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Influence of preparation methodology on solid-state properties of an acidic drug–cyclodextrin system

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Abstract

We have investigated the influence of processing variables on the solid-state of a model drug, flurbiprofen, in cyclodextrin-based systems and its effect on dissolution behaviour of the drug. The interaction between flurbiprofen and hydroxypropyl β-cyclodextrin (HP-β-CyD) was studied by NMR spectroscopy and phase solubility studies. Binary systems containing flurbiprofen and HP-β-CyD or povidone (polyvinylpyrrolidone) K30, prepared by various processes, were characterized by FTIR, DSC, XRD and dissolution studies. HP-β-CyD enhanced the solubility of flurbiprofen and increased dissolution rates from binary systems. It was found to be superior to povidone K30 in producing higher dissolution rates. The method of preparation of the binary systems and the agents used were found to have a major influence on the final solid-state of flurbiprofen. Solvents and processing conditions favouring greater interaction between flurbiprofen and the cyclodextrin during the preparation process resulted in greater extent of drug–cyclodextrin association and/or greater amorphization of the drug. Use of ammonia during the preparation of binary systems yielded solids from which very rapid drug dissolution was achieved, due to a higher extent of molecular dispersion of the drug. Processing variables therefore could significantly influence the solid-state of a drug in cyclodextrin-based formulations and thereby affect its dissolution behaviour. This could lead to significant effects on the in-vivo performance of the formulation.

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Introduction

The general techniques of preparation of cyclodextrin complexes i.e. co-precipitation, slurry method, paste method and dry mixing methods, have been described by Hedges (1998). During any process aimed at preparation of a complex, the fraction of total drug that would exist as a true inclusion compound, in the final preparation, would depend on a variety of factors. These would include: the strength of the complex formed (the association constant); the extent of interaction between the drug and the cyclodextrins during the process; the use of solvents during the process and their type; the solubility of the drug and the cyclodextrin in the solvent system used; and the duration of processing.

In most cases, the drug would not only exist within the cavity of the cyclodextrin but also between the cyclodextrin rings. In the case of crystalline complexes of β-cyclodextrin, these can be termed as "crystal lattice inclusion complexes" (Pitha et al 1983). For amorphous cyclodextrins such as hydroxypropyl β-cyclodextrin (HP-β-CyD), the drug would be dispersed in the cyclodextrin matrix and not necessarily within the cyclodextrin cavity. The drug need not be molecularly dispersed in the carrier and may be present in a crystalline or amorphous form within the matrix. These systems can therefore be appropriately termed as "solid dispersions". The solid state characteristics of the drug after preparation and during storage will depend on the processing variables as well as the characteristics of the system. When the objective of the formulation is to attain faster dissolution rates, presence of amorphous or disordered forms of the drug would be favourable. The higher apparent solubility of the drug and the improved dissolution rate would therefore be a result of presence of amorphous high energy forms of the drug as well as the ability of the cyclodextrin to form a soluble complex with the drug. The performance of the product over the shelf-life would

depend on the ability of the cyclodextrin to prevent crystallization of the amorphous drug to its stable crystalline form. The method of preparation of these systems would influence the final solid state of the drug and would therefore be a vital determinant of the performance of the system. The primary objective of this study was to investigate and demonstrate the influence of processing variables on the solid state of a model drug, flurbiprofen, in binary systems with HP-β-CyD and to correlate it with in-vitro dissolution performance.

Flurbiprofen is a weakly acidic drug possessing very low aqueous solubility. This results not only in slower dissolution and absorption rates on oral administration but is one of the causes for local irritation to the gastric mucosa leading to gastrointestinal side effects of the drug. Improvement in drug solubility would lead to enhancement of its bioavailability and reduction in local side effects. HP-β-CyD has been reported to form a complex with, and improve the solubility of flurbiprofen (Frijlink et al 1991; Junquera et al 1998; Kagkadis et al 1998). Flurbiprofen was therefore chosen as a model drug to study the influence of processing variables on the solid-state characteristics of the drug in HP-β-CyD-based systems.

Materials and Methods

Materials

Flurbiprofen was obtained as a gift sample from FDC Ltd, Mumbai. The sample of hydroxypropyl β-cyclodextrin was donated by Cerestar Ltd. Povidone K30 was obtained as a gift sample from Khandelwal Laboratories, Mumbai. All other materials used in the study were of analytical reagent grade.

Phase solubility studies (Higuchi & Connors 1965)

Excess of solid flurbiprofen was equilibrated with aqueous solutions of increasing concentration of HP-β-CyD (0–16 mM), on a mechanical shaker, for a period of 24 h at 27 ± 1°C. At the end of 24 h the solutions were filtered and analysed spectrophotometrically at 247 nm for flurbiprofen concentration.

NMR spectroscopy

The ^{1}H NMR spectra of pure drug, HP-β-CyD and a 1:1 molar mixture of drug and HP-β-CyD were recorded in D_2O on a Bruker AMX-500 FT-NMR at 298 K.

Preparation of binary systems

Binary systems containing flurbiprofen (MW 244.25) and HP-β-CyD (MW 1371.6) were prepared in a 1:1 molar proportion by various techniques. The codes used for the resulting systems are given in parentheses.

