RESULTS AND DISCUSSION

Typical chromatograms from the analysis of a human serum blank and a spiked serum standard are shown in Fig. 1. Under the analytical conditions described, the retention times of derivatized I and II were 4.0 and 8.7 min, respectively. Standard curves prepared from a series of duplicate serum standards were linear over two concentration ranges typically encountered in pharmacokinetic monitoring. Linear regression for a series of low concentration standard (0.05–2.0 μg/ml) curves produced a slope of 1.642 ± 0.068, an intercept of −0.043 ± 0.063 with a mean correlation coefficient of 0.992. Corresponding results for the high-concentration range (1–40 μg/ml) produced a slope of 0.106 ± 0.002, an intercept of −0.0119 ± 0.031, and a mean (r) of 0.998. This reflects excellent between-run reproducibility, which is further supported by the recovery data over a wide concentration range shown in Table I. Replicate analysis of two spiked serum control specimens resulted in between-run coefficients of variation of 6.3% ($\bar{x}$ = 0.107 μg/ml) and 3.8% ($\bar{x}$ = 4.54 μg/ml). The practical lower limit of sensitivity for this procedure, for which a signal–baseline noise ratio of 3:1 can be seen, was 50 ng/ml. This is the same as that reported using a GC–electron-capture detection procedure (7). The entire procedure requires ~4 hr and allows analysis of ~20 specimens/analyst/working day.

The identity of the derivatized I GC peak was confirmed by comparing its mass spectrum to that of authentic, synthesized pentafluorobenzoyl imidazopyrazole (III). These were identical and demonstrated a parent peak at m/z 303 and a base peak at m/z 195, which corresponded to the pentafluorobenzoyl fragment. Other characteristic peaks occurred at m/z 167, 108, and 81 as seen in the proposed fragmentation pattern in Fig. 2.

The pharmacokinetic behavior of I was followed in four pediatric patients receiving intravenous bolus doses in a Phase I clinical trial. The plasma time course for one patient who received 450 mg is shown in Fig. 3. The mean terminal phase half-life and volume of distribution for all patients was 4.4 hr and 1.5 liters/kg, respectively. These values were in reasonable agreement with those reported for adult patients (6) ($t_{1/2}$ = 3–10 hr, Vd = 5–15 liters), although other widely divergent values using less specific radioactivity detection methods have also been reported (8). No other data in pediatric patients have been reported to date.

In the course of studying the reproducibility of the assay, it was observed that significant decreases in measured concentrations of I occurred if the spiked serum sample was allowed to sit unfrozen over a few hours. This had not been reported in the previous literature and appears to have serious implications for I measurements in biological media. A limited stability study was performed of I in pH 7.4 buffer, human serum, and 5% purified serum albumin. The results are summarized in Table II and indicate a relatively rapid decline in I in the presence of protein, which appears to stabilize at a level of 65–70% of the initial level over time.

A preliminary experiment examining the possibility of rapid irreversible protein binding suggested that this did not explain the relatively rapid decline observed. Although this problem can be circumvented by either immediate analysis of samples or quick freezing in methanol–dry ice, the mechanism of this apparent instability merits further study. Lack of recognition of this problem could lead to altered serum concentrations and errors in pharmacokinetic analysis *in vivo*.

The method reported appears sufficiently sensitive, reproducible, and rapid to support extended pharmacokinetic studies of I in humans or animals provided caution is used in rapidly handling samples to avoid an apparent instability in biological specimens.

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Stability of Concentrated Aqueous Solutions of Pralidoxime Chloride

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Received August 18, 1981, from the *U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010.* Accepted for publication November 20, 1981.

Abstract □ Concentrated aqueous solutions of pralidoxime chloride degrade more rapidly than dilute solutions. The rate and degree of degradation is dependent on the initial and final pH as well as the container in which the solution is stored. The effects of glass, metal, plastic, and rubber stoppers on the stability of concentrated and dilute solutions are discussed. The stability and shelf lives of 50% aqueous concentrates at different temperatures were determined.

Keyphrases □ Pralidoxime chloride—stability of concentrated aqueous solutions, kinetics □ Stability—concentrated aqueous solutions of pralidoxime chloride, kinetics □ Kinetics—comparison of stability in concentrated aqueous solutions of pralidoxime chloride

Pyridinium oximes in combination with atropine provide effective therapy in poisoning by many organophosphorus cholinesterase inhibitors (1). More clinical data are available for pralidoxime salts than other pyridinium oximes, as this oxime is in wider use. Pralidoxime also reportedly produces few undesirable side effects (2, 3). It is generally accepted that, for therapeutic efficacy, pralidoxime plasma concentrations should be at least 4 μg/ml (4). A 600-mg im dose is required to produce this level. A dilute solution would require an injection of an impractical volume. Since pralidoxime chloride is very water soluble, 30–50% concentrates can be used to provide a 4-μg/ml plasma level (5, 6). The purpose of this study was to determine stabilities and the effect of containers on the shelf life of aqueous concentrates of pralidoxime chloride[1].

