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Preparation and pharmacokinetic evaluation of curcumin solid dispersion using Solutol® HS15 as a carrier

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ABSTRACT

Solubility of curcumin at physiological pH was significantly increased by forming solid dispersion (SD) with Solutol® HS15. Since curcumin undergoes hydrolytic degradation, chemical stability study was conducted in pH 1.2, 6.8 and 7.4 buffer media. Solutol® HS15 exhibited superior stabilizing effect to Cremophor® RH40 and Kollidon® 30. The physical state of the dispersed curcumin in the polymer matrix was characterized by differential scanning calorimetry and X-ray diffraction studies. SD preparation transformed curcumin into amorphous form and facilitated micellar incorporation, thereby preventing hydrolysis in aqueous medium. *In vitro* drug release in pH 6.8 buffer revealed that SD (1:10) improved the dissolution of curcumin with approximately 90% release of the drug within 1 h. Pharmacokinetic study of the solid dispersion formulation in rat showed that bioavailability of the drug was significantly improved as compared to pure curcumin. SD containing 1:10 ratio of drug and Solutol® HS15 resulted in approximately 5 fold higher $AUC_{0-12\,h}$. SD formulation was physically stable over the study period of 3 months.

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1. Introduction

Curcumin is a hydrophobic polyphenol derived from the rhizome of the herb *Curcuma longa* L. (turmeric). It is a principal component of turmeric, used for centuries in Asian countries as a spice and also an herbal remedy. Chemically, curcumin is a bis-α,β-unsaturated β-diketone (commonly called diferuloylmethane, Fig. 1), which exhibits keto-enol tautomerism having a predominant keto form in acidic and neutral solution and stable enol form in alkaline medium. Turmeric has been used traditionally for many ailments, particularly as an anti-inflammatory agent, because of its wide spectrum of pharmacological activity. Curcumin has been shown to exhibit antioxidant, anti-inflammatory, antimicrobial and anticarcinogenic activities (Kuttan et al., 1985; Reddy et al., 2005; Srimal and Dhawan, 1973). It also has hepato and nephro-protective, thrombosis suppressing, myocardial infarction protective, hypoglycemic, and antirheumatic activities (Babu and Srinivasan, 1997; Deodhar et al., 1980; Kiso et al., 1983; Nirmala and Puvanakrishnan, 1996; Srivastava et al., 1985; Venkatesan et al., 2000). Moreover, various animal models or human studies proved that curcumin is extremely safe even at very high doses (Shankar et al., 1980; Shoba et al., 1998). Due to its pharmacological efficacy and safety, curcumin has been investigated in a wide range of research area *in vitro* and *in vivo*, in animal and human studies.

In spite of its various pharmacological activities and high safety, poor oral bioavailability can be one of the limiting factors in the clinical development of curcumin. The main reasons for low bioavailability of curcumin are its extremely low solubility in water, acidic and physiological pH, and its rapid hydrolysis under alkaline conditions (Tønnesen and Karlsen, 1985). The aqueous solubility of curcumin can be improved by increasing the pH of the solution. However, this approach leads to an undesirable outcome: a high rate of degradation by alkaline hydrolysis (Wang et al., 1997). So, several other strategies such as nanoparticle, liposome, micelles, phospholipid complex, and solid dispersion have been evaluated to enhance the bioavailability of curcumin (Anand et al., 2007; Paradkar et al., 2004).

Despite the vast literature on strategies of increasing bioavailability of curcumin, there are few studies on physicochemical stability of curcumin. A lot of studies just focused on the solubility of curcumin but not both solubility and stability. Within this limited number of physicochemical stability studies, Tønnesen (2002) have investigated the chemical stability of curcumin at pH 5 and 8 in a number of surfactant solution, including sodium dodecyl sulfate (SDS), Triton X-100 (TX-100 and tetradecyl trimethylammonium bromide (TTAB)). The results indicate that SDS and TX-100 micelles are highly effective in stabilizing curcumin, which increases the chemical stability by nearly 1800 times relative to a solution where these micelles are absent. Mandy et al. (2008) has investigated

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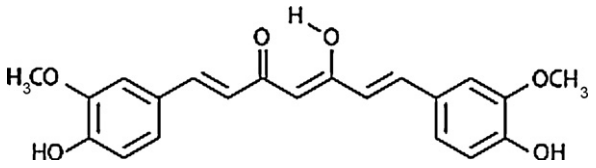

Fig. 1. Chemical structure of curcumin.

on suppressing alkaline hydrolysis of curcumin by encapsulation using cationic micelles composed of dodecyl trimethylammonium bromide (DTAB) and cetyl trimethylammonium bromide (CTAB) surfactants at pH 13. The ability of cationic micelles to stabilize the deprotonated curcumin is due to its attractive electrostatic interaction with the CTBA and DTAB head groups. As discussed earlier, in both stability studies, ionic surfactants were utilized and stability was evaluated in solution form. From drug delivery point of view, ionic surfactants are less favored owing to their relative higher toxicity compared with non-ionic surfactants.

Solid dispersion (SD) is one of the most promising strategies to improve the oral bioavailability of poorly water soluble drugs *via* the enhancement of their solubility and dissolution rate. In SD system, drug undergoes particle size reduction and the consequent increase in the surface area results in the improved dissolution (Graig, 2002). Moreover, no energy is required to break up the crystal lattice of a drug in the amorphous state during dissolution process, and drug solubility and wettability may be increased by surrounding hydrophilic carriers (Taylor and Zografi, 1997). Recently, it has been shown that the dissolution profile can be improved if the solid dispersion carrier has surface activity or self-emulsifying properties (Karata et al., 2005) Solid dispersions prepared by using surfactants are intended to achieve the highest degree of bioavailability for poorly soluble drugs and stabilize the solid dispersion, avoiding drug recrystallization. The use of surfactants such as Inutec® SP1, Gelucire® 44/14, poloxamer 407 as carriers is found to be effective in enhancement of bioavailability (Majerik et al., 2007; Van den Mooter et al., 2006; Yuksel et al., 2003).

