Development and Pharmacokinetic Evaluation of a Curcumin Co-solvent Formulation

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Abstract. *Poor solubility and bioavailability are limiting factors for the clinical application of curcumin. The objective of the current study was to develop a liquid formulation with increased solubility and systemic bioavailability. A co-solvent formulation with increased solubility of 20 mg/ml was developed and optimized. Pharmacokinetics of the new formulation were evaluated using rats receiving 30 mg/kg intravenous or 50 mg/kg intramuscular administration of co-solvent formulation, compared against a control group receiving 50 mg/kg of curcumin in DMSO through intramuscular injection. Plasma concentrations were measured using liquid chromatography-mass spectrometry (LC-MS/MS). The intramuscular injection of formulation resulted in 30% absolute bioavailability and provided sustained release by maintaining plasma concentrations of curcumin above 240 ng/ml for up to 4 h. A 29-fold increase in the maximum plasma concentration (C_{max}) and 28-fold increase in the area under the plasma concentration versus time curve (AUC) led to a 28-fold increase in relative bioavailability for the co-solvent formulation. The findings reported here suggest that the clinical application of curcumin can be better-exploited through an intramuscular administration of the co-solvent formulation developed in the present study.*

The phytopharmaceutical product Curcumin, a hydrophobic polyphenol, is derived from the rhizhome of the herb Curcuma Longa and has a wide spectrum of biological and pharmacological activities including anti-oxidant, anti-inflammatory, anti-hyperlipidemic, anti-carcinogenic effects and antiangiogenic effects (1-6). Furthermore, various animal models and human studies have proven that curcumin is extremely safe even at high doses of 12 g/day (7-12).

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Curcumin is classified as a biopharmaceutical classification system (BCS) class IV molecule on the basis of its poor aqueous solubility (11 ng/ml in aqueous buffer pH 5) and permeability through intestinal epithelial cells (12-14). Chemically, curcumin is a bis-a,β-unsaturated β diketone commonly called diferuloylmethane, and exhibits keto-enol tautomerism having a predominant keto form in acidic and neutral solutions and stable enol form in alkaline medium.

In spite of its promising pharmacological activities, high safety profile and efficacy, poor oral bioavailability of curcumin (reported to be less than 1%) (15) is one of the limiting factors regarding its clinical application. The major reasons contributing to low plasma and tissue levels associated with curcumin administration appear to be due to its poor aqueous solubility, poor absorption, rapid metabolism and rapid systemic elimination (16). Hence, the enhancement of solubility and the bioavailability of this phytochemical can potentially bring it to the forefront of therapeutic agents which can treat dreadful human ailments, as cancer.

Numerous approaches have been undertaken in recent years to improve the solubility and bioavailability of curcumin, including the use of adjuvants like piperine (16), solid dispersions (17-19) formulation of curcumin in liposomes (20), nanoparticles (21, 22) and formulation of structural analogues of curcumin (23). Even though many of these approaches have improved the bioavailability of curcumin to an extent by enhancing the solubility and permeability of this highly lipophilic phytochemical, most of the methods have limitations of their own. For instance, aggregation, insufficient cell uptake and a lack of evidence of efficient drug release from the nano-conjugates into tumor cells are the major disadvantages of nanoformulations (23); whereas insufficient stability, poor batch-to-batch reproducibility, sterilization difficulties and low drug loading are the major limitations of liposomes (23). Also, high molar ratios of various excipients to drug and comparably low drug loading efficiency are involved in these strategies. Therefore, these methods may not be suitable for the delivery of hydrophobic drugs in high doses (24, 25).

