[Chem. Pharm. Bull. 32(3)1113—1119(1984)]

Hydrolysis of Succinylcholine Chloride in pH Range 3.0 to 4.5

JENNIFER J. BOEHM[1] and ROLLAND I. POUST*

Burroughs Wellcome Co., P.O. Box 1887, Greenville, NC, U.S.A.

(Received July 15, 1983)

The influences of temperature, pH, buffer, ionic strength and initial drug concentration on the stability of succinylcholine chloride in aqueous solution were investigated. The following results were obtained:

(i) in unbuffered solutions—the reaction was apparent zero order; the pH fell during the course of reaction; the rate of hydrolysis was slowest in solutions initially adjusted to pH 3.75—4.50,

(ii) the rate of hydrolysis was significantly catalyzed by buffer,

(iii) the rate of hydrolysis increased modestly with an increase in ionic strength, and

(iv) the rate of hydrolysis increased with initial concentration of drug in solution.

Keywords——succinylcholine chloride; hydrolysis; buffer catalysis; pH dependency; ionic strength; initial concentration

The principal objective of the study was to determine the solution composition which is optimal for the stability of succinylcholine chloride. The pH limits set by the United States Pharmacopeia (USP) for Succinylcholine Chloride Injection are pH 3.0 to 4.5.[2] Outside these limits the rate of hydrolysis increases dramatically due to specific acid or base hydrolysis.[3] Preparations currently marketed in the USA are unbuffered.[4] The stability of succinylcholine chloride was therefore studied in unbuffered solutions using initial pH values within the USP limits.

Previous studies using solutions held at constant pH *via* buffers or a pH-stat to study stability concluded that the optimal pH for succinylcholine chloride in solution was pH 3.5 to 4.3, depending on the temperature studied.[3] In the present study the reaction was followed over the pH range 3.0 to 4.5 in solutions buffered with McIlvaine's buffer (0.1 M citric acid–0.2 M sodium monohydrogen phosphate) to determine the extent of buffer catalysis. Although buffer catalysis of succinylcholine chloride hydrolysis has been demonstrated in formate, acetate and phosphate buffers,[3,5] the question of whether catalysis outweighs the advantageous effect of preventing a fall in pH has not been resolved.

A further objective of the study was to clarify the nature of the relationship between rate of hydrolysis and initial drug concentration. Conflicting evidence concerning the relationship has been reported in the literature.[6,7] Gibb reported that succinylcholine chloride solutions having low initial concentrations were most stable, while Jensen–Holm found high concentration solutions of succinylcholine chloride to be more stable.[6,7]

Finally, the influence of ionic strength on the rate of hydrolysis of succinylcholine chloride was investigated. Information regarding ionic strength effects is necessary for meaningful comparison of literature results obtained at different ionic strengths.

Experimental

Preparation of Solutions——Bulk aqueous solutions of succinylcholine chloride were prepared using Anectine® Flo-Packs (succinylcholine chloride dihydrate, equivalent to 1000 mg succinylcholine chloride anhydrous per Flo-

NII-Electronic Library Service

TABLE I. Solutions Prepared for Succinylcholine Chloride Hydrolysis Study

Study	Initial pH	Buffer	Concentration (mg/ml)	Ionic strength	Storage temp. (°C)
(i)	3.00 4.00	None	20	0.5	40
	3.25 4.25				50
	3.50 4.50				60
	3.75				
(ii)	3.00 4.00	McIlvaine's buffer	20	0.5	40
	3.25 4.25				50
	3.50 4.50				60
	3.75				
(iii)	3.50	None	10 40	0.5	50
			20 50		
			30		
(iv)	3.50	None	20	0.25 0.75	50
				0.50 1.00	

Pack, Batches 1F1808 and 1C2197, Burroughs Wellcome Co., Research Triangle Park, North Carolina). The ionic strength was adjusted with sodium chloride and, in the case of unbuffered solutions, the pH was adjusted appropriately with either concentrated hydrochloric acid or 2 N sodium hydroxide solution. Buffered solutions were prepared by mixing appropriate volumes of 0.1 M citric acid and 0.2 M monohydrogen phosphate to achieve the desired pH. After adding the succinylcholine chloride, the pH of each solution was checked using a calibrated (Orion Ionalyzer, Orion Research, Inc.) pH meter. The solutions were filled into clean 10 ml glass ampuls which were then sealed and stored in constant temperature chambers. Formulae for the preparation of solutions used in each section of the study are presented in Table I.

