Int J Pharm Bio Sci 2015 Jan; 6(1): (P) 785 - 795

Research Article **Pharmaceutics**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

PHYSICOCHEMICAL CHARACTERIZATIONS, DISSOLUTION BEHAVIOR AND RELEASE KINETICS OF CURCUMIN AND β-CYCLODEXTRIN MOLECULAR INCLUSION COMPLEXES

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ABSTRACT

This paper presents physicochemical characterization, dissolution behavior and release kinetics of curcumin and its molecular inclusion complexes with β-cyclodextrin. Solid inclusion complexes of curcumin with β-cyclodextrin in 1:2 molar ratios were prepared by grinding, kneading and freeze drying methods. Complexes were characterized by differential scanning calorimetry, infra red spectroscopy and X-ray diffractometry. Prepared inclusion complexes were compared with pure curcumin for their dissolution behavior. The dissolution study demonstrated only 10.5% release from pure curcumin at 1 hour as opposed of approximately 30-35% release form curcumin complexes. 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 75-80% release was observed from all inclusion complexes with a maximum release of 80% exhibited by freeze dried complex. The release of the curcumin from all inclusion complexes was observed to follow the first order release kinetics, since the correlation coefficient for the first order was higher in comparison to other tested models.

KEYWORDS: **Curcumin, β-cyclodextrin, inclusion complexes, dissolution.**

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INTRODUCTION

Curcumin is a yellow colored natural pigment obtained from *Curcuma longa* (family. Zinziberaceae). It is a popular household spice. It is known to relieve the pain and inflammation and hence widely used since ancient times in traditional system [1-3]. Its mechanism of action is diverse. It is found to inhibit cyclooxygenase and lipoxygenage [4-5]. It also inhibits aggregation of platelets [6-8] and neutrophils [9] and reduces pro-inflammatory leukotriens synthesis during inflammatory states [10].Curcumin is found safe even upto a dose level of 8.0 gm/kg as evident from many pre-clinical trials [1-3, 11, 12] and clinical trials [13-18], however, no successful formulation is available on the market because of its low water solubility stability issues and poor bioavailability [19-21]. 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 [22, 23].Curcumin complexation with β-cyclodextrin is reported in few studies, however these report either solubility or stability or both of the yellow pigment to be used in food applications [24-27]. This paper presents the development and evaluation of solid inclusion complexes of curcumin with β-cyclodextrin its physicochemical characterization, solubility and dissolution enhancement and pattern to be employed in effective delivery of curcumin.

MATERIALS AND METHODS

Curcumin was purchased from Loba chemicals (Banglore, India.). β-cylodextrin 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 β-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 [28].

PREPARATION OF INCLUSION COMPLEXES

Based upon the stoichiometric ratio as obtained from phase solubility studies, inclusion complexes of curcumin with β-cyclodextrin were prepared in a 1:2 molar ratio by physical mixing, kneading and freeze drying methods.

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 with 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^{o} C for 30 minutes, powdered, passed through 100-mesh sieve and stored in a desiccator.

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 desiccators.

PHYSICOCHEMICAL CHARACTERIZATION OF COMPLEXES

X-ray diffraction of solid complexes

X-ray diffraction of curcumin and their inclusion complexes with β-cyclodextrin were studied by using continuous scanning on X-Ray diffractrometer (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 between 5-50□ with a step size of 0.020 per second.

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry of the pure curcumin, β-cyclodextrins 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 between 50-400 ^{o}C at a heating rate of 10 ^{o}C/min in nitrogen gas flowing at a rate of 20 ml/min.

Fourier Transform Infra Red spectroscopy (FT-IR)

The FT-IR spectra of curcumin, β-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 a pellet. Scanning was performed after base line correction setting a wave number range of 5000-500 cm^{-1}.

Dissolution rate profile of the solid complexes

Dissolution of pure curcumin (20 mg) and inclusion complexes (equivalent to 20 mg curcumin) were studies according to USP XII method with the apparatus II, in 900 ml of SGF without pepsin containing 1% SLS at 37 ± 5^{o}C and at a rotational speed of 75 rpm. The dissolution profiles of all the molecular inclusion complexes were subjected to the kinetic analysis to establish the drug-release mechanism. The release data were fitted to zero order, first order, matrix (Higuchi model), and Peppas models to ascertain the kinetic modeling of drug release [29, 30].

