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Original Research Article

Freeze-Dried Multicomponent Inclusion Complexes of Curcumin for Enhancement of Solubility and Anti-Inflammatory Activity

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ABSTRACT

Curcumin (CUR) is an anti-inflammatory agent which exhibits poor oral bioavailability due to its poor solubility. In the present study, freeze-dried multicomponent inclusion complexes (MICs) of CUR were prepared with cyclodextrins (CDs) such as *β*-cyclodextrin (*β*CD) and hydroxypropyl-*β*-cyclodextrin (HP*β*CD), using polyvinylpyrrolidone K30 (PVP) and poloxamer 188 (POLO) as the auxiliary substances in order to enhance the solubility and anti-inflammatory activity of CUR. Phase solubility study was performed in the absence and presence of auxiliary substances to evaluate their effect on the stability of complexes and complexation efficiency of *β*CD and HP*β*CD. The binary complexes and MICs were prepared by lyophilization method. The prepared complexes were characterized by DSC, ATR-FTIR, XRD and SEM analysis, and evaluated for drug content, saturation solubility and anti-inflammatory activity. The incorporation of auxiliary substances in complex systems resulted in an increased stability constants and complexation efficiency of CDs. DSC and ATR-FTIR analysis confirmed the strong interaction between CUR and CDs in the presence of PVP and POLO. XRD and SEM analysis revealed greater amorphization in case of MIC containing POLO. The incorporation of POLO in MIC enhanced the drug content, solubility and anti-inflammatory activity of CUR to a greater extent. The overall study indicated that freeze-dried MIC of CUR in the presence of POLO could be a cheap and effective way to enhance the solubility and biological activity of CUR than the binary complexes prepared using *β*CD and HP*β*CD.

***Keywords*:** Curcumin, Cyclodextrin, Polyvinylpyrrolidone, Multicomponent inclusion complexes.

Introduction

Curcumin [CUR], (1E,6E)-1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione is a polyphenol is obtained from the rhizomes of *Curcuma longa Linn*. (turmeric). CUR exhibits different activities such as anti-inflammatory, antioxidant, antiviral, antibacterial, antifungal, anticancer, hypocholesterolemic, wound healing, antispasmodic, anticoagulant, antitumor and hepatoprotective activities.[1,2] It is useful for treating the diseases such as arthritis, diabetes, malignant diseases, Alzheimer's disease, etc. Lot of research is focused on curcumin due to its wide range of biological activity.[3] Although CUR shows various pharmacological effects, its use as a therapeutic agent is limited because of its poor bioavailability upon oral administration. Its poor solubility in water and presystemic metabolism might be the main reason for its poor bioavailability.[4]
Cyclodextrins (CDs) belong to the family of cyclic oligosaccharides. The structure of CDs resembles a torus like ring formed from D-glucopyranose units. The basic CDs include α-cyclodextrin, *β*-cyclodextrin, and *γ*-cyclodextrin, which differ in the number of glucopyranose units i.e. six, seven, and eight, respectively.[5]

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The torus shape of CDs can be ascribed to the inhibition of free rotation by the bonds that link to the glucopyranose units. The narrow end consists of primary hydroxyl groups whereas the wider ring contains secondary hydroxyl groups.[6] CDs are pharmaceutical additives which have the ability to solubilize hydrophobic drugs by formation of water-soluble drug–CD complexes.[7] The arrangement of the hydroxyl groups imparts the hydrophilicity to the outer wall of the CDs and hydrophobicity to the inner cavity. The hydrophobic drug molecules can get entrapped in this cavity. In case of larger molecules, single molecule can be encapsulated by more than one CD molecules. Amongst the basic CDs, *β*-CD is readily available, cheap and highly applicable CD.[8] The unique feature of the CDs is to form inclusion complexes with any solid, liquid or gaseous substance which is non-polar in nature, by entrapping it within its lipophilic cavity. The complexation of CUR with CDs can improve its stability.[9]
There are issues associated with the stability of CUR. Its chemical degradation is observed in aqueous-organic solutions and it increases with increase in pH. It undergoes rapid degradation at alkaline pH due to hydrolysis. Also, CUR is degraded upon exposure to sunlight. Thus, poor aqueous solubility at acidic and neutral pH and stability issues restrict the therapeutic use of CUR.
There are reports available on complexation of CUR with *β*CD by co-precipitation, freeze-drying and solvent evaporation methods to increase its solubility and stability.[10] However, *β*CD exhibits low water solubility (18 mg/mL) due to the formation of a large number of intramolecular hydrogen bonds between the secondary hydroxyl groups. This leads to an increase in the structural rigidity of *β*CD which prevents its hydration.[10,11] To overcome this problem, hydrophilic derivatives of *β*CD have been synthesized by modifying the secondary hydroxyl groups of *β*CD.[13] The amount of *β*CD

derivatives in the pharmaceutical formulations are limited due to their toxic nature and high cost.[13,14] Therefore, scientists have enhanced the solubility of *β*CD along with its solubilizing efficiency by preparing the multicomponent inclusion complexes (MICs). It involves incorporation of hydroxyl carboxylic acids,[15–17] amino acids,[18] sugar alcohols[19] and hydrophilic polymers[20] during the preparation of the drug-CD inclusion complex. Till date, there are no reports related to the preparation and evaluation of MIC of CUR with CDs, using hydrophilic polymers as auxiliary substance.

