Effect of Buffer Constituents and Time on Drug Transfer across *In Vitro* Rat Intestine

LESLIE Z. BENET, JAMES M. ORR, ROBERT H. TURNER, and HOWARD S. WEBB

Abstract □ The *in vitro* absorption kinetics for salicylate ion and acetanilide were followed in six different buffer solutions over 2-hr. periods. Both everted and noneverted absorption rates were measured during four consecutive periods of 30-min. duration. Comparisons were made between initial rates, rates over the 4–30-min. periods, total amounts of drug transferred, lag time of initial runs, and increase of rates over the 2-hr. experiment (as percent of initial rate). By using this latter statistic as a measure of the loss in integrity of the intestinal tissue, Krebs bicarbonate pH 7.4 buffer was found to provide the best environment for maintenance of the *in vitro* membrane in a constant, viable condition. A sodium phosphate buffer and a tromethamine buffer were also found usable, although less effective than the Krebs buffer. In isotonic sodium chloride, serosal transfer rates of salicylate ion increased sharply while mucosal transfer rates remained constant over the 2-hr. experiment. Acetanilide, a nonionized drug, showed little increase in either mucosal or serosal transfer rates in this medium.

Keyphrases □ Salicylate, acetanilide transfer—rat intestine, *in vitro* □ Buffer, time, effects—drug transfer, rat intestine □ Intestine, everted, noneverted—absorption kinetics □ UV spectrophotometry—analysis

Recent studies by Turner *et al.* (1) and Turner and Benet (2) pointed out that drug ions show a difference in directional permeability coefficients in the *in vitro* rat intestine, with mucosal to serosal transfer occurring at faster rates than serosal to mucosal transfer. It appears that the difference in directional permeability coefficients might be related to a sodium-ion coupled transport; when sodium was completely replaced by potassium in phosphate buffer solutions, the difference in permeability coefficients tended to disappear. Nonionized drugs did not show a difference in directional permeability coefficients in either sodium or potassium phosphate buffers.

Mayersohn and Gibaldi (3, 4) showed that replacement of sodium ion by potassium ion in Krebs bicarbonate buffer significantly reduced the passive transfer of several water-soluble drugs (both ionized and nonionized) across the everted rat intestine. They also showed that: (*a*) ouabain (10^{-3} *M*) had no effect on the transfer process; (*b*) replacing sodium ion by ammonium ion or lithium ion in the buffer solution produced effects on selected drugs similar to those seen with potassium ion; and (*c*) the positively charged tromethamine[1] ion did not affect the rate of transfer. The inhibition of transfer with the various cations appeared to correlate well with the degree of tissue fluid uptake in the intestine.

Mayersohn and Gibaldi (3, 4) employed a modified Crane and Wilson technique (5), measuring the total amount of drug transferred in a 2-hr. period. The work in this laboratory measured the initial rates of absorption (during a 35-min. period) using a perfusion apparatus (1).

[1] Tris [2-amino-2-(hydroxymethyl)-1,3-propanediol].

The present study was undertaken to examine time effects on the rates of transfer through everted and noneverted segments of the rat intestine, and the effect of different buffer constituents on these rates.

EXPERIMENTAL

Materials—The following USP, NF, or reagent grade chemicals were employed: salicylic acid, acetanilide, tromethamine, monobasic sodium phosphate with one water of hydration, dibasic sodium phosphate anhydrous, monobasic potassium phosphate anhydrous, dibasic potassium phosphate anhydrous, sodium chloride, potassium chloride, calcium chloride, magnesium sulfate with seven waters of hydration, sodium bicarbonate, potassium hydroxide, a 100% oxygen gas, and oxygen–carbon dioxide gas mixture (95:5).

Procedure—Full strength Krebs–Henseleit buffer (Krebs bicarbonate buffer), pH 7.4, was prepared as described in the literature (6). The two modified Krebs bicarbonate buffers utilized were: (*a*) ½ Na^+ replaced by K^+, and (*b*) all of the Na^+ replaced by K^+. The pH 7.4 sodium phosphate buffer was prepared as previously described (1). The 0.154 *M* tromethamine buffer (pH 7.4) and the 0.9% sodium chloride (pH 6.6) solutions were prepared in the usual manner. The pH values of the solutions used were routinely checked at the beginning and end of each experiment on a Beckman research pH meter.