It is expected that using surfactant as a carrier for the preparation of the solid dispersion of curcumin could enhance both solubility and chemical stability of curcumin at physiological pH. The objective of this study is to enhance solubility, stability and bioavailability of curcumin *via* solid dispersion preparation.

2. Materials and methods

2.1. Materials

Curcumin was purchased from Sigma Co. (St. Louis, MO, USA, Purity: 94%, melting point: 175 °C). Lauroyl macrogolglycerides (Gelucire® 44/14, melting point: 44 °C) and stearoyl macrogolglyceride (Gelucire® 50/13, melting point: 55 °C) were obtained from Gattefosse Korea (Seoul, South Korea). Poloxamer 188 (Lutrol® F 68, melting point: 52 °C), poloxamer 407 (Lutrol® F 127, melting point: 56 °C), polyoxyl 40 hydrogenated castor oil (Cremophor® RH40, melting point: 28 °C), polyethylene glycol-15-hydroxystearate (Solutol® HS15, melting point: 30 °C) and polyvinylpyrrolidone K30 (Kollidon® 30, melting point: 150 °C) were obtained from BASF (Ludwigshafen, Germany). Polyethylene glycol 400 (PEG 400) and polyethylene glycol 20,000 (PEG 20000, melting point: 63 °C) were purchased from Junsei Chemical Co. (Tokyo, Japan). Hydroxypropyl methylcellulose 2910 (HPMC 2910, melting point: 180 °C) was purchased from Shin-Etsu Chemical Co. (Tokyo, Japan). All other chemicals were of reagent grade and used as received without further purification.

2.2. Methods

2.2.1. Analysis of curcumin by HPLC

The amount of curcumin was determined by using a high-performance liquid chromatography (HPLC) system (Shimadzu Scientific Instrument, MD, USA), consisting of a UV detector (SPD-10A), a pump (LC-10AD) and an automatic injector (SIL-10A). Samples in buffer solution were analyzed with the mobile phase consisting of acetonitrile and 1% (w/v) citric acid buffer (pH 3) at the ratio of 51:49% (v/v) with flow rate of 1 mL/min. Samples from animal study were analyzed with the mobile phase consisting of acetonitrile and 5% (v/v) acetic acid at the ratio of 52:48% (v/v) with the flow rate of 1 mL/min. The wavelength of the UV detector was 423 nm and a reversed-phase column (CAPCELL PAK C18 UG120 S5, Shiseido, Japan) was used. The samples were analyzed at a column temperature of 30 °C.

2.2.2. Screening of carriers

2.2.2.1. Preparation of solid dispersions. To determine the optimum carrier, solid dispersions (SDs) of curcumin with various surface active or non-surface active carriers, at the weight ratio of 1:10, were separately prepared by conventional solvent evaporation method. Curcumin and carriers were dissolved in minimum volume of acetone (10 mg curcumin/mL) except for HPMC 2901 and PEG 20000 which were dissolved in dichloromethane. After sonicating for 20 min, the solvent was removed under vacuum at room temperature.

2.2.2.2. Solubility studies. SDs of curcumin prepared with various surface active or non-surface active carriers at weight ratio of 1:10 was subjected for solubility test. SD equivalent to 5 mg of curcumin was added to 2 mL of pH 1.2, 6.8, 7.4 buffers and stirred at 400 rpm for 12 h at room temperature. The solubility of curcumin reached plateau value within 12 h. The samples were then centrifuged at 16,000 × *g* for 20 min and filtered through 0.45 μm pore-sized regenerated cellulose syringe filter (Target®, National scientific, USA). The filtrates were suitably diluted with methanol and analyzed by HPLC. The experiment was performed in triplicates.

2.2.2.3. Chemical stability studies. The chemical stability of curcumin was investigated in pH 1.2, 6.8 and 7.4 buffers. Four types of each buffer solution was used: buffer with no surfactant, buffer with 10% (w/v) Kollidon® 30, buffer with 10% (w/v) Solutol® HS15 and buffer with 10% (w/v) Cremophor® RH40. Stock solution of curcumin was prepared in methanol at a concentration of 1000 μg/mL. Each aliquot of the stock solution was 100 times diluted with one of the aforementioned buffer solutions. The samples were filtered through 0.45 μm pore-sized regenerated cellulose syringe filter and then filtrates were stored at 37 °C oven. Samples were withdrawn at predetermined time intervals and analyzed by HPLC.

2.2.3. Characterizations of solid dispersion

2.2.3.1. Effect of preparation method, pH of the medium and carrier ratio on solubility of curcumin. Solid dispersion (SD), physical mixture (PM) and melting mixture (MM) of curcumin with Solutol® HS15 at the weight ratio of 1:10 were prepared. SD was prepared as described before whereas PM was prepared by simply mixing curcumin and Solutol® HS15 in mortar and pestle. The mixing process was done as gentle as possible to minimize the possibility of conversion of curcumin into amorphous form. MM of curcumin with Solutol® HS15 were obtained by mixing curcumin with melted Solutol® HS15 with the help of stirrer at 40 °C for 1 h and solidifying at room temperature for 1 h. Solubility of curcumin from SD, PM and MM was determined at each of pH 1.2, 6.8 and 7.4 buffers by

taking 5 mg equivalent of curcumin in 2 mL of the medium. Solubility studies were conducted in same condition as described before. To study the effect of carrier ratio, SD and MM of curcumin with Solutol® HS15 were prepared at four different weight ratios of 1:5, 1:8, 1:10 and 1:20, respectively, and solubility from each sample was determined.