The aim of the present study was to prepare freeze-dried multicomponent complex system of CUR with *β*CD and HP*β*CD in the presence of PVP and POLO as auxiliary substances to increase the stability of the complexes and to enhance the solubility and anti-inflammatory activity of CUR. As the solid dispersions of CUR with PVP and POLO have shown promising results in terms of solubility and stability improvement of CUR in a recent study, it was decided to use PVP and POLO as auxiliary substances in our study.[22] Initial phase solubility studies were performed to establish stoichiometry between CUR and CDs. The prepared complexes were characterized by Differential scanning calorimetry (DSC), Attenuated total reflectance - Fourier transform infrared spectroscopy (ATR-FTIR), Scanning Electron Microscopy (SEM) and X-ray diffractometry (XRD). The complexes were evaluated for saturation solubility and drug content. The *in vivo* anti-inflammatory activity was evaluated using Carrageenan-induced rat paw oedema method.

Materials and Methods

Materials

CUR, *β*CD and polyvinylpyrrolidone K30 (PVP) were purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. HP*β*CD (DS: 4.69) was obtained as a gift sample from Gangwal Chemicals, Mumbai, India. Poloxamer 188 (POLO) was obtained from Signet Chemicals, Mumbai, India. The analytical grade reagents and distilled water were used throughout the experimental procedures.

Phase solubility studies

The phase solubility studies were performed in distilled water at room temperature (25 ± 2°C) according to the method reported by Higuchi and Connors.[23] Initially, *β*CD and HP*β*CD solutions were prepared in the concentration range of 0 moles/L to 0.01 moles/L. The excess quantity of CUR was added to 20 mL of *β*CD and HP*β*CD solutions in the presence and absence of auxiliary substances (0.5%w/v of PVP and POLO). The ratio of CDs to PVP and POLO was 1:1. The resultant suspensions were shaken for 72 h at 150 rpm using rotary shaker (Lab HOSP, India) to attain equilibrium. Thereafter, suspensions were filtered through membrane filter (0.45 µm) and appropriately diluted if necessary and analyzed to determine concentration of CUR using UV-spectrophotometer (Shimadzu 1800, Japan) at 425.5 nm. The stoichiometry between CUR and CDs was established from the phase solubility curves obtained by plotting the concentration of dissolved CUR (moles/litre) against the respective concentration of CDs (moles/L). The stability constants (K_S) of the binary and ternary complexes were calculated using the equation:

$$K_s = \frac{Slope}{S_o(1-Slope)} \quad (1)$$

Where, S_o is the solubility of CUR in absence of CDs (intrinsic solubility)

The complexation efficiency (C.E.)/solubilization efficiency (S.E.) of CDs was determined using the following equation.[24]

$$C.E. or S.E. = K_s S_o = \frac{Slope}{(1-Slope)} \quad (2)$$

Gibbs free energy change (ΔG_{tr}) in Joules/mole was also calculated for the phase solubility analysis to assess the thermodynamics of the solution and complexation process. using the equation.[25]

$$\Delta G_{tr} = -2.303RTlog\frac{S_c}{S_o} \quad (3)$$

Where, S_c is the molar solubility of CUR in aqueous solution of *β*CD/HP*β*CD in the presence or absence of auxiliary substance, S_o is molar solubility of CUR in distilled water in the absence of *β*CD/HP*β*CD as well as auxiliary substances (intrinsic solubility), *R* is gas constant and *T* is temperature in Kelvin.

Preparation of freeze-dried solid inclusion complexes

The method reported by Mangolim CS. *et al* (2014) was used with few modifications to prepare freeze-dried solid inclusion complexes of CUR.[10] The equimolar quantity (1:1) of CD (*β*CD or HP*β*CD) and CUR were weighed. In a 250 mL stoppered conical flask, CD was dissolved in distilled water (75 mL) with stirring to obtain a clear solution. In another conical flask, an equimolar quantity of CUR was dissolved in methanol (75 mL) and the resultant solution was added to the aqueous solution of CD. The reaction mixture was stirred at 180 rpm on rotary shaker for 48 h and filtered using a Whatman filter paper No. 41 (pore size: 0.45 µm). The obtained clear solution was subjected to freeze drying. The solid complex obtained was stored in desiccator till further use.

In case of ternary complex systems of CUR, above procedure was followed with the addition of the auxiliary substance, either 0.5 %w/w PVP or 0.5%w/w POLO, to the complexation media. Table 1 depicts the amount of CUR, *β*CD, HP*β*CD, PVP and POLO used for the preparation of complexes.

Table 1: Amount of CUR, *β*CD, HP*β*CD, PVP and POLO used for the preparation of complexes

Components	FBB	FHB	FBTP	FHTP	FBTPO	FHTPO
CUR (mg)	200	200	200	200	200	200
*β*CD (mg)	616	0	616	0	616	0
HP*β*CD (mg)	0	793	0	793	0	793
PVP (% w/w)*	0.5	0	0.5	0	0.5	0
POLO (% w/w)*	0	0.5	0	0.5	0	0.5

*Indicates concentration with respect to total weight of Curcumin (CUR) and CDs; FBB: Curcumin-*β*CD, FHB: Curcumin-HP*β*CD, FBTP: Curcumin-*β*CD-PVP, FHTP: Curcumin-HP*β*CD-PVP, FBTPO: Curcumin-*β*CD-POLO and FHTPO: Curcumin-HP*β*CD-POLO.

Characterization of complexes

Differential scanning calorimetry (DSC)

The DSC thermograms of pure CUR, *β*CD, HP*β*CD, PVP, POLO and all complexes were obtained using DSC analyzer (TA instruments, Q600 SDT USA). A sample (5 mg) was sealed in an aluminum pan and subjected to heating at a rate of 10°C/min in the scanning range of 30–400°C under dry nitrogen atmosphere (dry nitrogen flow rate = 25 mL/min).[26]

Attenuated total reflectance - Fourier transform infrared spectroscopy (ATR-FTIR)

Attenuated total reflectance (ATR)-Fourier transform infrared spectroscopy (FTIR) (BRUKER–ECO-ATR-ALPHA, Germany) was used for recording infrared spectra of CUR, *β*CD, HP*β*CD, POLO and prepared complexes. ATR-FTIR helps in direct analysis of the solid or liquid samples and does not require any complex sample preparation procedure. The samples were directly placed on ATR crystal and analyzed from 600 to 4000 cm^{-1} spectral range with 24 scans.

