous solubility, alkaline instability and speedy elimination.

***Objective*:** The present study is designed to prepare alginate polysorbate 80 nanoparticles to enhance aqueous solubility/dispersibility, hence bioavailability.

***Method*:** Curcumin-loaded alginate - polysorbate 80 nanoparticles were prepared by ionotropic gelation technique.

***Results*:** The optimized nano particles exhibited higher encapsulation efficiency (95%), particle size of 383 nm and Zeta potential of +200 mV. Formulations exhibited very low dissolution in Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF), but the major portion released in SCF which is attributed to the digestibility of alginate in Simulated Colonic Fluid (SCF) under the influence of colonic micro flora. FTIR and DSC observations revealed the successful entrapment of curcumin in alginate polysorbate-80 nanoparticles. The nanoparticles were more spherical, discrete and homogeneous. In healthy human volunteers, the oral bioavailability (AUC) of curcumin increased 5-fold after the consumption of curcumin nanosuspension compared to curcumin suspension. Maximum plasma concentration C_{max}- 636 ± 122 ng/ml was observed at t_{max}- 2h for nanosuspension, whereas C_{max}-87.7 ± 17.9ng/ml at t_{max}- 4h for suspension.

***Conclusion*:** Curcumin-loaded alginate - polysorbate 80 nanoparticles prepared by ionotropic gelation method, successfully entrapped curcumin. Both curcumin suspension and curcumin nanosuspension were safe and well tolerated and may thus be useful in the prevention or treatment of various inflammatory diseases of mankind.

Keywords: Bioavailability, curcumin, ionotropic gelation, nanoparticles, polysorbate 80, sodium alginate.

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1. INTRODUCTION

The turmeric (Curcuma longa) plant, a perennial herb of the ginger family, is an agronomic crop in the south and southeast tropical Asia. The rhizome is the most beneficial part of the plant for culinary and medicinal purposes. Turmeric an Indian yellow gold and universal spice is described in Ayurveda, an ancient treatise on longevity and quality life for the treatment of various inflammatory disorders [1-4]. It has been recommended to ameliorate various common ailments such as gastrointestinal problems (stomach ache, dysentery, ulcer *etc*.), hepatic disorders including jaundice, arthritis, sprains, wounds, acne, skin and eye infections [5]. Curcumin exhibits anti-tumor, anti-inflammatory and antioxidant activities [6]. This phytochemical has proved itself as a prophylactic for neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. It has an affinity for several biological proteins and inhibits various kinases. Curcumin modulates the activity of several transcription factors, regulates the functioning of inflammatory enzymes, cytokines, adhesion molecules, and apoptotic proteins [7]. Clinically, Curcumin is observed as safe even at very high doses but possesses limited pharmaceutical role as it has poor aqueous solubility, rapid systemic elimination, inadequate tissue absorption and degradation at alkaline pH, which restrains its bioavailability. Investigators have tried various strategies to enhance the bioavailability such as: (i) adjuvants like piperine which interfere with glucuronidation, (ii) Liposomal curcumin, (iii) nanoparticles, (iv) curcumin-phospholipid complex (v) structural analogues of curcumin. (vi) micronisation and nanonisation (vii) self-micro emulsifying drug delivery systems (SMEDDS), (vii) cyclodextrin inclusions, (ix) solid dispersions (x) nano emulsions, nano spheres, nano beads, and nanofibres [8-10]. The greater size of polymer molecules and their flexible, chain-like structures impart polymers certain exclusive and beneficial functions. Sodium alginate, a natural biopolymer derived from kelp and seaweed, is an important food adjuvant. Interest in nanocarriers for cancer chemotherapy is growing. Nanoparticle-based drug delivery strategies have the potentials of transforming hydrophobic agents like curcumin dispersible in aqueous media, thus eliminating the deficiencies of poor solubility.

The oral bioavailability of curcumin is low due to its hydrophobicity, hence poor solubility in the chime and their instability at physiological and alkaline pH [11]. The bioavailability of curcumin is additionally limited by the activity of transport proteins in the luminal membrane of enterocytes which transfer the intracellular conjugates back to the lumen. In rats and humans, only negligible amount of orally consumed curcumin is excreted as urinary metabolites [12, 13], while the larger portion is eliminated unchanged with faeces.

The present study is designed to prepare alginate polysorbate 80 nanoparticle composites to enhance aqueous solubility/dispersibility, dissolution and hence increased oral bioavailability of poorly soluble hydrophobic drug curcumin [14].

2. MATERIALS AND METHODS

2.1. Materials

Curcumin (97.0%) was obtained from HiMedia Laboratory (Mumbai, India). Sodium Alginate was purchased from Nice chemicals Pvt limited (Edappally, Cochin, Kerala, India). Polysorbate 80 was purchased from Rolex Chemical Industries (Mumbai, India). Calcium Chloride was obtained from Rolex Chemical Industries (Mumbai, India). Methyl Paraben was purchased from Merck Specialties Private Limited (Mumbai, India). Mannitol was purchased from Spectrum Reagents and Chemicals Pvt Limited (Edayar, Cochin, India).

2.1.1. Preparation of Plain Alginate - Polysorbate 80 Nanoparticles

Nanoparticles of Alginate and Polysorbate 80 were fabricated by cation (Bivalent, Ca^{2+}), induced controlled ionotropic gelation method. 3^2 factorial design was employed to optimize the Nanocarrier system.

2.2. Preparation of Stock Solutions of Polymers

2.2.1. 1% Sodium Alginate Solution Containing 0.1% Methyl Paraben was Prepared

1.00 g of sodium alginate was added to 100 ml of distilled water, stirred magnetically at 500 rpm for 15 min for solvation. The specified quantity of methyl paraben was added to the above solution and stirring continued for another 30 min to facilitate the dissolution of preservative.

2.2.2. 2% Polysorbate 80 Solution Containing 0.1% Methyl Paraben was Prepared

Measured 0.2ml Polysorbate 80 was added to 10 ml of distilled water, and stirred magnetically at 500 rpm. A specified quantity of methyl paraben was added to the above solution and stirring continued for another 10 min.

2.2.3. 0.1% $CaCl_2$ Solution Containing 0.1% Methyl Paraben was Prepared

0.05 g of $CaCl_2$was added to 50 ml of distilled water, stirred magnetically at 500 rpm for 5 min. A specified quantity of methyl paraben was added to the above solution and stirring continued for another 5 min.

All the above solutions were filtered through Whatman's filter paper to ensure particulate free, clear solutions.

2.3. Preparation of Plain Alginate-Polysorbate 80 Nanoparticles

Designated quantities of stock solutions of sodium alginate (1%) and polysorbate 80 (2%) were mixed to prepare polymer surfactant blend containing predetermined quantities of sodium alginate and polysorbate 80. The chosen quantities of sodium alginate, Polysorbate-80 and $CaCl_2$ were based on preliminary experiments in our laboratory. Five (5) ml of 0.1% $CaCl_2$ solution was added dropwise to 10 ml polymer surfactant blend prepared as per the formulation chart, and stirred magnetically (Tarson) at 500 rpm at room temperature. Nanoparticles generated were cured by stirring for an additional 45 min. Nanosuspension (opalescent preparation) was subjected to ultracentrifugation (Sorval Legend XTR, Courtesy - Vignan Bhavan, University of Mysore) at 15,000 rpm at 4°C for 30 min. Nano plug was washed twice with water to remove water-soluble components like free sodium alginate, Polysorbate 80 and calcium chloride.

