Comparative oral bioavailability advantage from curcumin formulations

Bhushan Munjal · Yogesh Bapurao Pawar · Sarsvatkumar Babulal Patel · Arvind Kumar Bansal

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Abstract The aim of the present study was to study the oral bioavailability of seven different formulations of curcumin (CRM). CRM formulations viz. aqueous suspension, micronized suspension, nanosuspension, amorphous solid dispersion, hydroxypropyl-β-cyclodextrin (HP-β-CD) inclusion complex, combination with piperine, and spray-dried CRM–milk composite were compared for oral bioavailability in male Sprague–Dawley rats at a CRM dose of 250 mg/kg body weight using a validated high-performance liquid chromatography method. Aqueous suspension provided a $C_{\text{max}}$ and $AUC_{(0-t)}$ of 28.9 ng/ml and 26.9 ng h/ml, respectively. In comparison, statistically significant increase in the oral bioavailability was obtained with the nanosuspension, HP-β-CD inclusion complex, and amorphous solid dispersion with 251%, 567%, and 446% increase in terms of $AUC_{(0-t)}$ and 405%, 415%, and 270% in terms of $C_{\text{max}}$. However, no significant increase in $AUC_{(0-t)}$ and $C_{\text{max}}$ was observed with piperine and micronized suspension. The milk composite reduced the oral bioavailability of CRM (10% and 37% in terms of $AUC_{(0-t)}$ and $C_{\text{max}}$). A statistically significant increase in the $T_{\text{max}}$ was observed with piperine and in HP-β-CD complex, while the $T_{\text{max}}$ was reduced for nanosuspension. The results provide interesting insights into the role of solubility enhancement and metabolism inhibition, for improving the oral bioavailability of CRM.

Keywords Curcumin · Oral bioavailability · Formulations

Introduction

Curcumin (CRM; Fig. 1), owing to diverse pharmacological actions and lack of major side effects, is undergoing clinical trials for several diseases like ulcerative colitis, colon cancer, pancreatic cancer, hypercholesterolemia, atherosclerosis, pancreatitis, psoriasis, Crohn’s disease, and neurological diseases [1]. Clinical development of CRM into a “medicine” has been hampered by its extremely poor oral bioavailability [2]. Reasons reported for its poor oral bioavailability include poor aqueous solubility, degradation in gastrointestinal tract (GIT) at neutral and alkaline pH, high pre-systemic metabolism in the intestinal wall, rapid metabolism to sulfate and glucuronide conjugates leading to short half-life, and rapid systemic elimination [2, 3, 4]. Recently, our research group assessed the permeability of CRM using Caco-2 cell model and found that CRM was poorly permeable with a $P_{\text{app}}$ (A→B) value of $2.93 \pm 0.94 \times 10^{-6}$ cm/s and can be classified as a Biopharmaceutic Classification System class IV molecule [5].

Numerous formulation approaches have been investigated to overcome delivery barriers of CRM. Various oral formulations like self-emulsifying drug delivery system [6, 7], phospholipid complexes [8, 9], solid lipid nanoparticles [10, 11], nanocrystal dispersion [12], cyclodextrin complex [13], polymeric nanoparticles [14, 15], nanoemulsions [16], Biocurcumax™ [17], amorphous solid dispersion [18], and Bioperine [19] have been investigated. Injectable formulations viz. polymeric implants [20], nanosuspension [21], polymeric nanoparticles [22], polymeric micelles [23], liposomes [24], and microspheres [25] have also been explored. Topical formulations of CRM like solid lipid nanoparticles [26] and nanoemulsions [27] are also reported.
Calculation of quantitative biopharmaceutical parameters

Quantitative biopharmaceutical parameters viz. dissolution time \( (T_{\text{disso}}) \) and absorbable dose \( (D_{\text{abs}}) \) were calculated using the following equations [28]:

\[
T_{\text{disso}} = \frac{h \rho}{3 D C_s}
\]

(1)

where \( h \) is the diffusion layer thickness, \( \rho \) is the density of the material, \( r \) is the radius of particle, \( D \) is the diffusion coefficient, and \( C_s \) is the solubility in milligrams per milliliter.

\[
D_{\text{abs}} = P_{\text{eff}} \times C_s \times A \times \text{MITT}
\]

(2)

where MITT is the mean intestinal transit time assumed to be 199 min and \( P_{\text{eff}} \) is the effective permeability and \( A \) is the intestinal area.