Co-grinding (CG)
The drug and cyclodextrin were passed through a sieve no-85 (pore size 180 μm). They were then ground well in a mortar to effect the formation of an homogeneous mixture. The mixture was passed through a sieve no-85 and stored over anhydrous calcium chloride (RH $\sim$ 0%). This system was prepared as a control, to determine the effect of HP-β-CyD uniformly mixed with the drug, without the aid of any solvent. The improved dissolution behaviour of this system was expected to be predominantly because of in-situ complexation of the drug with HP-β-CyD, in the dissolution medium.

Kneading with aqueous ethanol (KNE)
A mixture of flurbiprofen with HP-β-CyD, containing 500 mg flurbiprofen, was kneaded with 1 mL 50% aqueous ethanol, in a mortar. The mass was then dried in a tray dryer at a temperature not exceeding 50°C. The dried mass was then stored overnight in a vacuum desiccator.

Kneading with aqueous ammonia (KNA)
A mixture of flurbiprofen with HP-β-CyD equivalent to 500 mg flurbiprofen was kneaded with a mixture of 1 mL water and 0.2 mL 25% ammonia solution. The mass was processed in a manner similar to KNE.

Co-evaporation from aqueous ethanol (CEE)
HP-β-CyD was dissolved in 10 mL water and this solution was added to 10 mL ethanol containing 500 mg flurbiprofen. The mixture was stirred with heating at a temperature not greater than 50°C. The clear solution obtained was further heated with stirring, till a pasty mass was obtained. The residual solvent was removed under vacuum at room temperature in an Edwards Modulyo freeze dryer.

Co-evaporation from aqueous ammonia (CEA1 and CEA2)
Solid dispersions were prepared by this method in two different ways. CEA1 was prepared by addition of 10 mL of an aqueous solution of HP-β-CyD to 10 mL 25% ammonia solution in which 500 mg flurbiprofen was dissolved. CEA2 was prepared by addition of 0.2 mL 25% ammonia solution to 10 mL aqueous HP-β-CyD solution in which flurbiprofen was dispersed. Both the techniques led to formation of clear solutions, which were treated in a fashion similar to the mixture obtained during the preparation of CEE.

Freeze drying (FD)
An equimolar mixture (5 g) of HP-β-CyD and flurbiprofen was shaken for 24 h with 40 mL water. Ammonia solution (25%) was added to it drop wise till a clear solution was obtained. The volume was then made up to 50 mL. The solution was frozen overnight in Petri dishes at -40°C and lyophilized in an Edward Modulyo 4K freeze dryer at -40°C for 48 h. Secondary drying was carried out at room temperature.

Binary systems of flurbiprofen were prepared with povidone (polyvinylpyrrolidone) K30. This allowed comparison of the abilities of HP-β-CyD and povidone to enhance the dissolution of flurbiprofen. Povidone was used in the same weight ratio as HP-β-CyD to prepare CGP by co-grinding and CEP by co-evaporation from absolute ethanol. To

study the effect of the use of ammonia during preparation, 500 mg of the drug was dissolved in 10 mL water, containing 0.2 mL 25% ammonia solution, and processed, similar to the solution obtained during preparation of CEA2. The product obtained was coded as AMF.

The drug, and all the solid systems, were passed through sieve no-85 and stored in a desiccator over anhydrous calcium chloride.

Physical characterization

IR spectroscopy

Samples were mixed with KBr and compressed into discs. The IR spectra were recorded on a Jasco FT/IR 5300 spectrophotometer. The spectra were recorded with a resolution of 0.1 cm^{-1} and 16 spectra were added in the procedure. The spectra from two separate batches of each sample were recorded and there was good reproducibility in the peak positions ($\pm 0.5\ cm^{-1}$).

Differential scanning calorimetry (DSC)

Approximately 8–10 mg of each sample was heated in open aluminium pans from 30°C to 300°C at a rate of 10°C min^{-1}, under a nitrogen flow of 50 mL min^{-1} in a Shimadzu DT-40 Thermal Analyzer.

X-ray diffractometry (XRD)

Powder X-ray diffraction patterns of all the samples were recorded in a Phillips X-ray diffractometer (model PW 1130/00), using Cu Kα radiation (voltage 45 KV, current 30 mA). The samples were packed into powder sample holders and their XRD patterns were recorded between 5 to 40° 2θ at a scanning rate of 2° 2θ min^{-1}.

In-vitro dissolution

All the solid systems were assayed spectrophotometrically for drug content after dissolving in phosphate buffer, pH 7.2 (the dissolution medium for flurbiprofen tablets USP, United States Pharmacopoeia (2000)). The dissolution profile of flurbiprofen from plain flurbiprofen powder and the binary systems was studied in a USP Apparatus 2, at 50 rev min^{-1}, in 900 mL of simulated gastric fluid without pepsin (SGF, prepared as per USP 2000 and pH adjusted to 1.2 ± 0.05), by the dispersed powder technique (Chiou & Riegelman 1971). The amount of each sample used was equivalent to 50 mg flurbiprofen. The dissolution efficiency (%DE) was calculated over a 3-h period as reported by Khan (1975).

$$\%DE = (\text{Area under the dissolution profile (0–3 h)})/(\text{total area of the rectangle of the plot}) \times 100$$

The total area of the plot was calculated for the rectangle with x-axis and y-axis ranges from 0–3 h and 0–100% release, respectively.