Male Sprague-Dawley rats, weighing 220–250 g., were fasted for 24 hr. prior to the experiment but water was allowed *ad libitum*. The rats were rendered unconscious with carbon dioxide in an airtight chamber and were then sacrificed by a sharp blow at the base of the skull. The small intestine (from the pyloric junction to about the middle of the jejunum) was removed at once *via* a midline abdominal incision and flushed with 40 ml. of the buffer (warmed) to be used in the absorption study. The first 15-cm. segment was discarded and the next 2–12.5-cm. segments (stretched length using a 20-g. weight) were employed, with the proximal segment being everted alternately. The *in vitro* absorption kinetics for salicylic acid and acetanilide (in various buffers) were followed using perfusion devices and techniques previously described (1), except that each intestinal segment was used for four consecutive experiments with the solutions changed after each run. At appropriate intervals, 0.1-ml. samples were removed for assay.

For salicylate-ion transfer, the time for the experiment was 2 hr. and 5 min. For acetanilide transfer in 0.9% sodium chloride, rates were measured during four consecutive 15-min. periods. In the Krebs bicarbonate buffers, however, absorption measurements were made during the following time periods: initial rate, 0–15 min.; rate 2, 30–45 min.; rate 3, 60–75 min.; and rate 4, 105–120 min. Fifteen-minute transfer experiments were necessary in the acetanilide studies to correspond with requirements described previously (1) so that transfer would appear to follow unidirectional zero-order kinetics.

Assay Procedures—The 0.1-ml. samples were diluted and assayed as follows. Acetanilide was diluted with 1.0 ml. pH 7.4 isotonic buffer, and the concentration was determined by UV measurement at 239 nm. on a Beckman DB-G spectrophotometer. Salicylic acid was diluted with 5.0 ml. of 0.1 *N* sodium hydroxide or potassium hydroxide, and the drug concentration was determined on an Aminco-Bowman spectrophotofluorometer at an excitation wavelength of 300 nm. and an emission wavelength of 408 nm. (uncorrected). The choice of diluent depended upon the presence or absence of sodium ion in the environment. Standard curves for each drug were made using five known concentrations of the drug in the appropriate diluent. As in previous experiments (1, 5), no significant difference in transfer rate was observed between the first and second segments of intestine.

Table I—Summary of Transfer Data ± Standard Deviation for Salicylate Ion in Krebs Bicarbonate pH 7.4 Buffers Containing Varying Amounts of Sodium Ion

	No.	Everted (E)	Noneverted (NE)	Ratio	Level of Significance E *versus* NE
Full strength sodium ion					
A. Average rates—all runs (mcg./ml./min.)	7	1.65 ± 0.50	0.92 ± 0.28	1.90 ± 0.83	$p < 0.01$
B. Average initial rates (mcg./ml./min.)	7	1.59 ± 0.59	1.01 ± 0.30	1.62 ± 0.72	$p < 0.05$
C. Average rate increase (4th rate/initial rate) as %	6	109 ± 20	82 ± 19		N.S.[a]
D. Average lag time—initial runs (min.)	7	5.2 ± 1.7	6.5 ± 0.8		N.S.
F. Total amounts transferred —average (mg.)	6	3.12 ± 0.97	1.84 ± 0.59	1.82 ± 0.85	$p < 0.05$
One-half sodium replaced					
A.	12	1.26 ± 0.34	1.07 ± 0.24	1.17 ± 0.24	$p < 0.05$
B.	12	0.87 ± 0.36	0.64 ± 0.20	1.52 ± 1.02	N.S.
C.	12	201 ± 98	283 ± 179		N.S.
D.	12	7.6 ± 1.9	8.8 ± 1.4		N.S.
F.	12	2.36 ± 0.64	2.05 ± 0.43	1.16 ± 0.21	$p < 0.05$
All sodium replaced					
A.	8	0.62 ± 0.53	0.52 ± 0.27	1.26 ± 1.08	N.S.
B.	8	0.28 ± 0.40	0.22 ± 0.20	1.14 ± 0.55	N.S.
C.	8	660 ± 531	697 ± 446		N.S.
D.	8	—[b]	—[b]		
F.	8	1.55 ± 1.07	1.29 ± 0.99	1.42 ± 0.97	N.S.

[a] Not significant. [b] Because some of the initial rates approached zero, the lag times approached $-\infty$, and the averages appear meaningless.

