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ORIGINAL ARTICLE

Colon targeted curcumin microspheres laden with ascorbic acid for bioavailability enhancement

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ABSTRACT

An objective of the present study was to prepare colon-specific microspheres of curcumin (CUR) containing ascorbic acid (AA) for improved oral bioavailability. 3^2 factorial design was used to optimise chitosan microspheres (CSMS) containing CUR and AA. Subsequently, optimised CSMS were coated with Eudragit S-100, for delivery to colon. *In vitro* drug release, *in vivo* pharmacokinetics, and organ distribution studies were performed in Albino Wistar rats. Stabilisation of CUR in alkaline pH was successfully guarded by AA to the extent 98.5–100%. Results revealed complete amorphisation/molecular dispersion of CUR. Bioavailability enhancement of CUR and 90% of MS in colon at the end of 8 h in animals, deciphered successful design of colon-specific CUR MS. It can be concluded that AA in MS shielded the degradation of CUR. The developed double coat MS could be considered as a promising colon-targeted system for CUR aiming bioavailability enhancements.

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KEYWORDS
Ascorbic acid–drug interactions; bioavailability; curcumin; colon-specific; *in-vivo*

Introduction

Curcumin (diferuloylmethane; CUR) is the active constituent of turmeric (*Curcuma longa*). Traditional Indian and Chinese medicines have used turmeric for the treatment of a variety of diseases (Gupta *et al*. 2013). Besides, turmeric has been consumed as a food spice in Southeast Asian countries and said to be responsible for the low occurrence of colorectal cancer in these countries.

CUR, the active constituent of turmeric (*Curcuma longa*), is reported to possess antioxidant (Ak and Gulcin 2008), antiseptic, anti-inflammatory (Zhang *et al*. 1999), anti-cancer (Sharma *et al*. 2005), chemopreventive, and chemotherapeutic (Ayodele *et al*. 2017) activities. CUR inhibits carcinogenesis in a number of cancer types by its effect on different biological pathways that are involved in apoptosis, cell cycle regulation, proliferation, oncogene expression, and metastasis (Goel *et al*. 2008).

Although CUR has a well-established efficacy and safety profile, its medical usefulness has limitations because of poor oral bioavailability and stability (Goel *et al*. 2008). Reduced bioavailability of CUR can be attributed to its low aqueous solubility, degradation in gastrointestinal tract (GIT) at alkaline pH, high pre-systemic metabolism in the intestinal wall, and quick systemic elimination (Yang *et al*. 2007). In order to enhance its bio-availability, numerous approaches have been adopted inclusive of CUR in combination with additive like piperine (Shoba *et al*. 1998), liposome encapsulation, nanoparticle (Takahashi *et al*. 2009), phospholipid complexation (Maiti *et al*. 2007), and solid dispersions with polyvinyl pyrrolidone (PVP) (Xu *et al*. 2006). Practical utility of these methods have limitations because of effect of piperine on metabolism of co-administered drugs, complicated process of complexation and encapsulation, and hygroscopic nature of PVP. Moreover, scarce literature is available on stability of CUR in pharmaceutical formulations.

Interestingly, one of the findings has demonstrated that degradation of CUR in culture media or phosphate buffer above pH 7 can be completely blocked, in the presence of foetal calf serum, human blood, or addition of antioxidants such as ascorbic acid (AA) (Wang *et al*. 1997). It has been investigated that, during free radical reactions, the most easily abstractable hydrogen from CUR is phenol-OH group. Phenolic hydroxyl group results in the formation of phenoxyl radicals during free radical reaction. According to

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Jovanovic et al., peroxyl radicals, when reacted with CUR, result in CUR phenoxyl radicals. Regeneration reaction of phenoxyl radicals back to CUR is carried out by antioxidants like AA. Thus, the stability of CUR can be improved by lowering the pH or by adding antioxidants that can block its degradation in alkaline pH (Villegas *et al.* 2008). Moreover, a large intake of vitamin with antioxidant activity, such as AA, has been shown to decrease the risk of cancer by preventing DNA damage (Hunter *et al.* 1993). AA induces anti-proliferative, cytotoxic, and genotoxic effects on different colon cancer cell lines. (Pires *et al.* 2016). Also, it has been shown to stabilise p53, a protein involved in cell proliferation control (Reddy *et al.* 2001).

Mechanistic understanding of aforesaid has lead us to design of a colon specific, chitosan (CS) based, pH-sensitive, mucoadhesive, cross-linked polymeric microspheres (MS) of CUR. AA has been added to chitosan microspheres (CSMS) to prevent degradation of CUR, and will improve its stability and consequently bioavailability. For core MS preparation, CS a polysaccharide has been used because of its approved biological properties, like mucoadhesion, biocompatibility, non-toxicity, and degradation by colonic enzymes. Further, CSMS were coated with enteric polymers. The developed formulations were characterised by encapsulation efficiency, FTIR, DSC, *in vitro* CUR release, organ distribution studies, and bioavailability in rats.