Intramuscular injection is one of the most common and widely used routes of drug administration for the optimal

Table I. *Percentage composition of excipients in curcumin (20 mg/ml) co-solvent formulations.*

Excipient	Formula A	Formula B	Formula C	Formula D	Formula E	Formula F
NMP	15% (v/v)	15% (v/v)	15% (v/v)	15% (v/v)	15% (v/v)	15% (v/v)
PEG 400	20% (v/v)	30% (v/v)	30% (v/v)	30% (v/v)		
Water	65% (v/v)	55% (v/v)	45% (v/v)	35% (v/v)	50% (v/v)	40% (v/v)
Ethanol			10% (v/v)	20% (v/v	20% (v/v)	20% (v/v)
Tween 80					15% (w/v)	25% (w/v)
Visual grading	Precipitation after 24 h	Precipitation after 48 h	Precipitation after 60 h	Precipitation after 1 week	Precipitation after 2 weeks	No visual precipitation up to 8 weeks

delivery of liquid formulations such as co-solvent systems. The sustained release characteristics associated with the intramuscular route was the most important reason for our choice of route (26-28). Through intramuscular administration, the plasma concentration of curcumin can be maintained above optimum level for a prolonged period of time to better-exploit the therapeutic potentials of this phytochemical and ease its transition to the clinic. Therefore, an intramuscular injection can be considered an excellent choice for the administration of curcumin.

Although various approaches have been exploited to increase the solubility and bioavailability of curcumin to exploit its full therapeutic potentials, limited efforts have been undertaken to develop a liquid formulation which can be administered intramuscularly to sustain the tissue and plasma levels of curcumin. In the current article we report the development of a new curcumin co-solvent formulation with significantly increased solubility of curcumin. Bioavailability of the curcumin co-solvent formulation was evaluated after intramuscular administration to adult male Sprague-Dawley rats.

Materials and Methods

Materials. Curcumin (94% purity), Etravirine (Internal Standard), N-methyl pyrrolidinone (NMP), propylene glycol (PG), polyethylene glycol (PEG) 400, Tween 80, ethanol and dimethyl sulfoxide (DMSO) were purchased from Sigma Aldrich, (St. Louis MO, USA). High-performance liquid chromatography (HPLC)-grade methanol, acetonitrile and water were purchased from EMD chemicals, (Gibbstown, NJ, USA). All other chemicals used were of analytical grade from reputed suppliers.

Formulation development. Various co-solvent formulations of curcumin were evaluated (Table I) and an optimal liquid formulation was developed containing (a) NMP (15% v/v), (b) Tween 80 (25% w/v), (c) ethanol (20% v/v) and (d) water (40% v/v). The solubility of curcumin in the optimized formulation was found to be as high as 20 mg/ml without any visual precipitation of the solid drug. Pharmacokinetics of the optimized formulation was evaluated in male adult Sprague-Dawley rats.

Animal experiments. The experiments and protocols regarding animal usage were reviewed and approved by The Institutional Animal Care and Use Committee at Texas Southern University. All experimental procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, 8th Edition (NIH Publication 2011). Male adult Sprague-Dawley rats (Harlan Inc.; Indianapolis, IN, USA) weighing 225-250 g were acclimated for a minimum of 7 days in a temperature-controlled room with a 12-h light-dark cycle, given food and water *ad libitum* before the experiments. The day before the pharmacokinetic experiment, the right jugular veins of the rats were catheterized with a sterile cannula under a cocktail anesthesia containing ketamine (50 mg/ml), xylazine (3.3 mg/ml) and acetopromazine (3.3 mg/ml) in sterile water for injection through previously published procedures (29). After cannulation, intramedic PE-50 polyethylene tubing connected to the cannula was exteriorized through the dorsal skin. The cannula was flushed with 100 U/mL heparin. The animals were transferred to metabolic cages on the day of pharmacokinetic (PK) study.

Pharmacokinetic studies. Three groups of jugular vein-cannulated, male adult Sprague-Dawley rats were studied. One group (n=5) received 30 mg/kg of the optimized formulation (Formula F) through intravenous injection, while the second group (n=5) received 50 mg/kg of the optimized formulation through intramuscular injection. A third (control) group of rats (n=5) received 50 mg/kg of pure curcumin solution dissolved in DMSO at a 10 mg/ml concentration, intramuscularly.

Aliquots of plasma were collected from all the rats before dosing. The cannula was flushed with 20 U/ml heparin after each intravenous administration to remove any drug adhering to the tubing. After dosing, serial blood samples (approximately 250 μl) were collected from the cannula at 15, 30, 45, 60 and 90 min followed by 2, 4, 6 and 8 h collections. Each blood sample was collected into light-resistant polypropylene micro centrifuge tubes. After centrifugation, the plasma was collected and stored at –80˚C and analyzed within one week.