RESULTS

PHASE SOLUBILITY STUDY

Phase solubility diagram of curcumin with β-cyclodextrin was found to be non-linear limited solubility diagram (B_s type) indicating 2:1 ratio (Figure 1).

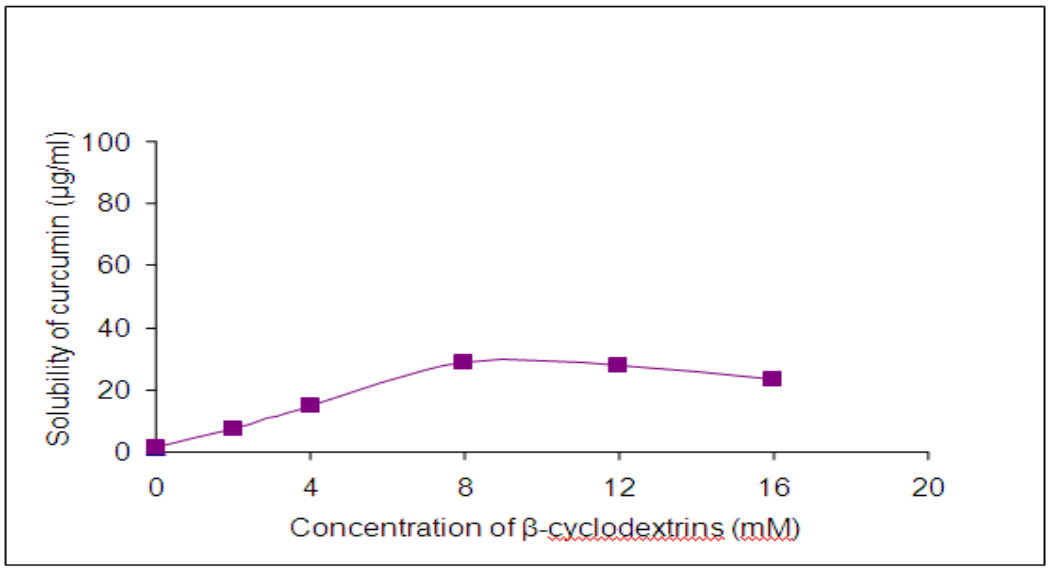


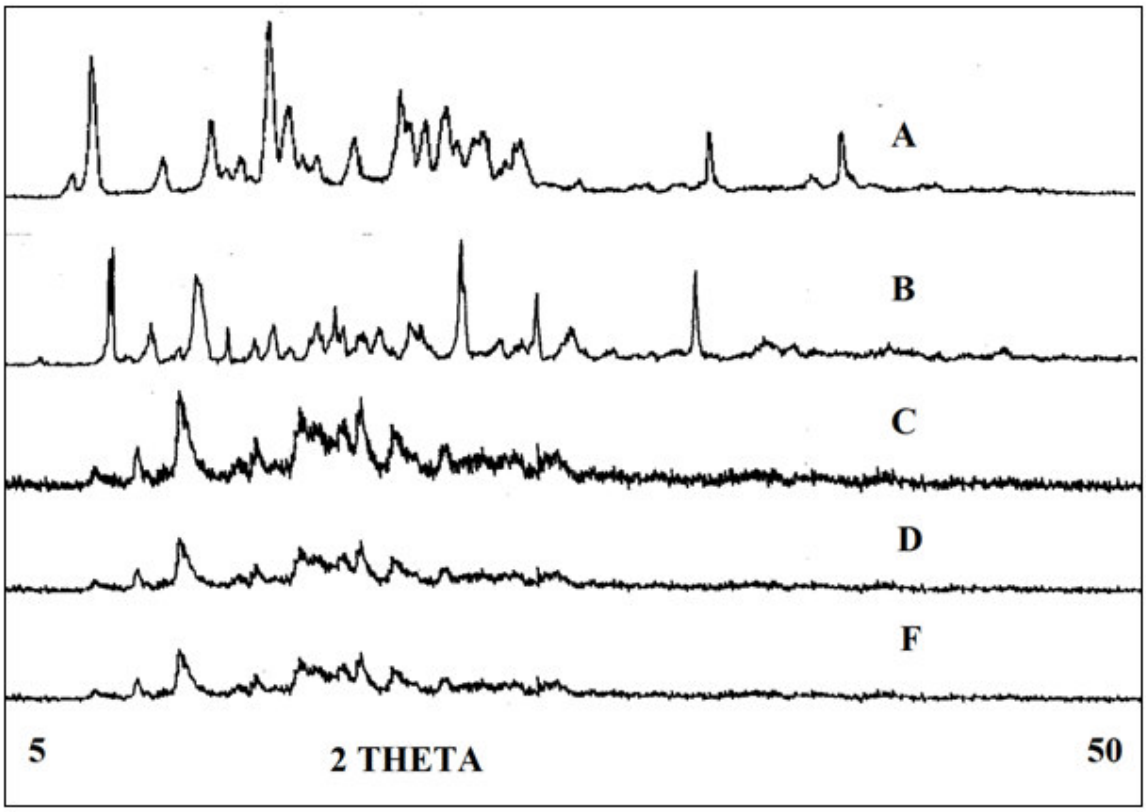
Figure 1
Comparative phase solubility diagrams of curcumin- β-cyclodextrin systems

