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Influence of pH and Temperature on Kinetics of Ceftiofur Degradation in Aqueous Solutions

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Abstract

The objective of this study was to evaluate the stability of ceftiofur (1 mg mL^{-1}) in aqueous solutions at various pH (1, 3, 5, 7·4 and 10) and temperature (0, 8, 25, 37 and 60°C) conditions. The ionic strength of all these solutions was maintained at 0·5 M. Ceftiofur solutions at pH 5 and 7·4 and in distilled water (pH = 6·8) were tested at all the above temperatures. All other solutions were tested at 60°C. Over a period of 84 h, the stability was evaluated by quantifying ceftiofur and its degradation product, desfuroylceftiofur, in the incubation solutions. HPLC was used to analyse these compounds.

At 60°C, the rate of degradation was significantly higher at pH 7·4 compared with pH 1, 3, 5 and distilled water. At both 60°C and 25°C, degradation in pH 10 buffer was rapid, with no detectable ceftiofur levels present at the end of 10 min incubation. Degradation rate constants of ceftiofur were $0{\cdot}79 \pm 0{\cdot}21$, $0{\cdot}61 \pm 0{\cdot}03$, $0{\cdot}44 \pm 0{\cdot}05$, $1{\cdot}27 \pm 0{\cdot}04$ and $0{\cdot}39 \pm 0{\cdot}01$ day^{-1} at pH 1, 3, 5, 7·4 and in distilled water, respectively. Formation of desfuroylceftiofur was the highest (65%) at pH 10. The rate of degradation increased in all aqueous solutions with an increase in the incubation temperature. At pH 7·4 the degradation rate constants were $0{\cdot}06 \pm 0{\cdot}01$, $0{\cdot}06 \pm 0{\cdot}01$, $0{\cdot}65 \pm 0{\cdot}17$, and $1{\cdot}27 \pm 0{\cdot}05$ day^{-1} at 0, 8, 25, 37 and 67°C, respectively. The energy of activation for ceftiofur degradation was 25, 42 and 28 kcal mol^{-1} at pH 5, 7·4 and in distilled water, respectively.

Desfurylceftiofur formation was the greatest at alkaline pH compared with acidic pH. Ceftiofur degradation accelerated the most at pH 7·4 and was most rapid at pH 10. The results of this study are consistent with rapid clearance of ceftiofur at physiological pH.

Ceftiofur (Figure 1), a third generation cephalosporin antibiotic, is currently being administered as an intramuscular injection for the treatment of certain respiratory diseases in beef cattle, dairy cattle, swine and day-old chicks, and to treat interdigital dermatitis in cattle. Ceftiofur is active against 515 bacterial isolates of pigs with various diseases from the USA, Canada, and Denmark (Salmon et al 1995). It is active against both Gram-positive and Gram-negative bacteria including beta-lactamase-producing bacterial strains (Ritter et al 1996). The bulky imino-methoxy side chain of ceftiofur is thought to be responsible for its broad spectrum of activity (Jaglan et al 1989). Ceftiofur is rapidly eliminated with an elimination half-life of 217 min in cattle (Soback et al 1989) and 381 min in sheep (Craigmill et al 1991). Ceftiofur is metabolized to desfuroylceftiofur and furoic acid in rats and cattle (Jaglan et al 1989). Desfuroylceftiofur is present as the only metabolite in the plasma of rats and cattle. Ceftiofur and its metabolite, desfuroylceftiofur, are equally effective in the control of most bacterial pathogens in-vitro (Salmon et al 1996). The minimum inhibitory concentration (MIC) of ceftiofur for several organisms is 2·0 $\mu g\ mL^{-1}$ or less (Brown et al 1991; Salmon et al 1995).