[1] The opinions or assertions contained herein are the private views of the author and not to be construed as reflecting the views of the Department of the Army or the Department of Defense.

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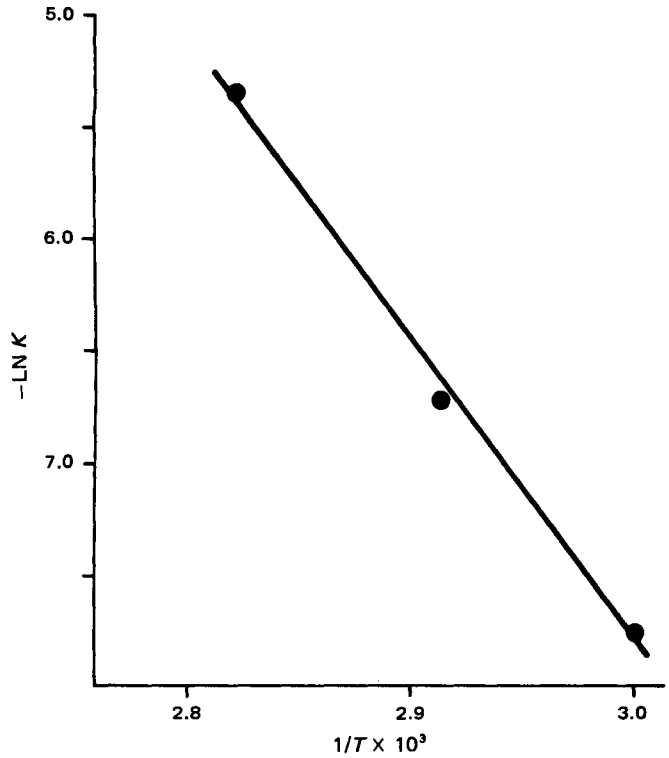

Figure 1—*Temperature Dependency of the Degradation of 50% Aqueous Solution of Pralidoxime in Glass Ampuls.*

EXPERIMENTAL

Twenty-five grams of pralidoxime chloride were transferred into a 50-ml volumetric flask and brought to volume with triple distilled water. Approximately 1.0-ml volumes were added to 2-ml glass ampuls which had been acid washed (0.01 *N* HCl), water-rinsed, and dried in an oven at 110°. The ampuls were sealed with an oxygen torch, then placed in hot air ovens at 60, 70, and 81°. Ampuls were removed at selected time periods. An aliquot was diluted with distilled water (0.2–500), then rediluted with 0.05 *N* NaOH (3.0–100) to obtain a final concentration of ~6 μg/ml. Samples were assayed by UV analysis at 336 nm (7).

Approximately 1.0-ml volumes were also added to glass, metal, stainless steel, and plastic (polypropylene) cylinders which were sealed at one end with a neoprene rubber stopper. After filling, the cylinder was closed with another neoprene rubber stopper, then weighed on a balance. Cylinders were placed in a holding device which consisted of a plastic base, 1.91-cm thick, containing holes slightly wider than the diameter of the glass cylinders. A plastic cap was placed over the top of each filled cylinder and a metal plate placed over the filled cylinders. The cylinders were secured by two screws. The holder containing the cylinders was placed in the oven, and the cylinders were removed as needed. Prior to analysis, cylinders were reweighed to determine possible losses due to evaporation or leakage. Pralidoxime chloride concentrations were determined as described above.

RESULTS

Stability of 50% Aqueous Solutions of Pralidoxime in Glass Ampuls—The rate of degradation of concentrates of pralidoxime at 60, 70, and 81° conforms to a first-order equation. As a 50% solution of pralidoxime at initial pH 3.65 is hypertonic, buffer was not added to maintain a constant pH. The rate constants at 60, 70, and 81° were 4.27×10^{-4}, 1.22×10^{-3}, and 4.68×10^{-3} hr^{-1}, respectively. The logarithm of velocity constants was plotted against the reciprocal of the absolute temperatures (Fig. 1). A linear relationship indicated the mechanism responsible for oxime decay is not altered by changes in temperature. The relationship is shown by the Arrhenius equation:

$$k = Ae^{-Ea/RT} \qquad \text{(Eq. 1)}$$

where Ea, the energy of activation, was 26,755 cal/mole; A was 4.08×10^{10} min^{-1}; R was the molar gas constant; and T the absolute temperature.