Preparation of formulations

The composition of the prepared formulations is depicted in Table 1. The AS was prepared by suspending CRM in citro-phosphate buffer pH 5.0 containing Na-CMC. The buffer was prepared by mixing 48.5 ml of 0.1 M citric acid solution with 0.2 M disodium hydrogen phosphate sufficient to bring the pH to 5.0. Micronization of CRM was achieved using air-jet mill (AS 50, Hosokawa Alpine Aktiengesellschaft, Germany) with the following parameters: compressed air pressure 6 kg/cm², propellant air pressure 2 bar, feed rate 0.9 g/min, and amplitude 25–30. Micronized CRM was suspended in citro-phosphate buffer of pH 5.0. NS was prepared by dispersing micronized CRM in citro-phosphate buffer of pH 5.0 containing poloxamer 188 using high-speed homogenizer (IKA-Ultraturrax; IKA-Werke GmbH & Co., Germany) at 8,000 rpm for 5 min, in

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity (mg)</th>
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<tbody>
<tr>
<td>CRM</td>
<td>50</td>
</tr>
<tr>
<td>Na-CMC</td>
<td>4</td>
</tr>
<tr>
<td>Poloxamer</td>
<td>4</td>
</tr>
<tr>
<td>PVP</td>
<td>-</td>
</tr>
<tr>
<td>HP-β-CD</td>
<td>315</td>
</tr>
<tr>
<td>Piperine</td>
<td>-</td>
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<tr>
<td>Milk</td>
<td>4,000</td>
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</table>

**Table 1 Composition of the CRM formulations**

**Materials and methods**

Materials

CRM (purity more than 99% by high-performance liquid chromatography (HPLC) assay) was extracted from *Curcuma longa* (turmeric). 4-Methoxychalcone (4-MC) and piperine were purchased from Sigma-Aldrich (Steinheim, Germany). HPLC grade methanol, ethyl acetate, acetonitrile (ACN), and tetrahydrofuran (THF) were purchased from RFCL Ltd. (New Delhi, India). Acetone and citric acid monohydrate were purchased from Merck (Mumbai, India), glacial acetic acid was procured from Qualigens Fine Chemicals (Mumbai, India), polyvinylpyrrolidone K-30 (PVP) was procured from ISP Technologies Inc. (Wayne, NJ, USA), sodium carboxymethyl cellulose (Na-CMC) was from Himedia Laboratories Ltd. (Mumbai, India), poloxamer 188 was from BASF (Ludwigshafen, Germany), and HP-β-CD was from Wacker Chemie (Munich, Germany). Pasteurized standardized milk (fat content 4.5 g/100 ml) was obtained from Verka (Mohali, India). Ultrapure water was produced by purification with Ultra pure® water system (USF Elga, England).
an ice bath. In the second step, the chilled formulation (temperature < 4°C) was passed through high-pressure homogenizer (Emulsiflex C3; Avestin, Canada). The suspension was given circulation at 500, 1,000, and 1,500 bar each for 5 min. The ASD of CRM was prepared by solvent evaporation method after dissolving CRM and PVP in miscible solvents; 3.0% (w/v) CRM solution was dissolved in acetone containing 0.1% (v/v) glacial acetic acid. PVP was dissolved in methanol and added to CRM solution with stirring. Solvents were removed by evaporating under vacuum at 50°C using a Rotavapor R-200 (Buchi, Switzerland). The dried product was passed through sieve BSS#200. HP-β-CD was dissolved in water (pH adjusted to 5.0 using glacial acetic acid) and added to 3.5% (w/v) CRM solution in methanol, with stirring. Stirring was continued for 8 h. The solvents were then removed by evaporation under vacuum at 60°C using a Rotavapor R-200 (Buchi, Switzerland). The formulation was then passed through sieve BSS#200. CRM and piperine were mixed with gentle trituration in mortar–pestle and suspended in citro-phosphate buffer containing Na-CMC. Slurry of CRM (1.2%, w/v) in hot milk (60–70°C) was prepared by stirring for 10 min. The slurry was then ultrasonicated in a bath sonicator for 5 min and spray-dried using mini-spray dryer B-191 (Buchi Labortechnik AG, Switzerland). The spray drying parameters were inlet temperature, 90°C; outlet temperature, 60–70°C; aspirator value, 80 m³/h; air flow rate, 600 l/h; and flow rate, 4 ml/min.