In cattle, infections of the bones and joints of the foot usually result from extension of the infection from interdigital dermatitis or infected sole or hoof wall lesions (Trent & Plumb 1991). For this reason, mixed infections with a wide variety of organisms are very common (Firth et al 1987; Smith et al 1989; Trent & Plumb 1991). Ceftiofur is active

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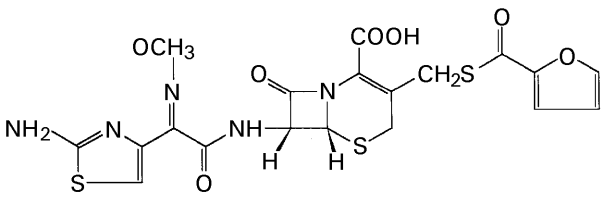
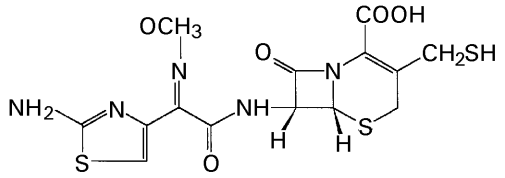
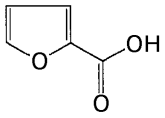


Ceftiofur



Desfuroylceftiofur



Furoic acid

Figure 1. Structures of ceftiofur and degradation products.

against many of the organisms commonly cultured from such infections (Brown et al 1991).

Ceftiofur has some structural resemblance to cefotaxime. Cefotaxime differs from ceftiofur as the 3-methyl dehydrothiazine ring has an acetate group in cefatoxime rather than the thiofurate group in ceftiofur. Desacetyl cefatoxime is the major hydrolysis product of cefotaxime (Fabre et al 1984). In acidic medium, desacetyl cefotaxime is converted to the corresponding lactone. Hydrolysis of ceftiofur sodium is complex; it is readily hydrolysed to desfuroylceftiofur and is further converted into more complex products (Koshy & Cazers 1997) such as the dimer of desfuroylceftiofur, corresponding thiolactone and various other forms. Many experiments such as pharmacokinetic and protein binding studies require a range of temperature and pH conditions in the handling and processing of biological samples. It was reported by earlier groups that the aqueous stability of cefotaxime is low, when compared with many other cephalosporins (Fabre et al 1984), despite its excellent biological activity and high resistance to beta-lactamases. Structural similarity of ceftiofur to cefotaxime prompted us to investigate the influence of temperature and pH conditions on the aqueous stability of ceftiofur. In this study, the influences of pH and temperature on ceftiofur degradation were assessed using a high-performance liquid chromatography (HPLC) assay. The findings of this study are useful in designing and interpreting the pharmacokinetics and pharmacodynamics of ceftiofur. The findings are also useful in developing solution dosage forms of ceftiofur.

Materials and Methods

Materials

Ceftiofur sodium (Naxcel), obtained as a sterile powder from Pharmacia & Upjohn Company (Kalamazoo, MI), was used as received. HPLC-grade acetonitrile and glacial acetic acid were obtained from Fischer Scientific (Fair Lawn, NJ). Potassium chloride, hydrochloric acid, sodium acetate, acetic acid, sodium dihydrogen ortho phosphate, disodium hydrogen phosphate, sodium hydroxide and citric acid, all of analytical reagent grade, were obtained from Sigma Chemical Company (St Louis, MO).

Solutions

In this study, HCl–KCl (pH 1), citric acid–Na_2HPO_4 (pH 3), acetate (pH 5), phosphate (pH 7·4) and borate (pH 10) buffers were used. The compositions of these solutions were: HCl–KCl buffer – 25 mL of 0·2 M KCl, 67 mL of 0·2 M HCl diluted to 100 mL with water; citric acid–Na_2HPO_4 buffer – 79·45 mL of 0·1 M citric acid and 20·55 mL of 0·2 M Na_2HPO_4; acetate buffer – 70 mL of 0·2 M sodium acetate and 30 mL of 0·2 M acetic acid; phosphate buffer – 40·5 mL of 0·2 M Na_2HPO_4 and 9·5 mL of NaH_2PO_4 diluted to 100 mL with water; borate buffer – 50 mL of a mixture 0·1 M with respect to both KCl and H_3BO_3 (7·455 g KCl and 6·184 g H_3BO_3) and 43·7 mL of 0·1 M NaOH, diluted to 100 mL with water. The ionic strength of all buffers was adjusted to 0·5 M by the addition of sodium chloride. The pH measurements were made using a Corning pH meter (Model 320). The pH meter was standardized at the temperature of study using standardized certified pH 4·0 and 7·0 buffer solutions (Fisher Scientific, Fair Lawn, NJ).