[2] Unpublished data.

Table I—Calculated Shelf Lives for 50% Aqueous Solutions of Pralidoxime Chloride in Glass Ampuls

Temperature°	Shelf Life[a] 10%	25%
10	37.0 *y*	101.0 *y*
15	16.0 *y*	44.0 *y*
20	7.3 *y*	20.0 *y*
25	3.4 *y*	9.3 *y*
30	1.6 *y*	4.4 *y*
35	286.0 *d*	2.1 *y*
40	142.0 *d*	1.1 *y*
45	72.0 *d*	198.0 *d*
50	38.0 *d*	103.0 *d*

[a] *y* = years; *d* = days.

Degradation of Concentrated Aqueous Solution of Pralidoxime—Glass ampuls and glass, metal, and plastic cylinders sealed with neoprene and butyl rubber stoppers were used. The degradation rates of 50% aqueous solutions of pralidoxime chloride were similar in plastic and glass cylinders sealed with neoprene stoppers and more rapid in metal cylinders when determined at 40°. The pH dropped with time, 3.6–3.2 after 40 days in glass and plastic, but increased in metal 3.6–4.2. When performed at 45 and 60° in glass with 30% solutions of pralidoxime chloride in cylinders stoppered with either neoprene or butyl rubber, the pH fell more rapidly in the butyl rubber than in the neoprene-stoppered containers[2].

DISCUSSION

The stability of 50% concentrates of pralidoxime chloride in unbuffered aqueous solutions in glass ampuls was studied under accelerated temperatures. Calculated 10 and 25% shelf lives are shown in Table I. At 25°, 10 and 25% shelf lives were found to be 3.4 and 9.3 years, respectively. The report also indicates the importance of considering the effects of material(s) in the container in which a formulation is packaged.

When results of the report are compared with the results of related investigations (5, 8), discrepancies in reported stabilities of pralidoxime might be assumed by the reader. However, on examination of data, one finds this is not so. Stability studies on 16% (w/v) solutions of pralidoxime methanesulfonate in sealed glass ampuls buffered at pH values 2.0–3.5 have been reported (5). Stability efforts on unbuffered solutions of 10–33% (w/v) pralidoxime chloride in glass ampuls and also glass vials sealed with butyl rubber stoppers have also been performed (8). Acid was added to obtain initial pH values of 1.3–2.0. In this study the stabilities of unbuffered, pH-unadjusted 50% solutions of pralidoxime chloride (w/v) in glass ampuls were investigated. In preliminary studies[3] with concentrates of pralidoxime chloride in cylinders made of glass, plastic, and metal sealed with rubber enclosures, significant variations in stability were found. Consequently, when comparing stability data, the reader must take into consideration different oxime salts, oxime concentrations, initial pH values, formulations, and storage containers. Fortunately, there are data where direct comparisons can be made. Stabilities for pralidoxime concentrates in sealed glass ampuls at 25 and 45° have been reported (5, 8).

Previous studies (9) on solutions (0.1%) of pralidoxime chloride showed that pralidoxime solutions possess maximum stability at pH 4.3 with a shelf life to 10 years at 25°. Here equilibria at pH 1–3 were noted but not considered in the shelf life estimations. If equilibria data had been included, the predicted shelf lives at these pHs would have been significantly greater. In later efforts 30% concentrated solutions were found to be less stable than 0.1% solutions, in that concentrated solutions of pralidoxime chloride degraded 2.5 times more rapidly than the dilute solution[2].

On examination of all data, one finds that: (*a*) oxime loss in a 50% solution of pralidoxime chloride and a 16% buffered solution of pralidoxime methanesulfonate, both stored in glass ampuls at 45°, are similar (a 16% solution of pralidoxime methanesulfonate is equivalent in oxime content to a 12% solution of pralidoxime chloride), (*b*) lowering of the initial pH in unbuffered concentrates results in greater stability (8), (*c*) use of buffer at pH 2.5 in 16% solutions of pralidoxime methanesulfonate decreased stability drastically when compared with 30% unbuffered solutions, (*d*) rubber closures affect the pH and stability of pralidoxime chloride concentrates stored in glass, plastic, and metal cylinders[2,3]. The drop in pH

[3] J. R. May, U.S. Chemical Research and Development Laboratories, Aberdeen Proving Ground, MD 21010, 1965.

was greater in the glass and plastic containers than in metal. When butyl rubber was used, a lower pH resulted than when neoprene rubber was tested. A lower pH is desirable because of the unique occurrence of equilibria.