Rat plasma analysis of curcumin by LC-MS/MS. Plasma concentration of curcumin was determined by a previously published and validated LC-MS/MS assay method (30). Briefly, curcumin was extracted from rat plasma by mixing 0.1 ml of plasma with 0.1 ml of internal standard (100 ng/ml of etravirine dissolved in 100% acetonitrile) to precipitate the proteins. After 1 min

vortexing for and 8 min centrifugation, 10 µl of the clear supernatant were injected on to a symmetry C_{18} column (50×4.6 mm, 3.5 µm). The Agilent1200 series HPLC chromatographic conditions were as follows. The mobile phase consisted of methanol (A) and 0.1% aqueous formic acid (B) using linear gradient elution ratio from A/B of 30/70 to 95/05 over a run time of 8 min. Flow rate was optimized at 0.3 mL/min. The mass spectrometry instrumentation was AB Sciex 3200 QTRAP operating in MRM scan in negative mode. The transitions (precursor to product) monitored were a *m/z* ratio of (369.1→177.1) for curcumin and a *m/z* ratio of (435.1→142.0) for the internal standard etravirine.

Pharmacokinetic analysis. Pharmacokinetic analysis was performed using data from individual rats. The mean and standard deviation (SD) were calculated for each group. C_{max}: The maximum plasma concentration of curcumin was determined from the plot of plasma curcumin concentration *vs*. time profile. AUC: The total area under the plasma concentration time curve was determined by the trapezoidal rule using plasma curcumin concentration *vs*. time data from time zero to the last sampling time, *i.e*. 8 h plus the extrapolated area (from the last experimental time to infinity). The extrapolated area was calculated as $1.44 \times t_{½} \times$ plasma concentration at the last experimental time. Half life: The terminal phase rate constant was determined from the slope of the terminal linear segment of a semi logarithmic plot of plasma curcumin concentration *vs*. time. CL/F: The apparent systemic clearance was determined as dose/AUC. V_d/F: The apparent volume of distribution at steady-state was calculated using the formula Dose [AUMC] / [AUC^2], where AUMC is the area under the first moment plasma concentration time curve. F_{abs}: Absolute bioavailability was calculated using $[(AUC_{im} \times Dose_{iv}) / (AUC_{iv} \times Dose_{im})] \times 100\%$. F_{rel}: Relative bioavailability was calculated using the equation $[(AUC_{formulation} \times Dose_{control}) / (Dose_{formulation} \times AUC_{control})]$.

Statistical analysis. Statistical analysis was performed using SYSTAT (version 12.0). A *p*-value of less than 0.05 was considered statistically significant. Statistical differences between the two mean pharmacokinetic parameter values were determined by both parametric unpaired *t*-tests and non-parametric Mann-Whitney tests followed by Post Hoc test for the homogenous parameters and the non-homogenous parameters respectively.

Results

Development of a co-solvent formulation of curcumin. Various percentage compositions of water-soluble organic solvents such as NMP, PEG 400, ethanol and the non-ionic surfactant such as Tween 80 were evaluated for an optimal curcumin formulation (Table I). Curcumin concentrations in these formulations were kept fixed at 20 mg/ml. Among these formulations (#A-E), curcumin was initially dissolved but subsequently exhibited various degrees of precipitation from 24 h storage at room temperature. However, the formulation #F with Tween 80, NMP, ethanol and water displayed clear solutions without any visual precipitation of the drug even after 8 weeks of storage. Hence, we decided to proceed with *in vivo* bioavailability studies for the formulation #F, which is composed of NMP, ethanol and Tween 80 at 15% (v/v), 20% (v/v), and 25% (w/v), respectively.

We observed that the solubility of curcumin in NMP, a pharmaceutically acceptable strong solubilizing agent, was quite high (125.07 mg/ml). Hence, NMP was chosen as the solubilizing agent in our formulation. There was a remarkable increase in the solubility of curcumin in the co-solvent formulation (20 mg/mL) even after the incorporation of 40% aqueous phase. Ethanol (at 20% v/v) was found to be a suitable additive in the formulation, as we observed a clear solution of high miscibility and compatibility with water (up to 40%). However at a lower concentration of 10% v/v, ethanol was found to be insufficient to prevent precipitation of free curcumin, observed after 60 h (Table I). Therefore, ethanol was fixed at a concentration of 20% v/v.