Drug analysis

The concentration of ceftiofur remaining in incubation solutions was monitored using a reversed-phase HPLC method. The HPLC system (Shimadzu) included a solvent delivery pump (LC-6A), an autoinjector (SIL-6B), a data processor (Chro-

matopac CR-501) and a UV detector (SPD-6A) set at 254 nm. A 25-cm long Microsorb C-18 column (Rainin Instruments, Emeryville, CA) with a particle diameter of 5 μm and a pore size of 100 Å was used in this study. The mobile phase consisted of 1% (v/v) glacial acetic acid, 69% (v/v) water, 30% (v/v) acetonitrile and 100 mg of heptane sulphonic acid per litre of the mobile phase. The mobile phase was delivered at a constant flow rate of 1 mL min^{-1}. The injection volume was maintained at 30 μL for all the samples. Under these conditions, the detection limits for ceftiofur and desfuroylceftiofur were 10 ng and 30 ng, respectively. The retention time of ceftiofur and desfuroylceftiofur were 14 min and 5·7 min, respectively. Ceftiofur loss was monitored as a function of time, as was the appearance of desfuroylceftiofur. Standard graphs were generated on the day of sample analysis and the relative standard deviation of the inter-day assay variation was observed to be 4%.

Preparation of desfuroylceftiofur

Desfuroylceftiofur was prepared by the hydrolysis of ceftiofur sodium in saturated potassium chloride solution under controlled experimental conditions according to the method of Koshy & Cazers (1997). Briefly, 50 mL of saturated KCl containing 0·5 g tetrasodium edetate and 0·5 g sodium bisulphite was de-aerated by sonication and cooled to 4°C. To this preparation, 1 g of sodium ceftiofur was added and dispersed by sonication. This mixture was cooled to 0°C while stirring and 3 mL of cold de-aerated 22·5% (w/v) KOH solution containing 0·5% (w/v) tetrasodium edetate was added in a drop-wise manner. This preparation was incubated for 1 h at 0°C under a nitrogen atmosphere. The pH of the hydrolysate was then adjusted to 2·5 with cold acetic acid (20% (v/v)). The precipitate formed was further washed with acetic acid and de-aerated water, resuspended in de-aerated water, lyophilized and purified by HPLC. The UV spectrum obtained for desfuroylceftiofur thus prepared was similar to the one reported by Tyczkowska et al (1993).

Stability studies

Kinetics of ceftiofur degradation were determined in aqueous buffer solutions at a constant ionic strength of 0·5 M at 60 ± 1°C. For each data time point, quadruplicate measurements were performed using various buffers (pH 1, 3, 5, 7·4, 10) and distilled water. In this study, all ceftiofur solutions were protected from light by covering the vials with aluminium foil.

A stock solution of ceftiofur (5 mg mL^{-1}) was prepared in distilled water and each stability study was initiated by adding 1 mL of stock solution to 4 mL of an appropriate buffer already adjusted to the reaction temperature, so as to produce an initial drug concentration of 1 mg mL^{-1}. Stock solutions and buffer solutions were sonicated before the start of the experiment. The solution was vortexed and an initial sample of 100 μL was withdrawn. Subsequently, 100-μL samples were removed from the reaction vessel at appropriate time intervals and immediately frozen at −4°C to quench the reaction. Before injection onto the HPLC, samples were diluted 20 times with the respective buffer. The temperature dependence of ceftiofur stability was conducted at 0, 8, 25, 37 and 60°C at pH 5 and 7·4 and in distilled water. Based on the time-course of ceftiofur levels under various conditions, the degradation rate constants were determined.

