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FORMULATION, OPTIMISATION AND EVALUATION OF MICROPARTICLES OF CURCUMIN-ZN COMPLEX

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ABSTRACT

In present study an attempt was made to prepare microparticulate drug delivery system of curcumin-Zn complex and evaluate it in albino rats spectroflurometrically. It was observed that curcumin-Zn complex was more soluble and stable than curcumin. Curcumin-Zn complex was prepared and encapsulated using sodium alginate. Characterization of prepared microparticles was done using FTIR, UV, SEM and DSC study. Microparticles thus obtained are further coated with various enteric polymers like eudragit S 100, eudragit L100, and cellulose acetate pthalate and ethyl cellulose at different coating thickness to control the release. Microparticles were evaluated for encapsulation efficiency, drug loading and in vitro drug release. Microparticles coated with cellulose acetate pthalate showed most satisfactory and controlled release with 502 min time for 60% cumulative release. Curcumin-Zn was estimated in serum after oral administration of microparticles to rats by using spectroflurometry. Estimation of curcumin in serum by spectroflurometry showed that drug concentration is maintained in the blood for longer time with t_{max} of 6 hours.

KEYWORDS: **Curcumin, curcumin-Zn complex, sodium alginate microparticles, enteric coating**

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INTRODUCTION

The natural product curcumin is a polyphenolic compound extracted from the rhizome of *Curcuma longa L*. In India, it is commonly used as spice, to add color and flavor to the food. In Ayurveda, use of curcumin is well documented for the treatment of various ailments[1]. Studies have shown that a new combination therapy with artemether and curcumin is unique, with potential advantages over the known ACTs[2]. Curcumin, in addition to having a direct killing effect as an antimalarial, is also able to activate the immune system against *Plasmodium berghei*[3]. An important issue with curcumin is its poor bioavailability, less stability, rapid metabolism and short half life[4]. Several attempts are underway to improve bioavailability through the use of preparations such as liposomes, phospholipid complexes, nanoparticles or microparticles[5,6,7]. Complexation of curcumin with transition metals is one of the useful ways to overcome the problems related to solubility, stability and bioavailability. Curcumin can chelate various metal ions to form metallocomplexes of curcumin, which shows greater effects than curcumin alone[8]. In present study an attempt was made to prepare microparticulate drug delivery system of curcumin-Zn complex which will maintain the concentrations of drug in blood throughout treatment period.

MATERIALS AND METHODS

Curcumin was purchased from Phytopharma actives Pvt. Ltd., Mumbai. Eudragit S-100, eudragit L-100, cellulose acetate pthalate (CAP) samples were gifted by US vitamin pharmaceutical ltd., Mumbai. Sodium alginate (Sigma Chemicals, UK), liquid paraffin heavy and liquid paraffin light, zinc sulphate (S.D. Fine Chemicals, Mumbai), calcium chloride (Ranbaxy Fine Chemicals, AR), di-sodium hydrogen phosphate, DMSO (E. Merck India ltd.), trisodium phosphate, acetone, petroleum ether (Poona Chemical Lab.), ethyl cellulose (Milton Chemicals, Mumbai), isopropyl alcohol, polyethylene glycol 4000 (Research lab, Mumbai), were procured from local market.

Animals

Inbred albino rats of either sex were obtained from the animal house of Pravara Medical College, Pravaranagar. The research was conducted in accordance with standard institutional guidance given by Institutional Animal Ethics Committee (IAEC). The Labs used for the purpose was approved by Committee for the purpose of control and supervision of experiments on animals, Ministry of social justice and empowerment, Govt. of India (Registration No.-448/01/c/CPCSEA).

Preparation of Curcumin- Zn Complex

Zinc sulfate ($ZnSO4 \cdot 7H2O$) was mechanically mixed in mortar with curcumin (Zn2+: Curcumin 1/1 mol) until homogenous powder mixture was obtained. Then glycerol/water (1: 1 v/v) solution was added to mixture, followed by mechanical shaking at 25◦C until pasty combination was obtained. Then, pasty product was dried at 50^0 C and free glycerol was eliminated by washing with distilled water. Dark color powder complex of Zn-curcumin was obtained[8].

