Stability and Blood Level Determinations of Cefaclor, a New Oral Cephalosporin Antibiotic

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Cefaclor solutions in pH 2.5 and 4.5 buffers contained at least 90% of their initial activity after 72 h at 4°C. Samples in pH 6.0, 7.0, and 8.0 buffers contained 70, 46, and 34%, respectively, of their initial activity after 72 h at 4°C. After 72 h at 25°C, samples prepared with pH 2.5, 4.5, 6.0, 7.0, and 8.0 buffers contained 95, 69, 16, 5, and 3%, respectively, of their initial activity. After 72 h at 37°C, cefaclor solutions in pH 2.5 buffer contained 80% of the initial activity, whereas samples prepared in pH 4.5, 6.0, 7.0, and 8.0 buffers contained less than 20%. Laboratory-prepared plasma and serum samples showed an 8% loss in activity when incubated for 6 h at 4°C, a 51% loss when incubated for 6 h at 25°C, and a 48% loss when incubated for 2 h at 37°C. Clinical samples demonstrated a similar stability pattern. Degradation rates for cefaclor in commercially prepared serum increased from 4- to 10-fold in comparison to rates obtained when samples were made in human serum freshly prepared in our laboratory. Consequently, serum standards should be made in freshly prepared human serum.

Cefaclor, 3-chloro-7-d-β-(phenylglycaminamido)-3-cephem-4-carboxylic acid, is an orally effective, broad-spectrum cephalosporin derivative that has been shown by Sullivan et al. (3) to be quantitatively absorbed as the intact antibiotic from the gastrointestinal tract of mice, rats, and dogs. Cefaclor is structurally related to other cephalosporins, in particular cefaloxin, but possesses stability characteristics different from those of cephalaxin. This study describes the stability characteristics of cefaclor and outlines procedures for the proper handling and evaluation of clinical samples.

MATERIALS AND METHODS

Stability of cefaclor in buffer. The stability of cefaclor in the following 0.1 M potassium phosphate buffers was examined: pH 2.5, 4.5, 6.0, 7.5, and 8.0. Samples were prepared at 1 mg of cefaclor activity per ml in the appropriate buffer and incubated at 37, 25, and 4°C. Samples were assayed at 0, 24, 48, and 72 h intervals by removing an appropriate amount of the dilution, 100-fold with 0.1 M pH 4.5 potassium phosphate buffer, and assaying by the AUTOTURB System (2) against a standard curve prepared in 0.1 M pH 4.5 potassium phosphate buffer at concentrations of 2.0, 5.0, 10.0, 20.0, and 40.0 μg of cefaclor per ml. *Staphylococcus aureus ATCC 9144 was used as the test organism.

In vitro stability of cefaclor in plasma and serum. Samples were prepared at 10 μg of cefaclor activity per ml in either pooled human serum obtained commercially or serum and plasma obtained from the same healthy volunteers. The fluids were tested before use to ensure that they were devoid of antimicrobial activity. The pH was adjusted to 7.45 by the addition of 2 N HCl. Samples were incubated at 4, 25, and 37°C, and the pH was maintained at 7.45 by overlaying the sample with an atmosphere of 95% O2-5% CO2. A 1-ml sample was removed at appropriate intervals up to 6 h, diluted 100-fold with pH 4.5 buffer, stored at 4°C, and assayed immediately after the 6-h sample was drawn. After each sampling period, the reaction vessel was flushed for 2 min with the 95% O2-5% CO2 gas mixture.

Samples were assayed by a modification of the microbiological agar diffusion assay described by Kavanagh and Dennin (1). Plastic petri plates (100 by 15 mm) contained a 10-ml layer of Antibiotic Medium no. 1 (Difco). The agar was seeded with a 0.2-ml suspension of a 1:10 dilution of *Sarcina lutea* ATCC 9341 (adjusted to a 30% light transmission at 530 nm with a Spectronic 20 spectrophotometer) for each 100 ml of agar. Standards for the assay were derived by volumetric dilution from a freshly prepared pH 4.5 buffer stock solution containing 1,000 μg of cefaclor activity per ml. Intermediate and final dilutions were made in pH 4.5 buffer containing 1% human serum or plasma. Standards containing 0.025, 0.05, 0.1, or 0.2 μg of cefaclor activity per ml were applied at a dosage of 0.1 ml/cylinder. Plates were incubated at 30°C for 16 to 18 h, and zones of inhibition were read to the nearest 0.1 mm with a Fisher-Lilly antibiotic zone reader.

Effect of sample handling on blood concentrations. In an attempt to relate the stability observed in laboratory-prepared samples to the handling of
clinical blood samples, 500 mg of cefaclor was given to a healthy volunteer and blood samples were collected at time intervals of 0, 10, 20, 30, 45, 60, 90, 120, and 180 min after the administration of the drug. Samples were collected in heparinized tubes and immediately centrifuged in a refrigerated centrifuge (4°C) at 1,000 x g for 10 min to separate the plasma from cellular fractions. Each sample was then divided, and one part was held at 4°C and the other part was held at 25°C for 4 h before assay.

Samples were assayed by a disk plate agar diffusion procedure. Plastic petri plates (150 by 15 mm) contained a 45-ml layer of Antibiotic Medium no. 1 inoculated with S. lutea ATCC 9341 as described earlier. Standards for the tests were prepared by volumetric dilution from a freshly prepared pH 4.5 buffer stock solution containing 1,000 µg of cefaclor activity per ml. The standards were diluted in control human plasma to contain 0.16, 0.63, 2.5, 10.0, and 20.0 µg of cefaclor activity per ml. The standard solutions were applied to each disk (6.35-mm, α-cellulose disk, BBL) by dipping the disk in the standard solution until fully saturated. Plates were incubated and zone diameters were read as described earlier.