Assay——Samples were assayed by a high performance liquid chromatography (HPLC) method[8] (Milton Roy minipump, Micromeritics model 725 injector, Tracor model 970 detector) at the following times:

(i) 40 and 50 °C samples—initial, 4. 8, and 12 weeks, (ii) 60 °C samples—initial, 1, 2, 3, and 4 weeks.

Results and Discussion

The study was divided into four sections, aimed at determining the effects of the following factors on the rate of succinylcholine chloride hydrolysis:

(i) initial pH of solution, for solutions in which the pH was not controlled, (ii) pH of solution, in buffered solutions, (iii) initial drug concentration, and (iv) ionic strength.

The Effect of pH on Hydrolysis of Succinylcholine Chloride in Unbuffered Solutions

A representative set of results (60 °C) for hydrolysis of succinylcholine chloride in unbuffered solutions at elevated temperatures is shown in Fig. 1. The approximately linear relationship between percentage remaining and time indicated that hydrolysis followed apparent zero-order kinetics. Although the underlying mechanism is first order with respect to succinylcholine chloride,[3] the production of succinic acid caused the pH of the solution to drop as the reaction proceeded. Succinic acid 20 mg/ml solution has a pH of 2.79 compared with pH 4.12 for an equivalent solution of succinylcholine chloride. As the reaction proceeded, the fall in pH of the solution caused the hydrogen ion-catalyzed hydrolysis to become increasingly important. This resulted in the rate of hydrolysis being maintained at a virtually constant value over the course of the reaction. The drop in pH was observed in all unbuffered solutions studied. A sample set of results is given in Table II.

Linear regression analysis was performed on results obtained for each initial pH at all three temperatures. The slopes calculated represent apparent zero-order rate constants in units of percentage lost per week. The constants are tabulated along with the correlation coefficients in Table III. At all three temperatures there appeared to be a plateau region

TABLE II. pH Change and Percentage Remaining in Unbuffered Solutions of Succinylcholine Chloride Stored for 4 Weeks at 50 and 60 °C

Initial pH	3.00	3.25	3.50	3.75	4.00	4.25	4.50
pH after 4 weeks, 50 °C	2.82	2.94	3.03	3.07	3.09	3.13	3.15
% remaining after 4 weeks, 50 °C	61.7	72.1	74.0	78.7	77.5	78.2	78.8
pH after 4 weeks, 60 °C	2.71	2.78	2.82	2.88	2.90	2.92	2.82
% remaining after 4 weeks, 60 °C	16.0	25.1	28.6	33.2	34.1	35.1	36.1

TABLE III. Rate Constants (k_0) and Correlation Coefficients (r) for Hydrolysis of Succinylcholine Chloride in Unbuffered Solutions Stored at 40, 50 and 60 °C

Temperature (°C)		pH 3.00	3.25	3.50	3.75	4.00	4.25	4.50
40 °C	k_0 (%/week)	3.98	3.05	3.23	2.79	2.89	2.56	2.75
	r	0.999	0.992	0.986	0.999	0.998	0.989	0.993
50 °C	k_0 (%/week)	7.17	6.54	6.54	6.19	6.28	6.10	5.99
	r	0.990	0.998	0.999	0.999	0.999	0.999	0.997
60 °C	k_0 (%/week)	21.12	19.02	18.20	17.07	17.05	16.51	16.43
	r	0.996	0.997	0.998	0.998	0.998	0.999	0.997

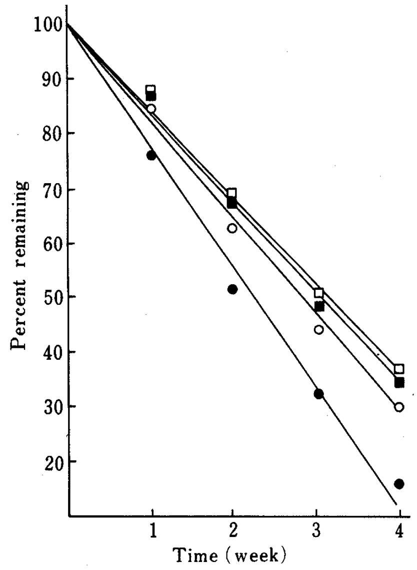

Fig. 1. Hydrolysis of Succinylcholine Chloride in Unbuffered Solutions Stored at 60 °C

pH values of solutions are represented as follows: □, pH 4.50; ■, pH 4.00; ○, pH 3.50; ●, pH 3.00.

