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Research Paper

Cefuroxime axetil solid dispersion with polyglycolized glycerides for improved stability and bioavailability

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Abstract

Objectives Cefuroxime axetil (CA), a poorly soluble, broad spectrum cephalosporin ester prodrug, is hydrolysed by intestinal esterase prior to absorption, leading to poor and variable bioavailability. The objective was therefore to formulate a stable amorphous solid dispersion of the drug with enhanced solubility and stability against enzymatic degradation.
Methods Spray drying was used to obtain a solid dispersion of CA with Gelucire 50/13 and Aerosil 200 (SDCAGA), and a solid dispersion of CA with polyvinyl pyrrolidone (SDCAP); amorphous CA (ACA) was obtained by spray drying CA alone. The formulations were characterized by differential scanning calorimetry, X-ray powder diffraction, scanning electron microscopy and Fourier transform infrared spectroscopy studies, and compared for solubility, dissolution and bioavailability in rats.
Key findings SDCAP and SDCAGA showed improved solubility and dissolution profiles owing to amorphization and formation of solid dispersions with hydrophilic carriers. The improved stability of amorphous CA in solid dispersions compared to ACA alone was attributed to hydrogen bonding interactions involving the amide of CA with the carbonyl of polyvinyl pyrrolidone in SDCAP, whereas in SDCAGA the interactions were at multiple sites involving the amide and carbonyl of CA with the carbonyl and hydroxyl of Gelucire 50/13. However, SDCAGA showed superior bioavailability compared to SDCAP, ACA and CA.
Conclusions Improvement in physical stability of solid dispersions was attributed to hydrogen bonding, while improvement in bioavailability of SDCAGA compared to SDCAP, in spite of comparable solubility and dissolution profile, may be attributed to Gelucire, which utilizes intestinal esterase for lipolysis, protecting the prodrug from enzymatic degradation to its non-absorbable base form.
Keywords bioavailability; enzymatic stability; lipid; solid dispersion

Introduction

Cefuroxime axetil (CA) is a prodrug of cefuroxime (C), a cephalosporin with broad-spectrum activity against Gram-positive and Gram-negative microorganisms. C has well-defined pharmacokinetics after intramuscular and intravenous administration in the form of its sodium salt. However, the oral absorption of C is lower than 1%, which restricts its use to the parenteral route only.[1] However, after oral administration the prodrug, CA, is absorbed and rapidly hydrolysed by esterases in the intestinal mucosa and portal blood to produce C. The 1-acetoxyethylester group in position 4 of CA ensures lipophilicity and promotes the intestinal absorption of C but at the same time compromises on the solubility. Hence the prodrug shows poor and variable bioavailability.[2]

Development of amorphous CA by spray drying is one of the widely used approaches for enhancing solubility.[3,4] The higher energy content of the amorphous or vitreous state is responsible for its higher solubility but it suffers from the drawback of recrystallization during storage. In addition, preparation of amorphous nanoparticles of CA has produced improved bioavailability owing to enhanced dissolution.[5] To date, several attempts have been made to obtain and stabilize drugs in the amorphous state. Solid dispersion (SD) with water-soluble carriers such as high molecular weight polyethylene glycols,[6] polyvinyl

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pyrrolidone (PVP),[7] hydroxypropylcellulose[8] and hydroxypropylmethylcellulose[9] have been reported. The use of lipid-based amphiphilic carriers with solubilizing properties like Gelucire 44/14[10] and Gelucire 50/13[11,12] has recently attracted much interest. Gelucire 50/13 (G50) is a saturated polyglycolized glyceride consisting of a well-defined mixture of mono-, di and tri-glycerides and mono- and di-fatty acid esters of polyethylene glycol. It has a hydrophilic–lipophilic balance value of 13.

Apart from poor aqueous solubility, hydrolysis of CA to non-absorbable C by intestinal esterases prior to its absorption is another reason for its low and variable bioavailability. However, CA shows a food effect, with 30% oral bioavailability in a fasted and 50% in a fed state.[2] The bioavailability is found to improve after a lipid-rich diet, preferably containing medium-chain triglycerides.[13] This is attributed to protection of the drug against hydrolysis, caused by competitive lipolysis of lipids. The potential to formulate cephalosporin prodrugs with an oily phase to prevent enzymatic hydrolysis has been reported. These take the form of a submicron emulsion of cefpodoxime proxetil with mixed medium-chain triglycerides.[14] Apart from enhancing solubility of poorly water-soluble drugs, lipid-based delivery systems are advantageous because of their ability to bypass some of the more resistant chemical and physical barriers associated with poorly absorbed drugs.[15]

The objective of this work was to develop a formulation that would increase drug solubility and decrease enzymatic degradation by esterase in the intestinal lumen by utilizing G50, an amphiphillic saturated polyglycolized glyceride, as the SD carrier.

G50 is a multifunctional excipient, which may compete with CA for hydrolysis, protecting it from enzymatic attack prior to its absorption. Even though the application of SDs containing low melting point lipids is advantageous, its utility is limited due to the difficulties in preparing the formulation due to spray-dried material sticking to the drying chamber. Passerini *et al*.[16] reported the formation of ultrasound-assisted spray-congealed carbamazepine-Gelucire 50/13 particles. However, the amount of lipid required in the formulation was particularly high and the process is very tedious and costly.

Aerosil 200 (A200) is an important carrier, which can result in free-flowing spray-dried product due to the high surface area, and which can cause faster drug dissolution due to the presence of surface silanol groups. Attempts have therefore been made by the authors to stabilize the spray-dried amorphous drug with G50 as the SD carrier with the aid of A200 as an adsorbent for improved stability and bioavailability.

