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Kinetic measurements of the hydrolytic degradation of cefixime: effect of Captisol complexation and water-soluble polymers

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Abstract

We have taken kinetic measurements of the hydrolytic degradation of cefixime, and have studied the effect of Captisol complexation and water-soluble polymers on that degradation. The phase solubility of cefixime in Captisol was determined. Kinetic measurements were carried out as a function of pH and temperature. High-performance liquid chromatography (HPLC) was performed to assay all the samples of phase-solubility analysis and kinetic measurements. Chromatographic separation of the degradation products was also performed by HPLC. FT-IR spectroscopy was used to investigate the presence of any interaction between cefixime and Captisol and soluble polymer. The phase-solubility study showed A_L-type behaviour. The pH–rate profile of cefixime exhibited a U-shaped profile whilst the degradation of cefixime alone was markedly accelerated with elevated temperature. A strong stabilizing influence of the cefixime–Captisol complexation and hypromellose was observed against aqueous mediated degradation, as compared with povidone and macrogol. The unfavourable effect of povidone and macrogol may have been due to the steric hindrance, which prevented the guest molecule from entering the cyclodextrin cavity, whereas hypromellose did not produce any steric hindrance.

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Introduction

Cefixime, a third generation oral cephalosporin (Ziv et al 1995), is a chemically unstable, poorly water-soluble drug, effective against a wide spectrum of bacterial pathogens (Montay et al 1991; Markham & Brogden 1995). Cefixime trihydrate is the crystalline form of cefixime. Its antibacterial activity is dependent on the presence of β-lactam functionality, and cleavage at any point of this ring results in complete loss of antibacterial activity. The most easily understood drug instability is the loss of drug through hydrolysis or any other degradation pathways resulting in a reduction of potency. In cephalosporins, the cyclic amides or β-lactams undergo rapid ring opening due to hydrolysis and there have been many studies on this hydrolytic degradation (Yamana & Tsugi 1976; Tsugi et al 1981, 1983; Fabre et al 1984; Namiki et al 1987; Okamoto et al 1996a, b).

Cyclodextrins are cyclic oligosaccharides containing a varying number of glucopyranose rings. Cyclodextrins possess a lipophilic cavity and a hydrophilic exterior, thus making them ideal for forming inclusion complexes with lipophilic drugs in relatively polar solvents. Cyclodextrins have been shown to increase the solubility of sparingly soluble drugs by forming an inclusion complex (Stella & Rajewski 1997; Ni et al 2001; Al-Marzouqi et al 2006), and are utilized for preformulation studies (Jumaa et al 2001; Loftsson et al 2001; Nguyen et al 2001). Considerable interest has been generated in the use of cyclodextrins to improve chemical stability and bioavailability, and reduce side effects and toxicity of drugs (Uekama et al 1981, 2001; Hirayama et al 1987; Cwiertnia et al 1999; Nagarsenker et al 2000; Kang et al 2002; Jumaa et al 2004; Perry et al 2006a, b; Mallick et al 2007a, b). Due to their unique chemical structure (a doughnut-like shape) cyclodextrins are capable of forming so-called 'inclusion' complexes with many drug molecules. The formation of an inclusion complex ordinarily by non-covalent bonds can lead to either stabilization of the drug or catalysis of its breakdown, depending on the nature of reaction and the orientation of the molecule within the cavity. If drug D is capable of undergoing chemical

degradation in solution, protection of the molecule by inclusion complexation with cyclodextrins is often possible (Figure 1). Uekama et al (1981) showed that, to a large extent, the addition of cyclodextrins stabilized the methyl ester of prostacyclin from its hydrolytic degradation in aqueous solution. The differential stabilizing effect of α-, β- and γ-cyclodextrins has been explained by the fact that ionization of the terminal carboxylic acid of prostacyclin inhibits complex formation. Dimethyl β-cyclodextrin shows a greater stabilizing effect on prostacyclin than does trimethyl β-cyclodextrin (Hirayama et al 1987). Prostaglandin E_1 in neutral and alkaline solution is destabilized by β-cyclodextrin but stabilized by carboxymethyl ethyl β-cyclodextrin (Adachi et al 1992). Cyclodextrins and their derivatives may enhance drug degradation, depending on the reaction mechanisms and the steric arrangement of the drug in the complex. For example, β-cyclodextrin inhibits the alkaline hydrolysis of benzocaine (Lach & Chin 1964) but enhances that of aspirin (Chin 1968). Drug–cyclodextrin complexations can reduce decomposition of drug by protecting the labile region from the potential reactants in the aqueous environment (Loftsson & Brewster 1996). The process of solubilization and stabilization of cephalosporin antibiotics by inclusion complexation using cyclodextrins was described by Kishinchand & Manilal (2005). Usuda et al (2000) have also shown that the presence of cyclodextrin slowed the rate of alkaline hydrolysis of artemisinin. Among the various cyclodextrins, sulfobutyl ether β-cyclodextrin has shown low toxicity in cell culture (Saarinen-Savolainen et al 1998) and in drug formulation (Rajewski et al 1995; Totterman et al 1997). Maffeo et al (2006) studied the positive effect of natural and negatively-charged cyclodextrins on the stabilization of β-lactam antibiotics towards β-lactamase degradation and found that the rate of hydrolysis of ampicillin was reduced to half in the presence of β-lactamase along with octakis(6-oxycarbonylethylthio-6-deoxy)-γ-cyclodextrin.