Characterization of formulations

High-performance liquid chromatography

The formulations were characterized for assay by HPLC method. The HPLC system (Shimadzu Corporation, Kyoto, Japan) comprised of SCL-10A VP system controller, LC-10AT VP liquid chromatograph, FCV-10AL VP flow control valve, DGU-14A degasser, SIL-10AD VP autoinjector, CTO-10AS VP column oven, SPD-10A VP ultra violet–visible (UV–Vis) detector, and a data acquisition Class-VP 6.10 software. The HPLC method was validated for linearity, precision, accuracy, and intra- and inter-day variability. The mobile phase was acetonitrile/tetrahydrofur- ran/1% (w/v) citric acid solution (aq.), pH 3.0 (56:14:30). All analyses were done using LiChrospher® C18 column (4.6 x 200 mm, 5 μm Merck KGaA, Darmstadt, Germany) under isocratic condition at a flow rate of 1.0 ml/min at 30°C with 20 μl injection volume, and effluent was monitored at wavelength 430 nm.

Particle size and shape

Particle size measurements of CRM in formulations AS and MS were carried out by optical microscopy (DMLP, Leica Microsystems, Germany). D_{10}, D_{50}, and D_{90} were calculated as the equivalent projected diameter such that 10%, 50%, and 90% of the particles are smaller than this number. All the samples were also observed under polarized light. The surface morphology of CRM, micronized CRM, ASD, and HIC was viewed under a scanning electron microscope (SEM; S-3400, Hitachi Ltd., Tokyo, Japan) operated at an excitation voltage of 25 kV. The samples were mounted onto steel stage using double-sided adhesive tape and sputter-coated with gold using ion sputter (E-1010, Hitachi Ltd., Tokyo, Japan), before analysis. The mean particle size of CRM in NS was determined by photon correlation spectroscopy (Zeta sizer, ZEN 3600, Malvern Instruments, UK), taking the average of five measurements.

Solid-state characterization

X-ray powder diffraction (XRPD) pattern of samples were recorded at room temperature using Bruker’s D8 Advance Diffractometer (Karlsruhe, West Germany) equipped with a 2θ compensating slit, using Cu Kα radiation (1.54 Å) at 40 kV and 40 mA passing through nickel filter. Samples were mounted on zero-background sample holder and subjected to a continuous scan over an angular range of 3° to 40° 2θ at a step size of 0.02° and scan rate of 1 s/step. Obtained diffractograms were analyzed with DIFFRACplus EVA (ver. 9.0) diffraction software.

Determination of oral bioavailability of formulations

Animals, dosing, and blood sampling

All animal experiments were performed in accordance with Committee for Purpose of Control and Supervision on Experiments on Animals guidelines, and the experimental protocols were approved by the Institutional Animals Ethics Committee. All the animals were bred and maintained in the institute’s central animal facility. Male SD rats weighing from 225 to 275 g were used and kept on fasting for 12 h before experimentation with free access to water. Six rats were randomly assigned to each treatment group. The calculated amount of formulation (equivalent to a dose of 250 mg/kg body weight of CRM) was administered to each group via oral gavage. After oral dosing, approximately 0.5 ml of blood samples was collected from retro-orbital
plexus at 0.25, 0.5, 1, 2, 3, and 5 h in heparinized (20 μl heparin solution (1,000 IU/ml), isotonic) microcentrifuge tubes. Plasma was separated immediately by cold centrifugation and stored at −80°C until processed and analyzed.

