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ENHANCEMENT OF CURCUMIN'S PHYSICOCHEMICAL PROPERTIES BY DEVELOPING ITS EUTECTIC MIXTURES

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ABSTRACT
Background: Curcumin is a compound obtained from the rhizomes of *Curcuma Longa*. It has various pharmacological properties like anti-inflammatory, anti-oxidant, anti-hyperlipidemic, etc. However, it also has some limitations that restrict its use as a medicine. **Material and method:** In the present study, a eutectic mixture of curcumin and glycine (35% and 65%) was prepared to improve its dissolution rate and photostability, characterized by DSC, FTIR, and XRD studies. **Result and discussion:** After 40 minutes, the eutectic mixture dissolved 10-fold more than the parent drug. The photostability studies were conducted according to ICH Q1B guidelines; after the seventh day of accelerated photostability studies, curcumin-glycine eutectic mixtures showed 36% degradation, 34% less than pure curcumin. **Conclusion:** The present study revealed that the physicochemical properties of BCS class II drugs can be improved by forming an eutectic mixture.

INTRODUCTION

It is well known that various drugs coming from drug discovery pipelines show problems like poor aqueous solubility, low stability, etc. Various approaches have been studied to improve the pharmaceutical properties of the drugs[1]. The most common approach is the preparation of solid dispersions, in which a drug substance is dispersed in a hydrophilic inactive ingredient [2]. Depending on the state of the drug, solid dispersions are of two types: amorphous or crystalline. Amorphous solid dispersions are formed when the drug and polymer are in amorphous form, or the drug is dispersed in a semi-crystalline polymer. These systems are unstable. Crystalline solid dispersions are formed when a drug and polymer are in the crystalline state, and these systems are thermodynamically stable. Formulations of this system are quite simple, so pharmaceutical scientists are attracted to crystalline solid dispersion [3]. Two components included in crystalline solid dispersion that are immiscible in solid state but completely miscible in liquid state are called eutectic mixtures. The eutectic mixture is a type of crystalline solid dispersion. According to thermodynamics, these systems are called intimately blended physical mixtures. Eutectic mixtures have a melting point lower than either of their components. Since the discovery of eutectic mixtures in the year, they have been used to make various metal blends in various

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metallurgical processes [4]. However, their use in medicine was not known before 1961 when first prepared a eutectic mixture of sulphathiazole-urea by Sekiguchi. Since then, eutectic mixtures have drawn the attention of researcher in the medical field due to their spontaneous preparation, easy scale-up, and promising improvement in aqueous solubility and stability [5]. The pharmaceutical industry, which previously viewed eutectic mixtures with slight aversion, is becoming more interested in eutectics for use in pharmaceutical formulations for the following reasons: (i) the preparation methods are easy to produce and scale up; (ii) eutectics do not require clinical trials because they are not considered novel chemical entities or new crystal forms; (iii) both ingredients exist in eutectic systems in the crystalline form, which is highly stable compared to amorphous materials; and (iv) the pharmaceutical industry has been paying more attention to the growing number of studies illustrating the benefits of using eutectic mixtures. Curcumin has shown various pharmacological properties like anti-inflammatory, antimicrobial, antioxidant, and antinociceptive properties, which make it a valuable compound in pharmacy. Due to Its coloring properties, it has been used as a food dye, and due to its pharmaceutical properties, it has been used in food supplements [6]. The US filed patents in the year 1995 on turmeric's healing properties, antiseptic properties, and surgical wound healing, but this was challenged by India, the main fact behind this challenge was that turmeric has been used in our traditional medicines, and there was no novelty in the patents. In Ayurveda, turmeric is the main constituent of various formulations, but its use in modern medicine is restricted because of its poor aqueous solubility and degradation, patents were canceled and geographical indication of turmeric was provided to India [7]. Curcumin has several drawbacks that restrict its use as a medication, including chemical instability, poor water solubility, limited bioavailability, and quick metabolism under physiological circumstances that cause a rapid systemic clearance. Its weak intrinsic physicochemical qualities, such as hydrolysis producing low bioavailability, photoinstability, and water insolubility, prevent it from being effectively exploited. It was discovered to be unstable at neutral pH and to degrade hydrolytically even under in vitro physiological conditions. The low bioavailability of curcumin is further attributed to photodecomposition in both solid and solution forms. Hence, despite curcumin's great performance and outstanding safety record, it is not yet recognized as a medicinal agent or medication for clinical usage. Adjuvants like piperine, which inhibit metabolic pathways, have improved the stability and bioavailability of curcumin.

Curcumin,(1E,6E)-1,7-Bis(4-Hydroxy-3methoxyphenyl)hepta-1,6-diene-3,5-dione belongs to Zingiberaceae family.this compound is bright yellow and is the main constituent of turmeric[8]. It is a polyphenol derived from the species of *Curcuma longa*. In the Biopharmaceutics Classification System (BCS), curcumin is classified under class II i.e., poorly water-soluble and highly permeable[6,9]. This compound is highly photo-degradable. These problems i.e. poor aqueous solubility, low dissolution profile, and photodegradability lead to poor clinical response of this compound or therapeutic failure. Therefore, improving these limiting properties is a key challenge in the development of formulation. Very less studies have reported innovations that modify the solubility, dissolution profile, and stability including the formation of solid dispersion, complexation, micronization, etc [10,11].