Characterisation of Curcumin and Curcumin-Zn complex[9, 10]

i. FT-IR Spectroscopy

FT-IR spectroscopy of curcumin and curcumin-Zn complex was performed on FTIR (Jasco FT/IR-4100) spectrophotometer. About 5mg of sample is mixed with 100 mg of KBr and compressed to form pellets. The spectra of sample were scanned from wave number range of 650 to 4000 cm−1.

ii. UV Spectroscopy

Solution of curcumin and its metal complex were prepared in DMSO and scanned on UV spectrophotometer in the range 400 to 650nm to find λ max.

iii. Calibration Curve

The calibration curve of curcumin and curcumin-Zn was developed using UV spectrophotometer (Thermoscientific evolution 201) in 0.1N HCL (pH 1.2), phosphate buffer (pH 6.8) and phosphate

buffer (pH 7.4) at 435 and 432 nm respectively. Various concentrations of curcumin were prepared by dissolving curcumin in 5ml of methanol and volume was then adjusted with one of above solvents.

iv. Stability Evaluation

Stability study of curcumin and curcumin-Zn was performed to analyze the kinetic degradation in phosphate buffer (pH 7.4) at 37^0C. Curcumin and curcumin-Zn complex solutions of known concentrations were prepared in phosphate buffer pH 7.4. Their degradation was studies by withdrawing samples at specified time interval and analyzing it for the drug content spectrophotomerically.

v. Solubility Evaluation

Solubility of curcumin and curcumin metal complex was evaluated by equilibrium solubility method. Excess amount of curcumin and curcumin- Zn complex was added to water and kept for stirring in orbital shaker for 6 hour. Solutions were filtered and filtrate was analyzed for the drug content spectrophotometrically.

Preparation of Microparticles

Microparticles of Curcumin-Zn were prepared by ionotropic gelation using sodium alginate and calcium chloride as cross-linking agent. SA and curcumin-Zn were dispersed in water at room temperature. Separately 200 ml of blend (50:50) of liquid paraffin (heavy and light) was placed in silicone treated round bottom flask. SA- curcumin-Zn dispersion was poured in liquid paraffin and stirred for 15 min., to it 100 ml of 4% calcium chloride solution was added slowly and stirring was continued for 20 min. Solidified microparticles were filtered, washed for several times with petroleum ether to remove traces of oil and dried under vacuum[11, 12]. Microparticles thus obtained are dried further for 24 hr. Four batches (1-4) were prepared with constant concentration of cross linking agent (CLA) and cross linking time (CLT) with varying stirring speed at SA-curcumin-Zn ratio 4:1 and stirring speed was optimized for 2000 rpm. Then SA- curcumin-Zn ratio was altered from batch A1 to A6 from 1:1 to 1:6. The effect of formulation variables on characteristics of microparticles is summarized in Table 1 and 2.

Entrapment Efficiency

50 mg of microparticles weighed accurately and crushed in glass mortar and pestle. Powdered microparticles were suspended in 5 ml of methanol for 24 hrs. After 24 hrs, the solution was filtered and volume is made with 50 ml with phosphate buffer pH 6.8. Filtrate was analyzed for drug content at 432 nm. Corresponding drug concentrations in samples were calculated from calibration plot generated by regression of data[13]**.**

Particle Size Analysis

The particle size of microparticles was determined by using optical microscopy method. The arithmetic mean diameter of total 300 microparticles for each sample was calculated by using Edmundson's general equation[14].

Table 1
Optimization of stirring speed

Batch	Stirring speed (rpm)	Volume of CLA (4% w/v $CaCl_2$) ml	% Entrapment	% Drug loading	Arithmetic mean diameter (μm)
1	1000	100	67.44 ± 1.25	16.12±0.74	268.42
2	2000	100	80.23± 2.31	18.53± 1.21	225.46
3	3000	100	73.24± 2.44	15.62± 1.02	194.28
4	4000	100	65.69±1.89	14.83 ±0.98	172.09