X-ray diffractometry (XRD)

The XRPD analysis of all samples were performed by using X-ray diffractometer (PW 1723, PHILIPS, Netherland) with tube anode Cu over the interval 05-60◦ (2θ). Generator tension (voltage) 40 kV,

Generator current 30 mA, and scanning speed 2◦/min were maintained during the operation.

Scanning electron microscopy (SEM)

Scanning electron microscope (SEM-JOEL Instruments, JSM-6360A, Japan) was used for studying surface morphology of CUR and all complexes. Samples were directly mounted on the aluminum stub and coated with a thin gold-ion layer using sputter coated unit, an acceleration voltage of 5 kV was applied and the micrographs were examined at ×300, ×1000 and ×3000 magnifications.

Determination of drug content

The content of CUR in the complexes was determined by adding the complex equivalent to 5 mg of CUR in 50 mL of methanol. The resultant mixture was stirred on the magnetic stirrer for 3 h. The solutions were filtered through Whatman filter paper No. 41, suitably diluted and analyzed using UV-visible spectrophotometer at 425.5 nm. The study was performed in triplicate.

Determination of saturation solubility

The saturation solubility of the pure CUR and the complexes was determined using the method described by Higuchi and Connors.[23] Excess quantity of pure CUR and complexes were added to the conical flasks each containing 10 mL of distilled water and sealed. The mixtures were shaken using rotary flask shaker for 72 h at room temperature to attain equilibrium. Appropriate fractions were withdrawn and filtered through Whatman filter paper No. 41 and analyzed using UV-visible spectrophotometer at 425.5 nm.

Evaluation of in vivo anti-inflammatory activity

The anti-inflammatory activity of CUR and its CD complex exhibiting high saturation solubility was evaluated *in vivo* as per the approval from Institutional Animal Ethics Committee (IAEC) of College of Pharmacy, Bhor, Maharashtra, India (Approval No. RDCOP/IAEC/Approval/2016-17/03 dated 08/08/2016). Carrageenan-induced rat paw edema method was used as reported previously.[27,26]

Experimental design:

The Wistar albino rats, 150-180 g of either sex were randomly divided into four groups of six rats in each. To produce acute inflammation, 0.1 mL of 1% freshly prepared Carrageenan solution in normal saline was injected in right hind paw of rats by sub-plantar route. Control group rats were treated with normal saline (orally), reference standard group with indomethacin (10 mg/kg body weight per oral), third group with pure CUR (100 mg/kg body weight per oral) and the test group with CUR-HPβCD-POLO ternary complex equivalent to 100 mg of CUR/kg body weight per oral, one hour prior to carrageenan injection. The paw volume was recorded using plethysmometer at an interval of 1, 2, 3, 4 and 5 hrs after Carrageenan injection and increase in mean paw volume in mL (Mean ± Standard Error) and % inhibition of paw inflammation was calculated. The percentage inhibition of paw inflammation was calculated using the formula:

$$\%\ inhibition\ of\ inflammation = \left(\frac{Control\ Mean - Treated\ Mean}{Control\ Mean}\right) \times 100 \qquad (4)$$

Statistical analysis

All values were expressed as Mean ± Standard Error of Mean (S.E.M.). For statistical analysis one-way analysis of variance (ANOVA) was used. $P < 0.05$ was considered statistically significant.

Results and Discussion

Phase solubility studies

A linear relationship was observed between the concentration of dissolved CUR and concentration of aqueous solutions of βCD or HPβCD which indicates formation of soluble complex between curcumin and βCD or HPβCD in all systems (see Figure 1). The shape of solubility curve indicated the stoichiometry of 1:1 for the inclusion complex formed. The slopes of all the phase solubility diagrams are less than 1. The increased complexation efficiencies as well as stability constants indicated that incorporation of auxiliary substance had positive effect on solubilization of CUR in aqueous solution of CDs. The higher value of K_s in case of ternary mixtures than those for corresponding binary mixtures indicated that the ternary complexes were more stable than binary complexes (see Table 2). A synergistic effect of addition of auxiliary substances can be observed in case of both βCD and HPβCD complex systems due to hydrogen bonding interactions between auxiliary substances, CDs and drug. The increase in complexation efficiency with respect to corresponding binary complex systems is also evident. The HPβCD ternary complex of CUR with 0.5% w/v POLO as auxiliary substance exhibited highest stability constant and complexation efficiency than the other systems.

An indication of the process of transfer of CUR from pure water to aqueous solution of βCD or HPβCD was obtained from the values of Gibbs free energy change. Table 3 depicts Gibbs free energy change (ΔG_{tr}) in Joules/ mole in the systems with increase in the concentration of CDs.

ΔG_{tr} values were all negative for βCD as well as HPβCD at various concentrations indicating exothermic nature of complexation process.[29] This suggests that solubilization of CUR is spontaneous. With the increase in concentration of βCD and HPβCD, increase in ΔG_{tr} values was evident. This signifies that the reaction became more favourable with increased βCD or HPβCD concentration.[30] Also, the ΔG_{tr} values of POLO were found to be highly negative as compared to the other systems which reveal that incorporation of POLO enhanced the solubilization of CUR to a greater extent than PVP.