Glycine, 2-aminoethanoic acid, is an amino acid that contains one hydrogen atom in its side chain. Its chemical formula is NH_2-CH_2-COOH and it is a stable amino acid. It has been previously used for the enhancement of drug solubility, and dissolution rate. Previously this amino acid has been used in the preparation of curcumin-glycine metal complexes for antimicrobial properties and improvement in drug solubility. Solid dispersions of curcumin with glycine were also prepared to enhance these properties. In the present context due to its charge separation, this molecule can be considered a suitable candidate for the possibility of the formation of a eutectic mixture with curcumin [12]. The compounds with distinct hydrogen bond donors such as −COOH, −NH2, and −OH—that may form intermolecular hydrogen bonds with curcumin were selected as coformers. This was then further examined for compounds that were considered to be generally regarded as safe (GRAS) and that have medicinal or nutritional value (such as vitamins, medicines, amino acids, and a small number of dicarboxylic acids). Among all these glycine was selected as coformer.

MATERIAL AND METHODS

Curcumin was purchased from Central Drug House Ltd. (Vardhan House, Daryaganj, New Delhi) and glycine was obtained from Nice Chemicals (P) Ltd. (Kerala). Ethanol and methanol were also used and these were HPLC-grade chemicals. All other chemicals used are of reagent grade.

Prediction of eutectic behavior of compound

It takes a lot of time and effort to prepare the samples, run them via Differential Scanning Calorimetry (DSC), and analyze the findings to determine the eutectic point. Van't Hoff has developed a concise and straightforward preformulation prediction approach that utilizes a modified version of his equation. The Ic index is used to calculate eutectic point with the help of melting points of components. This calculation is less time-consuming than other method discussed above. This index is based on the modified Van't Hoff equation. Nonetheless, the eutectic mixture will exhibit a melting or its solidification point that is significantly lower than its constituents in a particular ratio. The specific ratio of curcumin and glycine (35% and 65%) was selected based on the theoretical models but this was confirmed by experimental model. Above and below this specific ratio, physical mixtures were developed. In order to forecast the eutectic composition of drug-carrier binary systems, Law et al. created an index. This index was obtained by using the van der Hoff equation. In the investigation, compounds with different melting points and temperatures of fusion were employed. PEG was the selected carrier. Eight distinct drug EPs were successfully predicted by the model, with a maximum error of 10%. The drug's melting point and heat of fusion were related to the eutectic point. The fundamental presumptions of the paradigm were that systems were perfect, there was no deterioration, and there were no polymorph alterations. Despite the possibility of these presumptions being broken in practice, the model produced some respectable outcomes.

- A binary mixture of curcumin- glycine was prepared at different percentages. The percentage was calculated by using the Van't Hoff equation[13].
- $I_c=(T^f_d-T^f_p)\ \Delta H^f_d/R(T^f_d)^2$
- $T_{mix}=T^f_d-w_p[R(T^f_d)^2]/\Delta H^f_d$, where T^f_d= melting point of major component, W_p = weight fraction of minor component, R= molar gas constant, ΔH^f_d= molar heat of fusion of major component, T_{mix} = temperature along the liquidus line

Based on the above equation Law *et. al* proposed an index for the calculation of the composition of drug and conformer at the eutectic point. The Ic value calculated for glycine and curcumin is 0.49. Hence various percentages of curcumin and glycine were taken i.e., 35% curcumin and 65% glycine, 34% curcumin and 66% glycine, 36% curcumin and 64% glycine and 40% curcumin and 60% glycine were used for preparing binary mixtures. Table 1 represent Ic values and prediction of formation of eutectic mixture

Table1: I_c index value

Ic value	% (w/w) drug in PEG at the eutectic point
0.0≤ Ic <0.5	Approx. 35
0.5≤ Ic<1.5	Approx. 25
1.5 ≤Ic<2.5	Approx. 15
2.5<Ic	monotectic

Table 2: Conc. of drug and other components calculated by using the I_c index

S No	Drug + components	Ic value
1.	Curcumin + salicylic acid	0.876 (25% curcumin)
2.	Curcumin + tartaric acid	4.08(monotectic)
3.	Curcumin +nicotinamide	1.92 (15% curcumin)
4.	Curcumin +glycine	0.49(35% curcumin)

Preparation of Curcumin- Glycine (CG) binary mixtures

Binary mixtures were prepared by using the solvent evaporation method. Glycine was weighed accurately and placed in mortar, followed by the weighing of curcumin. These powders were mixed by using a spatula first, then a pestle was used for proper mixing. The ground powder was dissolved in ethanol. This experiment was done at room temperature i.e., 25±2^{0}C and relative humidity (RH) was 40±5%. The solutions were kept in a desiccator for solvent evaporation. After drying the binary mixtures were procured for further studies[3,14].

