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석사학위논문

Preparation of Solutol® HS15 based solid dispersion of curcumin for enhanced bioavailability

조선대학교 대학원

약 학 과

서 상 완

쿠르쿠민의 생체이용률 증가를 위한 솔루톨® HS15 기반의 고체분산체 제조

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지도교수 최 후 균

이 논문을 약학 석사학위신청 논문으로 제출함

2009 년 10 월

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서 상 완

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쿠르쿠민의 생체이용률 증가를 위한 솔루톨 HS15 기반의 고체분산체 제조

서 상 완

지 도 교 수 : 최 후 균

조선대학교 대학원 약학과

이번 연구의 목적은 계면활성제를 운반체로 한 쿠르쿠민 고체분산체를 제조하여 쿠르쿠민의 용해도와 안정성을 증가시키는 것이다. 다양한 계면활성제 검색을 통해 pH 1.2에서 솔루톨® HS15가 가장 효과적으로 쿠르쿠민의 용해도를 증가시킨다는 것을 확인하였다. 뿐만 아니라 pH 1.2, 6.8 그리고 7.4 에서 솔루톨® HS15에 의해 쿠르쿠민의 안정성이 증가되는 것을 확인할 수 있었다. 이에 솔루톨® HS15를 이용하여 쿠르쿠민의 고체분산체를 용매법으로 제조하여 보았다. 제조된 고체분산체의 용해도, 약물 결정성, 용출률 및 물리적

안정성을 시험하였다. 쿠르쿠민의 용해도는 고체분산체 중의 솔루톨® HS15의 양이 증가함에 따라 비례적으로 증가하였으며 X선 분석을 통하여 고체분산체 내의 약물이 모두 무결정 상태로 존재함을 알 수 있었다. 쿠르쿠민과 솔루톨® HS15을 1:10 무게 비율로 고체분산체를 제조하여 pH 6.8 용액에서 용출률을 조사하였고, 순수 쿠르쿠민 (약 0%), 물리적 혼합물 (약 10 %), 용융 혼합물 (약 80 %) 보다 뛰어난 용출률 (60분 이내에 90 % 이상 용출)을 나타냄을 알 수 있었다. 12주 동안의 보관에도 1:10 고체분산체의 용해도 감소는 관찰되지 않았다. 마지막으로 레트를 이용하여 *in vivo* 실험을 수행하였다. 1:10 고체분산체의 AUC_{0-12h} 와 C_{max} 가 순수 쿠르쿠민의 비해 훨씬 증가됨이 관찰 되었다. 결론적으로 솔루톨® HS15을 이용한 쿠르쿠민의 고체분산체는 쿠르쿠민의 생체이용률을 높이는데 유용한 제형임을 알 수 있다.

Abstract

Preparation of Solutol® HS15 based solid dispersion of curcumin for enhanced bioavailability

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The aim of this study was to improve the solubility and stability of curcumin by solid dispersion using surfactant as carrrier. Among the various surfactants screened, Solutol® HS15 was most effective to enhance the solubility of curcumin at pH 1.2. Moreover, enhanced stability of curcumin was observed at pH 1.2, 6.8 and 7.4 in the presence of Solutol® HS15. Thus, Solid dispersion (SD) of curcumin was prepared with Solutol® HS15 by the solvent method. Solubility, drug crystallinity, dissolution rate and physical stability of SD were then characterized. Solubility of curcumin was found to increase

proportionally with the amount of Solutol® HS15. X-ray diffraction (XRD) results indicated that the entire drug in SD was in amorphous state. SD of curcumin in weight ratio of 1:10 (w/w) enhanced dissolution rate (approx. 90% after 60 min) in pH 6.8 medium compared to that of pure curcumin (approx. 0%), physical mixture (approx. 10%) and melting mixture (approx. 80%). After 12 weeks of storage, solubility of curcumin from the optimized formulation was similar to that of fresh sample. Finally, *in vivo* experiment was also performed in rat. $AUC_{0\text{-}12h}$ and C_{max} of 1:10 SD were significantly greater than that of pure curcumin. In conclusion, SD of curcumin with Solutol® HS15 appeared to be useful for improving the bioavailability of curcumin.

1. Introduction

Curcumin is a hydrophobic polyphenol derived from the rhizome (tumeric) of the herb Curcuma *longa L*. It is a principal component of tumeric, 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. Tumeric 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, antimicrobiral and anticarcinogenic activities [1,2,3]. It also has hepato- and nephro-protective, thrombosis suppressing, mycrocardial infarction protective, hypoglycemic, and antirheumatic activities [4,5,6,7,8,9]. Moreover, various animal models or human studies proved that curcumin is extremely safe even at very high doses [10,11]. 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 efficacy and high safety, curcumin has not

yet been approved as a therapeutic agent due to its poor oral bioavailability. 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 [12]. 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 [13]. So, several other strategies such as nanoparticle, liposome, micelles, phospholipid complex, and solid dispersion have been evaluated to enhance the bioavailability of curcumin.

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 [14] 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), 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. Leung et al. [15] has investigated 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. But in both cases they used ionic surfactants and evaluated stability in solution form. Generally, ionic surfactants are more toxic than non-ionic surfactants. Moreover, Liquid (solution) dosage forms are difficult to package and offer poor portability.

Solid dispersion (SD) is one of the most promising strategies to improve the oral bioavailability of poorly water soluble drug 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 [16]. 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 [17]. Recently, it has been shown that the dissolution profile can be improved

if the carrier has surface activity or self-emulsifying properties [18]. 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 [19,20,21].