2.4. Preparation of Curcumin Loaded Alginate - Polysorbate 80 Nanoparticles

Alginate-Polysorbate 80 nanoparticles containing curcu-min were prepared similar to the procedure outlined in the preparation of plain nanoparticles. Here 10 mg of curcumin was levigated with 10 ml of polymer surfactant blend to which 5 ml of $CaCl_2$ solution was added dropwise [6]. Supernatant and washings were collected after ultracentrifugation and analyzed to estimate % encapsulation efficiency (% EE).

2.5. Preparation of Vacuum Concentrate of Nanopar-ticulate Plugs

The vanoparticulate plug to obtain the following ultracentrifugation of Nanosuspension was separated by decanting the supernatant to separate the free drug. This plug was redispersed in distilled water, ultra-centrifuged and the washings were mixed with the supernatant to estimate the free drug. The data was utilized for calculating % EE. The washed Nano plug obtained was redispersed in 5% mannitol solution (cryoprotectant) and the final volume of Nanoparticulate suspension was similar to that of the original Nanosuspension set aside for 4 hours, ultra-centrifuged and subjected to vacuum concentration at 0°C at high pressure (18.1 bars) for 3 hours (Savant, Speed vac concentrator, SPD 2010). The obtained vacuum concentrate was a free-flowing powder complying with water dispersibility test [15].

2.6. Nanoparticle Characterization

2.6.1. Particle Size and Zeta Potential

Dynamic light scattering (DLS) (Nanotrac wave w3231, Japan) was employed to assess the mean nanoparticle size and size distribution. DLS measurements were performed with a wavelength of 532 nm at 25°C with an angle of detection of 90°C after suitable dilution with distilled water. Volume (1 ml) of the sample was kept constant to eliminate the stray radiation effect amongst the samples. Zeta potential was recorded from the same instrument [16, 17].

2.6.2. Encapsulation Efficiency (%EE)

The encapsulation efficiency (EE) of the nanoparticles was evaluated by separating drug-loaded nanoparticles from free / unbound drug in the Nanosuspension using ultracentrifugation at 15,000 rpm, at 4°C for 30 minutes. The amount of curcumin loaded into the nanoparticles was calculated as the difference between the total drug added to prepare nanoparticles and the free drug present in the supernatant. Free curcumin in the su-

pernatant was quantified by UV-vis spectrophotometer (Shimadzu, Japan, UV- 2450) at 425 nm [6].

% EE of curcumin in the nanoparticles was calculated using the equation:

$$\% \text{ EE} = \frac{\text{Total amount of curcumin - Free drug}}{\text{Total amount of curcumin}} \times 100$$

2.7. Fourier Transform Infrared Spectroscopy (FTIR)

Cur-ALG nanoparticles were subjected for FTIR analysis using FT-IR-8300 spectrophotometer (Shimadzu, Japan). A total of 2% (w/w) of sample, with respect to the potassium bromide and curcumin were ground into fine powder using an agate mortar before compressing into KBR disc under a hydraulic press at 10,000 psi. Each KBr disc was scanned at 4 mm / s at a resolution of 2 cm over a wave number region of 400-4000 cm^{-1} at ambient temperature using IR solution software. Preformulation data on drug - polymer interactions are very critical in selecting appropriate polymers. FTIR spectroscopy was employed to ascertain the interaction of Curcumin with sodium alginate. FTIR Spectra of curcumin, sodium alginate plain nanoparticles, curcumin loaded nanoparticles and curcumin alginate physical mixture were taken.

2.8. Differential Scanning Calorimetry (DSC)

Differential scanning calorimetric (DSC) analysis was used to characterize the thermal behavior of the individual polymer, curcumin plain, curcumin physical mixture and curcumin loaded nanoparticles. DSC thermograms were obtained using an automatic thermal analyzer system. Temperature calibration was carried out using Indium Calibration Reference Standard (transition point: 156.60°C) as a standard. Samples were crimped in standard aluminum pans and heated from 0 to 400°C at a heating rate of 10°C / minutes under constant purging of dry nitrogen at 30 ml / minutes. An empty pan, sealed in the same way as the sample, was used as a reference. Differential scanning calorimetry was performed using DSC-60, Shimadzu instrument. 3 mg drug or equivalent (curcumin, ZC formulation, physical mixture) was used for the study. The thermograms were also recorded.

2.9. *In vitro* Drug Dissolution Studies

In vitro Curcumin release from the nano plug in Eppendorf tube was performed using Simulated Gastric Fluid (SGF) (without enzymes), containing 0.2% Polysorbate 80 for 2 hours, Simulated Intestinal Fluid (SIF) (without enzymes) with 0.2% Polysorbate 80% for 6 hours and Simulated Colonic Fluid (SCF) with 0.2% Polysorbate 80% and β-Galactosidase (0.13units / ml for 24hours) at 37 ± 0.5°C and, agitated in a shaker (100 cycles / minutes). Release medium was subjected to ultracentrifugation, supernatant collected, and the absorbance was measured at 425 nm to determine the released curcumin. The same set of tubes was used to study the release in SGF followed by SIF and SCF. All batches were run in triplicates.

2.10. Scanning Electron Microscope (SEM)

Surface and shape characteristics of pure curcumin and nanoparticle formulation were evaluated by scanning electron microscopy (Model Hitachi S3400N, Japan). The scanning electron microscopy samples were prepared by placing a drop on a double adhesive tape, which was stuck to an aluminum stub, and air dried. The stubs were then coated with gold to a thickness of ~300 Å using a sputter coater, and the photographs of samples were taken.

2.11. Water Dispersibility Test

Nanoparticulate powders obtained after the vacuum concentration of the optimized formulation (Zc) were dispersed (10 mg in 10 ml distilled water) and agitated for 2 minutes to test for facilitated homogeneous dispersion.

2.12. Human Subjects and Study Design

This study was reviewed and approved by the Institutional Human Ethics Committee of the Sarada Villas College of pharmacy, Mysuru, Karnataka, India. After a medical examination (Blood test, Urine test and Liver function test), six healthy male subjects aged 20-25 years with normal liver and kidney functions were recruited. Informed written consent was received from all the prospec-

tive volunteers. The selected subjects were apparently healthy, and not diagnosed for illness in the last 30 days, not presently taking any prescription medicine/s, and were not habitual users of tobacco and alcohol. Exclusion criteria include use of dietary supplements, >5 hours physical activity per week, or hypersensitivity to curcumin. The subjects were asked to avoid curcumin-containing foods starting one week before the first intervention and throughout the entire study [18]. Subjects were fasted for 12 hours prior to drug administration, three subjects received 150 ml of curcumin suspension containing 100 mg of curcumin and another three subjects received 150 ml of curcumin nanosuspension containing 100 mg of curcumin. To control the influence of the diet on curcumin pharmacokinetics, subjects received standardised meals during the entire intervention day. Blood was collected from a cephalic vein from each volunteer at 0, 1, 2, 4, 6, 8 and 24 hours post dose in EDTA tubes, centrifuged immediately and the plasma was stored at - 20°C till analysis for curcumin by validated UFLC method [19, 20].