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 β-CD also showed crystalline nature with peaks at 9.4°

(89%), 9.5° (96%), 12.8° (69%), 23° (100%) and 32° (75%) respectively whereas inclusion complex of curcumin-β-CD showed humps only, suggesting amorphous nature of the complex. 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.

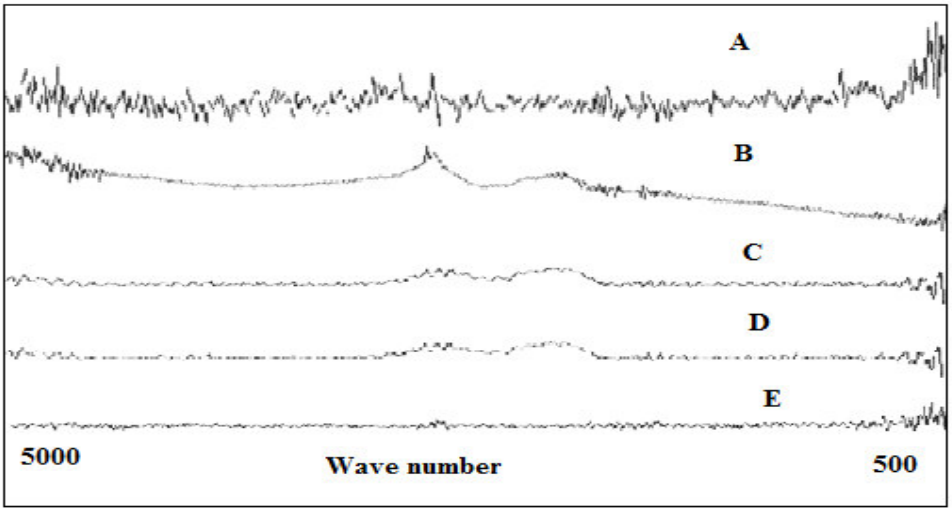


A)Curcumin, B) β-CD, C) curcumin-β-CD PM D) curcumin-β-CD Kneaded, E) curcumin-β-CD Freeze dried

FT-IR spectral analysis

Curcumin has a carbonyl-stretching band at 1629 cm^{-1} and –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 β-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- β 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- β CD products suggesting the inclusion of the drug in the cyclodextrin cavity.