Plots were made of concentration *versus* time; rates of transfer, intercepts, and ratios were obtained by the use of an unweighted least-squares program executed on a Hewlett-Packard model 9100A desk calculator. Although there was often an increase in the absorption rate (slope of concentration–time plot) during the four consecutive runs on each intestinal segment, the regression coefficients for all slopes were as high as those reported previously (1) in the single-run study, that is >0.980 in every case, with the majority >0.995. Using the everted (E) and noneverted (NE) data, the following calculations were made: A, the average E and NE rates and the E/NE ratios for all determinations on a particular drug in a particular buffer; B, the average initial rates (E and NE) and ratios (E/NE), *i.e.*, the first rate measured on each intestinal segment; C, the average increase in rates over the time of the experiment as a percent of the initial rate [*i.e.*, (4th rate/1st rate) × 100]; D, the average *X*-intercept or lag time observed with initial rate measurements; and F, the average total amount of drug transferred across the gut during the time for the experiment (four runs).

RESULTS AND DISCUSSION

The summary of the data is presented in Tables I and II for salicylate ion and in Table III for nonionized acetanilide. In one of the Krebs bicarbonate (full strength sodium ion) studies in Table I, the gut leaked during the fourth consecutive time period, thereby resulting in only six values for C (average rate increase) and F (total amounts transferred). The values for D (average lag time–initial runs) in the "all sodium replaced" studies in Table I have been omitted, since some of the initial rates yielded zero transfer of drug, resulting in an apparent lag time of $-\infty$, and, consequently, a meaningless average. The ratios reported in Tables I–III do not exactly correspond to the everted average values divided by non-

Table II—Summary of Transfer Data ± Standard Deviation for Salicylate Ion in Three Isotonic Solutions

	No.	Everted (E)	Noneverted (NE)	Ratio	Level of Significance E *versus* NE
Sodium phosphate pH 7.4 buffer					
A. Average rates—all runs (mcg./ml./min.)	9	2.13 ± 0.47	1.82 ± 0.47	1.20 ± 0.22	$p < 0.05$
B. Average initial rates (mcg./ml./min.)	9	1.79 ± 0.39	1.37 ± 0.25	1.32 ± 0.19	$p < 0.005$
C. Average rate increase (4th rate/initial rate) as %	9	140 ± 20	157 ± 27		N.S.
D. Average lag time for initial runs (min.)	9	6.1 ± 1.6	6.8 ± 3.1		N.S.
F. Average total amounts transferred (mg.)	9	4.04 ± 0.74	3.52 ± 0.82	1.17 ± 0.18	N.S.
0.154 *M* Tromethamine buffer, pH 7.4					
A.	5	1.33 ± 0.25	1.25 ± 0.20	1.09 ± 0.29	N.S.
B.	5	1.03 ± 0.22	0.83 ± 0.16	1.26 ± 0.21	$p < 0.05$
C.	5	160 ± 20	183 ± 55		N.S.
D.	5	4.9 ± 0.2	7.4 ± 1.7		$p < 0.05$
F.	5	2.51 ± 0.37	2.42 ± 0.42	1.07 ± 0.24	N.S.
0.9% Sodium chloride, pH 6.6					
A.	5	1.68 ± 0.19	1.20 ± 0.45	1.58 ± 0.59	N.S.
B.	5	1.68 ± 0.30	0.74 ± 0.36	2.68 ± 1.14	$p < 0.01$
C.	5	113 ± 21	242 ± 104		$p < 0.05$
D.	5	4.9 ± 3.0	6.7 ± 3.6		N.S.
F.	5	3.32 ± 0.45	2.41 ± 0.78	1.50 ± 0.50	N.S.

