Determination of Sulfonamides and Local Anesthetics with 9-Chloroacridine by Quenching Fluorometry

J. T. STEWART▲ and R. E. WILKIN

Abstract □ Sulfonamides and local anesthetics containing a primary aromatic amino group react with 9-chloroacridine to yield aminoacridine hydrochlorides. The formation of these derivatives results in quenching of fluorescence of the 9-chloroacridine reagent solution. Monitoring the fluorescence at activation and emission wavelengths of 385 and 420 nm., respectively, enables one to analyze drug in the 10^{-5}–10^{-6} *M* range. The method was applied to various sulfonamides, local anesthetics, and mixtures of pharmaceuticals containing these entities. A comparative study of this technique was made with other known fluorometric procedures.

Keyphrases □ Sulfonamides, containing primary aromatic amine—analysis, 9-chloroacridine, quenching fluorometry □ Anesthetics, local, containing primary aromatic amine—analysis, 9-chloroacridine, quenching fluorometry □ 9-Chloroacridine—analysis of sulfonamides and local anesthetics containing primary aromatic amine, quenching fluorometry □ Fluorometry, quenching—analysis, primary aromatic amine compounds with 9-chloroacridine

Fluorometric methods for the analysis of pharmaceuticals such as sulfonamides and local anesthetics containing a primary aromatic amino moiety were reviewed by various authors (1–4). These procedures involve derivation of the amino group with a reagent, which may or may not possess fluorescence itself, followed by measurement of the resulting fluorescent product. Amano and Mizukami (3) used *o*-phthaldehyde and 4,5-methylenedioxyphthaldehyde as reagents and obtained fluorescent substituted phthalimidine products that could be employed to determine sulfonamides in the 10^{-5}–10^{-6} *M* range. Recently, Dombrowski and Pratt (4) introduced the use of 2,6-diaminopyridine as a new fluorescence reagent and showed that the sensitivity was in the 10^{-6}–10^{-7} *M* range for sulfanilamide and benzocaine.

No additional information could be found in the literature regarding fluorescence methods for other local anesthetics. It is assumed that the Amano and Mizukami (3) method can also be employed for local anesthetics since it involves reaction with the amine function of the drug. In all the reported analyses, the reaction mixture possesses an increased fluorescence over that of the blank solution.

It was observed recently in this laboratory that the reaction of sulfonamides and/or local anesthetics with 9-chloroacridine to yield 9-substituted aminoacridines resulted in a decrease in fluerescence of the reaction mixture compared to that of the blank acridine solution. This condition was advantageous for the analysis of trace quantities of sulfa and/or local anesthetics based upon measurement of the quenching of fluorescence of the acridine solution caused by the presence of drug. Determination of the drug is obtained even in the presence of a fourfold excess of acridine reagent.

In this study, the method was applied to several sulfonamides and local anesthetics. Its usefulness in the analysis of these drugs in combination with other pharmaceuticals is shown. A comparative study of this technique was made with other known fluorometric procedures.

EXPERIMENTAL

Apparatus—Fluorescent spectra and measurements were made with a spectrophotofluorometer[1]. Clear, fused quartz cells (12.5 × 45 mm.) were used as sample cells, and the instrument supplier's slit arrangement number three was used throughout the work.

Reagents and Chemicals—9-Chloroacridine[2] was used as the fluorescent reagent. Powdered samples of sulfamethizole[3], sulfisoxazole[4], sulfamethoxypyridazine[5], sulfanilamide[6], benzocaine[2], butesin[7], butacaine sulfate[7], and metabutethamine hydrochloride[8] were used in the analytical procedure for preparation of standard curves. All other chemicals and pharmaceuticals used were the highest grade of the commercially available materials.

Solutions were prepared by dissolving weighed amounts of the sulfonamides, benzocaine, and butesin in ethanol. The remaining local anesthetics were dissolved in water. Solutions of 9-chloroacridine were prepared by dissolving the acridine in tetrahydrofuran and storing in a light-resistant volumetric flask[9].

Procedure—A quantity of an ethanolic or water solution of a sulfonamide and/or local anesthetic in the range 6.25–200 × 10^{-5} *M* was placed in a 50-ml. volumetric flask. If the drug was present as a hydrochloride salt, a 0.1 *N* sodium hydroxide solution was added dropwise until a neutral pH was obtained using indicator pH paper.

