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Development of amino acid salt-based curcumin@lysine acetate co-amorphous system using liquid-assisted grinding for improved solubility and dissolution

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ABSTRACT

Curcumin, multivalued phytoceutical, exhibits appreciable safety. However, its therapeutic utility is significantly compromised due to low aqueous solubility, and thus, poor absorption and low bioavailability become apparent. To surpass this limitation, the present work aims to develop amino acid salt-based curcumin@lysine acetate co-amorphous system for improved solubility and dissolution**.** Initially, screening of curcumin-amino acid mixtures was performed for saturation solubility assessment. Considering the outcome, lysine acetate was formulated to generate a co-amorphous mixture (COAM) by liquid-assisted grinding and evaluated for saturation solubility and different spectroscopical characterizations. Curcumin-lysine acetate COAM tablet formulation was developed by direct compression method and evaluated for appearance, thickness, hardness, weight variation, friability, drug content, disintegration, and *in vitro* dissolution studies. Further, curcumin-lysine acetate COAM and tablet formulation were screened for the accelerated stability study. Resultantly, curcumin-lysine acetate binary mixture demonstrated the highest saturation solubility among screened curcumin-amino acid binary mixtures that might be ascribed to the hydrotropic properties of lysine acetate. Moreover, 476-fold solubility enhancement in water was observed by curcumin-lysine acetate COAM. Later, the amorphization of the curcumin-lysine acetate COAM was confirmed using Fourier-transform infrared spectroscopy, differential scanning calorimetry, and powder X-ray diffraction. COAM tablet formulation showed optimum evaluation characteristics with improved drug dissolution. Therefore, the amino acid salt-based co-amorphous system can be used for solubility and dissolution improvement of curcumin and other multivalued phytoceutical.

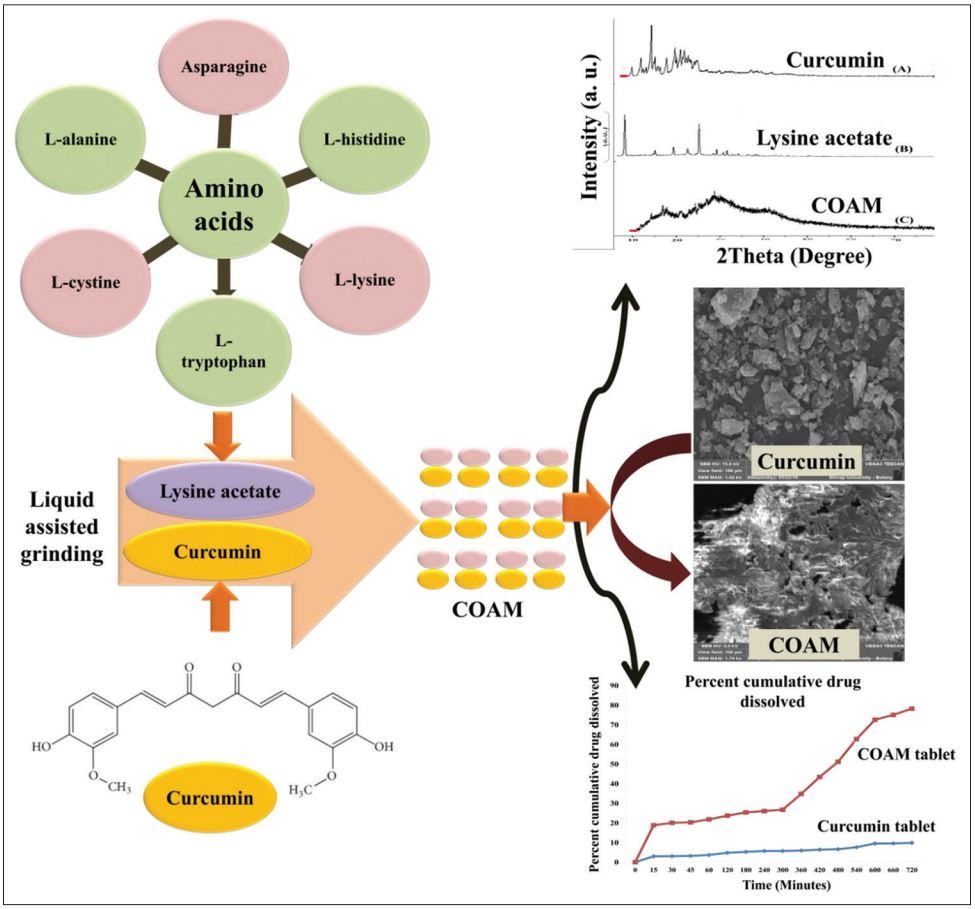
Keywords: Amino acid, co-amorphism, curcumin, dissolution, lysine acetate, solubility

Graphical Abstract

Development of lysine acetate-based curcumin co-amorphous system using liquid-assisted grinding for improved solubility and dissolution.