2.2.3.2. Differential scanning calorimetry (DSC). Thermal analysis was carried out using a DSC unit (Pyris 6 DSC, Perkin Elmer, Netherlands). Indium was used to calibrate the temperature scale and enthalpic response. Samples were placed in aluminum pans and heated at a scanning rate of 10 °C/min from 20 °C to 200 °C.

2.2.3.3. X-ray diffraction (XRD). X-ray powder diffraction was performed at room temperature with an X-ray diffractometer (X'Pert PRO MPD, PANalytical Co., Holland). The diffraction pattern was measured with a voltage of 40 kV and a current of 30 mA over a 2θ range of 3–40° using a step size of 0.02° at a scan speed of 1 s/step.

2.2.3.4. Dissolution tests. Dissolution tests of pure curcumin, PM and SD samples were performed in a dissolution tester (DST-810 and DS-600A, Labfine, Inc., Suwon, Korea) at the paddle rotation speed of 50 rpm in 900 mL of pH 6.8 phosphate buffer maintained at 37 ± 0.5 °C. Each formulation equivalent to 20 mg of curcumin was filled into size '2' hard gelatin capsule. Capsules were then placed inside the sinker and put into dissolution vessel. At the predetermined time intervals, 3 mL of the sample was withdrawn and the equal volume of fresh medium was added into dissolution vessel. The collected samples were filtered through regenerated cellulose syringe filters. Initial sample volume of 2 mL was discarded, and then final 1 mL was suitably diluted with methanol. Then samples were analyzed by HPLC.

2.2.3.5. Physical stability tests. The prepared SDs were stored in air tight container protected from light at room temperature. They were then analyzed for solubility at pH 6.8 phosphate buffer periodically. Physical stability was evaluated based on solubility of curcumin from solid dispersion with respect to time.

2.2.4. In vivo studies

2.2.4.1. Animal. Male Sprague–Dawley rats (240–280 g) were purchased from Samtako Bio Co. (Osan, Korea), and had free access to normal standard chow diet (Superfeed Company, Wonju, Korea) and tap water. All animal studies were performed in accordance with the "Guiding Principles in the Use of Animals in Toxicology" adopted by the Society of Toxicology (USA).

2.2.4.2. Dosing and sampling. Rats were fasted for 24 h prior to the beginning of experiments. The rats were divided into three groups. All groups received curcumin at a dose level of 50 mg/kg by p.o.: Group 1 [curcumin suspension in water with 5% (v/v) PEG 400, $n = 5$], Group 2 (1:5 SD, $n = 6$) and Group 3 (1:10 SD, $n = 6$). Blood samples were collected from the femoral artery at 0.25, 0.5, 0.75, 1, 2, 4, 8 and 12 h post-dose. Blood samples were centrifuged at 16,000 × *g* for 3 min and the obtained plasma was stored at −40 °C until analyzed.

2.2.4.3. Sample preparation. Plasma samples were thawed in a water bath at 37 °C. The plasma sample (100 μL) was transferred to a 2 mL eppendorf tube and hydrochloric acid (0.1 M; 10 μL) was added to plasma sample with thorough mixing. Then 950 μL acetonitrile was added and the mixture was vortex-mixed for 20 min. After centrifugation at 13,000 rpm for 10 min, all supernatant was transferred to a new 2 mL eppendorf tube and evaporated to dryness at 50 °C. The residue was reconstituted with 100 μL of the mobile phase and the solution was vortex-mixed. After centrifugation at 13,000 rpm for 3 min, 50 μL of the solution was injected into the HPLC system for analysis.

2.2.5. Pharmacokinetic parameters

Noncompartmental analysis was performed by using WinNonlin software version 5.2.1 (Pharsight Co., Mountain View, CA, USA). The area under the plasma concentration–time curve ($AUC_{0-12\,h}$) was calculated using the linear trapezoidal method. The peak plasma concentration (C_{max}) and the time to reach the peak plasma concentration (T_{max}) were observed from the values of experimental data. The elimination rate constant (K_{el}) was estimated by regression analysis from the slope of the line of best fit, and the half-life ($t_{1/2}$) of the drug was obtained by $0.693/K_{el}$.

2.2.6. Statistical analysis

All the means were presented with their standard deviation. The statistical significance of the difference in the parameters was determined using ANOVA followed by Dunnett's test or by a Student's *t*-test. *P* value < 0.05 was considered statistically significant.

3. Results and discussion

3.1. Screening of carriers

Carriers with better affinity and miscibility with drugs are known to bring better enhancement in solubility of the drug (Laitinen et al., 2009). In order to find optimum carrier for curcumin, solubility of curcumin was measured using SDs prepared with various surface active or non-surface active carriers at the weight ratio of 1:10. Low pH (1.2) buffer was used for solubility study since curcumin is more stable at lower pH (Tønnesen and Karlsen, 1985). Furthermore, based on results of solubility study, carriers were short-listed for chemical stability study of curcumin to select the optimum carrier for solid dispersion preparation.

