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# Influence of Temperature on Degradation Kinetics of Ceftriaxone in Diluted and Undiluted Human Serum

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**The stability of ceftriaxone in undiluted human serum and in human serum diluted 1/20 in 0.1 M phosphate buffer (pH 6.0) was measured at −70, −40, −20, 4, and 37°C. Ceftriaxone in diluted human serum contained at least 90% of the initial activity after 3 months of storage at −20, −40, and −70°C; unbuffered human serum contained 82.41, 84.92, and 88.96% of the initial activity, respectively. Ceftriaxone in unbuffered human serum and in diluted human serum showed 80.33 and 86.25% of the initial activity, respectively, after 55 days at 4°C. After 120 h at 37°C, this antibiotic in unbuffered human serum contained 33.08% of the initial activity, whereas samples of diluted human serum contained more than 60%. Consequently, the stability of antibiotic in human serum diluted 1/20 in 0.1 M phosphate buffer (pH 6.0) is increased.**

Ceftriaxone (Ro 13-9904), a 2-aminothiazoyl-methoxyimino semisynthetic cephalosporin, has been shown to have good in vitro and in vivo activities against many genera of bacteria (7). What distinguishes ceftriaxone from the other newer cephalosporins is its unusually long plasma half-life, which is 4 to 10 times longer than those of other cephalosporins (9). A simple method for buffering human serum at about pH 6.0 was developed by using 0.1 M phosphate buffer. This dilution method is useful when the samples, from clinical studies, cannot be shipped to the analyst after their collection or cannot be evaluated immediately. This study describes the stability characteristics of ceftriaxone sodium in undiluted human serum and in human serum diluted 1/20 in 0.1 M phosphate buffer (pH 6.0).

Ceftriaxone standard powder was kindly provided by Roche Products, S.A. We used batch 012037 with a potency of 820 μg as free acid per mg. Serum from healthy human subjects not receiving antibiotics was pooled. The final pH was 7.4. The fluid was tested before use to ensure that it was devoid of antimicrobial activity. The pooled human serum was diluted 1/20 in 0.1 M phosphate buffer (pH 6.0; 1.69 g of $Na_2HPO_4$ per liter, 11.69 g of $KH_2PO_4$ per liter). This dilution buffers the serum pH at about 5.9 to 6.1. Samples were prepared with 50 μg of ceftriaxone sodium per ml in both pooled human serum and diluted human serum. Of each sample, 0.5 ml was withdrawn and placed in a sterile glass vial (2 ml). All solutions were prepared in a laminar-flow hood by using aseptic techniques and then were incubated at 37 and 4°C for 120 h and 55 days, respectively, and at −20, −40, and −70°C for 3 months. Samples were removed at appropriate intervals and assayed after being thawed for 2 h at room temperature when they were conserved at freezing temperatures or after several minutes when they were stored at 37 and 4°C. After sampling, the specimens were discarded. The concentration of the antibiotic was determined, in triplicate, by a disk plate assay procedure using *Escherichia coli* SQ 12155 as the assay microorganism when we evaluated human serum samples and *Bacillus subtilis* ATCC 6633 spores to determine the stability of antibiotic in human serum diluted 1/20, because *B. subtillis* ATCC 6633 is serum susceptible and gives an inhibition zone in undiluted serum but not in diluted serum. Suspensions of spores were diluted 1/10 with sterile Ringer solution.