INTRODUCTION

Co-amorphism has been widely attempted for improving the physicochemical and technological properties of actives.[1,2] The co-amorphous mixture (COAM)



combines two or more low-molecular-weight ingredients into a homogeneous single-phase. Essentially, it generates an amorphous blend, wherein the stabilization is achieved by mutual interactions between drug-drug or drug-excipient (low-molecular-weight).[1,2] Basically, COAM surpasses the polymeric solid dispersion due to a small amount of coformer, improved processability, and robustness. The main benefit is reaped from excellent intermolecular interactions operating between drug and coformer, higher conformational flexibility, molecular mixing, and anti-plasticizing effect. Eventually, higher miscibility and prevention of crystallization and phase separation become apparent.[3] Moreover, high stability, improved solubility and dissolution can be viewed as predominant benefits of COAM, compared to amorphous compounds.[4-6] The gamut of solid-state interactions such as hydrogen, pi, and ionic, could mark a significant improvement in bioavailability and onset of action of drug/s in COAM.[7,8]

In recent times, amino acid-based COAM has been developed by workers due to prominent advantages such as biocompatibility, biodegradation, anti-plasticizer effect, and delayed recrystallization.[9] Improved physical stability and dissolution rate of COAM drugs have been reported through polymeric solid dispersions, due to the strong molecular interaction. Eventually, numerous amino acid-based COAM of drug/s have unfolded their potential applications, as evident from literature. Especially, studies have enunciated the enhanced dissolution of indomethacin due to its COAM with lysine.[10] Even, the COAM of indomethacin-arginine and carbamazepine-tryptophan have been prepared demonstrating improved stability.[11-13] The naproxen-arginine COAM has exhibited improved stability and a ten-fold faster dissolution rate compared to the crystalline form.[13] In another study, the selection of coformer amino acids based on ligand-receptor interaction made has deciphered improved physical stability for simvastatin-lysine and glibenclamide-serine. It divulged the prevention of the conversion of the amide group into an unstable tautomeric imide form.[14] The underlying reason for the addition of proline to naproxen-tryptophan and naproxen-arginine COAM increasing the dissolution rate along with stability improvement was found to be hydrogen bonding.[15] Interestingly, certain studies have reported amino acids for low Tg drugs, aiming to improve processability, along with solubility and dissolution.[16-18]

Reviewing incentives offered by amino acids, it was thought to prepare COAM for curcumin, which is a multifunctional phytoceutical extensively researched. Prospective pharmacotherapeutic potential despite pH-dependent instability has made curcumin a choice for many workers.[19] Each study on this polyphenolic active has been leveraged with its potential role as an antioxidant, antimicrobial, anticancer, anti-Alzheimer, etc.[20,21] The quest to improve the solubility and stability has enabled numerous curcumin COAM formulations. The COAM of curcumin-folic acid dihydrate has shown improved aqueous solubility, a higher dissolution rate, and improved stability.[22] Curcumin-piperazine COAM implied the hydrogen bonding

between the N-H group of piperazine and C=O group of curcumin, stabilizing the di-keto configuration of curcumin, and subsequently increasing the solubility of curcumin.[23] Reportedly, curcumin-artemisinin COAM showed two-fold higher bioavailability than curcumin-pyrogallol cocrystals, with minimal adverse effects.[24] Hence, we planned to screen amino acids for improving solubility and dissolution of curcumin by co-amorphization. The selection of an appropriate coformer for curcumin was made by screening various amino acids. Based on the solubility data and favorable structural interactions, L-lysine, a basic amino acid, was further utilized in the salt form, that is, lysine acetate. The curcumin-lysine acetate COAM was formulated using a liquid-assisted grinding technique. Subsequently, curcumin, lysine acetate, and COAM were evaluated using saturation solubility, Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), XRD, and scanning electron microscopy (SEM). The COAM tablet formulation was further evaluated for appearance, thickness, hardness, weight variation, friability, drug content, disintegration, *in vitro* dissolution, and accelerated stability.

MATERIALS AND METHODS

Materials

Curcumin was received as a gift sample from Naturite Agro Product Ltd., Hyderabad, Telangana, India. L-lysine acetate was supplied by Devson Impex Pvt. Ltd., Mumbai, Maharashtra, India. Acetic acid, methanol (analytical grade), and other non-drug components were purchased from Loba Chemie Pvt. Ltd., Mumbai, Maharashtra, India.

Methods

Screening of amino acids for improved solubility of curcumin

The screening of binary systems containing curcumin and each amino acid, namely, L-alanine, aspartic acid, L-cysteine, L-histidine, L-lysine, and L-tryptophan in equimolar quantity was performed for saturation solubility separately, by phase solubility method, in triplicate. An excess amount of curcumin-amino acid equimolar blend (10 mg) was dissolved separately in a conical flask containing 10 mL of distilled water. Further, all the solutions were stirred at 37°C for a time interval of 24 h using an orbital shaker. After equilibrium, centrifugation of samples was performed at 5000 rpm (15 min), the samples were filtered through Whatman filter paper no. 41, and absorbance was measured at λ_{max} 425 nm using a UV spectrophotometer (Shimadzu UV-1800 Spectrophotometer, Shimadzu, Japan).[25,26] For improvisation of solubility, an amino acid imparting the highest solubility to curcumin was further selected as amino acid salt to enable maximum favorable interactions within a binary mixture. Subsequently, a binary system comprising curcumin and lysine acetate was formulated into COAM.

Preparation and characterization of COAM

Saturation solubility

The COAM comprising curcumin and lysine acetate was prepared by a liquid-assisted grinding method. Accurately weighed equimolar quantities of curcumin and lysine acetate were ground in a mortar using a pestle for 15 min with a dropwise addition of methanol. The prepared COAM was further evaluated, as follows.[17] Saturation solubility for COAM was performed by phase solubility method, in triplicate. An excess amount of curcumin, lysine acetate, and COAM were dissolved separately in a conical flask containing 10 mL distilled water. Further, the dispersions were stirred at 37°C for 24 h in an orbital shaker. After equilibrium, centrifugation was performed at 5000 rpm (15 min) followed by supernatant filtration using Whatman filter paper no. 41 and absorbance was measured using a UV visible spectrophotometer (Shimadzu UV-1800 Spectrophotometer, Shimadzu, Japan).[25,26]

FTIR

The FTIR spectra of neat curcumin, lysine acetate, and COAM (1:1) were recorded by the FTIR spectrophotometer (Bruker Alpha-T, India) to observe physicochemical interactions. Samples (approximately 2 mg) were thoroughly ground with KBr and placed in a sample holder. All the samples were scanned between the wavenumber of 4000 and –400 cm^{-1} with 2 cm^{-1} resolution.