Characterization of complexes

Differential scanning calorimetry (DSC)

Figure 2 shows the DSC thermograms of CUR and the freeze-dried complexes. The DSC of CUR showed a characteristic sharp endothermic peak at 175.10°C close to its melting range (179-182°C) indicating crystalline nature of CUR. The DSC thermogram of βCD displayed a broad endotherm at 99.95°C due to loss of water of hydration. An endothermic peak at 335.32°C corresponded to its melting point. In case of HPβCD a broad endothermic peak at 75.48°C indicating loss of water was observed.[25] It showed melting endothermic peak at 366.62°C which may be related to the thermal decomposition. A broad endotherm between 70°C to 100°C due to presence of residual moisture in the DSC thermogram of PVP K 30 was observed; while thermogram of POLO showed a sharp endothermic peak at 60.28°C indicating its crystalline nature.

The DSC thermogram of freeze-dried binary complex of CUR and βCD (FBB) displayed a broad endotherm at 90.64°C, indicating loss of water of hydration of βCD. Another small endothermic peak at 335.91°C was observed corresponding to melting endotherm of βCD. The sharp endothermic peak at 175.10°C of curcumin was completely diffused. The DSC thermogram of freeze-dried binary complex of CUR and HPβCD (FBH) displayed a broad endotherm at 89.13°C, indicating loss of water of hydration of HPβCD. An endothermic peak at 343.87°C indicated decomposition of HPβCD. The complete disappearance of melting endotherm of CUR was observed indicating a change in the physical form or crystallinity of CUR.

The DSC thermogram of CUR/βCD/PVP (FBTP) displayed a broad endotherm at 89.13°C, indicating loss of water of hydration of βCD. Another small endothermic peak at 343.19°C was observed corresponding to melting endotherm of βCD. The sharp endothermic peak at 175.10°C of CUR was completely disappeared thus suggesting presence of amorphous form of CUR.

The DSC thermogram of CUR/βCD/POLO (FBTPO) displayed a broad endotherm at 105.05°C, indicating loss of water of hydration of βCD. An endothermic peak at 336.29°C indicated decomposition of βCD. In the thermogram, complete disappearance of endothermic peaks of CUR as well as POLO was observed. The crystalline nature of CUR has been changed to amorphous form.

The broad endotherm of HPβCD in CUR/HPβCD/PVP (FD) (FHTP) was shifted to 91.02°C which indicated loss of water of hydration. It showed melting endotherm at 352.21°C occurred due to shifting of

endotherm at 366.62°C. The thermogram displayed no peak corresponding to CUR as well as PVP.
The DSC thermogram of CUR/HP*β*CD/POLO (FD) (FHTPO) exhibited a broad endotherm at 87.23°C corresponding loss of water of hydration of HP*β*CD and at 346.53°C. The complete disappearance of sharp endothermic peak of CUR at 175.10°C suggested the presence of amorphous phase. No peak corresponding to POLO was observed.
In case of freeze-dried complexes, complete disappearance of characteristic endothermic peak of CUR at 175.10°C and shift in the endotherms of *β*CD and HP*β*CD clearly indicate the formation of inclusion complex between CUR and CDs. In case of ternary complex systems, no characteristic endothermic peaks of PVP as well as POLO were obtained. This clearly confirms the interaction of auxiliary substances with CUR and CDs. The disappearance of the thermal features of the CUR was suggestive of the inclusion of CUR within the cavity of the cyclodextrin cavity. This phenomenon suggested a strong interaction between CUR and CDs.[7,2] In case of ternary complexes, complete disappearance of peak corresponding to CUR, shifting of peaks of *β*CD and HP*β*CD and no peak corresponding to PVP and POLO were observed. This revealed the involvement of PVP and POLO in stabilizing the binary inclusion complexes via hydrogen bonding.[25,29,30] Thus, DSC thermogram study revealed that incorporation of the auxiliary substances led to increase in the interaction between CDs and CUR which may contribute to the solubility and stability enhancement of CUR.

Attenuated total reflectance - Fourier transform infrared spectroscopy (ATR-FTIR)
The ATR-FTIR spectra of CUR and freeze-dried complexes is displayed in Figure 3. The IR spectrum of CUR showed characteristic absorption peaks at 3506.35 cm^{-1} (stretching phenolic and enolic band of O-H), 2916.16 cm^{-1} (C-H stretching), 1628.13 cm^{-1}, 1626.16 cm^{-1} (C=C symmetric aromatic ring stretching),1504.65 cm^{-1} (C=O), 1427.09 cm^{-1},1273.32 cm^{-1}(enol C-O bending;), 1150.07 cm^{-1}((C-C-H) of aromatic rings) , 1126.20 cm^{-1}, 1023.98 cm^{-1}, 994.52 cm^{-1}, 961.79 cm^{-1} (benzoate trans-C-H vibration),and 854.96cm^{-1}((CH) of aromatic and skeletal C-CH).[31-33]
In case of freeze-dried complexes, FBB displayed shifting of peaks of *β*CD which appeared at 3293.30 cm^{-1} (OH stretching) and 1022.47 cm^{-1} (C-O-C stretching). The peak at 2916.16 cm^{-1} of CUR is shifted to 2919.19 cm^{-1}. The peak at 1150.07 cm^{-1} was shifted to 1151.32 cm^{-1} and appeared with reduced intensity. All other major peaks in CUR have either disappeared or shifted with reduced intensities. For FHB, the broad peak at 3348.45 cm^{-1} of HP*β*CD was shifted to 3302.22 cm^{-1}. The typical peak at 1026.41 $cm^{-1}$of HP*β*CD was shifted to 1023.35 $cm^{-1}$and is prominent in the spectrum. The peak at 2916.16 $cm^{-1}$of CUR was shifted to 2919.47 cm^{-1}. The peak at 1150.07 cm^{-1} was shifted to 1151.50 $cm^{-1}$and appeared with reduced intensity.
In case of FBTP, the peaks of *β*CD were shifted to 3302.85 $cm^{-1}$and 1023.93 cm^{-1}. The typical peak at 1023.99 cm^{-1} of *β*CD was shifted to 1023.93 $cm^{-1}$and is prominent in the spectrum. The peak at 2916.16 $cm^{-1}$of CUR is shifted to 2917.54 cm^{-1}. The peak at 1150.07 cm^{-1} was shifted to 1151.65 cm^{-1} and appeared with reduced intensity.
The FBTPO spectrum displayed shifting of peaks of *β*CD to 3265.79 $cm^{-1}$and 1023.08 $cm^{-1}$and were prominent in the spectrum. The peak at 2916.16 $cm^{-1}$of CUR was shifted to 2914.23 cm^{-1}. The peak at 1150.07 $cm^{-1}$was shifted to 1151.35 $cm^{-1}$and appeared with reduced intensity.
The spectra of FHTP exhibited shifting of the broad peak at 3348.45 cm^{-1} of HP*β*CD to 3290.92 cm^{-1}. The peak at 1023.82 $cm^{-1}$was prominent in the spectrum. The peak at 2916.16 cm^{-1} and 1150.07 cm^{-1} of CUR were shifted to 2915.39 cm^{-1} and 1151.60 cm^{-1}, respectively which appeared with reduced intensity.
In case of FHTPO, the broad peak at 3348.45 $cm^{-1}$and characteristic peak at 1026.41 $cm^{-1}$of HP*β*CD were shifted to 3295.02 cm^{-1} and 1022.85 cm^{-1}, respectively and were prominent in the spectrum. The peak at 2916.16 $cm^{-1}$of CUR was shifted to 2913.39cm^{-1}. The peak at 1150.07 $cm^{-1}$was shifted to 1151.39 $cm^{-1}$and appeared with reduced intensity.