Characterization

Differential scanning calorimetry (DSC)

As with DSC2-00347 (192.168.10.2), differential scanning calorimetry was used. Experiments were conducted with samples in aluminium pans that were vented but still crimped. A 3-5 mg sample was used. The samples were heated at a rate of 5˚C/min and purged in a stream of dry nitrogen running at a rate of 80mL/min. The thermogram's temperature range was 30–30°C[15]. However this technique has some limitations i.e. it is destructive method, it does not provide structural information and molecular interations cannot be studied by this method[16].

Fourier-Transform Infrared (FTIR)Spectroscopy

FTIR spectroscopy was performed on the (FT-IR Bruker 1206 0280, Germany) instrument by KBr disc technique. The range of scans was 4000- 400 nm [14].

Hot stage Microscopy

Hot-stage microscopy was performed on LINKAM (DSE600). Samples were placed in the pan. Temperature range was 1-200^{0}C. This technique is for thermal analysis and microscopy. It was used to support DSC studies [17].

Preparation of calibration curve

The stock solution had a concentration of 100µg/ml after curcumin was dissolved in ethanol, which was utilised as a solvent. After further diluting the stock solution, final concentrations of 2,4,6,8, and 10 µg/ml were obtained. Plotting the calibration curve involved figuring out the absorbance at 425 nm [18].

Dissolution studies

The dissolution test of the binary mixture (CG) was performed using the paddle method mentioned in USP (United States Pharmacopoeia) on the ELECTROLAB dissolution test apparatus. Each test was performed by using 500 mL of 40% ethanol-water mixture as a dissolution medium. Since curcumin is insoluble and prone to breakdown in different buffer solutions, choosing an appropriate release medium was important. This issue was resolved by combining ethanol with the buffer solutions, yet it was discovered that curcumin deteriorated in these solutions. As a result, ethanol:water was chosen as an appropriate release medium. Different ethanol concentrations (10–50%) were tested, but only 40% ethanol in water was able to dissolve enough curcumin to guarantee sink conditions[19]. Before the dissolution test binary mixtures were filled in empty capsule shells. Marketed formulation, pure drug, physical mixture, and binary mixtures (each 500 mg) were dissolved in a dissolution medium. 150 rpm was set for stirring of solution and temperature was set to 37±0.5°C. After every 20 min 5mL of aliquots were withdrawn and replaced by fresh medium to maintain constant level. The samples were diluted 10 times and then filtered through a membrane filter (0.45µm). Further analysis of samples was done by UV spectroscopy [14].

Photostability studies

Curcumin is a highly photounstable compound. It degrades into vanillin and ferulic acid after exposure to light. The photostability for CG eutectic mixtures and curcumin was performed in a photostability chamber (Thermolab ES2000UV) having a near fluorescent lamp and cool fluorescent lamp, according to ICH Q1B guidelines for photostability testing [20]. Irradiance power was set to an overall illumination of 1.2 million lux h^{-1}. The relative humidity and temperature were maintained at 40% and 25°C respectively. The samples (5mg) were spread on the Petri plates made up of glass with 6cm diameter. Then these plates were placed in the stability chamber for seven days, samples were taken from both(eutectic mixture and pure curcumin) at a specific interval of time and analyzed by HPLC [21]. Understanding the photochemical activity of pharmaceuticals can help with product handling, packaging, and labeling. Using the right packing materials and containers may shield the goods from light's harmful effects.A drug's sensitivity to a certain light spectrum might change depending on its chemical composition[19,22].

HPLC instrumentation and chromatographic conditions

Shimadzu high-performance liquid chromatography system that includes a 10µL Hamilton syringe, SPD-M20A prominence array detector, DGU-20A5 prominence Degasser, and CTO-10As column. Software called LC Solution was utilized to collect the data. A 400C water Spherisorb ODS2 column (4.6 x 250 mm, 5 µm particle size) was employed. Methanol-water (77:33, v/v) optimized to pH 3 at a flow rate of 1 ml/min, run time of 10 min, and spectroscopic detection at 425 nm made up the optimized mobile phase [23].

Standard stock solution

Stock solutions of curcumin and eutectic mixtures of curcumin(100µ/ml) were prepared in methanol, stored in amber-colored bottles, and kept in the refrigerator[19].

Method validation

ICH guidelines Q2(R1) were followed for the validation of the developed HPLC method and various parameters were evaluated [24]

Sensitivity: The method's sensitivity was assessed concerning the limits of quantification (LOQ) and detection (LOOD). To ascertain LOQ and LOD, several drug solution concentrations, ranging from 0.05 to 10µg/ml, were added to the column and examined. The signal-to-noise ratio for determining the limit of detection is 3, and for LOQ, it is 10^{22} [25]. In general, it is thought that a signal-to-noise ratio of 3:1 or 2:1 is suitable for determining the detection limit [26].