**** Data is expressed as mean ± SD, (n = 3)***
For all batches drug : polymer ratio kept constant - 1:4.

Table 2
Optimization of drug: polymer ratio

Batch No.	Drug: Polymer ratio	% Entrapment efficiency	% Drug loading	Average Particle size (μm)	Characteristic of microparticles
A1	1:1	41.24 ± 2.45	28.57± 0.78	147.65	Irregular
A2	1:2	55.33 ± 1.97	22.91± 1.02	182.26	Spherical free flowing
A3	1:3	62.58 ± 2.35	17.22± 1.54	210.78	Spherical free flowing
A4	1:4	80.14 ± 2.01	17.39± 0.89	232.12	Spherical free flowing
A5	1:5	73.67 ± 1.25	13.03± 0.29	269.76	Irregular
A6	1:6	62.08 ± 0.88	11.06± 0.77	289.07	Fibrous and irregular

**** Data is expressed as mean ± SD, (n = 3)***

Enteric Coating

Optimized microparticles were coated with different enteric polymers to varying coating thickness. Coating solutions were prepared using different concentrations of polymers used for coating. Eudragit S-100 and L-100 solution were prepared using isopropyl alcohol and PEG 4000 (20:1) as plasticizer. CAP was dissolved in acetone and propylene glycol was used as plasticizer. For EC, acetone is used as solvent and PEG 4000 as plasticizer .Microparticles were coated with one of different polymers at two different concentrations in pan coater. The microparticles were coated and dried with the help of inlet air (temperature 35-45°C). The coating process was repeated till the desired level of coating was achieved. The increase in percent mass of microparticles upon coating was taken as an indication of coat thickness[15].

Determination of Surface Morphology

The microparticles were observed under scanning electron microscope (SEM, Hitachi, Japan). The microparticle samples were observed at 20 kV by sprinkling sample on the aluminum stubs having double adhesive tape and subsequent evaporation of gold palladium alloy in the ion sputter unit[16].

Differential Scanning Colorimetry (DSC)

The DSC analysis of pure curcumin, curcumin-Zn complex and curcumin Zn-loaded microparticles was carried out using a DSC (DSC Q 100 V9.9 Build 303) to evaluate any possible drug-polymer interaction. The analysis was performed at a rate 10^0 C min**-1** from 0^0 C to 300^0 C temperature range under nitrogen flow of 30 ml min -1[13].

In-vitro Dissolution Studies

Microparticles equivalent to 50 mg of curcumin-Zn were filled in hard gelatin capsules and used for dissolution test. The USP 24 (8) method for enteric coated tablets (basket method, 75 rpm, 37±0.5°C) was used for all experiments. For the initial 2 hr, the study was conducted in 750 ml of 0.1 mol L^{-1} HCl, followed by dissolution at a pH of 6.8 (adjusted by addition of 250 ml of 0.2 M trisodium phosphate). At suitable interval 5 ml aliquot was removed and 5 ml of fresh media was added to maintain original volume. Dissolution was conducted in 0.1M HCl for 2 hrs and while after two hour dissolution was conducted in phosphate buffer pH 6.8 and aliquots were analyzed for curcumin-Zn at a λ_{max} 432 nm[16.]

Estimation of Curcumin-Zn Complex in Serum by Spectrofluorometry

The spectrofluorimetric study was carried out with a Shimadzu RF 5301 PC spectrofluorimeter, to determine the level of fluorescence of the sample in a stationary state. The light source used was a xenon 150 W lamp with an optical system composed of two automatic monochromators, one for excitation and the other for emission.

Preparation of Standard Curve of Curcumin-Zn Complex in Serum

Curcumin-Zn was estimated in goat serum, by spectrofluorimetric method for determination of its concentration in biological fluids. For this purpose a standard curve of curcumin-Zn was prepared in goat serum. Buffer solution (PBS, pH 6.5) was used for dilution of the serum to maintain the acidic medium during the estimation process. Goat blood was collected from a slaughterhouse and serum was separated by centrifugation at 3000 rpm for 15 min. The isolated clear serum was diluted with buffer solution (PBS, pH 6.5) to 10 %. Then, 10 mg curcumin-Zn was weighed and dissolved in a minimum volume of methanol. Then, the volume was made up to 100 ml with diluted serum and this stock solution was used to prepare required dilutions containing 50 ng/ml to 500 ng/ml curcumin-Zn. The samples were analyzed in the spectrofluorimeter against solvent blank (diluted serum). The wavelength and intensity of each sample was recorded. A standard curve for curcumin was prepared in serum (diluted with pH 6.5 buffer) at excitation and emission wavelengths of 232 nm and 614 nm.