So, we can expect enhanced solubility and chemical stability of curcumin at physiological pH, if solid dispersion of curcumin is prepared by using surfactant as carrier. Solutol® HS15 (polyethylene glycol 660 hydroxystearate) is a non-ionic surfactant and semi-solid state at room temperature that becomes liquid above 30°C. Solutol® HS15 is regarded as relatively safer (Rat oral LD_{50} is approx. 20g/kg) and commonly used in intravenous formulations. Therefore, Solutol® HS15 can be used as a carrier for enhancement of stability and bioavailability of curcumin.

The objective of this experiment is to enhance solubility, stability and bioavailability of curcumin via solid dispersion preparation with Solutol® HS15 as carrier.

2. Materials and methods

2.1 Materials

Curcumin was purchased from Sigma Co. (St. Louis, MO, USA). PEG-8 glyceryl capryl/caprate (Labrasol®) and stearoyl macrogol glyceride (Gelucire® 50/13) were obtained from Gattefosse Korea (Seoul, South Korea). PEG sorbitan monoleate (Tween® 80), sorbitan monoleate (Span® 80) were purchased from Junsei chemicals (Tokyo, Japan). Glyceryl cocoate/citrate/lactate (Imwitor® 380) was obtained from Sasol Germany GmbH (Hamburg, Germany). Poloxamer 188 (Lutrol® F 68), Poloxamer 407 (Lutol® F 127), polyoxyl 40 hydrogenated castor oil (Cremophor® RH40) and Polyethylene glycol 660 hydroxystearate (Solutol® HS15) was obtained from BASF (Ludwigshafen, Germany). Polyethylene glycol 400 (PEG 400) was purchased from Junsei 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^{o}C.

2.2.2 Screening of surfactants

5 mg of curcumin was added to 2 mL of pH 1.2 buffer containing 2 %w/v respective surfactant and stirred at 400 rpm for 12 h at room temperature. The samples were then centrifuged at 13000 rpm for 20 min and filtered through 0.45 μm pore-sized regenerated cellulose syringe filter (Target®, National scientific, USA), suitably diluted with

methanol and analyzed by HPLC. The experiment was performed in triplicates.

2.2.3 Influence of Solutol® HS15 on physico-chemical properties of curcumin

2.2.3.1 Effect of Solutol® HS15 concentration on solubility

5 mg of curcumin was added to 2 mL of 2, 4, 6, 8, 10 %w/v Solutol® HS15 solution at pH 1.2 buffer and stirred at 400 rpm for 12 h at room temperature. The samples were then processed for HPLC analysis as mentioned in section 2.2.2.

2.3.3.2 Effect of pH on solubility

5 mg of curcumin was added to 2 mL of 10 %w/v Solutol® HS15 solution at pH 1.2, 6.8, 7.4 buffer respectively, and stirred at 400 rpm for 12 h at room temperature. The samples were then processed for HPLC analysis as mentioned in section 2.2.2

2.2.3.3 Chemical stability studies

The chemical stability of curcumin was investigated in pH 1.2, 6.8

and 7.4 buffers with and without 10 %w/v Solutol® HS15. Stock solutions of curcumin were prepared in methanol at a concentration of 100 μg/mL. This solution was 100 times diluted with respective buffer solutions with and without Solutol® HS15. 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 and analyzed by HPLC at various time intervals.

2.2.4 Preparation of solid dispersion, physical mixture and melting mixture

Solid dispersions (SDs) of curcumin with Solutol® HS15 at different ratios (1:5, 1:8, 1:10, 1:20) were separately obtained by conventional solvent evaporation method. Curcumin and Solutol® HS15 were dissolved in minimum volume of acetone and solvent was removed under vacuum at room temperature.

Physical mixture (PM) of curcumin with Solutol® HS15 was obtained by simply mixing curcumin and Solutol® HS15 using pestle and mortar.

Melting mixture (MM) of curcumin with Solutol® HS15 was obtained by melting the physical mixture at 40°C for 1h and then

solidifying at room temperature for 1 h.

2.2.5 Characterizations of solid dispersion

2.2.5.1 Solubility tests

Solubility of curcumin from SDs, PMs and MMs was determined by taking amount equivalent to 5 mg of curcumin in 2 mL of pH 1.2, 6.8, 7.4 buffers and stirring at 400 rpm for 12 h at room temperature. The samples were then centrifuged at 13000 rpm for 20 min and filtered through 0.45 μm pore-sized regenerated cellulose syringe filter, suitably diluted with methanol and analyzed by HPLC. The experiment was performed in triplicates.

2.2.5.2 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^{o}C/min from 20^{o}C to 200^{o}C.

2.2.5.3 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 2-θ range of 3-40^{o} using a step size of 0.02^{o} at a scan speed of 1 s/step.

2.2.5.4 Dissolution tests

Dissolution tests of pure curcumin 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^{o}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 the dissolution vessel. At the predetermined time intervals, 3 mL of the samples were withdrawn and the equal volume of fresh medium was added into the dissolution vessel. The collected samples were filtered through regenerated cellulose syringe filters. Initial sample volume of 2 mL was discarded and final 1mL was collected and then suitably diluted with methanol. Samples were analyzed by HPLC.

2.2.5.5 Physical stability tests

The prepared SDs was stored in air tight container protected from light at room temperature. They were then analyzed for solubility at pH 6.8 phosphate buffer periodically.