Table III—Summary of Transfer Data ± Standard Deviation for Acetanilide in Three Isotonic Solutions

	No.	Everted (E)	Noneverted (NE)	Ratio	Level of Significance E *versus* NE
0.9% Sodium chloride, pH 6.6					
A. Average rates—all runs (mcg./ml./min.)	3	4.42 ± 0.65	4.11 ± 0.65	1.08 ± 0.09	N.S.
B. Average initial rates (mcg./ml./min.)	3	4.26 ± 0.75	3.94 ± 0.55	1.08 ± 0.07	N.S.
C. Average rate increase (4th rate/initial rate) as %	3	115 ± 25	101 ± 6		N.S.
D. Average lag time for initial runs (min.)	3	0.74 ± 0.36	0.87 ± 0.49		N.S.
F. Average total amounts transferred (mg.)	3	4.16 ± 0.58	3.93 ± 0.62	1.06 ± 0.05	N.S.
Krebs bicarbonate—full strength sodium ion					
A.	5	3.88 ± 0.75	3.49 ± 0.34	1.11 ± 0.19	N.S.
B.	5	3.79 ± 0.64	3.66 ± 0.42	1.05 ± 0.27	N.S.
C.	5	111 ± 10	107 ± 17		N.S.
D.	5	0.93 ± 2.0	0.53 ± 2.1		N.S.
F.	5	4.48 ± 0.65	4.19 ± 0.53	1.08 ± 0.21	N.S.
Krebs bicarbonate—all sodium replaced					
A.	4	3.82 ± 0.37	4.79 ± 1.23	0.83 ± 0.16	N.S.
B.	4	3.08 ± 0.26	4.22 ± 0.92	0.75 ± 0.15	N.S.
C.	4	148 ± 32	124 ± 42		N.S.
D.	4	0.45 ± 1.32	−1.30 ± 2.94		N.S.
F.	4	4.45 ± 0.60	6.07 ± 1.10	0.74 ± 0.06	$p < 0.02$

everted average values, since the ratio values reported are the average of ratios calculated for each individual run. As explained previously (1, 2), it is hoped that this method of treatment will cancel out the biological variability of differences in absorption rates due to the individual physiological characteristics seen in a number of different rats.

The level of significance for each of the five averages (A through F) used to compare transfer through everted and noneverted segments is also reported in Tables I–III. It can be seen that significant differences (at 95% or greater confidence levels using Student's *t* test for paired data) are observed for salicylate ion in Krebs bicarbonate (full strength sodium ion), 0.9% sodium chloride, and sodium phosphate buffers. No significant difference is seen between everted and noneverted rates for the nonionized acetanilide. These results are in accord with those reported previously (1, 2), where differences in directional permeability coefficients were observed in initial rate studies for ionized drugs in the presence of sodium ions. However, in this study, a significant difference in directional transport for salicylate ion was also seen in the initial rate studies with tromethamine buffer, suggesting that a sodium couple is not involved in the difference in directional transport and that the results seen previously (1) with sodium and potassium phosphate buffers might be better explained in terms of the effects of phosphate on the intestinal mucosa (2).

Qualitatively, it can be seen that when the values in row C are close to 100% (*i.e.*, no change between initial and fourth rate), the ratios calculated by all three methods (A, B, and F) are almost identical. If one considers the change in rates (percent of initial) over the four experiments as a measure of the loss of biologic integrity of the intestinal tissue, it would appear that the best buffer for maintenance of the membrane in a constant condition is the Krebs bicarbonate (full strength sodium) buffer. In comparison, the sodium phosphate and tromethamine buffers appear to have less ability to maintain gut viability but are superior to unbuffered 0.9% sodium chloride solutions or the modified Krebs buffers employed. In unbuffered 0.9% sodium chloride solution, there is a sharp rise in the serosal (NE) transfer of salicylate ions as compared to mucosal (E) transfer (242 and 113% of initial, respectively, as shown in Table II), while for acetanilide, a nonionized drug, there is very little change in the rate of passage in either direction (Table III).

The lag time before absorption of salicylate ion begins falls into the range of 5–7 min. for all of the buffers, with the exception of the Krebs bicarbonate buffers where potassium replaced sodium. However, the nonionized acetanilide yields lag times that are not significantly different than zero (at 95% confidence levels) for all three of the buffers studied. The results indicate that the lag time for salicylate-ion absorption is 7 to 8 times greater than that for acetanilide.