Materials and methods

Materials

Chitosan and CUR are the one those were gifted from Central Institute of Fisheries Technology, Kochi, India, and Sami laboratories Bangalore, India, respectively. Eudragit-S100 was kindly provided by Rohm Pharma (Darmstadt, Germany). L-AA (98%) was obtained from SD Fine Chemicals, Mumbai, India. Other chemicals and solvents were of analytical grade.

Methods

Stability studies of CUR in presence of AA

Degradation of CUR was measured at different pH values of phosphate buffer (pH 6.8 & 7.4). A stock solution of CUR (100 μg/mL) was prepared in methanol. Then 1 ml of this solution was added to the 24 ml of respective buffer solutions to obtain the CUR concentration of 4 μg/mL solution and stored in amber colour volumetric flask (Yeh 2000). The absorbance values were measured by using UV Visible Spectrophotometer (JASCO, V-550 Japan) against the pure medium as blank. The percentage amount of CUR remaining at different time interval 0.5, 2, 4, 6, and 8 was estimated.

Separately, AA was added to prevent the degradation of CUR at three different concentration level (based on molar mass of CUR and AA) to the CUR solution at mentioned pH. Molar ratio of CUR to AA was maintained to 1:0.5, 1:1, and 1:1.5. The absorbance of the resultant solution was measured using UV Visible Spectrophotometer (JASCO, V-550 Japan) at different intervals (Table 1).

Preparation of MS

Step I – preparation of CSMS

For optimisation of CSMS, a 3^2 full factorial design was employed using CS concentration (X_1) and volume of sodium tripolyphosphate (TPP) (X_2) as independent variables, and entrapment efficiency, *in vitro* CUR released up to 8 h (t_8) as dependent variables as shown in Table 2. The responses were analysed using analysis of variance (ANOVA). RSM plots for each response were generated using the DESIGN EXPERT (STAT-EASE) demo version software 9.0.3.

CSMS were prepared by a spray-drying technique. Briefly, CS solution was prepared in distilled water that contains 4.0% v/v glacial acetic acid and 11.95 mg of AA (so that molar ratio of CUR to AA was maintained to 1:1). Then 25 mg CUR was dissolved in ethanol

Table 1. Stability of CUR in the absence and presence of AA at pH 6.8 and 7.4.

		% CUR remained			
		Without AA		CUR:AA (1:1)	
Sr. No	Time (hr)	Phosphate buffer (pH 6.8)	Phosphate buffer (pH 7.4)	Phosphate buffer (pH 6.8)	Phosphate buffer (pH 7.4)
1	0	100.12 ± 0.04	100.02 ± 0.04	100.17 ± 0.3	100.05 ± 0.13
2	0.5	96.58 ± 0.54	14.64 ± 1.15	100.15 ± 0.3	100.02 ± 0.4
3	2	92.59 ± 1.24	0.42 ± 0.06	99.84 ± 0.03	99.97 ± 0.01
4	4	89.08 ± 0.91	ND	99.25 ± 0.12	99.29 ± 0.15
5	6	88.24 ± 0.91	ND	99.02 ± 0.26	99.04 ± 0.35
6	8	85.43 ± 1.06	ND	98.98 ± 0.25	98.80 ± 0.53

All data are expressed as mean ± SD; $n = 3$. ND: not detected.

Table 2. A 3^2 full factorial experimental design layout and response variables for CSMS.

	Independent variables		Dependent variables*	
Batch code	CS (% w/v)	TPP (mL)	Y_1:% Entrapment efficiency	Y_2: % Drug release pH 6.8 After 8 hrs
A	0.5	1	37.08 ± 1.45	88.27 ± 1.63
B	0.5	2	30.89 ± 0.41	84.18 ± 1.13
C	0.5	3	25.16 ± 1.45	77.27 ± 1.31
D	1.0	1	57.05 ± 1.11	86.88 ± 1.74
E	1.0	2	47.15 ± 2.36	78.34 ± 0.65
F	1.0	3	40.64 ± 0.54	67.32 ± 1.61
G	1.5	1	59.23 ± 0.58	72.13 ± 1.20
H	1.5	2	52.65 ± 1.05	71.33 ± 2.11
I	1.5	3	40.08 ± 2.40	62.20 ± 1.23

*Values are in average of three determinants, $n = 3$.

and added to the CS solution; various volumes of a 1% w/v aqueous solution of TPP were added to CS solution, slowly under constant stirring at 1500 rpm for 10 min. This solution was spray-dried using LU-222 ADVANCED lab spray drier (Labultima, Mumbai, India) for making CSMS under standard conditions.