Among the different percentage compositions of the non-ionic surfactants considered, including PEG 400 and Tween 80, the latter surfactant was picked as an ingredient in the optimal formulation at a concentration of 25% w/v. This was purely based on the solubility, clarity and miscibility of Tween80 at this concentration with the rest of the additives in the formulation. It was also found that a percentage concentration of 15% w/v is insufficient to prevent the precipitation of the free curcumin, which as we observed precipitatation after 2 weeks. Hence we decided to optimize the formulation with Tween80 at a percentage concentration of 25% w/v.

Among the different co-solvent formulations (#A-F) of curcumin we developed, a co-solvent system (Formulation F) consisting of NMP (15% v/v), ethanol (20% v/v), Tween80 (25% w/v) showed a high solubility of 20 mg/ml curcumin in 40% of aqueous medium with no precipitation upon storage at room temperature for the entire study period of 8 weeks. Therefore, this formulation was selected for *in vivo* bioavailability studies and further evaluations.

In vivo pharmacokinetic behavior of curcuminc co-solvent formulation. Figure 1 shows mean ± SD plasma concentration versus time profile of curcumin in Sprague-Dawley rats. Figure 2 shows the mean plasma concentration *vs*. time curve of curcumin in male Sprague-Dawley rats after 50 mg/kg intramuscular administrations of curcumin co-solvent formulation (open circles) and controlled curcumin solution (solid black circles). The mean plasma concentration of curcumin was significantly higher in the co-solvent formulation group (open circles) in comparison with the controlled pure curcumin solution group (solid black circles) throughout the entire 8-h sampling period. The pharmacokinetic parameters are summarized in Table II and statistical comparisons are performed after dose normalization. Following intravenous administration, curcumin displayed a multi-exponential disposition with a rapid decline of the initial plasma concentration from 192.41 µg/ml to less than 100 ng/ml within 1 h post-dose administration. There was a 29-fold increase in the maximum plasma concentration (C_{max}) and a 28-fold increase in the

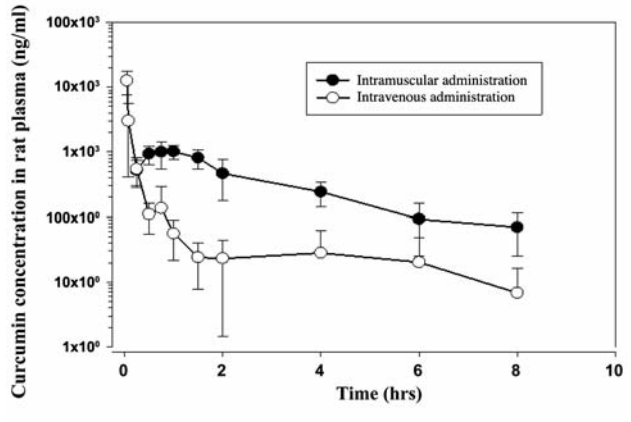

Figure 1. *Mean±SD plasma concentration-time profile of curcumin in male Sprague-Dawley rats after 50 mg/kg intramuscular administration of curcumin co-solvent formulation and 30 mg/kg intravenous administration of curcumin co-solvent formulation.*

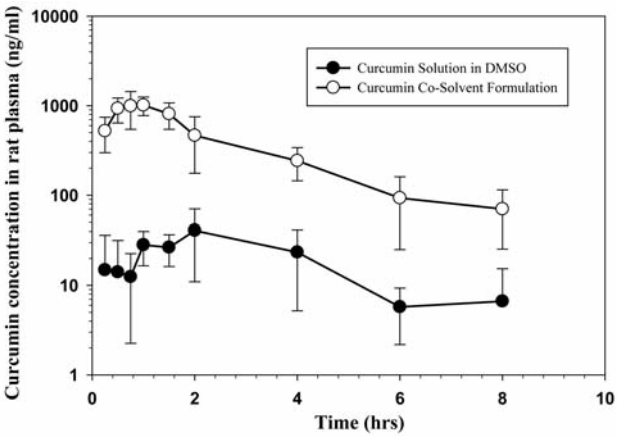

Figure 2. *Mean±SD plasma concentration-time profile of curcumin in male Sprague-Dawley rats after 50 mg/kg intramuscular administration of curcumin co-solvent formulation and curcumin solution in DMSO (Control).*