Parent cyclodextrins (α- and β-forms) are not suitable for systemic formulations because of renal toxicity, but nephrotic damage is not observed with sulfobutyl ether β-cyclodextrin (Thompson 1997).

We have investigated the kinetic measurements of cefixime degradation in aqueous solution as a function of pH and temperature. The effect of Captisol complexation and water-soluble polymers on cefixime degradation was examined. In this method, the formation of inclusion complex between cefixime and Captisol was assessed using phase-solubility techniques. The influence of water-soluble polymer such as hypromellose (hydroxypropyl methylcellulose) on stability/hydrolysis kinetics of substances has been studied by several authors (Maggi et al 2003; Werawatganone & Wurster 2007). Loftsson et al (1994) reported the use of water-soluble polymers to increase the cyclodextrin–drug complexation and improve the stabilizing effect. Solubility of the drug could be increased by incorporating solid drug in Captisol solution (20–40%) containing water soluble polymers such as povidone (polyvinylpyrrolidone), carmellose (carboxymethylcellulose) or hypromellose (0.1–2%) (Information package 2003, CyDex Inc.). This study has also described the influence of water-soluble polymers such as hypromellose, povidone and macrogol on hydrolysis of cefixime. The changes in enthalpy (ΔH), entropy (ΔS) and free energy (ΔG) were then determined from a standard thermodynamic equation (van't Hoff equation). FT-IR spectra of the binary system and ternary system of cefixime, Captisol and polymer were obtained to investigate the type of interaction. The number of major decomposition products on the accelerated condition was determined by HPLC analysis.

Materials and Methods

Materials

Cefixime trihydrate ($C_{16}H_{21}N_5O_8S_2$; MW 507.5) was generously provided by Orchid Chemicals and Pharmaceuticals Ltd, Chennai, India. Captisol, a brand of sulfobutyl ether β-cyclodextrin sodium, an amorphous white solid (average molecular weight 2163, based on average degree of substitution of 6.5) was kindly provided by CyDex, Inc. (Overland Park, KS). Hypromellose E5 (Methocel E5), povidone (K30) and macrogol 4000 (polyethylene glycol 4000) were purchased from Scientific Traders, Balasore, India. All other solvents used were HPLC grade.

Phase-solubility analysis

Phase-solubility measurements were performed according to Higuchi & Connors (1965). Excess amounts of cefixime were added to 10 mL aqueous solutions containing various concentrations of Captisol (0–92 mM). The suspensions were sonicated for 10 min and were shaken in a water bath at 28°C for 6 h to minimize degradation. Samples were withdrawn with a syringe equipped with a Millipore membrane (0.45 μm) filter.

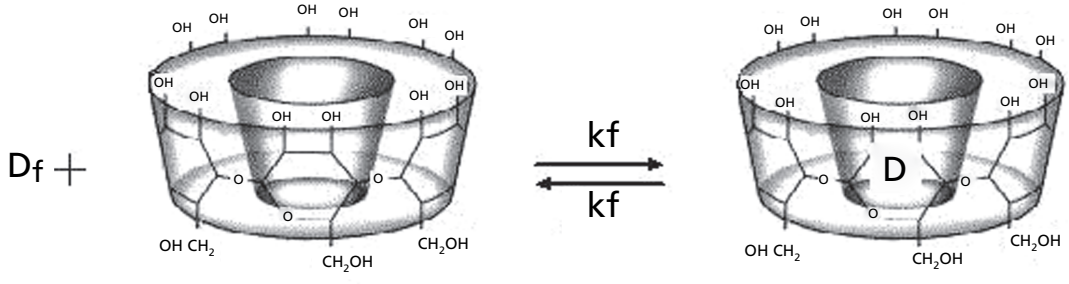

Figure 1 The interaction of free drug molecules (D_f) with the cavity of cyclodextrins. The binding constant, $K = k_f/k_r$ (k_f = forward rate constant and k_r = reverse rate constant).