Step II – preparation of Eudragit-coated CSMS

Optimised CSMS were dispersed in Eudragit S100 solution made in organic phase (6 ml of acetone: ethanol taken in ratio 2:1). Further, this mixture was slowly added into 80 ml light liquid paraffin containing 1% w/v Span 80, with stirring at 1000 rpm (Remi Labs, Mumbai, India). Mixture was stirred for 3 h to evaporate organic solvent (oil-in-oil solvent evaporation method. Finally, the coated CSMS were collected, rinsed with n-hexane, and freeze-dried at −80 °C using Mini-Lyodel freeze dryer (Chennai, India).

Similar CUR formulation excluding AA (formulation G) was prepared and evaluated to support the stability of CUR in the presence of AA.

Characterisation of microspheres

Fourier transform infra-red spectroscopy (FTIR)

Fourier transform infra-red spectroscopy (FT-IR) analysis was performed for CUR, AA, CS, and CSMS (batch D) on FTIR spectrophotometer (JASCO FT/IR-410 Japan) using KBr pellet method. The scanning range was 400–4000 cm^{-1}, and the resolution was 4 cm^{-1}.

X-ray powder diffraction (XRPD) and differential scanning calorimetry (DSC)

The powder X-ray diffraction study was performed to verify polymorphic forms of CUR and CSMS using differential scanning calorimeter (Bruker D8 AXS Advance, Germany). The studies were performed by exposing samples to CuK2 α rays with a voltage of 40 kV and 30 mA of current on a flat plate with θ/2θ geometry, where 2θ ranged from 5 to 50°, with a step width of 0.020. To study thermal changes in MS, differential scanning calorimetry (Mettler Toledo 823e, Switzerland) was used. The DSC measurements were performed under nitrogen flow (40 ml/min) at scanning rate of 10 °C/min from 30 °C to 300 °C (Madgulkar *et al.* 2009).

Entrapment efficiency (EE)

CSMS were triturated followed by dispersion in 10 ml methanol and kept overnight for drug extraction. The MS were then centrifuged at 1000 rpm for 10 min to remove any insoluble solids; the supernatant layer decanted. Absorbance was measured by using UV-Visible Spectrophotometer (JASCO, V-550 Japan) at 418 nm. Entrapment efficiency was calculated using the following formula (Pavani *et al.* 2013):

$$EE = \frac{\text{Mass of drug in MS}}{\text{Initial mass of drug}} \times 100 \quad (1)$$

Scanning electron microscopy (SEM)

Surface characteristics of the CSMS and coated MS were examined using scanning electron microscope (SEM). Dried MS were analysed at 20 kV after coating with platinum using sputter coater. Samples were analysed with the help of SEM (JEOL JSM-6303, Japan) (Chowdary KPR and Srinivasa Rao 2003).

In vitro *drug release and kinetic studies*

In vitro release studies of prepared MS were carried in USP-II dissolution apparatus (USP Dissolution apparatus Type II, LABINDIA) using 900 ml of dissolution medium (pH 6.8) at a paddle rotation of 100 rpm and 37 °C ± 0.5 °C. Samples were withdrawn using a pipette fitted with a microfilter at regular intervals and analysed using UV spectrophotometer (JASCO, V-550 Japan).

In vitro drug release studies of Eudragit-coated CSMS (25 mg) were performed, using USP-II dissolution apparatus at 37 °C ± 0.5 °C rotated at constant speed

of 100 rpm. The simulation of gastrointestinal transit condition was achieved by changing the pH of dissolution medium. The pH of dissolution medium for first 2 h was maintained at 1.2 with 0.1 N HCl. After 2 h, 1.7 g KH_2PO_4 and 2.2 g Na_2HPO_4 were added to the dissolution medium, and pH was adjusted to 4.5 using 0.1 N NaOH and the release study was performed for next 2 h. Finally, the pH was adjusted to 6.8 with 0.1 N NaOH and maintained up to 24 h (Paharia *et al.* 2007). With the help of pipette fitted with microfilter, samples were withdrawn at suitable time interval from the dissolution medium and analysed by UV spectrophotometer (JASCO, V-550 Japan) at 420 nm.

Release kinetics

To understand the mechanism of CUR release from MS, data of the *in-vitro* dissolution study were analysed using different kinetic model like zero-order, first-order, Higuchi models, and Korsmeyer model (Mathew *et al.* 2007). The rate constants for the respective models were also calculated. For selecting the most appropriate model criterion was based on R^2 values. *In vitro* release results were further fitted into the following Korsmeyer and Peppas equation to explore the kinetic behaviour:

$$\frac{M_t}{M_\infty} = Kt^n \quad (2)$$

Linear regression analyses were made for zero-order ($M_t/M_0 = K_0 \times t$), first order ($\ln (M_0 - M_t) = K_1 \times t$), Higuchi ($M_t/M_0 = K_H \times t^{1/2}$), and Korsmeyer and Peppas ($M_t/M_0 = K_{KP} \times t_n$) models, where K is the kinetic constant and M_t/M_0 is the fraction of drug released at time t.