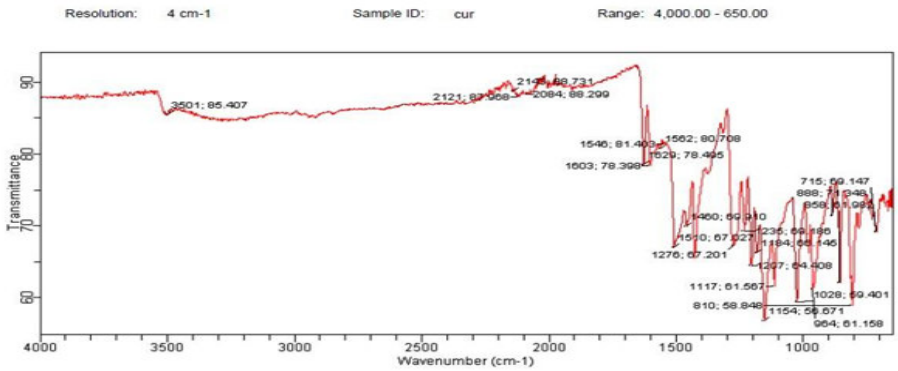
Estimation of Curcumin-Zn in Serum after Oral Administration to Rats

Three albino rats (Wistar strain) were each given 50 mg/kg curcumin-Zn microparticles orally. Blood samples were collected from the retro orbital plexus at intervals. Blood was allowed to clot at room temperature for about 1 hr, then centrifuged at 3000 rpm for 15 min and serum was separated. Estimation of curcumin-Zn was performed by spectrofluorimetry after dilution of serum with buffer solution (pH 6.5)[17].

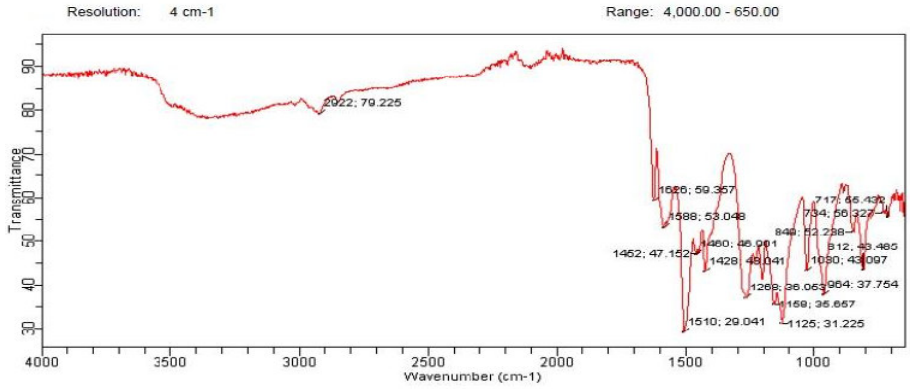
RESULTS AND DISCUSSION

Curcumin and curcumin-Zn complex in powdered form was scanned from a wavelength of 4000cm^{-1} to 650cm^{-1}. The resultant spectrum obtained has been shown in graph 1 and 2. From the FTIR and UV Spectroscopy it was confirmed that curcumin Zn complex is successfully prepared. The 1629 and 1603 cm^{-1} bands correspond to the mixtures of stretching vibrations of (C=C) and (C=O) in curcumin were red shifted to 1625 and 1588 cm^{-1} in the curcumin-Zn complex respectively.