area under the curve (AUC) for the co-solvent formulation group as compared to the reference group, which was administered with 50 mg/kg intramuscular dose of control curcumin solution in DMSO. Moreover, the intramuscular injection maintained plasma concentrations above 240 ng/ml for up to 4 h after drug administration. The absolute bioavailability of curcumin in our co-solvent curcumin formulation was determined to be as high as 30% after an intramuscular dose of 50 mg/kg. The relative bioavailability of our co-solvent formulation was 2,818% compared to the reference curcumin solution in DMSO.

Acute toxicity. No signs of discomfort or any cardiovascular or respiratory disorders were observed in animals after the administration of the co-solvent formulation or curcumin solution in SMSO solution and throughout the study period. There was no change in body weight monitored for a 7-day period after drug administrations.

Discussion

We have developed an optimal curcumin co-solvent formulation with a high solubility, significantly increased bioavailability and sustained plasma concentrations of curcumin. This formulation is unique in its attempt to formulate curcumin as a liquid co-solvent formulation suitable for intramuscular administration sustaining its plasma levels. Intramuscular injection of curcumin co-solvent formulation resulted in a sustained release of curcumin in rat plasma. This is evident from the fact that the intramuscular injection maintained plasma concentrations above 240 ng/ml for up to 4 h after drug administration and the mean plasma concentration of curcumin was significantly higher in the co-solvent formulation-group compared to the control group throughout the entire sampling time. Even after oral and intravenous administration of novel drug delivery systems of curcumin such as nanoparticles, there was a rapid decline of the plasma levels of curcumin to 100 ng/mL or lower, within 1 h post-dose (31, 32). Similarly, after an intravenous administration of our co-solvent formulation, there was a rapid decline in the initial plasma concentration from 192.41 μg/mL to less than 100 ng/ml within 1 h post-dose. This is in agreement with the concentration-time profile reported in pharmacokinetic studies of advanced drug delivery systems of curcumin by Mohanty *et al.* and others (32, 33). Curcumin displayed a bi-exponential disposition after intravenous administration and the observed pharmacokinetics in the dose-range used were linear.

Intramuscular route is a well-known and widely used route of drug administration. It improves patient compliance by decreasing frequency of administration, with reduced gastrointestinal side effects. It also avoids the first-pass effect, which is the primary cause of low oral bioavailability of curcumin (15, 16). Intramuscular injection delivers medication into dense muscle below subcutaneous tissues. In comparison with adipose tissues, skeletal muscles absorb larger volumes of fluid due to the rapid uptake of the drug into the blood stream via muscle fibers (34). Thus, intramuscular injections can be used to inject concentrated drugs that could damage subcutaneous tissue. Moreover, inter-subject variability in the extent of oral absorption could also be avoided by intramuscular administration (27).

Previous studies have demonstrated that intramuscular administration of novel drug delivery systems can be used for the sustained delivery of many drugs with various therapeutic activities (26-28). In a similar study, Wei *et al.* has reported

Table II. *Comparative pharmacokinetic parameters for curcumin in male Sprague-Dawley rats.*

PK Parameter	Co-solvent formulation	Curcumin solution in DMSO	Co-solvent formulation
Route	Intravenous	Intramuscular	Intramuscular
Dose (mg/kg)	30	50	50
Number of rats	5	5	5
C_{max} (µg/ml)	192.41±110.24	0.043±24.7	1.252±25**
AUC (µg h/ml)	5.91±2.7	0.103±86.8	2.903±0.43**
Half-life (h)	2.78±2.0	2.47±0.67	1.90±0.4
Vd/F(l/kg)	7.02±4.4	986.39±554.19	43.95±12.9**
Cl/F (l/min/kg)	28.35±2.0	270.45±115.78	16.42±2.6**
MRT (h)	0.13±0.1	3.00±0.8	2.18±0.5
F_{abs}			30%
F_{rel}			2818%

