(4) W. P. Larson and E. Nelson, *ibid.*, **21**, 278 (1924).
(5) W. P. Larson R. D. Evans, and E. Nelson, *ibid.*, **22**, 194 (1924).
(6) T. B. Hartzell, *J. Am. Dent. Assoc.*, **12**, 1452 (1925).
(7) W. P. Larson and H. Eder, *J. Am. Med. Assoc.*, **86**, 998 (1926).
(8) H. E. Jones, *Br. J. Dent. Sci.*, **70**, 171 (1927).
(9) A. S. Hopkins, *Dent. Cosmos*, **72**, 830 (1930).
(10) S. F. Seeley, *Ann. Surg.*, **96**, 350 (1932).
(11) S. V. Mead, *J. Dent. Res.*, **16**, 41 (1937).
(12) W. F. Dossenback and H. R. Muhlemann, *Helv. Odontol. Acta*, **5**, 25 (1961).
(13) H. E. Schroeder, T. M. Marthaler, and H. R. Muhlemann, *ibid.*, **6**, 6 (1962).
(14) D. G. Demmers and C. M. Belting, *J. Periodontol.*, **38**, 294 (1967).
(15) W. van Betteray and P. Riethe, *Caries Res.*, **7**, 85 (1973).
(16) R. J. Fitzgerald and P. H. Keyes, *J. Am. Dent. Assoc.*, **61**, 9 (1960).
(17) R. J. Fitzgerald, H. V. Jordan, and H. R. Stanley, *J. Dent. Res.*, **39**, 923 (1960).
(18) B. Krasse, *Arch. Oral Biol.*, **11**, 429 (1966).
(19) N. W. Littleton, S. Kakehashi, and R. J. Fitzgerald, *ibid.*, **15**, 461 (1970).
(20) J. D. de Stoppelaar, J. van Houte, and O. Backer Dirks, *Caries Res.*, **3**, 190 (1969).
(21) T. Ikeda, H. J. Sandham, and E. L. Bradley, *Arch. Oral Biol.*, **18**, 555 (1973).
(22) S. Duchin and J. van Houte, *ibid.*, **23**, 779 (1978).
(23) B. Kohler, B. M. Pettersson, and D. Bratthall, *Scand. J. Dent. Res.*, **89**, 19 (1981).
(24) R. A. Coburn, *J. Dent. Res.*, **58**, 2396 (1979).
(25) R. M. McCabe, P. H. Keyes, and A. Howell, Jr., *Arch. Oral Biol.*, **12**, 1653 (1967).
(26) J. M. Tanzer, Y. Reid, and W. Reid, *Antimicrob. Agents Chemother.*, **1**, 376 (1972).
(27) J. M. Tanzer, A. M. Slee, B. Kamay, and E. Scheer, *ibid.*, **12**, 107 (1977).
(28) *Idem.*, **15**, 408 (1979).
(29) R. T. Evans, P. J. Baker, R. A. Coburn, and R. J. Genco, *J. Dent. Res.*, **56**, 559 (1977).
(30) N. Tinanoff, J. M. Tanzer, and M. L. Freedman, *Infect. Immun.*, **21**, 1010 (1978).
(31) H. V. Jordan, R. J. Fitzgerald, and A. E. Bowler, *J. Dent. Res.*, **39**, 116 (1960).
(32) J. A. Washington, II., and A. L. Barry, in "Manual of Clinical Microbiology," 2nd ed., E. H. Lennette, E. H. Spaulding, and J. P. Truant, Eds., American Society for Microbilogy, Washington, D.C., 1974, p. 414.
(33) J. M. Tanzer, A. M. Slee, and B. A. Kamay, *Antimicrob. Agents Chemother.*, **12**, 721 (1977).
(34) E. Freese, C. W. Sheu, and E. Galliers, *Nature (London)*, **241**, 321 (1973).