All samples were diluted in 60% aqueous acetonitrile to within the range 0.04–6.0 μg mL^{-1} cefixime for analysis by HPLC.

HPLC

Assay of all the samples from the phase-solubility analysis and the chromatographic separation of degradation products were performed by HPLC using a described and validated process (Tsuji et al 1979; Rolando et al 2001). The HPLC system consisted of a Waters 1515 Isocratic HPLC pump, a Rheodyne injector and a Waters 2487 Dual (λ) absorbance detector. A reverse-phase column (Zorbax, L1-C18, 4.6 × 75 mm, 3.5 μm) was used in the HPLC analysis.

Mobile phase preparation

To prepare the mobile phase for the analysis of cefixime the following reagent solutions were prepared. Phosphoric acid solution (1.5 M) was prepared by adding 6.8 mL phosphoric acid to 300 mL distilled water, the pH was adjusted to 7.0 with 10 M NaOH and the volume was made up to 1000 mL with distilled water. To prepare the tetrabutylammonium hydroxide solution, 25 mL 0.4 M was added to an adequate amount of distilled water, the pH was adjusted to 7.0 with 1.5 M phosphoric acid and the volume was made up to 1000 mL with distilled water. The mobile phase consisted of acetonitrile and tetrabutylammonium hydroxide solution in a 260:90 v/v ratio and detection was made at 254 nm. The HPLC analysis was carried out at ambient temperature and with a flow rate of 1.0 mL min^{-1}. All the samples were analysed for drug content against freshly prepared standard samples and the calibration curve was linear in the range of 0.04–6.0 μg mL^{-1}. Assay validation was performed for inter-day variability and on-system stability by the use of quality control replicates. Analysis of decomposition products of cefixime solutions stored at 353 K for 5 h and at an alkaline pH of 9.2 and an acidic pH of 2.3 at laboratory ambient temperature for 24 h was performed by the HPLC method.

Kinetic measurements

Preliminary studies indicated that degradation of cefixime in 60% (v/v) aqueous acetonitrile at or below 40°C was not detected for at least 48 h. Therefore, samples of the degradation reaction were quenched by the addition of acetonitrile (to make 60% v/v) and then stored in a refrigerator until analysis by HPLC within 48 h unless otherwise specified.

pH–rate profile

Cefixime alone was weighed and dissolved in aqueous solutions of various pH values (1.45–11). The initial cefixime concentration in the solution was varied (10–20 mg/500 mL). To assess the kinetics of degradation as a function of pH in aqueous solution, the flasks containing the drug were placed in the dark at the ambient temperature of the laboratory. Apparent first-order rate constants for the degradation of cefixime were determined at various pH values ranging from 1.45 to 11, using HCl, phosphate buffers (0.1 M) and NaOH, for pH values 1.45–2.30, 4.50–8.20 and 9.20–11.00, respectively. All pH measurements were made with a digital pH meter (Systronics). After definite time intervals the respective samples were withdrawn and a volume of acetonitrile (to make 60% v/v) was added before storage in a refrigerator until analysis. The samples were analysed by HPLC within 24 h of sampling. In the pH range 4.50–8.20, a minimum of three buffer concentrations for each buffer were used to enable appropriate extrapolation to zero buffer concentration to examine the possibility of buffer catalysis. The rate of hydrolytic decomposition of cefixime was determined from the plot of the logarithm of remaining drug concentration vs time. To prepare a comparative study of cefixime degradation in aqueous solution as a function of pH, the logarithm of the rate of hydrolysis was plotted vs pH. The corresponding time to reach 50 and 10% degradation of cefixime (t50 and t90, respectively) was calculated from the slope of the pH–kinetic curves.

Temperature–rate profile

Cefixime was weighed and dissolved in glass-distilled water (pH 6.40) and in hypromellose, povidone and macrogol aqueous solutions (1% w/v). Cefixime–Captisol complex (1:1 molar ratio) was weighed and dissolved in glass-distilled water and in hypromellose solution (1% w/v). The initial cefixime concentration in each solution was varied (10–20 mg/500 mL). To assess the kinetics of degradation of cefixime in aqueous solution as a function of temperature, the flask containing the studied substance was placed in an incubator previously adjusted to 303, 333, 343 or 353 K. After definite time intervals the respective samples were withdrawn and the drug content was analysed by HPLC. To determine the relative rate of hydrolytic decomposition of cefixime, the % logarithm of remaining drug concentration (% ln c) was plotted vs time. The linearity of the dependence was investigated using linear regression. The t50 and t90 of the hydrolysis were calculated from the slope of the kinetic curves.