In vivo *study*

Pharmacokinetic study

All experiments were conducted according to the protocol approved by the Institutional Animal Ethics Committee (Approved protocol no- IAEC/ABCP/05/2014–15). Albino rats (Wistar strain; 4 groups with 6 animals; average weight: 200–250 g) were housed in polypropylene cages under standard laboratory conditions. The animals were divided into the following four groups CUR (dose, 100 mg/kg) and were administered by oral gavage:

Group I –control group
Group II –suspension of CUR in water
Group III –Eudragit-coated MS without AA (G)
Group IV –Optimised Eudragit-coated MS with AA (G_2)

At pre-determined time intervals, blood samples were withdrawn by retro-orbital plexus technique into centrifugation tubes. Plasma was separated by centrifugation at 2000 rpm for 10 min and stored at −20 °C until analysis. Ethyl acetate was added to extract CUR from plasma and all tubes were centrifuged at 2000 rpm for 10 min. Organic layer was separated and evaporated to dryness, dissolved in 1.0 ml of methanol and processed to determine content of CUR by HPLC according to validated procedure (Suwannateep *et al.* 2011). Various pharmacokinetic parameters were calculated using data obtained through HPLC analysis.

Organ distribution study

Similar animal protocol mentioned under pharmacokinetic study was used organ distribution studies. After 2, 4, 6, and 8 h, animals were sacrificed. Stomach, small intestine, and colon were isolated. These isolated organs were homogenised along with a small amount of phosphate buffer saline (pH 6.8) by Micro Tissue Homogenizer (Mac, Mumbai, India). One microliter of ethyl acetate was added to homogenate. The contents were centrifuged at 2000 rpm for 10 min. Separated organic phase was dried, and reconstituted with mobile phase (Rahman *et al.* 2008). The CUR content in stomach, small intestine, and colon was determined by HPLC method.

Statistical analysis

All the means were represented as the mean ± SD. Statistical analysis was performed employing ANOVA (Graph-pad Prism 7) software to compare different groups. The level of significance was set at $p < 0.05$.

Results

Stability studies of CUR in presence of AA

When CUR was alone in alkaline buffer, it rapidly undergoes degradation. Its stability was dramatically improved in presence of AA. *In vitro* stability study has shown that 98.80 ± 0.22% and 98.98 ± 0.25% CUR remains stable when molar ratio of CUR to AA was maintained to 1:1 after 8 h at pH 7.4 and pH 6.8. The degradation of CUR was almost completely inhibited at this concentration (Table 1), and no significant difference was obtained at ratio 1:1.5 (data not shown).

Characterisation of microspheres

Fourier-transform infra-red spectroscopy

The FTIR of CS, AA, pure CUR, and optimised formulation D are presented in Figure 1(a). FTIR of CS shows absorption band at 3429 cm^{-1} (O-H stretching overlapping the N-H stretching), 2923.56 (C-H stretching), 2848 (C-H stretching), and 1653.66 (amides I). In CUR, a peak at 3508 cm^{-1} indicates the presence of OH. The strong peak at 1627.63 cm^{-1} has a mixed (C = C) and (C = O) character. Another band at 1602.56 cm^{-1} indicates aromatic ring stretching vibrations (C = C ring). In MS, the absorption band at 3429 cm^{-1}, and in CS was broadened by the physical interactions with TPP. Also in MS, 1653 cm^{-1} and 1580 cm^{-1} peaks of NH_2 bending vibration shifted to 1636.3 cm^{-1} and 1559.17 cm^{-1}.

XRPD and DSC analysis

X-ray diffraction patterns of CUR and various CSMS were studied. The characteristic peaks of pure CUR disappeared in CSMS (Figure 1(b)); it suggests that crystalline CUR get converted to amorphous state. Pure CUR used in DSC study showed a sharp melting endothermic peak at 185.80 °C indicates crystalline state. Sharp peak of CUR was absent in the DSC curve of MS. It may be due to reduced drug crystallinity (Figure 1(c)). It suggests amorphous phase of CUR in CSMS or solid solution state in polymeric matrix.

