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ORIGINAL ARTICLE

Design and Development of Microparticulate Delivery System for Curcumin

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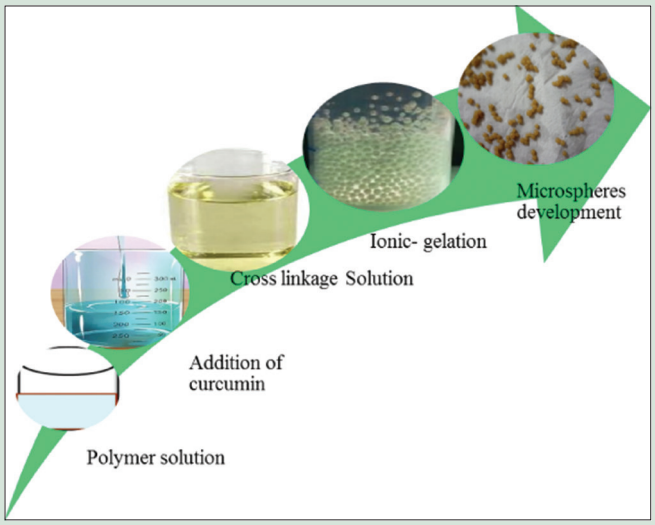
ABSTRACT

Background: Curcumin has been associated with remarkable beneficial effects; despite having a broad spectrum of activities, curcumin is characterized by poor water solubility and low bioavailability. **Objective:** The objective of this study is to enhance the solubility of curcumin using polyethylene glycol 400 (PEG400) and establish a microparticulate drug delivery system in a sodium alginate polymer matrix (microspheres containing curcumin triturated with PEG400 [Cur-PEGMS]) to improve the bioavailability of curcumin. **Materials and Methods:** The microspheres were formulated using the ionic gelation technique. Nine batches (F1 to F9) were prepared using 3^2 factorial design. The amount of sodium alginate and calcium chloride was selected as a formulation variable. The prepared Cur-PEGMS were characterized by Fourier-transform infrared spectroscopy, scanning electron microscope, differential scanning calorimeter, ultraviolet spectrophotometer, and high-performance thin-layer chromatography (HPTLC). The pharmacokinetics of curcumin was characterized in rats using high-performance liquid chromatography, and calculations were performed using WinNonlin standard edition version 1.1 software. **Results:** We found that solubility was affected by the use of cosolvent. All F1–F9 batches were investigated for entrapment efficiency, and batch F5 was found to be optimum batch which was further evaluated. HPTLC chromatogram of Cur-PEGMS shows peak retention for curcumin. The particle size of microspheres was found to be in the range of 384–468 μm. Remarkable improvement in maximum plasma concentration and bioavailability was observed. Maximum the time after administration of a drug when maximum plasma concentration is reached (T_{max}) was found to be 0.5 h, and AUC_{0-24} was found to be 374.75 ng/ml and 622 ng/ml for curcumin suspension and curcumin microspheres, respectively. **Conclusion:** The developed formulation enhances the bioavailability of the drug. This enhanced oral absorption of Cur-PEGMS may provide a practical formulation to conduct a correlative pharmacodynamic study.

Key words: Curcumin, factorial design, microspheres, pharmacokinetics, sodium alginate, polyethylene glycol 400

SUMMARY

- *Curcuma longa* (turmeric) is widely used Indian spice that imparts color and flavor to food. Curcumin is a polyphenol and principal curcuminoid present in turmeric. Curcumin has been reported to have wide range of pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial, anticancer, antioxidant, antidiabetic, antihyperlipidemic, and hepatoprotective activities. Despite number of pharmacological activities, curcumin exhibits low aqueous solubility. The earlier pharmacokinetic studies indicated poor bioavailability and instability of curcumin. To overcome these problems, a number of novel delivery systems have been tried. Based on the solubility studies, in this study, curcumin solubility was enhanced with polyethylene glycol 400 and then it was encapsulated in sodium alginate matrix. The concentration of polymer and the concentration of cross-linker were selected as formulation variables to prepare nine batches of formulation using 3^2 factorial design. The batch F5 was found to be optimum batch and was further characterized by scanning electron microscopy, high-performance thin-layer chromatography, Fourier-transform infrared spectroscopy, and particle size analysis. The pharmacokinetic parameters were determined in rats using WinNonlin standard edition version 1.1 software. An increase in AUC_{0-24} and maximum plasma concentration of curcumin in microspheres compared to curcumin in suspension was observed after oral administration. The solubilization method and the enhanced absorption of curcumin in formulation may provide a solution to overcome the limitation of curcumin and offer an opportunity for efficient use of it.



Abbreviations Used: PEG: Polyethylene glycol, PEG200: Polyethylene glycol 200, PEG400: Polyethylene glycol 400, PEG600: Polyethylene glycol 600, FTIR: Fourier-transform infrared spectroscopy, SEM: Scanning electron microscopy, UV: Ultraviolet spectrophotometer, Cur-PEGMS: Microspheres containing curcumin triturated with PEG400, HPLC: High-performance liquid chromatography, HPTLC: High-performance thin-layer chromatography, DSC: Differential scanning calorimetry, %DEE: Percentage of drug entrapment efficiency, SMEDDS: Self-microemulsifying drug delivery system, PLGA: Poly (lactic-co-glycolic acid), Std.: Standard, AUC: Area under curve, C_{max}: Maximum plasma concentration, T_{max}: Maximum the time after administration of a drug when maximum plasma concentration is reached, Plasma Conc.: Plasma concentration, %EE: Percentage of entrapment efficiency, SD: Standard deviation, IS: Internal standard, R_f value: Retention factor value, ng: Nanogram, μg: Microgram, μm: Micrometer, mg: Milligram, ml: Milliliter, mm: Millimeter, cm: Centimeter, m: Meter, g: Gram, Kg: Kilogram, i. d.: Internal diameter.