Determination of free energy of transfer and stability constant

The equation for the free energy of transfer of cefixime from aqueous solution to the cavity of sulfobutyl ether β-cyclodextrin (Captisol) was derived from the thermodynamic relationship for the chemical potential of a solute according to the analysis of Nozaki & Tranford (1963), Gekko et al (1998) and Porras et al (2003). Due to the low solubility of the drug in water, the standard free energy of transfer, Δ_{trans} G°, the free energy of transfer of the solute (cefixime) from water to Captisol solution (or cyclodextrin cavity) could be approximated as (Al-Marzouqi et al 2006):

$$\Delta_{trans} G^\circ = -RT\ln(S/S_0) \quad (1)$$

where S_0 and S are the solubility of cefixime in the absence and presence of cyclodextrin, respectively. The apparent stability constant (K_s) was calculated according to Higuchi & Connors (1965) from the solubility diagram of cefixime using the following equation:

$$K_S = \text{slope}/(S_0(1-\text{slope})) \quad (2)$$

Where, slope = slope of the phase solubility diagram and S_0 = the intrinsic solubility of cefixime in water in absence of Captisol, which was determined independently. The correct S_0 value was an important factor for reliable calculation of the apparent stability constant. It is important to note that the sincerely measured S_0 value in absence of Captisol was used for the calculation of apparent stability constant instead of using the intercept obtained from the least-squares analysis.

Preparation of the cefixime–Captisol inclusion complex without and with water-soluble polymers (kneading method)

The inclusion complex of cefixime (MW 507.5 g mol^{-1}) with Captisol (MW 2163 g mol^{-1}), a brand of sulfobutyl ether β-cyclodextrin sodium, was prepared by a kneading method (1:1 molar ratio). Captisol (85 mg) was wetted with methanol in an agate mortar and kneaded to form a paste. Cefixime (20 mg) and methanol were added. The sample was kneaded for approximately 60 min and vacuum dried to constant mass at ambient temperature.

Similarly dispersions of cefixime–Captisol complex and polymer were prepared with the water-soluble polymers (drug:polymer 1:1, w/w) by the kneading method to examine the interaction using FT-IR study.

FT-IR

The FT-IR spectra were obtained using a type IR necolate instrument (Model: JASCO FT-IR 410). The prepared samples of binary and ternary systems of cefixime, Captisol and polymer were previously ground and mixed thoroughly with KBr and disks were formed by compressing the powder. The scans were executed at resolution of 2 cm^{-1} from 4000 to 400 cm^{-1} and accumulated more than 50.

Statistical methods

The objective of the study was to evaluate the relevance of differences in the degradation profiles of cefixime alone and in the presence of Captisol and/or different hydrophilic polymer variants. The degradation rate constant at different temperatures was tested by two-way analysis of variance. For cases of significant differences, analysis of variance was followed by post-hoc test for pair-wise comparisons. The level of significance was $P < 0.05$.

Results and Discussion

Phase solubility and free energy of transfer

Phase solubility of cefixime in Captisol was constructed by plotting the evaluated equilibrium concentrations of cefixime against concentration of Captisol. Aqueous solubility of cefixime was linearly increased as a function of Captisol concentration with a slope of 0.0154 ($r^2 = 0.9853$) over the concentration range investigated, suggesting the formation of an A_L-type inclusion complex. Since the slope of the phase solubility line was smaller than 1, the formation of 1:1 cefixime–Captisol complex was suggested in an aqueous solution of Captisol (Ma et al 2000; Liu & Zhu 2006). In an aqueous solution of cefixime–Captisol complex, the free drug molecules are in equilibrium with the drug molecules entrapped in the cavity. Thus, on increasing the concentration of Captisol more cefixime molecules will transfer from the aqueous solution to the hydrophobic cavity of Captisol. Therefore, it can be expected that more cefixime will dissolve in water in the presence of Captisol than in the absence of Captisol and consequently, the aqueous solubility of cefixime increases with increasing concentration of Captisol in the concentration range of 0–92 mM cefixime.