Amorphous CA (ACA), SD with PVP (SDCAP) and SD with G50 and A200 (SDCAGA) were obtained by spray drying. Physicochemical characterization of freshly prepared samples as well as aged samples was performed using scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), differential scanning colorimetry (DSC) and X-ray powder diffraction (XRPD). To confirm the stabilization in terms of enzymatic degradation, SDCAGA was compared with SDCAP for in-vivo bioavailability in Wistar rats.

Materials and Methods

Materials

Crystalline CA and indapamide were generous gift samples from Lupin Research Park (Pune, India). Gelucire 50/13 (Stearoyl Macrogoglycerides EP, Gattefosse, France) was supplied by Colorcon India (Mumbai, India). PVP K-30 (BASF, Ludwigshafen, Germany) and Aerosil 200 (Degussa, Dusseldorf, Germany) were supplied by Get-Rid Pharmaceuticals Ltd (Pune, India). All other chemicals and solvents were of analytical grade.

Preparation of ACA and SDCAP

CA, alone or in combination with PVP (1 : 1 parts by weight), was dissolved in dichloromethane (DCM) (7–10% w/v). The clear solution was spray dried on a spray drier (Jay Instruments & Systems Pvt. Ltd, Mumbai, India) under the following set of conditions: flow rate: 5–6 ml/min; inlet temperature: 70°C; outlet temperature: 40°C; aspiration: –1.471 kPa and atomization air pressure: 1961 kPa. The product was stored in a vacuum dryer at 30°C and –0.66 kPa for 24 h.

Preparation of SDCAGA

CA in combination with G50 (1 : 0.5, 1 : 1 and 1 : 1.5 parts by weight) was dissolved in DCM (10% w/v). To this clear solution, A200 was added as an adsorbent and the dispersion was spray dried on a spray drier keeping the conditions the same as above. The resulting product was stored in a vacuum dryer for 24 h.

Physical mixtures (PMs) of drug and carrier in the same ratio were prepared by mixing CA and PVP (PMCAP) or CA, G50 and A200 (PMCAGA) thoroughly for 5 min in a mortar until a homogeneous mixture was obtained. Samples were stored in a desiccated environment until required for further study.

Thermogravimetric analysis

To determine the residual solvent in ACA and SD samples, thermogravimetric analysis was performed using a TA-60WS thermogravimetric analyzer (Shimandzu, Japan). Samples (approximately 30–40 mg) were heated in a platinum crucible in a nitrogen atmosphere and the loss of mass as a function of temperature was recorded.

Saturation solubility

Saturation solubility measurements of CA, ACA, PMs and SDs were carried out by adding a known excess amount of different formulations in 10 ml 0.1 M HCl. Samples were stirred at 20 rpm in a water bath (37 ± 0.5°C) for 24 h. Samples were then filtered through Whatman filter paper 41 (Spring Field Mill, UK), diluted and analysed spectrophotometrically at 278 nm.

Scanning electron microscopy

Scanning electron microscopy (SEM) samples were mounted on the aluminum stubs and coated with a thin gold–palladium layer using an auto fine coater unit (Jeol, JFC, Tokya, Japan). The surface topography was analysed with a Jeol scanning

electron microscope (JSM-6360A, Tokya, Japan) operated at an acceleration voltage of 10 kV.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) studies were carried out using a Mettler-Toledo DSC 821^e instrument equipped with an intracooler (Mettler-Toledo, Tokyo, Japan). Indium and zinc standards were used to calibrate the DSC temperature and enthalpy scale. The samples were hermetically sealed in aluminum pans and heated at a constant rate of 10°C/min over a temperature range of 25–200°C. An inert atmosphere was maintained by purging with nitrogen gas at a flow rate of 50 ml/min.

X-ray powder diffraction

The X-ray powder diffraction (XRPD) patterns were recorded on an X-ray diffractometer (PW 1729, Philips, The Netherlands). Samples were irradiated with monochromatized CuKα radiation (1.542 Å) and analysed at 5–50°2θ. The voltage and current used were 30 kV and 30 mA, respectively.

DRIFT spectroscopy

The diffuse reflectance infrared Fourier transform (DRIFT) spectra were obtained, after appropriate background subtraction, using an FT-IR spectrometer (FT/IR-4100, Jasco, Japan). Samples were mixed with dry potassium bromide and scanned from 4000–400 cm^{-1}. Jasco spectra manager Version 2 was used for data acquisition and analysis.

In-vitro drug release

The dissolution studies were performed using USP 24 type II dissolution test apparatus (TDT-06P, Electrolab, India). All samples were placed in a dissolution vessel containing 900 ml 0.1 M HCl, maintained at 37 ± 0.5°C and stirred at 100 rpm. Samples were collected periodically and replaced with a fresh dissolution medium. After centrifugation, filtration through 0.45 μm membrane filter and suitable dilution, the concentration of CA was determined spectrophotometrically at 278 nm. Data were analysed using PCP-Disso software (V3, Poona College of Pharmacy, Pune, India).

Bioavailability study

The rat has been reported as a suitable model for assessing the intestinal absorption of drugs in humans.[17] The rat has also been utilized for determining the absorption and degradation (hydrolysis) kinetics of CA.[18] Thus the rat was selected as an experimental animal for the present study. All studies were approved by the Institutional Animal Ethics Committee of Poona College of Pharmacy (Pune, India), and were conducted under the provisions of the approved protocol (CPCESA/70/07).