Statistical methods

The dissolution data was statistically evaluated by the Kruskal Wallis test to study the effect of different processing conditions on the amount of drug released at 10, 30 and 180 min during the dissolution test. A single factor analysis of variance test (Microsoft Excel) was performed to compare and evaluate differences between individual binary systems. In all the cases a significance level of $P < 0.05$ denoted significance.

Analytical technique

Drug concentrations were estimated by UV spectrophotometry using a Shimadzu UV-160 spectrophotometer. This method was used for the estimation of the drug concentrations in the phase solubility studies, drug content of the binary mixtures and the amount of drug released in the dissolution studies. Details of the analytical method validation experiments are provided in the Results and Discussion section.

Results and Discussion

Analytical method validation

The UV spectrum of drug solution in water, SGF and phosphate buffer, pH 7.2 exhibited a peak at 247 nm. The absorbance at this wavelength was used for quantification of the drug. Standard plots relating the drug concentration to the absorbance were constructed in all the three media (Figure 1). A linear relationship was obtained

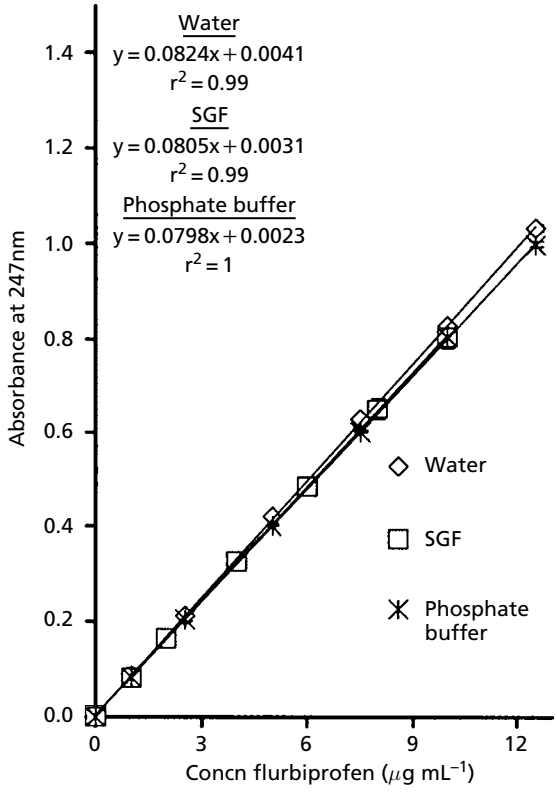

Figure 1 Standard plots relating concentration of flurbiprofen to absorbance at 247 nm, in different media.

in the concentration range of 0–10 $\mu g\,mL^{-1}$ (in SGF) and 0–12.5 $\mu g\,mL^{-1}$ (in water and phosphate buffer). The equations and the r^2 values are provided in Figure 1. The standard deviations in these measurements were very small (the error bars being smaller than the legends in Figure 1), indicating the precision of the analytical technique. The lowest concentration evaluated in the above experiments was 1 $\mu g\,mL^{-1}$ and this was used as the lower limit of quantification. All solutions analysed for drug content were diluted, if required, to a concentration range between 1–10 $\mu g\,mL^{-1}$, before measurement.

The other components used in the binary systems i.e. HP-β-CyD and povidone, did not exhibit any UV absorbance at 247 nm and hence did not interfere in the estimation of the drug as such. However, presence of cyclodextrin in solution was found to cause a reduction in the extinction coefficient of the drug due to formation of a complex. The position of the peak however remained unchanged. The molar extinction coefficient was calculated over a range of drug:HP-β-CyD molar ratios (1:0 to 1:4), keeping the drug concentration constant at 6 $\mu g\,mL^{-1}$. This was done in all the three media. A linear fit was obtained between the molar extinction coefficient of the drug and the HP-β-CyD:drug molar ratio (Table 1). Although the equations in Table 1 were obtained at a flurbiprofen concentration of 6 $\mu g\,mL^{-1}$, they were used to estimate concentrations in the range of 5.5–9.0 $\mu g\,mL^{-1}$. Any errors due to this were expected to be very small and this approach would yield more accurate values of drug concentration than by totally neglecting the effect of complex formation. The specific approaches used have been discussed in the relevant individual sections.

Phase solubility studies

As discussed earlier the reduction in the molar extinction coefficient of the drug due to complexation would introduce an error in the calculation of drug concentration. The solutions obtained in the phase solubility studies were appropriately diluted and the absorbance was measured at 247 nm. Since the concentration of the cyclodextrin in the solution was known, the concentration of the drug could be obtained based on the equation in Table 1. The general equation used was as follows:

Flurbiprofen concn (mM) = ((absorption at 247 nm) × (dilution factor) + 0.30124 × (HP-β-CyD concn (mM)))/ 20.472