2.2.6 *In vivo* studies

2.2.6.1 Animal

Male Sprague-Dawley rats (240-280g) 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).

2.2.6.2 Dosing and sampling

Rats were fasted for 24 h prior to the beginning of experiments. The rats were divided into three groups of six animals each. 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), Group 2 (1:5 SD), Group 3 (1:10 SD). Blood samples were collected from the femoral artery at 0.25, 0.5, 0.75, 1, 2, 4, 8 and 12 hr post-dose. Blood samples were centrifuged at 13000 rpm for 3 min and the obtained plasma was stored at -40°C until analyzed.

2.2.6.3 Sample preparation

Plasma sample 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, and then 950 μL acetonitrile was added and the mixture was vortex-mixed for 20 min. After centrifugation at 13000 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 in 100 μL of the mobile phase and the solution was vortex-mixed. After centrifugation at 13000 rpm for 3 min, 50 μL of the solution was injected into the HPLC system for analysis.

2.2.7 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 ($AUC_{0\text{-}12h}$) 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 values from the experimental data.

2.2.8 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 Surfactants

Since surfactants can enhance the solubility of poorly water soluble drugs, we investigated the effect of surfactants on the solubility of curcumin in pH 1.2 buffer containing 2 %w/v of various surfactants. Low pH (1.2) buffer was used for screening because curcumin is more stable at lower pH. Nine different surfactants consisting Span® 80,

Imwitor® 380, poloxamer 188, poloxamer 407, Labrasol®, Tween® 80, Solutol® HS15, Cremophor® RH40 and Gelucire® 50/13 were screened. Results are summarized in Table 1. As shown in the table, Solutol® HS15 was found to be most effective in enhancing the solubility of curcumin. Since, Solutol® HS15 is a surfactant, solubilization of curcumin is due to interfacial tension reduction and micelles formation. Carriers with better affinity and miscibility with drugs can bring better enhancement in solubility of the drug [22]. Hence, Solutol® HS15 may have similar solubility parameters with curcumin that produced better miscibility and resulted in higher enhancement of solubility of curcumin. So, Solutol® HS15 was selected for the further studies.

3.2 Influence of Solutol® HS15 on physico-chemical properties of curcumin

Effect of Solutol® HS15 concentration on solubility is illustrated in Fig. 2. As concentration of Solutol® HS15 is increased, solubility of curcumin increased linearly. This indicated that solubility of curcumin was dependent on the amount of Solutol® HS15. As every samples used in this study had Solutol® HS15 concentration more than its critical micelles concentration (CMC) (0.021 %w/v) [23] the increase in

curcumin solubility linearly with increase in Solutol® HS15 was due to increase in micelles number in the solutions. Curcumin, being hydrophobic and having good affinity with Solutol® HS15, partitioned into micelles to enhance the solubility.

Solubility of curcumin is affected by pH of the medium. Solubility of curcumin is extremely low at acidic pH and it can be improved by increasing the pH. But hydrolysis rate of curcumin also increases with the increasing pH [12]. Curcumin solubility in various pH buffers was determined. As seen in Table 2, at pH 1.2 buffer, solubility was extremely low (60 ng/mL). In pH 6.8 and 7.4 buffers, no any curcumin peak was detected from samples during HPLC analysis. As mentioned above, degradation rate of curcumin at pH 6.8 and 7.4 was faster than in pH 1.2 and hence all dissolved curcumin degraded in the higher pHs samples within 12 h. Effect of Solutol® HS15 on the solubility of curcumin was also investigated in pH 1.2, 6.8 and 7.4 buffers, respectively. Solubility of curcumin was significantly higher than that in the absence of Solutol® HS15 in all pH buffers. It meant Solutol® HS15 has potential to enhance the solubility of curcumin at physiological pH. Interestingly, considering the fact that curcumin were not detected in pH 6.8 and 7.4 buffers due to rapid hydrolysis,

solubility of curcumin should be higher in pH 1.2 buffer than in pH 6.8 and 7.4 buffers, but solubility of curcumin was slightly higher in pH 6.8 and 7.4 buffers than in pH 1.2 buffer (Table 2). It indicated that Solutol® HS15 might protect hydrolysis of curcumin by forming micelles. Moreover, this small difference in solubility at various pHs indicated that micelles formation was little affected by various pHs and was major player in solubilization of curcumin.

Based on the findings of solubility of curcumin at various pH, the hydrolytic degradation of curcumin was examined in pH 1.2, 6.8 and 7.4 buffers, respectively, at various time intervals. Furthermore, effect of 10 %w/v Solutol® HS15 on the stability of curcumin was investigated. Fig. 3(a) shows the stability of curcumin in pH 1.2, 6.8 and 7.4 buffers. As can be seen from the results, curcumin was more stable at lower pH. 12 % of curcumin degraded in 6 h at pH 1.2 buffer whereas 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, and keto-enol form 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 [24]. So, higher rate of

degradation is observed at high pH. Fig. 3(b) showed that curcumin was stable at pH 1.2, 6.8 and 7.4 buffers containing 10 %w/v Solutol® HS15. In all the cases, below 5 % of curcumin were 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 buffer medium was 4.2, 2.4 and 1.3 % of curcumin, respectively. This might be due to the encapsulation of curcumin by Solutol® HS15 micelle, which protected the keto-enol moiety of curcumin from the attack of nucleophilic OH^- ion. From the results of these two stability tests, we can see the potential use of Solutol® HS15 as a SD carrier.