Poloxamer 188, poloxamer 407, Solutol® HS15, Cremophor® RH40, Gelucire® 50/13 and Gelucire® 44/14 were used as surface active carriers whereas non-surface active carriers screened included Kollidon® 30, PEG 20000 and HPMC 2910. As shown in Table 1, Kollidon® 30 and Solutol® HS15 were found to be most effective in enhancing the solubility of curcumin. In general, improved solubility of drug from solid dispersion depends on the capability of carrier to disperse drug in amorphous form or to arrest the particle size growth of crystals to minimum, and to increase the saturation solubility of the drug in aqueous state. Transparent mass were obtained from SDs with Kollidon® 30, HPMC 2910, Solutol® HS15 and Cremophor® RH40, indicating that the drug was dispersed in the carriers molecularly. Despite transparent appearance of SD prepared with HPMC 2910, low solubility of curcumin in pH 1.2 buffer was observed. This indicated inability of HPMC 2910 to maintain high supersaturation concentration of curcumin in the medium. Cremophor® RH40, however, showed relatively better

Table 1
Solubility of curcumin from SD in pH 1.2 buffer (mean ± S.D., $n = 3$).

	Carriers	Solubility (μg/mL)
Surface active	Solutol® HS15	560.7 ± 55.8
	Cremophor® RH40	371.9 ± 35.2
	Gelucire® 50/13	319.8 ± 15.9
	Gelucire® 44/14	311.4 ± 26.3
	Poloxamer 407	138.4 ± 14.8
	Poloxamer 188	15.1 ± 4.5
Non-surface active	Kollidon® 30	575.0 ± 33.8
	PEG 20000	13.1 ± 3.8
	HPMC 2910	9.3 ± 2.3

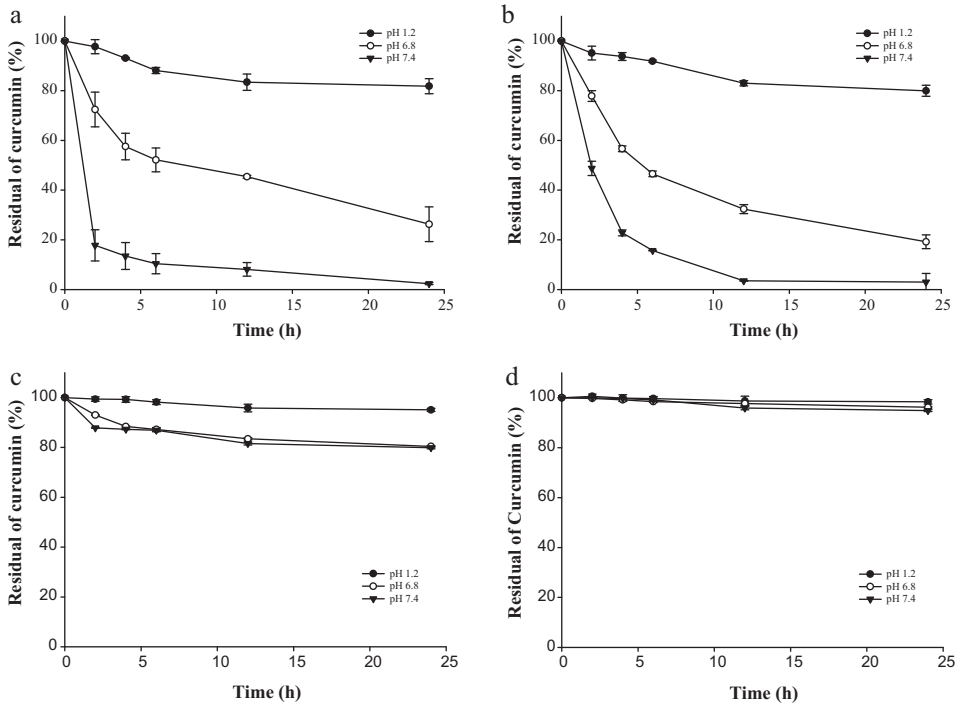

Fig. 2. Chemical stability of curcumin in (a) buffers. (b) buffers containing 10% (w/v) Kollidon® 30, (c) buffers containing 10% (w/v) Cremophor® RH40 and (d) buffers containing 10% (w/v) Solutol® HS15 (mean ± S.D., $n = 3$).

solubilization of the drug as compared to HPMC 2910 but not to that extent as compared to Kollidon® 30 and Solutol® HS15. Solubility enhancement brought by Kollidon® 30 and Solutol® HS15 were similar and significantly higher than other carriers screened. And, it was also observed that SDs prepared with surface active carriers provided higher solubility than non-surface active carriers except for Kollidon® 30. Surface active carriers have additional solubilizing effect due to lowering of interfacial tension and micelle formation. Physicochemical properties of the carriers are influential and preferred characteristics of the carriers vary with nature of the drug used. The results obtained from surface active carriers suggested that solubility can be affected by HLB value or more specific carrier–drug interactions. Surface active carriers with high HLB value (HLB value of poloxamer 188 and 407 are 29 and 22, respectively) provided lower solubility of curcumin, however, higher solubility of curcumin obtained from Solutol® HS15 and other surface active carriers with similar HLB value (HLB of Solutol® HS15, Cremophor® RH40, Gelucire® 50/13 and Gelucire® 44/14 are 14-16, 14-16, 13 and 14, respectively), suggesting the role of more specific carrier–drug interaction.