To determine activation energies for the degradation of ceftiofur under various conditions, plots of log k versus 1/T were constructed and their slopes determined. The slope of the Arrhenius plot (m) was related to the energy of activation as per the expression, $m = -Ea/2{\cdot}303R$.

The results in this study are expressed as mean ± s.d., unless otherwise stated. Statistical significance of the difference between means was evaluated using an unpaired Student's *t*-test. $P < 0{\cdot}05$ was considered statistically significant.

Results and Discussion

Effect of pH on ceftiofur degradation at 60°C

To determine the stable pH region for ceftiofur in aqueous solutions, the stability of ceftiofur was determined under various pH conditions at 60°C. HPLC analysis of ceftiofur degradation products showed, in each case, the formation of an early eluting peak, at ~5·7 min. The peak eluting at 5·7 min was collected and characterized for UV spectrum and it was similar to the spectrum previously reported (Tyczkowska et al 1993).

The pH rate profiles (percent ceftiofur remaining vs time) were plotted on a semi-logarithmic scale (Figure 1). The rate constants at 60°C, as a function of pH, are shown in Table 1. It was observed that ceftiofur is extremely unstable at pH <5 and pH >6·8. This could be due to the cleavage of thioester bonds and formation of desfuroylceftiofur, which can further degrade into various other complex products. Ceftiofur was relatively stable at pH 5·0 for all the conditions tested and the stability was equally good in distilled water (pH 6·8). From

Table 1. Effect of various pH conditions on the rate constant for ceftiofur degradation at 60°C.

pH	[b]Rate constant (10^{-2} day^{-1})
1	78·87 ± 2·09
3	61·07 ± 2·62
5	43·90 ± 4·56
7·4	126·85 ± 4·11
[a]Distilled water	38·63 ± 1·32

[a]pH = 6·8, ionic strength was not maintained. [b]Data are expressed as mean ± s.d. for n = 4.

Table 2. Rate constants and activation energies for ceftiofur degradation.

	pH	Temp (°C)	[c]K_{pH} (10^{-2} day^{-1})	Ea (kcal mol^{-1})
[a]Distilled water	6·8	0	3·14 ± 0·34	28
		8	5·98 ± 0·23	
		25	6·24 ± 0·22	
		37	9·96 ± 2·67	
		60	38·64 ± 1·44	
[b]Citric acid–NaH_2PO_4	5·0	0	7·02 ± 0·47	25
		8	7·02 ± 0·27	
		25	22·05 ± 0·78	
		37	24·61 ± 6·60	
		60	43·69 ± 1·63	
[b]Phosphate buffer	7·4	0	6·45 ± 0·70	42
		8	6·41 ± 0·25	
		25	39·61 ± 1·40	
		37	64·61 ± 17·32	
		60	127·04 ± 4·73	

[a]pH = 6·8, ionic strength was not maintained. [b]0·5 M ionic strength was maintained. [c]Data is expressed as mean ± s.d. for n = 3.

Table 2, it can be seen that the rate constant (day^{-1}) at pH 7.4 is approximately 3-fold that at pH 5 ($P < 0·05$) and 2-fold that at pH 3 ($P < 0·05$), which explains the instability of ceftiofur in biological fluids at pH 7·4. Also, these results are consistent with the low plasma levels of ceftiofur in the sheep following intravenous administration (Craigmill et al 1991). Low plasma levels of ceftiofur may be attributed not only to extensive metabolism by the enzymatic systems but also to its high rate of degradation at physiological pH.