3.3 Characterizations of solid dispersion

3.3.1 Solubility tests

. Solubility of curcumin from PM, MM and SD in the weight ratio of 1:10 was investigated at pH 1.2, 6.8 and 7.4 buffers. As can be seen from Fig. 4, SD showed highest solubility at all pH. This may be due to conversion of crystalline form of curcumin into amorphous form in SD system. Also, 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. During preparation of MM, some curcumin dissolved in melted Solutol® HS15 and dispersed in molecular state.

In general, drug solubility and crystallinity of SD can be governed by the carrier-to-drug ratio. The more the amount of carrier added, the higher the conversion of crystalline drug into amorphous form, resulting in increased solubility and dissolution rate of drug [25]. Effect of amount of Solutol® HS15 on the solubility of curcumin is shown in Fig. 5. In both cases, MM and SD, solubility was increased by increasing the concentration of Solutol® HS15 at all pH mediums. SD showed almost 2 times higher solubility than MM except for the formulation with 1:20 weight ratio. In case of MM, solubility of curcumin was more enhanced at 1:20 ratio compared with other lower ratios. This may be due to the fact that more amount of curcumin dissolved in higher amount of melted Solutol® HS15 at 1:20 ratio to exist in amorphous state.

At higher ratio of Solutol® HS15 (1:20), the difference in the ratio of solubility of curcumin from MM and PM was narrowed compared with lower ratio. Hence, considering the amount of Solutol® HS15 in the SD and respective solubilities, SD with weight ratio of 1:10 was

found to be optimum for further investigations.

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3.3.2 Differential scanning calorimetry (DSC)

The DSC thermograms of curcumin, Solutol® HS15, PMs, MMs and SDs are shown in Fig. 6. 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 [26]. Interestingly, the DSC thermograms of PM and MM were similar to those from SD. This result might be explained by the fact that curcumin in PM and MM dissolved in the melted Solutol® HS15 when thermal analysis was carried out. If temperature increases, solubility of solvate in solution also increases. So, all curcumin dissolved in melted Solutol® HS15 and thus melting peak of curcumin disappeared.

3.3.3 X-ray diffraction (XRD)

Crystallinity of curcumin from PM and MM could not be identified using DSC analysis. So, 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. 7. Pure curcumin has several dominant peaks at 2-θ 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 any characteristic peaks, these three peaks came from crystalline form of curcumin. It meant that curcumin was partially present in crystalline form in the PM. This could be due to the partial melting of Solutol® HS15 during grinding. Drug might have dissolved in the melted Solutol® HS15 and existed in partial amorphous form. Melting mixture also showed similar peaks, but one peak (at 17.8°) disappeared, and other two peaks became smaller with increasing Solutol® HS15 ratio. In case of SDs, characteristic peaks of curcumin completely disappeared in all samples. This indicated that all curcumin was in amorphous state in the SD system.

3.3.4 Dissolution tests

Fig. 9 provides the dissolution profiles of pure curcumin, 1:10 PM, 1:10 MM and 1:10 SD formulations. For dissolution test of MM and SD formulations, fresh samples were used. As shown in the Fig. 8, 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, MM and SD showed a significant high release rate. This is due to the fact that curcumin existed predominantly in amorphous state in MM and SD, and hence had higher solubility. Between MM and SD, SD showed a higher release rate. This is mainly because in the SD, curcumin was completely transformed into an amorphous state whereas in MM, the drug partially existed in amorphous state (Fig. 7). 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 is 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 might have insignificant role in decline of dissolved % of curcumin in dissolution test.

3.3.5 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. 9, 1:10 SD showed solubility of curcumin above 495 μg/mL (0 day) which is similar till experimental period of 12 weeks. It means 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.

3.4 *In vivo* studies

Given the good solubility and dissolution results obtained for SD, 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. 10 and pharmacokinetic parameters are summarized in Table 3, together with the corresponding significance levels (*p*-values) found between the different formulations. 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 being approx. 6 folds from 1:10 SD as compared to pure

curcumin. Except for the $AUC_{0\text{-}12h}$ in the case of pure curcumin vs 1:5 SD, all differences in $AUC_{0\text{-}12h}$ are significant. $AUC_{0\text{-}12h}$ increased by approx. 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 prevention of degradation of curcumin in GIT may 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 [27].

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 an experimental period of 12 weeks. The SD (1:10) improved the dissolution of curcumin with approx. 90 % dissolved within 1 h. *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.

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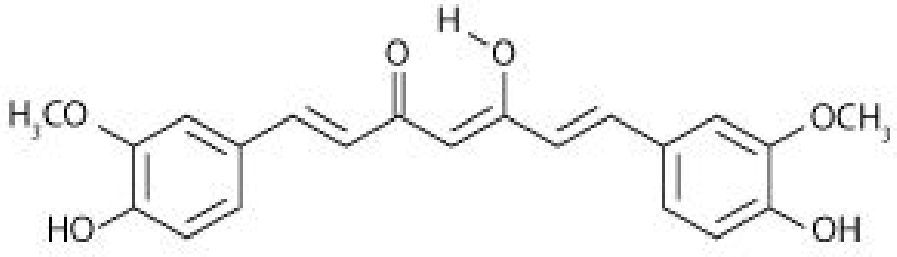


Fig.1. Chemical structure of curcumin.

Table 1. Solubility of curcumin in pH 1.2 buffer containing 2 %w/v surfactant (mean ± S.D., n=3).