Linearity and calibration curve: To verify that the procedure is linear, the calibration curve was plotted. To create the standard

solution, the stock solution was diluted with methanol to a concentration range of 1–10 µg/ml. Plotting the peak area against a concentration of curcumin eutectic mixtures allowed for the construction of the calibration graph. The regression equation was calculated [26].

Accuracy: Recovery experiments were conducted to assess the method's accuracy. Samples were again spiked with 50, 100, and 150% standard curcumin, and the suggested procedure was used for the analysis. Drug recovery was assessed at several stages**.** [26].

Precision: Both intraday and interday precisions were used to assess precision. The study employed three distinct concentration levels (2.5, 5, and 7.5 µg/ml) to ascertain intra- and inter-day fluctuations.

Robustness: The HPLC method's robustness was assessed using a variety of purposefully altered parameters. The mobile phase's pH, flow rate, and column temperature were all adjusted. Modest adjustments were made to the predetermined parameters, and the impact on the outcomes was evaluated.

In vitro anti-inflammatory activity: Protein denaturation is the primary cause of inflammation. Therefore, protein denaturation can be used as an in vitro anti-inflammatory activity screening paradigm. A 1% bovine albumin solution and a curcumin-glycine eutectic combination at various concentrations make up the reaction mixture. The pH was adjusted with 1N HCl. Samples were heated to 57°C for 20 minutes after being incubated for 20 minutes at 37°C. Samples were examined spectroscopically at 660 nm after cooling. Three copies of the experiment were conducted [27,28]. Percent inhibition was calculated by:

$$\%\ inhibition = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

***In Vivo* anti-inflammatory activity**

A well-established model of acute inflammation, the carrageenan-induced paw edema is generated by a range of inflammatory mediators and has been widely used to assess the anti-edematous activity of natural products [29,30]**.** Carrageenan-induced paw edema method: in vivo studies were performed under prescribed conditions as per guidelines of IAEC and approval of the IAEC committee (MDU, Rohtak). For in vivo studies, curcumin (pure drug) was employed as the standard drug (100mg/kg) while the equivalent dose (100mg/kg) and half dose (50mg/kg) of eutectic mixtures were employed to study anti-inflammatory activity. Half a dose of eutectic mixtures was used because there was an improvement in the dissolution profile of the drug after preparing the eutectic mixture. Starving of rats was done overnight. Rats were divided into 4 groups [31,32].

Table 3: Scheme for in vivo anti-inflammatory activity

Group	Treatment	Dose	Route of administration
Group 1	Carrageenan	0.05ml sol of 1% sol	Injected in the planter side of left hind paw
Group 2	Curcumin	100 mg/kg	Oral route (suspension in 0.5% solution of carboxymethyl cellulose)
Group 3	Curcumin + glycine (eutectic mixture)	100 mg/kg	Oral route (suspension in 0.5% solution of carboxymethyl cellulose)
Group 4	Curcumin + glycine (eutectic mixture)	50 mg/kg	Oral route (suspension in 0.5% solution of carboxymethyl cellulose)

RESULT AND DISCUSSION

Differential scanning colorimetry: DSC is the only technique to reveal information about the eutectic phase and this is the most informative technique DSC analysis at a heating rate of 5 ◦C/min showed that the product obtained by solvent evaporation method. The mixture of CG was prepared in 5 proportions i.e. curcumin and glycine, 33% curcumin and 67% glycine, 34% curcumin and 66% glycine 35% curcumin and 65% glycine, , 36% curcumin and 64% glycine. and 38% curcumin and 62% glycine and were analyzed through DSC. Thermograms were compared. 35% curcumin and 65% glycine have a lower melting point (CUR–GLY 172.09◦C) than that of the starting materials (CUR 181.4 ◦C, glycine 233 ◦C). The rest of the preparations were neglected as there was slight or no depression in the melting points.