Values are expressed as the Mean±SD. Cmax: maximum plasma concentration; $AUC_{0-\infty}$: total area under the plasma concentration *vs*. time curve; Half-life: terminal elimination half-life; V_d: apparent volume of distribution; Cl: systemic plasma clearance; MRT: mean residence time; F_{abs}: absolute bioavailability; F_{rel}: relative bioavailability. **$p<0.01$.

that intramuscular administration of a nanosuspension containing curcumin didecanoate, a pro-drug of curcumin, can increase the bioavailability and sustain the therapeutic levels of curcumin (35). However, after an intramuscular dose of 100 mg/kg of the aforementioned nanosuspension formulation, C_{max} and AUC were 69 ng/ml and 239 ng/mL, respectively, whereas our co-solvent formulation resulted in a much higher C_{max} (1,252 ng/ml) and AUC (2,903 ng/mL) values after an intramuscular dose of 50 mg/kg. Our statistically significant increase in C_{max} and AUC compared to control curcumin solution can be attributed to the superior solubilizing efficiency of co-solvents such as NMP and ethanol as well as surfactants such as Tween 80, which prevent precipitation of the drug in aqueous media and enhance its absorption into the tissues. This resulted in a 28-fold increase in the relative bioavailability of our co-solvent formulation compared to pure curcumin solution in DMSO.

The objective of this study was to develop a liquid formulation of curcumin with enhanced solubility, bioavailability and sustained therapeutic efficacy. The use of water-soluble organic solvents, non-ionic surfactants and lipids can enhance the solubility and permeability of poorly-soluble drugs (36). This strategy has also been widely used to increase the bioavailability of drugs such as ritonavir and saquinavir, propofol and of chemotherapeutics like paclitaxel (36). Among these, PEG, ethanol and Tween 80 are some of the major water-miscible organic solvents widely used in commercially available formulations. In addition NMP is a major pharmaceutical co-solvent widely used in oral and parenteral medications (37). It is a biodegradable solubilizing agent with a high safety profile (38). We observed a significant increase in the solubility of curcumin from 11 ng/ml to 125 mg/ml when NMP was used as a solubilizing agent. NMP's solubilization maybe due to its simultaneous reaction as a co-solvent and a complexing agent (39). This mechanism enables it to solubilize even highly lipophilic compounds like curcumin at low concentrations compared to other common co-solvents. This was evident from the 10,000-fold increase in aqueous solubility of curcumin when NMP was used as a co-solvent at a concentration of 15% (v/v) in our formulation. Even though NMP is used in a high percentage composition (55-65%) in marketed pharmaceutical products such as leuprolide acetate, we could successfully solubilize curcumin at a percentage composition of 15% (v/v) without any visual precipitation in a 40% aqueous phase. Although PEG could solubilize curcumin effectively, it was excluded during the optimization process due to the formation of precipitate upon mixing with the other ingredients in the formulation. Ethanol and Tween 80 at a concentration of 20% (v/v) and 25% (w/v), respectively, were chosen due to their superior solubility, clarity and miscibility when combined with the other ingredients in the formulation. Hence, the excipients used in our co-solvent formulation enhance the desired solubility and permeability for curcumin to reach the systemic circulation.

This novel co-solvent formulation with relatively simple processing steps was found beneficial in increasing the aqueous solubility of this highly lipophilic molecule which comes under the BCS IV. This optimized formulation could circumvent the major disadvantages of encapsulation-based drug delivery systems such as poor drug loading, poor release characteristics and batch-to-batch variabilities. Also, relative to coarse dispersions like suspensions and emulsions, aqueous solutions such as co-solvent solutions allow for control over the drug release and can be more easily drawn into a syringe (syringe-ability) and more readily ejected from the syringe (injectability) (40). The significant improvement in the

bioavailability of our formulation could allow for a decrease in the dose and frequency of administration required to achieve and maintain therapeutic levels of curcumin.