Figure 3
Comparative FT-IR spectra of curcumin and inclusion complexes

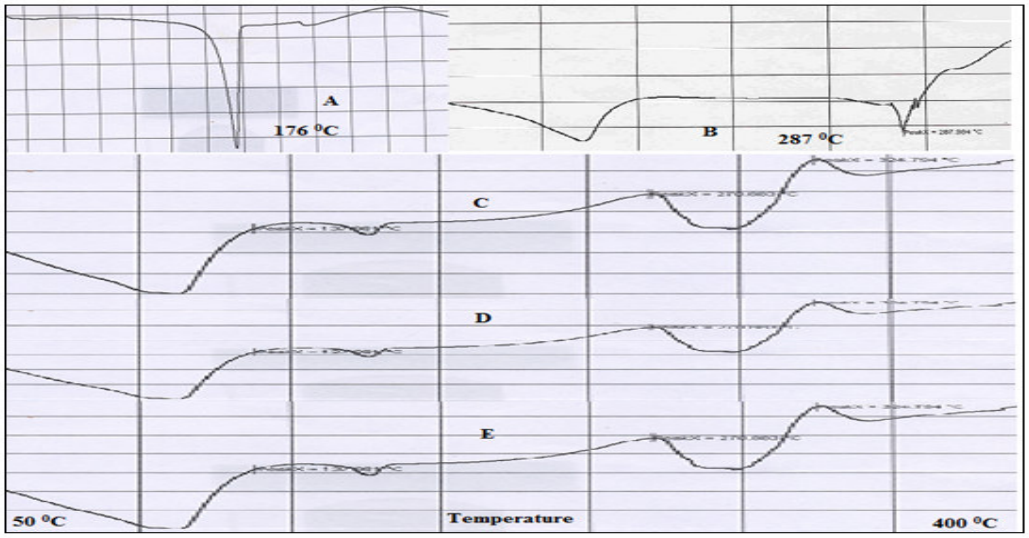


A)curcumin, B) β-CD, C) curcumin-β-CD physical mixture, D) curcumin-β-CD Kneaded complex, E) curcumin-β-CD Freeze dried complex

Differential Scanning Calorimetry (DSC)

The thermal curve of pure curcumin was typical of a crystalline anhydrous substance with a sharp endothermic peak at 176 ^{o}C corresponding to the melting point of the drug. The DSC curve of cyclodextrin showed the liberation of crystal water as an endothermal effect peaked between 80-150°C, followed by a peak at 287°C corresponding to melting point of β-cyclodextrin. The complete disappearance of the drug endothermal effect was observed with all curcumin-β-cyclodextrin complexes suggesting the 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) β-CD, C) curcumin-β-CD physical mixture, D) curcumin-β-CD Kneaded complex, E) curcumin-β-CD Freeze dried complex

Dissolution rate profile and release kinetics 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 medium. A stock solution of curcumin in methanol (10 µg/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 were 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 simulated gastric fluid without pepsin with 1% (w/v) sodium lauryl sulphate (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 the 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. The kinetics of the *in vitro* release of the curcumin and its inclusion complexes were depicted in Figure 6-9. The release of the curcumin from all inclusion complexes was observed to follow the first order release kinetics, since the correlation coefficient for the first order was higher in comparison to other tested models (Table 1).

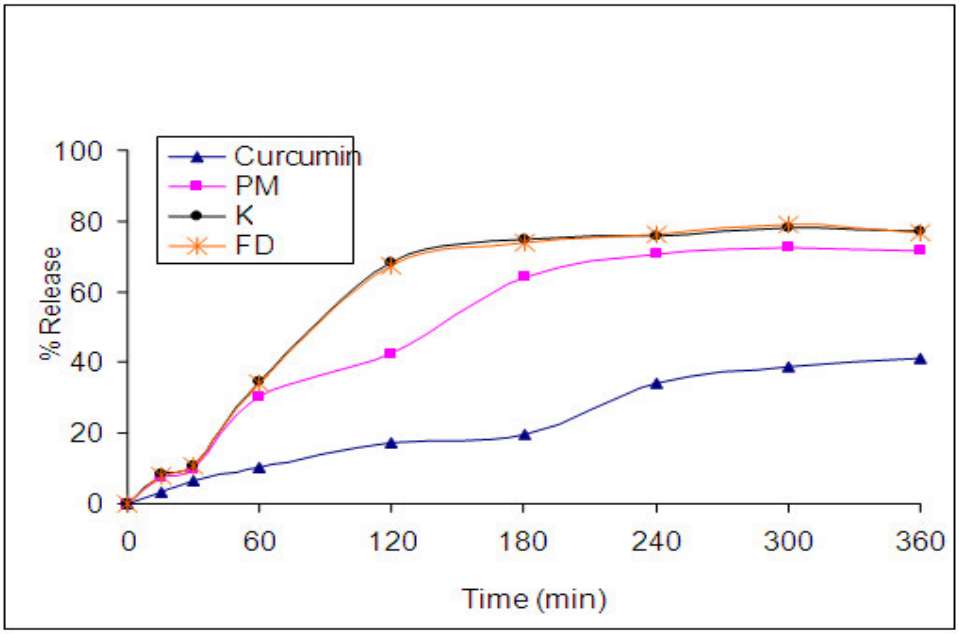


Figure 5

Release profile of curcumin and curcumin-β-CD complexes in simulated gastric fluid without pepsin with 1% (w/v) of sodium lauryl sulphate.