Entrapment efficiency

EE of CSMS varies from 25.16 to 59.23%. By increasing the CS concentration, the entrapment efficiency was increased (Table 2). Polynomial equation proposed by the model for percentage drug entrapment is as follows:

$$EE = +\ 48.51 + 9.81X_1 - 7.91X_2 - 1.80X_1X_2 - 7.43X_1^2 - 0.35X_2^2 \quad (3)$$

In this case, X_1, X_2, and X_1^2 are significant model terms. By considering all design factors at a time, the quality of the entire model is predicted by F-value. As model F-value is larger (81.46), it indicates that model is significant. Percentage drug entrapment and yield of formulation G_2 was found to be 91.20 ± 0.88% and 85.61 ± 2.66%, respectively (Table 3).

Scanning electron microscopy

The MS prepared by spray drying (Formulation D) as well as solvent evaporation method (Formulation G_2) have proved a good sphericity with smooth surface

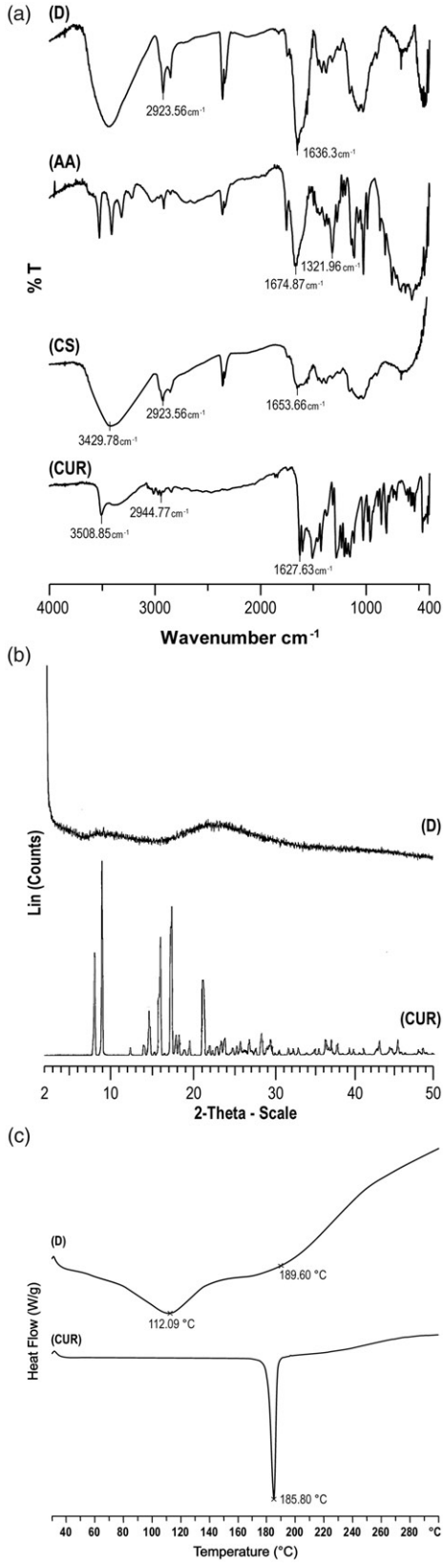

Figure 1. (a) FTIR spectra of CUR: plain curcumin, CS: chitosan, AA: ascorbic acid and D: formulation D, (b) X-ray diffractogram of CUR: pure curcumin and D: formulation D, (c) DSC thermogram of CUR: pure curcumin and D: formulation D.

Table 3 Effect of core–coat ratio on yield, entrapment efficiency, and release of CUR from Eudragit coated CSMS.

Coated CSMS	Core:coat ratio	% Entrapment efficiency	%Yield	% Drug release after 24 hrs
G_1	1:1	81.7 ± 2.51	86.13 ± 1.80	91.52 ± 0.31
G_2	1:2	91.2 ± 0.88	85.61 ± 2.66	88.74 ± 0.82
G_3	1:3	92.1 ± 2.58	70.07 ± 3.00	85.9 ± 0.55

*Values are in average of three determinants, $n = 3$.