The difference between average initial rates (B) and the average of all rates (A) is presented in Table IV. A good correlation may be seen between the values taken as representing intestinal integrity (C values in Tables I–III) and the level of significance of the differences between the two sets of measurements compared in Table

Table IV—Comparison of Levels of Significance for Differences between Average Initial Rates and Average Rates for Four Consecutive Runs

Drug	Buffer	Level of Significance: Between Everted Rates	Level of Significance: Between Noneverted Rates
Salicylate	Krebs bicarbonate—full strength sodium ion	N.S.	N.S.
	Krebs bicarbonate—one-half sodium ion replaced	$p < 0.001$	$p < 0.001$
	Krebs bicarbonate—all sodium ion replaced	$p < 0.01$	$p < 0.001$
	Sodium phosphate, pH 7.4 buffer (1)	$p < 0.002$	$p < 0.005$
	0.154 *M* Tromethamine buffer, pH 7.4	$p < 0.002$	$p < 0.05$
	0.9% Sodium chloride, pH 6.6	N.S.	N.S.
Acetanilide	Krebs bicarbonate—full strength sodium ion	N.S.	N.S.
	Krebs bicarbonate—all sodium replaced	$p < 0.05$	N.S.
	0.9% Sodium chloride, pH 6.6	N.S.	N.S.

Table V—Level of Significance for Differences between Average Initial Everted Transfer Rates and between Total Amounts Transferred for Salicylate Ion and Acetanilide in Different Buffers

Drug	Buffer 1	Buffer 2	Average Initial Everted Rates	Total Amounts Transferred (E)
Salicylate	Krebs bicarbonate—full strength sodium	Krebs bicarbonate—one-half sodium replaced	$p < 0.01$	$p < 0.1$
	Krebs bicarbonate—full strength sodium	Krebs bicarbonate—all sodium replaced	$p < 0.001$	$p < 0.02$
	Krebs bicarbonate—full strength sodium	Sodium phosphate, pH 7.4	N.S. ($p < 0.8$)	$p < 0.1$
	Krebs bicarbonate—full strength sodium	0.154 *M* Tromethamine, pH 7.4,	$p < 0.05$	N.S. ($p < 0.2$)
	Krebs bicarbonate—full strength sodium	0.9% Sodium chloride, pH 6.6	N.S. ($p < 0.5$)	N.S. ($p < 0.5$)
	Sodium phosphate, pH 7.4	0.154 *M* Tromethamine, pH 7.4	$p < 0.001$	$p < 0.001$
	Sodium phosphate, pH 7.4	0.9% Sodium chloride, pH 6.6	N.S. ($p < 0.5$)	$p < 0.05$
	0.154 *M* Tromethamine, pH 7.4	0.9% Sodium chloride, pH 6.6	$p < 0.005$	$p < 0.02$
Acetanilide	Krebs bicarbonate—full strength sodium	Krebs bicarbonate—all sodium replaced	$p < 0.02$	N.S. ($p < 0.5$)
	Krebs bicarbonate—full strength sodium	0.9% Sodium chloride, pH 6.6	N.S. ($p < 0.2$)	N.S. ($p < 0.2$)
	Krebs bicarbonate—all sodium replaced	0.9% Sodium chloride, pH 6.6	$p < 0.05$	N.S. ($p < 0.5$)

IV. For example, in Krebs bicarbonate (full strength sodium ion) where intestinal membrane integrity is assumed to be the best, there is no difference between average rates obtained from initial rate–30-min. experiments or from 2-hr. experiments. However, whenever the sodium ion is replaced (in the buffers studied here), the rates increase with time, and the absolute values of the rates differ significantly between the 30-min. and 2-hr. experiments. A significant difference is also observed in the comparison of everted rates for acetanilide in Krebs bicarbonate buffer where all the sodium was replaced, indicating that the presence of potassium has a deteriorating effect on the membrane which affects the transport of the unionized acetanilide as well as the ionized salicylate.

Table V presents a comparison between two methods utilized to determine the effect of buffer constituents on *in vitro* drug absorption [*i.e.*, initial rates used in this laboratory (1, 2) and total amounts transferred as used by Mayersohn and Gibaldi (3, 4)]. From the data in Table V, it appears that under the conditions used in this work, the average of initial rates is a more sensitive measure than the total amounts transferred, since the average initial rate is not influenced to the same degree by the *in vitro* degradation of the gut membrane. When there is a significant difference between rates in any two buffers, the level of significance is generally greater for the average initial rate data (values of $p < 0.1$ considered significant here). The cases where there is no significant difference also seem to be better demonstrated by the average initial rate data, as seen by comparison of the values in parentheses in Table V. One particularly interesting comparison is the rate of salicylate-ion transfer in Krebs bicarbonate (full strength sodium-ion buffer) *versus* the rate in 0.154 *M* tromethamine buffer. Mayersohn and Gibaldi (4) reported no significant difference between total amounts of salicylate transferred in these two buffers. Similarly, in the present work, no significant difference was found using total amounts transferred. However, there was a difference between the two buffers at the 95% confidence level when initial transfer rates were employed. It must be emphasized, however, that these discrepancies may be a result of the methodology employed in this work, since relative values of the standard deviations reported for total amounts transferred in this work are greater than those reported by Mayersohn and Gibaldi (3, 4).