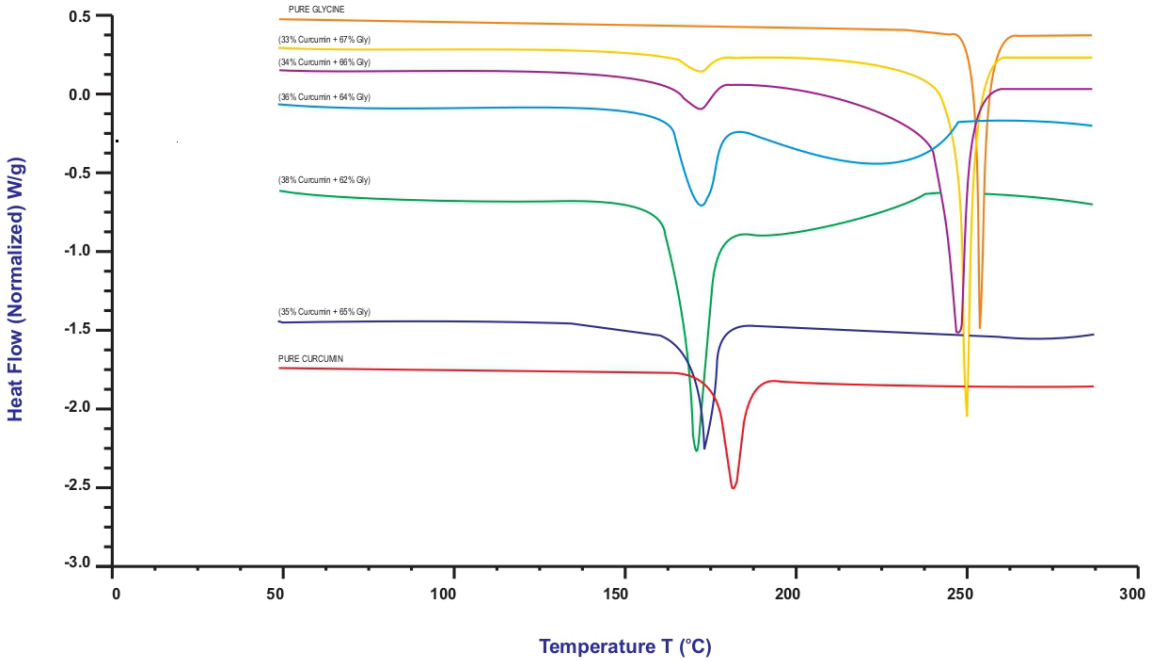

Figure 1: Overlay of DSC thermograms i.e., orange shows pure glycine, red shows pure curcumin. Dark blue shows thermogram of 35% curcumin and 65% glycine (EM), green shows 38% curcumin and 62% glycine, blue shows 36% curcumin and 64% glycine (EM), Purple shows thergram of 34% curcumin and 66% glycine (EM), yellow shows 33% curcumin and 67% glycine (EM),