Surfactants	**Solubility (μg/mL)**
Solutol® HS15	197.36 ± 3.22
Gelucire® 50/13	173.18 ± 0.22
Tween® 80	169.01 ± 4.28
Cremophor® RH40	167.27 ± 2.33
Poloxamer 407	89.04 ± 1.48
Labrasol®	18.55 ± 6.42
Poloxamer 188	2.48 ± 1.64
Imwitor® 380	0.11 ± 0.12
Span® 80	0.08 ± 0.04

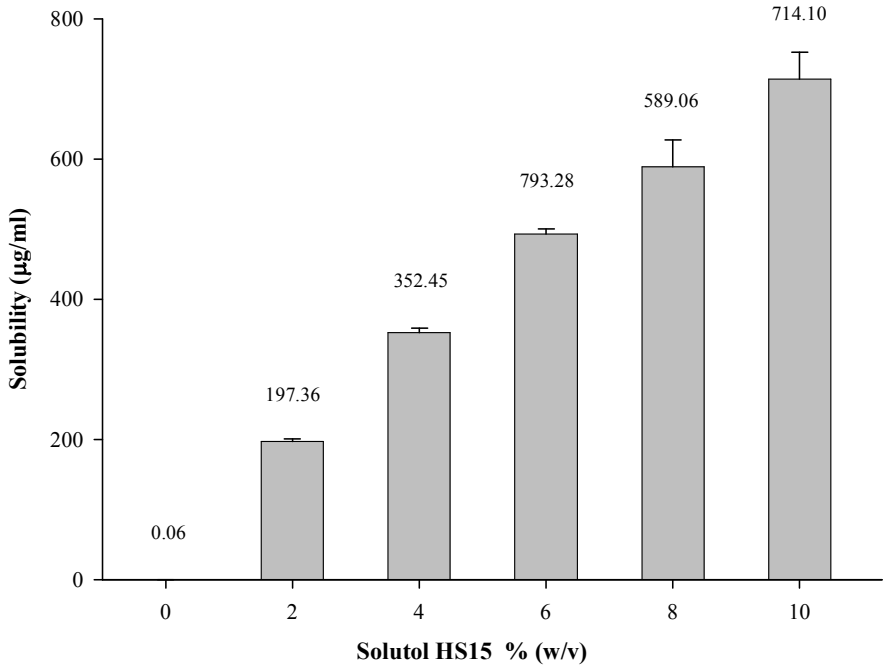
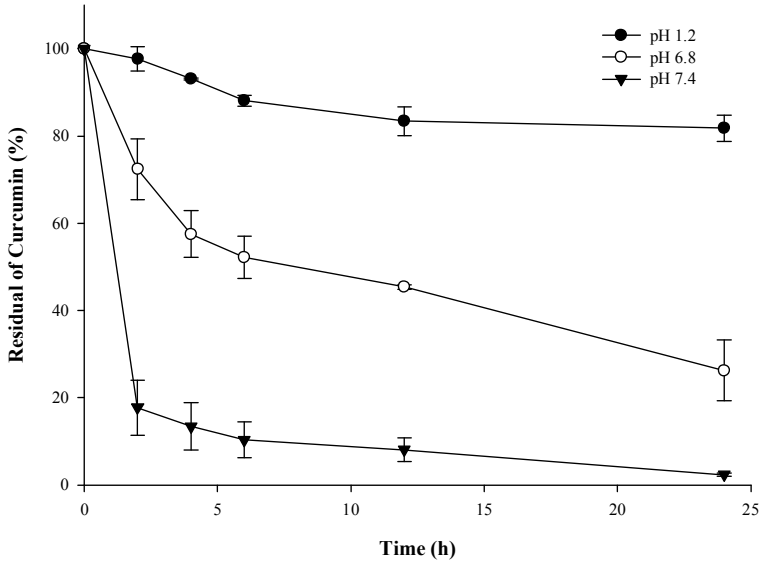


Fig. 2. Solubility of curcumin in various concentration of Solutol® HS15 at pH 1.2 (mean ± S.D., n=3).

Table 2. Solubility of curcumin in various pH buffers (mean ± S.D., n=3).

Medium	Solubility (μg/mL)
pH 1.2	0.06 ± 0.01
pH 6.8	N.D.
pH 7.4	N.D.
pH 1.2 containing 10 %w/v Solutol® HS15	714.10 ± 38.64
pH 6.8 containing 10 %w/v Solutol® HS15	814.61 ± 15.69
pH 7.4 containing 10 %w/v Solutol® HS15	770.38 ± 52.79

(a)



(b)

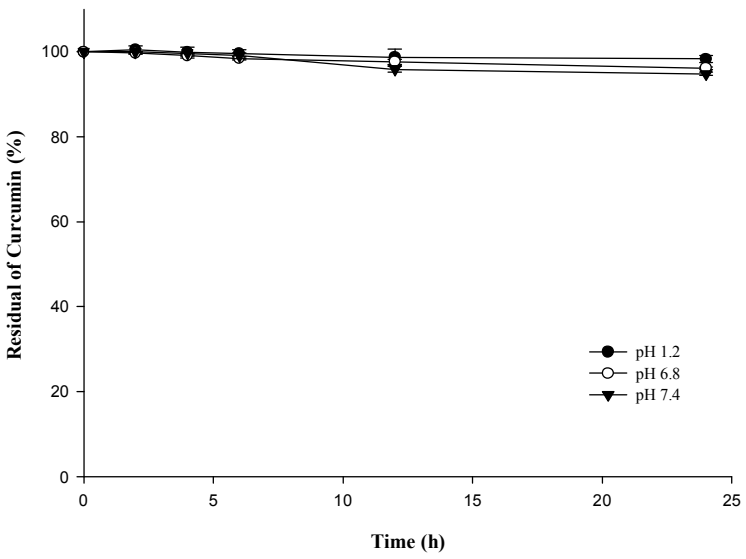


Fig. 3. Chemical stability of curcumin in (a) buffers without Solutol® HS15 (b) buffers containing 10 %w/v Solutol® HS15 (mean ± S.D., n=3).