DSC

DSC (Shimadzu) was used to analyze the thermal behavior of curcumin, lysine acetate, and COAM. Approximately, 3 mg of each sample was placed in an aluminium pan. Herein, an empty aluminium pan was considered as a reference standard. Hermetically sealed aluminium pans eliminated the possibility of environmental influence. Nitrogen gas was continuously purged (50 mL/min) to retain the inert atmosphere throughout the process. Further, melting nature was recorded in triplicate from ambient temperature to 220°C with a heating rate of 10°C/min.

X-ray diffraction (XRD)

XRD pattern (XRD) of neat curcumin, lysine acetate, and COAM was screened at room temperature using an X-ray diffractometer (D2 Phaser Bruker) equipped with a 2θ compensating slit. Considering the size of the sample, either a polymethyl methacrylate holder (25 mm) or a zero background small sample holder was utilized to mount the sample. The analysis was performed using Cu Kα (λ = 1.5418Å) source of X-rays with a continuous mode, 0.001° step size, 1 sec step time at 40 kV and 40 mA. The diffraction data were collected in a 2θ range of 10–90°.

SEM

Neat curcumin, lysine acetate, and COAM were characterized for morphology assessment by scanning electron microscopy (VEGA3 TESCAN). The stubs were coated with gold to a thickness of ~ 300 Å under an argon atmosphere using a gold sputter coater in a high vacuum evaporator. The coater was operated at 0.1 torrs (argon) for 90 s at an accelerating voltage of 15 kV. Further, the coated sample was randomly examined by SEM at a suitable magnification of ×2000 and ×5000.

Formulation and evaluation of tablets

The COAM tablet formulation was prepared using excipients such as lactose monohydrate, ethylcellulose, microcrystalline cellulose, polyethylene glycol 6000, magnesium stearate, and talc by direct compression method and evaluated.

Appearance, thickness, and hardness

The appearance of the COAM tablets was assessed to determine color, nature, and shape. The thickness of the randomly selected 20 tablets was determined individually using a vernier caliper.[27]

The hardness of the randomly selected 20 tablets was determined individually using a hardness tester (Monsanto). The breaking point of each tablet was recorded and further, the average hardness was calculated.[27]

Weight variation and friability test

Twenty tablets were selected randomly, and the individual weight of each tablet was determined. Then, the average weight was assessed and compared with individual tablet weight. The friability test for tablets was performed by Roche Friabilator to determine weight loss due to friability. Ten tablets were weighed initially and transferred into a friabilator. The friabilator was operated at speed of 25 rpm for 4 min. Then, after weighing again, % friability was calculated using the formula:

$$\% \text{ weight loss} = \frac{(\text{Initial weight of the tablet} - \text{Final weight of the tablet})}{\text{Initial weight of the tablet}} \times 100 \quad (1)$$

Drug content

Three tablets were weighed, crushed separately and 10 mg equivalent powder was dissolved in 0.1N hydrochloric acid (HCl) (10 mL) and filtered by Whatman filter paper no. 41. Then, the drug content was calculated from the absorbance taken at 425 nm by a UV spectrophotometer.

Disintegration and *in vitro* dissolution studies

The disintegration time assessment was performed for COAM tablets using USP Disintegration test apparatus, (model ED-2L, Electrolab, India) in HCl (0.1 N) as a disintegrating media maintained at 37 ± 2°C. Each tablet was placed separately in each of the six tubes of the apparatus and the time at which each tablet disintegrates were noted. *In vitro* dissolution of COAM tablets was carried out by USP Type-II dissolution test apparatus. Nine hundred ml HCl (0.1 N) and phosphate buffer (6.8 pH) were used as a dissolution media. Both media were maintained at 37 ± 0.5°C. The paddle speed was set at 75 rpm. A sample (5 mL) was withdrawn with a specific time interval from each vessel and analyzed using UV spectroscopy at the wavelength maxima of 425 nm. The same volume of fresh 0.1 N HCl and phosphate buffer pH 6.8 was utilized for buffer replacement. Further, the dissolution profile of COAM tablets and conventional tablets comprising neat curcumin was compared.[28-30]