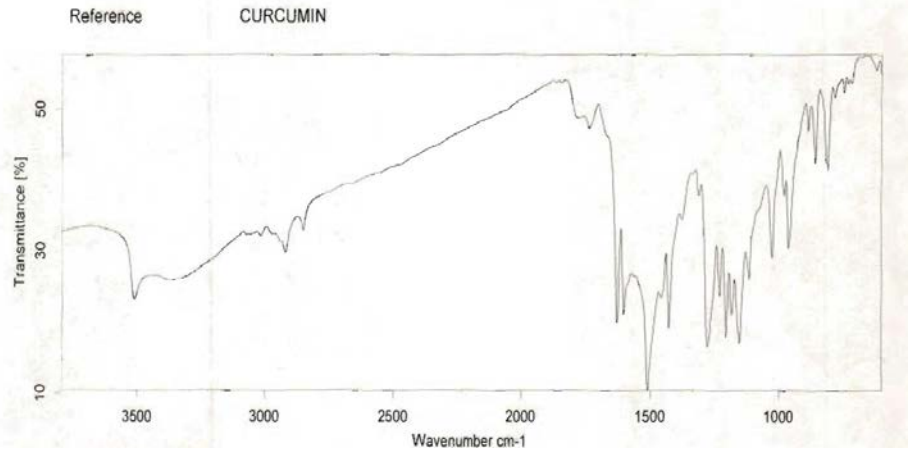

Figure 2: FTIR of pure curcumin

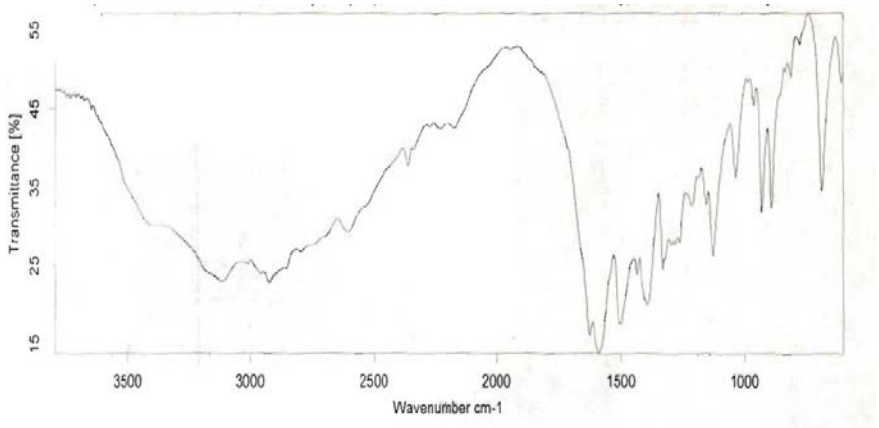

Fig3: FTIR of CG eutectic mixture

Fourier-Transform Infrared (FTIR) Spectroscopy

The FT- IR spectrum of pure Curcumin revealed stretching vibrations 3200–3500 cm^{-1} due to phenolic hydroxyl groups, stretching vibration at 1490 cm^{-1} associated with the aromatic C=C bond, and a bending vibration at 1246 cm^{-1} due to the presence of the phenolic C-O group. The CG eutectic blends' FT-IR investigations: The CG eutectic mixture's FT-IR spectra showed that the peaks for O-H (H Bonded), C-H Stretch, C=O Stretch, and =C-H Stretch were located at 3310–3457, 2816–3088, 1615–1911, and 848–1065, respectively. The drug-glycine interaction may be the cause of some of the peaks moving in the eutectic mixture's spectrum. Compared to the drug and conformer spectra, the eutectic mixture spectrum exhibits an absence characteristic at peaks 2617–2810. This verified that a eutectic mixture had formed. Both graphs are provided for comparative analysis.

Hot stage microscopy

With the help of HSM, one can visualize the melting of either component in a multicomponent system with the gradual increment in the temperature of sample. This study reveals the melting of the mixture of curcumin and glycine at 172°C, which supports the DSC study and proves that a eutectic mixture was formed. The melting of the curcumin is at 183°C and that of glycine is at 233°C which is greater than the melting point of eutectic mixture.

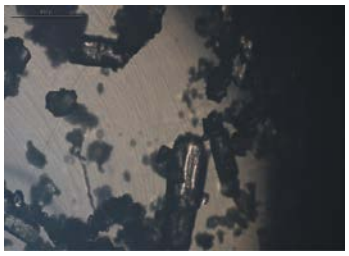

Figure 4: Micrograph of CG eutectic mixture at 150°C

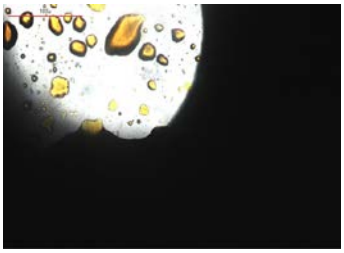

Figure 5: Micrograph of CG eutectic mixture at 172°C

Both micrographs were obtained by Hot-stage microscopy and these clearly show that there is no interaction at 150°C but both components melted at 172°C. This confirms the formation of eutectic mixtures of curcumin and glycine.

Standard curve of curcumin: Multiple concentration of curcumin in an ethanol-water mixture was taken. Absorbance was determined at 425nm. A graph was plotted between absorbance and concentration. The R-value was calculated based on the calibration curve and it was found to be 0.974. table 4 shows concentration *vs* absorbance data.

Table 4: Concentration *v/s* absorbance data

Concentration (µg/ml)	Absorbance
2	0.129±0.22
4	0.289±0.42
6	0.33±0.32
8	0.48±0.17
10	0.634±0.38

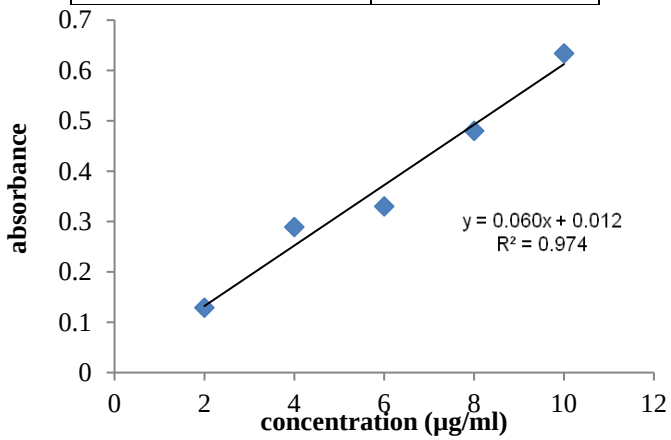

Figure 6: calibration curve of curcumin

Dissolution studies: In vitro, dissolution profile of pure curcumin, CG eutectic mixtures, CG physical mixture, and marketed formulation (Dr. Morepen curcumin veg capsule) of curcumin shown in the following table and graph. Curcumin showed about 13% drug release while physical mixture, eutectic mixture, and marketed formulation exhibit about 15%, 17%, and 18% respectively. At 60 min there was a burst effect in the dissolution profile of CG eutectic mixtures, this is just because of the high solubility of the eutectic mixture. The outcomes of this in vitro drug release study demonstrated enhancement of the dissolution profile of CG eutectic mixture than that of pure drug and marketed formulation. The results show that eutectic mixture has improved dissolution properties than that of pure drug, physical mixture, and marketed formulation. The readings were taken in triplicate. Table 5 Percentage drug release profile of the pure drug, eutectic mixture, physical mixture, and marketed formulation.