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INTRODUCTION

Curcumin is a yellow-colored, hydrophobic polyphenolic pigment derived from the rhizomes of spice–herb turmeric or *Curcuma longa* L., belonging to the family, Zingiberaceae.[1] Curcumin (1,7-bis-[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione: Diferuloyl methane) is insoluble in water and ether but is soluble in ethanol, dimethyl sulfoxide, and other organic solvents. *C. longa* L. contains curcuminoids; the three major components of curcuminoids are curcumin (77%), demethoxycurcumin (17%), and bisdemethoxycurcumin (3%).[1-3] Turmeric is a food additive, especially in India and Indian subcontinent. It is used as a coloring and flavoring agent.[3,4] Traditionally, turmeric has been used for the treatment of skin wounds, inflammations, and tumor.[4] Curcumin has been reported to exhibit various activities[5] such as antioxidant,[6] anti-inflammatory,[7,8] antimicrobial,[9,10] anticancer,[11,12] antioxidant,[13] antidiabetic, antihyperlipidemic, and hepatoprotective activities.[14-17]

However, previous studies indicated that low water solubility, rapid metabolic elimination, resulting in poor bioavailability after oral administration.[18] Various formulation techniques have been designed to improve the oral bioavailability of curcumin. For example, curcumin-loaded liposomes with an average size of 100–150 nm were prepared that showed improved activity compared to free curcumin.[19] In another study,[20] curcumin liposomes result in a reduction in tumor growth. The self-microemulsifying drug delivery system for curcumin exhibits increased dissolution and improved bioavailability.[21] Various different polymeric nanoparticles were prepared with the aim to increase the efficiency of curcumin and have versatile pharmacokinetics, and it includes curcumin encapsulated poly (lactic-co-glycolic acid) (PLGA)[22] and polyethylene glycol (PEG).[23,24] Curcumin-loaded PLGA nan-particles[25] were studied for the kinetics of tissue distribution and blood–brain barrier penetration. Colon-targeted microspheres of curcumin[26] showed prolonged drug release. Another gastroretentive floating drug delivery system was designed for curcumin[27] and tested for chronic and acute inflammation. Carrier-mediated drug delivery system where chitosan and alginate blended with cloisite 30B[28] was reported for controlled release of curcumin. A micronized form of curcumin exhibits significantly improved bioavailability.[29] Calcium alginate beads for colon-specific delivery containing self-emulsifying curcumin were developed, and cytotoxic activity was evaluated against human colon adenocarcinoma cell lines (HT-29).[30] Curcumin-PEG complex followed by encapsulation with chitosan–gelatin nanoparticles[31] was designed that exhibits improved bioavailability. Recently, curcumin was encapsulated to improve the stability and solubility in water using supercritical antisolvent technology.[32]

The entrapment of poorly water-soluble drug in the microparticulate drug delivery system has been extensively studied using various polymers and techniques.[33] Sodium alginate is a natural occurring hetero-polysaccharide from marine brown algae. It is a hydrophilic, high molecular weight, nontoxic, and biodegradable polymer composed of 1,4-linked-β-D-mannuronic acid and α-L-guluronic acid residues. Sodium alginate gels in the presence of divalent cation like calcium, and this technique is known as ionotropic gelation technique or cross-linking mechanism.[34,35]

Based on the investigations, in the present study, solubility of curcumin in PEGs was first optimized and then curcumin-PEG complex was encapsulated in alginate beads (microspheres containing curcumin triturated with PEG400 [Cur-PEGMS]). The process and formulation variables were optimized so as to obtain the highest curcumin encapsulation. The statistical design was used in this study to analyze the influence of these variables on properties of formulation. In this work, the studied variables were concentration of polymer and concentration of cross-linker. Further, the physicochemical parameters including morphology, particle size, percentage of drug entrapment, and cumulative drug release were described. The pharmacokinetic parameters of Cur-PEGMS were also investigated in rats, and samples were analyzed by high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Authentic samples of curcumin purchased from Changsha Staherb Natural Ingredients Co., Ltd., China, and internal standard (IS) emodin was obtained from Natural Remedies Pvt. Ltd. Bengaluru, India, used as such without further purification. Acetonitrile (HPLC grade) and acetic acid (Analytical grade) were purchased from Merck Specialties Pvt. Ltd. (Mumbai, India), and HPLC grade water obtained from ELGA LabWater (Model: PURELAB UHQ-II) was used in the analysis. Other chemicals such as sodium alginate, calcium chloride, and PEG400 were purchased from LOBA Chemie Pvt. Ltd. Mumbai, India.

Determination of solubility of curcumin in water

To determine the amount of curcumin solubilized in water, curcumin (100 mg) was triturated with the increasing proportion of PEG200, PEG400, and PEG600 (0.2, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ml). The physical mixture (0.5 g) was dissolved in 10 ml of water and filtered, and the absorbance of the resulting solution was read [Figure 1] at 424.5 nm on ultraviolet (UV)–visible spectrophotometer (Shimadzu UV-1800 instrument). The optimum proportion of the drug to the polymer was established from the proportion yielding the maximum amount of solubilized curcumin in water.

Preparation of curcumin–polyethylene glycol–alginate microspheres

The Cur-PEGMS were prepared by ionic cross-linking technique. Ionic gelation involves cross-linking in the presence of multivalent counterions. The stock solution was prepared by dispersing 0.5 g of curcumin and previously triturated with 2 ml of PEG400 into appropriate volumes of sodium alginate (2% w/v) in deionized water. The Cur-PEGMS were prepared by dropwise addition of sodium alginate dispersion via 20-gauge hypodermic needle fitted with a 10 ml syringe into 100 ml of 3% w/v of cross-linking agent, calcium chloride solution, with a distance of 5 cm. The process continued for 10 min with a stirring rate of 200 rpm (Remi Motors, Mumbai, India). The droplets from the dispersion instantaneously gelled. The formed microspheres were further allowed to stir in the solution of cross-linking agent for 15 min. The beads were washed with 3 × 50 ml volume of deionized water and were thereafter air-dried until a constant weight was obtained.