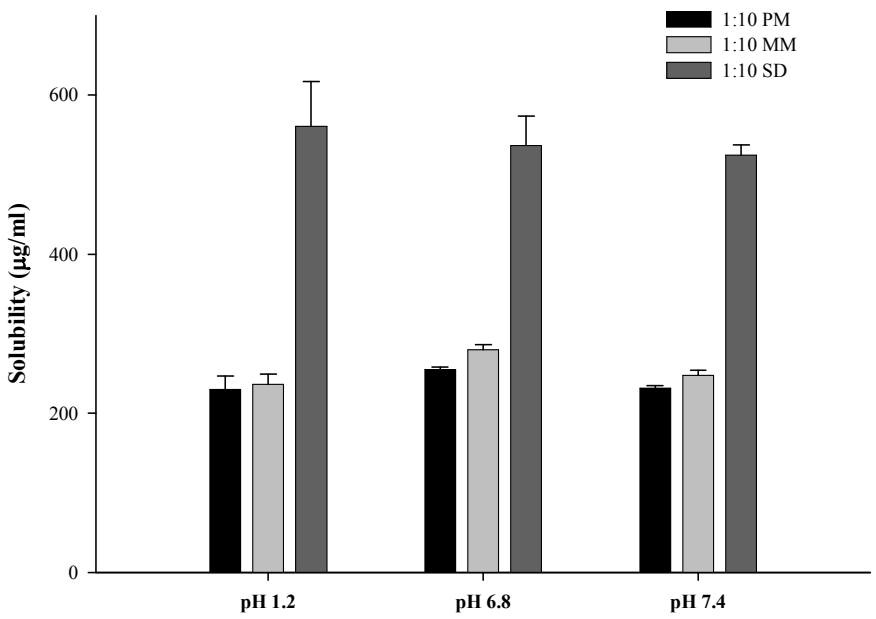


Fig. 4. Solubility of curcumin from physical mixture, melting mixture and solid dispersion at various pH buffers (mean ± S.D., n=3).

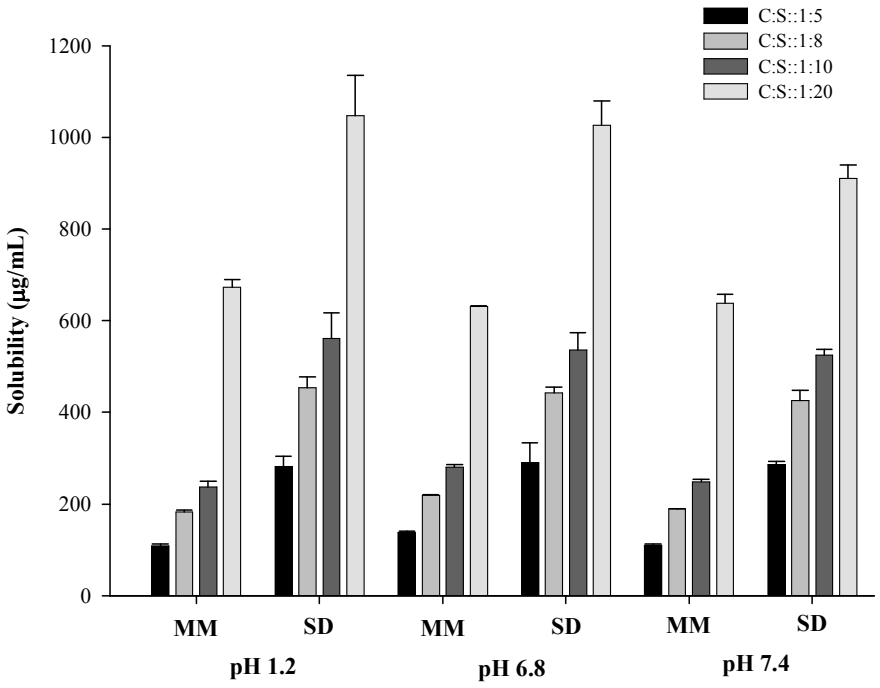


Fig. 5. Solubility of melting mixture and solid dispersion at various curcumin and Solutol® HS15 ratios (C:S) (mean ± S.D., n=3).