The free energies of transfer (Δ_{trans} G°) of cefixime from aqueous solution to the cavity of sulfobutyl ether β-cyclodextrin (Captisol) have been calculated from equation 1. As shown in Table 1, Δ_{trans} G° values were negative and increased negatively with increasing Captisol concentration. Negative values of Δ_{trans} G° indicated that Captisol was a more favourable environment than water for cefixime. The percentage increase in the solubility of cefixime and corresponding free energy of transfer of cefixime from aqueous solution to the lipophilic cavity of Captisol in the presence of an excess amount of cefixime at 28°C have been shown in Table 1. The stability constant (K_s) for cefixime in aqueous solution of Captisol calculated from equation 2 was $11.5 \pm 1.04\ M^{-1}$.

Decomposition products

The numbers of decomposition products of cefixime in aqueous solution stored at 353 K for 5 h, at alkaline pH 9.2 for 24 h, and at acidic pH 2.30 for 24 h were determined by HPLC analysis. The retention time (RT) of pure cefixime was 2.3 min. The chromatogram of aqueous samples stored at 353 K revealed the presence of three major degradation products, retention times being 0.9, 1.1 and 1.5 min. The chromatogram of aqueous samples stored at alkaline pH 9.2 suggested the presence of three major decomposition products (RT = 0.9, 1.5 and 1.7 min). At acidic pH 2.3 there were three major degradation products (RT = 0.9, 1.1 and 1.5 min). The maximum contributions of two decomposition products were common in all the three conditions (RT 0.9 and 1.5 min). The peak attributed to cefixime at 2.3 min was observed to disappear almost completely under accelerated conditions.

Table 1 Increase in solubility and free energy of transfer of cefixime from aqueous solution to the cavity of sulfobutyl ether β-cyclodextrin (Captisol) in the presence of an excess amount of cefixime at 28°C

Captisol (mM)	Solubility of cefixime (mM)	Increase in solubility of cefixime (%)	$-\Delta_{trans}$ G° (cal mol^{-1})
0	1.36 ± 0.087	–	–
5.75	1.52 ± 0.078	11.76 ± 0.735	65.8 ± 4.064
11.50	1.59 ± 0.158	16.91 ± 1.595	92.5 ± 8.843
23.00	1.87 ± 0.043	37.50 ± 1.930	188.5 ± 8.316
46.00	2.22 ± 0.153	63.24 ± 4.130	290.1 ± 19.601
92.00	2.80 ± 0.017	105.88 ± 0.612	427.5 ± 8.397

Values are mean ± s.d., n = 3.

pH–rate profile

Since the β-lactam ring of cefixime is known to be sensitive to hydrolytic degradation (Namiki et al 1987), a wide range of pH values (1.45–11) was chosen on purpose to cover acid, neutral and alkaline conditions, and to allow comparison among these conditions. The susceptibility of cefixime to hydrolytic decomposition was determined as a decrease of the concentration of cefixime during the time course of the experiment. When a reaction dependent on hydronium and hydroxide ion activity is performed at a particular pH, it usually follows pseudo-first-order kinetics, which can be described by first-order rate constant k_{obs}. A reaction in which hydronium and hydroxide ion, and water catalysis are observed can be described by:

$$k_{obs} = k_{H^+}a_{H^+} + k_{H_2O} + k_{OH^-}a_{OH^-} \quad (3)$$

where k_{obs} is the sum of specific rate constants and activity for each parallel pathway. k_{H^+}, k_{OH^-} and k_{H_2O} are hydronium ion-, hydroxide ion- and H_2O-catalysed rate constants, respectively, whilst a_{H^+} and a_{OH^-} are the activity of hydronium and hydroxide ion, respectively. Comparative study of cefixime degradation in aqueous solution as a function of pH from 1.45 to 11 is depicted in Table 2. The pH rate profile of cefixime exhibited a U-shaped profile. It indicated that all the terms of equation 3 contributed significantly, and water catalysis could compete with hydronium ion and hydroxide ion catalysis. The hydrolysis of cephalothin, cephaloridine and cefotaxime were the three reported examples of cephalosporin which exhibited U-shaped pH–rate profiles (Yamana & Tsugi 1976; Berge et al 1983; Fabre et al 1984). Although these drugs have an ionizable carboxylic group at the 4-position, the apparent pH–rate profile was U-shaped because there was no major difference in degradation rate between the ionized and un-ionized forms of these drugs, and the same was also true for cefixime. The kinetic rate and corresponding time to 50 and 90% of degradation of cefixime (t50 and t90, respectively) as a function of pH are shown in Table 2. t50 indicates the half-life of the drug, whereas t90 or shelf-life for a drug at 'use' condition is the time period the product will remain satisfactory, assigning the expiration date. Relatively fast degradation of cefixime was observed in the alkaline solution rather than the acidic solution. The rate of hydrolysis was slow at pH 6.4 ($k = 0.3 \times 10^{-3}$ h^{-1}). Relatively fast degradation was observed at alkaline solution ($k = 9.1 \times 10^{-3}$ h^{-1} at pH 11) compared with acidic solution at pH 1.45 ($k = 1.0 \times 10^{-3}$ h^{-1}).