Accelerated stability study

The stability study of the COAM mixture and tablet was performed at the accelerated condition of relative humidity (75% RH) and temperature (40°C) as per ICH guidelines. COAM and tablet formulation were positioned in a stability chamber for 3 months. Samples were withdrawn every month from the time of placing up to 3 months. XRD and DSC were used to analyze the COAM mixture, whereas COAM tablets were evaluated for drug content and *in vitro* dissolution. The diffractogram of COAM was recorded on the 0th, 30th, 60th, and 90th days to assess the nature of the mixture. COAM was analyzed by D2 Phaser Bruker diffractometer with beta filtered Cu Kα (λ = 1.5418Å) source of X-rays in a 2θ range of 10–90° with a step size of 0.02°. The thermogram of COAM was recorded using DSC (Shimadzu) on the 0th, 30th, 60th, and 90th day for evaluation of thermal changes occurring during the stability study. Approximately 3 mg COAM sample was analyzed from ambient temperature to 210°C with a heating rate of 10°C/min by DSC (Shimadzu) in the presence of nitrogen gas. Three tablets were weighed, crushed separately and a quantity of powder equivalent to 10 mg was dissolved in 10 mL of 0.1N HCl and filtered through Whatman filter paper no. 41. The curcumin content was calculated by measuring absorbance at 425 nm by UV spectrophotometer.[28] *In vitro* dissolution of COAM tablets was performed with the USP Type-II dissolution test apparatus. In brief, 900 mL of 0.1 N HCl and phosphate buffer (pH 6.8) were used as dissolution media. Both media were maintained at 37 ± 0.5°C and paddle speed was kept constant at 75 rpm. Herein, a 5 mL sample was withdrawn at a specific time interval from each basket and analyzed by UV spectroscopy at 425 nm. The same volume of fresh 0.1 N HCl and phosphate buffer (pH 6.8) was utilized for buffer replacement.[28]

RESULTS

Screening of Amino Acids for Improved Solubility of Curcumin

Saturation solubility data demonstrated the highest solubility of curcumin in the curcumin-L-lysine blend. As depicted in Figure 1, a statistically significant difference was observed between the solubility of curcumin-L-lysine, and curcumin-L-alanine, curcumin-aspartic acid, curcumin-histidine, curcumin-tryptophan, and curcumin-L-cystine ($P < 0.05$).

Preparation and Characterization of COAM

Saturation solubility

The saturation solubility of neat curcumin was found to be 0.0010 ± 0.0002 mg/mL in distilled water, while lysine acetate had a saturation solubility of

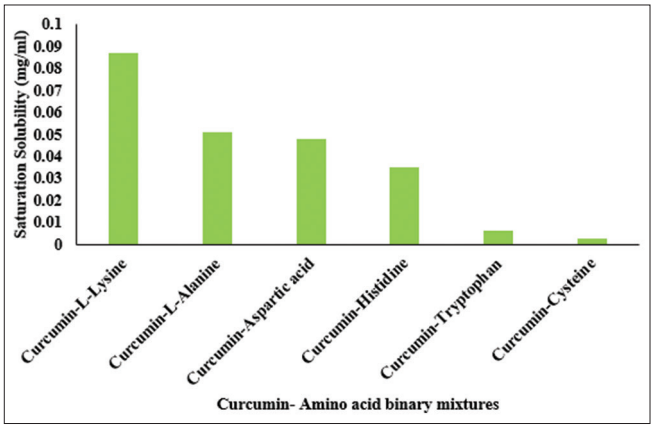


Figure 1: Saturation solubility profile of curcumin-amino acid binary mixtures

0.228 ± 0.0107 mg/mL. The saturation solubility of COAM was found to be 0.476 ± 0.0359 mg/mL in distilled water. In this study, the COAM showed significant improvement in solubility over neat curcumin as well as curcumin-amino acid binary systems ($P < 0.05$). Mainly, it may due to hydrotropic properties of lysine acetate.[31]

FTIR

The FTIR spectrum of curcumin showed the presence of characteristic peaks at 3281.57 cm^{-1}, 1707.62 cm^{-1}, 1625.05 cm^{-1}, and 1454.25 cm^{-1} representing the presence of phenolic OH stretching vibration, C=O stretching vibration, C=C stretching vibration, and aromatic C=C stretching vibration, respectively [Figure 2a]. The FTIR spectrum of lysine acetate showed the presence of amine (3367.98 cm^{-1}), ester (C-C (O) – C) (1169.20 cm^{-1}), and carboxylic acid (-OH) stretch (2644.51 cm^{-1}) [Figure 2b]. The FTIR of the COAM showed an altered pattern. The characteristic peaks were observed at 1204.35 cm^{-1}, 1574.81 cm^{-1}, 1505.17 cm^{-1}, and 2649.49 cm^{-1} representing the presence of ester C-C (O) – C stretching vibration, aromatic C=C stretching vibration, C=C stretching vibration, and carboxylic acid (-OH) stretching vibration, respectively [Figure 2c]. It was observed in COAM that characteristic peaks at wavenumber 1454.25 cm^{-1} (aromatic C=C) and 1625.05 cm^{-1} (C=C) in the FTIR of curcumin were shifted to 1574.81 cm^{-1} and 1505.17 cm^{-1}, respectively. Characteristic peaks observed at wavenumber 1169.20 cm^{-1} (ester) and 2644.51 cm^{-1} (carboxylic acid) in the FTIR of lysine acetate were shifted to1204.35 cm^{-1} and 2649.49 cm^{-1}, respectively. Here, the majority of peaks were observed to be shifted to a higher wavenumber.

DSC

The DSC thermograms of curcumin, lysine acetate, and COAM have been depicted in Figure 3. The thermogram of neat curcumin showed the presence of a sharp endothermic peak at 183.62°C [Figure 3a], while, lysine acetate, being salt, showed a relatively broad endothermic peak at 180.45°C [Figure 3b], both corresponding to melting points. These thermograms were suggestive of the crystalline nature of curcumin and lysine acetate. However, in the COAM, the endothermic peak was remarkably broadened and shifted to a lower melting point (175.98°C) [Figure 3c].