The formation profile of desfuroylceftiofur at different pH conditions was plotted as a function of time at 60°C (Figure 2). It can be observed that the formation of desfuroylceftiofur is most rapid at pH 10. The ceftiofur peak was not observed at or beyond 6 h at pH 10. At 6 h, desfuroylceftiofur accounted for about 65% and the remaining 35% can possibly be accounted by other hydrolytic and oxidative products as described by previous reports

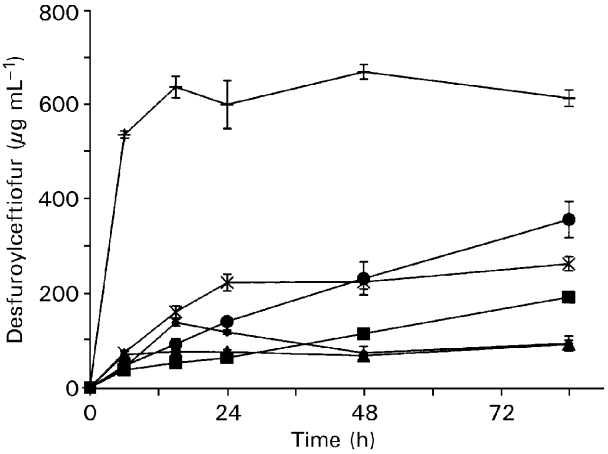


Figure 2. Formation of desfuroylceftiofur at 60°C as a function of pH. ● pH 1, ■ pH 3, ▲ pH 5, × pH 7·4, pH 10, ◆ distilled water (pH 6·8). Mean ± s.d. (n = 4)

(Koshy & Cazers 1997). Additional studies indicated that ceftiofur disappeared completely at the end of 10 min incubation at pH 10. After 24 h, the conversion of ceftiofur to desfuroylceftiofur at 60°C and pH 7·4 was 22·5%, whereas it was 12% in distilled water (pH 6·8), 8% at pH 5·0, 7% at pH 3 and 14% at pH 1. Thus, compared with conditions of acidic pH, alkaline pH is more likely to induce the formation of desfuroylceftiofur. The percentage of ceftiofur remaining after 24 h at 60°C and pH 7·4 was 27·2%, whereas it was 76·2% in distilled water and 64·7%, 41·7% and 40·1% at pH 5, 3 and 1, respectively. Considering this, a relatively stable pH region (pH 5 and distilled water) was selected and then subjected to various temperature conditions to investigate the stability of the compound under the regular sample storage conditions and experimental conditions (pH 7·4) in which pharmacokinetic, protein binding and cellular uptake studies are conducted.

The above findings are illustrated by the typical chromatograms shown in Figure 3. The parent compound and the degradation products are shown at the end of 24 h incubation at 60°C. At pH 10, the ceftiofur peak was absent. At pH 10, as well as at pH 7·4, desfuroylceftiofur was seen as a major product. At lower pH values (pH 3 and pH 5) multiple degradation peaks including desfuroylceftiofur at 5·7 min were observed. The peak at 4·5 min is postulated to be desfuroylceftiofur thiolactone.

Effect of temperature on ceftiofur degradation

The effect of temperature on ceftiofur stability was studied with buffer solutions at pH 5 and 7·4 and in