Table 2: Phase solubility parameters

UR/CD systems	Phase solubility parameters		
	Slope	**$K_{c,1:1}$ (M^{-1})**	**C.E.**
CUR/*β*CD	2.83×10^{-4}	123.63	2.84×10^{-4}
CUR/*β*CD/PVP (0.5%w/w)	7.15×10^{-4}	293.08	7.15×10^{-4}
CUR/*β*CD/POLO (0.5%w/w)	9.73×10^{-4}	351.18	9.74×10^{-4}
CUR/HP*β*CD	7.50×10^{-4}	326.84	7.50×10^{-4}
CUR/HP*β*CD/PVP (0.5%w/w)	8.95×10^{-4}	367.24	8.96×10^{-4}
CUR/HP*β*CD/POLO (0.5%w/w)	1.05×10^{-3}	379.12	1.05×10^{-3}

Table 3: Gibbs free energy change (ΔG_{tr}) in Joules/ mole

Moles of CDs	CUR/*β*CD	CUR/*β*CD/ PVP (0.5%w/w)	CUR/*β*CD/ POLO(0.5%w/w)	CUR/ HP*β*CD	CUR/HP*β*CD/ PVP(0.5%w/w)	CUR /HP*β*CD/POLO (0.5%w/w)
0.002	-153.63	-915.34	-1538.21	-1099.21	-1270.23	-1661.95
0.004	-656.65	-1721.55	-2417.39	-1855.23	-2265.0	-2669.85
0.006	-1198.40	-2602.29	-3300.80	-2602.29	-3005.59	-3431.18
0.008	-1538.21	-3108.00	-3793.66	-3085.61	-3411.57	-3818.80
0.01	-1910.36	-3391.80	-4049.39	-3545.67	-3916.86	-4280.92

The major peaks of CUR in the spectra of freeze-dried complexes have either disappeared or shifted with reduced intensities. All these changes in the IR spectra suggest possible interaction between curcumin and *β*CD/HP*β*CD in the presence or absence of ternary component i.e. PVP or POLO. The changes like shifting or disappearance of peaks, reduction in the peak intensities, attenuation or peak broadening with no new peaks suggest the host-guest interaction indicating formation of inclusion complexes. Many peaks in CUR were masked by those of *β*CD/HP*β*CD and predominantly the peak corresponding to *β*CD/HP*β*CD had been obtained. This indicated the formation of inclusion complexes where the CUR molecule might have been included in the CD cavity.

X-ray diffractometry (XRD)
Figure 4 depicts the X-ray diffractograms of the CUR and the prepared freeze-dried complexes. The intensity of the characteristic peaks of CUR in the diffractogram of all freeze-dried complexes was observed to be reduced considerably. The binary complex with HP*β*CD (FHB) showed maximum reduction in intensities indicating marked reduction in crystallinity. A marked reduction in the peak intensities of pure CUR in case of ternary complexes of both *β*CD and HP*β*CD with POLO was observed indicating reduction in crystallinity of CUR. The change in crystallinity might be a contributing factor for increase in solubility and stability of CUR.[34]

Scanning electron microscopy (SEM)

SEM was used to study the surface morphology of pure drug and complexes (see Figure 5). The micrographs of pure CUR exhibited the presence of somewhat spherical or irregular shaped crystals. The images of freeze-dried inclusion complexes showed tendency of aggregation of particles. A change in the morphology of particles was observed in both, HP*β*CD and *β*CD based binary as well as ternary complexes. The microphotographs of freeze-dried complexes exhibited the change in the morphology of CUR and characteristic irregular pieces of aggregates were observed.

The decrease in the particle size of the complexes reveals an apparent interaction in the solid-state. POLO played an important role in reducing the crystallinity of the CUR in presence of HP*β*CD. XRD results were in good agreement with that of SEM analysis for the binary and ternary complexes.

Thus, changes in particle shape with reduction in crystallinity and close contact between hydrophilic CD and curcumin in the binary and ternary complexes might be responsible for improved aqueous solubility of CUR in presence of CDs and auxiliary substances during phase solubility study.[25] A greater reduction of crystallinity was noticed in case of CUR/ HP*β*CD/ POLO than other complexes, which may lead to an increase in the solubility of CUR to a greater extent.