Curcumin being susceptible for hydrolysis, stability in aqueous medium is crucial for the bioavailability of curcumin (Tønnesen and Karlsen, 1985). The hydrolytic degradation of curcumin was examined in pH 1.2, 6.8 and 7.4 buffers, respectively, at various time intervals. Furthermore, the effect of 10% (w/v) Kollidon® 30, 10% (w/v) Solutol® HS15 and 10% (w/v) Cremophor® RH40 on the stability of curcumin at pH 1.2, 6.8 and 7.4 buffers was also investigated. Kollidon® 30, Solutol® HS15 and Cremophor® RH40 were selected for the chemical stability study based on the results of solubility test. Fig. 2(a) shows the stability of curcumin in pH 1.2, 6.8 and 7.4 buffers. Curcumin was more stable at lower pH. 12% of curcumin degraded in 6 h at pH 1.2 buffer, however, almost 50% and 90% of curcumin degraded in 6 h at pH 6.8 and pH 7.4 buffers, respectively. Curcumin exists in equilibrium between the diketo- and keto-enol form, which is strongly favored by intramolecular H-bonding. Hydrolytic degradation of curcumin starts with an attack from the nucleophilic OH^- ion to the carbonyl carbon in the keto-enol moiety (Tønnesen et al., 2007). Therefore, higher rate of degradation is observed at higher pH. Degradation profiles of curcumin in pH 1.2, 6.8 and 7.4 buffers containing 10% (w/v) Kollidon® 30 (Fig. 2(b)) were similar with that in buffers without Kollidon® 30. It showed that Kollidon® 30, a non-surface active polymeric carrier, could not prevent degradation of curcumin. On the contrary, curcumin was relatively stable at pH 1.2, 6.8 and 7.4 buffers containing 10% (w/v) Cremophor® RH40 and 10% (w/v) Solutol® HS15 as illustrated in Fig. 2 (c) and (d), respectively. This result confirmed that encapsulation of curcumin by surfactant micelles prevents hydrolytic degradation due to protection of the keto-enol moiety of curcumin from the attack of nucleophilic OH^- ion. Comparatively, Solutol® HS15 exhibited superior stabilizing effect to Cremophor® RH40 at all pH tested. This observation suggested the formation of more effective micellar barrier by Solutol® HS15 as compared to Cremophor® RH40. In case of buffers containing 10% (w/v) Solutol® HS15, below 5% of curcumin was degraded in 12 h and better stability was observed at lower pH. The amount of curcumin degraded in pH 7.4, 6.8 and 1.2 buffers was 4.2%, 2.4% and 1.3%, respectively.

Chemical stability study of curcumin inferred that degradation of curcumin is complex mechanism involving various underlying factors. There had been some attempts to pin point the order of chemical degradation kinetics of curcumin with varying outcomes. A study by Tønnesen and Karlsen (1985) suggested that curcumin followed pseudo second-order reaction kinetics at pH range of 1.23–7.98 when incubated at temperature of 31.5 °C. On the other hand, Wang et al. (1997) showed that degradation kinetic of curcumin followed first-order reaction at the pH range of 3–10 when incubated at temperature of 37 °C. The linear regression analysis of data obtained by present chemical stability study of curcumin indicated relatively closer resemblance to pseudo second-order reaction kinetics. Hence, degradation rate constants (k) and half life ($t_{1/2}$) of curcumin at various pH buffers were calculated based on second-order kinetics and enlisted in Table 2.

Based on the results of solubility and stability tests, Solutol® HS15 was chosen as the best carrier for development of solid

Table 2
The rate constant and half-lives for the overall % degradation of curcumin from various samples according to second order kinetics at various pH values (mean ± S.D., $n = 3$).

Mediums	pH of the medium	Rate constant (k, h^{-1})	Half life ($t_{1/2}$, h)	Correlation coefficient (R^2)
Blank buffers	1.2	9.3×10^{-5}	108.0	0.81
	6.8	1.2×10^{-3}	8.6	0.97
	7.4	1.7×10^{-2}	0.6	0.94
Buffers (10% (w/v) PVP K-30)	1.2	1.0×10^{-4}	95.8	0.91
	6.8	1.8×10^{-3}	5.7	1.00
	7.4	2.8×10^{-2}	0.4	0.88
Buffers (10% (w/v) Cremophor® RH40)	1.2	2.2×10^{-5}	464.2	0.89
	6.8	1.0×10^{-4}	98.6	0.79
	7.4	1.0×10^{-4}	95.5	0.68
Buffers (10% (w/v) Solutol® HS15)	1.2	7.0×10^{-6}	1423.1	0.84
	6.8	1.7×10^{-5}	604.2	0.97
	7.4	2.3×10^{-5}	441.9	0.90

dispersion of curcumin. In addition, Solutol® HS15 is commonly used in intravenous formulations and regarded as relatively safe (rat oral LD_{50} is approximately 20 g/kg).

3.2. Characterizations of solid dispersion

3.2.1. Effect of preparation method, pH of the medium and carrier ratio on solubility of curcumin

In order to elucidate the mechanism of solubilization and to find the optimum preparation method of curcumin SD, solubility of curcumin from PM, MM and SD at the weight ratio of 1:10 was investigated in pH 1.2, 6.8 and 7.4 buffers. As can be seen from Fig. 3, among three different samples, SD showed highest solubility at all pH tested. This may be due to conversion of crystalline form of curcumin into amorphous form in SD system. MM showed slightly better solubility of curcumin compared with that of PM. It may be due to partial transformation of crystalline curcumin into amorphous form in MM. During preparation of MM, some part of curcumin dissolved in melted Solutol® HS15 and may be dispersed in amorphous state. It should also be noted that some of curcumin prepared by physical mixture may be converted to amorphous form during mixing process by mortar and pestle.

Minimal effect of pH on solubility of curcumin from PM, MM and SD was observed, indicating that the extent of micellar solubilization of curcumin from each of PM, MM and SD was similar in pH 1.2, 6.8 and 7.4 buffers, respectively. This also implied that non-micellar solubilization of curcumin was minimal, hence, varying degree of hydrolysis of the drug at various pH was only slightly manifested. Moreover, transformation into amorphous state improved micellar incorporation of curcumin thereby significantly improving solubility in the case of SD.