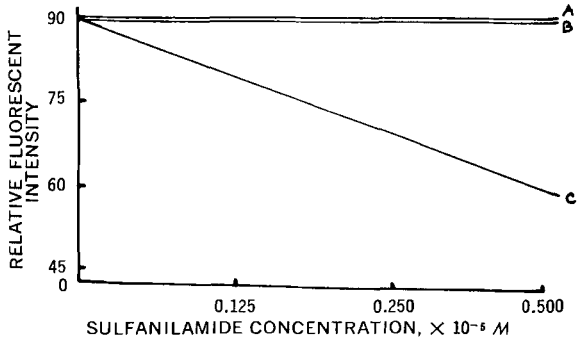

Figure 1—*Relative fluorescent intensity readings of sulfanilamide solutions* versus *blank and background solutions. Key: A, blank solution containing 9-chloroacridine reagent; B, background solution containing succinylsulfathiazole, tetracycline hydrochloride, and 9-chloroacridine; and C, sample solution containing sulfanilamide along with succinylsulfathiazole, tetracycline hydrochloride, and 9-chloroacridine.*

[1] Aminco-Bowman.
[2] Eastman Organic Chemicals.
[3] Ayerst Laboratories, New York, N. Y.
[4] Hoffmann-La Roche, Inc., Nutley, N. J.
[5] Parke-Davis and Co., Detroit, Mich.
[6] American Pharmaceutical Co., New York, N. Y.
[7] Abbott Laboratories, North Chicago, Ill.
[8] Novocal Chemical Manufacturing Co., Inc., Brooklyn, N. Y.
[9] Low actinic volumetric flask (Corning No. 55640).

Table I—Analysis of Known Sulfonamide and/or Local Anesthetic Mixtures for Sulfonamide and Local Anesthetic

Mixture	Components, Concentration of 4×10^{-5} *M*	Found, $M \times 10^{-5}$	Percent of Theory
I[a]	Sulfisoxazole Succinylsulfathiazole[b]	4.00	100.0
II	Sulfisoxazole Tetracycline hydrochloride[c]	4.12	103.0
III[d]	Sulfanilamide Succinylsulfathiazole Tetracycline hydrochloride	3.86	96.6
IV	Metabutethamine hydrochloride Chlorpromazine hydrochloride[e] Phenobarbital[f]	4.00	100.0
V	Butacaine sulfate Ephedrine sulfate[g] Oxazepam[h]	3.87	96.7
VI	Benzocaine Aspirin[i]	4.00	100.0

[a] Mixture analyzed for sulfisoxazole content. [b] Marketed as Sulfasuxidine by Merck, Sharpe & Dohme, Rahway, N. J. [c] Chas. Pfizer and Co., Inc., New York, N. Y. [d] Mixture analyzed for sulfanilamide content. [e] Marketed as Thorazine Hydrochloride by Smith Kline & French, Philadelphia, Pa. [f] Mallinckrodt Chemicals, St. Louis, Mo. [g] Ruger Chemical Co., Inc., Irvington-on-Hudson, N. Y. [h] Marketed as Serax by Wyeth Laboratories, Philadelphia, Pa. [i] Merck, Sharpe & Dohme, Rahway, N. J.

To this solution was added a volume of the acridine-tetrahydrofuran solution equivalent to an approximate one- to fourfold molar excess. Then the pH was adjusted to 4 using indicator pH paper with 10% v/v aqueous hydrochloric acid. The mixture was heated at 60° for 10 min. and cooled, and ethanol was added to volume. The decrease in the fluorescence of the reaction mixture compared to the corresponding reagent blank was measured using activation and emission wavelength settings of 385 and 420 nm., respectively.

RESULTS AND DISCUSSION

In the analytical procedure, a sulfonamide and/or local anesthetic containing a primary aromatic amino group reacts with 9-chloroacridine to yield highly colored aminoacridine hydrochlorides. Previous reports from this laboratory showed the reaction to be applicable to colorimetric determinations of sulfonamides and local anesthetics as well as most primary aromatic amines (5-7). It was observed that the formation of these derivatives resulted in a quenching of the fluorescence of the 9-chloroacridine reagent solution. Monitoring the disappearance of fluorescence caused by the reaction of drug and acridine at the activation and emission wavelengths (385/420 nm.) of 9-chloroacridine is the basis of the fluorometric determination described here. The fluorescence-quenching effect observed in this study is shown in Fig. 1 in terms of relative fluorescent intensity readings for blank and background solutions *versus* sample solutions containing sulfanilamide. Samples of local anesthetics also gave analogous results. Differences in fluorescent intensity readings between blank and sample solutions plotted against various concentrations of drug give linear logarithmic relationships for standard curves. The analysis of unknown concentrations of sulfonamide and/or local anesthetic in this procedure requires the direct comparison of the fluorescence quenching of the unknown to that of a known concentration under the same conditions. A drug concentration range of $0.125\text{–}4 \times 10^{-5}$ *M* can be successfully assayed using this method, which makes it comparable to other fluorometric determinations for drugs containing a primary aromatic amino group. For best results, no more than a fourfold excess quantity of acridine reagent should be employed in the procedure.