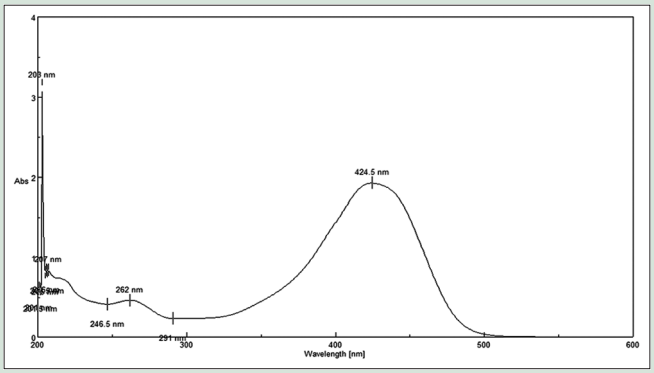

Figure 1: Ultraviolet spectrum of standard curcumin (10 μg/ml)

Drug-excipient compatibility study

Visual inspection: Drug and polymer were mixed, and color of their physical mixture was observed.

Fourier-transform infrared spectroscopy studies

The Fourier-transform infrared spectroscopy (FTIR) spectra of curcumin, sodium alginate, and prepared Cur-PEGMS were taken by 4 cm^{-1} resolution FTIR spectrophotometer (Shimadzu R prestige 21) [Figure 2] over the spectral range of 450–4000 cm^{-1}. Each sample was first mixed with fused potassium bromide in a ratio 1:100, and the prepared pallet was placed to determine FTIR spectra.

Differential scanning calorimetry of curcumin formulation

The formulation was analyzed by differential scanning calorimeter (Mettler-Toledo star 822^e system, Switzerland). The thermograms of curcumin and Cur-PEGMS were obtained [Figure 3] over a temperature range 50°C–200°C at a scanning rate of 10°C/min.

Statistical design

To evaluate the effects of variables and interrelationship among them, a 3^2 factorial design was studied. The factorial design helps to attain maximum information and reduce the number of trials. The amount of polymer, sodium alginate (1–3 g), and cross-linking agent, calcium chloride (1–5 g), was defined as independent variable that varied at low level (−1), medium level (0), and high level (1). The percentage of drug entrapment efficiency (%DEE) was selected as a dependent variable (response). The response surface regression analysis generates a mathematical equation using Design-Expert® (version 10.0.6; StatEase Inc., USA) software [Figure 4].

Percentage of drug entrapment efficiency

The loaded Cur-PEGMS (100 mg) were ground in a mortar and dispersed in methanol. The mixture was placed in a sonicator for about 3 h. Then, the solution was filtered through a 0.45-µm membrane filter. The dilutions were prepared, and drug content was then determined [Figure 5] using UV–visible spectrophotometer (Shimadzu UV-1800) at 424.5 nm. The regression equation derived from standard graph was then used to calculate %DEE with the following formula:

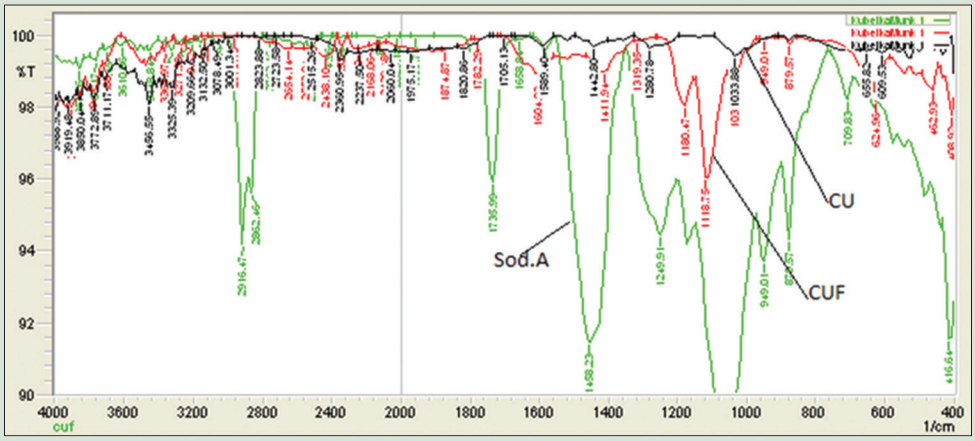

Figure 2: Fourier-transform infrared spectroscopy spectra of curcumin, sodium alginate, and curcumin formulation (microspheres containing curcumin triturated with polyethylene glycol 400)

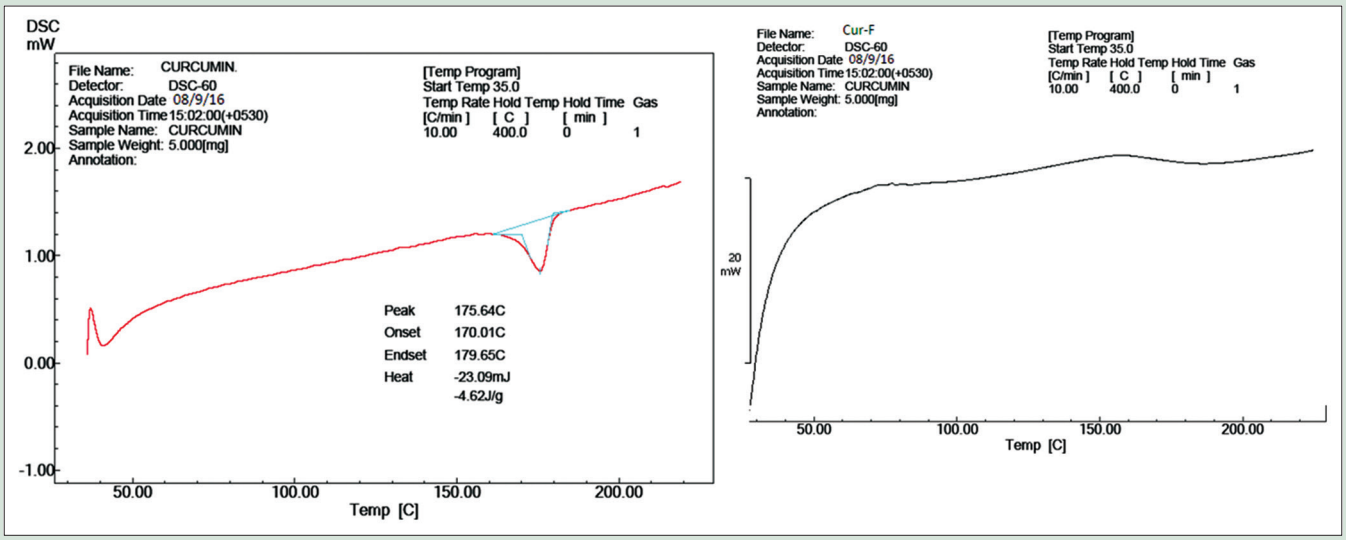

Figure 3: Differential scanning calorimetry of curcumin (standard) and prepared microspheres containing curcumin triturated with polyethylene glycol 400

Drug entrapment efficiency = (Actual drug content/Theoretical drug content) × 100

Imaging through high-resolution digital scanning microscope

The images of Cur-PEGMS were taken using a digital microimaging adaptor with SAGLO soft software (SAGLO Research Equipments, India). Randomly chosen microparticles were observed [Figure 6] for their individual shape and morphology. Microparticles were visualized under ×25 magnification.

