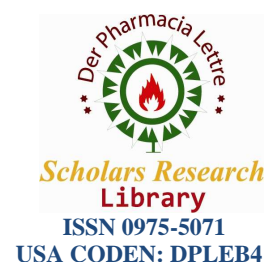




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Physicochemical characterizations and dissolution behavior of curcumin and α -cyclodextrin molecular inclusion complexes

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ABSTRACT

The aim of present work was to enhance the aqueous solubility and dissolution rate of the curcumin, a water insoluble yellow colored phenolic pigment with good anti-inflammatory property obtained from dried rhizomes of plant *Curcuma longa*. This paper presents physicochemical evaluations of curcumin and its molecular inclusion complexes with α -cyclodextrin. Phase solubility studies revealed linear relationships (AL type) indicating 1:1 ratios of curcumin and α -cyclodextrin. Based upon the stoichiometric ratio as obtained from phase solubility studies, solid inclusion complexes were prepared by grinding, kneading and freeze drying methods and then investigated by differential scanning calorimetry (DSC), infra red spectroscopy (IR) and X-ray diffractometry (XRD). All tested curcumin complexes behaved as amorphous solid mixture in XRD and FT IR investigations. Prepared inclusion complexes were compared with pure curcumin for their dissolution behavior. Dissolution study revealed 10.5% release of curcumin at 1 hour and a maximum of 40.9% release was obtained by the end of 6 hours, which was due to poor water solubility of curcumin. In contrast, curcumin complexes demonstrated faster and better release behaviors. At least 3 fold increases in dissolution of all tested complexes were observed at one hour as compared to pure curcumin, with a maximum increase of approximately 3.5 fold as shown by kneaded and freeze dried complexes. At the end of 6 hours, approximately 60-65% release was observed from all inclusion complexes with a maximum release of 65% exhibited by freeze dried complex. Cyclodextrin complexation of curcumin enhanced dissolution of practically insoluble curcumin.

Keywords: Curcumin, α -cyclodextrin, inclusion complexes, solubility and dissolution.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used of all therapeutic agents. They are frequently prescribed for long-term treatment of rheumatic musculo-skeletal complaints. The major drawback to anti-inflammatory drug use is the occurrence of gastrointestinal side effects with majority of agents. Curcumin, a natural polyphenol obtained from *Curcuma longa* (family, Zinziberaceae), has been used to relieve the pain and inflammation since ancient times in traditional system [1-3]. It inhibits cyclooxygenase and lipoxygenase [4-5], platelet aggregation [6-8], neutrophils aggregation [9] and reduces pro-inflammatory leukotriens synthesis [10] during inflammatory states.

Many pre-clinical trials [1-3, 11, 12] and clinical trials [13-18] have revealed that curcumin is safe even upto a dose level of 8.0 gm/kg but no successful formulation is available on the market because of its poor bioavailability. Oral absorption of curcumin is dissolution rate limited due to very low aqueous solubility and rapid biotransformation leading to very low bioavailability [19-21]. Curcumin is a weak base and pK_a values for three acid protons have been

determined to be 7.8, 8.5 and 9.0 respectively [22]. It was found unstable at basic pH and undergoes hydrolytic decomposition even in *in vitro* physiological condition along with photodecompositions in solid as well as solution form which further contribute in poor bioavailability of curcumin [23]. Effect of α -cyclodextrin on the solubility *in vitro* and *in vivo* evaluations of the curcumin in solution has been reported [24]. This paper presents the development and evaluation of solid inclusion complexes of curcumin with α -cyclodextrin.

MATERIALS AND METHODS

Curcumin was purchased from Loba chemicals (Banglore, India.). α -cyclodextrin was purchased from S. D. Fine Chemicals (India). All other compounds and solvents used in this study were of analytical reagent grade.

