Semiautomated Fluorometric and Colorimetric Methods for the Determination of Reserpine in Single Tablets

B. N. KABADI, A. T. WARREN, and C. H. NEWMAN

Abstract □ Semiautomated fluorometric and colorimetric methods for the determination of reserpine in single tablets are described for 0.25- and 0.5-mg. potency tablets. A simple practical manual method for the extraction of reserpine from a large number of tablets is outlined. The yellow-green fluorogenic product formed by the reaction of reserpine and nitrous acid is analyzed in the automatic analyzer system. Using excitation maximum of 390 mμ the fluoresence is measured at 510 mμ. In the colorimetric determination the absorbance is measured spectrophotometrically at 390 mμ. Using the colorimetric method, the effect of temperature on the reaction of reserpine and nitrous acid, and the effect of nitrogen gas on the recordings are investigated. In the fluorometric method there is no interference by the excipients and no effect of nitrogen gas on the recordings is noted. Further, this is more sensitive than the colorimetric method.

Keyphrases □ Reserpine—single tablet analysis □ Automated procedure—reserpine tablet analysis □ Diagram—automated analysis procedure □ Nitrous acid—reagent □ Fluorometry—analysis

For assurance of quality and as part of a program for testing content uniformity of single tablets, the authors have undertaken the task of developing new automated methods for the determination of reserpine in compressed tablets.

The reaction of reserpine with nitrous acid to produce a yellow-green fluorescent product and its subsequent fluorometric determination is a well-known pharmaceutical analytical procedure (1–3). The mechanism of the yellow-green fluorogen formation has been extensively studied (4). This forms the basis of the authors fluorometric and colorimetric methods. The colorimetric method is the official procedure to determine reserpine in tablets (5, 6). The fluorometric method is recommended to determine trace of reserpine in feeds (7, 8).

In this work reserpine tablets[1] of 0.25- and 0.5-mg. potency have been analyzed using an automatic analyzer[2] method.

EXPERIMENTAL[3]

Reagents—*Sulfuric Acid Reagent*—Two and $^{8}/_{10}$ ml. of spectral grade sulfuric acid was dissolved in 1 l. of 20% (v/v) methanol in distilled water.

Sodium Nitrite Reagent—One hundred milligrams of sodium nitrite[4] was dissolved in 1 l. of distilled water.

Ammonium Sulfamate Reagent—Seven hundred milligrams of ammonium sulfamate[5] was dissolved in 1 l. of distilled water.

Nonionic Surfactant Reagent—One-half gram of polysorbate 80 was dissolved in 100-ml. distilled water at room temperature.

Standard Preparation—In the colorimetric determination the reserpine standards were prepared as follows: 25 mg. of USP reserpine Reference Standard, previously dried at 60° under reduced pressure for 3 hr., was transferred to a 100-ml. volumetric flask. The reserpine standard, dissolved in a few drops of chloroform, was diluted to volume with methanol.

Ten milliliters of the above reference stock solution, equivalent to the potency of 10 tablets, was transferred to another 100-ml. volumetric flask and diluted to volume with methanol. A placebo mixture containing all the ingredients in a 0.25-mg. potency tablet equivalent to the weight of 10 tablets except reserpine and 1-ml. distilled water was added to the 100-ml. volumetric flask containing 100 ml. of reference solution. It was stoppered and agitated vigorously until the mixture formed a slurry. To this slurry, 2 ml. chloroform and a few solid glass beads (4-mm. diameter size) were added. The whole suspension was agitated vigorously again and centrifuged. To make 90 and 110% standard reserpine solutions, to represent the lower and upper limits of acceptance, identical procedures were carried out, except that 9- and 11-ml. reserpine stock solutions were used, respectively. These were the standards for 0.25-mg. potency tablets.

This procedure was repeated for the standards of 0.5-mg. reserpine tablets using their placebo equivalent to 10 tablet weights.

In the fluorometric determination of reserpine, 12.5 mg. USP reserpine Reference Standard, dried at 60° for 3 hr. under reduced pressure, was dissolved in 100 ml. methanol. The three (90, 100, and 110%) standard solutions were prepared by diluting 9, 10, and 11 ml. of the above reserpine stock solution to 100 ml., respectively.

Sample Preparation—Due to the low absorbance the amounts of solvents used in the extraction of the reserpine were limited to small volumes. Utilization of a Solid Prep Sampler module was undesirable. The following manual method was developed for the preparation of the sample.