Experimental procedure

The improvement in bioavailability of SDCAGA and SDCAP in comparison with CA and ACA was determined in healthy Wistar rats of both sexes, each weighing between 200 and 250 g. The animals were fasted overnight prior to dosing. Water was allowed *ad libitum.* The animals were randomly divided into three groups of six animals each. Bioavailability was assessed after a single oral dosing of the sample (50 mg/kg) in the form of an aqueous suspension (prepared in 0.25% w/v carboxymethyl cellulose just before dosing). After mild anaesthesia of animals, serial blood samples (0.5–1 ml each) were collected using the retro-orbital puncture technique, at predetermined time intervals (0, 15, 30, 45, 60, 90, 180, 240, 300, 1200 and 1440 min). Plasma was separated by centrifugation at 3000 rpm and 4°C for 15 min (Cryocentrifuge 2810R, Eppendorf, USA) and promptly analysed by HPLC.

Assay of plasma concentration

Plasma (200 μl) was transferred to a stoppered test-tube, to which 200 μl of internal standard (indapamide, stock solution 10 μg/ml in mobile phase) was added. The sample was mixed by vortexing for 5 min. Deproteinization and extraction of CA and the internal standard was carried out using 600 μl of acetonitrile with vortex mixing for 5 min. The solution was filtered through a 0.45 μm membrane filter and analysed by HPLC.

The HPLC system specifications were as follows: pump: PU-1580 (JASCO, Japan); injector: auto sampler (AS-1555; JASCO); column: RP C_{18}, 250 × 4.6 mm, 5 μm (Thermo Electron Corporation, USA); detector: UV/visible (UV-1575; JASCO). Data acquisition and analysis was carried out using Borwin/HSS 2000 software (LG 1580–04; JASCO). The chromatographic conditions were as follows: mobile phase 0.01 M potassium dihydrogen phosphate and methanol (60 : 40, pH 5); flow rate: 1.5 ml/min; wavelength: 278 nm. The calibration curves of CA covered a concentration range of 0–25 μg/ml. The ratio of peak area of CA/indapamide was used for quantification of plasma samples.

Stability studies

All samples were packed in aluminum foil and monitored for up to 3 months at ambient temperature and relative humidity (30°C/65% RH). Samples were removed periodically (1 month and 3 months) and characterized as to (1) the extent of dissolution and (2) the presence of crystallinity, in comparison with the initial sample, using DSC and XRPD studies.

Statistical analysis

Statistical analysis of the effects of different formulations (CA, ACA, PMs and SDs) on the solubility of drug was performed using one-way ANOVA. Individual differences between various formulations were examined using Dunn's post hoc test (GraphPad Prism, version 4.03.354, GraphPad Software, Inc., San Diego, CA, USA). Statistical analysis of the effects of different formulations (CA, ACA, PMs and SDs) on the percentage dissolved for each formulation at each time point was performed using the Kruskal–Wallis test. Individual differences between various formulations were examined using Dunn's post-hoc test. Statistical analysis of the effects of different formulations (CA, ACA, SDCA and SDCAGA) on pharmacokinetic parameters (C_{max} and AUC) was performed using one-way ANOVA. Individual differences between various formulations were examined using Dunnet's post-hoc test. A significance level of $P < 0.05$ denoted a significant difference while $P < 0.01$ denoted a very significant difference.

Results and discussion

Spray-drying process variables were optimized on the basis of production yield and powder characteristics. ACA was obtained by spray drying the solution of CA in DCM, with a product yield of 55% w/w. Spray drying of CA and PVP in a 1 : 1 ratio w/w resulted in SDCAP with an 85% w/w yield.

The product of SD with G50 could not be obtained even at the lowest proportion of lipid (1 : 0.5) and the lowest possible temperature (30°C) due to the sticking of the product to the walls of the drying chamber, indicating a limitation of spray drying. In the literature, the spray-freezing technique has been reported to allow dispersion of drugs in Gelucire or other lipids, but such a technique lacks commercial feasibility.[16] A combination of SD and the adsorption technique has therefore been attempted in order to overcome the limitations of spray drying. The outlet temperature should not exceed the melting temperature of Gelucire, so DCM was the only solvent possible because of its low boiling point. Moreover, G50 and the drug were completely soluble in this medium.

A200, colloidal silicon dioxide, was used as an adsorbent. Initially CA, G50 and A200 in various proportions were attempted for the feasibility of spray drying. Based on the powder characteristics, encapsulation efficiency and saturation solubility, an optimum ratio of 1 : 1 : 1 parts by weight of CA, G50 and A200 was determined. SD in the ratio of 1 : 1 : 1 was a free-flowing powder with 90 ± 2% (w/w) yield and 98.1 ± 1% (w/w) encapsulation efficiency.

The saturation solubility increased from 0.41 ± 0.12 mg/ml for CA to 1.28 ± 0.11 mg/ml for ACA, 3.23 ± 0.21 mg/ml for SDCAP and 3.31 ± 0.19 mg/ml for SDCAGA (1 : 1 : 1), whereas, PMCAP and PMCAGA showed saturation solubilities of 0.54 ± 0.13 and 0.61 ± 0.24 mg/ml, respectively (Table 1). When the solubility of various formulations was compared statistically with the solubility of CA, formulations of ACA and PMs did not represent a significant improvement ($P > 0.05$). However the solubility of all SDs was a significant improvement ($P < 0.05$) over the solubility of CA. The difference between the saturation solubility of PMs and SDs may be attributed to effect of the carrier, the more intimate contact between carrier and drug in SDs, and also to the presence of an amorphous form of the drug in SDs (the drug being present in crystalline form with PMs). The presence of a hydrophilic carrier prevents aggregation or agglomeration of individual drug particles and creates a microenvironment in which the drug solubility is high. The effect of the spray-drying process on the chemical stability of the product was examined by HPLC. Chromatograms of spray-dried CA did not show any additional peak, indicating an absence of chemical degradation during processing. The amount of residual organic solvent in the samples was below the detection limit of thermogravimetric analysis (< 0.05% w/w). The spray-drying method used in this study therefore appears applicable for the preparation of SDs with high encapsulation efficiency.