Particle size analysis

Particle size measurement of prepared formulation, Cur-PEGMS, was carried out with an optical microscope. Stage micrometer was used to calculate the calibration factor. The particle size was calculated by measuring randomly chosen 100 particles with the help of calibrated ocular micrometer.

Scanning electron microscopy

The surface morphology of the Cur-PEGMS was examined by scanning electron microscopy (SEM) [Figure 7]. The dry microspheres were placed on carbon stub coated with gold in an ion sputter and scanned using Carl Zeiss Supra 5 model (Germany). The voltage provided was between 5 and 10 kV. SEM shows almost spherical particles with rough and nonporous surface.

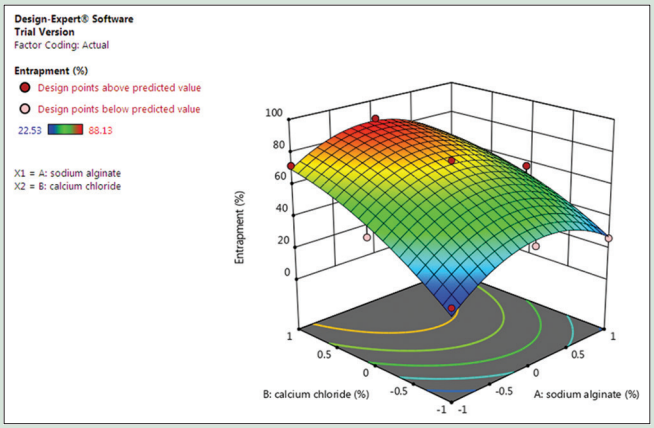

Figure 4: Contour plot showing combined effect of sodium alginate and calcium chloride on percentage of entrapment

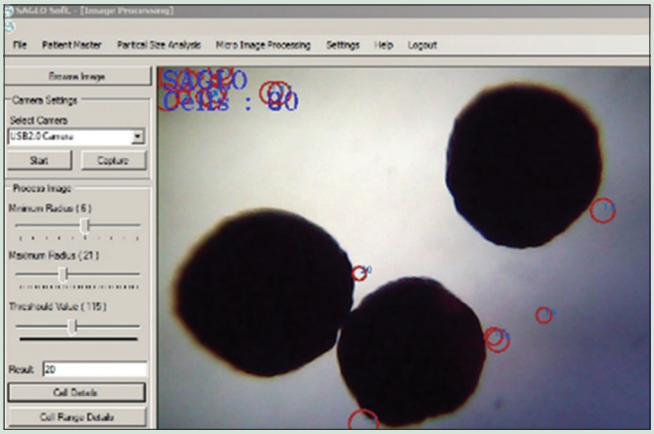

Figure 6: High-resolution digital imaging of microparticle at ×25

Curcumin estimation in formulation by high-performance thin-layer chromatography

The samples were spotted in the form of bands of a width of 6 mm with space between bands of 8.2 mm, with a 100 µL sample syringe (Hamilton Bonaduz, Switzerland) on precoated silica gel aluminum plate 60 F254 (10 cm × 10 cm) with 250-µm thickness (E. Merck, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The slit dimensions of 5 mm × 0.45 mm and the scanning speed of 20 mm/s were employed. The linear ascending development was carried out in 10 cm × 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using ethyl acetate: methanol (8.5:1.5 v/v) as mobile phase. TLC plates were dried in a current of air with the help of a hair drier. After the development of plate, densitometric scanning was performed [Figure 8a and b] on CAMAG thin-layer chromatography scanner at 425 nm for all developments operated by winCATS software version 1.4.2 (CAMAG, Switzerland).

Pharmacokinetic analysis

A pharmacokinetic study was performed in male albino Wistar rats (approximately 200 g). According to the standard ethical conditions, all the rats were provided with *ad libitum*. A free access to standard laboratory diet and water was provided throughout the experiment. The ethical clearance was obtained from the Institutional Animal Ethical Committee, and the procedures were performed according to a protocol. Two groups of rats (each group had at least six rats) were administered curcumin (5 mg/kg) and curcumin formulation suspended in 2% gum acacia (equivalent to 5 mg/kg of curcumin). Blood samples (0.5 ml) were collected in ethylenediaminetetraacetic acid-coated bottles through retro-orbital route during a dosing interval at the following times: 0 (prior to drug administration), 0.5, 1, 2, 4, 6, 10, 12, and 24 h postdose. Samples were centrifuged for 30 min at 1300 rpm to collect plasma and

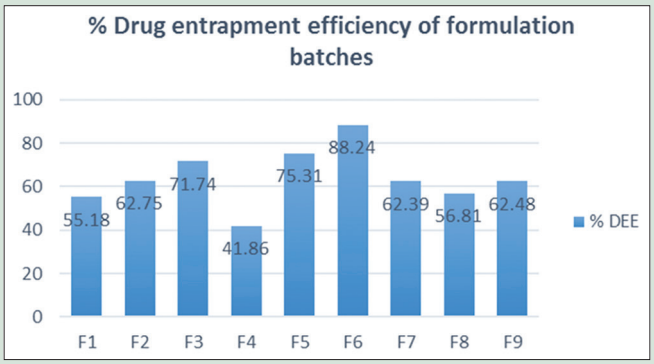

Figure 5: Percentage entrapment efficiency of different formulation batches (F1–F9)