In summary, a co-solvent formulation containing 20 mg/ml curcumin was developed and optimized. This formulation increased the aqueous solubility of curcumin. Furthermore, intramuscular administration of the co-solvent formulation resulted in 30% absolute bioavailability, a significantly increased relative bioavailability and sustained plasma levels of curcumin for a prolonged period of time in male adult Sprague-Dawley rats. However further studies including clinical trials are warranted for the transition of this herb from bench to bedside.

References

1 Aggarwal BB, Sundaram C, Malani N *et al*: Curcumin the Indian solid gold. Adv Exp Med Biol *595*: 1-75, 2007.

2 Shukla PK, Khanna VK, Khan MY *et al*: Protective effect of curcumin against lead neurotoxicity in rat. Hum Exp Toxicol *22*: 653-658, 2003.

3 Abe Y, Hashimoto S and Horie T: Curcumin inhibition of inflammatory cytokine production by human peripheralblood monocytes and alveolar macrophages. Pharmacol Res *39*: 41-47, 1999.

4 Pari L and Murugan P: Antihyperlipidemic effect of curcumin and tetrahydrocurcumin in experimental type 2 diabetic rats. Ren Fail *29*: 881-889, 2007.

5 Aggarwal BB, Kumar A and Bharthi AC: Anticancer potential of curcumin: preclinical and clinical studies. Anticancer Res *23*: 363-398, 2003.

6 Liu D, Schwimer J, Liu Z *et al*: Anti-angiogenic effect of curcumin in pure versus in extract forms. Pharm Biol *46*: 677-682, 2008.

7 Shankar TN, Shantha NV, Ramesh HP *et al*: Toxicity studies on turmeric (Curcuma longa): acute toxicity studies in rats, guinea pigs and monkeys. Indian J Exp Biol *18*: 73-75, 1980.

8 Qureshi S, Shah AH and Ageel AM: Toxicity studies on Alpiniagalanga and Curcuma longa. Planta Med *58*: 124-127, 1992.

9 Lao CD, Demierre MF and Sondak VK: Targeting events in melanoma carcinogenesis for the prevention of melanoma. Expert Rev Anti-cancer Ther *6*: 1559-1568, 2006.

10 Lao CD, Ruffin MT, Normolle D *et al*: Dose escalation of a curcuminoid formulation. BMC Complement Altern Med *6*: 10, 2006.

11 Cheng AL, Hsu CH, Lin JK *et al*: Phase I clinical trial of curcumin, a chemo preventive agent in patients with high-risk or pre-malignant lesions. Anticancer Res *21*: 2895-2900, 2001.

12 Wahlang B, Pawar YB and Bansal AK: Identification of permeability-related hurdles in oral delivery of curcumin using the Caco-2 cell model. Euro J Pharm Biopharm *77*: 275-282, 2011.

13 Tonnesen HH: Solubility, chemical and photochemical stability of curcumin in surfactant solutions. Studies of curcumin and curcuminoids XXVII. Pharmazie *57*: 820-824, 2002.

14 Tonnesen HH, Masson M and Loftsson T: Studies of curcumin and curcuminoids XXVII. Cyclodextrin complexation: Solubility, chemical and photochemical stability. Int J Pharm *244*: 127-135, 2002.

15 Yang KY, Lin LC, Tseng TU *et al*: Oral bioavailability of curcumin in rat and the herbal analysis from Curcuma longa by LC-MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci *853*: 183-189, 2007.

16 Shoba G, Joy D, Joseph T *et al*: Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. Planta Med *64*: 353-356, 1998.

17 Onoue S, Takahashi H, Kawabata Y *et al*: Formulation design and photochemical studies on nanocrystal solid dispersion of curcumin with improved oral bioavailability. J Pharm Sci *99*: 1871-1881, 2010.

18 Seo SW, Han HK, Chun MK *et al*: Preparation and pharmacokinetic evaluation of curcumin solid dispersion using Solutol® HS15 as a carrier. Int J Pharm *424*: 18-25, 2012.

19 Vasconcelos T, Sarmento B and Costa P: Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs. Drug Discov Today *12*: 1068-1075, 2007.