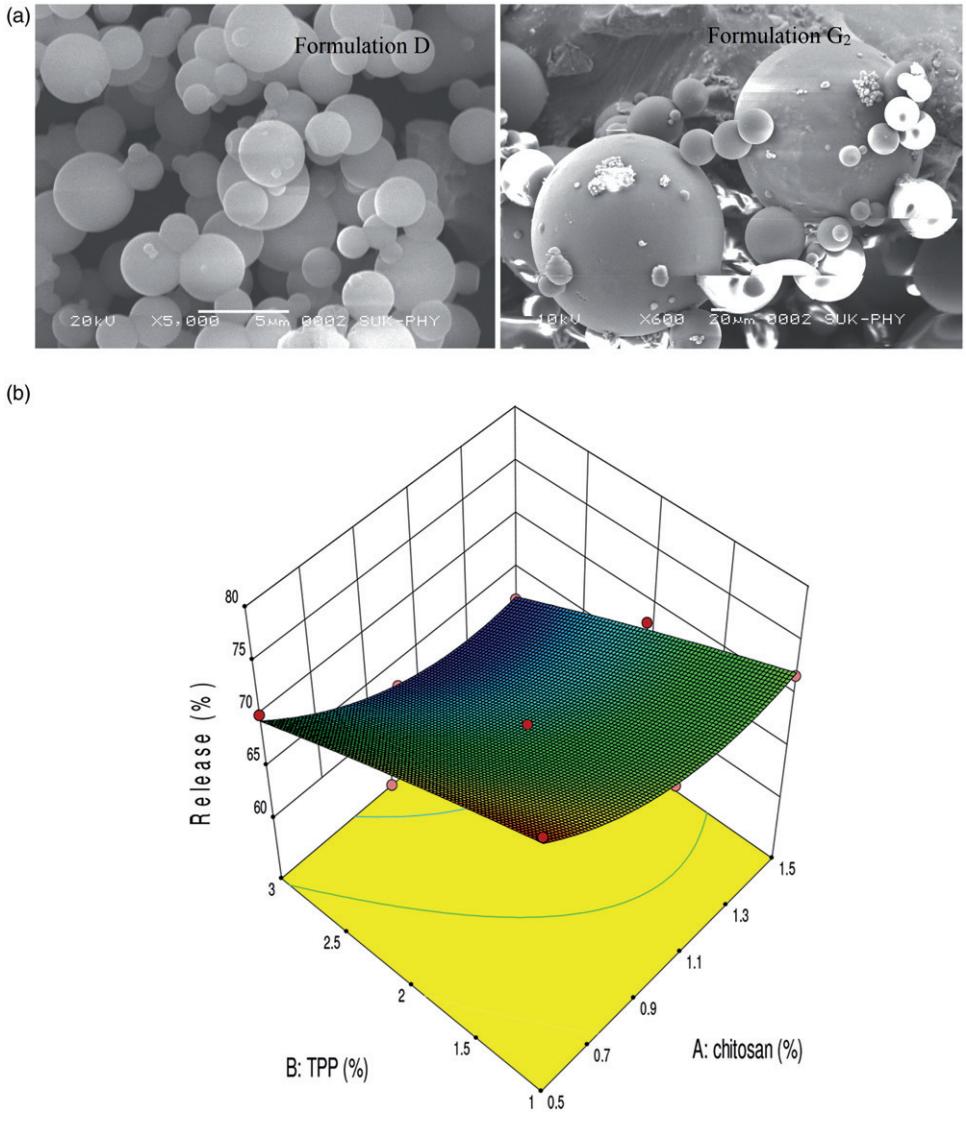

Figure 2. (a) Scanning electron microphotographs of core MS (formulation D) and Eudragit-coated MS (formulation G_2) and (b) Response surface graph showing effect of variables on *in vitro* drug release from core MS.

(Figure 2(a)), and the particles were distributed uniformly without forming any clumps.

In vitro *drug release studies*

Figure 2(b) represents response surface that explains the effect of two formulation factors on % drug release at pH 6.8 at 8th hour. Release profile of CUR from CSMS followed a biphasic kinetic mechanism. Initially release rate was fast which may be due to some CUR particles that have migrated at the surface during the drying of MS. This was followed by slow release of CUR which was controlled by swelling of CS and diffusion of CUR from the interior of the MS. Factorial equation for *in vitro* release at 8 h is:

$$\text{Release} = +67.94 - 3.45X_1 - 4.74X_2 - 0.30X_1X_2 + 2.97X_1^2 - 0.25X_2^2 \quad (4)$$

The F-value predicts the quality of the entire model considering all design factors at a time. The Model F-value is larger (61.13) indicates the model is

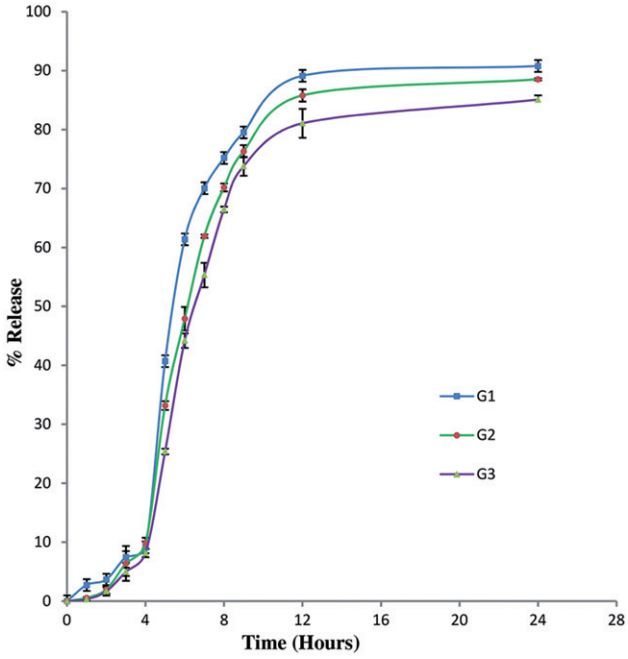

Figure 3. Percentage cumulative *in vitro* CUR release from Eudragit-coated MS in simulated gastro-intestinal fluids of different pH.