The reason(s) that high concentrations of pralidoxime chloride degrade more rapidly than dilute concentrations is not known. To resolve this problem, studies should be carried out to determine and quantitate degradation products and their rate of formation. By establishing the proper mechanism(s) one should be able to design stable formulations of concentrated solutions of drugs.

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Growth and Characterization of Calcium Oxalate Dihydrate Crystals (Weddellite)

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Received July 14, 1980, from the *Faculté de Pharmacie, Université de Montréal, Montreal, Quebec, Canada.* Accepted for publication November 25, 1981.

Abstract □ Conditions are given for the growth of calcium oxalate dihydrate crystals (weddellite) in aqueous solution. The crystals obtained were characterized by scanning electron microscopic, spectroscopic, and thermal methods. The dissolution kinetics and electrophoretic mobility were determined; the thermodynamically unstable calcium oxalate dihydrate had a higher dissolution rate and a lower zeta potential than the monohydrate and underwent a phase transformation into the more stable calcium oxalate monohydrate. The results obtained on the chemical stability and the surface charge of calcium oxalate dihydrate offered additional information for assessing the current theories on the formation of calcium oxalate renal stones.

Keyphrases □ Crystals—calcium oxalate dihydrate, growth and characterization □ Dissolution, kinetics—determination, calcium oxalate dihydrate crystals, growth and characterization □ Electrophoretic mobility—determination, calcium oxalate dihydrate crystals, growth and characterization

In recent years there has been much discussion on the role of different hydrated calcium oxalate crystals in the formation of calcium oxalate stones (1–4). Chemically, two varieties of calcium oxalate crystals occur in renal calculi: calcium oxalate monohydrate (whewellite) and calcium oxalate dihydrate (weddellite). The variable amount of water of crystallization is a direct consequence of the urine composition. The formation of these phases and the possible transformation between them is important from the urinary calcification standpoint.

The growth of calcium oxalate dihydrate in natural urine, synthetic urine, and in a mixture of both has been a subject of a number of investigations (5–7). In a recent study from this laboratory, it has been noticed that the calcium oxalate dihydrate was formed in the rat kidney after the injection of 4-hydroxy-L-proline. These crystals transformed gradually into the more stable calcium oxalate monohydrate (8). Previously it was reported that it is possible to grow calcium oxalate dihydrate crystals from a medium consisting of natural and synthetic urine (9). The chemical reaction between the sodium oxalate (0.005 *M*) and calcium chloride (1 *M*) at 37° in the previously described medium produced the octahedral dipyramidal calcium oxalate dihydrate. The crystals obtained were mixed with ~5% monohydrate. This work is intended to describe an improved technique for the growth of calcium oxalate dihydrate in aqueous solution and to study the dissolution kinetics and electrophoretic mobility of this form. Some aspects of the structure-dependent properties of both whewellite and weddellite crystals will be discussed.

EXPERIMENTAL

Materials—The following materials were used: synthetic urine [an aqueous medium containing various electrolytes present in normal urine which has been described previously (5, 9)], calcium chloride dihydrate[1], sodium oxalate[1], and calcium oxalate monohydrate[1] (high purity reagent grade), and freshly bidistilled water.

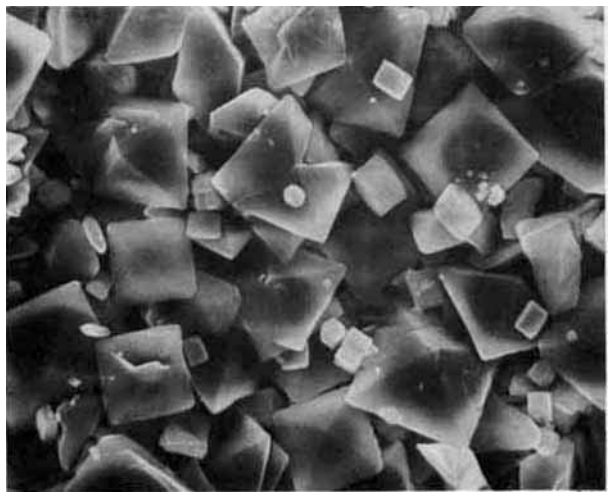

Figure 1—*Scanning electron micrograph of calcium oxalate dihydrate (2000×).*

[1] Fisher Scientific Co., Fair Lawn, N.J.

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