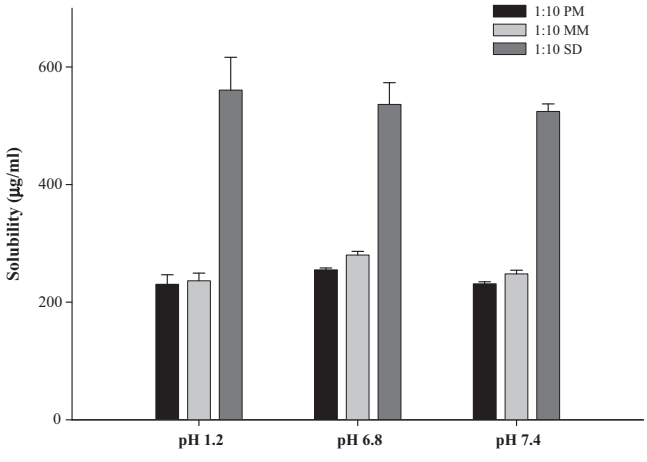

Fig. 3. Solubility of curcumin from physical mixture, melting mixture and solid dispersion at various pH buffers (mean ± S.D., $n = 3$).

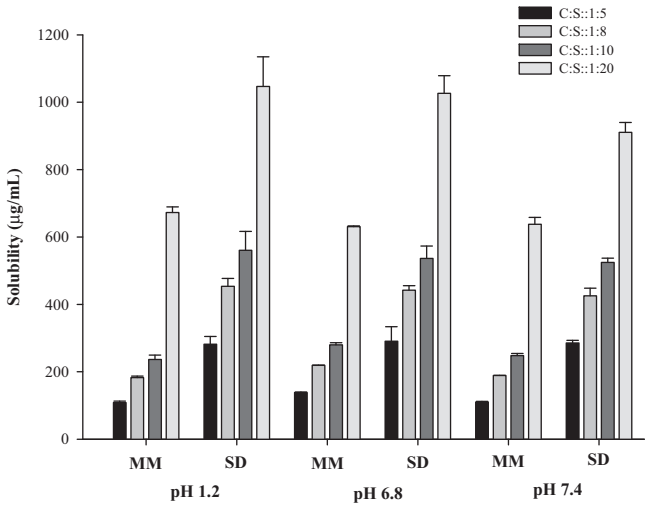

Fig. 4. Solubility of melting mixture and solid dispersion at various curcumin and Solutol® HS15 ratios (C:S) (mean ± S.D., $n = 3$).

In general, carrier to drug ratio affects crystallinity of drug in SD and solubility of drug in aqueous medium. The more amount of the carrier added, the higher is the conversion of crystalline drug into amorphous form, resulting in increased solubility and dissolution rate of drug up to a certain point (Tantishaiyakul et al., 1999). Effect of amount of Solutol® HS15 on the solubility of curcumin is shown in Fig. 4. In both cases, MM and SD, solubility was increased by increasing the concentration of Solutol® HS15 at all pH buffers tested. SD showed almost twice higher solubility than MM except for the formulation with 1:20 weight ratio. In case of MM, solubility enhancement rate of curcumin was higher at 1:20 ratio compared with other lower ratios. At this ratio, higher amount of curcumin may be transformed into amorphous form as increased ratio of melted Solutol® HS15 in MM solubilized higher amount of curcumin. Consequently, the size of remained non-dissolved crystals may be significantly reduced as compared to relatively larger crystal size in lower Solutol® HS15 ratios. The reduced-size crystals may exhibit higher saturation solubility. Therefore, in addition to carrier dependent transformation of curcumin to amorphous form, additional curcumin may be dissolved in aqueous phase due to sufficient size reduction. Net effect was increased rate of micellar incorporation of curcumin from MM in 1:20 weight ratio compared with lower ratios. Therefore, at higher ratio of Solutol® HS15 (1:20), the difference in the ratio of solubility of curcumin from MM and SD was narrowed compared with that from lower ratios. Considering the amount of Solutol® HS15 in the SD and respective solubility, SD with weight ratio of 1:10 was found to be optimum for further investigations.

3.2.2. Differential scanning calorimetry (DSC)

The DSC thermograms of curcumin, Solutol® HS15, PMs, MMs and SDs are shown in Fig. 5. The DSC curves of pure curcumin and Solutol® HS15 exhibited endothermic peaks around 182 °C and 30 °C, respectively, which corresponded to their intrinsic melting points. However, no curcumin peak was observed from PM, MMs and SDs, indicating that curcumin might have dispersed in molecular form in the carrier (Leuner and Dressman, 2000). This unexpected results might be explained by the fact that

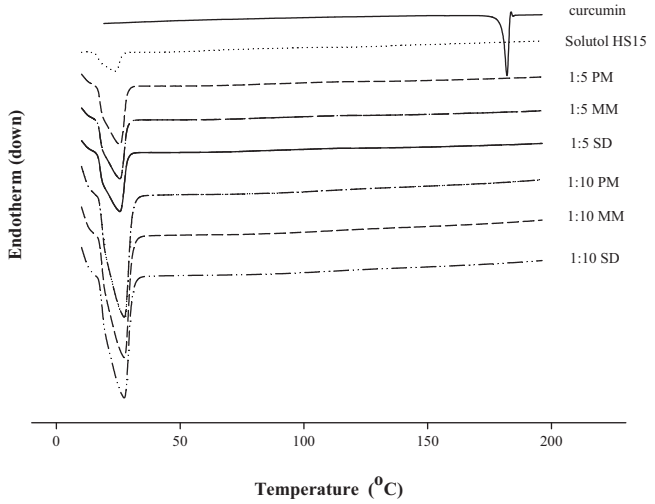

Fig. 5. DSC thermograms of curcumin, Solutol® HS15, physical mixtures, melting mixtures and solid dispersions.