Graph 1
FTIR spectrum of curcumin



Graph 2
FTIR spectra of Curcumin Zn complex



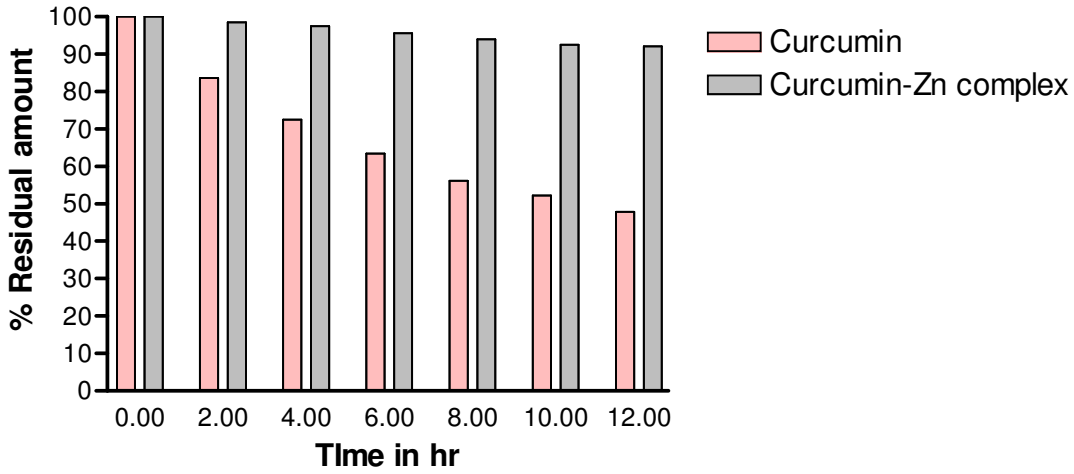
UV Spectrophotometric Determination

UV spectra of curcumin and its metal complex are developed by dissolving it in DMSO. Their spectra are deconvoluted with absorption band at 435 nm for curcumin, at 432 nm for curcumin-Zn complex.

Kinetic Stability

The results of kinetic stability studies showed that curcumin degraded extensively with in 12 hr while its complex with zinc showed good stability in the similar conditions (Graph 3).

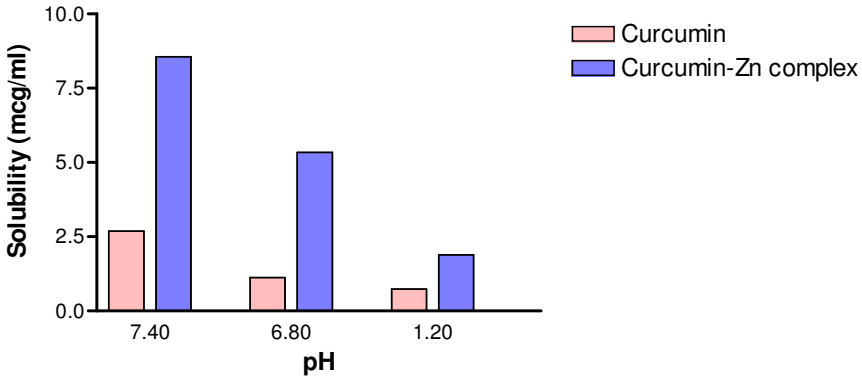
Graph 3
Kinetic stability of Curcumin-Zn complex compared to Curcumin



Solubility Determination

Solubility of Curcumin and Curcumin metal complex was evaluated by equilibrium solubility method in orbital shaker. The result of solubility study is shown in Graph 4.

Graph 4
Comparative solubility of curcumin & curcumin-Zn



SEM Study

The SEM photographs of microparticles at low and moderate magnification indicated (Figure 1) that the microparticles were discrete, spherical with little variation in size.