Determination of drug content

The CUR content in the freeze-dried complexes is shown in Table 4. The values of drug content were found to be less in case of complexes because during complex preparation, the mixing of alcoholic solution of CUR and aqueous solution of CDs with/without PVP and POLO leads to the precipitation of some amount of CUR which gets removed during filtration. As a result, the quantity of CUR in the filtrate gets reduced resulting in a lesser drug content. However, from the values of drug content, it can be noticed that the incorporation of PVP and POLO led to increase in the drug content. This may be due to the increase in the solubility and minimization of precipitation of CUR during mixing of alcoholic solution of CUR and aqueous solution of CDs. FHTPO showed maximum drug content as compared to the other complexes confirming that POLO facilitated superior drug loading onto the binary and ternary complexes relative to PVP.

Saturation solubility studies

All the complexes showed marked enhancement in solubility with respect to pure CUR (see Table 5). The ternary complexes with both PVP and POLO exhibited greater solubility in comparison to the respective binary complexes of *β*CD and HP*β*CD. The ternary complex system CUR-HP*β*CD-POLO exhibited highest solubility with 856 fold increase in solubility of CUR.

The enhancement of solubility of complexes mainly attributed due to the formation of a stable inclusion complex of CUR, *β*CD and HP*β*CD. The stability constant suggests that *β*CD, HP*β*CD and CUR have sufficient affinity towards each other to form a stable inclusion complex. In ternary systems, incorporation of auxiliary substances enhance their complexation efficiencies, binding potential towards *β*CD and HP*β*CD as well as increase the stability of the complexes.

The ternary complex system of CUR with HP*β*CD and POLO exhibited the highest solubility which can be attributed to amorphization of CUR due to the formation of CUR-HP*β*CD inclusion complexes, improved complexation efficiency due to incorporation of POLO and formation of stable inclusion complexes.[35-37]

Evaluation of in vivo anti-inflammatory activity

The results for anti- inflammatory activity with respect to change in paw volume and % inhibition of paw inflammation (Mean ± Standard Error) obtained with Carrageenan-induced rat paw edema method are summarized in Table 6.

The positive control i.e indomethacin decreased the paw volume by 26.08% after two hours; while after tw hours the percent inhibition for pure CUR was 22.50%. The percent inhibition of paw volume for FHTP is 27.47% which is more as compared to pure CUR and indomethacin, however, indomethacin showed better anti-inflammatory effect after three hours.

Thus, FHTPO displayed significant increase ($p<0.01$) in the anti-inflammatory activity than pure CUR. This finding suggested that the formulation of ternary complexes containing curcumin, HP*β*CD and POLO may offer superior curcumin bioavailability relative to other studied binary and ternary complex systems.

Table 4: Drug content (%) of CUR in freeze-dried complexes

Complex	Drug content (%) (mean ± SD)
FBB	5.65 ± 0.43
FHB	8.11 ± 0.75
FBTP	10.39 ± 0.24
FHTP	7.02 ± 0.33
FBTPO	11.52 ± 0.61
FHTPO	13.85 ± 0.22

Table 5: Solubility of CUR and its CD-complexes

Complex	Saturation solubility (μg/mL)*	Fold increase in solubility in comparison to pure curcumin
CUR	0.83 ± 0.08	-
FBB	291.76 ± 1.22	351
FHB	334.05 ± 0.89	402
FBTP	403.82 ± 1.54	486
FHTP	534.63 ± 1.89	644
FBTPO	668.19 ± 2.18	805
FHTPO	710.54 ± 1.63	856

*Values indicates Mean ± Standard deviation of three readings

Table 6: In *vivo* anti-inflammatory activity of CUR and FHTPOC

Time	Mean paw volume (mL)*			
	Carrageenan	Indomethacin	CUR	CUR-HP*β*CD-POLO (FHTPOC)
0h	1.202 ± 0.064	1.138 ± 0.031	1.193 ± 0.038	1.143 ± 0.029
1h	1.543 ± 0.115	1.435 ± 0.093	1.47 ± 0.041	1.353 ± 0.018
2h	2.013 ± 0.081	1.488 ± 0.057	1.56 ± 0.027$^{\#}$	1.46 ± 0.033$^{\#‡}$
3h	1.925 ± 0.077	1.193 ± 0.123	1.448 ± 0.015†	1.248 ± 0.035$^{\#}$
4h	1.813 ± 0.0427	1.025 ± 0.123	1.38 ± 0.010†	1.178 ± 0.037$^{\#}$
5h	1.735 ± 0.047	0.937 ± 0.107	1.32 ± 0.009†	1.108 ± 0.025$^{\#}$
% inhibition at 2 h		**26.08**	**22.50**	**27.47**

*Values are expressed as Mean ± S.E.M.; N=6 animals per group; †: $p<0.01$ as compared to carrageenan; #: $p < 0.001$ as compared to carrageenan; ‡: $p<0.01$ as compared to CUR.