Standard curves were prepared by using appropriate concentrations of drug in the same fluids as those used for drug solutions stored under the assay. Five working dilutions of standard solution were made, containing 10, 7, 5, 3, and 2 μg/ml when *B. subtilis* ATCC 6633 was used and 1, 0.8, 0.5, 0.4, and 0.2 μg/ml with *E. coli* SQ 12155. The detection limits of the bioassay were 0.05 μg/ml for *E. coli* SQ 12155 and 1 μg/ml for *B. subtilis* ATCC 6633. To determine the precision of the microbiological assay, nine spiked samples ranging in concentration from 10 to 2 μg/ml for *B. subtilis* ATCC 6633 and 1 to 0.2 μg/ml for *E. coli* SQ 12155 were prepared and subsequently measured on three different agar plates in triplicate. For each concentration, the coefficient of variation was then determined from this set of three measurements. This was repeated on three different occasions. Coefficients of variation between days averaged 0.34% (range, 0.19 to 0.45%) for *B. subtilis* ATCC 6633 and 0.19% (range, 0.13 to 0.23%) for *E. coli* SQ 12155; within-day coefficients of variation ranged from 0.27 to 0.45% for *B. subtilis* ATCC 6633 and from 0.45 to 0.56% for *E. coli* SQ 12155. Petri dishes were incubated at 37°C for 18 h, and zones of inhibition were read to the nearest 0.05 mm with a vernier caliper. Zones of inhibition of standard solutions were used to construct a standard curve derived by linear regression. Only standard curves with correlation coefficients ($r$) greater than 0.996 were used. The remaining percentage of the initial ceftriaxone concentration, at each time interval, was determined by comparing the initial concentration with the ceftriaxone concentration measured just after the initial reconstitution. These values were obtained by interpolation of the standard curve. Semilogarithmic plots of the residual ceftriaxone concentrations versus time were constructed to determine the rate and order of ceftriaxone degradation.

Differences between results obtained in the two fluids at each temperature were measured using a Scheffé test for paired comparisons to see whether the differences were significant. The a priori level of significance was $P \leq 0.05$.

Ceftriaxone sodium was found in this study to be more

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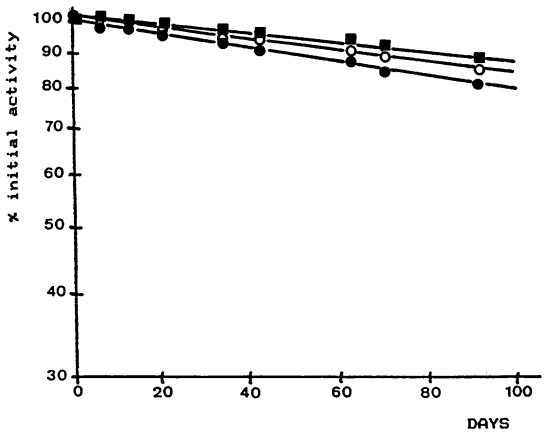


FIG. 1. Stability of ceftriaxone in undiluted human serum at −20°C (●), −40°C (○), and −70°C (■).

stable in human serum diluted 1/20 in 0.1 M phosphate buffer (pH 6.0) than in unbuffered human serum under a variety of storage temperatures.

After storage for 91 days at −20°C, ceftriaxone in undiluted human serum contained 82.41% of its initial activity; it contained 84.92% at −40°C and 88.96% at −70°C (Fig. 1). In diluted human serum, ceftriaxone contained 96.61, 99.09, and 99.56% at −20, −40, and −70°C, respectively, for the same period. Loss of activity was accelerated by storage at 4°C. After 55 days, samples prepared with undiluted and diluted human serum contained 80.33 and 86.25% of their initial activity, respectively (Fig. 2). After 120 h at 37°C, ceftriaxone solutions in undiluted human serum contained 33.08% of the initial activity, whereas samples in diluted human serum contained 62.49% (Fig. 3).

These results indicate that the degradation of ceftriaxone in both fluids has first-order kinetics and is faster at higher temperatures. Consequently, the regression line represents an average of the undiluted and diluted human serum data at each time interval for three determinations. The degradation rate kinetics of ceftriaxone sodium, the times at which concentrations fell to 90% of the original concentration, and the correlation coefficients in unbuffered and diluted human serum are shown in Table 1. Significant differences in stability were observed between unbuffered and diluted human serum at each temperature, as the test of Scheffé proved.

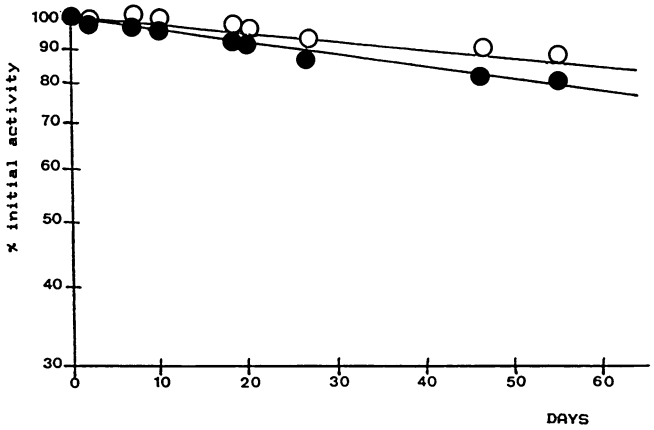


FIG. 2. Stability of ceftriaxone in undiluted (●) and diluted (○) human serum at 4°C.