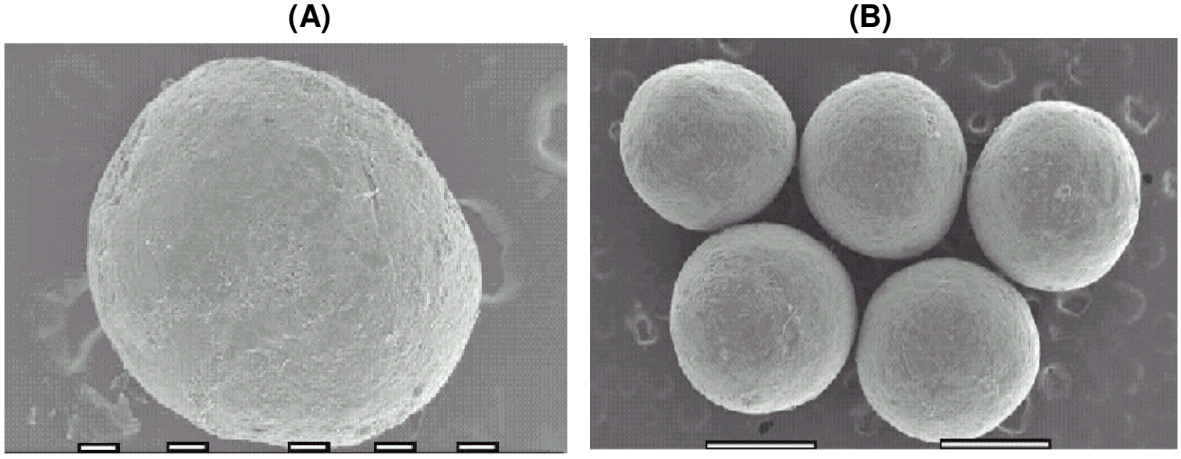
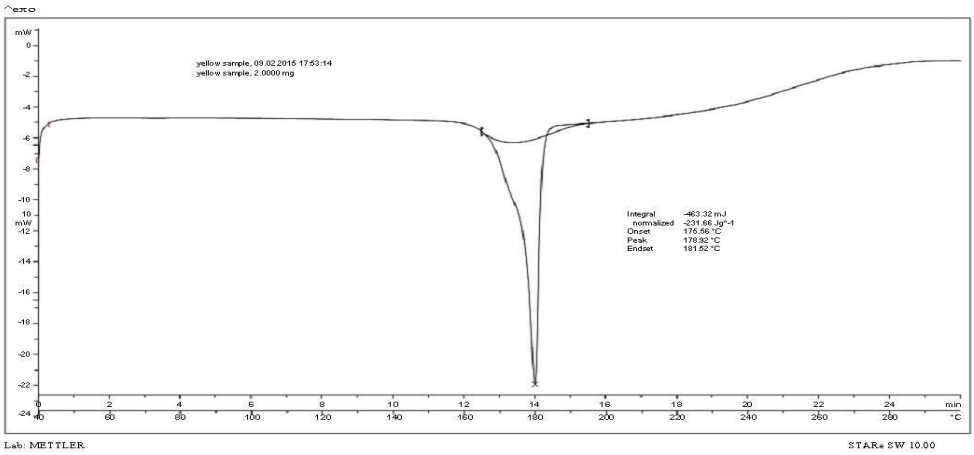


Figure 1
SE micrograph of microparticles of Curcumin-Zn
(A) Scale bars denotes 10 µm (B) Scale bars denotes 100 µm

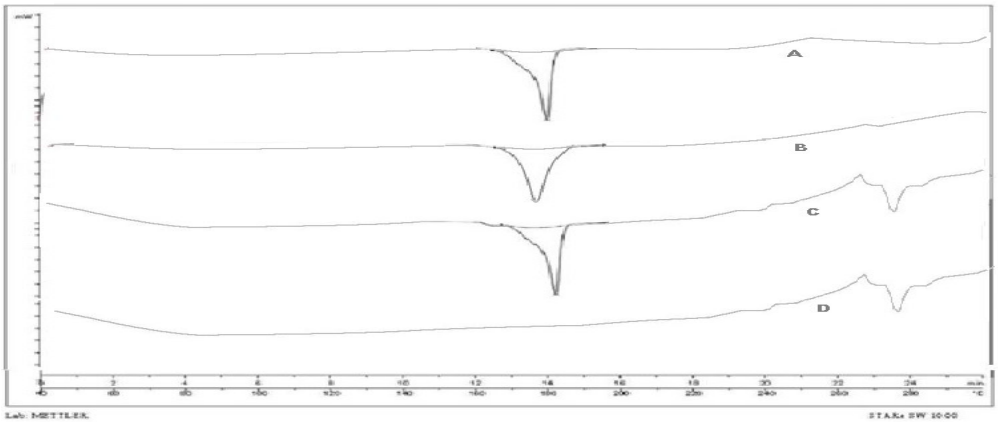
DSC study

DSC analysis was performed for the curcumin, curcumin-Zn complex and sodium alginate and microparticle formulation. DSC study revealed that the thermograms of the curcumin Zn complex, sodium alginate and its formulation were almost identical. This indicates that there were no changes in thermal behavior of drug and the formulation[11].