Table 5: percentage drug release profile of curcumin, CG eutectic mixture, physical mixture, and marketed formulation

Time	Pure drug	Curcumin+ glycine	Curcumin +glycine pm	Marketed formulation
0	0	0	0	0
20	3.753205±0.03	8.865156±0.02	9.94564±0.03	6.652779±0.04
40	7.899723±0.02	12.67405±0.03	10.36786±0.02	10.84278±0.03
60	8.357315±0.01	13.9435±0.04	10.62366±0.04	12.74516±0.05
80	8.789013±0.02	13.62344±0.02	10.88093±0.03	13.54331±0.06
100	9.138136±0.01	14.10351±0.04	11.28624±0.02	14.05318±0.03
120	9.471495±0.03	14.40915±0.02	11.92999±0.04	14.47853±0.04
140	10.00055±0.02	15.00441±0.03	12.90404±0.03	15.14573±0.02
160	10.75131±0.03	15.38785±0.02	13.18769±0.02	15.58182±0.02
180	11.33496±0.02	15.85277±0.02	14.11776±0.01	16.02134±0.01
200	12.03685±0.04	16.67926±0.03	14.39772±0.06	16.37486±0.06
220	12.27995±0.02	17.18766±0.04	14.9044±0.04	17.41584±0.03
240	13.08005±0.02	17.64086±0.03	15.33925±0.05	18.14216±0.01

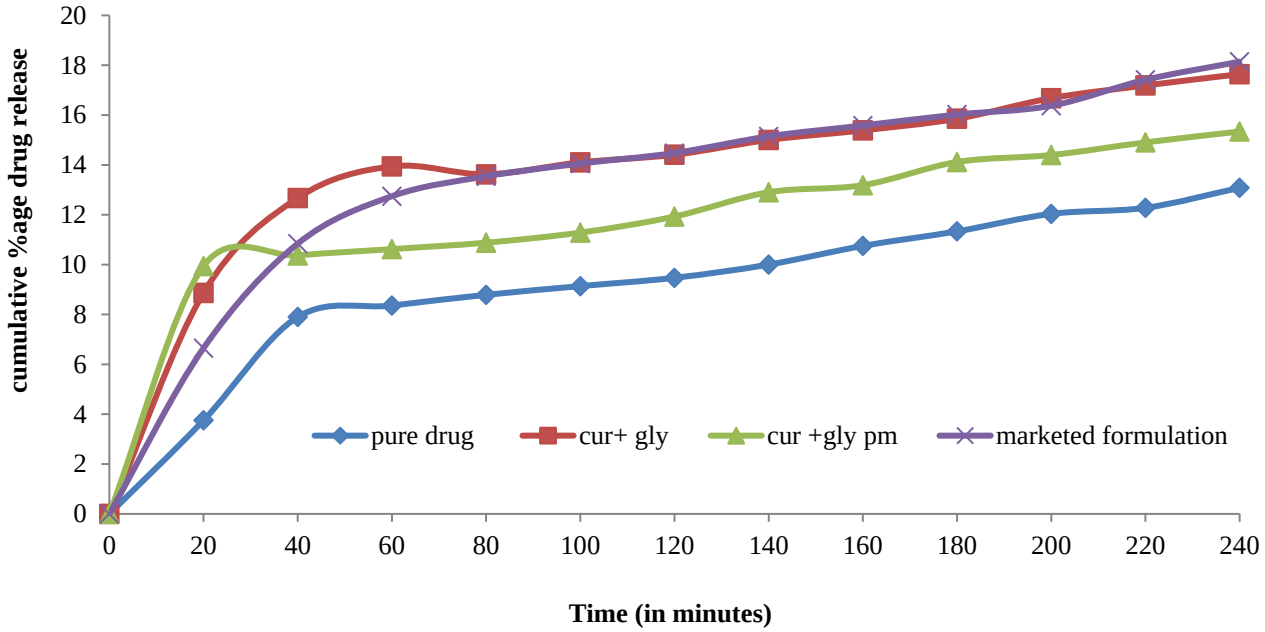

Figure 7: % drug release graph

Stability studies: In this study, the eutectic mixture (35%:65%, curcumin:glycine) and pure curcumin (gm) were subjected to accelerated photodegradation in a photostability chamber (1.2 million lux h^{-1}). The samples were withdrawn at 24 hr, 48hr, 72hr, 96hr, 120hr, 144hr, and 168hr and were analyzed by HPLC. After the 7^{th} day of study, pure curcumin degraded up to 70.5% while the eutectic mixture was 36% which is twofold less than the pure curcumin. The degradation follows first-order kinetics and R^2 value is 0.968 (pure curcumin) and 0.979 (CG eutectic mixture). Table 6 represent the percent degradation of eutrectic mixture and pure curcumin *versus* time.