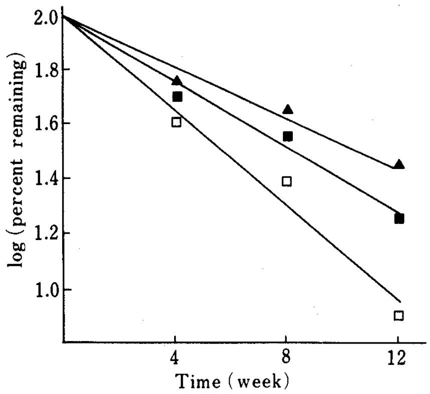

Fig. 2. Hydrolysis of Succinylcholine Chloride in Buffered Solutions Stored at 40 °C

pH values of solutions are represented as follows: □, pH 3.25; ■, pH 4.00; ▲, pH 4.50.

between pH 3.75 and 4.50 over which hydrolysis was virtually independent of initial pH. Below pH 3.75 there was a significant increase in reaction rate, with the rate at pH 3.00 about 25 percent faster than in the plateau region. From these results it appears that the optimal initial pH for unbuffered aqueous solutions of succinylcholine chloride lies in the pH range 3.75 to 4.50.

The Effect of pH on Hydrolysis of Succinylcholine Chloride in Buffered Solutions

Hydrolysis of succinylcholine chloride in buffered solutions followed pseudo-first order kinetics at 40 and at 50 °C (see Fig. 2). At 60 °C the reaction was first-order at pH 4 and above, but below pH 4 the reaction order appeared to be mixed based on the fact that neither zero- nor first-order kinetics could be used to describe the data with a reasonable degree of correlation.

Hydrolysis in buffered solutions was much faster under all conditions studied than in the corresponding unbuffered solutions. A sample comparison of results for unbuffered and buffered solutions is shown in Fig. 3. It can be seen that the buffer effect was much more pronounced at the higher pH. This suggested that catalysis by the dihydrogenphosphate ($H_2PO_4^-$) species was responsible for the increase in rate compared with unbuffered solutions. Analysis of results at 40 °C (refer to Table IV) supported this hypothesis. There was a drop in half-life of almost four weeks per pH unit over the range pH 3.75 to 4.5 in buffered solutions. Since the rate is virtually independent of pH in the corresponding unbuffered solutions over this pH range, the drop in half-life in the buffered solutions can be attributed entirely to buffer effects.

The strongly correlated ($r=0.988$) relationship between increase in reaction rate and concentration of $H_2PO_4^-$, depicted in Fig. 4, indicated that catalysis was due to $H_2PO_4^-$. Buffer catalysis has previously been reported in the literature for both phosphate and acetate ion.[3] Degradation of succinylcholine chloride in presence of physiological buffers such as

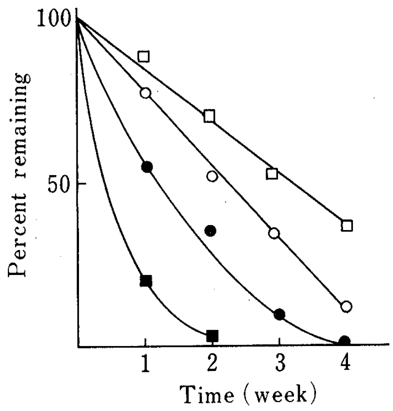

Fig. 3. Comparison of Hydrolysis of Succinylcholine Chloride in Buffered and Unbuffered Solutions at 40 °C

Initial pH values of solutions are represented as follows: □, pH 4.50 (unbuffered); ■, pH 4.50 (buffered); ○, pH 3.00 (unbuffered); ●, pH 3.00 (buffered).

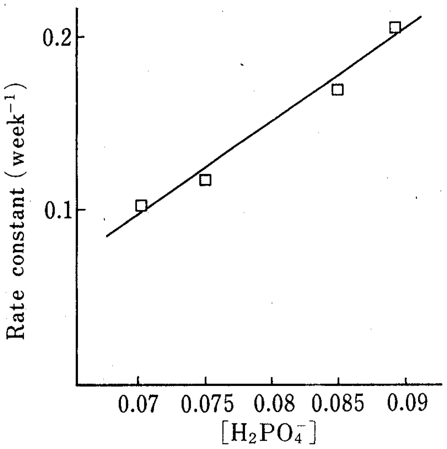

Fig. 4. Relationship between First-Order Rate Constant and Concentration of Dihydrogen Phosphate Ion ($H_2PO_4^-$) in Solution at 40 °C