The drug possessed a low aqueous solubility of 0.134 mM. Increase in the concentration of HP-β-CyD in the range of 0–16 mM resulted in a linear increase in the apparent aqueous solubility of the drug due to the formation of a soluble drug–cyclodextrin complex (Figure 2). The apparent association constant of a 1:1 complex was calculated as $K_{1:1} = m/S_0\,(1 - m)$, where S_0 was the aqueous solubility of the drug (0.134 mM) and m was the slope of the line in Figure 2. The apparent $K_{1:1}$ was 5200 M^{-1}. This indicated the formation of a strong complex between the drug and the cyclodextrin. It was, however, much lower than the association constants of 18 300 M^{-1} and 12 500 M^{-1} reported for a flurbiprofen–HP-β-CyD complex, in water (Frijlink et al 1991; Kagkadis et al 1998). The slope of the line reported by Kagkadis et al (1998) i.e. 0.4144 and the slope of the line in the solubility plot reported by Frijlink et al (1991) (calculated ~0.4) agreed well with the slope obtained in this study (m = 0.411). This indicated a similar solubilizing effect of HP-β-CyD in all the studies. The large differences in the association constants obtained could therefore be attributed to differences in the S_0 term used in the calculation of the association constant. For example the S_0 value taken as the y-intercept of the solubility line by Kagkadis et al (1998) was 0.04 mM at 25°C (approximately threefold lower than the solubility of the drug obtained by us, which accounted for an approximately threefold higher value of $K_{1:1}$ reported by them). The temperature of the study is one of the factors that will affect the measured value of S_0. This could explain the differences in the apparent

Table 1 Effect of HP-β-CyD:drug ratio, in different media, on the molar extinction coefficient of the drug at 247 nm. For each medium, the equations were generated based on spectral data at HP-β-CyD:drug ratios of 0:1, 0.5:1, 1:1, 2:1 and 4:1. The drug concentration was 6 $\mu g\,mL^{-1}$.

Medium	Equation	r^2
SGF	E = −405.05n + 19639	0.97
Water	E = −301.24n + 20472	0.98
Phosphate buffer, pH 7.2	E = −276.82n + 19601	0.99

All measurements were made in triplicate. E is the calculated molar extinction coefficient of the drug (lit.$mol^{-1}\,cm^{-1}$) and n is equal to the molar ratio of HP-β-CyD:drug.

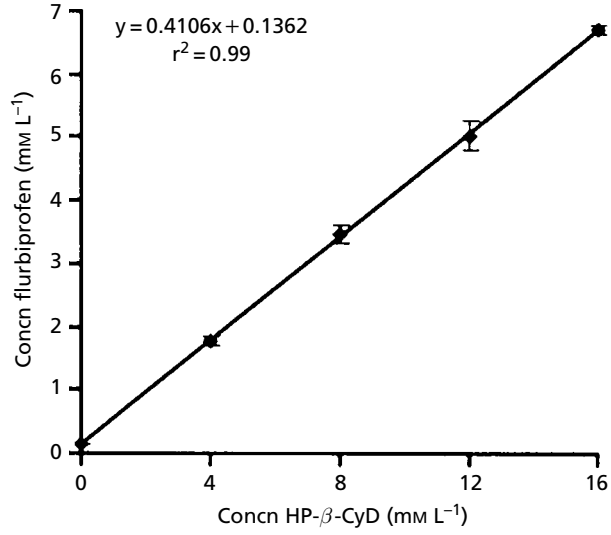

Figure 2 Phase solubility plots for flurbiprofen in presence of increasing concentrations of HP-β-CyD. Each data point represents a mean of three determinations.

$K_{1:1}$ values obtained in different studies, despite similar solubilizing effects (slope) seen.

NMR spectroscopy

The chemical shifts in the ^{1}H NMR spectrum of the drug were assigned to flurbiprofen protons (Figure 3), as reported by Wade et al (1990). The aromatic protons between 7.2 and 7.6 ppm showed upfield shifts in the presence of HP-β-CyD (Figure 3). The signals for the protons at positions 2′, 3′, 4′, 5′, 6′ showed significant shifts indicating a possible inclusion of the unsubstituted phenyl ring into the torus of the cyclodextrin. This is the preferred mode of the drug–cyclodextrin interaction, with the hydrophobic phenyl group of the drug molecule included within the hydrophobic cavity of HP-β-CyD.