Table 2 Time (h) to reach t50 and t90 for the degradation of cefixime in aqueous solution as a function of pH

pH	k[a]	t50	t90
1.45	1.0 ± 0.080	693.0	104.0
2.30	0.6 ± 0.018	1155.0	173.3
4.50	0.4 ± 0.010	1732.5	260.0
6.40	0.3 ± 0.012	2310.0	346.7
8.20	0.7 ± 0.011	990.0	148.5
9.20	1.1 ± 0.062	630.0	94.5
11.00	9.1 ± 0.102	76.1	11.4

Values are mean ± s.d., n = 3. [a] $\times 10^{-3}$ h^{-1}. t50 indicates half-life, whereas t90, or shelf-life, is assigned for expiration date.

Temperature–rate profile

The apparent first-order rate constant for degradation of cefixime in aqueous solution was studied as a function of temperature. The rate of degradation of cefixime alone was markedly accelerated with elevated temperature (Figure 2). The speed of reaction increased approximately two times with each 10°C rise in temperature. The effects of Captisol complexation and polymer such as hypromellose, povidone and macrogol on aqueous degradation rate were also examined at 303, 333, 343 and 353 K. Figure 3 depicts the effects on degradation at 353 K. Influence of Captisol complexation and hypromellose on degradation rate of cefixime and corresponding t50 and t90 of degradation as a function of temperature

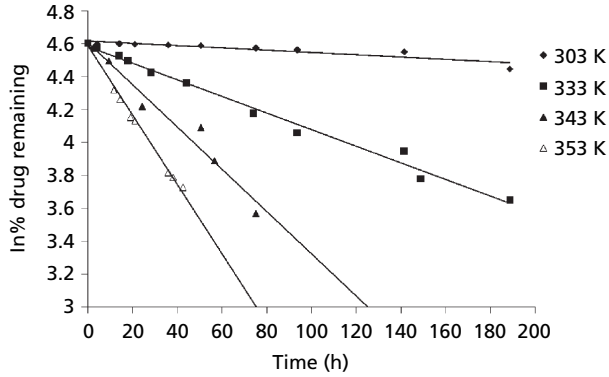

Figure 2 Effect of temperature on hydrolytic degradation of cefixime in aqueous solution. The solid lines represent best fit using linear functions.

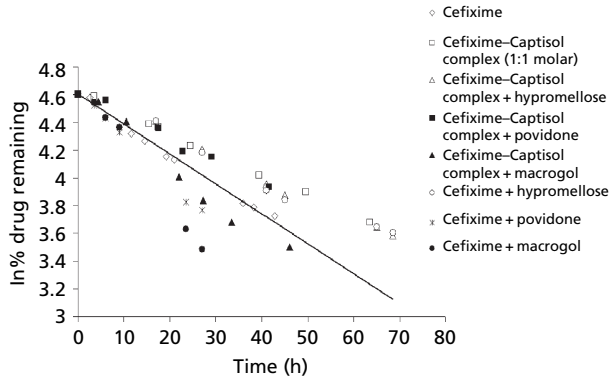

Figure 3 Effect of Captisol-complexation and polymers on degradation of cefixime at 353 K. The solid line represents the degradation profile of cefixime alone. The points above the solid line indicate stabilizing influence and points below the line indicate destabilization.

are shown in Table 3. The results indicated a strong stabilizing influence exerted by Captisol (drug complex in a molar ratio 1:1) and hypromellose, and combination of Captisol complex and hypromellose. The degradation rate of cefixime alone at 353 K was significantly increased when compared with the value when complexed with Captisol and/or in the presence of hypromellose at 353 K. Povidone and macrogol did not show any stabilizing effect against aqueous-mediated degradation. In contrast, a greater degradation of cefixime was observed in solutions containing povidone and macrogol. The degradation rate of cefixime in the presence of Captisol (1:1 molar complex) and hypromellose also increased approximately twofold with each 10°C rise in temperature. Since temperature is one of the primary factors affecting drug stability, van't Hoff developed and described the effect of temperature on rate processes as:

$$\ln k = \ln A - \frac{\Delta H}{RT} \quad (4)$$

$$\ln K = \frac{\Delta S}{R} - \frac{\Delta H}{RT} \quad (5)$$

$$\Delta G = \Delta H - T\Delta S \quad (6)$$

Where, A = coefficient of frequency, ΔH = enthalpy change, ΔS = entropy change, ΔG = free energy change, R = universal gas constant, T = Absolute temperature in K. The plot of lnK vs 1/T has been traditionally used to describe the temperature dependency for various equilibrium processes (Mitra & Mikkelson 1988; Akaho & Fukumori 2001; Perry et al 2006b; Mallick et al 2007b). The effect of temperature on the equilibrium rate constants was obtained by using the van't Hoff equation. The rate constants were employed for the assessment of thermodynamic parameters, in the presence and absence of hypromellose. Various thermodynamic parameters were calculated (Table 4). The rate constants were independent of drug concentration. The large positive value of ΔH (14.48 kcal mol^{-1}) indicated that the degradation of cefixime proceeded as the temperature was elevated. This statement was supported by the increasing value of degradation constants with increased incubation temperature. ΔH and ΔS decreased in the presence of Captisol and hypromellose, indicating that the cefixime was protected from its degradation in aqueous solution. ΔG decreased with the rise in temperature, which meant more and more cefixime molecules were being degraded. ΔG increased in the presence of Captisol and hypromellose indicating that the drug molecules were being degraded to a lesser extent in aqueous solution.

FT-IR spectroscopy

FT-IR spectroscopy was used to investigate the presence of any interaction between cefixime and sulfobutyl ether β-cyclodextrin (Captisol) and soluble polymer. FT-IR spectra of cefixime (Figure 4) showed strong absorbance bands for carbonyl stretching at 1770, 1669, 1592 and

Table 3 Effect of Captisol and hypromellose on the degradation rate constant of cefixime in aqueous solution as a function of temperature

Temperature (K)	Cefixime alone			Cefixime–Captisol inclusion complex			Cefixime with hypromellose			Cefixime–Captisol complex in hypromellose		
	k[a]	t50	t90	k[a]	t50	t90	k[a]	t50	t90	k[a]	t50	t90
303	0.66 ± 0.02	1050	157.6	0.44 ± 0.01	1575	236.4	0.59 ± 0.03	1332.7	176.3	0.52 ± 0.01	1174.6	200
333	5.1 ± 0.15	135.9	20.4	3.5 ± 0.16	198	29.7	4.1 ± 0.21	169	25.4	3.9 ± 0.31	177.7	26.7
343	12.9 ± 0.44	53.7	8.0	9.6 ± 0.41	72.2	10.8	9.1 ± 0.41	76.2	11.4	8.8 ± 0.39	78.7	11.8
353	21.1 ± 0.58	32.8	4.9	14.8 ± 0.20	46.8	7.0	15.2 ± 0.65	45.6	6.8	15.3 ± 0.71	45.3	6.8

Values are mean ± s.d., n = 3. [a] × 10^{-3} h^{-1}. t50 and t90 are the corresponding time in h to 50% (half-life) and 10% (shelf-life) of degradation, respectively.

Table 4 Effect of Captisol and hypromellose on the thermodynamic parameters for the degradation of cefixime in aqueous solution

	Slope	lnA	r^2	ΔH (kcal mol^{-1})	ΔS (e.u.)	ΔG (kcal mol^{-1})			
						303 K	333 K	343 K	353 K
Cefixime	−7286 ± 1472	16.64 ± 3.63	0.9951	14.48 ± 3.11	33.1 ± 3.01	4.48 ± 0.98	3.49 ± 0.59	3.16 ± 0.33	2.83 ± 0.01
Cefixime with hypromellose	−6555 ± 1148	14.22 ± 5.01	0.9976	13.03 ± 4.81	28.3 ± 2.35	4.67 ± 1.55	3.63 ± 0.81	3.35 ± 0.15	3.07 ± 0.02
Cefixime–Captisol inclusion complex	−7215 ± 1022	16.07 ± 2.59	0.9924	14.34 ± 2.08	31.9 ± 2.89	4.49 ± 0.91	3.72 ± 0.09	3.40 ± 0.29	3.08 ± 0.09
Cefixime–Captisol inclusion complex with hypromellose	−6838 ± 1601	15.01 ± 6.21	0.9983	13.59 ± 5.10	29.8 ± 4.02	4.56 ± 0.28	3.66 ± 0.39	3.37 ± 0.07	3.07 ± 0.07

Values are mean ± s.d., n = 3. e.u., entropy units.