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COMMUNICATIONS

Use of Continuous Withdrawal Technique to Estimate the Initial Area Under the Curve

Keyphrases □ Continuous withdrawal technique—estimation of the initial area under the plasma concentration–time curve □ Pharmacokinetics—use of the continuous withdrawal technique to estimate the initial area under the plasma concentration–time curve □ Clearance—use of the continuous withdrawal technique to estimate the plasma concentration–time curve

To the Editor:

Recently it was pointed out (1) that the instantaneous input hypothesis of the conventional compartmental models may overestimate the area under the plasma concentration *versus* time curve (*AUC*). It was shown that the *AUC* of furosemide could be overestimated, using extrapolation to time zero, by as much as 20% in the dog. This is consistent with data found for Evans blue when used to measure cardiac output (2). In the first 12 sec after an intravenous bolus injection, no dye could be found in the arterial blood; then, the concentration rose gradually, not reaching a peak for several more seconds. Thereafter, it declined for 10 sec before it rose again to a second, lower peak at ~38 sec.

Based on physiological considerations, the delay could be explained as the time needed to reach the sampling site from the administration site, with the second peak perhaps resulting from recirculation of the dye. This phenomenon is important for drugs with a fast initial decline, particularly where the initial phase potentially contains a significant portion of the *AUC*. It is the purpose of this communication to illustrate an ideal method for determination of the *AUC* in the initial period which reflects accurate values, regardless of distribution and elimination rates and sampling sites. This method is based upon the continuous sampling technique described previously (3) to determine various pharmacokinetic parameters, and has been used in our laboratory for several years with good success (4).

A peripheral vein was cannulated prior to the start of the study to facilitate constant blood withdrawal. The drug was then administered in another vein after initiating the constant withdrawal.

The amount of drug in collected plasma, A_w, withdrawn over time t, when a constant blood withdrawal at rate $\dot{V}$ is carried out, can be obtained from:

$$A_w = (1 - H)\dot{V} \int_0^t C dt \qquad \text{(Eq. 1)}$$

where C is the concentration of drug in the plasma at any time, and H is the hematocrit. The area under the curve, $\int_0^t C dt$, in the time period, t, therefore, can be obtained by the ratio $A_w/[\dot{V}(1 - H)]$. Because the plasma drug amount withdrawn is equal to the total volume withdrawn, V_w, multiplied by the plasma concentration in the withdrawn sample, C_w, and because:

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$$(1 - H)V = V_w/t \qquad \text{(Eq. 2)}$$

the following is obtained:

$$\int_0^t Cdt = C_w t \qquad \text{(Eq. 3)}$$

By keeping a constant withdrawal of blood and measuring the concentration of drug in the sample and the time of withdrawal, the *AUC* can readily and accurately be determined without the problems of extrapolation.

This method is especially valuable in the time just after administration of an intravenous bolus dose, because no assumptions regarding the distribution and elimination need to be made. At later time points, when it is more desirable to determine the various rate constants, the continuous withdrawal can be terminated and individual blood samples then can be taken.

We have used this method extensively for indocyanine green clearance in rabbits, where the initial half-life after a 0.1-mg/kg iv. bolus dose is ~0.8 min. Approximately 40–60% of the total area can be estimated to be located between 0 and 1 min if an extrapolation is carried out when the first sample is taken at 1 min. However, when a continuous withdrawal is carried out, only 20–30% of the total area is obtained in the first minute. Therefore, as much as a 1.5- to twofold underestimation of the clearance can be made when using single venous plasma concentration time points with extrapolation, rather than a continuous withdrawal technique for indocyanine green clearance in rabbits.

(1) W. L. Chiou, G. Lam, M.-L. Chen, and M. G. Lee, *J. Pharm. Sci.*, **70**, 1037 (1981).

(2) I. J. Fox and E. H. Wood, in, "Medical Physics," vol. 3, O. Glasser, Ed., Year Book Publisher, Chicago, Ill., 1960, pp. 155–163.