2.12.1. Extraction and UFLC Analysis of Curcumin in Plasma

Appropriate volumes of working standard (10 µg / ml) were spiked into 0.5 ml plasma (to get varying concen-trations of curcumin in plasma (300 to 1600 ng / ml), added 0.5 ml acetonitrile (protein precipitant) and centrifuged at 13,000 rpm for 10 minutes below 10°C. The supernatant was decanted and filtered. Chromatographic separation was done using Phenomenex C18 column (250 X 4.6 mm, 5µ ID). The mobile phase consisted of methanol and Acetonitrile (20:80, v/v). The flow rate was adjusted to 1.0 ml/minutes and run time was 10 minutes. Curcumin was detected at a wavelength of 420 nm using a PDA detector with retention time of 3.47 minutes. 10 µl volume was used for injection onto UFLC column. Optimizations of the chromatographic conditions were carried out by changing the methanol concentration, strength and acetonitrile concentration.

2.12.2. Statistical Analysis

The AUC was calculated using the software package GraphPad Prism 7.04 for Windows OS (GraphPad Software, San Diego, CA, USA). C_{max} and T_{max} were estimated by visual inspection of the graphs. The plasma concentration data of nanosuspension was compared with the conventional suspension and analysed by unpaired Student t-test. Differences were considered significant at $p < 0.01$. Reported values are arithmetic mean with Standard Deviation (SD) [21].

3. RESULTS AND DISCUSSION

3.1. Preparation of Curcumin Loaded Alginate-Polysorbate 80 Nanoparticles

The technique used for preparation is based on controlled ionotropic gelation involving the addition of a volume of a specific concentration of $CaCl_2$ solution into curcumin levigated in a polymeric blend (sodium alginate + Polysorbate 80) at room temperature as per formulation chart (Table **1**). Varying concentrations were chosen, which resulted in the appearance of turbid/opalescent solutions but not aggregates. We observed a fine dispersion (suspended particles were seen) of curcumin in levigated polymeric blend. But during the dropwise addition of $CaCl_2$ with stirring, the suspended curcumin particles got dispersed and translucent Nanosus-pension formed. This could be due to the incorporation of dissolved hydrophobic curcumin into Nanostructures / micelles produced by polysorbate 80 stabilized by alginate. Das *et al.* utilized ethanol to prepare alginate - chitosan- pluronic composite nanoparticles. There is a variation in our procedure. We could get good Nanosuspension using alginate-polysorbate 80, comparatively a simpler formulation. They used 0.1% pluronic to enhance EE. They attributed this to the self-assembling property of pluronic (nonionic surfactant) in aqueous environment with a hydrophobic core in which curcumin is encapsulated. We could achieve > 90% EE in our formulation, while Das *et al.* showed % EE only up to 12%. XA, XB, XC formulations containing 0.4% sodium alginate were not stable physically even for a day, implying insufficient hydrogel structure at the interface of Nanosuspension, while Y and Z series were relatively stable for 6 days and 11 days, respectively. All the Y and Z formulations exhibited excellent entrapment efficiency (Table **2**), good nano size and satisfactory thermodynamic stability.

Table 1. Formulations of curcumin loaded alginate-polysorbate 80 nanoparticles using ionotropic gelation by 3^2 factorial design.

Ingredients	Formulation Code								
	XA	**XB**	**XC**	**YA**	**YB**	**YC**	**ZA**	**ZB**	**ZC**
Curcumin (mg)	10	10	10	10	10	10	10	10	10
Sodium alginate (mg)	**40**	**40**	**40**	**60**	**60**	**60**	**80**	**80**	**80**
Polysorbate 80 (mg)	10	20	40	10	20	40	10	20	40
Methyl paraben (mg)	10	10	10	10	10	10	10	10	10
Calcium chloride (mg) (0.1%)	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**

Table 2. Data for the characterization of curcumin loaded sodium alginate nanoparticles.

Evaluation Parameters (± S.D)	XA	XB	XC	YA	YB	YC	ZA	ZB	ZC
Physical stability of nanodispersion * mean ± S.D	<1	<1	<1	4	6	6	8	9	11
% Drug loading	6.1	6.2	6.0	6.2	6.2	6.3	6.1	6.3	6.2
%Encapsulation Efficiency mean ± S.D	92±5.2	94±4.3	91±3.1	94±4.2	93±2.9	95±4.5	92 ±3.6	95±4.6	93 ±2.5
Mean particle Size nm ± S.D	NE	NE	NE	105±3.0	292±0.40	380±6.15	107±3.15	285±5.15	383±7.10
Zeta potential mv ± S.D	NE	NE	NE	150±1.15	150 ±1.15	200 ±2.15	200 ±2.15	200 ±2.15	200 ±2.15
%CDR SGF 2hours	NE	NE	NE	1.5±0.100	1.7±0.110	1.5±0. 100	1.60 ±0.017	1.5±0.100	1.7±0.110
%CDR SIF 6hours	NE	NE	NE	2.9±0.120	2.6±0.110	28±0.130	2.4±0.101	2.6±0.110	2.6±0.110
% CDR SCF 24 hours	NE	NE	NE	95±0.16	97±0.19	94 ±0.92	95 ±0.16	96 ±0.17	94±0.18

NE - Not evaluated

*Number of days of no settling in Nanosuspension: n=3.

Investigators have prepared curcumin Nano-delivery systems with a higher concentration of polysorbate 80 (up to 10%). Primarily, there are two types of emulsifiers / dispersing agents employed in foods and pharmaceuticals; low molecular weight polysorbate 80 and high molecular weight proteins or polysaccharides. Low molecular weight nonionic surfactant ensures a reduction in interfacial tension and formulation of self-assemblies of micelles while high molecular weight hydrophilic colloids stabilize the dispersion by forming viscoelastic interfacial film [22]. Zeta potential approximates the surface charge of dispersed particles. Higher values result in improved physical stability of heterogeneous systems owing to repulsive forces. The higher zeta potential of

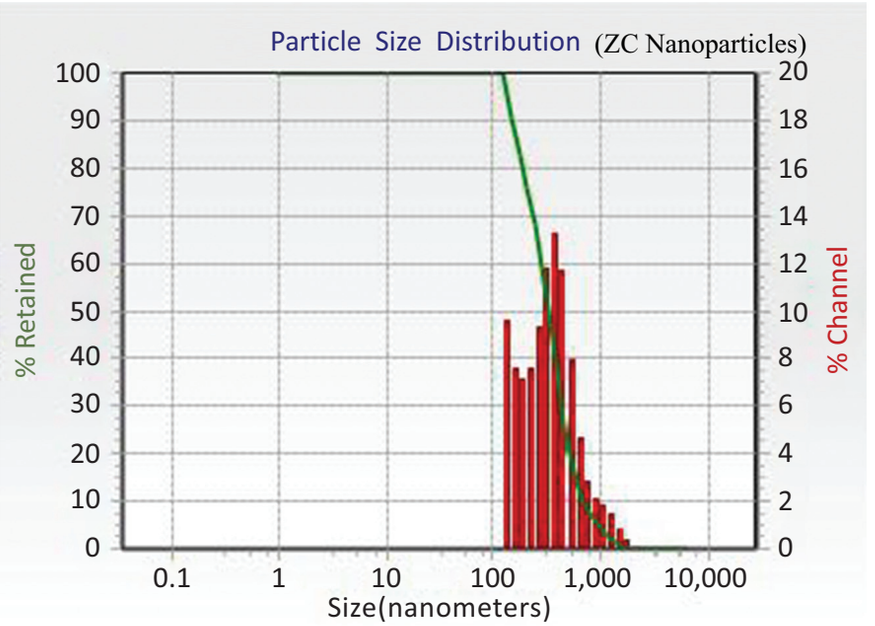


Fig (1). Particle size analysis of ZC nanoparticles.