Table1
Release kinetics of inclusion complexes of curcumin

Curcumin inclusion complexes	Correlation Coefficient Value			
	Zero Order	**First Order**	**Higuchi**	**Peppas**
CUR	0.9695	0.951	0.969	0.9114
PM	0.9388	0.939	0.9388	0.7858
KD	0.8694	0.895	0.8671	0.7609
FD	0.8671	0.894	0.8694	0.7710

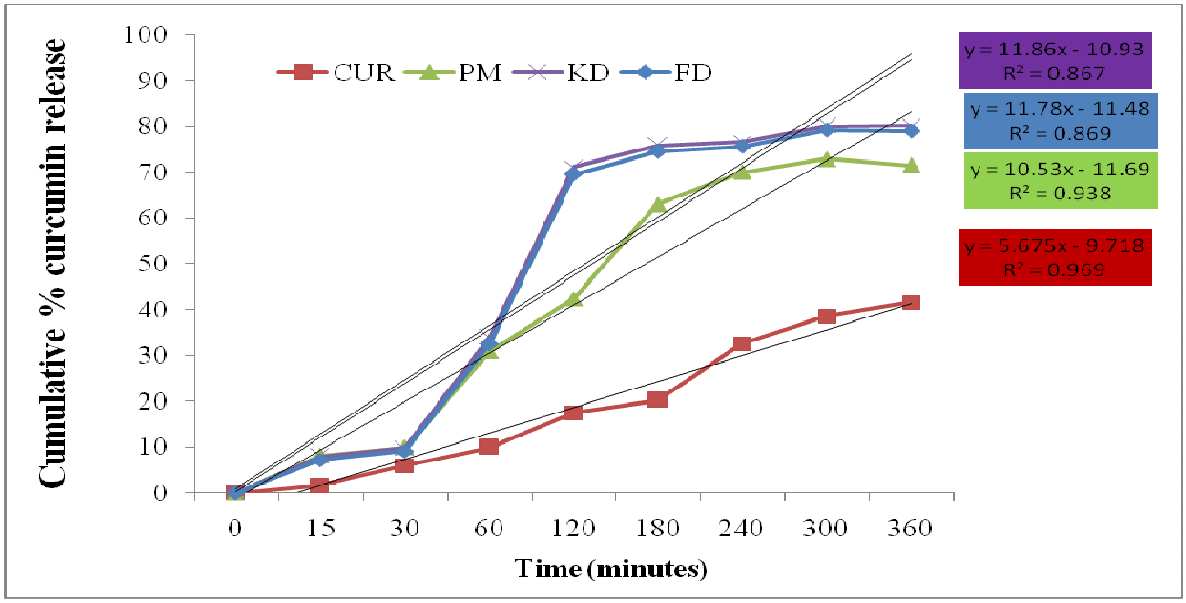