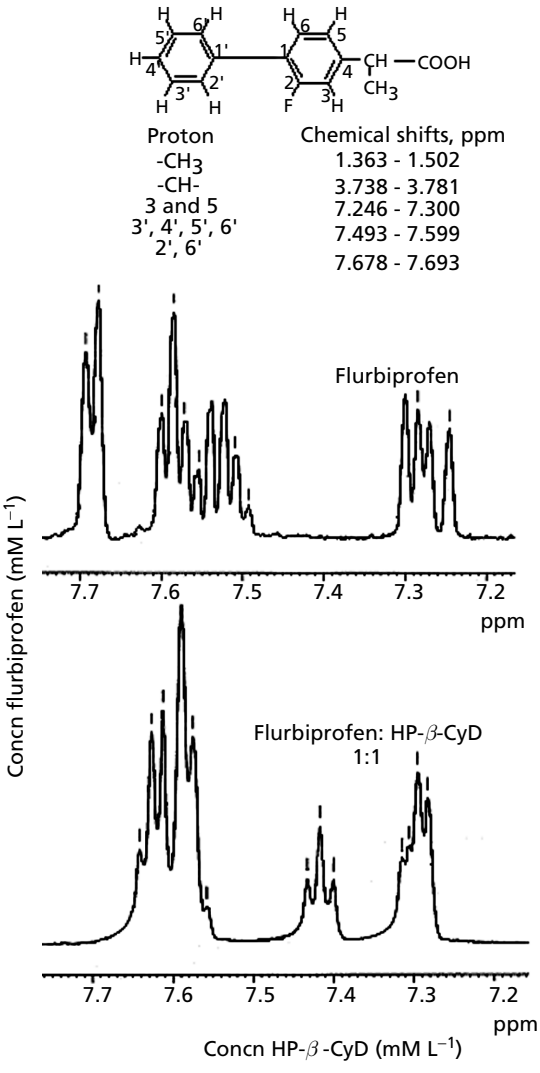

Figure 3 Numbering of flurbiprofen protons and assignment of the ^{1}H NMR chemical shifts based on Wade et al (1990). The lower panel shows the upfield shifts for the aromatic flurbiprofen protons in presence of HP-β-CyD.

Physical characterization

IR spectroscopy

The IR spectra of CG and CGP exhibited all the characteristic drug peaks. The position of the C=O stretching vibration of flurbiprofen (1701 cm^{-1}) showed changes in the IR spectra of some binary systems. In HP-β-CyD systems prepared using ethanol (KNE and CEE), this peak was seen at 1703 cm^{-1}. For those prepared with the aid of ammonia (FD, CEA1, CEA2, and KNA), the C=O stretching vibration was very weak and was shifted to a higher frequency of 1724 cm^{-1} to 1726 cm^{-1}. Representative spectra are shown in Figure 4.

Carboxylic acids normally exist as dimers in the solid state. Monomers exist only in extremely dilute solutions where the intermolecular hydrogen bonding between two molecules is minimized or absent. Hydrogen bonding in the dimer decreases the force constant of the C=O bond and therefore the C=O stretching is seen at a lower frequency. A change from dimer to monomer will therefore result in a shift in the C=O stretching vibration to a higher

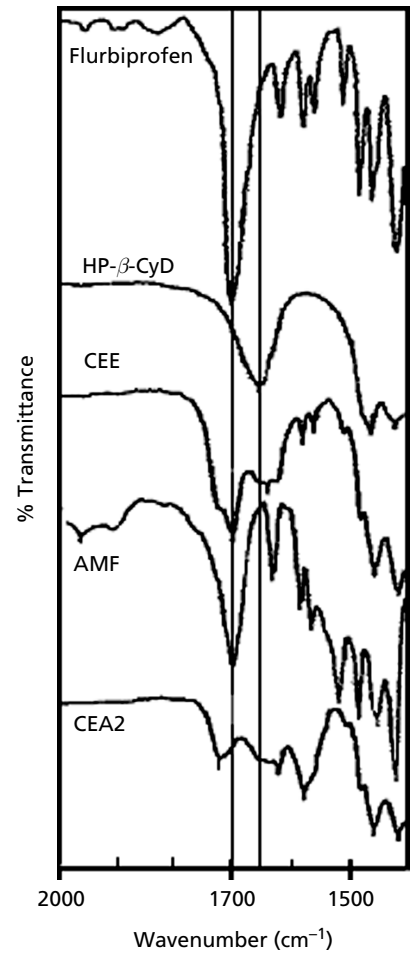

Figure 4 Overlaid FTIR spectra of flurbiprofen, HP-β-CyD, AMF and representative binary systems. See text for explanation of abbreviations.

frequency (Silverstein & Webster 1998). The shift in this stretch to a higher frequency for FD, CEA1, CEA2 and KNA could therefore indicate conversion of flurbiprofen to a monomerized state. During preparation, ammonia retains the acidic drug in solution (as a salt). This increases the extent of interaction (inclusion complex formation) between the drug and HP-β-CyD in solution. Therefore a higher proportion of the molecules would exist in the complexed form. Besides, there would be greater extent of molecular dispersion of the drug in the cyclodextrin matrix. This leads to a reduction in the presence of the flurbiprofen dimer in the final solid. As co-evaporation proceeds, the ammonia present in the system, either free or as an ammonium salt of flurbiprofen, leaves the system and the free acid of flurbiprofen is left behind. The presence of significant amounts of ammonium salt in the final solid is ruled out, as this would have resulted in a shift in the C=O stretch to a lower frequency.

For solid dispersions prepared by co-evaporation using aqueous ethanol, initial loss of ethanol from the solvent system resulted in precipitation of flurbiprofen, which was poorly soluble in water. This would reduce the extent of association of flurbiprofen with the cyclodextrin during the process. Flurbiprofen could precipitate out in an amorphous or crystalline form wherein it would exist as a dimer.

Presence of the C=O stretch at 1701 cm^{-1} in the IR spectrum of AMF indicated existence of the free acid dimer. Although ammonia resulted in complete dissolution of the drug during the preparation of the binary systems or AMF, it was lost during the process leaving back the free acid form of flurbiprofen. However, it helped in improving the extent of the interaction of the drug with the carrier.

In the spectrum of CGP, the C=O stretch of the dimer at 1701 cm^{-1} overlapped with the broader lactam carbonyl stretching of povidone. In CEP, the C=O stretching peak of the drug was suppressed, which could indicate interactions (dipole–dipole) between the drug and polymer, involving the carbonyl group of the drug molecule.