Table 6: Stability study of curcumin & CG eutectic mixture

No. of days	% Degradation of CG eutectic mixtures	% Degradation of pure curcumin
0	0	0
1	9.76	11.56
2	13.44	31.2
3	17.09	41.04
4	22.64	44.72
5	30.00	58.56
6	31.84	66.83
7	36.00	70.5

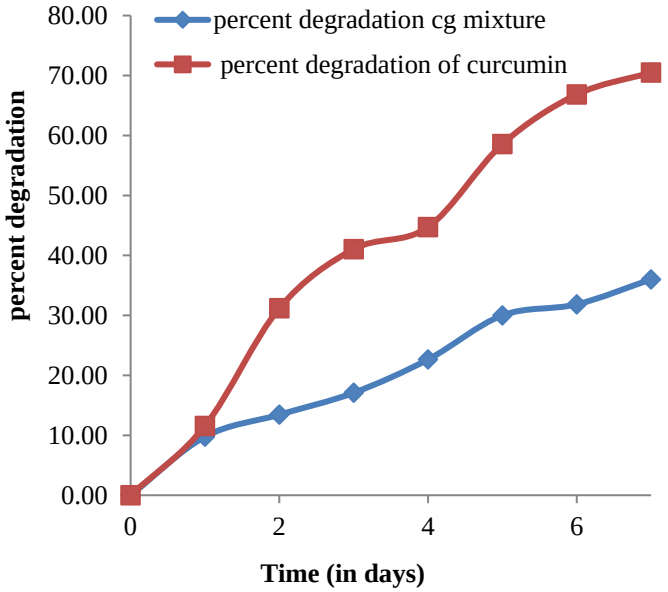

Figure 8: Percentage of drug degradation profile

Table 7: Parameters calculated during photostability study

Parameter	Pure curcumin	Eutectic Mixture
K value	1.4	0.09
R^2 value (first order)	0.961	0.979
R^2 value (Zero order)	0.934	0.968
Shelf life	2days	12 days

In vitro anti-inflammatory activity: Research conducted in vitro has shown that denaturation of proteins is the primary cause of inflammation. The effectiveness of curcumin in denaturing proteins was investigated in order to elucidate the mechanism behind its anti-inflammatory properties. Curcumin-glycine eutectic combination was shown to reduce heat-induced denaturation of protein at varying doses. At 500µg/ml, the maximum inhibition of 71.53 ±0.26% was noted. 119.35±1.99 µg/ml was determined to be the IC50 value. At 250µg/ml, aspirin, a common anti-inflammatory medication, had the highest level of inhibition, measuring 76.23± 0.54%.

Table 8: Percentage inhibition of denaturation of protein by curcumin and standard drug

Concentration of sample	% Inhibition	Control (aspirin) concentration	% Inhibition
100µg/ml	11.6±0.88	50 µg/ml	16.94±0.50
200µg/ml	28.08±0.21	100 µg/ml	29.88±0.46
300 µg/ml	43.07±0.52	150 µg/ml	43.52±025
400µg/ml	55.61±0.82	200 µg/ml	60.47±0.36
500µg/ml	71.53±0.26	250 µg/ml	76.23±0.54

In vivo anti-inflammatory activity:

In vivo, anti-inflammatory activity: Anti-inflammatory activity of eutectic mixtures on carrageenan-induced paw edema in rats' hind paws is presented in the table 9. The dose of the eutectic mixture to group 3 was kept at half compared to the parent drug curcumin and in group 4 equivalent dose was given. There is no reduction in inflammation was found in group 1. However, all dose-treated groups showed a reduction in inflammation but the CG eutectic mixture showed maximum reduction. The values of reduction of paw volume in each group, 0.531±0.01, 0.363±0.05, 0.260±0.04, and 167±0.05 were found respectively after 5 hour of carrageenan administration

Table 9: Paw volume data of animals

Time	Group 1	Group 2	Group 3	Group 4
1	0.463±0.02	0.106±0.02	0.217±0.03	0.310±0.03
2	0.521±0.03	0.162±0.03	0.223±0.06	0.321±0.02
3	0.513±0.03	0.183±0.02	0.225±0.04	0.333±0.01
4	0.478±0.05	0.179±0.04	0.235±0.06	0.336±0.01
5	0.509±0.06	0.152±0.06	0.245±0.05	0.342±0.04
6	0.531±0.01	0.167±0.05	0.260±0.04	0.363±0.05

Group 1 – Control; Group 2 – Pure drug (100mg/kg); Group 3 – Eutectic mixture 50mg/Kg; Group 4 – Eutectic mixture 100mg/Kg

CONCLUSION

The outcome proves that curcumin can make an eutectic mixture with glycine with an increased dissolution rate. The in vivo activity supports the hypothesis i.e., increase in the dissolution of the eutectic mixture (curcumin 35% and glycine 65%) when compared to pure drug correlates the decrease in inflammation of rat paw. Physicochemical properties of curcumin can be improved by the formation of eutectic mixture. This approach is cost effective and less time consuming. Animals used in this study was approved by IAEC MDU, Rohtak vide letter no.CAH/2023/157-162 dated 21/08/2023

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CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR CONTRIBUTION

All authors contributed to the conception of study design by Vikas Budhwar. Materials and lab data results are prepared by Sunita Ahlawat. Pharmacological activity was analysed by Manjusha Choudhary. Sunita Ahlawat wrote drafts of manuscript and all authors read and approved manuscript.

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