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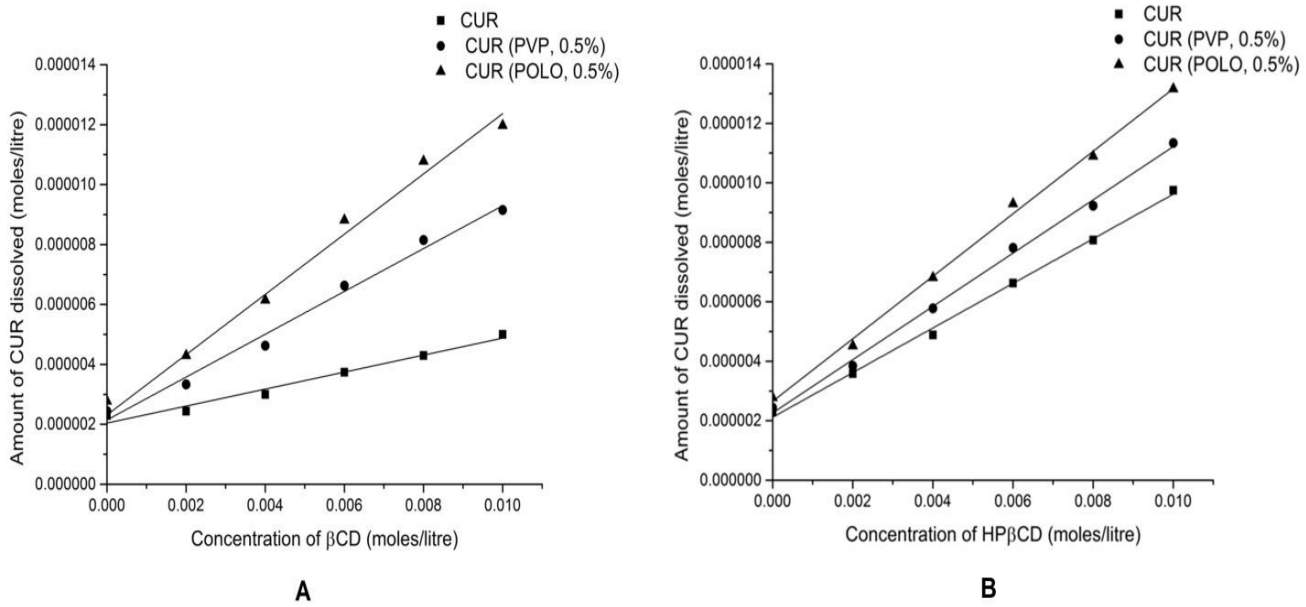

Figure 1: Phase solubility diagram of binary and ternary systems with *β*CD (A) and HP*β*CD (B). CUR: Curcumin; PVP: Polyvinylpyrrolidone K30; POLO: Poloxamer 188.