Differential scanning calorimetry (DSC)

The DSC curve of the drug showed the presence of a sharp melting endotherm at 117°C (Figure 5). All HP-β-CyD- and povidone-containing systems exhibited a shallow endotherm corresponding to water loss between 35°C and 100°C. HP-β-CyD and povidone showed no thermal change thereafter till a temperature of 300°C. The melting endotherm of the drug was seen in the thermograms of CG, KNE and CEE, which indicated the presence of uncomplexed flurbiprofen crystals.

The DSC curves of KNA, CEA1, CEA2 and FD showed complete absence of the melting endotherm of the drug, which could indicate complete loss of drug crystallinity. Drug crystallization during storage and during the DSC experiment would be inhibited by the presence of HP-β-CyD (Uekama et al 1992). Compared with the sharp melting peak for the pure drug, AMF showed a broad endotherm between 94°C and 129°C, with a peak at 111°C. This could possibly indicate some disorder or changes in the crystal lattice.

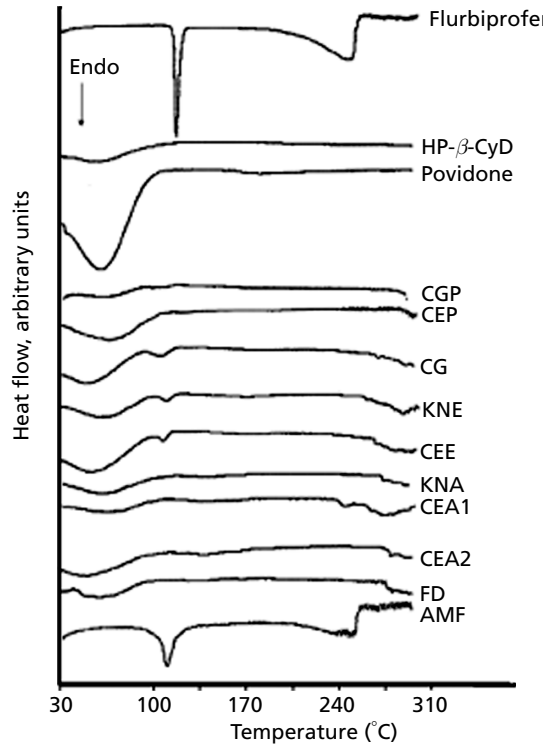

Figure 5 DSC heating curves of flurbiprofen, povidone, HP-β-CyD, all binary systems and AMF. See text for explanation of abbreviations.

The DSC curve of CGP showed a small and shallow endothermic segment in the melting range of the drug, indicating reduction in drug crystallinity due to grinding with povidone. The complete disappearance of the melting endotherm in the case of CEP indicated complete amorphization, due to inhibition of crystallization of the drug by povidone, during co-evaporation.

X-ray diffractometry (XRD)

The XRD pattern of the drug was found to match well with the pattern reported for the stable crystal form of flurbiprofen (Henck & Kuhnert-Brandstatter 1999). HP-β-CyD and povidone were X-ray amorphous (Figure 6).

CG showed all characteristic peaks corresponding to the drug, but with lower intensity due to the lower drug concentration and a possible reduction in crystallinity due to grinding (Figure 6). The peak intensities were further reduced for CGP, CEE and KNE indicating greater loss of crystallinity. CEP, KNA, CEA1, CEA2 and FD were X-ray amorphous (Figure 6).

The X-ray pattern of AMF was different from that of the pure drug (Figure 6) and a comparison with reported patterns of flurbiprofen polymorphs (Henck & Kuhnert-Brandstatter 1999) indicated that it could contain a mixture of different polymorphic forms. The DSC technique employed, however, did not detect any thermal events associated with polymorphic transitions, or crystallization of the stable form in the DSC heating curve of AMF (Figure 5).

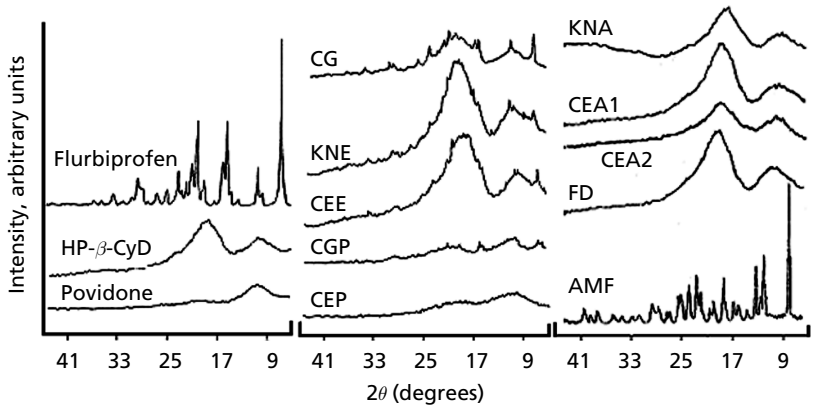

Figure 6 X-ray diffraction patterns of flurbiprofen, povidone, HP-β-CyD, all binary systems and AMF. See text for explanation of abbreviations.