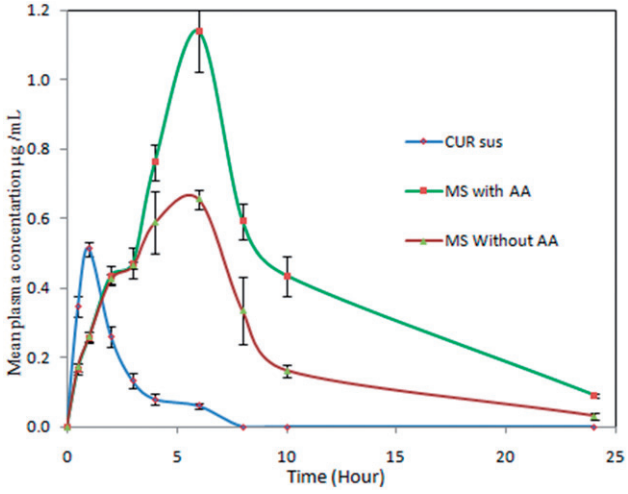

Figure 4. Plasma concentration – time profiles of CUR following oral administration of CUR suspensions, CUR-loaded Eudragit MS without ascorbic acid (formulation G) and CUR-loaded Eudragit MS with ascorbic acid (formulation G_2) (mean ± SD, $n = 3$).

significant (Datta and Kumar 2012). A smaller *p* values and a larger F-value indicate the effect of the higher degree of significance on the dependent factors (Tang *et al.* 2012).

The MS of formulation D exhibited higher drug entrapment efficiency and maximum drug release (t_8). To obtain desired release profile with low dose, this formulation was coated with Eudragit S 100. Figure 3 indicates *in vitro* release profiles of CUR from the Eudragit-coated CSMS. The result showed that all the Eudragit-coated formulations released negligible quantity of drug (5%) in the initial 4 h; thereafter, the release rate was increased.

Release kinetics

Release data were fitted to various kinetic models to investigate the drug-release kinetics. The release exponent (***n***) is an indicative of the mechanism of release because it indicates relative rates of diffusion and polymer relaxation. The values of *n* of formulations G_1, G_2, and G_3 ranging from 0.9669 to 1.3279 (data not shown) indicated that the drug release mechanism was case II or super case II. The higher correlation coefficient values indicated that the drug release followed by zero-order and Peppas kinetics, i.e. the drug release from MS governed by combining the effects of diffusion, swelling, and erosion. The exponential value (*n*) for optimised formulation greater than 1, signified drug release follows non-Fickian super case II mechanism. It means the drug release rate does not change over the time, and the drug is released according to zero-order mechanism. This phenomenon can generally attributed to structural changes induced in the polymer by the penetrate (Divesh *et al.* 2011).

Pharmacokinetic parameters

On the basis of *in vitro* release performance and entrapment efficiency, formulation G_2 was selected for pharmacokinetic study and organ distribution study. The absorption was rapid with pure CUR as shown by the low t_{max} value (1 h), whereas the CSMS exhibited delayed absorption as indicated by the high t_{max} (6 h) values. The C_{max} values for pure CUR, coated MS without AA (G), and with AA (G_2) were 0.512 ± 0.020, 0.655 ± 0.028, and 1.139 ± 0.118 µg/mL, respectively. The AUC_{0-24} of coated MS with AA (G_2) 15.597 µg h/mL was significantly higher ($p < 0.05$) than that of pure CUR 2.128 µg. h/mL and MS without AA (G) 8.867 µg h/mL (Figure 4). Formulation G_2 showed 7.33-fold increase in the blood concentration than CUR suspension. The C_{max} and AUC values obtained after administration of CUR in suspension form and formulation G were significantly lower than those found after administration of formulation G_2. It suggested low absorption and/or an alkaline degradation of CUR *in vivo* and the presence of AA increased pharmacokinetic profiles by suppressing chemical degradation of CUR.