An interesting comparison can be made with respect to the effects of replacing sodium by potassium on the transport of the two drugs studied. In Table I it can be seen that the replacement of sodium with potassium in Krebs bicarbonate causes a 60–80% reduction (depending on whether the A or B values are compared) in the transport of salicylate ion, while in Table III only a 15–30% reduction is observed for nonionized acetanilide. As was pointed out initially by Bosackova and Crane (7) and recently by Mayersohn and Gibaldi (4), the uptake of water by the intestinal membrane increases as a function of the potassium ion causing a marked increase in the swelling of the tissues. In the present work, this led to a very significant decrease in the transport of the ionized salicylate but only to a slight decrease in the transport of the nonionized acetanilide. These results might be intepreted as indicating that the two drugs were transported through different pathways. Possibly the salicylate ion diffuses through aqueous pores, as is suggested by Crone and Keen (8) for the pyridinium aldoximes, and that in the presence of potassium where the membrane is swollen, these pores are effectively blocked. However, acetanilide might diffuse through the lipid portions of the membrane and be affected to a lesser extent by the amount of water in the tissues. In either case, an increase in transfer rates with time is seen as the membrane deteriorates in the presence of potassium. These suggestions are made with some hesitation, however; although Mayersohn and Gibaldi (4) found a similar percent inhibition of the salicylate transfer in the presence of large amounts of potassium as was found in this work for the 2-hr. experiments, they found even greater inhibition for the nonionized drugs, riboflavin and sulfanilamide. Work is continuing in this area in an attempt to explain these differences.

Recently, Levine *et al.* (9) pointed out that everted intestinal rat sacs are morphologically intact after inversion but that they progressively lose structural integrity in an oxygenated Krebs–Henseleit buffer, pH 7.4, containing 1 g. glucose/l. Histological studies indicate that there is total disruption of the epithelial border after 1 hr. Since no significant increase in the everted transfer rates of either salicylate or acetanilide was observed in this buffer (without glucose) in the present work, it would appear that the epithelial border may not be the rate-limiting barrier to transport across the *in vitro* rat intestinal segment. If this was true, the use of the everted intestinal sac as a screen for the possible absorption characteristics of a new drug would appear questionable. This was pointed out in the previous report (1) with respect to some ionized drugs.

REFERENCES

(1) R. H. Turner, C. S. Mehta, and L. Z. Benet, *J. Pharm. Sci.*, **59,** 590(1970).

(2) R. H. Turner and L. Z. Benet, to be published.

(3) M. Mayersohn and M. Gibaldi, *J. Pharm. Sci.*, **58,** 1429 (1969).

(4) M. Mayersohn and M. Gibaldi, *Biochim. Biophys. Acta*, **196,** 296(1970).

(5) S. Feldman and M. Gibaldi, *J. Pharm. Sci.*, **58,** 425(1969).

(6) H. A. Krebs and K. Henseleit, *Hoppe-Seyler's Z. Physiol. Chem.*, **210,** 33(1932).

(7) J. Bosackova and R. K. Crane, *Biochim. Biophys. Acta*, **102,** 423(1965).

(8) H. D. Crone and T. E. B. Keen, *Brit. J. Pharmacol.*, **35,** 304 (1969).

(9) R. R. Levine, W. F. McNary, P. J. Kornguth, and R. LeBlanc, *Eur. J. Pharmacol.*, **9,** 211(1970).

ACKNOWLEDGMENTS AND ADDRESSES

Received March 25, 1970, from the *School of Pharmacy, University of California, San Francisco, CA 94122*

Accepted for publication August 26, 1970.

Presented in part to the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Washington, D. C. meeting, April 1970.

This investigation was supported by PHS Research Grant No 17243 from the National Institute of General Medical Sciences, by a grant-in-aid from the research funds of the Academic Senate, San Francisco Division, University of California, and by General Research Support Grant FR-05435 from the National Institutes of Health.