Nanodispersions could be due to the higher percentage of sodium alginate employed in our study compared to other investigators. Physical stability of prepared or reconstituted dispersion is one of the important attributes. Bigger particles expose smaller surface area, hence surface energy for particle aggregation, whereas smaller particles with the larger surface area have a greater tendency for aggregation and instability. Physical stability of nano-particles is influenced by zeta potential as well as steric effect conferred by the adsorbed polymeric surface of nano-particles. Several batches of alginate polysorbate 80 nanoparticles were prepared in our lab. All the times we noted positive zeta potential. This may be explained as alginate ions carry a negative charge which is neutralised by $CaCl_2$ during the cross-linking process. The amount of adsorbed Ca^{2+} determines the zeta potential of nanoparticles.

3.2. Encapsulation Efficiency (EE)

The percentage encapsulation efficiency of the prepared nanoparticles was found to be in the range of 91 % to 95% (Table **2**). High Encapsulation Efficiency could be attributed to curcumin alginate interactions (Hydrophilic and Hydrophobic) [23]. Higher EE values obtained in our formulations imply that formulation composition and protocol aresatisfactory and suitable for industrial scale up. EE seems to be not much dependent on either alginate or polysorbate-80 concentrations.

3.3. Particle Size

The mean particle size of the nanoparticle formulations was found to be in the range of 105 to 383 nm as recorded in Table **2** and Fig. (**1**). Increasing concentration of Polysorbate 80 appears to increase the nanoparticles size, which might be due to bigger micelles produced with increasing concentration. Zc, optimized formulations have an average of 383 ± 7 nm. The Polydispersity index (PDI) is 0.2, PDI nearer to zero implies homogeneity of dispersions and greater than 0.3 indicates heterogeneity [23]. The observations suggest that concentration of sodium alginate and polysorbate 80 can be tailored to get the desired particle size. This observation provides a clue that calcium chloride concentration may have to be tuned to optimize zeta potential for better stability. The zeta potential (ζ) is dependent on surface charge, which is developed when a solid powder is dispersed in a liquid. The zeta potential quantifies the degree of repulsion between like charge neighboring particles in dispersion [24]. Zeta potential determines the physical stability of nanoparticle formulation [25]. Lower values nearer to zero indicate agglomeration / aggregation resulting in physically instability [26]. The zeta potential of our nanopar-

ticulate formulation was found to be in the range of 150-200mV, hence the better physical stability of Zc formulation.

3.4. FTIR Spectroscopy

FTIR spectrum of curcumin exhibits a characteristic peak at 3511cm^{-1} due to the presence of –OH (Fig. **2**), the strong peak at 1602 cm^{-1} due to (C- C) and (C - O), 1510 cm^{-1} (C-C) stretch (aromatic ring), while (C- O) peak for enol was obtained at 1281 cm^{-1}, (C -O- C) peak at 1027 cm^{-1}, benzoate trans-(C-H) vibration at 962 cm^{-1} and cis (C-H) vibration of aromatic ring at 810 cm^{-1} (Fig. **2a**). In the spectrum of alginate, the broadband at 3471 cm^{-1}corresponds to hydroxyl groups, the peaks near 1625 cm^{-1}and 1417 cm^{-1}were caused by symmetric and asymmetric stretching vibrations of (COO-) groups, respectively (Fig. **2b**). The bands around 1030 cm^{-1} (C-O-C stretching) are attributed to its saccharide structure. In the spectra of physical mixture, similar characteristic peaks were observed as obtained in the spectra of curcumin and sodium alginate *i.e.* 3510cm^{-1}, 1627 cm^{-1}, 1028 cm^{-1}, which con-firmed the presence of the drug and the polymer (Fig. **2c**). Unloaded spectra exhibit two prominent peaks at 1732 cm^{-1}due to the presence of sodium alginate (Fig. **2e**). The spectra of curcumin loaded alginate nanoparticles exhibited prominent peaks at 2341 cm^{-1} and 1154 cm^{-1}due to the presence of asymmetric CH stretching, symmetric-CH stretching, carbonyl group, NH bending (amide II band) and -CH deformation (scissoring in CH_2, respectively, in the spectra of curcumin and sodium alginate *i.e.* 1028 cm^{-1},which confirms the presence of the drug and the polymer, benzoate trans-(C-H) vibration at 963 cm^{-1} and cis (C-H) vibration of aromatic ring at 811 cm^{-1} which confirmed the entrapment of curcumin in the alginate nanoparticles successfully (Fig. **2d**).

3.5. Differential Scanning Calorimetry

The DSC profiles of curcumin, pure sodium alginate, physical mixture of curcumin and sodium alginate and loaded optimized formulation (ZC) are shown in Fig. (**3**). In the pure curcumin, there was a sharp endothermic peak around 177.59°C, which is attributed to melting of curcumin crystals [27, 28]. In physical mixture, there was a broad endothermic peak at 101°C and a sharp endothermic peak around 178.14°C due to sodium alginate and curcumin respectively [29] as shown in Fig. (**3**). The broad peak can be attributed to water evaporation from sodium alginate [30], whereas the sharp peak corresponds to the melting of curcumin crystals [27, 28], which suggests that the curcumin is in a crystalline form in the physical mixture. In optimized formulation (ZC) of curcumin-sodium alginate nanoparticles, there is anevidence of a sharp endothermic peak around 178.89°C, which suggests that the curcumin is in crystalline form rather than amorphous form. Reduction in the intensity of the peak in physical mixture and optimized formulation Zc might be attributed to the dilution effect contributed by the excipient that is polymer present.

3.6. *In-vitro* Drug Release Studies

Based on several references [29-31], we have devised an *in-vitro* release procedure which is more simple, comprehensive, integrated / taking several factors into consideration / and economical to screen various formulations. In this study, drug release from various formulations at 2 h from Simulated gastric fluid (SGF) containing 0.2% polysorbate 80, 6 h from Simulated Intestinal fluid (SIF) and 24 h from Simulated Colonic fluid (SCF), containing 0.2% polysorbate 80 is recorded in Table **2**. All formulations exhibited very low dissolution in SGF and SIF, but major portion released in SCF which is attributed to the digestibility of alginate in SCF under the influence of colonic microflora which is not present in the stomach or small intestine [29]. Release profile of Zc formulations was compared with curcumin (Fig. **4**). Native curcumin exhibited immediate / burst release of nearly 95% in 2 h in SGF followed by a decline in the concentration after 2 h in SIF and SCF, attributed to significantly enhanced alkaline degradation. Our observations supported the hypothesis that nanoparticles are promising delivery devices for protection against physiological and chemical degradation and release sustenance for continuous absorption throughout the Gastro-Intestinal Tract (GIT). Sustenance of the release is due to the crosslinked polymer which resists water attack and swelling of polymer leading to water diffusion, dissolution of drug and consequent