**Bioanalytical method**

Sample preparation was carried out by extracting thawed plasma (200 μl) spiked with 10 μl of 4-MC (12 μg/ml, in methanol) with ethyl acetate/methanol mixture, 95:5% (v/v). The samples were then centrifuged at 12,500×g for 5 min, and the upper organic layer (800 μl) was separated and evaporated to dryness by centrifugal evaporation under vacuum (MAXI dry lyo, Heto). The dried residue was reconstituted with 100 μl of the mobile phase and an aliquot injected directly into the HPLC system. The HPLC system consisted of LC-10AT VP pump fitted with a degasser unit DGU-14A, a SIL-10AD VP auto sampler with refrigeration unit, a dual wavelength SPD-10A VP UV–Vis detector, and a CTO-10AS VP column oven (Shimadzu, Japan). The system was controlled by a SCL-10A VP system controller with Class-VP 6.10 chromatography software. The analytical column was a LiChrospher® C18 column (4.6×200 mm, 5 μm Merck). The mobile phase consisted of a mixture of 1% (w/v) citric acid monohydrate (pH adjusted to 3.0±0.05 using 45% (w/v) potassium hydroxide), ACN, and THF (45:35:20) at a flow rate of 1 ml/min. The detection wavelengths were 425 and 319 nm for CRM and 4-MC (internal standard, Fig. 2), respectively. The injection volume was 40 μl and the column temperature was kept at 30°C. CRM was quantified by ratio of the peak area of CRM to that of the 4-MC using weighted (1/√x) linear regression. The method was validated as per USFDA guidance [29] for bioanalytical method validation.

**Data analysis**

The mean plasma CRM concentration profiles were generated and the standard non-compartmental pharmacokinetic parameters were calculated using PCNONLIN version 4.0 Professional Data analysis SCI software (Lexington, KY, USA). Statistical comparisons were performed using SigmaStat for Windows Version 2.03 (SPSS Inc.). Statistical testing between two mean values for statistical significance with α=0.05 was performed using two-sided unpaired t test for samples with unequal variance where each formulation was compared with the conventional formulation, AS.

**Results and discussion**

**Formulation characterization**

**Particle size and shape**

The particle size of micronized CRM (D<sub>10</sub>=10 μ, D<sub>50</sub>=17 μ, and D<sub>90</sub>=63 μ) was significantly smaller than that of untreated CRM (D<sub>10</sub>=40 μ, D<sub>50</sub>=80 μ, and D<sub>90</sub>=144 μ). The average particle size of NS was found to be of 464±40 nm with a polydispersity index of 0.233. CRM and micronized CRM showed characteristic birefringence when viewed under polarized light that was absent in the ASD, HIC, and MC indicating the absence of crystallinity in these three formulations. SEM of pure CRM showed flat-broken needle-shaped crystals of varying sizes with well-developed edges. In contrast, micronized CRM showed irregular-shaped particles. ASD and HIC showed irregular particles of varying sizes with smooth surface (Fig. 3).

**Solid-state characterization**

Pure CRM and micronized CRM showed characteristic diffraction peaks at 2θ positions of 9.1, 17.2, 21.5, and 24.9. An additional peak at 2θ position of 13.5 was observed for micronized CRM, characteristic of another polymorph of CRM (unpublished data from our lab), which indicates that the micronized material was a mixture of two crystalline forms of CRM. These peaks were absent and halo pattern was observed for formulations ASD, HIC, and MC indicating the absence of crystallinity (Fig. 4). The DSC thermogram of CRM showed a sharp melting endotherm at 179.32°C (onset); however, this endotherm was absent in the thermogram of ASD indicating the amorphous nature with T<sub>g</sub> of 69°C (Fig. 5).