The reserpine tablets to be analyzed were placed separately in 2.5 × 15-cm. screw-cap tubes. Three drops of distilled water were added to the tablet in each tube. After 5 min. standing each tube was shaken by hand with swirling motion to disintegrate the tablet to a smooth paste. To this paste three glass beads (4-mm. diameter) and 0.2 ml. chloroform were added. The tubes were agitated vigorously on a mixer[7] to form a white creamy suspension. For colorimetric determination exactly 10 ml. absolute methanol was added. The tubes were covered with a piece of aluminum foil and tightened with the screw caps. These were placed in a liter beaker containing 150 ml. water. The water was then heated to a temperature not exceeding 60° during a 5-min. period and cooled. The tubes were agitated on a mixer and centrifuged. The clear supernate from each tube was transferred to an 8.5-ml. automatic analyzer sample cup. The final analysis was made in the automatic analyzer. In the fluorometric determination the sample was diluted to one-half its concentration with methanol.

Effect of Temperature—The effect of temperature on the color reaction of reserpine and nitrous acid was studied using the flow system shown in Fig. 1. The reaction mixture consisted of 12.5 ml. of 0.04% reserpine reference standard, 6 ml. sodium nitrite reagent, 3 ml. sulfuric acid reagent, and 3.5 ml. methanol to make 25 ml. solution.

The reaction mixture in small aliquots was transferred to the special reservoir flask connected to the flow system, Fig. 1. The solution was slowly introduced into the flow system by means of the

[1] Marketed as Rau-sed by E. R. Squibb and Sons, Inc., New Brunswick, N. J.

[2] AutoAnalyzer, Technicon Corp., Tarrytown, N. Y.

[3] Equipment: Technicon liquid sampler II, Technicon constant temperature bath with double delay coils, Technicon proportional pumps, Technicon Turner fluorometer, model 111, Technicon line recorder, additional 20 feet and 10 feet delay coils, Hitachi Perkin-Elmer Spectrophotometer, model 139, Photovolt scale expander, model 7632, Sargent recorder, model SRL with variable MV range, and Thomas 10-mm. light path Flow Cell with spectrophotometer adapter.

[4] Merck and Co., Inc., Rahway, N. J.

[5] Matheson Coleman and Bell, Rutherford, N. J.

[6] Marketed as Tween 80 by Atlas Chemical Industries, Inc., Wilmington, Del.

[7] Vortex-Genie, Scientific Industries, Mass.

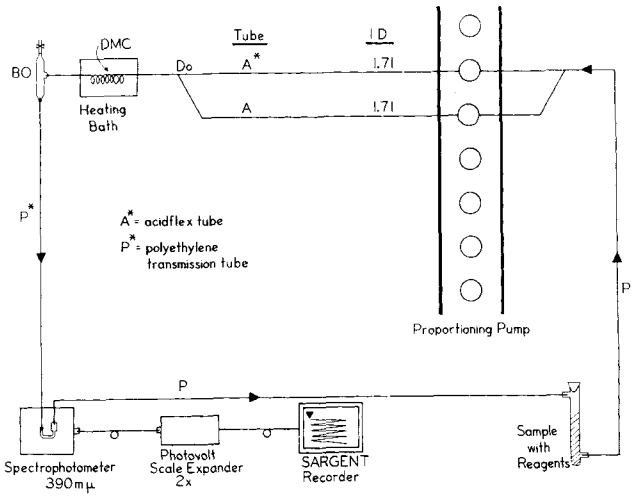

Figure 1—*Flow diagram to determine the effect of temperature on the reaction of nitrous acid and reserpine.*

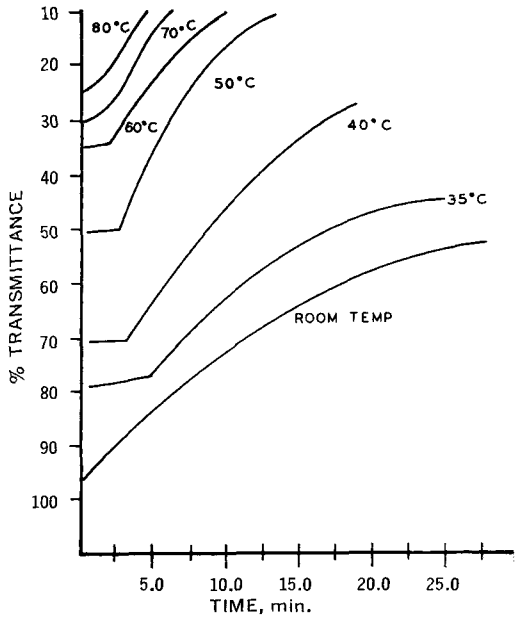

Figure 2—*The Effect of temperature on the reaction of nitrous acid and reserpine.*

pump. With passage of the reaction mixture through the heating coil, the solution became colored. After the solution emerged from the heated mixing coil, it was debubbled by an intermittent opening of the pinch clamp on the acidiflex tubing attached to the BO separator. The unsegmented colored solution was pumped through the continuous flow cell and fed back into the flow system to be recirculated. This experiment was repeated at various temperatures. Increase in temperature increased the absorbance. The results of these observations are reported in Fig. 2.