Phase solubility studies

Excess amount of curcumin was placed in separate amber colored bottles containing 0-20 mM aqueous solutions of α -cyclodextrin. Suspensions were stirred continuously for five days, filtered using 0.45 micron membrane filter and then analyzed spectrophotometrically at a λ_{max} of 428 nm by using aqueous solutions of cyclodextrin as blank [25].

Preparation of inclusion complexes

The inclusion complexes of curcumin with α -cyclodextrin were prepared in a 1:1 molar ratio based on phase solubility studies by various methods such as physical mixing, kneading and freeze drying.

Physical mixture

A physical mixture was prepared by gentle mixing of accurately weighed equimolar quantities of curcumin and cyclodextrin for 5 minutes in a clean dry glass pestle and mortar.

Kneading

Equimolar quantities of curcumin and cyclodextrin was wetted by ethanol and triturated for 30 minutes in a clean dry glass pestle and mortar to get a paste like consistency. Trituration was continued until the product started drying on the walls of mortar. The products were further dried in the hot air oven at 60°C for 30 minutes, powdered, passed through 100-mesh sieve and stored in a dessicator.

Freeze-drying

Accurately weighed quantities of curcumin and cyclodextrin were dissolved in distilled water with a small amount of ammonia (27%) to aid dissolution of curcumin and sonicated for 15 min to get clear solutions. The solutions were frozen in ultra freezer by keeping over night and freeze-dried over 8 hours in a Lyph-lock 6 apparatus (Labconco). The resulting amorphous products were powdered in glass mortar, passed through 100-mesh sieve and stored in a dessicator.

Physicochemical Characterization of the solid complexes

X-ray diffraction of solid complexes

X-ray diffraction of curcumin and their inclusion complexes with α -cyclodextrin were studied by using continuous scanning on X-Ray diffractometer (PW 1830, Phillips, Japan). The X-RD traces of pure curcumin, physical mixture and inclusion complexes were compared with regard to peak position and relative intensity, peak shifting and presence or lack of peaks in certain regions of 2θ values. Run conditions are given in the Table I.

Table I. Conditions used in X-ray diffractometry

Parameters	Unit
Generator tension	30 (kV)
Generator current	25(mA)
Start angle (2θ)	5.0
End angle (2θ)	50
Step size (2θ)	0.020
Time per step	1.000 (s)
Type of scan	Continuous

Differential scanning calorimetry (DSC)

Differential scanning calorimetry of the pure curcumin, α -cyclodextrin and prepared inclusion complex of curcumin was performed using Perkin Elmer Pyris 6 DSC. Samples equivalent to 1 mg of curcumin were accurately weighed and crimped in the aluminium pans (Perkin Elmer) to get pallets. All the samples were then scanned according to the specifications listed in the Table II.

Table II. Conditions used in differential calorimetry.

Parameters	Unit
Sample size	Equivalent to 1 mg of curcumin
Temperature range	50-400 °C
Heating rate	10 °C/min
Atmosphere	Inert nitrogen gas
Flow of gas	20 ml/min

Fourier Transform Infra Red spectroscopy (FT-IR)

The FT-IR spectra of curcumin, α -cyclodextrin and inclusion complex of curcumin were recorded on the Win-IRrez (Bio-Rad) using the potassium bromide (KBr) disc technique. Sample was mixed with potassium bromide in a clean glass pestle and mortar and compressed to get pellet. Scanning was performed after base line correction setting a wave number range of 5000-500 cm^{-1}