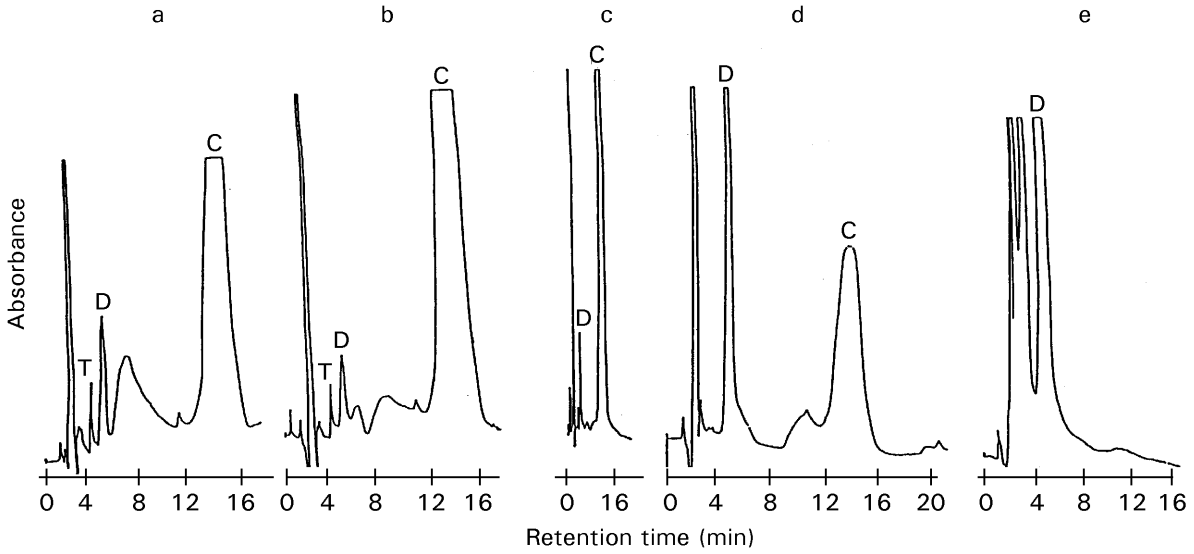


Figure 3. HPLC chromatograms showing the degradative products of ceftiofur after 24 h incubation at 60°C, a pH 3·0, b pH 5·0, c pH 6·8, d pH 7·4 and e pH 10. Peak C is ceftiofur, D is desfuroylceftiofur and T is postulated to be desfuroylceftiofur thiolactone.

distilled water, by measuring the degradation rates at 0°C, 8°C, 25°C, 37°C and 60°C. The temperature–time course profiles of ceftiofur at different pH conditions are shown in Figure 4. These plots are semi-logarithmic plots between percentage remaining versus time, as a function of temperature, at different pH conditions. The values of rate constants at different temperatures and the Arrhenius activation parameter (kcal mol^{-1}) estimated from the Arrhenius plot are given in Table 2. The degradation of ceftiofur was temperature dependent and an increase in the temperature resulted in an increase in the rate of degradation at all pH conditions tested. The rate of degradation was significantly higher at pH 7·4 when compared with distilled water at a temperature of 25°C or above (Table 1). Furthermore, the degradation rate constant of ceftiofur at 37°C and pH 7·4 was 2·62-fold that at pH 5 and 6·5-fold that in distilled water (Table 2). This high degradation rate at pH 7·4 is consistent with the instability of ceftiofur in biological fluids (Tyczkowska et al 1993). A major metabolite of ceftiofur, desfuroylceftiofur, eluted before ceftiofur on a reversed-phase HPLC column (Figure 3) indicating that the metabolite is more hydrophilic than the parent compound. Such polar compounds are more likely to undergo rapid renal clearance. Thus, low plasma levels of ceftiofur following intravenous administration (Craigmill et al 1991) may be attributed in part to chemical hydrolysis of ceftiofur to desfuroylceftiofur.

Activation energies determined are interpreted by transition state theory. According to this theory, the reactant molecules are in equilibrium with an activated complex of these molecules. For a molecule to degrade, a bimolecular reaction must occur whereby the reactant molecule in its activated state is able to degrade. The frequency factor (A) indicates the frequency with which an activated complex goes to its decomposition product. The activation energy can be used as a measure of the transition state. It was assumed that for ceftiofur to degrade, a transition state requiring some activation energy for formation is required. The observed activation energies for ceftiofur degradation are consistent with solvolysis and are comparable with those observed for other cephalosporins (Fabre et al 1984).

The observed rate constants for 1 mg mL^{-1} sodium ceftiofur reconstituted in distilled water (pH 6·8) and in buffers (pH 5 and 7·4) were similar at 8°C (Table 2). These results indicate that the rate constants are independent of pH in this range. Also, the rate constant was independent of the ionic strength (0·5 M for the buffers and ionic strength was not maintained for the product reconstituted in distilled water). The maximum stability for a solution occurred in the pH range 5·0–6·8. The buffer used at pH 5 did not accelerate ceftiofur hydrolysis.