Table 1 Saturation solubility of different formulations of cefuroxime axetil

Type of formulation	Saturation solubility (mg/ml)
CA	0.41 ± 0.12
ACA	1.28 ± 0.11
PMCAP	0.54 ± 0.13
PMCAGA	0.61 ± 0.24
SDCAP (1 : 1)	3.23 ± 0.21
SDCAGA (1 : 1 : 0.5)	3.17 ± 0.26
SDCAGA (1 : 1 : 1)	3.31 ± 0.19
SDCAGA (1 : 1 : 1.5)	3.33 ± 0.16

Values are mean ± SD; $n = 3$.

Morphology

Scanning electron microphotographs of CA, ACA, SDCAP and SDCAGA are shown in Figure 1. The unprocessed CA as provided by the manufacturer was a mixture of some large crystals and a few microparticles, which might have been generated by micronization or other size reduction process at the time of manufacturing. The ACA particles were hollow microspheres with a smooth surface. The reduced particle size and hollow nature of the particles therefore may be responsible for the increased dissolution rate of ACA. SDCAP showed smooth, spherical particles with concave depressions. SDCAGA, on the other hand, appeared as irregularly shaped matrices.

SDs revealed significant changes in particle shape and surface topography due to the impact of the spray-drying process. It is therefore possible that the reduced particle size, increased surface area and the close contact between the hydrophilic carrier and the drug may be responsible for the enhanced dissolution rate of SDs.

Differential scanning calorimetry and X-ray powder diffraction

DSC thermograms of pure CA, PMs and SDs are shown in Figure 2. Unprocessed CA exhibited an endothermic band at around 181°C, indicating a crystalline nature. ACA did not show an endothermic peak corresponding to melting; instead,

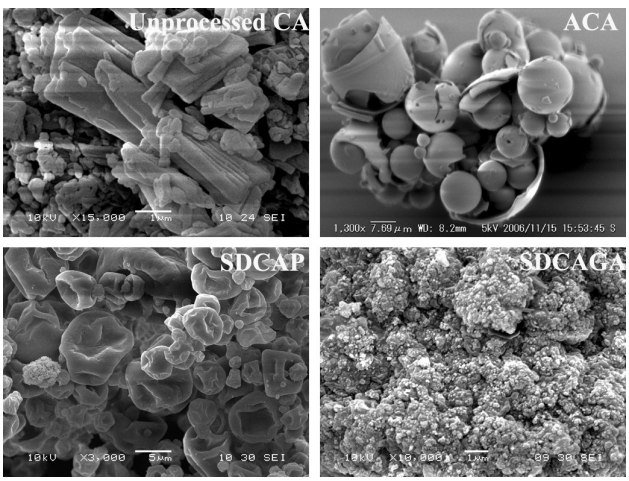

Figure 1 Scanning electron photomicrographs of cefuroxime axetil preparations. Magnifications: unprocessed CA at 15 000×, ACA at 1300×, SDCAP at 3000× and SDCAGA at 10 000×.

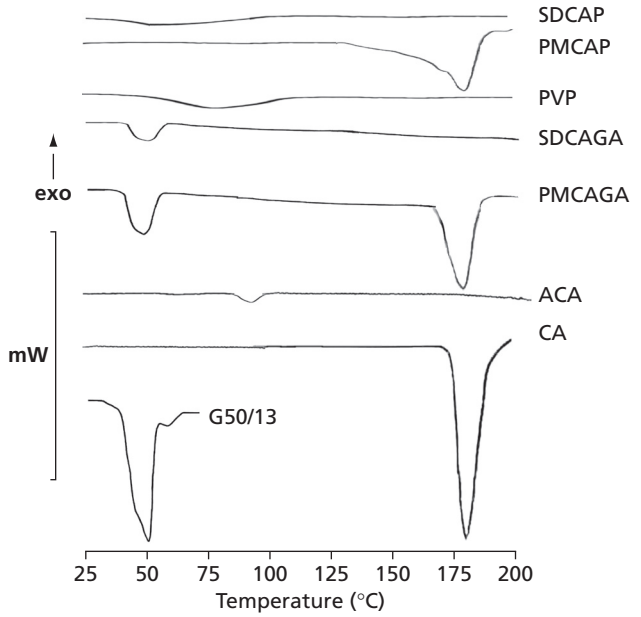

Figure 2 Differential scanning calorimetry thermograms of cefuroxime axetil preparations