The results of the studies were in agreement with each other. The use of ammonia retained the drug in solution during the initial stages of preparation. This would increase the interaction of the drug with HP-β-CyD. During the later stages, crystallization was inhibited due to the complexing ability of HP-β-CyD and the viscosity of the medium. In the absence of HP-β-CyD however (AMF), the loss of ammonia during the process resulted in crystallization of the free acid as a dimer, although in different crystal forms.

The results indicated complete loss of crystallinity in systems prepared using ammonia where the drug would exist partially or almost completely complexed with HP-β-CyD. It may also exist to varying extents in the amorphous form or may be molecularly dispersed in the HP-β-CyD matrix, not necessarily within the cyclodextrin cavity. There was partial loss of drug crystallinity in CG, CGP, KNE and CEE, and crystallization in different polymorphic form(s) in AMF.

During the preparation of CEP, flurbiprofen, being soluble in ethanol, was retained in solution. During the later stages, due to the inhibition of crystallization by povidone, flurbiprofen was retained in its amorphous state in the final co-evaporate. In the preparation of CEE however, initial loss of ethanol resulted in early crystallization of the drug in the water-rich system, leading to incomplete amorphization.

Drug content

UV spectrophotometry was used to determine the drug content of the binary systems. The HP-β-CyD:drug molar ratio was 1:1 in all the systems. Samples of the binary systems were dissolved in phosphate buffer, diluted to obtain a drug concentration of $\sim 6\,\mu g\,mL^{-1}$ and the absorbance was measured at 247 nm. The HP-β-CyD:drug molar ratio would therefore remain 1:1 in the final solution. The equation in Table 1 for phosphate buffer was used to calculate the drug content, using the value of n as 1.

For systems not containing HP-β-CyD, the drug contents were calculated using the standard plots in Figure 1. The drug content in all the systems studied was between 14.9% w/w to 15.4% w/w (98.5% to 101.8% of theoretical content).

In-vitro dissolution

The dissolution medium used (900 mL SGF) did not provide sink conditions (due to extremely low drug solubility) to qualify as an ideal medium. It was, however, suitable to distinguish and discriminate the ability of the different systems to promote enhanced drug dissolution in the acidic environment of the stomach. This was expected to provide an insight into possible effects of the binary systems, on the local irritant effects of the drug in the stomach, which is one of the factors contributing to ulcerogenicity of the drug.

Since the presence of HP-β-CyD was found to decrease the extinction coefficient of the drug, the calculation of drug release was based on the equation in Table 1. Since HP-β-CyD is highly water soluble, it was expected to instantaneously dissolve in the medium under the conditions of the dissolution test. The concentration of the cyclodextrin in the dissolution medium would therefore be 0.2274 mM (280.78 mg in 900 mL medium). This was assumed to be the concentration of cyclodextrin in the medium throughout the study. Although the dissolution test involved withdrawal of samples and replenishment with fresh medium, the decrease in concentration of the cyclodextrin in the medium would be small and would result in an extremely small error in the final value of % drug released. The lowest concentration of drug analysed from the dissolution medium, for a HP-β-CyD containing system, was for CG at the 10-min time point (see Figure 7). At this concentration the HP-β-CyD:drug molar ratio was $\sim$2.5:1. This ratio fell within the range over which the equations in Table 1 were generated (0:1 to 4:1). Using the equation in Table 1 and Beer-Lamberts law and substituting the value for molar concentration of HP-β-CyD in the dissolution medium, the final equation was obtained:

$$\text{Flurbiprofen concn } (\mu g\,mL^{-1}) = (\text{dilution factor} \times \text{absorbance at } 247\text{ nm})/0.08041 + 1.146$$

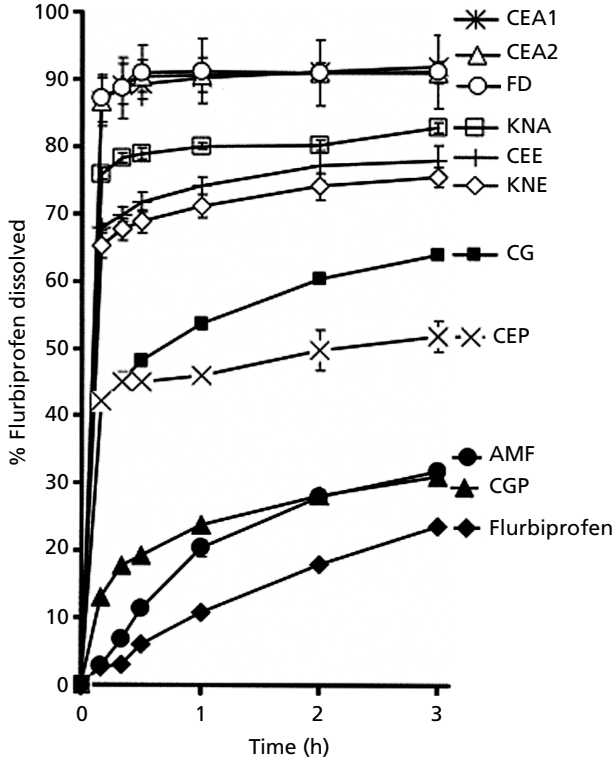

Figure 7 Dissolution profiles of drug from plain drug powder and different solid systems. Dissolution conditions: USP Apparatus 2, 900 mL simulated gastric fluid, $37 \pm 0.5\,^{\circ}C$, 50 rev min^{-1}. Each data point is a mean of six determinations from the same and multiple batches. See text for explanation of abbreviations.