**Bioanalytical method validation**

The calibration curve was linear (10–1,280 ng/ml; y = 0.002127x + 0.000299, CV=14.036, n=6) with mean correlation coefficient, R<sup>2</sup> > 0.9921 (<0.5% CV, n=9). The LLOQ and LOD were found to be 10 and 5 ng/ml, respectively. No significant interference was observed with blank plasma. The intra- and inter-day accuracy and precision for QC samples were below 15% and 10.7% (CV), respectively. The mean extraction efficiency was
96.67±4.76%. No significant change was found in the CRM spiked rat plasma samples after 1 month at −80°C, after three freeze–thaw cycles and in the processed samples for 7 h at 4°C in the autosampler.

Determination of oral bioavailability of formulations

CRM has been reported to have an oral bioavailability of less than 4% [4] in rats. In human beings, also undetectable plasma levels were obtained even after administration of doses as high as 8 g [30]. To theoretically assess the rate-limiting factors for oral bioavailability, we calculated quantitative biopharmaceutical parameters. Calculations gave values of $1.81 \times 10^6$ min and $9.16 \times 10^{-8}$ mg for $T_{\text{disso}}$ and $D_{\text{abs}}$, respectively. $T_{\text{disso}}$ value of $1.81 \times 10^6$ min which was far greater than 199 min and $D_{\text{abs}}$ value of $9.16 \times 10^{-8}$ mg which was far lesser than administered dose indicated that both the solubility and dissolution rate are the rate-limiting parameters in oral bioavailability of CRM.

Fig. 3 Scanning electron microscopy image of a curcumin (CRM), b micronized CRM, c amorphous solid dispersion (ASD), d HP-β-CD inclusion complex (HIC)

Fig. 4 XRPD pattern of curcumin (CRM), micronized CRM (MS), amorphous solid dispersion (ASD), HP-β-CD inclusion complex (HIC), and milk-composite (MC)
The mean plasma profiles obtained after oral administration of the formulations to SD rats are presented in Fig. 6, and the results of the calculated pharmacokinetic parameters have been tabulated in Table 2. AS of CRM, that was administered as the conventional formulation, achieved maximum plasma concentration ($C_{\text{max}}$) of only 28.9 ng/ml, and the AUC$_{(0-\infty)}$ was found to be 26.9 ng h/ml. $C_{\text{max}}$ was achieved within 0.5 h and the plasma levels were reduced to below limit of quantification (BLOQ) within 2 h. The half-life ($t_{1/2}$) was calculated and found to be only 0.8 h. Rapid clearance and high metabolism have been reported in the literature as the major reasons responsible for the short $t_{1/2}$ of CRM [30]. The results were comparable to a study conducted by Yang et al. [4], wherein after oral administration of a single dose of CRM in SD rats at a dose of 500 mg/kg, $t_{1/2}$ and $T_{\text{max}}$ of 0.8 and 0.695 h, respectively, were achieved.

In this study, we employed six more different formulation strategies and evaluated the extent of bioavailability advantage achieved with these formulation strategies with respect to the conventional formulation, AS. A comparative assessment of the percentage increase in the mean AUC$_{(0-\infty)}$ and $C_{\text{max}}$ as compared to AS (considered as 100%) is presented in Fig. 7.

Particle size reduction by the micronization and nanonization approaches is known to enhance the dissolution rate and bioavailability of molecules with poor aqueous solubility [31, 32]. These two approaches were therefore screened for their capacity to improve the oral bioavailability of CRM in MS and NS, respectively. For MS, the mean compared with that of AS to male SD rats ($n=6$). The error bars represent standard error of means.
AUC\(0−t\), C\(\text{max}\), and T\(\text{max}\) were found to be 27.8 ng h/ml, 32.2 ng/ml, and 0.25 h, respectively. Micronization of CRM did not provide significant improvement in oral bioavailability, as compared to AS. NS, however, provided a statistically significant improvement in mean AUC\(0−t\), C\(\text{max}\), and T\(\text{max}\), with values of 67.4 ng h/ml, 117.1 ng/ml, and 15 min, respectively. NS, among all formulations, provided the highest C\(\text{max}\) and shortest T\(\text{max}\). A previous study [12] also reported a reduction in the T\(\text{max}\) due to nanonization of CRM.