XRD

The confirmation of crystalline curcumin was revealed from the appearance of high-intensity diffraction peaks (2θ) at 12.16°, 14.51°, 15.11°, 15.74°, 17.24°, and 18.13 [Figure 4a]. In the XRD pattern of lysine acetate [Figure 4b], high-intensity diffraction peaks were observed at 2θ 12.59°, 18.62°, 23.15°, 26.69°, and 27.17° confirming the crystalline nature of lysine acetate. The diffractogram of COAM showed the reduced intensity of diffraction peaks [Figure 4c].

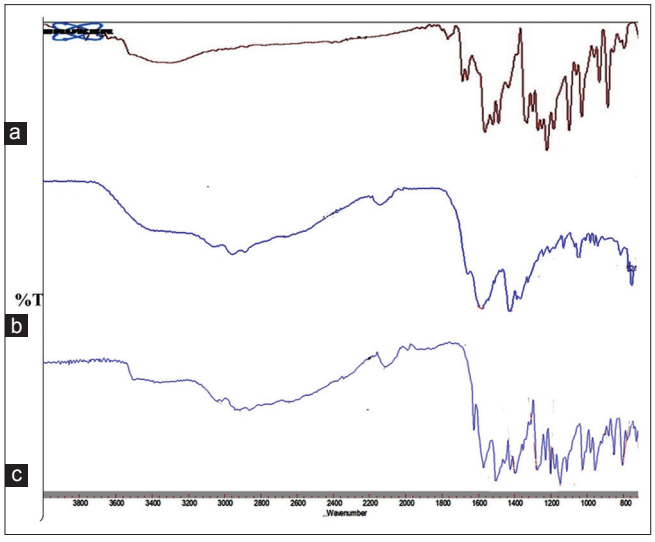


Figure 2: Fourier-transform infrared spectroscopy spectra for (a) curcumin, (b) lysine acetate, and (c) co-amorphous mixture

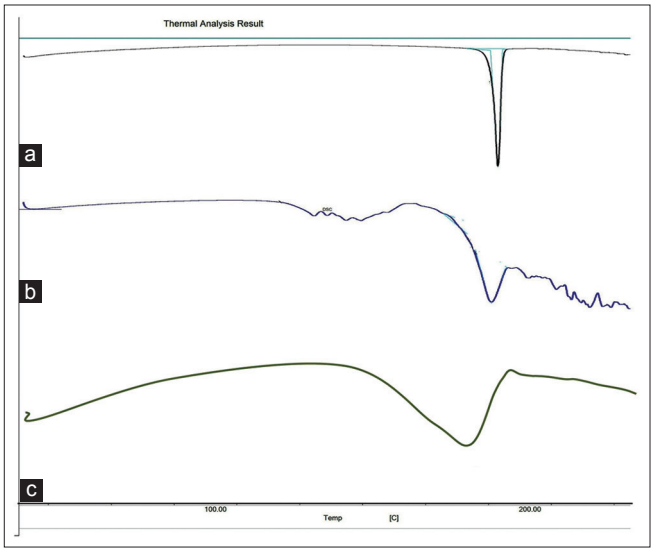


Figure 3: Differential scanning calorimetry thermograms of (a) curcumin, (b) lysine acetate, and (c) co-amorphous mixture

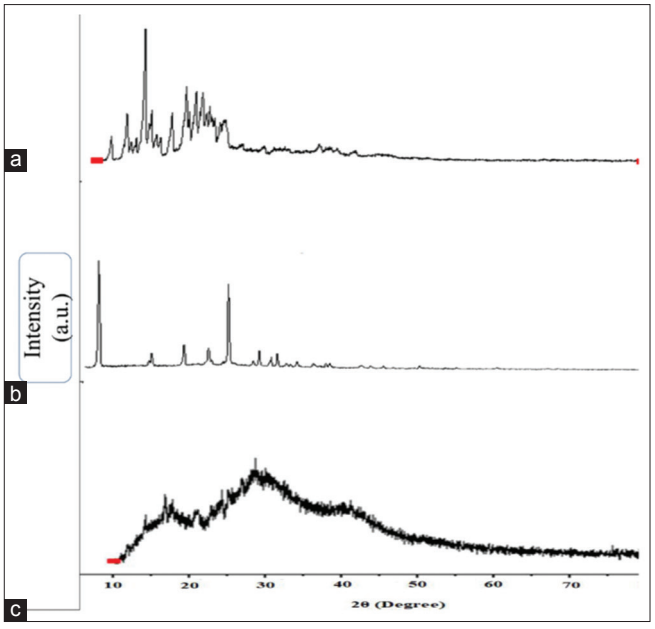


Figure 4: X-ray diffractograms for (a) curcumin, (b) lysine acetate, and (c) co-amorphous mixture

SEM

SEM photographs of curcumin [Figure 5a] and lysine acetate [Figure 5b] showed that both had crystal surface morphology. Whereas, the SEM photograph of the COAM exhibited disrupted crystallinity of curcumin along with porous morphology [Figure 5c and d].

Formulation and Evaluation of Tablet

COAM tablets were successfully prepared using the direct compression method, according to the formulation composition provided in Table 1.

Appearance, thickness, and hardness

Tablets were yellow-colored having a smooth texture and disk in shape. An average thickness of 20 tablets was recorded to be 2.34 ± 0.2 mm. An average hardness for randomly selected 20 tablets was valued as 4.56 ± 0.12 Kg/cm^2. The values of both thickness and hardness lie within the acceptable limit.