RESULTS

Temperature and pH stability. After storage for 72 h at 4°C, cefaclor solutions in pH 2.5 buffer contained 95% of their initial activity, in pH 4.5 buffer contained 93%, in pH 6.0 buffer contained 71%, in pH 7.0 buffer contained 46%, and in pH 8.0 buffer contained 34% (Fig. 1A). Loss of activity was accelerated by storage at 25°C (Fig. 1B). After 72 h, samples prepared with pH 2.5, 4.5, 6.0, 7.0, and 8.0 buffers contained 95, 69, 16, 5, and 3%, respectively, of their initial activity. After 72 h at 37°C (Fig. 1C), cefaclor solutions in pH 2.5 buffer contained 80% of the initial activity, whereas samples in pH 4.5 and 6.0 buffers contained 15 and 5%, respectively. pH 7.0 and 8.0 solutions contained only 3% of the initial activity after 24 h.

In vitro stability of cefaclor in plasma and serum. The effect of temperature on the stability of cefaclor in freshly prepared human serum and plasma is shown in Fig. 2. No difference in stability was observed between serum and plasma samples. Consequently, the regression line represents an average of the serum and plasma data at each time interval for five separate determinations. Plasma and serum samples showed an 8% loss in activity when incubated for 6 h at 4°C, a 51% loss when

![Fig. 1. Stability of cefaclor in pH 2.5, 4.5, 6.0, 7.0, and 8.0 0.1 M potassium phosphate buffers at (A) 4°C, (B) 25°C, and (C) 37°C.](http://aac.asm.org/content/1/1/50.f2)

![Fig. 2. In vitro stability of cefaclor in freshly prepared human serum and plasma at 4, 25, and 37°C.](http://aac.asm.org/content/1/1/50.f3)
incubated for 6 h at 25°C, and a 48% loss when incubated for 2 h at 37°C.

A dramatically different stability pattern was observed when cefaclor was added to commercially prepared pooled human serum. The loss of activity as the percentage of the initial activity at 4, 25, and 37°C is shown in Fig. 3A. Losses of approximately 15% per h at 4°C, 45% per h at 25°C, and 68% per h at 37°C were observed.

Effect of sample handling on apparent blood concentrations. In view of the effect that temperature and pH had on the stability of laboratory-prepared samples of cefaclor, the effects of storage temperature on the recovery of cefaclor from clinical blood samples were examined. As indicated above, all samples were prepared as plasma, primarily because of the time involved in preparing serum from whole blood. Results of the study involving the storage of samples at room temperature and 4°C are shown in Table 1. Those samples that were kept at 4°C throughout the test procedure and assayed 4 h after they were drawn gave values that were 20 to 40% higher than those values obtained from the same samples held at room temperature and assayed within the same time period.

DISCUSSION

Results obtained from pH studies showed cefaclor to be markedly more stable under acid conditions than under alkaline conditions. Temperature influenced the stability of cefaclor, and increased stability was achieved at lower temperatures. Such a stability pattern indicated that stock standards should be prepared fresh daily in pH 4.5 buffer.

As would be predicted from the pH buffer stability data, cefaclor was unstable in vitro in human serum and plasma. Over 50% of the initial antimicrobial activity was lost when prepared serum and plasma samples were held at 25°C for 6 h (Fig. 2). Conversely, only 5 to 10% of the activity was lost when the same samples were held at 4°C for the same time period (Fig. 2). At 37°C, the degradation was even greater, and approximately 50% of the initial activity was lost in 2 h.

Similarly, clinical samples stored at room

<table>
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<th>Time sampled (min)</th>
<th>Samples held at room temp for 4 h before assay</th>
<th>Samples held at 4°C for 4 h before assay</th>
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<tr>
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* S. lutea disk plate assay.

![Fig. 3. In vitro stability of cefaclor at 4, 25, and 37°C in (A) commercially prepared human serum and (B) freshly prepared human serum.](image-url)
temperature for 4 h before assay gave results that averaged 35% lower than results from the same samples held at 4°C for 4 h. These findings are in close agreement with the laboratory-prepared samples discussed earlier, which demonstrated a 30 to 40% loss in activity after 4 h at 25°C.

Of additional interest was the large difference in stability observed when samples were prepared in two different lots of human serum obtained commercially. Degradation rates increased from 4- to 10-fold in comparison to rates obtained when samples were made in human serum freshly prepared in our laboratory. Storage, pH, and handling conditions were the same as those used for freshly prepared serum, and no reason for the difference could be determined. However, such a difference in serum stability is of concern since standard curves prepared in serum obtained commercially would give erroneously high assay values for blood samples. Human sera used for cefaclor assays should be prepared fresh from healthy volunteers as required.

In summary, of those conditions tested, cefaclor is most stable when in the acid pH range at a temperature of 4°C. For analytical work, cefaclor stock solutions should be prepared fresh daily in pH 4.5 buffer. When dealing with biological fluids, such as plasma or serum, which by nature do not possess this desired acidic pH, the optimum stability of samples can be obtained by keeping the samples cold at 4°C and assaying them on the same day they are collected. Samples that are to be assayed at a later time should be stored at −70°C.

If serum is desired, clotting and serum preparation should be performed at 4°C. In addition, serum standards should be made in freshly prepared human serum and not in serum obtained commercially.

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LITERATURE CITED