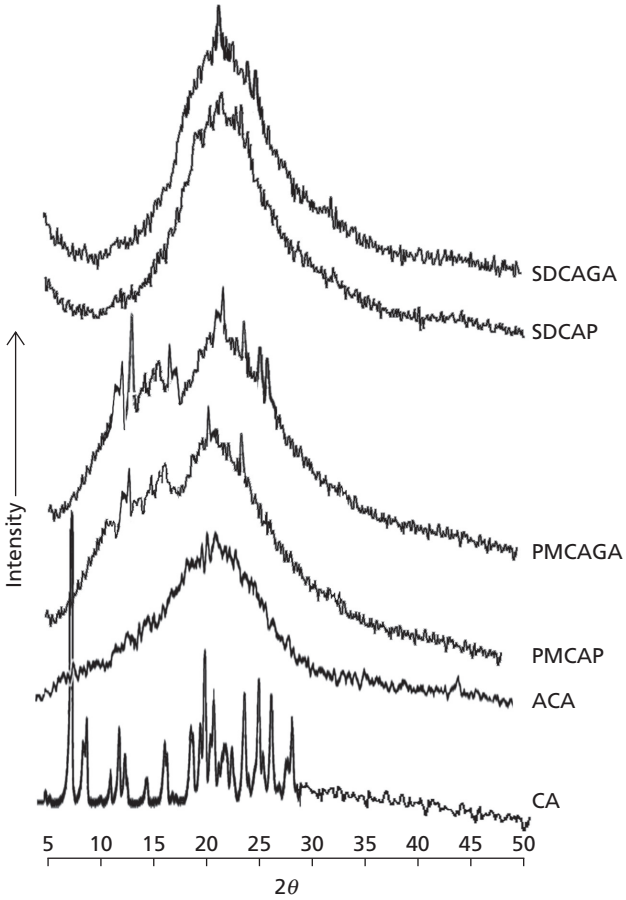

Figure 3 X-ray powder diffraction patterns of cefuroxime axetil preparations

a T_g was observed at 90–95°C, indicating amorphization of CA. The DSC curves of PMCAP also showed an endothermic peak corresponding to the melting of CA, indicating the presence of crystallinity. In contrast, no endotherm corresponding to pure CA was observed in SDs prepared using PVP. Thermograms of PMCAGA revealed the endothermic peak of the corresponding polyglycolized glyceride carriers, along with a slight shift in the melting endotherm of the drug, and a significant decrease in enthalpy of fusion, indicating dissolution of some crystalline CA in molten G50. SDCAGA did not show any melting endotherm of CA, possibly due to the presence of the amorphous form of CA in the SDCAGA. Previous studies have shown that PVP used in SDs can inhibit the crystallization of drugs, resulting in the formation of the amorphous form of the drug in SDs.[19–23] We have also reported the crystallization inhibition of drugs by G50 in the form of SDs.[11,12] The crystallization inhibition by PVP and G50 is often due to interactions with the drug, such as hydrogen bonding. DSC analysis therefore suggests that CA might be present in amorphous form within SDs, owing to crystallization inhibition of the drug by PVP and G50.

In order to further confirm the physical state, XRPD studies were performed in order to analyse unprocessed and processed drug samples. The XRPD patterns of pure CA, PMs and SDs are shown in Figure 3. The XRPD patterns of CA show characteristic high intensity diffraction peaks, indicating that CA is highly crystalline in nature. PMs with PVP and G50 were also crystalline, as indicated by the numerous characteristic diffraction peaks of CA, but there was a significant decrease in intensity of some major CA crystalline peaks. In general this partial loss of crystallinity may be due to the physical presence of amorphous excipients. PMCAGA showed additional low-intensity peaks corresponding to G50 at 12.5, 26 and 27°.[11] On the other hand, the XRPD patterns of SDs prepared using spray drying were completely different to those of pure CA and PMs, with a hollow, broad and diffused pattern caused by random arrangement of the constituent molecules. The XRPD patterns of SDs did not show any characteristic diffraction peaks corresponding to pure CA, confirming that CA is present in amorphous or non-crystalline form. It is evident that there is probably an important interaction, such as hydrogen bonding, between pure CA and carriers during the preparation of SDs.

FT-infared spectroscopy

To study the possible interaction of CA with the carriers, DRIFT spectroscopy was carried out. The FT-IR spectra of CA, PMs and SDs are shown in Figure 4. The spectrum of CA was characterized by peaks at 3480–3210 cm^{-1} (NH, NH_2 complex), 1782 cm^{-1} (β-lactam), 1760 cm^{-1} (acetate), 1720 cm^{-1} (4-ester group) and 1676 cm^{-1} and 1534 cm^{-1} (7-amido). The FT-IR spectrum of PVP showed a characteristic absorption band at 1660 cm^{-1} (C=O) and a very broad absorption band at 3435 cm^{-1} due to the presence of water. The absorption bands corresponding to crystalline CA were also observed for the physical mixtures with PVP, suggesting that there is no interaction between pure CA and PVP in PMs. In contrast, absorption bands corresponding to the C=O