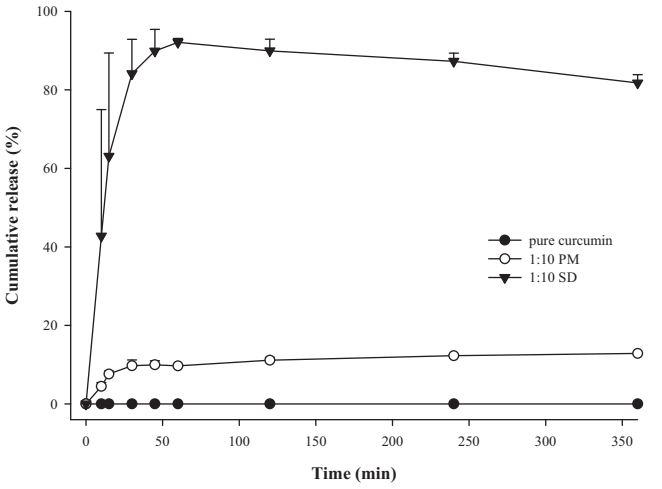

Fig. 7. Dissolution profiles of curcumin, physical mixture, melting mixture and solid dispersion in pH 6.8 buffer (mean ± S.D., $n = 3$).

curcumin in PM and MM dissolved in the melted Solutol® HS15 when thermal analysis was carried out. As temperature increases, solubility of curcumin in melted Solutol® HS15 also increases. So, all curcumin dissolved in melted Solutol® HS15 before it reached melting point of curcumin. Consequently, we were not able to elucidate the physical state of curcumin by DSC.

3.2.3. X-ray diffraction (XRD)

Since crystallinity of curcumin from PM and MM could not be identified using DSC analysis, XRD was used to confirm the loss of drug crystallinity. X-ray diffractograms of pure curcumin, Solutol® HS15, PM, MMs and SDs at various weight ratios are provided in Fig. 6. Pure curcumin showed several characteristic peaks at 2θ angles within 30°. In case of PM with 1:10 ratio, although most of the peaks disappeared, peaks at 8.9°, 17.2°, 17.8° were still observed. Since Solutol® HS15 provided no characteristic peak, these three peaks must originate from crystalline form of curcumin. The results indicated that curcumin was partially present in crystalline form in the PM. Partial melting of Solutol® HS15 during PM preparation dissolved some of curcumin, resulting in partial transformation into amorphous form. Melting mixture also showed similar peaks, but the peak at 17.8° disappeared, and other two peaks became smaller with increasing Solutol® HS15 ratios. In case of SDs, characteristic peaks of curcumin completely disappeared in all the samples. This indicated that all curcumin was in amorphous state in the SD.

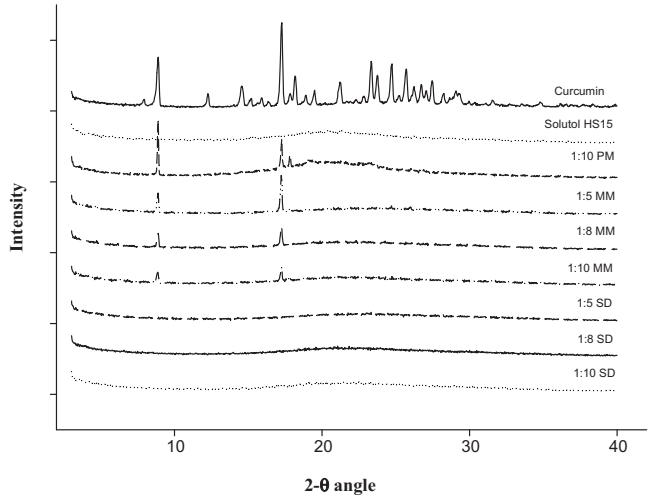

Fig. 6. X-ray diffractograms of curcumin, Solutol® HS15, physical mixture, melting mixtures and solid dispersions.

3.2.4. Dissolution tests

Fig. 7 shows the dissolution profiles of pure curcumin, 1:10 PM, and 1:10 SD formulations. For dissolution test of SD formulation, fresh samples were used. As shown in Fig. 7, pure curcumin practically remained undissolved in dissolution medium for 6 h. The dissolution of curcumin was slightly higher from PM as compared to pure curcumin due to solubilization of the drug by micellization. Compared to the PM, SD showed a significantly higher release rate. This implies that curcumin existed predominantly in amorphous state in SD, and hence had higher solubility. In case of SD, above 90% dissolution rate was observed within 60 min. But it decreased to 81% at 6 h. The decrease in dissolved % of curcumin with increased time interval was predominantly due to precipitation of the drug that existed in supersaturated state in buffer. Precipitated drug was visually observed after completion of dissolution test. As demonstrated in chemical stability study, degradation of curcumin have insignificant role in decline of dissolved % of curcumin in dissolution test.

As illustrated by dissolution test, values obtained from solubility test did not correlate with release % of curcumin in dissolution test. Solubility test revealed that solubility of curcumin from each sample was above 250 μg/mL in pH 6.8 buffer, however, the dissolution test did not show 100% release of curcumin from PM and SD even though theoretical concentration of curcumin subjected for dissolution test was around 22 μg/mL. This may owe to the fact that solubility test and dissolution test were conducted at different conditions. Major differing parameters between solubility test and dissolution test were: concentration of Solutol® HS15 (25 mg/mL *vs* 222 μg/mL), rpm (400 *vs* 50) and time duration (12 h *vs* 6 h). Hence, based on net effect of differing parameters, it can be predicted that solubility of curcumin from PM and SD was significantly lower in dissolution test as compared to that in solubility test.