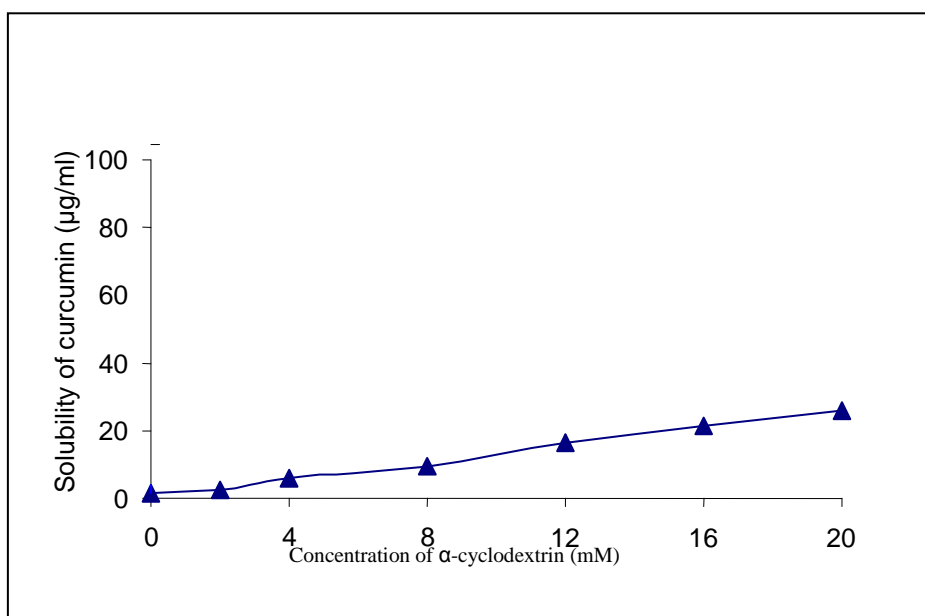


Fig 1: Comparative phase solubility diagrams of curcumin- α -cyclodextrins systems

Dissolution rate profile of the solid complexes

Dissolution of pure curcumin (20 mg) and inclusion complexes (equivalent to 20 mg curcumin) were studied according to USP XII method with the apparatus II, in 900 ml of SGF without pepsin containing 1% SLS at $37 \pm 5^\circ\text{C}$ and at a rotational speed of 75 rpm.

RESULTS

Phase solubility study

Phase solubility diagrams of curcumin with cyclodextrin were found to be linear (A_L type) indicating 1:1 ratio (Figure 1).

X-Ray diffraction of solid complexes

X-ray diffractogram of curcumin showed various peaks at different angles with most intense one at an angle of 17.68° (100%) followed by 17.62° (92%) and 9.22° (80%) respectively, revealing the crystalline nature of curcumin. X-ray diffractogram of α -CD showed the peaks at 10.2° (100%), 14.7° (65%) and 22° (91%) respectively, revealing the crystalline nature of α -cyclodextrin whereas inclusion complex of curcumin- α -cyclodextrin showed no significant peak suggesting formation of amorphous compound. A comparative X-ray diffractograms of curcumin and inclusion complexes are shown in the Figure 2.