The Noyes–Whitney equation (Eq. 3) describes the dissolution rate, \(\frac{dc}{dt}\) of a drug:

\[
\frac{dc}{dt} = \frac{D_f}{\theta_D} S_c (C_s - C_t)
\]

(3)

where \(D_f\) is the diffusion coefficient of the solute, \(\theta_D\) is the thickness of diffusion boundary layer, \(V\) is the volume of dissolution medium, \(S_c\) represents the interfacial area, \(C_s\) is the saturation solubility, and \(C_t\) represents concentration in the bulk medium at time \(t\) [33]. Micronization enhances the \(dc/dt\) owing to an increase in \(S_c\) caused by a reduction in the particle size. Nanonization, however, may also enhance \(C_s\) as explained by Ostwald–Freundlich equation [34]. The increase in \(dc/dt\) in the case of nanosuspension will thus be even more pronounced and is caused by an increase in the \(C_s\) as well as \(S_c\). The higher C\(\text{max}\) and shorter T\(\text{max}\) obtained with NS thus establish the role of enhanced solubility and dissolution rate in oral absorption of CRM.

Though significant enhancements of C\(\text{max}\) (405%) and AUC\(0−t\) (251%) were obtained (Table 2), the plasma levels diminished to below the LOQ after 2 h, thus preventing significant improvement in the mean residence time (MRT). NS thus provided enhanced and rapid entry into systemic circulation, but could not sustain this advantage. This indicates role of rapid metabolism and/or distribution to peripheral compartment, in achievement of overall bioavailability advantage. Interestingly, blood plasma profile of NS showed a biphasic response. CRM is known to undergo enterohepatic recirculation which could have contributed to the biphasic response [35]. A very rapid tissue distribution may also give similar results. A previous study by our group has supported the evidence of accumulation of CRM in tissues [5]. This is a novel finding with respect to CRM, and further studies are needed to unveil its contribution to pharmacokinetics of CRM.

ASD was evaluated based on previous reports of enhancement of dissolution rate and bioavailability of CRM, using amorphous solid dispersion [12, 18].

Table 2 Comparison of various pharmacokinetic parameters of CRM formulations (the values are reported as mean±standard error of means)

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Formulation</th>
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<tbody>
<tr>
<td></td>
<td>AS</td>
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<tr>
<td>AUC(0−t) (ng h/ml)</td>
<td>26.9±8.1</td>
</tr>
<tr>
<td>C(\text{max}) (ng/ml)</td>
<td>28.9±7.1</td>
</tr>
<tr>
<td>T(\text{max}) (h)</td>
<td>0.50±0.1</td>
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<tr>
<td>AUMC(0−t) (ng h^2/ml)</td>
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<tr>
<td>MRT(0−t) (h)</td>
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</table>

*P<0.05, statistically significant difference in comparison with formulation AS

AS aqueous suspension, MS micronized suspension, NS nanosuspension, ASD amorphous solid dispersion, HIC HP-β-CD inclusion complex, WP with piperine, MC milk composite

Fig. 7 Percentage enhancement in pharmacokinetic parameters for various formulations in comparison with aqueous suspension (AS). MS micronized suspension, WP with piperine, HIC HP-β-CD inclusion complex, ASD amorphous solid dispersion, NS nanosuspension, MC milk-composite
mean $AUC_{(0→)}$, $C_{max}$, and $T_{max}$ were found to be 119.8 ng h/ml, 78.1 ng/ml, and 0.5 h, respectively. The percentage increase in the $AUC_{(0→)}$ (446%), $C_{max}$ (270%), $AUMC_{(0→)}$ (981%), and mean residence time, $MRT_{(0→)}$ (220%) were significant (Table 2). The increase in the $AUC_{(0→)}$ and $C_{max}$ can be explained by the enhancement of the aqueous solubility and the dissolution rate of CRM in the solid dispersion. An important observation, however, was that the plasma levels were detectable till 5 h. This led to an increase in the $AUMC_{(0→)}$ and $MRT_{(0→)}$. In a previous study, when amorphous solid dispersion of CRM was administered to male Wistar rats at oral dose of 200 mg/kg, CRM was detectable in plasma even after 10 h [36]. ASD consist of molecular dispersion of CRM in PVP matrix. This is a step ahead of particle size reduction, as drug is already present in “molecularly dispersed” state and contribution of crystal lattice energy on dissolution gets minimized. The solid dispersion of CRM with PVP is, therefore, capable of enhancing the biological half-life and oral bioavailability of CRM.