Graph 6
DSC thermogram of Curcumin Zn complex



Graph 7
A. Curcumin B. Curcumin Zn Complex C. Microparticles of Curcumin- Zn and sodium alginate D. Sodium Alginate



In vitro Dissolution study

Cumulative percent release of curcumin-Zn from different enteric-coated microparticles is given in Table 3. Time to release 60% ($t_{60\%}$) for different batches of polymers were compared and reported in Table 4**.** It could be seen that dissolution rate was retarded with the increase in coat thickness. There was significant difference in $t_{60\%}$ value for different batches of enteric coated microparticles coated to different coating thickness. Dissolution data for various batches of microparticles were analyzed by fitting it into various models. CAP 10% w/v showed most satisfactory (zero order), controlled release in its group and passes the entire model test. The batch with CAP 10%w/v coating was tested for *in vivo* study for determination of concentration in serum after oral administration.

Table 3
In-vitro dissolution data (cumulative percent release) of curcumin-Zn

Sr. No.	Time in hrs.	Eud. S-100 4%w/	Eud. S-100 6%w/v	Eud. L-100 4%w/v	Eud. L-100 6%w/	CAP 6% w/v	CAP 10%w/v	EC 6% w/v	EC 10% w/v
Dissolution data in 750 ml of 0.1M HCl:									
1	**0.5**	2.1± 0.2	2.2± 0.14	1.9± 0.54	2.00± 0.4	1.9± 0.33	1.6± 0.09	2.0± 0.12	1.9± 0.09
2	**1**	3.2± 0.3	3.6± 0.22	2.8± 0.28	2.70± 0.52	2.5± 0.74	2.8± 0.8	3.3± 0.2	2.5± 0.5
3	**2**	5.7± 0.21	5.9± 0.58	6.9± 0.35	5.80± 0.41	3.5± 0.21	3.8± 0.17	5.1± 0.54	4.6± 0.61
Dissolution data in phosphate buffer pH 6.8 (+ 250 ml of 0.2 M tri-sodium phosphate)									
4	**2.5**	21.05± 1.02	19.8± 0.68	30.25± 1.44	26.90± 2.04	22.8± 0.82	20.16± 0.87	18.52± 0.9	18.58± 1.07
5	**3**	27.25± 2.12	24.4± 0.86	36.55± 1.57	33.96± 1.00	27.35± 1.27	24.93± 1.48	26.79± 1.12	25.88± 1.53
6	**4**	37.5± 2.28	36.2± 1.67	42.98± 2.56	40.27± 1.40	38.57± 1.11	34.09± 2.19	34.81± 1.80	33.12± 1.35
7	**6**	53.06± 1.87	45.6± 1.78	57.79± 1.41	53.8± 1.88	53.95± 2.54	48.52± 1.99	46.96± 1.77	47.66± 1.57
8	**8**	62.89± 2.51	57.22± 2.49	66.35± 0.7	63.66± 2.54	60.12± 1.89	58.15± 1.52	51.23± 1.28	52.03± 1.09
9	**10**	70.28± 1.99	66.10± 1.14	73.84± 0.65	71.75± 1.21	74.18± 1.66	68.57± 2.04	57.49± 2.08	56.38± 2.11
10	**12**	77.18± 1.48	72.18±1 .58	79.5± 1.97	77.29± 1.59	82.52± 2.08	79.90± 1.51	61.95± 1.04	59.02± 1.24
11	**14**	84.03± 2.05	79.76± 2.01	86.95± 1.75	84.39± 1.29	90.77± 1.67	89.42± 2.31	70.05± 1.26	66.95± 0.94
12	**16**	89.87± 2.41	85.47± 0.85	94.27± 2.13	90.24± 1.57	98.9± 1.10	98.11± 1.59	79.72± 1.97	76.59± 1.43

n=3±S.D.