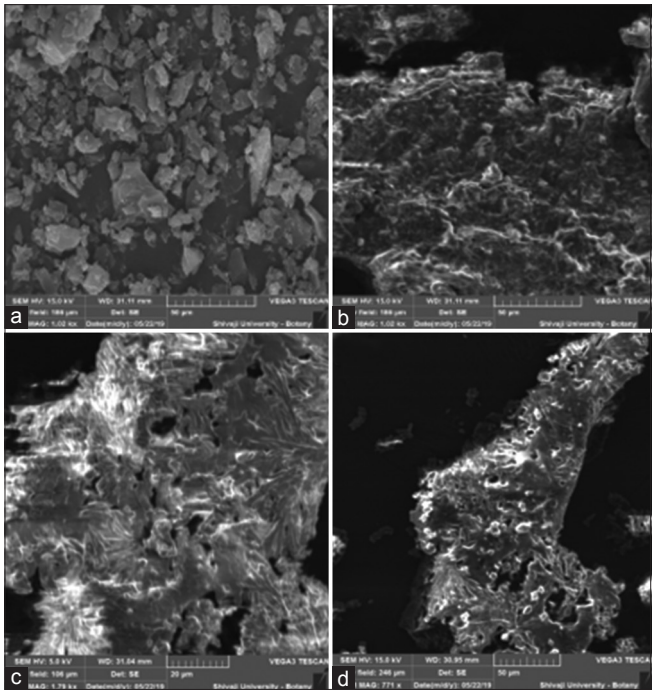


Figure 5: Scanning electron microscopy images of (a) curcumin, (b) lysine acetate, and (c and d) co-amorphous mixture

Table 1: Composition of curcumin-Lysine acetate COAM tablet formulation

S. No.	Ingredients	Quantity (mg)
1.	COAM	100
2.	Lactose monohydrate	100
3.	Ethylcellulose	60
4.	Microcrystalline cellulose	20
5.	Polyethylene glycol 6000	10
6.	Magnesium stearate	5
7.	Talc	5

COAM: Co-amorphous mixture

Weight variation and friability test

Weight variation for 20 tablets was found to be 300 ± 4.36 mg. As the variation was below 5%, it could be considered as acceptable. The average value of % friability for randomly selected ten tablets was found to be 0.23 ± 0.04%. As the value is below 1%, it lies within the acceptable limit.

Drug content

The average curcumin content in COAM tablets was found to be 99.93 ± 2.86 %. This confirmed presence of intact curcumin in COAM tablets.

Disintegration test and In vitro dissolution test

The average disintegration time for six tablets was observed to be 12 ± 0.81 min. As depicted in Figure 6a, the *in vitro* dissolution study demonstrated that neat curcumin tablets dissolved 9.82 ± 0.48% of curcumin in 12 h, while 78.23 ± 3.96% of curcumin was dissolved from COAM tablets, in 0.1N HCl at the end of 12 h. In phosphate buffer pH 6.8, the neat curcumin tablets showed 32.98 ± 1.62% drug dissolution, whereas the COAM tablets showed 92.37 ± 4.59% drug dissolution at the end of 12 h [Figure 6b]. Herein, the amorphization of curcumin has been attained in the COAM mixture that increased dissolution rate as compared to the pure curcumin. In addition, the use of lysine acetate acts as a hydrotropic agent that enhances the solubility of curcumin in dissolution media. Literature survey reported that the pure curcumin gets degrade at higher pH that is restricted in the presence of lysine acetate. Principally, curcumin is converted into phenolic radicals that can be regenerated into curcumin by donating two electrons from double bond.[31,32]

Accelerated stability study

XRD

Significant variations in the peak intensities were noted during the stability study [Figure 7]. Herein, the peak intensities were observed to be increased. However, the values of 2θ did not change, ascertaining curcumin presence.

DSC

The DSC thermograms of the binary mixture at the 0th, 30th, 60th, and 90th days are depicted in Figure 8. DSC thermograms revealed significant variations in the thermal behavior of COAM during the stability study, that is, narrowing of the endothermic peak was observed.

Drug content

An accelerated stability study indicates the drug content at the exaggerated conditions of temperature and humidity. In the case of COAM, curcumin content was decreased with time scale. Initially, curcumin content was 99.93 ± 2.86%, which further decreased to 94.53 ± 3.31%, 81.90 ± 2.53%, and 78.35 ± 2.13% on the 30th, 60th, and 90th day, respectively.

In vitro dissolution

In vitro dissolution correlates with the *in vivo* performance of the dosage form. The *in vitro* dissolution of COAM tablets was observed as 78.23 ± 3.96%, 55.90 ± 2.73%, 51.39 ± 2.80%, and 48.57 ± 2.19%; in 0.1 N HCl, on 0th, 30th, 60th, and 90th day, respectively [Table 2]. Whereas, in phosphate buffer (pH 6.8), on the 0th, 30th, 60th, and 90th day, it was observed