Bioavailability enhancement via improved solubility and stability has been among one of the frequent applications of cyclodextrins. Poor stability in the GIT and the poor aqueous solubility of CRM are partially responsible for the poor oral bioavailability of CRM; thus, this strategy seemed to have potential in enhancing its oral bioavailability. HP-β-CD inclusion complex of CRM is reported to enhance its aqueous solubility and stability [37]. However, no in vivo studies on CRM oral bioavailability were reported.

HIC provided $AUC_{(0→)}$, $C_{max}$, and $T_{max}$ of 152.2 ng h/ml, 120 ng/ml, and 60 min, respectively. The percentage enhancement in the $AUC_{(0→)}$ (567%) and $C_{max}$ (415%) was significantly higher than AS. The increase in the $AUC_{(0→)}$ and $C_{max}$ can be ascribed to the increase in the aqueous solubility of CRM by cyclodextrin complexation [38]. However, it was interesting to note that the $T_{max}$ increased significantly to 1 h. This might be explained by the increased hydrolytic stability of CRM in the GIT due to complexation [37]. This may increase the duration of apparent absorption phase by making stable CRM available for absorption, thus causing an increase in the $T_{max}$. CRM undergoes hydrolysis at intestinal pH and intestinal enzymatic degradation (e.g., glucuronidation), and cyclodextrins can help in maintaining CRM concentration in the intestine by stabilization. However, absorption phase was succeeded by a rapid elimination phase, and plasma levels were reduced to below LOQ after 3 h. The biological half-life of CRM was unaltered as no significant difference in the $MRT_{(0→)}$ was observed.

For this study, WP was chosen as concomitant administration of CRM with piperine is reported to enhance its oral bioavailability significantly by inhibiting its metabolism [14, 19]. The mean $AUC_{(0→)}$, $C_{max}$, and $T_{max}$ were found to be 45.6 ng h/ml, 40.3 ng/ml, and 1 h, respectively.

$T_{max}$ was significantly delayed. This observation was similar to the results obtained by Shaikh et al. and Shoba et al. In both the studies, the $T_{max}$ found to be significantly enhanced. The delay in the $T_{max}$ might be caused by prolongation of the apparent absorption phase of CRM by reducing its intestinal cell wall metabolism via glucuronidation inhibition by piperine. However, $AUC_{(0→)}$ and $C_{max}$ were not significantly higher in comparison to AS. The increase in the $AUC_{(0→)}$ (169.8%) was comparable to that reported by Shoba et al. (154%). The increase in the $C_{max}$ was also comparable in all the three studies 139.4% in the present study as compared to 133.3% and 134% in studies performed by Shaikh et al. and Shoba et al., respectively. However, in all the three studies, the $P$ value was more than 0.05 indicating no statistically significant difference. Since piperine does not have any effect on the solubility of CRM, hence $C_{max}$ may not be expected to be significantly increased.

**Table 3** Classification of the prepared formulation approaches based upon the statistical analysis of the pharmacokinetic data

<table>
<thead>
<tr>
<th>Class</th>
<th>Formulation approach</th>
<th>Effect on pharmacokinetics of CRM</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>$AUC_{(0→)}$ $C_{max}$ $T_{max}$ $MRT_{(0→)}$</td>
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<tr>
<td>I</td>
<td>HIC</td>
<td>↑**</td>
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<td>NC</td>
</tr>
<tr>
<td>III</td>
<td>MC</td>
<td>↓*</td>
</tr>
</tbody>
</table>