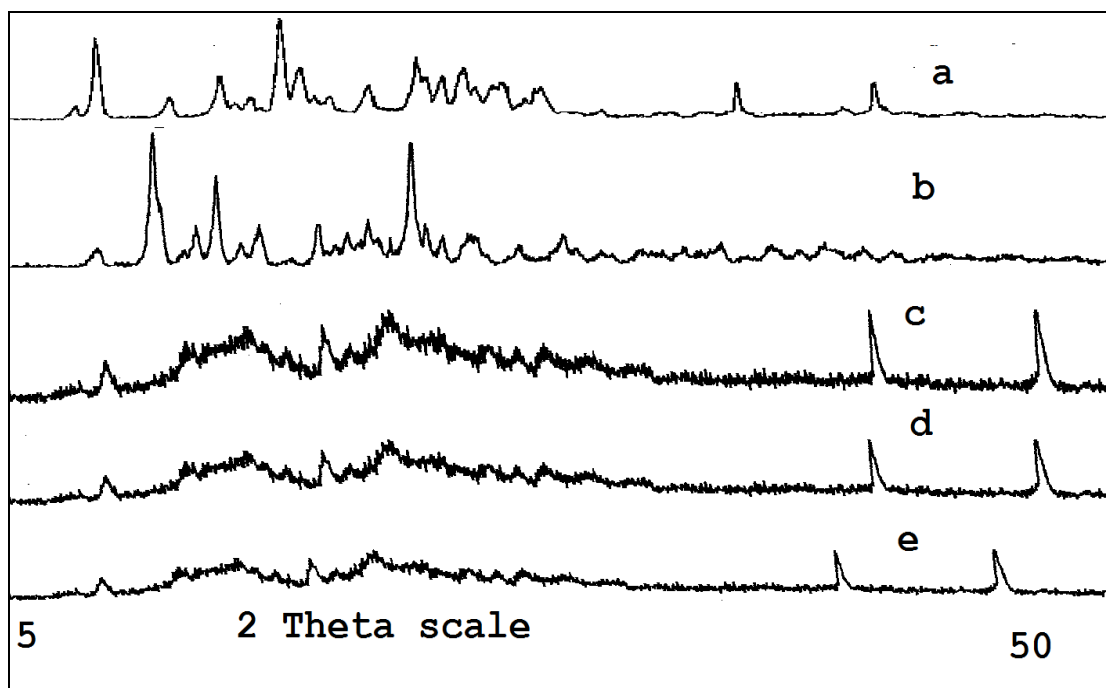


Figure 2: Comparative X-ray diffractograms of curcumin and inclusion complexes
a)curcumin, b) α-CD, c) curcumin-α-CD PM, d) curcumin-α-CD Kneaded, e) curcumin-α-CD Freeze dried

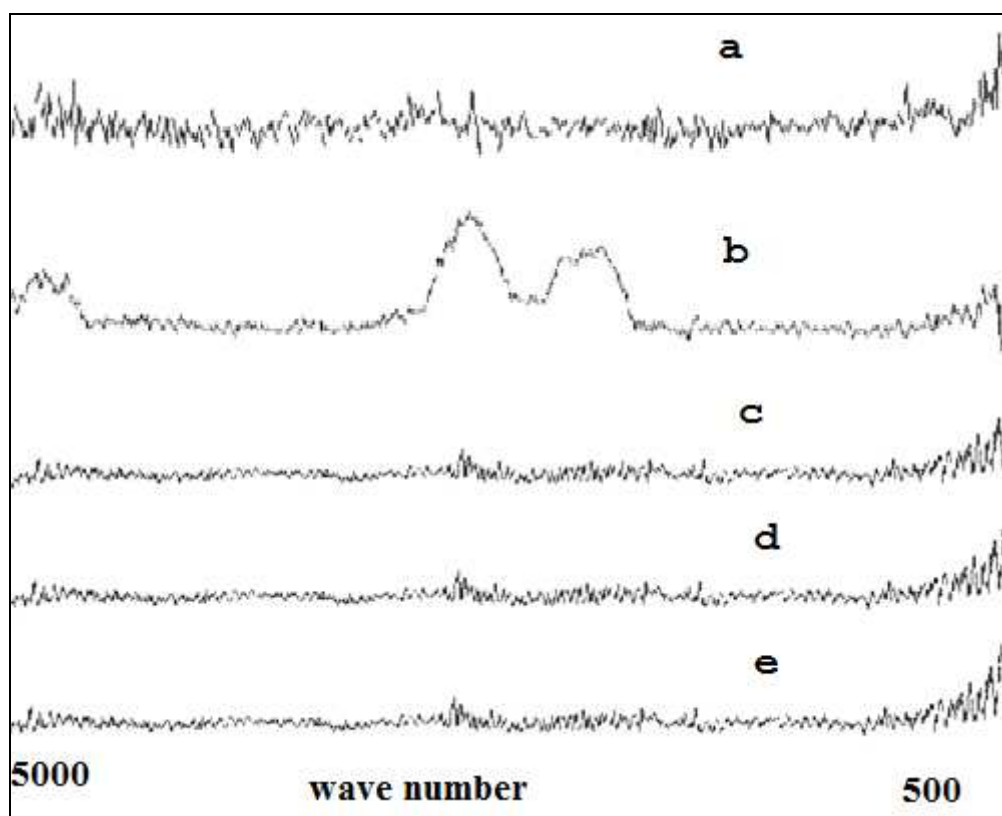


Figure 3: Comparative FT-IR spectra of curcumin and inclusion complexes
a)curcumin, b) α-CD, c) curcumin-α-CD PM, d) curcumin-α-CD Kneaded, e) curcumin-α-CD Freeze dried