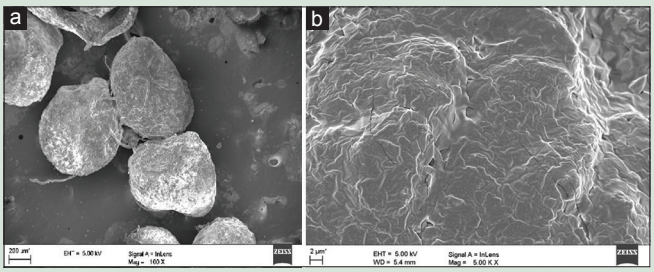

Figure 7: (a) Scanning electro micrograph of prepared microspheres (b) surface morphology of prepared microspheres

then frozen at −20°C until analysis. The stored plasma samples were analyzed for curcumin concentrations by HPLC. with UV (Jasco HPLC system consisting of Jasco PU-2080 Plus HPLC pump and PU 2075 Plus detector and Jasco Borwin PDA 1.2 version software) detection under the above-mentioned conditions. All values were expressed as mean ± standard deviation (SD).

High-performance liquid chromatography

The standard stock solution of curcumin and emodin was prepared (1000 μg/ml). The stock solution of curcumin was further diluted with acetonitrile to get series of working standard solutions having concentration 10, 20, 50, 100, 200, 300, 400, and 500 ng/ml. Emodin stock solution was further diluted to 10 ml with acetonitrile to get standard solution of concentration 2 μg/ml. A volume of 0.25 ml of each working standard solution of curcumin (0.2–2 μg/ml) was transferred into a series of Eppendorf tubes (Eppendorf-Netheler-Hinz, Hamburg, Germany) containing 0.25 ml of rat plasma, separately. In each flask, 0.25-ml stock solution of emodin (2 μg/ml) was added and 0.25 ml of acetonitrile was added prior to HPLC analysis [Figure 9a and b]. Such pretreatment is required to remove protein and potential interferences. Tubes were vortexed for 10 min on vortex mixer and then centrifuged for 30 min at 3500 rpm. The peak area ratios of curcumin to emodin were calculated, and the calibration curve was plotted of response factor against concentration of drug.

RESULTS AND DISCUSSION

Curcumin is a polyphenolic compound with number of pharmacological activities, but it exhibits poor bioavailability. One of the crucial factors is its poor solubility that limits the utilization of curcumin. The improvement in oral bioavailability of curcumin was found to be associated with improved aqueous solubility.[18,36] The past few years witnessed a continuous progress in the research of incorporation of curcumin in novel delivery systems such as liposomes, microemulsifying delivery systems, nanoparticles, solid dispersion, polymeric formulations, nanodisks, and nanotubes.[33,37] However, in the present work, an attempt was made to use PEG as a cosolvent system for improvement in the solubility of curcumin and subsequent incorporation of curcumin into alginate matrix. PEG is a nontoxic, biocompatible cosolvent. Here, PEGs with

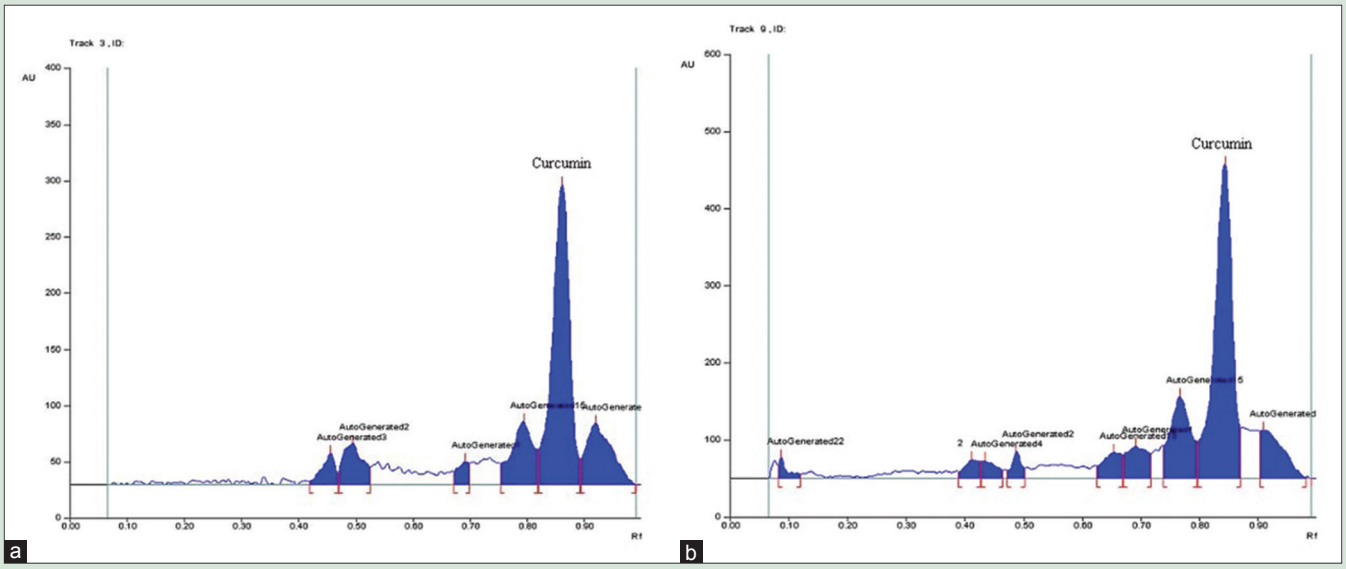

Figure 8: (a) 2D densitogram of Track 3 (standard curcumin, 400 ng/band). (b) 2D densitogram of Track 9 (curcumin in sample, microspheres containing curcumin triturated with polyethylene glycol 400)