Assay Procedure—The sampling probe of the sampler II was aligned so that satisfactory aspiration from the cups of the turntable tray and the wash cup occurred. A zero base line was established with all instruments operating according to the flow diagram of Figs. 3 and 4. The assembly of the colorimetric system is shown in Fig. 5. The sampling rate was set at 20 samples per hour. Before the aspiration of the samples, the Thomas flow cell was removed from the spectrophotometer and gently tapped. The Teflon tubings of the flow cell were straightened and properly positioned to release trapped air. In the fluorometer both primary and secondary filters were properly positioned to the flow cell. The fluorometer slit was set at Position 1. After a 0.5 hr. of conditioning of the flow systems, the samples were introduced.

The set of three theory standards (90, 100, and 110%) in duplicate, were sampled initially and the absorbances were determined. Ten samples were then assayed, followed by the 100% standard in duplicate. If the readings of the duplicate standards fell within the range of 2%, to the original set of 100% standards, the standard values were considered as appropriate to be used in the calculation for the subsequent group of samples. This assumes no base line drift.

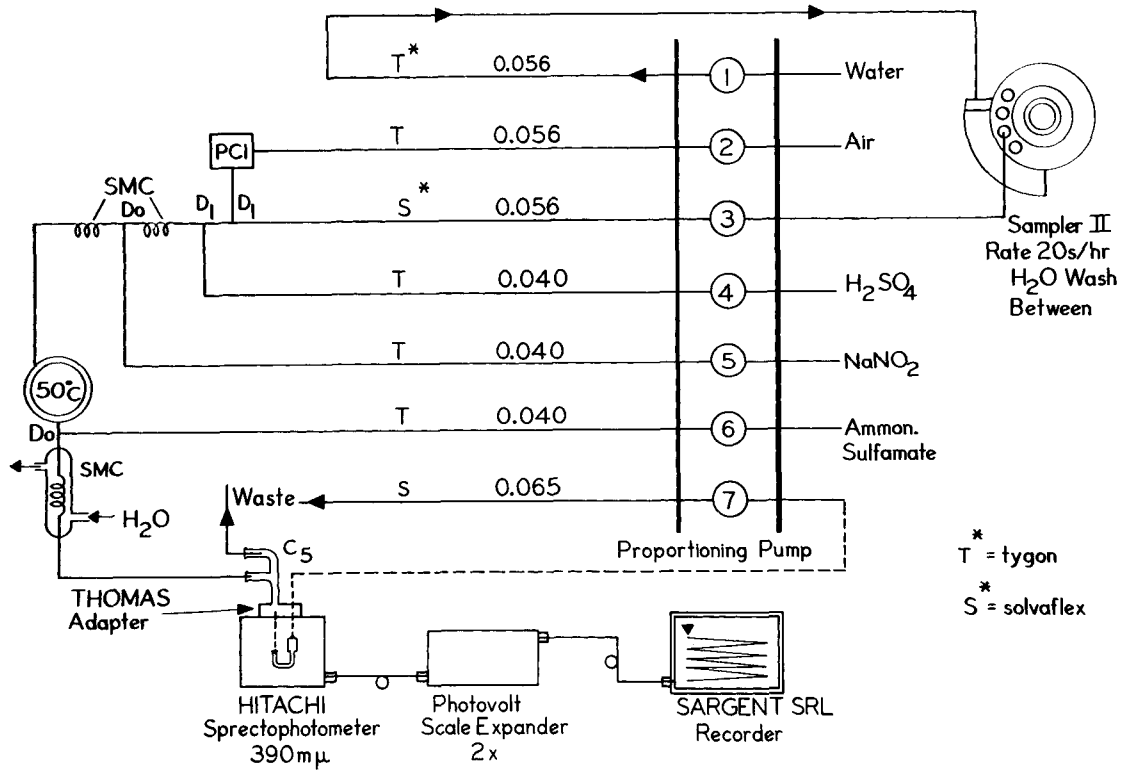

Figure 3 — *Flow diagram for the colorimetric determination of reserpine.*

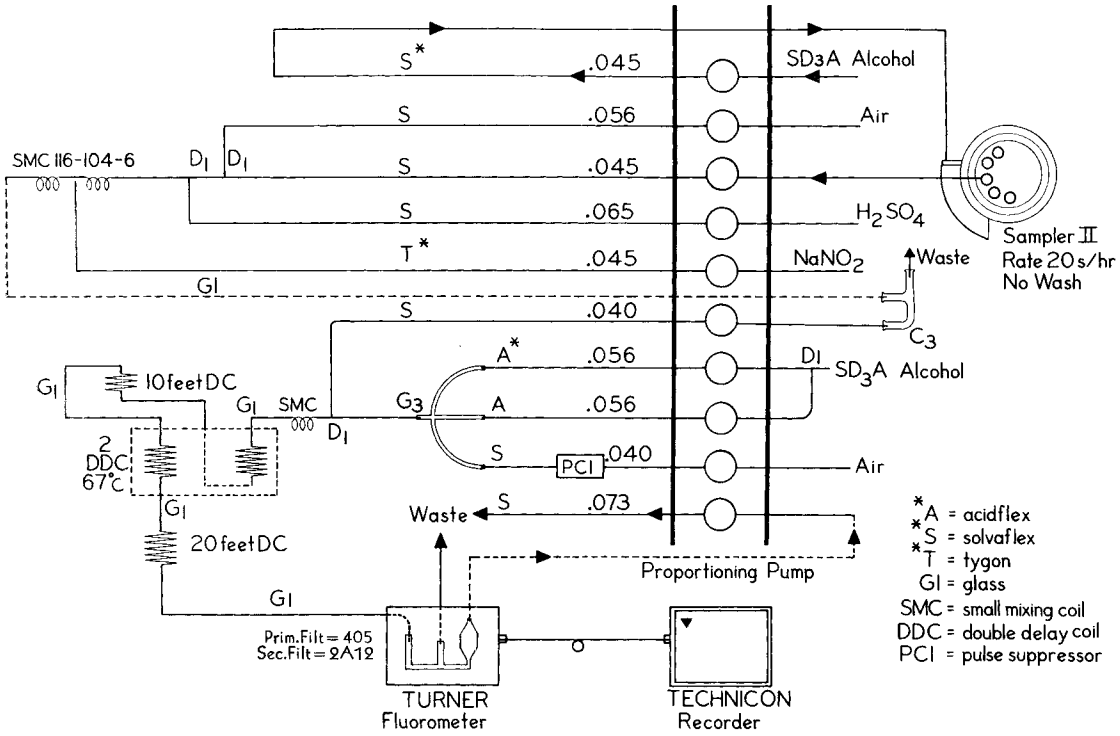

Figure 4 — *Flow diagram for the fluorometric determination of reserpine.*

RESULTS AND DISCUSSION

Vigorous shaking of the tablets in chloroform, following disintegration with a small amount of water, and then dilution with methanol gave both a simple and efficient manual extraction of reserpine.