Table IV. Half-life of Succinylcholine Chloride in Buffered and Unbuffered Solutions at 40 °C

Initial pH	3.00	3.25	3.50	3.75	4.00	4.25	4.50
$t^{1/2}$ (weeks) buffered	9.6	6.9	6.8	6.0	5.9	4.1	3.4
$t^{1/2}$ (weeks) unbuffered	12.6	16.4	15.5	17.9	17.3	19.5	18.2

Ringer's solution, lactated Ringer's solution, sodium lactate, stored serum, and protein hydrolysate has also been reported.[6]

Although addition of buffering agents prevented a fall in pH and hence prevented increased hydrogen ion catalysis as the reaction proceeded, this advantageous effect was outweighed by the catalytic effect of the buffer. Succinylcholine chloride is therefore more stable in unbuffered solutions than in a variety of buffered solutions over the pH range 3.00 to 4.50.

The Effect of Ionic Strength on the Rate of Hydrolysis of Succinylcholine Chloride in Unbuffered Solutions

For bimolecular reactions, the effect of ionic strength on reaction rate is given by

$$\log k = \log k_0 + 1.02\ Z_a Z_b (\mu)^{1/2}$$

where k_0 is the rate constant extrapolated to zero ionic strength
Z_a is the charge on reactant A
Z_b is the charge on reactant B, and
μ is the ionic strength

The equation is best suited to solutions of low (less than 0.1) ionic strength. Since the ionic strength of solutions studied was much greater than 0.1, the equation merely gives a guideline to the effects of ionic strength on rate of hydrolysis in the present study.

Figure 5 shows a plot of log (rate constant) *versus* square root of ionic strength at an initial pH of 3.50. As ionic strength increased, there was a modest increase in the rate of hydrolysis of succinylcholine chloride. The plot exhibited some curvature as the ionic strength approached one. The curvature may be explained by the secondary salt effect on the dielectric constant of the medium which is known to occur at higher ionic strengths. The slope of the line was used to obtain information regarding charges on the reacting species, since slope $=1.02\ Z_a Z_b$. Neglecting the $\mu=1$ datum, the slope was found to be 0.219 ($r=0.999$). Several factors may have resulted in the fractional slope value. First, the relationship between log (rate constant) and square root of ionic strength is only approximate at higher ionic strengths. Second, the reaction may have proceeded *via* dual pathways at pH 3.50 *i.e.*,

(i) simple hydrolysis of succinylcholine chloride by water

$$Z_a=+2, \quad Z_b=0$$

$$Z_a Z_b=0, \quad \text{yielding a slope of zero}$$

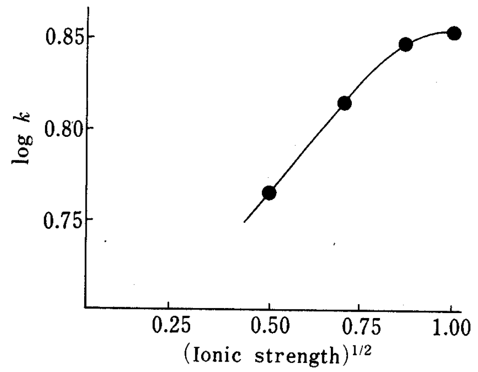

Fig. 5. Dependency of Succinylcholine Chloride Hydrolysis in Unbuffered Solution on Ionic Strength at 50 °C at an Initial pH of 3.50

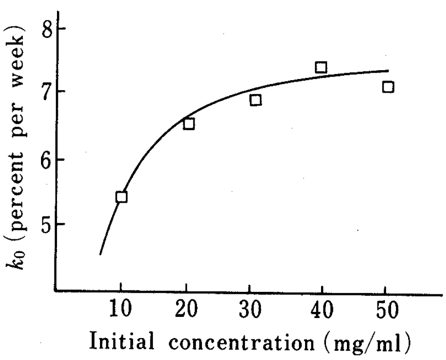

Fig. 6. Relationship between Rate of Hydrolysis of Succinylcholine Chloride in Unbuffered Solution (Initial pH 3.50) and Initial Concentration of Succinylcholine Chloride at 50 °C

TABLE V. pH of Unbuffered Solutions Containing Various Initial Concentrations of Succinylcholine Chloride after Storage at 50 °C at an Initial pH of 3.50