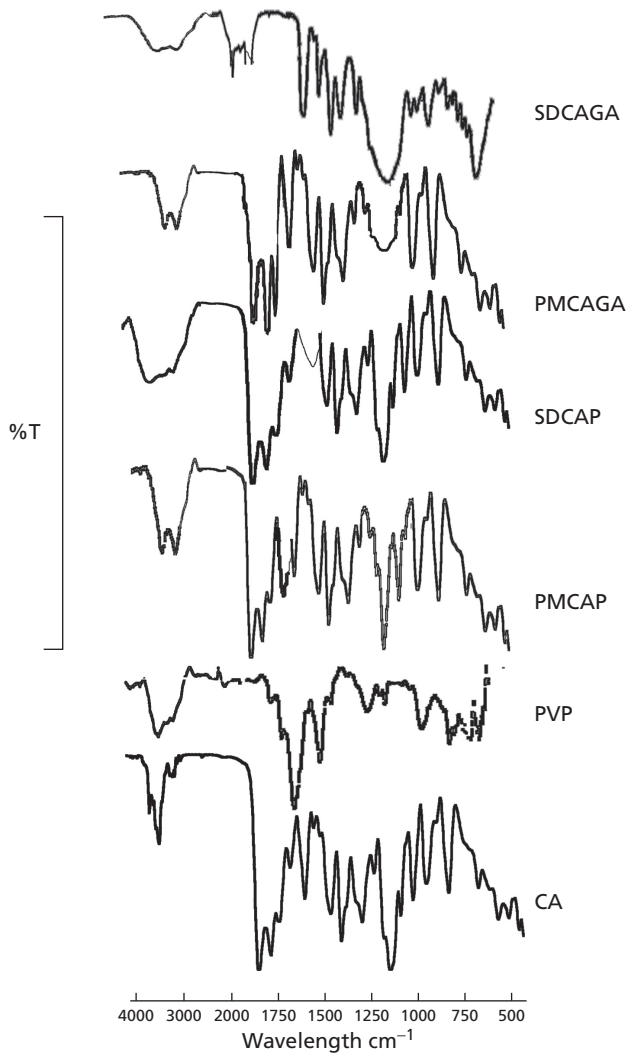

Figure 4 FT-infrared spectra of cefuroxime axetil preparations and polyvinyl pyrrolidone

stretching bands at 1650 cm^{-1} were not observed, and the symmetrical and asymmetrical N–H stretching vibrations of the primary amide groups of CA at 3410 and 3270 cm^{-1} were broadened in the SDCAP spectra. Taylor and Zografi[24] reported that PVP has two functional groups, i.e. =N– and C=O, that can potentially form hydrogen bonds with the drug. However, stearic hindrance precludes the involvement of nitrogen atoms in intermolecular interactions, thus making the carbonyl group more favourable for hydrogen bonding. These results suggest that the N–H functional groups of CA act as a hydrogen donor while the C=O of PVP is a hydrogen acceptor, forming hydrogen bonds in SD and resulting in the formation of stable amorphous or non-crystalline forms of CA.

Ideally, the DRIFT spectra of PMs should be equivalent to the addition spectrum of excipients and the crystalline drug. However, the overall spectrum of and PMCAGA appeared to be influenced by the incorporation of silicon dioxide. The presence of a broad prominent peak at 1107 cm^{-1} (strong Si–O linkage) is characteristic of silicon dioxide.[11] As stated earlier, the hydrogen bonding potential of silanol groups in the local environment of silica is well documented, so there is always a possibility that during preparation of PMs the amide group of CA can form very weak hydrogen bonds with the silanol groups of A200.

All major peaks of carbonyl stretching vibrations were absent and amide peaks were broadened in the DRIFT spectra of SDCAGA. This indicated the possibility of hydrogen bonding between CA and the carriers. However, the site of interaction of Gelucire would be expected to be in the C=O group, affecting the N–H vibration, as reported by Passerini *et al*.[16] In fact, the interaction may possibly also occur between the C=O group of CA and the –OH group of Gelucire as reported by Shimpi *et al*.[12] This was supported by the significantly decreased intensity of peaks corresponding to the carbonyl stretching vibrations. This was the reason etoricoxib was reported to form hydrogen bonds with Gelucire but not with PVP, as PVP acts only as a proton acceptor in the hydrogen bonding interaction.[12] Although both the carriers show formation of a stable SD with CA by hydrogen bonding, the functional groups involved are different. While in hydrogen bonding of CA with PVP an amine is involved, with Gelucire both carbonyl and amine functional groups are involved.

A200 may also take part in hydrogen bonding with the amine group of CA. However, no such interaction was observed in SDCAGA. Since A200 is dispersed in DCM it is not freely available for hydrogen bonding interactions. This is in accordance with the findings of Raghavan *et al*.[25]

Dissolution

Figure 5 shows in-vitro drug-release profiles of different formulations of CA. Pure CA was characterized by only 45% drug release within 150 min in 0.1 M HCl. Seventy per cent of the drug was released during the 150 min testing period for ACA. The improved drug dissolution could be attributed to the presence of an amorphous form of CA, as confirmed by DSC and XRPD studies (Figures 2 and 3, respectively). PMs showed a slight improvement in the dissolution rate but were still less than ACA. This was in accordance with the observations of saturation solubility (Table 1). The increase in dissolution of the drug in PMs is probably attributable to improved wetting and local solubilization of the drug by the excipients in the diffusion layer.[26] However, the improvement was not statistically significant ($P > 0.05$) for ACA and PMs, while both the SDs showed statistically significant improvement ($P < 0.05$) in dissolution compared to CA. Possible mechanisms for the increased dissolution rates of SDs include conversion of the CA to the amorphous state, as confirmed by DSC and XRPD studies (Figure 2 and 3, respectively), reduction of particle size, a solubilization effect of the carriers, absence of aggregation of drug particles, improved wettability and dispersibility of drug from the dispersion,[27,28] physical interaction of CA with carriers, such as hydrogen bonding, as confirmed by IR spectroscopy (Figure 4), dissolution of the drug in the hydrophilic carrier[16,29] and, finally, combinations of these effects.[30]