FT-IR spectral analysis

Curcumin has a carbonyl-stretching band at 1629 cm^{-1} and $-\text{OH}$ band at 3511 cm^{-1} , therefore, FT-IR could be used to detect guest interactions. The carbonyl-stretching region of IR spectra of curcumin and its different systems with $\alpha\text{-CD}$ are presented in Fig. 3. The IR spectra of cyclodextrin showed the peaks corresponding to the nature and position of functional groups present. The spectra of all curcumin- CD binary products did not show new peaks

indicating that no chemical bonds were created in the formed compounds. Though, IR C=O stretching band was instead highly diminished, broader and shifted to lower frequencies in all spectral patterns of curcumin-CDs products suggesting the inclusion of the drug in the cyclodextrin cavity.

Differential Scanning Calorimetry (DSC)

The thermal curve of pure curcumin was typical of a crystalline anhydrous substance with a sharp endothermic peak at 176°C corresponding to the melting point of the drug. The DSC curve of α -cyclodextrin showed the liberation of crystal water as an endothermal effect peaked between 80-150°C, followed by a peak at 288°C corresponding to melting point of α -cyclodextrin. The complete disappearance of the drug endothermal effect was observed with all curcumin- α cyclodextrin complex suggesting inclusion of the drug and formation of amorphous compounds. Comparative DSC thermo grams of curcumin and inclusion complexes are shown in the Figure 4.