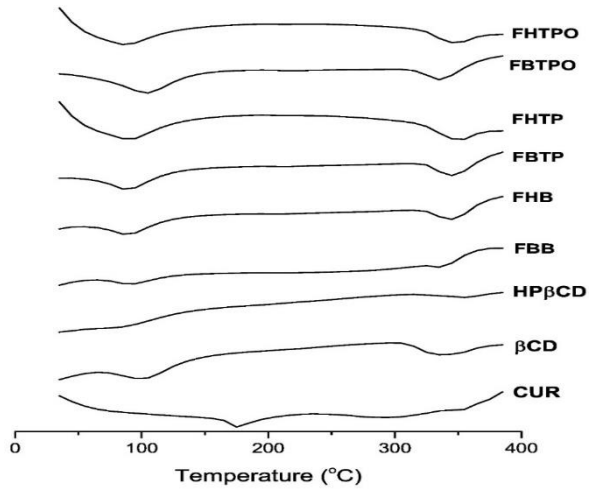

Figure 2: DSC thermograms of CUR, βCD, HPβCD and freeze dried complexes

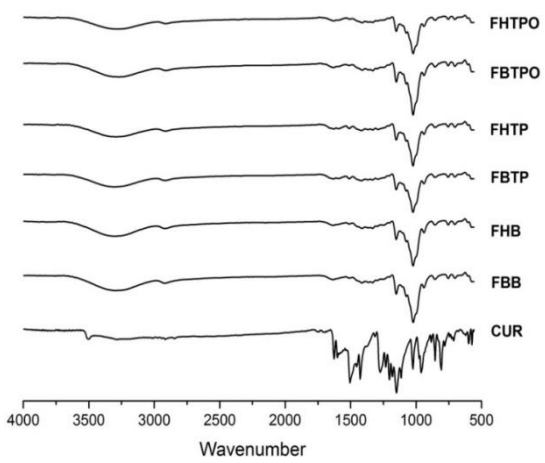

Figure 3: ATR-FTIR spectra of CUR and freeze dried complexes

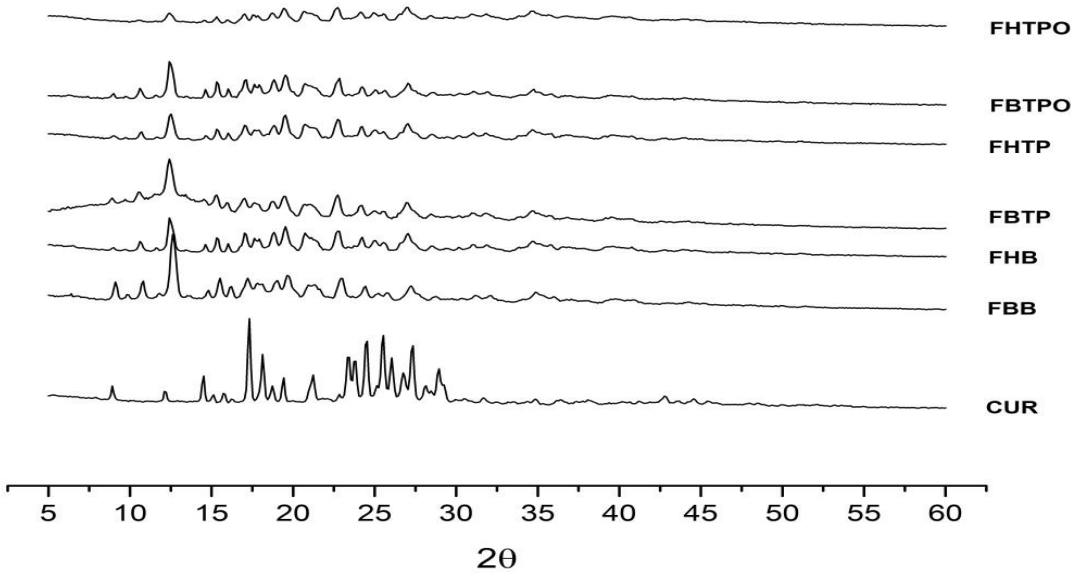

Figure 4: XRD diffractogram of CUR and freeze-dried complexes

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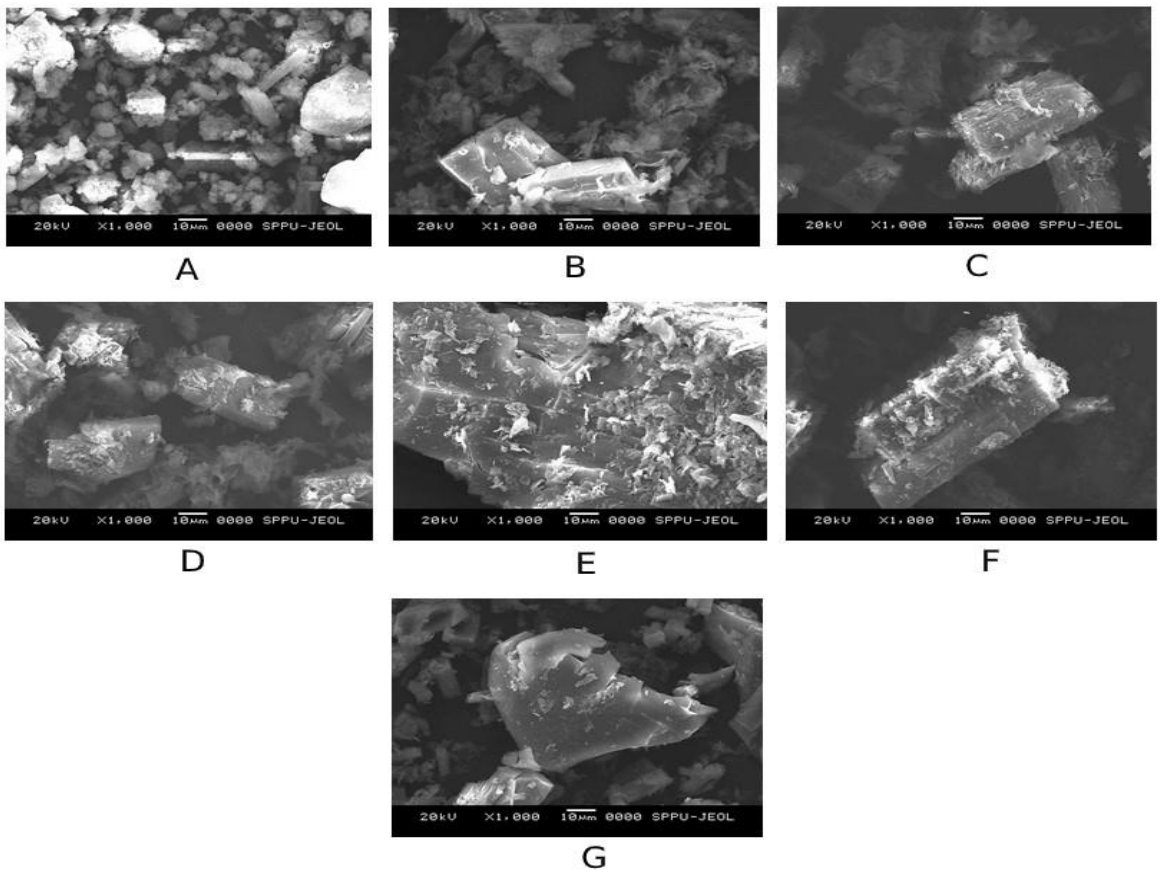

Figure 5: SEM images of CUR **(A)** and freeze-dried complexes- FBB **(B)**, FHB **(C)**, FBTP **(D)**, FBTPO **(E)**, FHTP **(F)**, FHTPO **(G)**

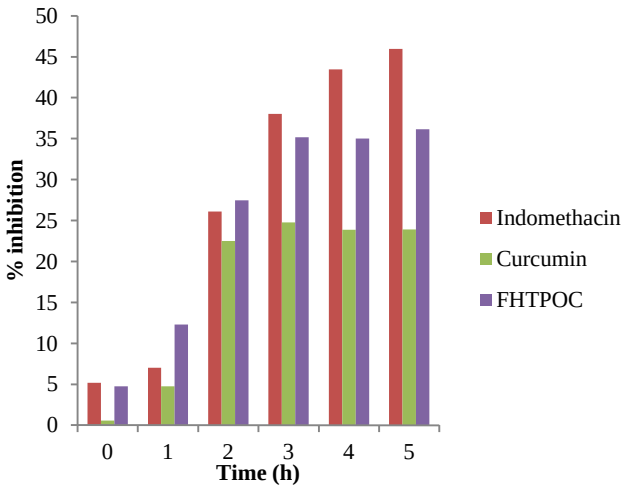

Figure 6: The paw oedema-inhibitory properties of anti-inflammatory agents over 5 h

Conclusion

Stable multicomponent inclusion complexes of CUR containing *β*CD or HP*β*CD with or without PVP and POLO can be effected through lyophilization technique, resulting in improved physicochemical properties and anti-inflammatory activity in comparison to the pure drug. The ternary complex comprised of CUR, HP*β*CD and POLO (FHTPO) exhibited superior anti-inflammatory effect over indomethacin for up to 2 h after oral administration. The increased in *vivo* anti-inflammatory activity of CUR/HP*β*CD/POLO confirms the formation of stable complexes showing increased bioavailability as compared to pure CUR. HP*β*CD ternary system with 0.5% w/w POLO exhibited superior physicochemical and anti-inflammatory activity relative to other studied inclusion complex systems.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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