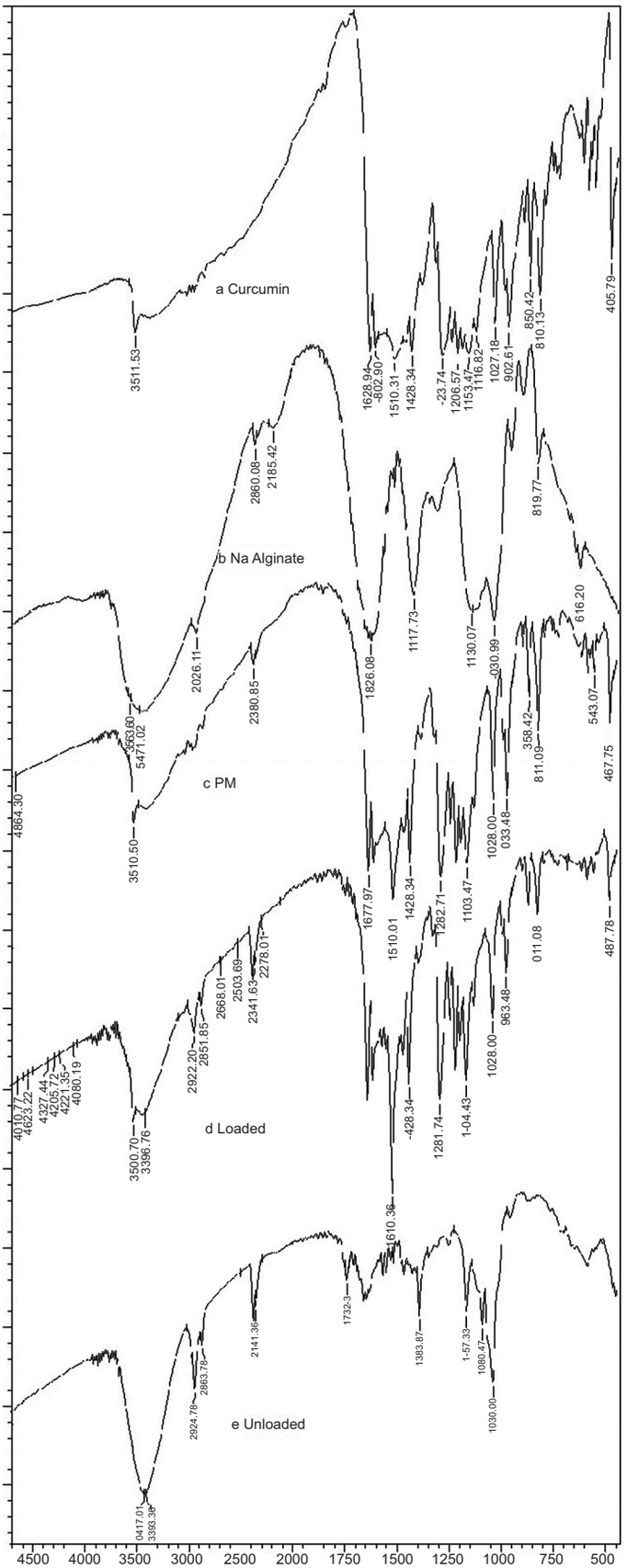


Fig (2). 2a. FT-IR spectra of curcumin, **2b**. Sodium alginate, **2c**. Physical mixture, **2d**. Drug loaded formulation, **2e**. Unloaded formulation.

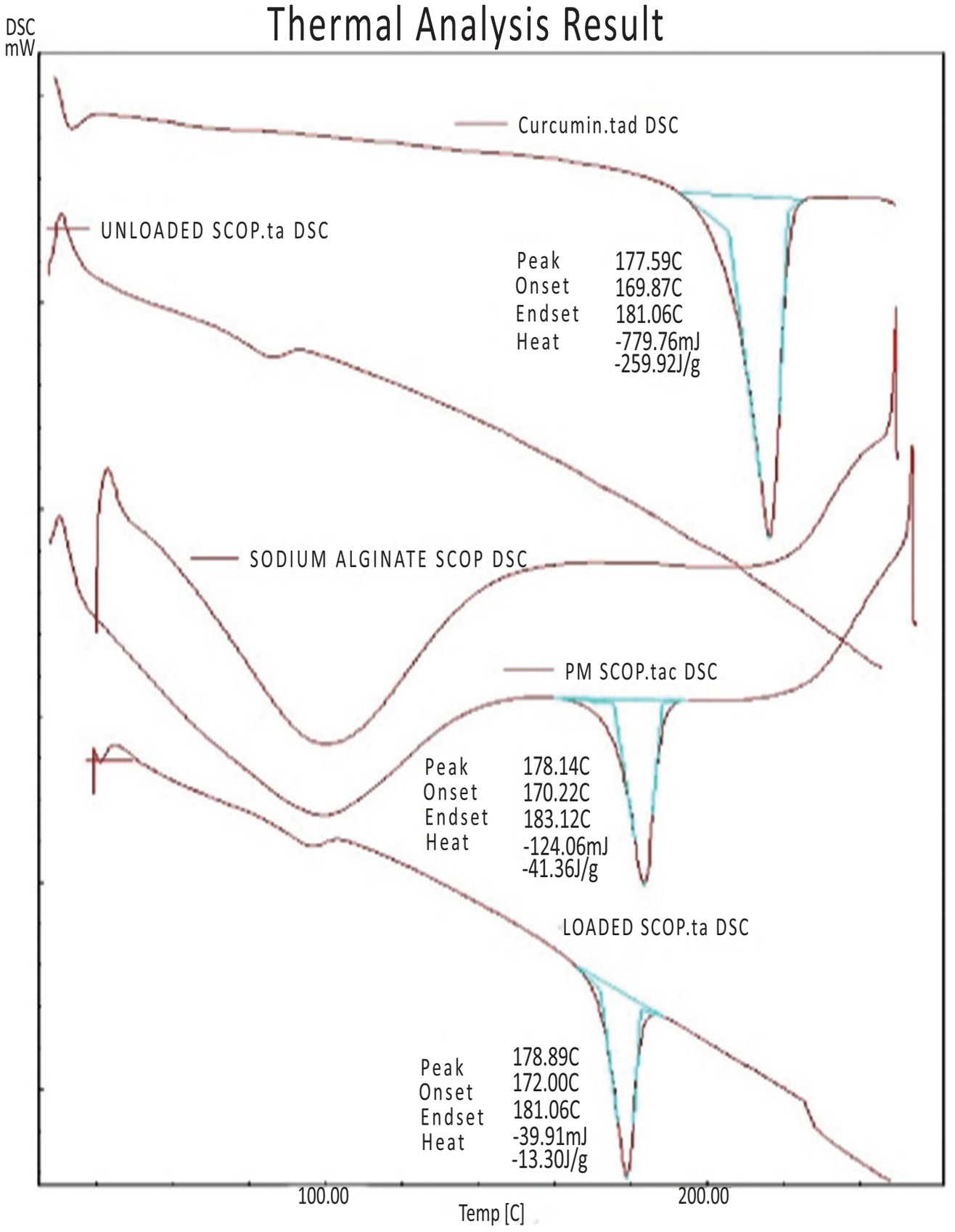


Fig (3). DSC thermogram of curcumin, Sodium alginate, Physical mixture, Drug loaded (ZC), unloaded.