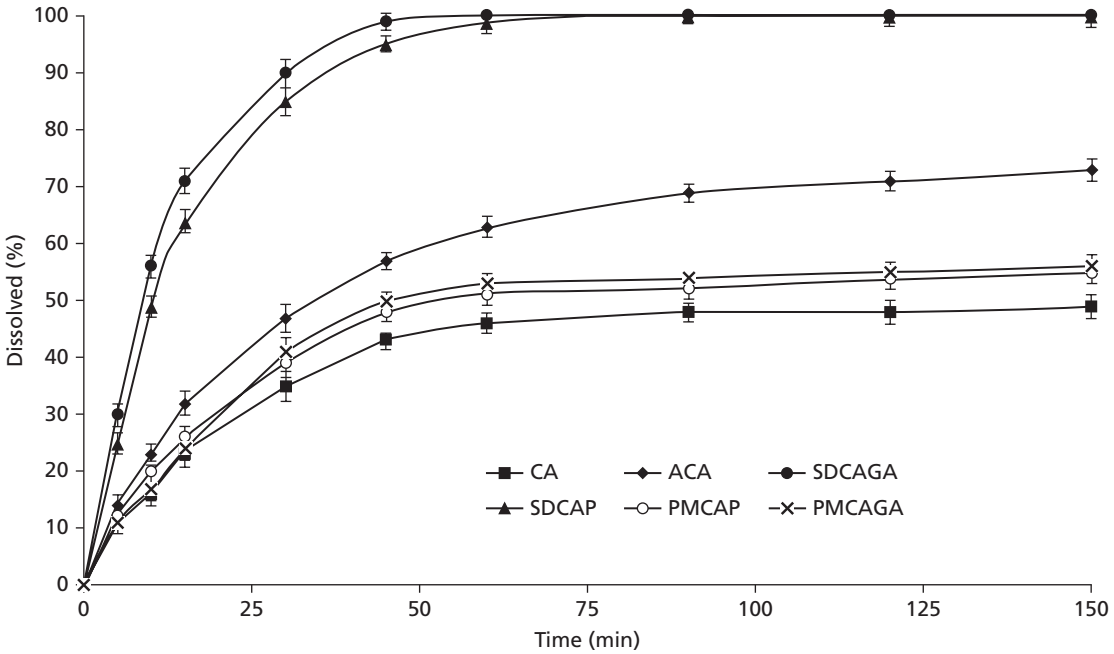

Figure 5 Dissolution profiles of cefuroxime axetil preparations

Stability studies

It is well known that amorphous drugs formulated in the form of SDs tend to recrystallize on storage. The identification of appropriate storage conditions for each amorphous formulation, using an accelerated stability study, is necessary to predict their long-term stability.[31] The temperature during storage has been reported to influence the rate of transformation of the amorphous to the crystalline form. The humidity during storage is also extremely important in view of the hygroscopic nature of hydrophilic polymers. Moreover, Gelucire tends to absorb moisture and undergo physical transformation at increased temperature and humidity. Absorbed moisture can act as a plasticizer and reduce the T_g of amorphous substances and lead to further instability.[32,33] For the present study, ambient temperature and relative humidity (30°C/60% RH) were selected. During stability, there was a gradual decrease in the dissolution rate of ACA over the period of 3 months. This can be explained on the basis of DSC and XRPD studies (Figure 6 and Figure 7, respectively). ACA showed gradual changes in the DSC thermograms and XRPD patterns, indicating the inability of amorphous CA to resist the recrystallization.

The DSC thermograms and XRPD patterns of both the SDCAP and SDCAGA after 1 month were identical, indicating the presence of the amorphous form of CA. The DSC thermogram of SDCAP showed a broad endotherm around 90–100°C, which can be attributed to the loss of absorbed moisture by the polymer during storage at higher relative humidity. Similarly, at the end of 3 months, DSC and XRPD observations of SDs were identical to those of fresh samples, indicating its physical stability. The amorphous nature of CA in SDs was further evidenced by there being no significant decrease in the dissolution rate over the period of 3 months compared to freshly prepared SDs. Thus, the improved stability of SDCAP could be due to hydrogen bonding between the drug and PVP, as evidenced by the DRIFT spectra (Figure 4), and the anti-plasticizing property of PVP, whereas SDCAGA showed improved stability owing to the hydrogen bonding between CA and G50 and adsorption on the surface of the silicon dioxide.

Bioavailability study

Even though results of in-vitro drug release and stability studies were promising for both of the SDs, the in-vivo performance of SDCAGA was expected to be better than SDCAP owing to its ability to protect the hydrolysis of the drug prior to absorption. To prove this hypothesis an in-vivo

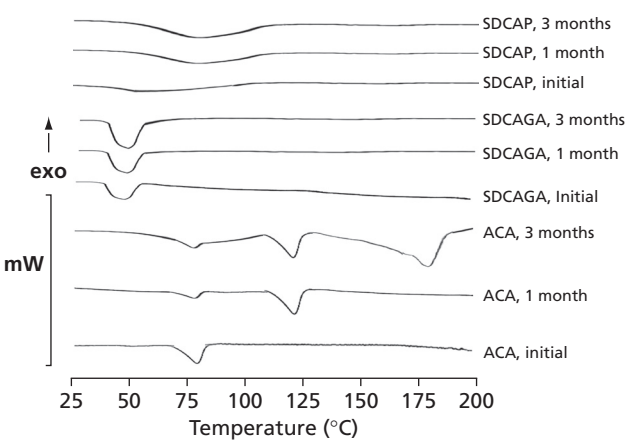

Figure 6 Differential scanning calorimetry thermograms of stability samples of cefuroxime axetil preparations