For the purpose of this study, shelf-life was defined as the time during which ceftiofur con-

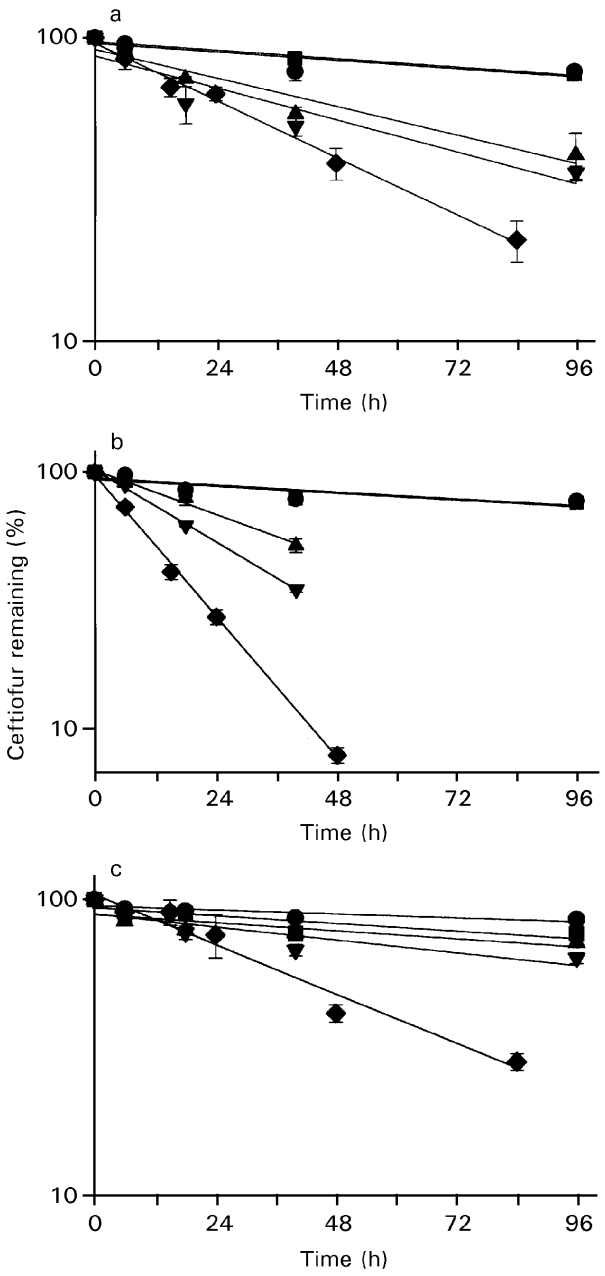


Figure 4. Effect of temperature on percent ceftiofur remaining in incubation solution at different time points. Ceftiofur was incubated in buffers maintained at pH 5 (a), pH 7·4 (b) or distilled water (c, pH 6·8). Initial concentration of ceftiofur in the incubation mixture was 1 mg mL^{-1}. ● 0°C, ■ 8°C, ▲ 25°C, ▼ 37°C, ◆ 60°C. Mean ± s.d. (n = 3).

centration equalled or exceeded 90% of its initial concentration (T_{90}). Based on the observation that ceftiofur is most stable in the pH range 5·0–6·8, shelf-life predictions were made at pH 5 using the expression, $T_{90} = 0{\cdot}105/k$. The predicted shelf-life for sodium ceftiofur in distilled water is 1·05 (37°C), 1·68 (25°C) and 1·75 (8°C) days and in pH 5 buffer, it is 0·42 (37°C), 0·48 (25°C) and 1·5 (8°C) days. The corresponding shelf-lives at pH 7·4 were 0·16, 0·27 and 1·64 days, respectively.

Conclusions

Ceftiofur rapidly degraded in aqueous solutions. Desfuroylceftiofur was identified as a major degradation product. Formation of desfuroylceftiofur as well as ceftiofur degradation was most rapid at pH 10. With an increase in pH, the degradation process was most accelerated at pH 7·4. The decomposition was defined by a first-order mechanism. The reaction rate increased with increasing temperature. The linearity of Arrhenius plots indicates that there was no change in the reaction mechanism within the temperature range studied.

Acknowledgements

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