For systems which did not contain HP-β-CyD, the standard plots in Figure 1 were used for estimation of the amount of drug dissolved.

Comparison of the amount of drug dissolved at 10, 30 and 180 min of the dissolution test, from all the systems, by the Kruskal Wallis test, indicated significant differences between at least two of the systems ($P < 0.05$). All individual pair wise comparisons and determinations of differences between two systems, at all the above time points, were carried out by the single factor analysis of variance test at 95% confidence level.

The drug being poorly soluble under the acidic conditions of SGF dissolved very slowly with only ~6% dissolving in the first 30 min and 24% at the end of 3 h (Figure 7).

For the povidone systems, the presence of a water soluble carrier which improved wettability and reduced aggregation and agglomeration of drug particles, the possible existence of amorphous form of the drug, and a reduction in the size of the drug particles increased the rate and extent of drug dissolution. CGP improved the dissolution of the drug, as compared with the plain drug, with 19% dissolving in the first 30 min and up to 31% in 3 h ($P < 0.05$). In addition to the above factors, complete amorphization of the drug in CEP, and a solubilizing effect of povidone in the microenvironment surrounding the drug particles resulted in 51% dissolution in 3 h. This was significantly higher than the amount of drug released from CGP ($P < 0.05$).

In the case of the HP-β-CyD systems, in addition to the factors which resulted in better dissolution properties of the povidone systems (mentioned above), the formation of a strong and highly water soluble complex between the drug and the carrier resulted in higher rate and extent of drug dissolution. Complex formation not only resulted in faster dissolution but also retained the drug in solution (in the form of a complex).

Completely amorphous drug, dispersed in a highly water soluble polymer i.e. CEP, offered a lower dissolution advantage as compared with all the cyclodextrin systems, some of which even retained some drug crystallinity (e.g. CG, KNE). This was due to the formation of a strong water soluble complex either during the preparation process itself or in-situ in the dissolution medium. The amount dissolved from these samples represented both free and complexed drug in solution.

Dissolution of 64%, 75% and 78% of the drug in 3 h was seen from CG, KNE and CEE, respectively. The use of solvent in preparation of KNE and CEE, as compared with plain co-grinding (CG) caused greater amorphization of drug and could lead to better molecular association of the drug with the cyclodextrin, thereby leading to better dissolution. Thus, dissolution from CEE was significantly better compared with KNE, which was better than the dissolution from CG ($P < 0.05$).

Complete amorphization of the drug and significant molecular dispersion of the drug, due to complex formation in FD, CEA1, CEA2, and KNA resulted in greater rate and extent of drug dissolution. Flurbiprofen dissolved rapidly from FD, CEA1 and CEA2, with ~85% dissolution in the first 10 min. The dissolution profiles from these systems were very similar ($P > 0.05$). Dissolution from KNA was lower than that from FD, CEA1 and CEA2 ($P < 0.05$), with 73% of the drug dissolving in 10 min and 80% in 3 h. Although there was complete amorphization of the drug in KNA, there could be a lower extent of association of the drug with the HP-β-CyD cavity as compared with CEA1, CEA2 and FD.

In this study, the process of kneading used limited solvent and did not involve complete dissolution of the solids. However, co-evaporation, which involved complete dissolution of both components in the solvent system before evaporation, resulted in greater extent of interaction of the drug with the cyclodextrin and greater amorphization. Hence the effect of solvent use on drug dissolution would be more pronounced in the case of co-evaporates than in the case of the kneaded systems prepared using similar solvents.

Although the amount of drug which dissolved in the first 10 min from plain drug powder and AMF were similar ($P > 0.05$), a higher amount (32%) of flurbiprofen dissolved from AMF in 3 h ($P < 0.05$). The slightly better dissolution as compared with plain flurbiprofen could be due to disorder in the solid-state or existence of the drug in higher-energy polymorphic forms in AMF.

The % dissolution efficiency values indicated the ability of the preparations to enhance drug dissolution

and were in the order flurbiprofen (13.6) < AMF (21.5) < CGP (24.4) < CEP (46.5) < CG (51.8) < KNE (67.5) < CEE (70.3) < KNA (75.3) < CEA1 (85.3) = CEA2 (85.4) = FD (85.6).

The results of the in-vitro dissolution studies agreed with the inferences drawn from the physical characterization. Although the formation of a drug–HP-β-CyD complex in-situ in the dissolution medium resulted in an increase in the overall dissolution rate, the presence of the drug molecularly dispersed in the binary system itself further enhanced drug dissolution.

Conclusions

The method of preparation of the binary systems was found to have a profound influence on the final solid-state and hence the product performance. Use of ammonia during the preparation of flurbiprofen–HP-β-CyD solid dispersions yielded systems from which very rapid drug dissolution was achieved.

Maximum improvement in the performance of a dosage form of a poorly soluble drug is therefore not ensured solely by incorporation of an agent which can form a soluble complex. The manufacturing process used, solvents used, processing times, and processing conditions could significantly influence the performance of the final formulation. Choosing these variables based on the properties of the system, strength of the complex formed and the solubility of the complex forming species could aid in significantly increasing the advantages provided by the cyclodextrin-based systems.

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