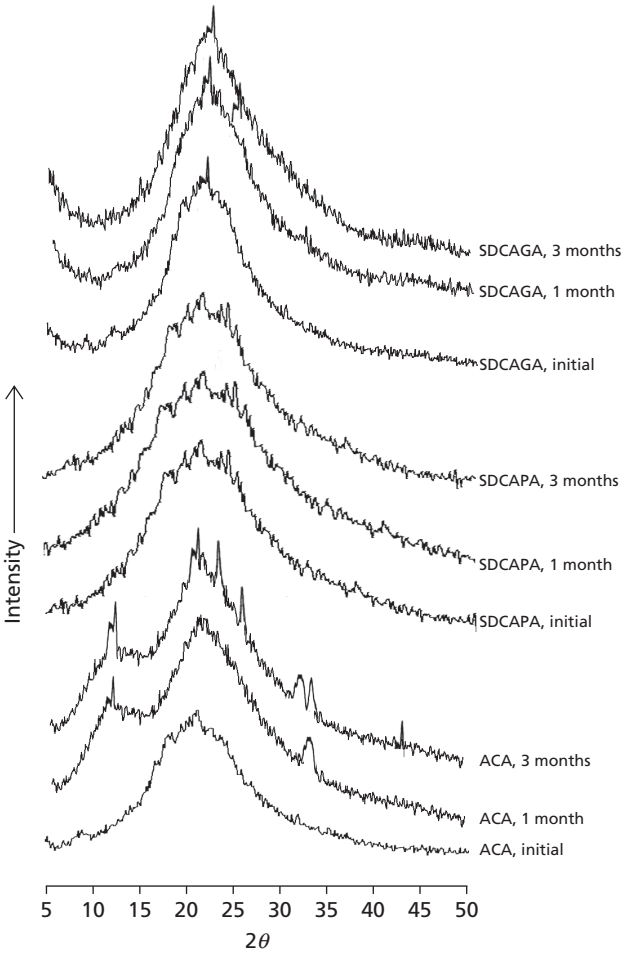

Figure 7 X-ray powder diffraction patterns of stability samples of cefuroxime axetil preparations

study was carried out in Wistar rats to quantify C after oral administration of CA (prodrug) formulations in suspension form. The HPLC method and the extraction process used were well validated. The calibration curves of CA covered a concentration range of 0–25 μg/ml. The ratio of CA to indapamide (internal standard) concentration was linear. The plasma profiles of C in adult Wistar rats following oral administration of CA, ACA, SDCAP and SDCAGA were compared. A summary of pharmacokinetic parameters and plasma concentration versus time curves is shown in Table 2. The plasma concentration profile of C for ACA was significantly greater ($P < 0.01$) than CA owing to the increased solubility of the substantially amorphous form over CA. This was in accordance with earlier reports. SDs showed significantly enhanced ($P < 0.01$) bioavailability as compared to crystalline CA and ACA because of its greater solubility and dissolution rate as observed during in-vitro studies. CA in its crystalline form is only slightly soluble in water and can form a thick gel on contact with an aqueous medium, indicating poor dissolution and bioavailability. By spray drying CA in a substantially amorphous form, increasing solubility and bioavailability was obtained. However, the amorphous form is unstable and undergoes recrystallization on storage for 3 months. The stable amorphous form in SD with PVP and Gelucire was obtained and had enhanced solubility. However, both the C_{max} and $AUC_{0-24\ h}$ values of SDCAGA were remarkably higher than those of the SDCAP, indicating a remarkable improvement in oral absorption of CA when administered with G50 in the form of an SD. Apart from poor aqueous solubility, hydrolysis of CA by intestinal esterase prior to absorption leads to formation of the unabsorbable form, C, thus reducing the bioavailability. The improvement in bioavailability can be explained by the fact that the esterase is now hydrolysing not only CA but also Gelucire. Gelucire therefore competes with CA for esterases, preventing the hydrolysis of CA to non-absorbable C prior to absorption. This is the reason for the enhanced bioavailability of SDCAGA over SDCAP in spite of their having comparable in-vitro solubility and dissolution. The absorption window for CA is located in the proximal part of the gastrointestinal tract (GIT) and any of the drug that misses this window is hydrolysed, is unabsorbed and is lost in the faeces. Immediate release of the drug in the stomach through use of SDs makes it available at the site of absorption, avoiding its passage to the distal segments of the GIT where it may be hydrolysed into non-absorbable C.

Conclusions

Stable amorphous SDs of the poorly water-soluble drug CA were successfully prepared by a spray-drying technique using PVP as well as G50. Free-flowing SDCAGA was obtained with the aid of A200 as an adsorbent. The stability of the amorphous state was attributed to hydrogen bonding between the drug and carriers as revealed by FT-IR spectroscopy. The amide functional group of the drug is involved in hydrogen bonding with the C=O group of PVP, whereas both the amide and C=O groups of the drug are involved in hydrogen bonding with the C=O and –OH of Gelucire. An in-vivo study in Wistar rats indicated the improvement in bioavailability in the following order SDCAGA > SDCAP > ACA > CA. The enhanced bioavailability of ACA was attributed to improved solubility owing to the amorphous nature of the

Table 2 Pharmacokinetic parameters of CA, ACA, SDCAP and SDCAGA after oral dose in Wistar rats

Pharmacokinetic parameter	CA	ACA	SDCAP	SDCAGA
C_{max} (μg/ml)	5.5 ± 1.03	7.1 ± 1.13	10.1 ± 1.56	11.9 ± 1.13
T_{max} (min)	90	60	45	45
$AUC_{0-24\ h}$ (μg h/ml)	65 ± 1.56	81 ± 2.21	125 ± 1.25	138 ± 1.78

Values are mean ± SD; $n = 6$.

drug. The enhanced bioavailability in SDs is attributed to the presence of the drug in amorphous form, improved wettability and dispersibility of drug from SD, the solubilization effect of the carriers and hydrogen bonding of CA with the carriers. The better bioavailability of solid dispersions prepared with Gelucire compared to those prepared with PVP, in spite of comparable solubility and dissolution profiles, may be due to the ability of Gelucire to prevent the hydrolysis of the prodrug (CA) to its non-absorbable base form (C). This promising technique should therefore be further exploited for enhancing the bioavailability of drugs that have poor aqueous solubility and enzymatic degradation.

Declarations

Conflict of interest

The authors declare that they have no conflicts of interest to declare.

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