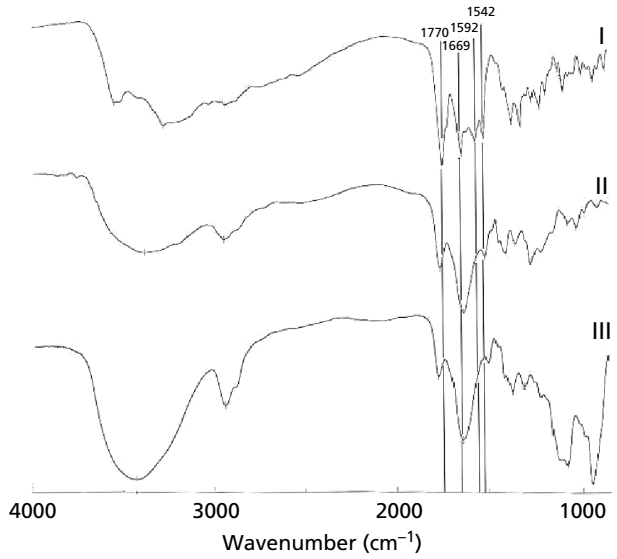

Figure 4 FT-IR spectra of the binary system and ternary system of cefixime, Captisol and polymer to investigate the type of interaction: cefixime (I), cefixime–povidone solid dispersion (1:1, w/w) (II), cefixime–Captisol inclusion complex (1:1 molar) and povidone dispersion (1:1 w/w, drug–polymer) (III).

1542 cm^{-1}. The absorption band at 1770 cm^{-1} was due to C = O stretching vibration of the β-lactam ring of cefixime and it was slightly shifted to 1779 cm^{-1} in both the binary and ternary systems with Captisol and polymer. The absorption band observed at 1669 cm^{-1} was assigned to Ar-CONH and was shifted to 1657 and 1653 cm^{-1} in the solid dispersion systems of cefixime–povidone and cefixime–Captisol–povidone, respectively. Other bands, such as 1592 cm^{-1} suffered, probably due to the influence of overlapping with cyclodextrin/povidone in the same zone. The band at 1542 cm^{-1} shifted to a lower wave number, to 1538 cm^{-1} in the binary system of cefixime–povidone and still shifted to 1535 cm^{-1} in the ternary system of cefixime–Captisol–povidone. This result implied that intermolecular hydrogen bonding occurred in the crystalline drug, whereas in the solid dispersion the binding was spread out. The C = O functional group of cefixime might have interacted with the functional group as, for example, hydroxyl (OH) group of povidone and sulfobutyl ether β-cyclodextrin at the molecular level in the solid dispersion. The solid dispersion system of nifedipine and hypromellose (Sujimoto et al 1982; Suzuki & Sunada 1998) also improved the dissolution of nifedipine that contained such moieties. This interaction between cefixime, Captisol and hypromellose markedly protected cefixime in the higher energy amorphous state. The stabilization of amorphous drugs has been reported for the systems of excipients with povidone and povidone–covinyl acetate and drugs with hydrogen donor groups such as indometacin, lacidipine, nifedipine and tolbutamide (Forster et al 2001). Miyazaki et al (2004) suggested that the stronger stabilizing effect of carbomer (polyacrylic acid) was due to the stronger interaction with paracetamol in solid dispersions.

Conclusions

The aqueous degradation of cefixime appeared to be pH and temperature dependent. Since Δ_{trans} G° of cefixime increased negatively with increasing Captisol concentration (complex formed in 1:1 molar ratio), Captisol created a more favourable environment than water for cefixime in its (Captisol) increased concentration. The rate of hydrolysis of cefixime alone was slow at pH 6.4, slightly faster at acidic pH (pH 2.3 and 1.45) and was quite rapid at alkaline pH (pH 9.2 and 11). Although cefixime has an ionizable carboxylic group at the 4-position, the apparent pH–rate profile was found to be U-shaped because there was no major difference in degradation rate between the ionized and un-ionized forms of the drug. The rate of cefixime alone was markedly accelerated with elevated temperature. A strong stabilizing influence of the cefixime–Captisol complexation was observed against aqueous-mediated degradation. To a lesser extent hypromellose alone and in combination with Captisol drug complex also played a role in the stabilization of cefixime. The degradation of cefixime was slightly accelerated in solutions containing povidone and macrogol. The unfavourable effect of povidone and macrogol may have been due to the steric hindrance of the polymers (povidone and macrogol), which prevented the guest molecule from entering the cyclodextrin cavity, whereas hypromellose did not produced any steric hindrance. ΔH and ΔS decreased, whilst ΔG increased in the presence of Captisol and hypromellose, indicating that cefixime molecules had been protected to some extent from degradation in aqueous solution.

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