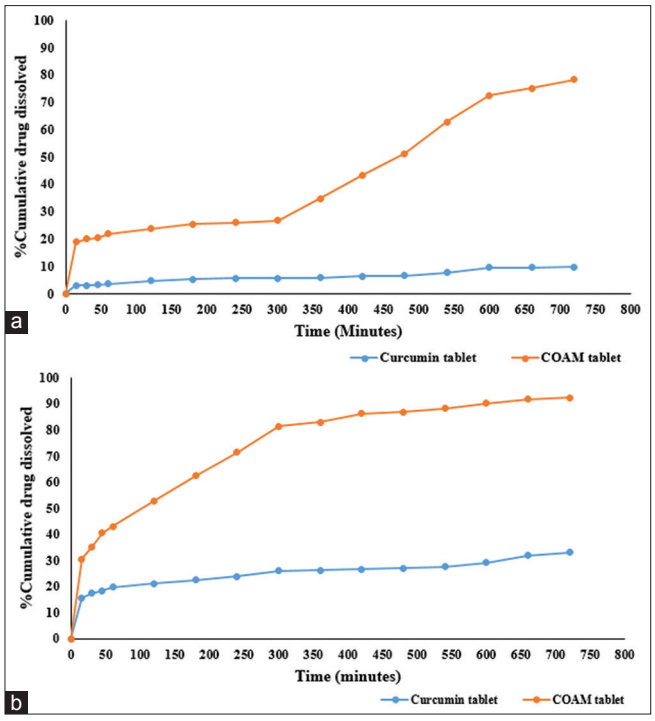


Figure 6: (a) *In vitro* dissolution profile for curcumin-lysine acetate co-amorphous mixture (COAM) tablet in 0.1N Hydrochloric acid. (b) *In vitro* dissolution profile for curcumin-lysine acetate COAM tablet in phosphate buffer pH 6.8

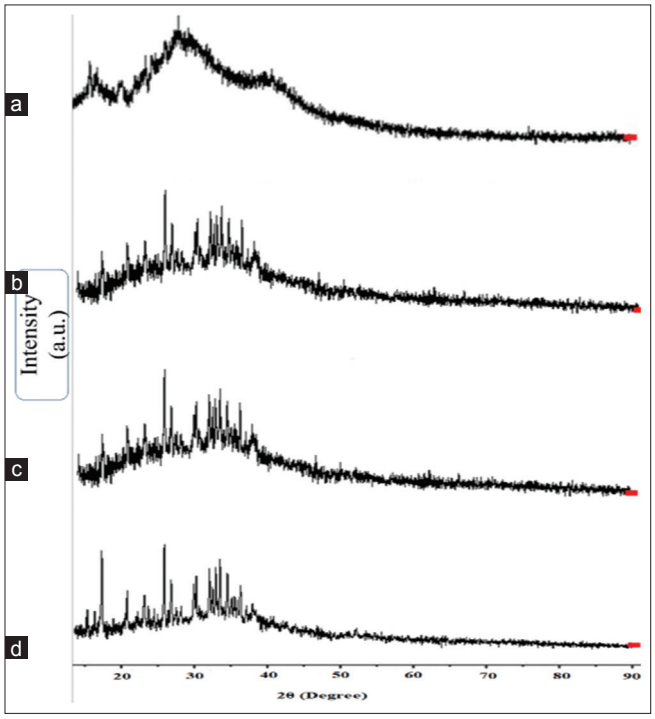


Figure 7: X-ray diffractograms of co-amorphous mixture for stability study (a) 0th day, (b) 30th day, (c) 60th day, and (d) 90th day

as 92.37 ± 4.59%, 72.32 ± 3.54%, 63.09 ± 3.09%, and 57.96 ± 2.63%, respectively. Percentage cumulative curcumin dissolved was reduced during the accelerated stability study.

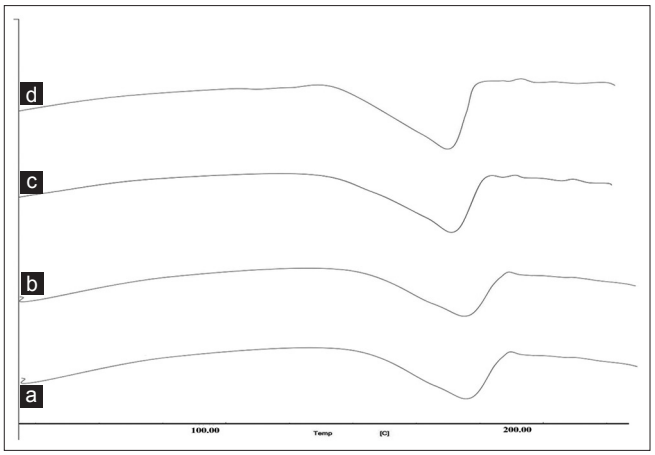


Figure 8: Differential scanning calorimetry thermograms of co-amorphous mixture for stability study (a) 0th day, (b) 30th day, (c) 60th day, and (d) 90th day

DISCUSSION

In this study, we emphasized improving solubility and dissolution of curcumin by co-amorphization; a simple and cost-effective approach.[1-4] Initially, curcumin-amino acid binary mixtures were screened to assess the saturation solubility of curcumin. The hydrotropic property of amino acids plays an important part in solubility enhancement.[31] Herein, saturation solubility data demonstrated the highest solubility of the curcumin in curcumin-L-lysine blend compared to curcumin-L-alanine, curcumin-aspartic acid, curcumin-histidine, curcumin-tryptophan, and curcumin-L-cystine due to stronger molecular interactions and binding affinity. Interestingly, the solubility of curcumin was found to be enhanced further (476 folds) in COAM form compared to neat curcumin. This might be attributed to stronger molecular level interactions and amino acid salt-induced crystallinity disruption of Curcumin, thus resulting in a higher energetic amorphous composite leading to enhanced solubility. The system was, further, analyzed by FTIR, DSC, XRD, and SEM to confirm the formation of COAM.[11,12] The shift in the majority of peaks to higher wavenumber in the FTIR spectrum was suggestive of the possibility of hydrogen bonding between curcumin and lysine acetate, usually a prominent feature of co-amorphous systems. Furthermore, broadened DSC endothermic peak revealed disruption of curcumin crystallinity, suggestive of strong interactions, implying a change in the solid-state. Moreover, the XRD diffractogram of COAM showed the reduced intensity of diffraction peaks greatly divulging the amorphous nature.[11,12] SEM of COAM showed disrupted crystallinity with porous morphology signifying amorphization. Interestingly, COAM tablet formulation showed significant improvement in the dissolution profile of curcumin regardless of pH. During accelerated stability study, the peak intensities in XRD spectra were observed to be increased which might be due to the devitrification process during which the quantum of amorphous COAM might have been converted to crystalline form, leading to the appearance of high-intensity peaks. Furthermore, narrowing of the endothermic peak was observed in the DSC spectrum which might be due to the transformation of irregularly ordered COAM to the partially