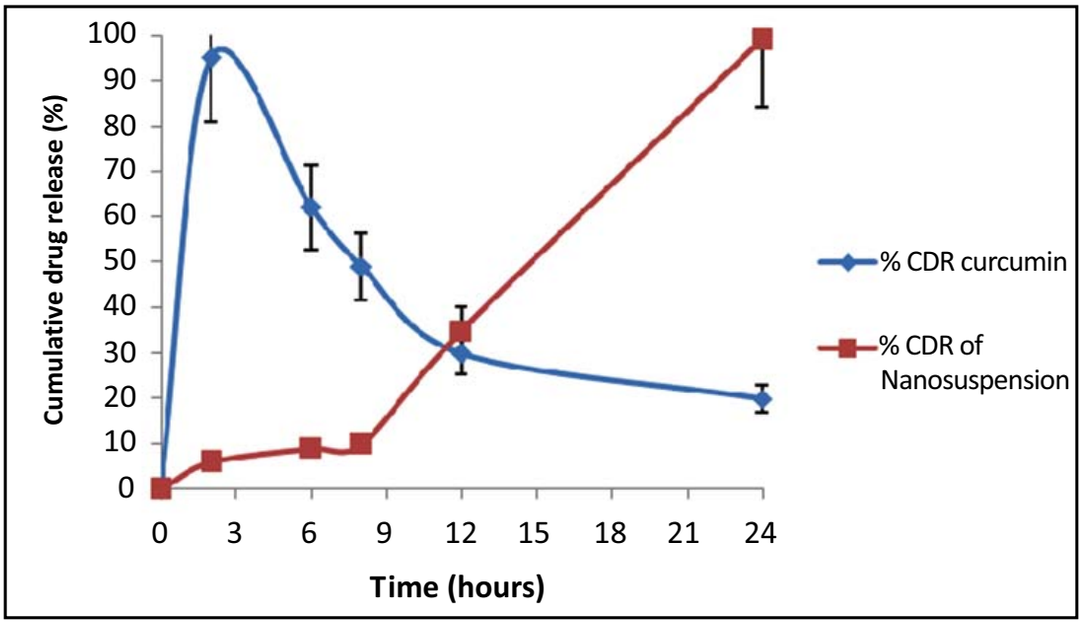


Fig (4). % Cumulative drug release of ZC formulation.

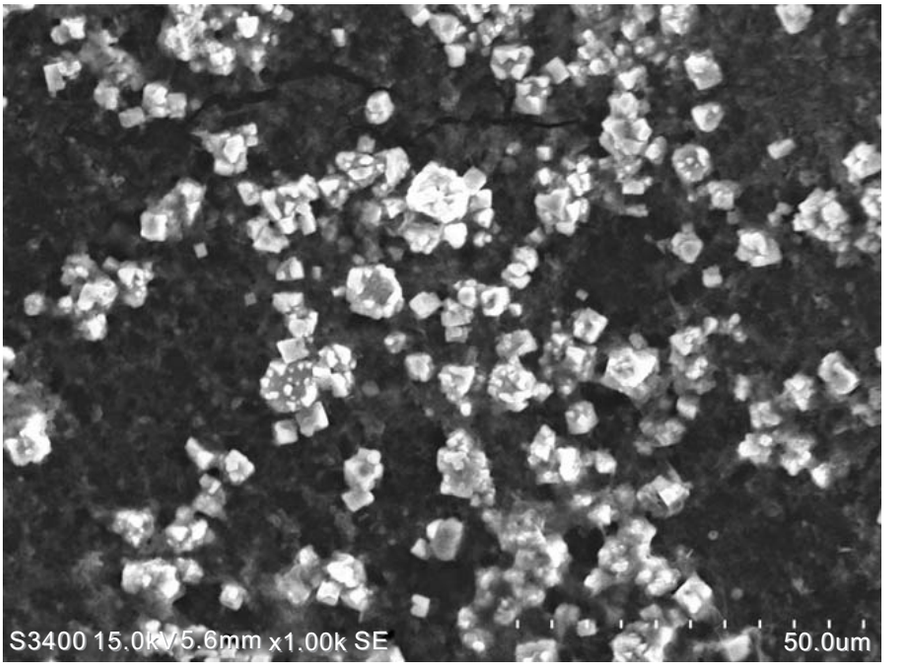


Fig (5). SEM image of ZC formulation.

diffusion of drug molecules for release into the medium. Dissolution medium SGF containing 0.2% Polysorbate 80 is intended to provide sink conditions for release process, as curcumin is sparingly soluble in water / aqueous medium. In SIF which is alkaline, the cross-linking of nanoparticle is expected to get broken or ruptured facilitating water permeation into the matrix of polymeric nanoparticles initiating swelling with the resultant dispersion and diffusion of curcumin into the dissolution medium.

3.7. Surface Morphology Studies

SEM imaging of the nanosuspension formulation Zc (Fig. **5**) shows the morphological characteristics of the prepared nanoparticles. As observed, the nanoparticles were more spherical, discrete and homogeneous.

3.8. Water Dispersibility Test

Curcumin is poorly water-soluble and dispersible, hence nanoparticulate strategies were explored

Table 3. Biochemical characteristics (mean ± SD) of the participants on medical examinations.

S. No.	Variables	Normal Range	Men (n=6)
1)	Age (Years)	-	25 ± 3
2)	Body Height (m)	-	1.76 ± 0.03
3)	Body weight (Kg)	-	70 ± 6.1
4)	BMI (Kg/m^2)	<25	23.8 ± 1.8
5)	Total Cholesterol (mg/dL)	0-200	105 ± 30.1
6)	LDL Cholesterol (mg/dL)	0-150	120 ± 7.6
7)	HDL Cholesterol (mg/dL)	40-70	58 ± 9.2
8)	Triacylglycerols (mg/dL)	0-200	128 ± 25
9)	Fasting Plasma glucose (mg/dL)	70-110	95 ± 6
10)	Haematocrit (%)	40-45	42 ± 0.2
11)	Blood Hb (g/dL)	13-17	14.5 ± 2.1
12)	Systolic Blood Pressure (mmHg)	120	120 ± 2
13)	Diastolic Blood Pressure (mmHg)	80	80 ± 2

to improve this essential physicochemical property. The optimized nanoparticle (Zc formulation) got easily dispersed in water at 0.1% level and appeared translucent similar to original nanodispersion which is confirmed by DLS analysis also (Particle size -396 nm and zeta potential -200 mV).

3.9. Bioavailability Studies

Curcumin is a potential nutraceutical for the prophylaxis therapy of many challenging diseases in humans. Because of its poor aqueous solubility and bioavailability, low concen-tration of curcumin is absorbed into systemic circulation which hinders its utility as a drug or medicine. Several stra-tegies have been explored by the investigators to improve bioavailability. One of the strategies is to increase plasma concentrations of curcuminoids by the concomitant use of adjuvants to inhibit their metabolism and elimination [32]. Yet another strategy proved that the simultaneous ingestion of native curcumin with phytochemicals and micellar curcu-minoid formulation increased the AUC for total curcumin of 8-fold and 88-fold respectively [18]. In another human study, a single oral dose of 30 mg curcumin formulated as colloidal nanoparticle dispersion ("Theracurmin") resulted in a 27-fold increased AUC relative to native curcumin [33]. In another report, in 11 subjects receiving 2 g curcumin alone or in combination with turmeric essential oils (BCM-95®CG), the AUC of curcumin increased by 7-fold [34]. The co-administration of 2 g curcumin with 20 mg piperine (n = 8) in another study increased the AUC of free curcumin to 20-fold compared to native curcumin [32].