Figure 6
Zero order release kinetics of curcumin and curcumin-β-CD complexes

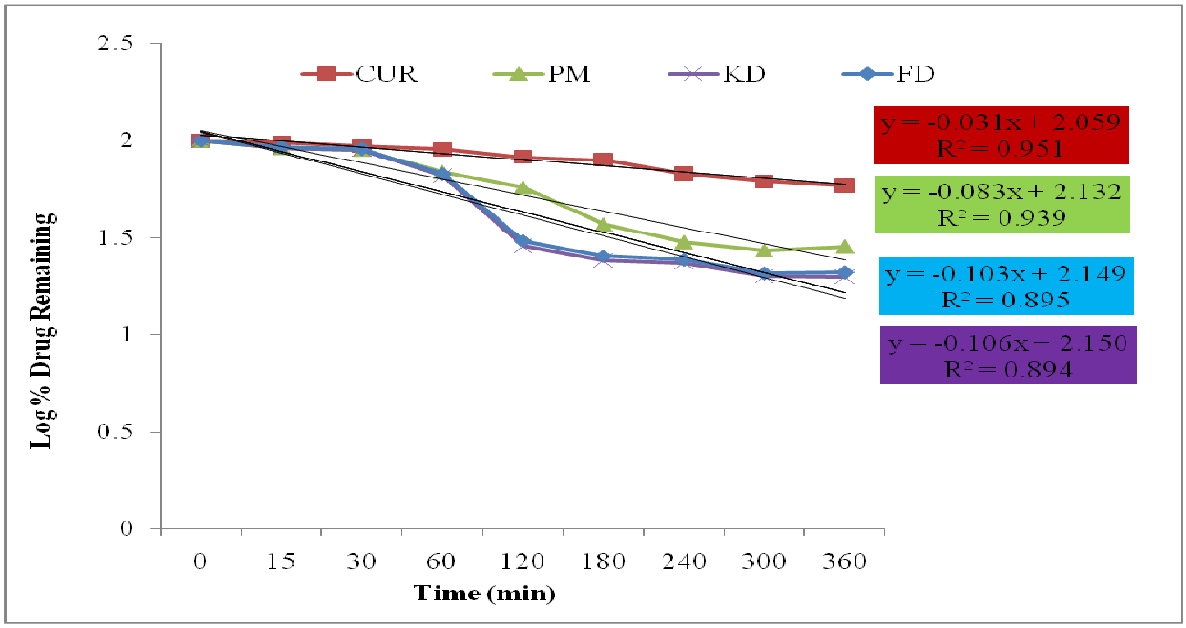


Figure 7
First order release kinetics of curcumin and curcumin-β-CD complexes

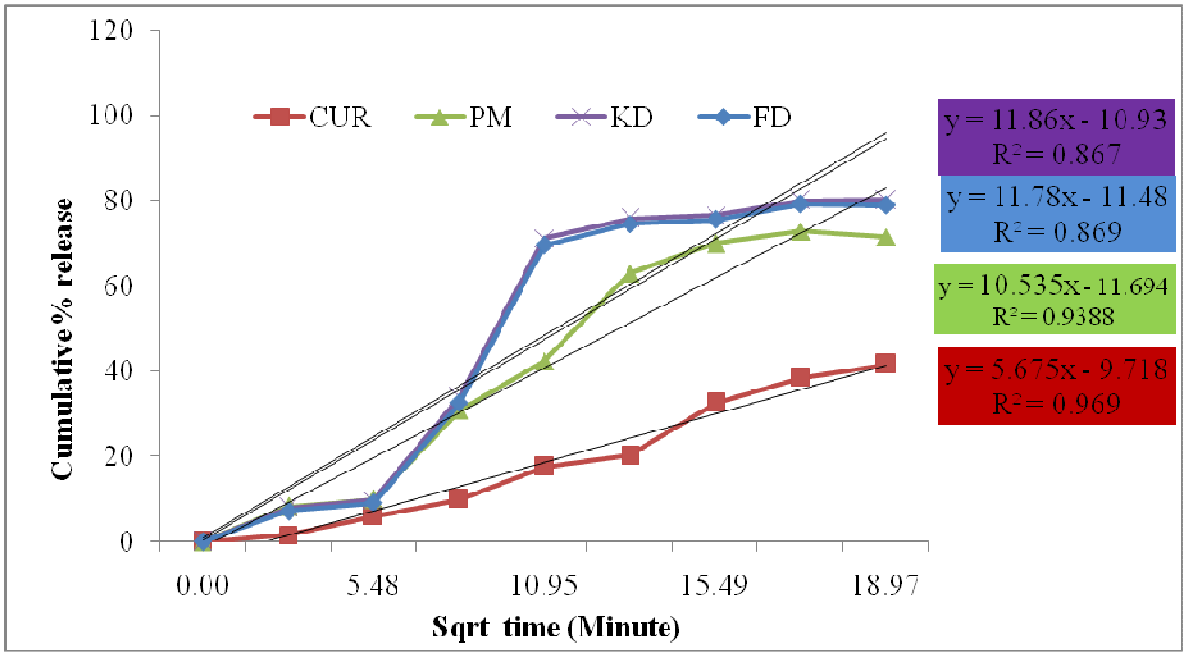


Figure 8
Higuchi model of release behavior of curcumin and curcumin-β-CD complexes