The authors thank Miss Marilyn Huckleberry for technical assistance.

Solubility Parameter of Selected Sulfonamides

CHIMIN SUNWOO* and HENRY EISEN

Abstract □ This investigation was primarily concerned with the application of Hildebrand's solubility equation to the determination of the solubility parameter of selected sulfonamides. The vapor pressure of several selected solvent systems was determined by an isoteniscopic method. These data were used to calculate the solubility parameter of the mixed solvents. The heat of fusion of each sulfonamide was determined by differential thermal analysis. The solubility of the sulfonamides was measured in a series of solvent blends varying in polarity, and the solubility parameter of the sulfonamides was determined from their solubility data. An attempt was made to correlate the solubility parameter with the dielectric constant. The results were found to be linear with respect to the solvent blends of alcohol–water, alcohol–water–propylene glycol, water–glycerin, and dimethylacetamide–water–glycerin. Hildebrand's solubility concept appears to be a useful tool for predicting drug solubility where the solubility parameters of drug and solvent system are close to each other.

Keyphrases □ Sulfonamides—solubility parameter □ Solubility, solubility parameter—sulfonamides □ Isoteniscopic method—solvent vapor pressure determination □ Differential thermal analysis—heat of fusion, sulfonamides □ UV spectrophotometry—analysis

The wide application of liquid dosage forms used in pharmaceuticals is demonstrable proof of the importance of solutions in formulation. A convenient and reliable means of determining enhanced solubility of pharmaceutical substances has long been sought by formulation workers. The concept of solubility parameters has been found to be useful, particularly in guiding the selection of solvents for film formers and in the formulation of paints, varnishes, and printing inks (1). Hildebrand's solubility equation (2) was applied by Chertkoff and Martin (3) and by Restaino and Martin (4) to determine the solubility of benzoic acid

Table I—Heat of Vaporization and Solubility Parameter of Solvents Employed

Solvent	Slope	ΔH_v, kcal./mole at 25°, Determined	ΔH_v, kcal./mole at 25°, Reported Value	δ at 25°, Determined	δ at 25°, Reported Value
Water, double-distilled	−2250 ± 6	10.296	9.730[a]	23.2	23.4[c]
Absolute alcohol	−2136 ± 5	9.765	9.22[a]	12.5	—
Absolute alcohol, distilled	−2233 ± 5	10.217	10.08[b]	12.8	12.7[c] 13.0[d]
Propylene glycol	−2700 ± 7	12.357	—	12.6	—
Dimethyl-acetamide	−2297 ± 4	10.454	—	10.3	10.6[e]
2-Ethoxy-ethanol	−2516 ± 7	11.748	—	10.7	9.9[c]

[a] *Reference 13.* [b] *Reference 14.* [c] *Reference 15.* [d] *Reference 4.* [e] *Reference 16.*

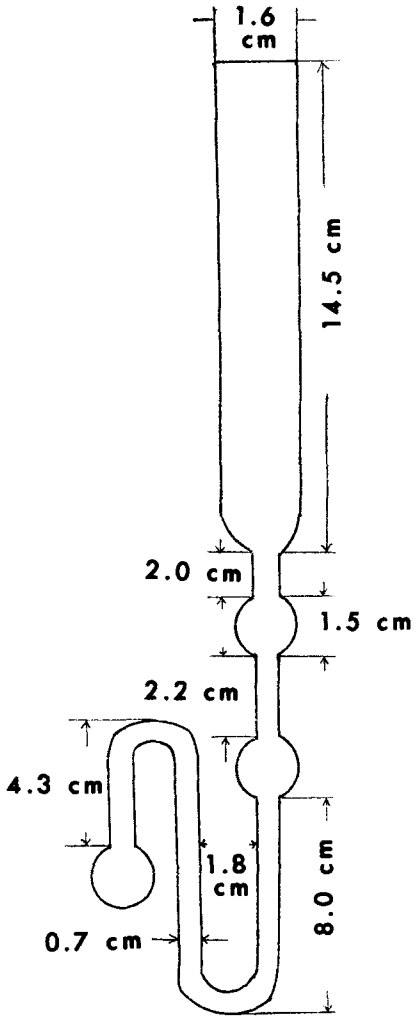

Figure 1—*Schematic diagram of the isoteniscope used for vapor pressure determination.*