In our work, no adverse events / reactions were observed as anticipated in the volunteers like (increased stool frequency, diarrhea, tiredness, headache, heartburn, regurgitation and nausea) as we have chosen the very small dose of curcumin (100mg / volunteer). UFLC chromatograms for standard curcumin spiked in human plasma and those of volunteer's plasma matched satisfactorily with the retention factor of 3.47 (Figs. **6a** and **6b**). The biochemical characteristics of the participants were observed and recorded in Table **3**. The data for the plasma concentration -time profiles of curcumin suspension and nanosuspension in human volunteers is recorded in Table **4** and the data of log plasma concentration of curcumin suspension and nanosuspension is recorded in Table **5**. The mobile phase consisted of methanol and Acetonitrile in the ratio of 20:80. Curcumin-loaded polysorbate 80 nano-particles exhibited a shorter t_{max} owing to expedited absorption and intestinal

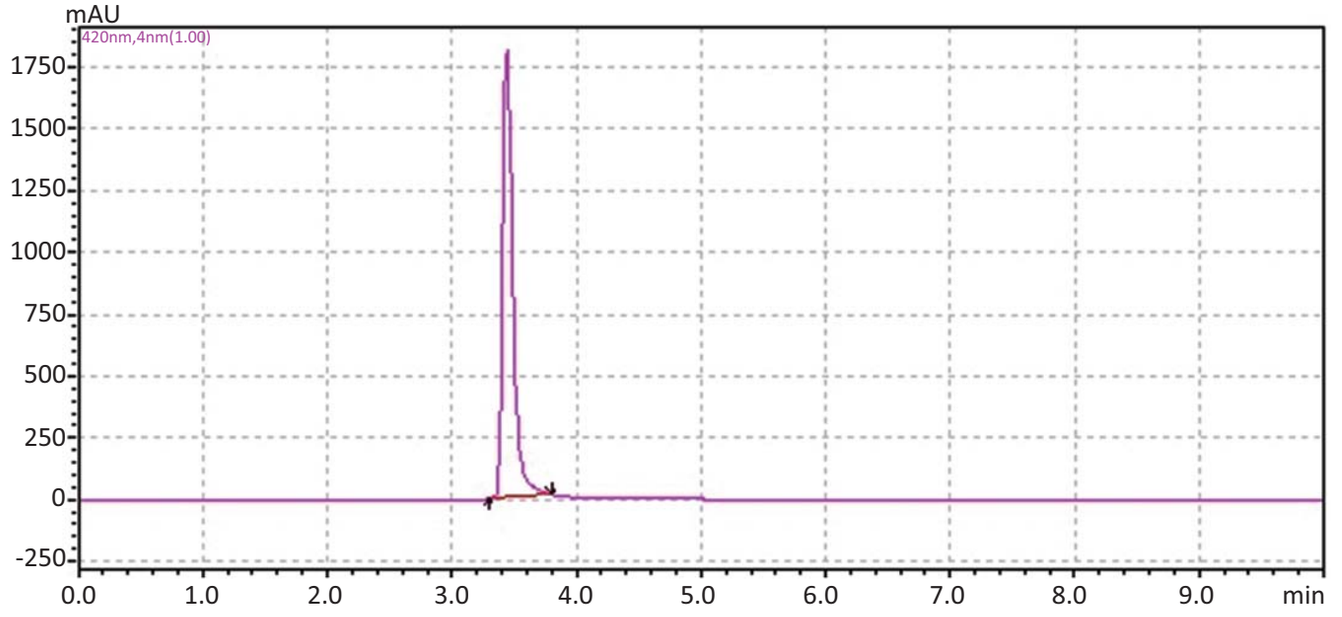


Fig (6a). UFLC Chromatogram of curcumin spiked in human plasma.

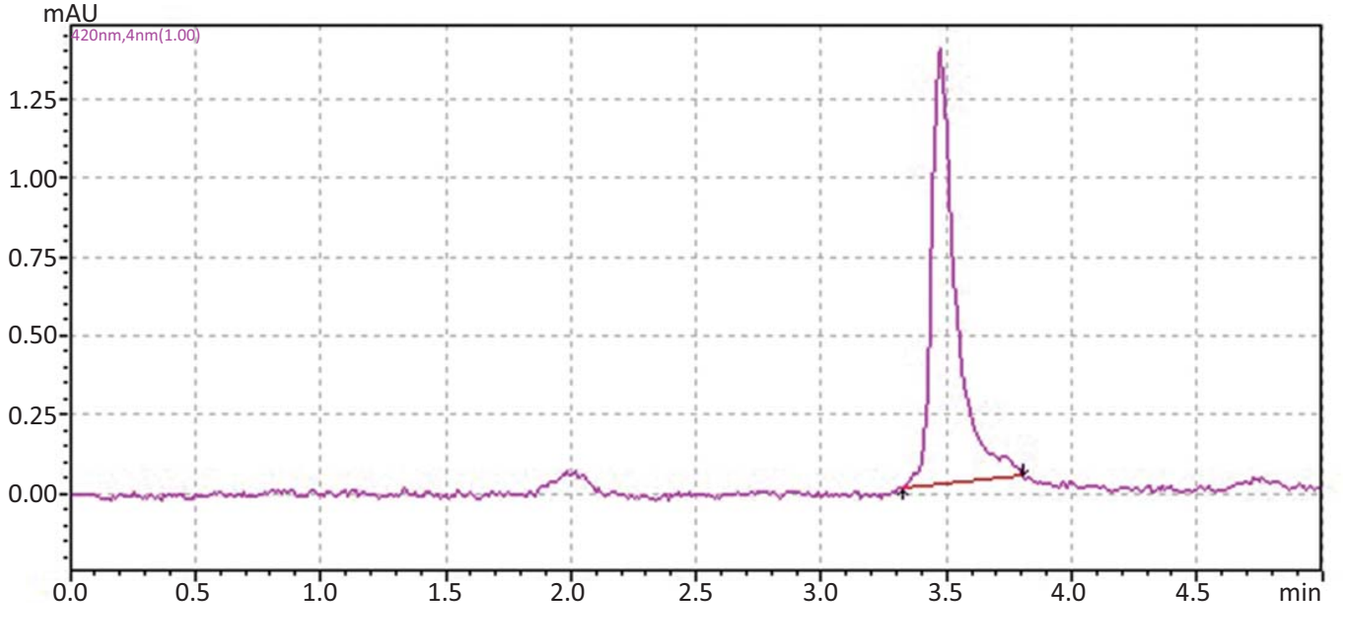
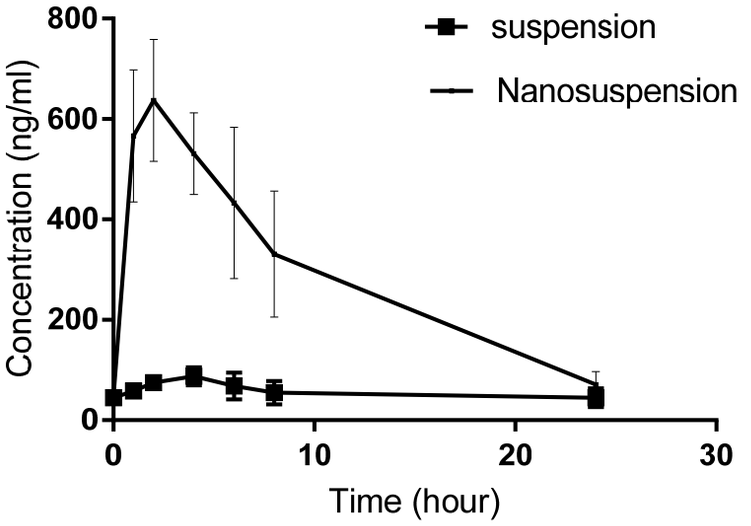


Fig (6b). Sample UFLC Chromatogram of human volunteer's blood plasma Characterizing curcumin.

permeability mechanized by cellular uptake of the nanosized particles. The conventional suspension possesses micron size particle, the absorbtion of which is dissolution rate dependent. The concentration-time profiles of curcumin loaded nanosuspension and suspension are displayed in Figs.(**6c-6e**). The values are statistically analyzed by unpaired student T-test using graph pad prism version 7.04 and the difference between the two formulations is significant at $p < 0.01$ [35, 36]. The oral bioavailability (AUC) of curcumin increased 5-fold after intake of curcumin nanosuspension compared to curcumin suspension. In our study, the higher AUC values observed for suspension havebeen attributed to the presence of Polysorbate 80 (0.4%), a surfactant and micronzed status of curcumin as we had levigated curcumin in the vehicle while preparing the suspension.



curcumin suspension 100mg/kg, AUC**= 1360 ± 18.4

curcumin nanosuspension 100mg/kg, AUC**= 7021± 92.7

Values are mean ± SD (n=6) ***p*<0.01.