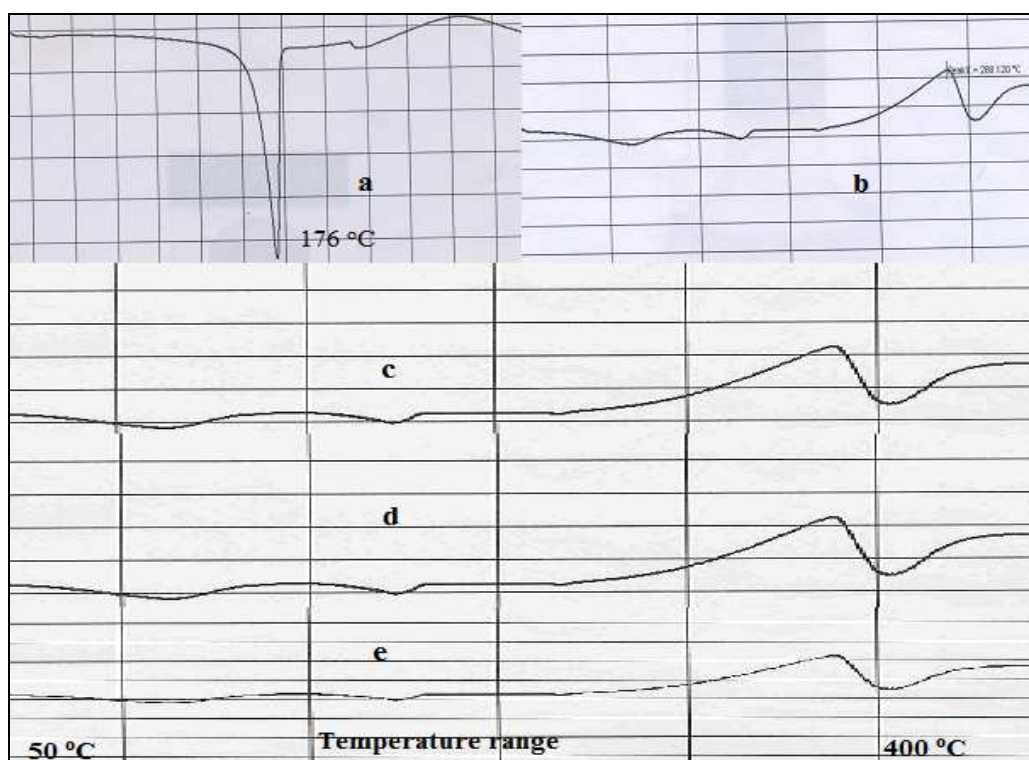


Figure 4: Comparative DSC thermo grams of curcumin and inclusion complexes
a) curcumin, b) α -CD, c) curcumin- α -CD PM, d) curcumin- α -CD Kneaded, e) curcumin- α -CD Freeze dried

Dissolution rate profile of the solid complexes

Dissolution performed in 0.1 N HCL gave only 20-30 % release at the end of 6 hours suggesting that either curcumin is tightly bound to cyclodextrin or dissolution media was saturated. A preliminary study was therefore done to get a suitable dissolution media. A stock solution of curcumin in methanol (10 μ g/ml) was prepared, diluted with only distilled water and distilled water containing 30% alcohol, 1% SLS, 0.1% tween 20 and 0.1% tween 80 separately and analyzed spectrophotometrically against the similar blank solutions at 430 nm. Based on preliminary study, 1% SLS was used as the co-solvent that was found to be compatible with dissolution media as no interaction was seen in the UV scans study. Standard plot of curcumin in SGF without pepsin with 1% (w/v) SLS (Regression equation $Y=0.1744X$, Correlation coefficient $R^2. 0.9911$) was then prepared for the calculation of dissolution at different time intervals. Dissolution study revealed 10.5% release of curcumin at 1 hour and a maximum of 40.9% release was obtained by the end of 6 hours, which was due to poor water solubility of curcumin. In contrast, curcumin complexes demonstrated faster and better release behaviors. At least 3 fold increases in dissolution of all tested complexes were observed at one hour as compared to pure curcumin, with a maximum increase of approximately 3.5 fold as shown by kneaded and freeze dried complexes. At the end of 6 hours, approximately 60-65% release was observed from all inclusion complexes with a maximum release of 65% exhibited by freeze dried complex. A comparative dissolution profile is given in the Figure 5.