Quantitative data from several systems shown in Table I reveal that use of this procedure permits the determination of sulfonamides and/or local anesthetics in the presence of miscellaneous pharmaceuticals representing a variety of chemical structures.

Table II—Comparison of 4×10^{-5} *M* Solutions of Sulfanilamide and Butesin by the 9-Chloroacridine Method and Other Known Fluorometric Methods

Compound	—Standard Deviation of Mean, %— 9-Chloroacridine Method	*o*-Phthaldehyde Method[a]	2,6-Diaminopyridine Method[b]
Sulfanilamide	0.50	3.42	2.34
Butesin	0.29	0.38	2.54

[a] See *Reference 3*. [b] See *Reference 4*.

Favorable characteristics of the method are that it requires less than 0.5 hr. for a complete analysis of sulfonamide and/or local anesthetic and only one reagent solution is required. These are distinct advantages over the diazotization procedure of Dombrowski and Pratt (4), which requires many manipulative steps and numerous reagents, and over the aldehyde method of Amano and Mizukami (3), which requires reaction times of 30–45 min.

Heating of the analytical solution is essential to ensure a more complete reaction. Maximum yield of product can be obtained by heating the solutions at 60° for 10 min. Repeated readings for a series of different samples indicated that the solutions were stable for up to 1 day. Great care was taken to prevent overexposure of the samples to the xenon source of the fluorometer as well as to prevent contamination of solutions and cells used in the analysis.

Comparison of the 9-chloroacridine method to other known fluorometric procedures for sulfonamides and/or local anesthetics is shown in Table II in terms of percent standard deviation of the mean for 4×10^{-5} *M* concentrations of sulfanilamide and butesin (8). Each literature method was tested in this laboratory and was in good agreement with reported data. It can be seen that the acridine method is comparable to the existing procedures and, in most cases, reproducibility of the acridine procedure is better than the other methods listed.

In summation, determinations with 9-chloroacridine provide a relatively simple and rapid means of determining sulfonamides and/or local anesthetics in the presence of various other drugs. Comparison with other fluorometric procedures demonstrates its advantages and effectiveness. The method differs from other widely used techniques in that it involves quenching of fluorescence rather than increased fluorescence intensity as the basis for the fluorometric measurements. The procedure should be applicable in the analysis of any primary aromatic amine.

REFERENCES

(1) C. E. White, *Anal. Chem.*, **42**, 66R(1970).

(2) J. A. F. deSilva and L. D'Arconte, *J. Forensic Sci.*, **14**, 189 (1969).

(3) T. Amano and S. Mizukami, *J. Pharm. Soc. Jap.*, **85**, 1035 (1965).

(4) L. J. Dombrowski and E. L. Pratt, *Anal. Chem.*, **43**, 1042 (1971).

(5) J. T. Stewart, T. D. Shaw, and A. B. Ray, *ibid.*, **41**, 360 (1969).

(6) J. T. Stewart, A. B. Ray, and W. B. Fackler, *J. Pharm. Sci.*, **58**, 1261(1969).

(7) J. T. Stewart and D. M. Lotti, *ibid.*, **59**, 838(1970).

(8) K. A. Connors, "A Textbook of Pharmaceutical Analysis," Wiley, New York, N. Y., 1967, p. 572.

ACKNOWLEDGMENTS AND ADDRESSES

Received September 20, 1971, from the *Analytical Laboratory, Department of Medicinal Chemistry, School of Pharmacy, University of Georgia, Athens, GA 30601*

Accepted for publication November 30, 1971.

Supported in part the National Science Foundation Research Grant GY-8777.

The authors acknowledge the technical help of Mrs. Javotte Driggers and Mr. Don Griffin.

▲ To whom inquiries should be directed.