20 Takahashi M, Uechi S, Takara K *et al*: Evaluation of an oral carrier system in rats: bioavailability and antioxidant properties of liposome-encapsulated curcumin. J Agric Food Chem *57*: 9141-9146, 2009.

21 Mohanty C and Sahoo SK: The *in vitro* stability and in-vivo pharmacokinetics of curcumin prepared as an aqueous nanoparticulate formulation. Bio-materials *25*: 6597-6611, 2010.

22 Anand P, Kunnumakkara AB, Newman RA *et al*: Bioavailability of curcumin: problems and promises. Mol Pharm *4*: 807-818, 2007.

23 Bansal SS, Goel M, Aqil F *et al*: Advanced drug delivery systems of curcumin for cancer chemoprevention. Cancer Prev Res (phila) *4*: 1158-1171, 2011.

24 Savjani KT, Gajjar AK and Savjani JK: Drug solubility: Importance and enhancement techniques. ISRN Pharm 195727, 2012.

25 RabinowBE: Nanosuspensions in drug delivery. Nat Rev Drug Discov *3*: 785-796, 2004.

26 Salem HF: Sustained-release progesterone nanosuspension following intramuscular injection in ovariectomized rats. Int J Nanomedicine *5*: 943-954, 2010.

27 Rhee YS, Sohn M, Woo BH *et al*: Sustained-release delivery of octreotide from biodegradable polymeric microspheres. AAPSPharmSciTech *4*: 1293-1301, 2011.

28 Ahmed TA, Ibrahim HM, Ibrahim F *et al*: Development of biodegradable *in situ* implant and microparticle injectable formulations for sustained delivery of haloperidol. J Pharm Sci *101*: 3753-3762, 2012.

29 Xiong MP, Yariez JA, Remsberg CM *et al*: Formulation of a geldanamycinprodrug in mPEG-b-PCL micelles greatly enhances tolerability and pharmacokinetics in rats. J Control Release *129*: 33-40, 2008.

30 Liu A, Lou H, Zhao L *et al*: Validated LC/MS/MS assay for curcumin and tetrahydrocurcumin in rat plasma and application to pharmacokinetic study of phospholipid complex of curcumin. J Pharm Biomed Anal *40*: 720-727, 2006.

31 Shaikh J, Ankola DD, Beniwal V *et al*: Nanoparticle encapsulation improves oral bioavailability of curcumin by at least 9-fold when compared to curcumin administered with piperine as absorption enhancer. Eur J Pharm Sci *37*: 223-230, 2009.

32 Anand P, Nair HB, Sung B *et al*: Design of curcumin-loaded PLGA nanoparticles formulation with enhanced cellular uptake, and increased bioactivity *in vitro* and superior bioavailability in-vivo Biochem Pharmacol *79*: 330-338, 2010.

33 Mohanty C and Sahoo SK: The *in vitro* stability and *in vivo* pharmacokinetics of curcumin prepared as an aqueous nanoparticulate formulation. Bio-materials *25*: 6597-6611, 2010.
34 Walters MC and Furyk J: Paediatric Intramuscular Injections for Developing World Settings: A Review of the Literature for Best Practices. J Transcult Nurs *23*: 406-409, 2012.
35 Wei XL, Han YR, Quan LH *et al*: Oily nanosuspension for long-acting intramuscular delivery of curcumin didecanoate prodrug: Preparation characterization and *in vivo* evaluation. Eur J Pharm Sci *49*: 286-293, 2013.
36 Strickley RG: Solubilizing excipients in oral and injectable formulations. Pharm Res 21: 201-300, 2004.
37 Avdeef A: Solubility of sparingly-soluble ionizable drugs. Adv Drug Deliv Rev *59*: 568-590, 2007.
38 Chow ST and Ng TL: The biodegradation of N-methyl-2-pyrrolidone in water by sewage bacteria. Water Res *17*: 117-118, 1983.
39 Jouyban A, Fakhree MA and Shayanfar A: Review of pharmaceutical applications of N-methyl-2-pyrrolidone. J Pharm Pharm Sci *13*: 524-535, 2010.
40 Singh R and Lillard JW Jr.: Nanoparticle-based targeted drug delivery. Exp Mol Pathol *86*: 215-223, 2009.

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