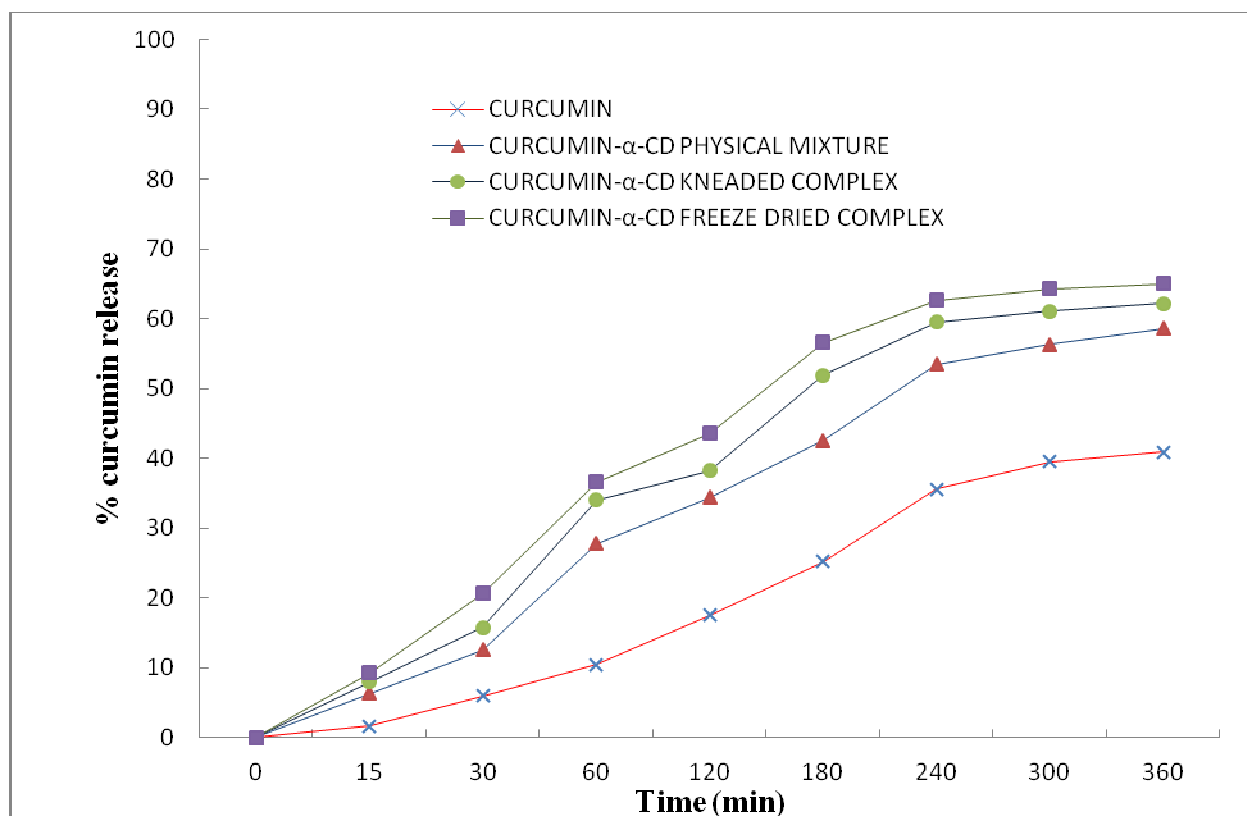


Fig 5: Release profile of curcumin and curcumin- α -CD complexes in SGF without pepsin with 1% (w/v) of SLS

DISCUSSION

Curcumin is one of the most widely studied herbal drugs. A lot of publications are available which cover pharmacological activity of curcumin ranging from anti-inflammatory to antibacterial to anti cancer and anti AIDS, neuroprotective and cardioprotective. In spite of extensive researches on this miraculous drug it is still beyond the market as a result of poor inherent physicochemical properties like water insolubility, photo-instability and hydrolysis. Absorption and tissue distribution of curcumin in albino rats revealed that curcumin is partly (about 60%) absorbed and percentage of curcumin absorbed remained constant regardless of the dose administered [26]. After oral administration of 400 mg of curcumin to rats, about 90% of the dose was present in the stomach and small intestine at the end of 30 min [26]. It was also found that whatever dose was being absorbed underwent very rapid biotransformation [27]. Following an oral dose of (^3H) curcumin, about 90% dose was excreted via faeces and 7% in the urine in 72 hours [28]. After an oral dose of 80 mg of curcumin to rat, about 99% radioactivity was excreted in faeces over 72 hours [29].

Previously preparation of curcumin alpha cyclodextrin inclusion complex by pulverization method followed by its *in vitro* and *in vivo* evaluations has been reported [24]. In this project we prepared curcumin alpha cyclodextrin inclusion complex by different methods that follows physicochemical characterization using various techniques and comparative evaluation of their dissolution behaviors. Prepared solid inclusion complexes showed promising results with approximately 3-3.5 folds increase in dissolution of practically insoluble curcumin.

CONCLUSION

The results obtained in the present investigation are significant from the point of view that curcumin-CD complexes have much better dissolution, which can be further explored for industrial purposes. Inclusion complex formation resulted in amorphous compounds with improved solubility and dissolution of curcumin. Freeze dried complexes have greater solubility than kneading one but it is much costlier than the later.

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