3.2.5. Physical stability tests

Monitoring the physical stability of the solid dispersion is imperative, as they might convert to lower energy state (*e.g.* through recrystallization). To investigate the rate of recrystallization and its effect on the solubility of curcumin, physical stability study of the SD samples were carried out. As shown in Fig. 8, 1:10 SD

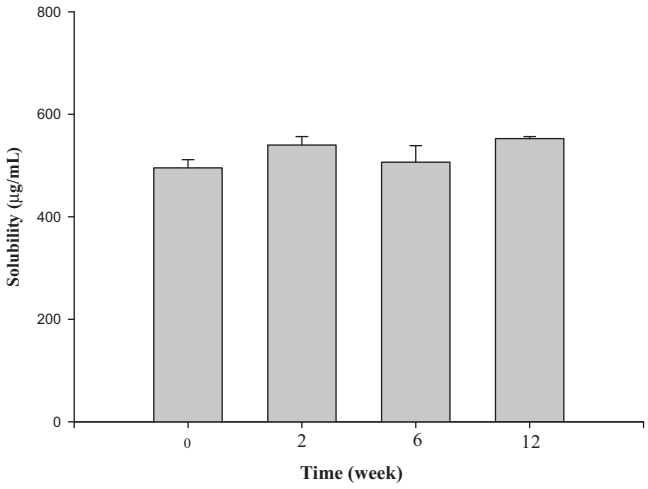

Fig. 8. Solubility of curcumin from solid dispersion (1:10) with respect to storage time (mean ± S.D., $n = 3$).

showed solubility of curcumin above 495 μg/mL (0 day) which was similar till experimental period of 12 weeks. It meant that amorphous state of curcumin in SD did not change into crystalline state significantly within a period of 12 weeks. Physical stability of curcumin in 1:10 SD can be explained by higher drug solubilization capacity of Solutol® HS15.

3.3. *In vivo* studies

SD exhibited significantly improved solubility and dissolution and, therefore, the *in vivo* performance of 1:5 SD and 1:10 SD formulation was compared to that of pure curcumin. Plasma-concentration profiles for curcumin are depicted in Fig. 9 and pharmacokinetic parameters are summarized in Table 3. From the results, it can be seen that there is significant statistical difference between the C_{max} value of the pure curcumin and 1:10 SD ($p < 0.01$). The increment is approximately 6 folds from 1:10 SD as compared to pure curcumin. Except between pure curcumin and 1:5 SD, all differences in $AUC_{0-12\,h}$ were significant. $AUC_{0-12\,h}$ was increased by approximately 5 folds in case of 1:10 SD as compared to pure curcumin. It indicated that enhanced bioavailability of curcumin can be obtained by using 1:10 SD. Effective solubilzation and

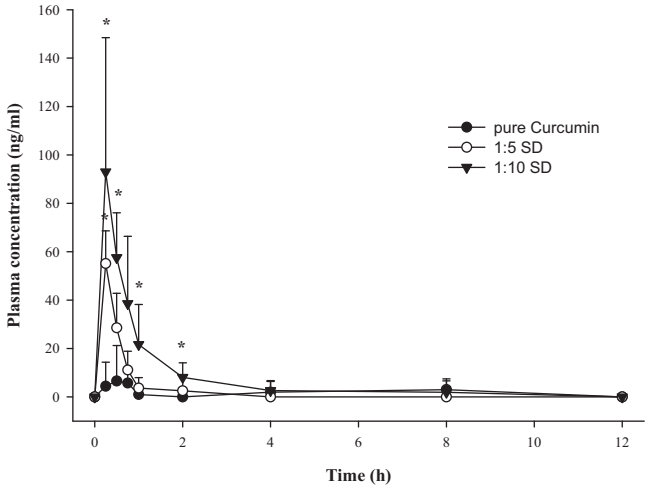

Fig. 9. Plasma profiles of pure curcumin ($n = 5$) and solid dispersions of curcumin with Solutol® HS15 at the weight ratio of 1:5 and 1:10 ($n = 6$) (mean ± S.D). * $p < 0.01$, compared to the control (pure curcumin).

Table 3
Pharmacokinetic parameters following an oral administration of curcumin (50 mg/kg) in three different formulations to rats (mean ± S.D., $n = 5$–6).

Parameter	Pure curcumin (control)	1:5 SD	1:10 SD
C_{max} (ng/mL)	15.65 ± 12.6	55.15 ± 13.4*	95.60 ± 53.8*
T_{max} (h)	0.45 ± 0.3	0.25 ± 0.0	0.33 ± 0.1
$AUC_{0-12\,h}$ (ng/mL h)	15.31 ± 19.7	24.62 ± 9.4	72.84 ± 36.4*
$t_{1/2}$ (h)	NA	0.44 ± 0.49	1.89 ± 1.47

NA: not available.
* $p < 0.01$, compared to the control.

prevention of degradation of curcumin in GI tract might be the possible reasons for improved bioavailability of the drug from the SD. Moreover, the carrier, Solutol® HS15 is a surfactant which may have induced increased permeability of intestinal epithelium, opening of the tight junction to allow paracellular transport and inhibition of P-gp and/or CYP450 to increase intracellular concentration and residence time (Kommuru et al., 2001).

4. Conclusion

Solutol® HS15 was found to be effective in enhancing the solubility and stability of curcumin. Curcumin in the SD prepared with Solutol® HS15 at the weight ratio of 1:10 existed in amorphous form and was physically stable for experimental period of 12 weeks. *In vivo* study in rat revealed that SD was effective in enhancing bioavailability significantly. Hence, the present formulation offers an effective means of overcoming problems related to solubility, stability and bioavailability of curcumin. Moreover, present approach is simple and safe and can be designed as oral dosage form filled into soft-gelatin or hard-gelatin capsules.

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