(3) B. Vogelstein, A. A. Kowarski, and P. S. Lietman, *Clin Pharmacol. Ther.*, **22**, 131 (1977).

(4) T. W. Guentert and S. Øie, *J. Pharmacol. Exp. Ther.*, **215**, 165 (1980).

Jin-ding Huang
Svein Øie ˣ
Department of Pharmacy S-926
University of California
San Francisco, CA 94143

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Schiff Base Formation with Nitrogen of a Sulfonamido Group

Keyphrases □ Benzothiadiazones—Schiff base formation with the nitrogen of a sulfonamido group □ Schiff bases—formation with the nitrogen of a sulfonamido group □ Colored complexes—Schiff base formation with the nitrogen of a sulfonamido group

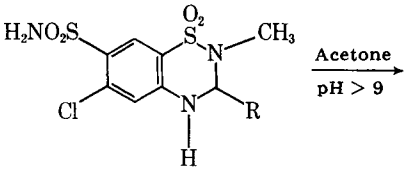
To the Editor:

Recently an unexpected result was reported (1): A violet-colored compound was reported to have formed on TLC plates when benzothiadiazines (I) were sprayed with acidified *p*-dimethylaminobenzaldehyde (II). Hennig *et al.* (1) stated that the type of colored complex formed between I and II was difficult to postulate, since there was no apparent record of Schiff base formation with the nitrogen of a sulfonamido group. We have an explanation that attempts to rationalize the formation of this colored complex.

Our studies with polythiazide (I*a*) have shown that II as well as acidified vanillin (II*a*) form a violet-colored compound. We have identified this compound to be a product of a double aldol condensation between acetone, used as the solvent for spotting I, and II or II*a* to form in each case, respectively, substituted distyryl ketone (V).

Bahner and Schultze (2) reported the formation of divanillylidene acetone (V*a*) in a photometric estimation of acetone. Compound V*a* is yellow in alkaline solutions and develops into a pink-violet color when acidified with hydrochloric acid.

We have noted that at pH 9 polythiazide partially decomposes into 4-amino-2-chloro-5-(methylsulfamyl)-benzenesulfonamide (VI). Compound VI reacts with acetone to form a Schiff base (see Scheme). The ionized form of this Schiff base has an R_f = 0.55 in an ethyl acetate–benzene (8:2) system. When sprayed with acidified vanillin, the Schiff base hydrolyzes and the acetone thus liberated reacts with vanillin to give a violet-colored spot on the chromatograms. A similar reaction takes place with acidified *p*-dimethylaminobenzaldehyde used as a spray reagent.



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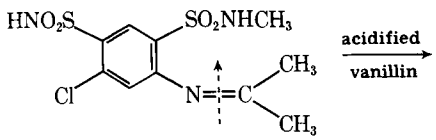
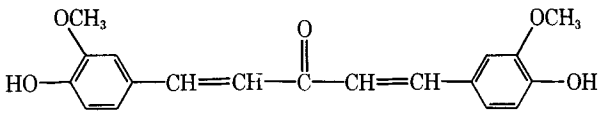


VI schiff base



V*a*

Vanillin is often used as flavoring to mask the unpleasant taste of polythiazide tablets. If the alcohol that is used as a granulating liquid contains acetone as an impurity, the reaction in the Scheme may cause an incompatability problem resulting in colored granules.

(1) U. G. Hennig, R. E. Moskalyk, L. G. Chatten, and S. F. Chan, *J. Pharm. Sci.*, **70**, 317 (1981).

(2) F. Bahner and G. Schultze, *Z. Klin. Chem.*, **3**, 10, 1965; through Chem. Abstr., 16616-b, 1965.

A. B. Thakur
*S. Dayal*ˣ
Pharma Research and Development Department
Pfizer Ltd., Express Towers
Nariman Point
Bombay, India

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