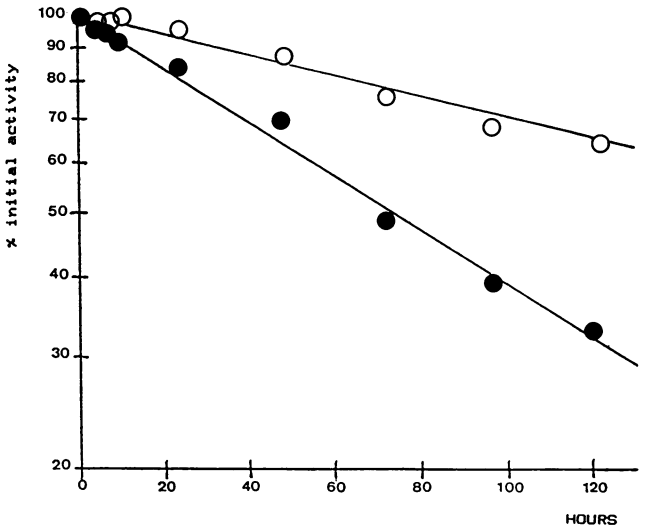


FIG. 3. Stability of ceftriaxone in undiluted (●) and diluted (○) human serum at 37°C.

These studies showed that ceftriaxone stability in human serum was increased when serum was diluted 1/20 in 0.1 M phosphate buffer (pH 6.0) and that the temperature influenced the stability of ceftriaxone. An increase in stability was achieved at lower temperatures, in agreement with the theory of Arrhenius.

The difference in stability observed in the fluids could be due to several factors, such as pH, macromolecular components, and metallic cations of the serum, among others. Studies of pH show that β-lactams are more stable under acid conditions (1, 2, 5); unbuffered serum has pH 7.4. Several authors (3, 4, 8) think that the instability and reduction of biological activity of β-lactam antibiotics in serum could be due to macromolecular components such as lipoproteins and albumin. The components are bound irreversibly and destructively to serum proteins. The concentrations of these components in diluted human serum are lower, so the antibacterial activity will be less affected and stability will be increased. Metallic cations such as Cu(II), Zn(II), and Mg(II) that can be found in serum could accelerate the degradation of ceftriaxone, as Maskell and Milles (6) found with other antibiotics. Our data suggest that samples of

TABLE 1. Degradation rate kinetics of ceftriaxone sodium in undiluted human serum and in human serum diluted 1/20 in phosphate buffer (pH 6.0) at controlled temperatures

| Fluid | Temp (°C) | Degradation rate constant | $t_{90}$[a] | Correlation coefficient |
|---|---|---|---|---|
| Undiluted human serum | −70 | $-0.001157\ Day^{-1}$ | 91 Days | −0.904 |
| | −40 | $-0.001416\ Day^{-1}$ | 74 Days | −0.817 |
| | −20 | $-0.002362\ Day^{-1}$ | 44 Days | −0.979 |
| | 4 | $-0.004102\ Day^{-1}$ | 26 Days | −0.988 |
| | 37 | $-0.009756\ h^{-1}$ | 11 h | −0.995 |
| Diluted human serum | −70 | | — | |
| | −40 | | — | |
| | −20 | | — | |
| | 4 | $-0.002976\ Day^{-1}$ | 35 Days | −0.979 |
| | 37 | $-0.000385\ h^{-1}$ | 27 h | −0.995 |

[a] $t_{90}$, Time at which concentrations fell to 90% of the original concentration.
—, No degradation in this period.

human serum should be diluted 1/20 in phosphate buffer (pH 6.0) and stored at low temperatures to increase the stability of the drug.

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