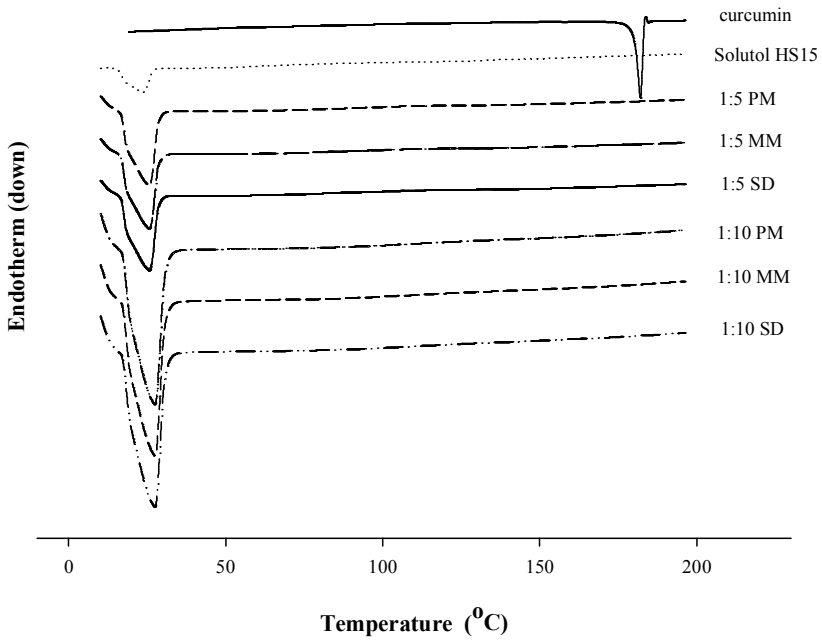


Fig. 6. DSC thermograms of curcumin, Solutol® HS15, physical mixture, melting mixture and solid dispersion.

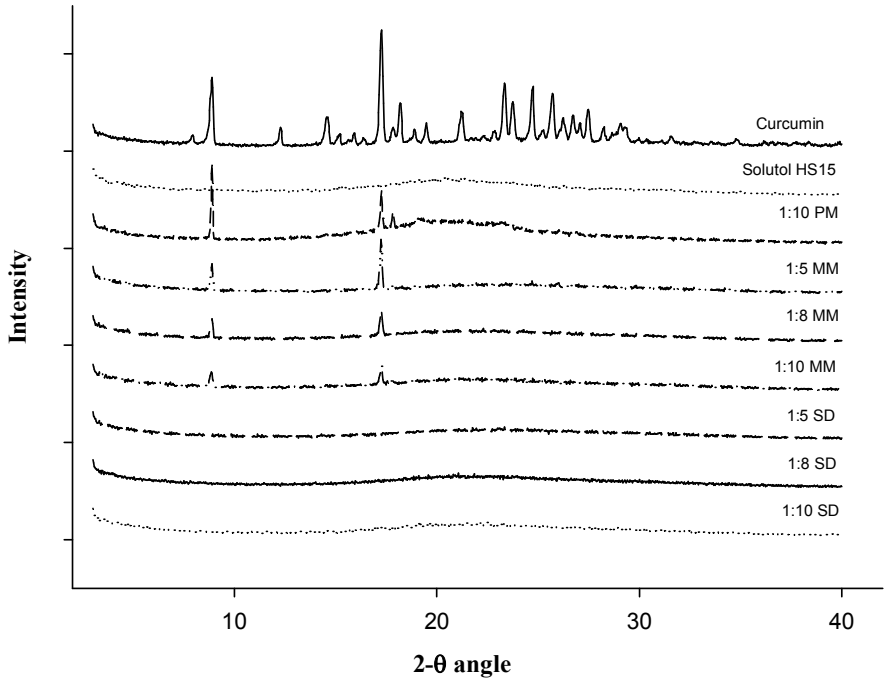


Fig. 7. X-ray diffractograms of curcumin, Solutol® HS15, physical mixture, melting mixtures and solid dispersions.

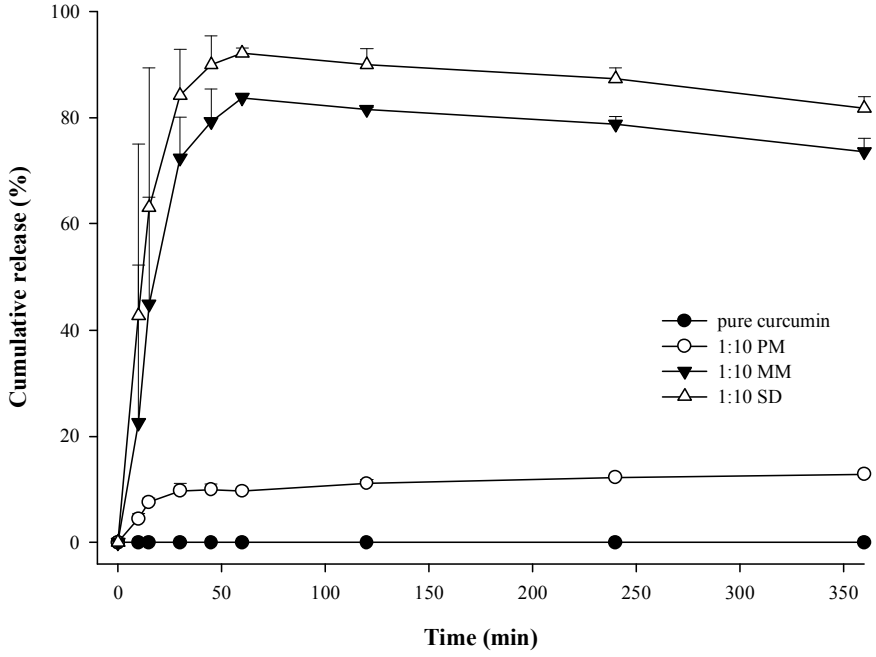


Fig. 8. Dissolution profiles of curcumin, physical mixture, melting mixture and solid dispersion in pH 6.8 buffer (mean ± S.D., n=3).