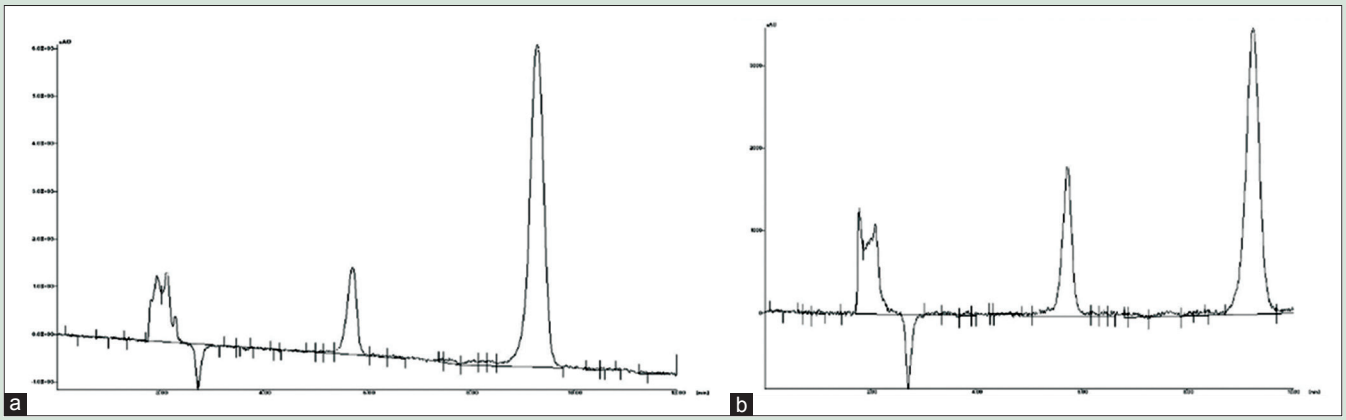

Figure 9: (a) Representative chromatograph for plasma sample at 0.5 Hr (curcumin microspheres) retention time emodin – 5.762 min and curcumin – 9.291 min. (b) Representative chromatograph for plasma sample at 0.5 Hr (curcumin suspension) retention time emodin – 5.778 min and curcumin – 9.255 min

different molecular weights have been used. A homogeneous solution of curcumin-PEG was obtained with PEG200, PEG400, and PEG600. As shown in Table 1, the curcumin concentration in solution was found to be improved with increasing percentage of PEG. The PEG400 and PEG600 have shown comparable solubilization potential. However, PEG600 cosolvent system after entrapment in the alginate matrix shows leaching, so, for further studies, PEG400 cosolvent system was optimized and subjected for preparation and evaluation of formulations.

Fourier-transform infrared spectroscopy

The chemical structure of curcumin contains the functional group OH which is indicated by its peak at 3496.16 cm^{-1} [Figure 2]. It also contains the functional group C = O at 1615.75 cm^{-1}. Peak at 1497.34 cm^{-1} indicates the presence of aromatic C = C group. Other characteristic peaks of curcumin include 1033.88 cm^{-1} and 1280.78 cm^{-1}. In the sodium alginate spectrum, 1735.99 cm^{-1} corresponds to stretching vibrations of C = O and The band around 1028 cm^{-1} corresponds to C-O-C stretching vibrations. The peak 1458.23 cm^{-1} was indicating asymmetric starching vibrations. After loaded with curcumin, the formulation resembles similar peak pattern of sodium alginate; however, characteristic peaks of curcumin were found to be masked. Overlying spectra of curcumin, polymer, and its formulation indicate no physical interaction and thus were found to be compatible. This indicates entrapment of curcumin in polymer matrix.

Differential scanning calorimetry

The differential scanning calorimetry (DSC) studies were demonstrated on pure curcumin and encapsulated curcumin beads [Figure 3]. The DSC study indicates sharp endothermic peak for pure curcumin at 175.64°C, while such sharp peak was not observed for the formulation. This may be due to the encapsulation of curcumin into polymeric matrix.

Statistical analysis

The preliminary batches were prepared to obtain distinct microsphere. The concentration of polymer and the concentration of cross-linking agent were selected as independent variables. The formulations were evaluated for percentage of drug entrapment. On the basis of this preliminary study, further 9 formulation batches (F1–F9) were prepared by applying 3^2 factorial design. It was found that increasing concentration of sodium alginate resulted in increased viscosity and that affects encapsulation process. The increased entrapment efficiency was observed in the formulations (F3, F5, and F6) with an increase in the polymer concentration [Table 2]. However, manufacturing of microspheres became difficult as the concentration of alginate increases to 3%. This may be due to the increase in the viscosity of alginate solution. At low calcium chloride concentration (1% w/v), the formulation showed deformed particles whereas, at a concentration of 3% w/v (F5), the particles gelled rapidly with 84.24% entrapment efficiency [Figure 5]. A higher concentration of calcium chloride (5% w/v) solution as a cross-linking agent adversely affects entrapment efficiency [Figure 4]. This may indicate that above a certain level of increase in concentration of cross-linking agent does not improve entrapment efficiency.[35,38]

Particle size and surface morphology by scanning electron microscopy

The particle size distribution was found in microrange 384–468 μm. The shape and surface morphology was studied by SEM. The SEM micrographs [Figure 7] indicate almost spherical surface beads with the rough surface area.[39]

High-performance thin-layer chromatography

Various compositions of mobile phase were tried; the mobile phase, ethyl acetate: methanol (8.5:1.5 v/v), gave a dense and compact spot. The identification of curcumin in Cur-PEGMS was carried out by matching the R_f values of curcuminoids in formulation with standard tracks. The Rf value for standard curcumin was 0.87, whereas the R_f value for curcumin in Cur-PEGMS was 0.85. No chemical interaction with the polymer or due to entrapment was confirmed from retention of the peak in the chromatogram [Figure 8a and b].

Pharmacokinetic analysis

Plasma samples were analyzed for curcumin concentrations by HPLC with UV detection under the above-mentioned conditions. All values were expressed as mean ± SD. To determine the pharmacokinetic parameters, the plasma concentration and time profiles were compared.