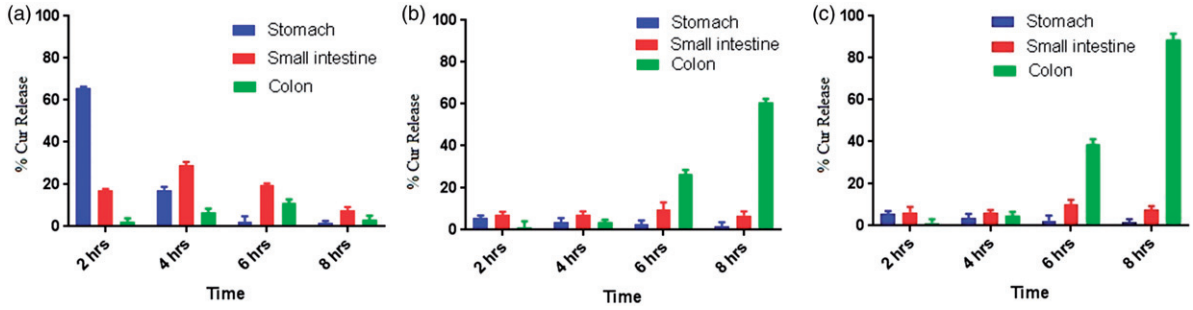

Figure 5. CUR content in isolated organs of albino rats after oral administration of (a) plain CUR (b) CUR-loaded Eudragit-coated MS without ascorbic acid (formulation G), and (c) CUR-loaded Eudragit-coated MS with ascorbic acid (formulation G_2).

Organ distribution study

The results showed that following oral administration of plain CUR, 65.52% concentration of CUR was observed in stomach after 2 h and a small amount was found in the small intestine and colon (Figure 5(a)). The Eudragit-coated CSMS (with and without ascorbic acid) were observed relatively intact, and approximately 3– 6% of total drug was released during its transit through the upper GI tract. After 6–8 h of administration, maximum percentage of CUR was observed in the colon from MS containing AA (G_2) (Figure 5(c)) which indicated that AA decisively improved the bioavailability of CUR. These results suggested that degradation of CUR occurred *in vivo* and presence of AA suppressed this process and increased plasma concentration of CUR.

Discussion

CUR is a promising dietary compound for disease prevention and/or treatment. CUR undergoes chemical degradation in aqueous-organic solutions and the degradation increases as the pH is increased, which is of a serious concern in its applications (Priyadarsini 2009). Previous research has suggested important role of the phenolic radical mechanism in degradation of CUR where it is first converted to a phenolic radical. AA produces the regeneration reaction of these free radicals back to CUR (Oetari *et al.* 1996). AA is an electron donor which donates two electrons from a double bond between the second and third carbons of the 6-carbon molecule (Padayatty *et al.* 2003). In the absence of AA, CUR rapidly degraded, while co-addition of AA significantly increased CUR stability. It indicated that co-addition of AA could serve as a practical strategy to enhance stability and biological activities of CUR.

Prepared core MS were free flowing, non-aggregate, and yellowish brown. FTIR studies suggested linking of ammonium groups of CS in MS with phosphoric groups of TPP. Same results had been reported by the previous studies on CS-TPP nanoparticles. XRD of CUR and formulation D was obtained and compared. In contrast to plain CUR, no typical peaks appeared in the cross-linked CUR-encapsulated CSMS indicates that CUR from a highly crystalline structure has been converted to an amorphous state. The results obtained from XRD studies were further confirmed by DSC thermogram. CS concentration and volume of TPP had significant effect on EE. As the volume of TPP solution increased, EE of CSMS decreased and it was increased when concentration of CS was increased. Previous research has shown that increase in viscosity of aqueous phase increases polymer concentration that helps to stabilise the droplets and also prevent out flow of drug during the hardening phase. Eudragit coating shield CUR from premature release in upper part of gastrointestinal part. After reaching small intestine, Eudragit will continuously and slowly dissolve, thus leaving the CSMS. CS will adhere to colonic membrane; an environment rich in bacterial enzymes that degrade CS and most of the drug was released in the colon.

Oral bioavailability of CUR was enhanced to a great extent with MS containing AA compared to pure CUR and MS without AA. Presence of AA in CSMS might shield it from pH mediated chemical degradation, thus improving the fraction of CUR to be absorbed. The results obtained from *in vivo* organ distribution study indicated protection of CUR in upper gastrointestinal part. CUR was released after reaching to colon due to dissolution of Eudragit and degradation of CS by microbial flora residing in the colon. Our studies have shown that co-addition of AA enhanced pharmacokinetics profile and stability of CUR.

Conclusions

A novel approach for delivery of curcumin laden MS to colon has been successfully attempted. CSMS consisting AA as a pH-regulating agent imparts stability to curcumin in alkaline milieu of colon, and exercises a spatial and temporal control over curcumin release. As hypothesised, 7-fold enhancements in bioavailability of curcumin have been noted. Even, work also enunciates

further, a great promise for treatment of colonic diseases by curcumin in presence of AA as an antioxidant.

Disclosure statement

The authors report no conflict of interest.

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