Table 2: *In vitro* dissolution data for the accelerated stability study samples (COAM tablet)

Time (min)	% cumulative drug dissolution in 0.1N HCl				% cumulative drug dissolution in phosphate buffer pH 6.8			
	0th Day	30th Day	60th Day	90th Day	0th Day	30th Day	60th Day	90th Day
0	0	0	0	0	0	0	0	0
15	18.81±0.84	15.81±0.71	15.68±0.73	15.61±0.61	30.53±1.37	13.89±0.56	11.39±0.48	10.48±0.45
30	20.04±0.95	16.36±0.82	16.5±0.78	16.09±0.63	35.08±1.41	16.86±0.61	14.35±0.53	13.94±0.50
45	20.31±1.03	17.45±0.87	16.77±0.81	16.36±0.66	40.55±1.59	21.87±0.73	20.05±0.66	18.91±0.53
60	21.81±1.05	18.81±0.93	17.14±0.86	17.04±0.71	43.06±1.63	29.84±0.99	24.37±1.09	22.55±0.66
120	23.72±1.13	20.86±0.95	22.90±1.09	22.80±0.98	52.86±1.80	33.49±1.06	29.62±1.16	28.70±1.07
180	25.36±1.20	22.90±1.03	24.40±1.14	24.40±1.01	62.43±1.99	38.05±1.34	33.26±1.39	32.12±1.21
240	26.04±1.22	27.40±1.09	27.40±1.31	25.63±1.12	71.31±2.21	43.29±1.58	37.13±1.48	35.08±1.33
300	26.72±1.35	34.09±1.59	30.81±1.44	27.54±1.30	81.34±2.63	46.48±1.84	40.10±1.67	40.01±1.63
360	34.77±1.49	37.22±1.64	34.09±1.73	32.45±1.33	82.93±2.71	50.12±1.96	44.43±1.89	44.11±1.68
420	43.36±2.12	39.27±1.88	38.59±1.89	35.18±1.41	86.35±4.23	55.36±2.03	47.16±2.12	46.62±2.01
480	51.13±2.45	43.90±1.95	40.63±1.99	40.63±1.86	86.81±4.39	61.74±2.55	55.36±2.26	52.63±2.13
540	62.72±2.94	47.31±2.03	46.09±2.04	43.5±1.97	88.17±4.40	64.48±2.76	57.18±2.40	53.08±2.16
600	72.54±3.23	54.95±2.25	49.22±2.16	45.68±2.01	90.22±4.51	66.07±2.99	59.46±2.59	54.91±2.21
660	75.04±3.62	55.22±2.64	50.18±2.58	46.22±2.13	91.82±4.53	69.72±3.01	60.37±2.96	55.36±2.30
720	78.23±3.96	55.90±2.73	51.39±2.80	48.57±2.19	92.37±4.59	72.32±3.54	63.09±3.09	57.96±2.63

*All readings are average±SD (n=3)

ordered crystalline form, which supports the devitrification of COAM. Percentage cumulative curcumin dissolved was reduced during the accelerated stability study due to the acceleration of the devitrification process. COAM being in the highly amorphous state, on day zero, which showed the highest % cumulative curcumin dissolution. After devitrification, the % cumulative curcumin dissolved got impaired. Hence, the need for a ternary system comprising acidifier in curcumin COAM has been emanated, as investigated in our earlier studies. The ascorbic acid seems to be a promising acidifier for curcumin, which might synergistically enhance the solubility of curcumin with lysine acetate in addition to the stabilization of curcumin.[32]

CONCLUSION

Solubility study of curcumin-amino acid (L-alanine, asparagine, L-cysteine, L-histidine, L-lysine, and L-tryptophan) binary system showed improved solubility of curcumin in L-lysine, compared to other amino acids due to stronger binding affinity. The salt form of L-lysine, lysine acetate, was successfully used to augment interactions and amorphization of curcumin, which, further, improved solubility. The FTIR, DSC, and XRD confirmed the amorphous nature of curcumin-lysine acetate COAM. The COAM demonstrated enhanced solubility of curcumin by 476 folds in water. The COAM tablet formulation showed optimum evaluation characteristics with improved solubility and dissolution of curcumin. The COAM of tablet formulation showed 7-fold higher drug dissolution in 0.1 N HCl and 2-fold higher drug dissolution in phosphate buffer of pH 6.8, compared to neat curcumin tablet. Stability study revealed devitrification of COAM. Thus, it can be concluded that lysine acetate can be prominently employed as a coformer for the amorphization of curcumin. However, the reason for devitrification needs to be investigated further. In the future, the incorporation of a suitable acidifier might assist in the stabilization of COAM.

CONFLICTS OF INTEREST

None to declare

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