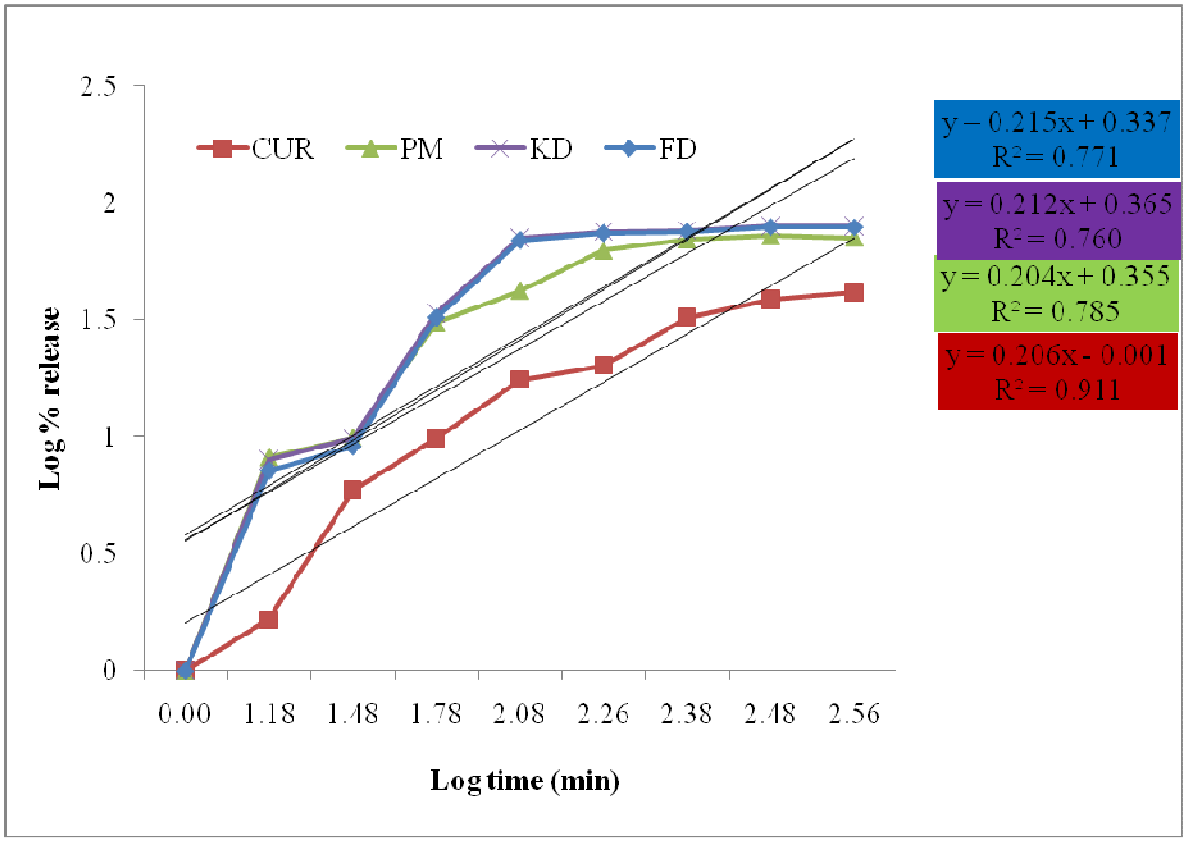


Figure 9
Korsemeyer-Peppas model of release behavior of curcumin and curcumin-β-CD complexes.

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 [31]. 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 [31]. It was also found that whatever dose was being absorbed underwent very rapid biotransformation [31]. Following an oral dose of (^{3}H) curcumin, about 90% dose was excreted via faeces and 7% in the urine in 72 hours [33]. After an oral dose of 80 mg of curcumin to rat, about 99% radioactivity was excreted in faeces over 72 hours [34]. Previously preparation of curcumin β-cyclodextrin inclusion complex and it's *in vitro* and *in vivo* evaluations has been reported [24]. In this project we prepared curcumin β-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 approximately 3.5 folds increase in the dissolution of practically insoluble curcumin.

CONCLUSION

The results obtained in the present investigation are significant from the point of view that curcumin-βCD 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.

ACKNOWLEDGEMENT

The authors are grateful to college of pharmacy Jamia Hamdard New Delhi for providing facilities and instrumentation to carry out this research project.

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