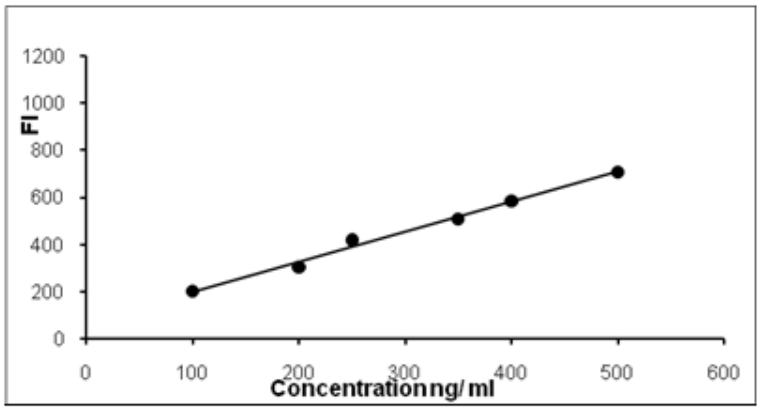
Table 4
$t_{60\%}$ for different enteric coated batches of microparticles

Time	Eud. S-100 4% w/v	Eud. S-100 6% w/v	Eud. L-100 4% w/v	Eud. L-100 6% w/v	CAP 6% w/v	CAP 10% w/v	EC 6% w/v	EC 10%w /v
$t_{60\%}$ in Min	470	530	407	435	479	502	714	725

Estimation of curcumin-Zn in rat serum after oral administration

A standard curve for curcumin was prepared at excitation and emission wavelengths of 232 nm and 614 nm, using a spectrofluorimeter. The plot of concentration versus intensity exhibited a linear relationship.

Graph 8
Standard curve of curcumin-Zn in serum



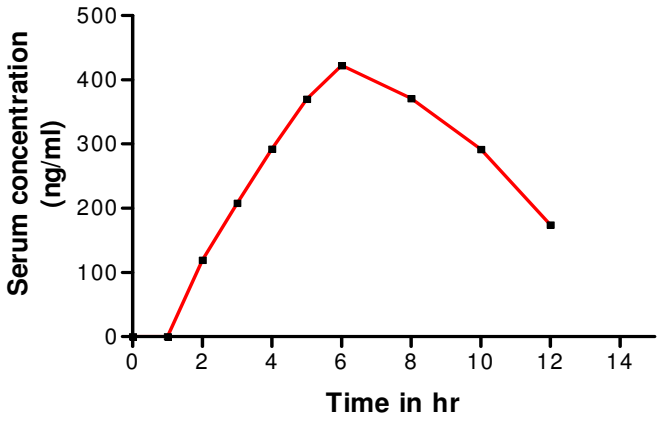
Curcumin was also estimated in serum of rats after oral administration of curcumin Zn microparticles at a dose equivalent to 50 mg/kg of curcumin. The peak serum concentration (422.41 ng/ml) was observed after 6 hr of administration and concentration of curcumin was maintained in the serum of rats for longer period. The results are shown in Table 5.

Table 5
Concentration of curcumin-Zn in rat serum after oral administration

Sr. No.	Time (hr)	Concentration of curcumin-Zn(II) in serum for microparticles (ng/ml)
1	1	--
2	2	119.47± 6.89
3	3	208.11 ± 11.88
4	4	292.15 ± 8.23
5	5	370.24 ± 21.27
6	6	422.41 ± 13.25
7	8	371.15± 19.56
8	10	291.77 ± 16.32
9	12	174.24 ± 12.32

All values are mean ± S.D. (n = 3)

Graph 9
Concentration of curcumin-Zn in rat serum after oral administration



CONCLUSION

Curcumin is naturally occurring polyphenolic compound having broad spectrum of therapeutic and prophylactic potential. It was observed from the study that both solubility and stability of curcumin were improved by preparing it as zinc complex. Curcumin-Zn complex tend to dissociate in acidic pH. Microparticles of sodium alginate was prepared and coated with enteric polymers to avoid the dissociation in acidic pH. Sodium alginate microparticles coating with Cellulose acetate pthalate (10% w/v) showed most satisfactory release. *In vivo* study conducted on albino rats has showed that concentration of curcumin was maintained in the serum for longer time after oral administration of curcumin-Zn microparticles to rats. Studies have shown that a new combination therapy with artemether and curcumin is unique, with potential advantages over the known ACTs. But both artemether and curcumin suffers from disadvantages of short life and faster elimination. Future study can be focused to evaluate microparticulate drug delivery system of curcumin-Zn complex in combination with artemether for antimalarial activity, which will maintain the concentrations of drug in blood throughout treatment period and reduce the chances of developing drug resistance.

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