↑ increase, ↓ decrease, NC no statistically significant difference, AS aqueous suspension, MS micronized suspension, NS nanosuspension, ASD amorphous solid dispersion, HIC HP-β-CD inclusion complex, WP with piperine, MC milk composite

$**P<0.05$, statistically significant difference; $**P<0.01$, statistically significant difference
enhanced. Plasma samples from rats of this group showed an additional peak at retention time of 4.8 min. The peak was found to be of piperine as confirmed by retention time of standard piperine (Fig. 8). The observed AUC and $C_{\text{max}}$ of CRM in the individual rats were found to be directly related to the AUC observed for piperine. The $T_{\text{max}}$ was also found to be similar for both the molecules (data not shown). These findings point out that the co-administration of piperine with CRM provides only a marginal improvement in bioavailability. However, since piperine enhanced the $T_{\text{max}}$ of CRM, addition of piperine in combination with an approach to increase the solubility may provide additive improvement. One such strategy can be administration of piperine with the NS or ASD or HIC.

Indian traditional system of medicine, Ayurveda, recommends administration of turmeric (major source of CRM) in milk. This concept was investigated in MC. However, except for two rats, where the concentration measured at 0.25 h was found to be 25.4 and 59.1 ng/ml, plasma levels obtained in all the rats were below LOQ. Although the nature of CRM in MC was amorphous, yet a significant decrease in the AUC$_{(0\rightarrow\infty)}$ was observed. More mechanistic studies are needed to understand the exact mechanism responsible for reduction in the oral bioavailability of CRM in this formulation. In contrast, Shishu and Maheshwari recently found out that administration with milk enhanced the permeability of CRM by 2.5-fold as compared to that of aqueous suspension in permeability studies done using non-everted rat intestinal sac model. The rate of permeation of CRM from milk was reported to be 7-fold as compared to that of aqueous suspension [39]. However, the reasons for this behavior were not explored.

These results brought interesting insight into the bioavailability problem of CRM. From the statistical analysis of the results, it was found that all the six formulations behaved differently when compared with the AS, as presented in Table 3. The evaluated formulations can be classified into three classes based upon the bioavailability advantage achieved for CRM: class I, formulations that significantly enhanced the oral bioavailability and include formulations HIC, NS, and ASD; class II, the formulations incapable of providing significant increase in oral bioavailability and includes formulations WP and MS; and class III, formulation that decreased the bioavailability of CRM and includes MC. The effect of combination of strategies which increase the $C_{\text{max}}$ and AUC (NS and ASD) with piperine (which increases the $T_{\text{max}}$) on the oral bioavailability of CRM may be explored.

The highest $C_{\text{max}}$ in serum achieved for CRM in rats (dose 2 g/kg) has been reported to be around 1.5 μg/ml [2], which is unfortunately below therapeutic efficacy, whereas the six formulations in this study were already predicted not to exceed the levels. Therefore, it is reasonable to say that comprising among the most promising formulations in a single study makes the best sense, although technically it is difficult due to proprietary nature of other formulations such as those mentioned in the “Introduction”. Regardless of variations across independent investigations, the benchmark of such studies in future could be set at the highest $C_{\text{max}}$ reported at ∼1.5 μg/ml.

**Conclusions**

The present study provided interesting insights into the capacity of various formulations approaches in enhancing the oral bioavailability of CRM. A clear impact of particle size reduction could be observed, wherein micronization improved $T_{\text{max}}$ marginally, whereas nanonization provided significant improvement in AUC$_{(0\rightarrow\infty)}$ $C_{\text{max}}$, and $T_{\text{max}}$. Molecular dispersion of CRM in solid dispersion with PVP provided significant improvement in AUC$_{(0\rightarrow\infty)}$ $C_{\text{max}}$, and MRT. Formulation approach involving incorporation of metabolism inhibitor, piperine, delayed the $T_{\text{max}}$, indicating slower metabolism.

This work underlines the importance of addressing multiple delivery hurdles like solubility, dissolution kinetics, and intestinal first pass metabolism, to achieve significant oral bioavailability enhancement of CRM.

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**References**