Table 1: Effect of increasing the concentration of polyethylene glycol 400 on solubility of curcumin in water

Curcumin: PEG200 (mg: ml)	Concentration of curcumin in water (mg/ml)	Cur: PEG400 mg: ml	Concentration of curcumin in water (mg/ml)	Cur: PEG600 (mg: ml)	Concentration of curcumin in water (mg/ml)
100:0.0	0.89	100:0.0	0.93	100:0.0	0.91
100:0.2	2.42	100:0.2	5.93	100:0.2	6.54
100:0.5	10.95	100:0.5	10.56	100:0.5	15.31
100:1.0	18.31	100:1.0	32.99	100:1.0	35.31
100:1.5	38.30	100:1.5	54.31	100:1.5	61.31
100:2.0	44.34	100:2.0	75.14	100:2.0	81.34
100:2.5	51.32	100:2.5	81.01	100:2.5	86.30

PEG: Polyethylene glycol

Table 2: Statistical design

Formulation batches	Level	Amount of sodium alginate (g) X1	amount of CaCl2 (g) X2	Percentage of EE±SD
F1	−1	1	1	55.18±0.16
F2	0	1	3	62.75±0.88
F3	1	1	5	71.74±0.34
F4	−1	2	1	41.86±0.07
F5	0	2	3	88.24±0.09
F6	1	2	5	75.31±0.08
F7	−1	3	1	62.39±0.18
F8	0	3	3	56.81±0.07
F9	1	3	5	62.48±0.24

SD: Standard deviation; EE: Entrapment efficiency

The chromatograms [Figure 9a] showed that retention times for curcumin (in microspheres) and emodin (as IS) were 9.291 and 5.672 min, respectively, whereas retention times for curcumin in suspension and for IS were 9.255 and 5.778 min, respectively [Figure 9b]. No obvious interference peaks were located at the retention times of analyses in the blank chromatograms. Thus, HiQ Sil C18 (250 mm × 4.6 mm internal diameter, 5 m) column with the mobile phase of acetonitrile: 5% acetic acid (70:30 v/v) provided optimum separation for curcumin with these analytical parameters. There is an improvement in maximum plasma concentration in curcumin microspheres compared to curcumin suspension. Maximum the time after administration of a drug when maximum plasma concentration is reached (T_{max}) was found to be 0.5 h [Figure 10]. AUC_{0-24} was found to be 374.75 ng/ml [Table 3] and 622 ng/ml [Table 4] in curcumin suspension and curcumin microspheres, respectively, indicating marked improvement in bioavailability of the formulation of 165.87%.

It can be concluded that better bioavailability of formulated curcumin may be attributed to better solubility and low first-pass metabolism. Similar conclusions were noted in the literature.[21-23,40]

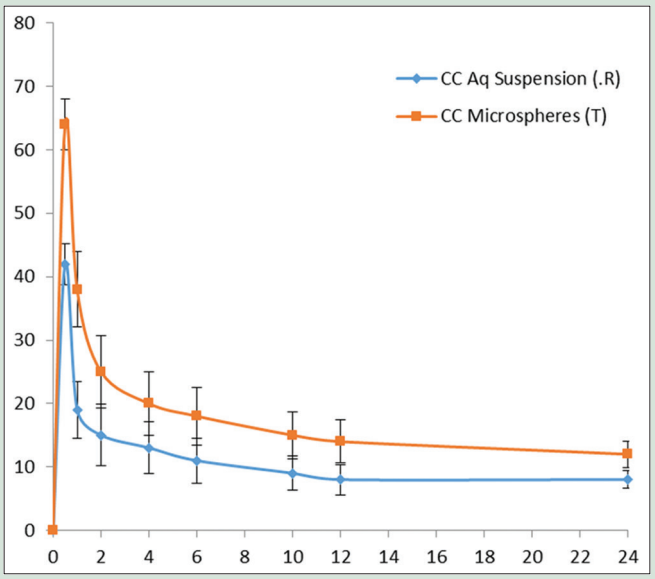

Figure 10: Pharmacokinetic profile (concentration vs. time) of curcumin and curcumin microspheres

Table 3: Area under the concentration calculations for curcumin suspension

Time (h)	Plasma concentration (ng/mL)	AUC	AUC_{0-24}
0	0	0	
0.5	42	10.5	10.500
1	19	25.75	36.250
2	15	26.5	62.750
4	13	43	105.750
6	11	37	142.750
10	9	62	204.750
12	8	26	230.750
24	8	144	374.750

AUC: Area under curve

Table 4: Area under the concentration calculations for curcumin in microspheres containing curcumin triturated with polyethylene glycol 400

Time (h)	Plasma concentration (ng/mL)	AUC	AUC_{0-24}
0	0	0	
0.5	64	16	16.000
1	38	41.5	57.500
2	25	50.5	108.000
4	20	70	178.000
6	18	58	236.000
10	15	102	338.000
12	14	44	382.000
24	12	240	622.000

AUC: Area under curve

CONCLUSION

In this study, curcumin microspheres were prepared using ionic gelation technique. Our studies showed that the formulation offers better bioavailability and such formulation provide practical solution to overcome limitations and can be extrapolated for further studies.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. World Health Organization. WHO Monograph of Selected Medicinal Plants. Vol. 1. Geneva: World Health Organization; 1999. p. 115-24.
2. Duke JA, Bogenschutz-Godwin M, du Cellier J, Duke P. Handbook of Medicinal Herbs. 2nd ed. Boca Raton, FL: CRC Press; 2002. p. 740-4.
3. The Wealth of India. A Dictionary of Indian Raw Materials and Industrial Products. 2nd Supplement Series, Vol. 1. A-F. New Delhi: National Institute of Science Communication and Information Resources, Council of Scientific and Industrial Research; 2006. p. 258-9.
4. Nadkarni KM, editor. Dr. K. M. Nadkarni's, Indian Materia Medica. Vol. 2. Bombay: Popular Prakashan; 1908. p. 414-6.
5. Ammon HP, Wahl MA. Pharmacology of *Curcuma longa*. Planta Med 1991;57:1-7.
6. Kunchandy E, Rao MN. Oxygen radical scavenging activity of curcumin. Int J Pharmacogn 1990;58:237-40.
7. Aggarwal BB, Harikumar KB. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. Int J Biochem Cell Biol 2009;41:40-59.
8. Jurenka JS. Anti-inflammatory properties of curcumin, a major constituent of *Curcuma longa*: A review of preclinical and clinical research. Altern Med Rev 2009;14:141-53.
9. Gupta A, Mahajan S, Sharma R. Evaluation of antimicrobial activity of *Curcuma longa* rhizome extract against *Staphylococcus aureus*. Biotechnol Rep (Amst) 2015;6:51-5.
10. Gul P, Bakht J. Antimicrobial activity of turmeric extract and its potential use in food industry. J Food Sci Technol 2015;52:2272-9.
11. Kuttan R, Bhanumathy P, Nirmala K, George MC. Potential anticancer activity of turmeric (*Curcuma longa*). Cancer Lett 1985;29:197-202.
12. Mukerjee A, Vishwanatha JK. Formulation, characterization and evaluation of curcumin-loaded PLGA nanospheres for cancer therapy. Anticancer Res 2009;29:3867-75.
13. Ak T, Gülçin I. Antioxidant and radical scavenging properties of curcumin. Chem Biol Interact 2008;174:27-37.
14. Rezq AM. Curcumin derivatives in experimental diabetes. Endocrinol Metab Synd 2014;3:120.
15. Choudhary N, Sekhon BS. Potential therapeutic effect of curcumin – An update. J Pharm Educ Res 2012;3:64-71.
16. Shrishail D, Handral H, Handral R, Tulsianand G, Shruthi SD. Turmeric: Nature's precious medicine. Asian J Pharm Clin Res 2013;6:10-6.
17. Ali Hussain HE. Hypoglycemic, hypolipidemic and antioxidant properties of combination of Curcumin from *Curcuma longa*, linn, and partially purified product from *Abroma augusta*, linn. in streptozotocin induced diabetes. Indian J Clin Biochem 2002;17:33-43.

18. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: Problems and promises. Mol Pharm 2007;4:807-18.
19. Vishwanath G, Jithan A, Reddy VM. Development of new drug delivery strategies to increase bioavailability of curcumin. Int J Pharm Sci Nanotechnol 2009;1:335-40.
20. Malam Y, Loizidou M, Seifalian AM. Liposomes and nanoparticles: Nanosized vehicles for drug delivery in cancer. Trends Pharmacol Sci 2009;30:592-9.
21. Cui J, Yu B, Zhao Y, Zhu W, Li H, Lou H, *et al.* Enhancement of oral absorption of curcumin by self-microemulsifying drug delivery systems. Int J Pharm 2009;371:148-55.
22. Yongmei H, Qingshan L, Hong W, Xing G, Jiang Q, Lv W. Use of novel polyurethane microspheres in a curcumin delivery system. Spectrosc 2014;2014:1-7. [http://dx.doi.org/10.1155/2014/926268].
23. Zhongfa L, Chiu M, Wang J, Chen W, Yen W, Fan-Havard P, *et al.* Enhancement of curcumin oral absorption and pharmacokinetics of curcuminoids and curcumin metabolites in mice. Cancer Chemother Pharmacol 2012;69:679-89.
24. Modasiya MK, Patel VM. Studies on solubility of curcumin. Int J Pharm Life Sci 2012;3:1490-7.
25. Tsai YM, Chien CF, Lin LC, Tsai TH. Curcumin and its nano-formulation: The kinetics of tissue distribution and blood-brain barrier penetration. Int J Pharm 2011;416:331-8.
26. Vajpayee A, Suresh F, Singh A, Jha S. Formulation and evaluation of colon targeted curcumin microspheres using natural polymers. J Pharm Res Opinion 2011;1:108-12.
27. Kumar K, Rai AK. Development and evaluation of floating microspheres of curcumin. Trop J Pharm Res 2012;11:713-9.
28. Malesu VK, Sahoo D, Nayak PL. Chitosan–sodium alginate nanocomposites blended with cloisite 30b as a novel drug delivery system for anticancer drug curcumin. Int J Appl Biol Pharm Technol 2011;2:402-11.
29. Schiborr C, Kocher A, Behnam D, Jandasek J, Toelstede S, Frank J. The oral bioavailability of curcumin from micronized powder and liquid micelles is significantly increased in healthy humans and differs between sexes. Mol Nutr Food Res 2014;58:516-27.
30. Sookkasem A, Chatpun S, Yuenyongsawad S, Wiwattanapatapee R. Alginate beads for colon specifc delivery of self-emulsifying curcumin. J Drug Deliv Sci Technol 2015;29:159-66. [doi: 10.1016/j.jddst. 2015.07.005].
31. Farnia P, Mollaei S, Bahrami A, Ghassempour A, Velayati A, Ghanavi J. Improvement of curcumin solubility by polyethylene glycol/chitosan-gelatin nanoparticles (CUR-PEG/CS-G-nps). Biomed Res (Aligarh) 2016;27:659-65.
32. Arango-Ruiz Á, Martin Á, Cosero MJ, Jiménez C, Londoño J. Encapsulation of curcumin using supercritical antisolvent (SAS) technology to improve its stability and solubility in water. Food Chem 2018;258:156-63.
33. Dutta AK, Ikiki E. Novel drug delivery systems to improve bioavailability of curcumin. J Bioequiv Availab 2013;6:1-9.
34. Del Gaudio P, Colombo P, Colombo G, Russo P, Sonvico F. Mechanisms of formation and disintegration of alginate beads obtained by prilling. Int J Pharm 2005;302:1-9.
35. Voo WP, Ooi CW, Islam A, Tey BT, Chan ES. Calcium alginate hydrogel beads with high stiffness and extended dissolution behavior. Eur Polym J 2016;75:343-53.
36. Cretu R, Dima C, Bahrim G, Dima S. Improved solubilization of curcumin with a microemulsification formulation. AUDJG Food Technol 2011;35:46-55.
37. Wang S, Tan M, Zhong Z, Chen M, Wang Y. Nanotechnologies for curcumin: An ancient puzzler meets modern solutions. J Nanomaterials 2011;2011:1-8. [doi: 10.1155/2011/723178].
38. Nagpal M, Maheshwari D, Rakha P, Dureja H, Goyal S, Dhingra G. Formulation development and evaluation of alginate microspheres of ibuprofen. J Young Pharm 2012;4:13-6.
39. Nayak AK, Malakar J, Pal D, Hasnain M, Beg S. Soluble starch-blended Ca2þ-Zn2þ-alginate composites-based microparticles of aceclofenac: Formulation development and *in vitro* characterization. Future J Pharm Sci 2018;4:63-70.
40. Jamwal R. Bioavailable curcumin formulations: A review of pharmacokinetic studies in healthy volunteers. J Integr Med 2018;16:367-74.