Initial concentration mg/ml	10	20	30	40	50
pH after 4 weeks/50 °C	3.14	3.03	2.90	2.82	2.77
pH after 8 weeks/50 °C	2.92	2.90	2.72	2.66	2.61

(ii) hydrogen ion-catalyzed hydrolysis

$Z_a = +2, \quad Z_b = +1$

$Z_aZ_b = +2$, leading to a slope of +2

A reaction proceeding *via* parallel pathways with different Z_aZ_b values would be expected to yield a fractional slope for the ionic strength plot. Between pH 3.75 and 4.50, the rate of succinylcholine chloride hydrolysis by water is virtually independent of pH, suggesting that simple hydrolysis is the predominant pathway in that region. At pH values below 3.75, hydrogen ion catalysis becomes increasingly important as pH is lowered. Both pathways would threfore contribute to the overall rate of hydrolysis at pH 3.50, the initial pH of the solutions prepared for this section of the study. The value obtained for the slope of the ionic strength plot suggests that water-catalyzed hydrolysis is the more important decomposition pathway at pH 3.50. In solutions more acidic than pH 3.5 it is predicted that ionic strength effects would become more important since the hydrolysis would be proceeding *via* the hydrogen ion-catalyzed mechanism to a greater extent.

The Effect of Initial Drug Concentration in Solution on the Rate of Hydrolysis of Succinylcholine Chloride in Unbuffered Solutions

The relationship between rate of succinylcholine chloride hydrolysis and initial concentration of drug in solution is shown in Figure 6. A modest increase in rate with increase in initial drug concentration was observed, in agreement with the findings of Gibb.[6] The increase in rate with concentration is probably ascribable to the greater production of hydrogen ion in more concentrated solutions. This would lead to a greater drop in pH and therefore to a more significant contribution by the hydrogen ion-catalyzed pathway in solutions containing a higher initial concentration of succinylcholine chloride. The pH measurements taken after 4 and 8 weeks of storage at 50 °C (shown in Table V) support this argument.

Conclusions

The hydrolysis of succinylcholine chloride in unbuffered solutions adjusted to pH values between 3.00 and 4.50 follows apparent zero-order kinetics. There is a plateau region between pH 3.75 and 4.50 in the pH-rate profile for hydrolysis of succinylcholine chloride in unbuffered solutions. In this region the water-catalyzed reaction appears to be the predominant mechanism of hydrolysis. Below pH 3.75, the rate of hydrolysis increases with decrease in initial pH as the hydrogen ion-catalyzed pathway becomes more important.

In buffered solutions, the reaction appears to follow first-order kinetics. The hydrolysis of succinylcholine chloride is catalyzed to a significant extent by addition of McIlvaine's (citric acid–sodium monohydrogenphosphate) buffer.

The rate of hydrolysis of succinylcholine chloride increases modestly with increasing ionic strength or initial concentration of drug in solution.

NII-Electronic Library Service

Optimal stability conditions for aqueous solutions of succinylcholine chloride appear to exist when concentration and ionic strength are low, the solution is unbuffered, with the initial pH adjusted to a value in the range 3.75 to 4.50.

Acknowledgement The following students participated in this study as part of Burroughs Wellcome Co. Summer Pharmacy Intern Program: A. Johnson and E. Parker, University of North Carolina (Chapel Hill), P. Mitchell, University of Montana, and R. Smith, University of Cincinnati. The excellent technical assistance of Ms. K. Elks CLA (ASCP) is acknowledged.

References and Notes

1) Present adress: *University of Michigan College of Pharmacy, Ann Arbor, MI, U.S.A.*
2) United States Pharmacopeia, 20th Ed., U. S. Pharmacopeial Convention, Rockville MD, 1980, p. 743.
3) T. Suzuki, *Chem. Pharm. Bull.*, **10**, 912 (1962).
4) B. B. Huff (ed.), "Physician's Desk Reference," 34th Ed., Litton Industries, Oradell NJ, 1980, p. 743.
5) T. Suzuki and Y. Tanimura, *Chem. Pharm. Bull.*, **15**, 674 (1967).
6) D. Gibb, *Anaesth. Int. Care*, **1**, 109 (1972).
7) J. Jensen-Holm, *J. Pharmacol. Toxicol.*, **41**(Suppl. IV), 55 (1977).
8) United States Pharmacopeia, 20th Ed. (Third Suppl.), U. S. Pharmacopeial Convention, Rockville MD, 1982, p. 256.

NII-Electronic Library Service