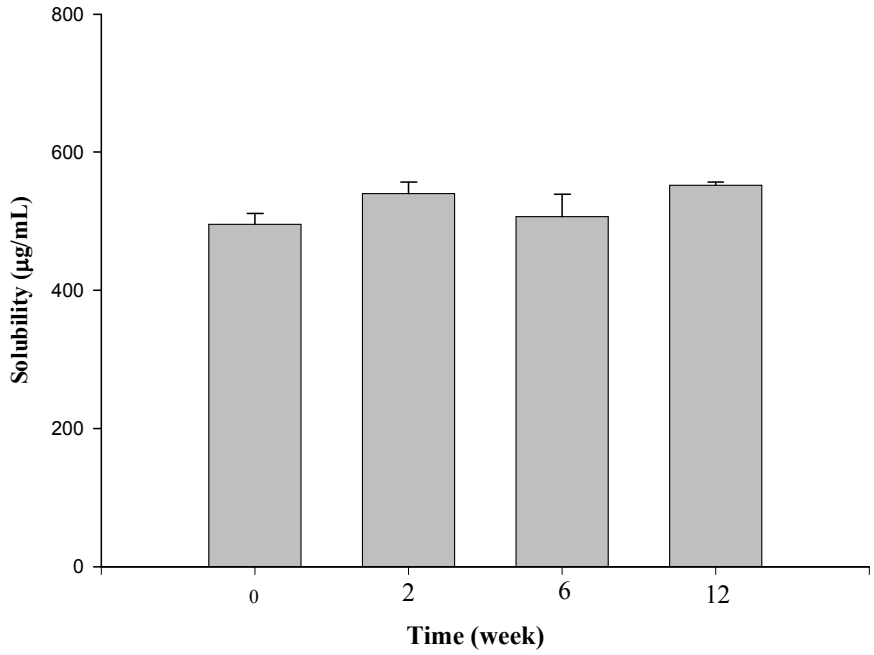


Fig. 9. Solubility of curcumin solid dispersion (1:10) with respect to storage time (mean ± S.D., n=3).

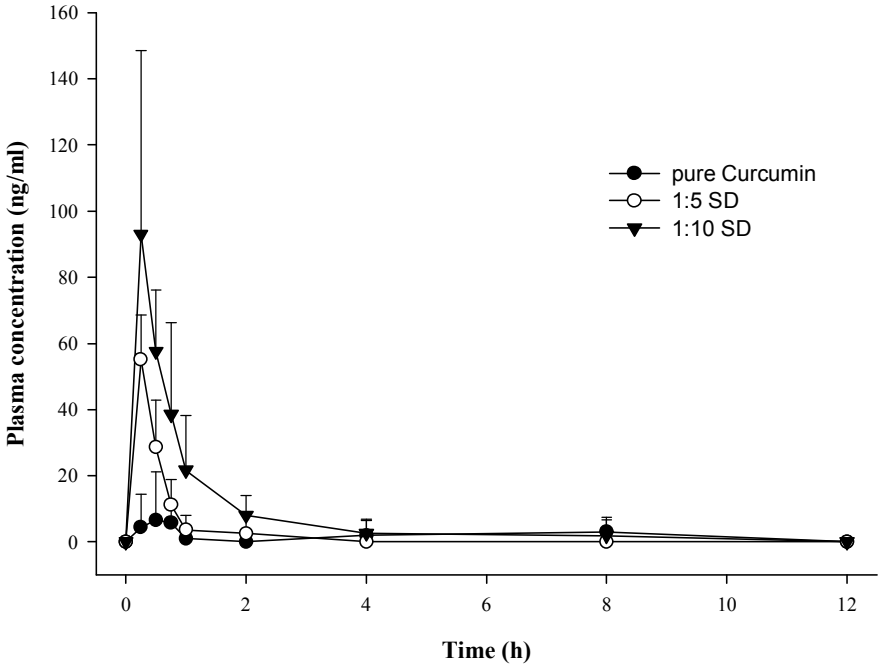


Fig. 10. Plasma profiles of pure curcumin (n=5) and solid dispersions of curcumin with Solutol® HS15 at the weight ratio of 1:5 and 1:10 (n=6) (mean ± S.D).

Table 3. Pharmacokinetic parameters.

Parameter	Pure curcumin	1:5 SD	1:10 SD	*p*-Values, one-way ANOVA		
				Pure curcumin Vs 1:5 SD	Pure curcumin Vs 1:10 SD	1:5 SD Vs 1:10 SD
C_{max} (ng/mL)	15.65±12.6	55.15±13.4	95.60±53.8	$p > 0.05$	$p < 0.01$	$p > 0.05$
$T_{max,}$ (h)	0.45±0.3	0.25±0.0	0.33±0.1	$p > 0.05$	$p > 0.05$	$p > 0.05$
$AUC_{0–12h}$ (ng/mL· h)	15.31±19.7	24.62±9.4	72.84±36.4	$p > 0.05$	$p < 0.01$	$p < 0.05$

C_{max}: peak plasma concentration

T_{max}: time to reach peak plasma concentration

$AUC_{0\text{-}12ht}$: area under the plasma concentration-time curve from 0h to12h.

저작물 이용 허락서

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논문제목	한글: 쿠르쿠민의 생체이용률 증가를 위한 솔루톨® HS15 기반의 고체분산체 제조 영문: Preparation of Solutol® HS15 based solid dispersion of curcumin for enhanced bioavailability				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 -조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB 구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5 년으로 하고, 기간종료 3 개월 이내에 별도의 의사표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1 개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의( ) 반대(○)

2010 년 2 월 25 일

저작자: 서 상 완 (서명 또는 인)

조선대학교 총장 귀하