Fig (6c). Mean plasma concentration-time profiles of curcumin suspension and Nanosuspension following Oral administration in human volunteers.

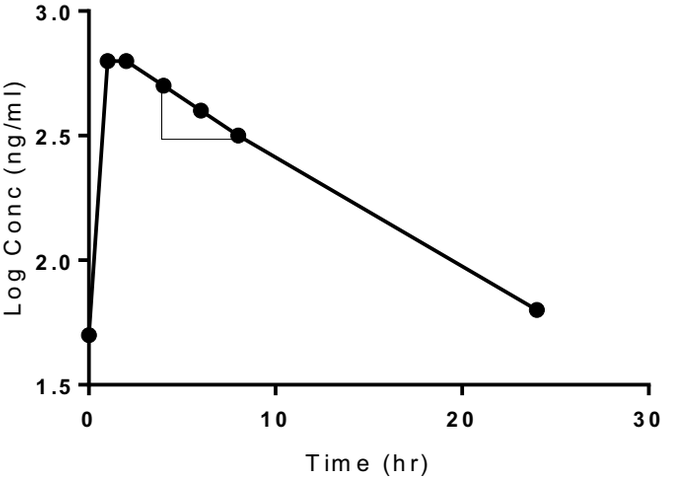


Fig (6d). Plasma log concentration-time profile of curcumin suspension.

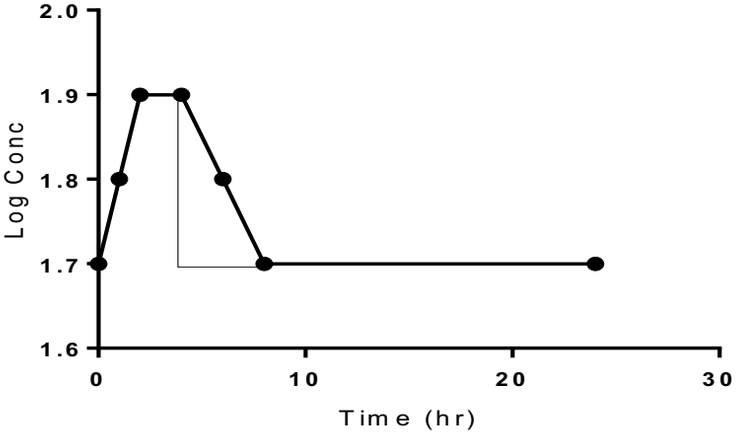


Fig (6e). Plasma log concentration-time profile of curcumin nanosuspension**.**

Table 4. Data for the plasma concentration time profiles of curcumin suspension and nanosuspension in human volunteers.

Time (h)	Mean Curcumin Concentration (ng/ml) in Plasma	
	Curcumin Suspension	Curcumin Nanosuspension
0	46 ± 14	47 ± 12
1	58 ± 14	566 ± 132
2	75 ± 15	637 ± 122
4	87 ± 17	531 ± 82
6	68 ± 26	433 ± 151
8	55 ± 23	331 ± 126
24	45± 18	70 ± 26

Values are mean of 3 volunteers (n=6).

Table 5. Data of log plasma concentration *vs.* time of curcumin suspension and nanosuspension.

Time (h)	Log Concentration (ng/ml)	
	Curcumin Suspension	Curcumin Nanosuspension
0	1.7	1.7
1	1.8	2.8
2	1.9	2.8
4	1.9	2.7
6	1.8	2.6
8	1.7	2.5
24	1.7	1.8

Table 6. Pharmacokinetic parameters of curcumin suspension and nanosuspension.

Parameters	Curcumin Suspension	Curcumin Nano Suspension
C_{max}(ng/ml)	87.7 ± 17.9	636 ± 122
T_{max}(h)	4	2
$t_{1/2}$ (h)	6.01	6.01
k_e (h^{-1})	0.115	0.115
AUC $_{0-24}$ (ng.h/ml)	1360 ± 18.4	7021± 92.7

Still higher / better pharmacokinetic profile of nanosuspension could be attributed to the colloidal micellar status of curcumin in nanosuspension [12]. C_{max} of nanosuspension is found to be nearly 7 times greater than that of curcumin suspensions (Table **6**). Maximum plasma concentration was observed at 2 hours with nanosuspension compared to 4th h in suspension [18]. Assuming linear Ist order pharmacokinetics [34], k_e- Elimination rate constant for the two products in our study

was found to be similar for suspension ($0.115h^{-1}$) and nanosuspension ($0.115h^{-1}$) implying unaltered elimination profiles irrespective of the formulation design. Polymeric nanoparticle production is comparatively more simpler than liposomes or niosomes and less expensive in addition to better physicochemical stability and *in-vivo* performance to modulate better programmable pharmacokinetic profile.

CONCLUSION

Curcumin-loaded alginate - polysorbate 80 nanoparticles prepared by ionotropic gelation method, successfully entrapped curcumin, exhibiting higher encapsulation efficiency nearing - 95%. Nanoparticles produced were in the size range 105-383 nm and zeta potential was found to be in the range of 150-200mV. Formulations exhibited very low dissolution in Simulated Gastric fluid (SGF) and Simulated Intestinal fluid (SIF), but the major portion released in SCF was attributed to the digestibility of alginate in Simulated Colonic fluid (SCF) under the influence of colonic microflora which is not present in the stomach or small intestine. Based on the physical stability of nanosuspension (number of days of no settling), we have chosen ZC as the optimized formulation. FTIR and DSC observations revealed the successful entrapment of curcumin in alginate polysorbate-80 nanoparticles. The nanoparticles of Zc formulation were more spherical, discrete and homogeneous. Nanoparticles could be transformed into easily redispersible powder form either by freeze drying/ vacuum concentration or spray drying for application in nutraceuticals and controlled drug delivery systems. In healthy human volunteers, the oral bioavailability (AUC) of curcumin increased 5 -fold after the consumption of curcumin nanosuspension compared to curcumin suspension. Maximum plasma concentration C_{max}- 636 ± 122ng/ml was observed at t_{max}- 2h for nanosuspension, whereas Cmax-87.7 ± 17.9ng/ml at t_{max}- 4h for suspension. Elimination rate constant was found to be similar for both curcumin suspension and curcumin nanosuspension which signified unaltered elimination profiles irrespective of formulation design. Both curcumin suspension and curcumin nanosuspension were safe and well tolerated, and may thus be useful in the prevention or treatment of various inflammatory diseases of mankind.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Human studies in this work were carried out under an Institutional Ethics Committee (Human Studies) at Sarada Vilas College of Pharmacy, Mysuru.

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All human procedures were followed in accordance with the ethical standards of Institutional Ethics Committee (Human Studies) and with the *Helsinki Declaration* of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

Informed written consent was received from all the prospective volunteers.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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