The complete analytical systems are outlined in the flow diagrams, Figs. 3 and 4. The sample solution, aspirated from the Liquid Sampler was air-segmented and joined by the sulfuric acid reagent. This acidified solution was passed through the mixing coil, as it emerged, it was joined by the sodium nitrite reagent. The combined solutions of acidic sodium nitrite and reserpine were passed through the heated double delay coils. The hot yellow-green fluorescent stream was cooled in the single delay coil before entering the flow cell of the fluorometer. The recordings were made as previously reported (4), at the excitation maximum for nitrous acid induced fluorescence of 390 mμ and the fluorescent maximum of 510 mμ.

In the colorimetric determination of reserpine the hot yellow-green fluorescent solution after emerging from the heating bath was joined with ammonium sulfate reagent, mixed and cooled in the jacketed mixing coil. This cooled segmented stream was debubbled and the absorbance was measured at 390 mμ in the spectrophotometer. The flow cell was intermittently washed with acetone when flakes from manifold or sleeve tubings appeared in the flow cell; this prevented the minute gas bubbles being trapped on the surface of the flow cell. The entrapped gas produced noisy pointed peaks.

The reaction between nitrous acid and ammonium sulfamate evolved nitrogen (9). This nitrogen and nitrous acid gas generated in the system affected the recordings. Increased temperature and reagent concentrations increased gas evolution. To reduce the noise on the tracings, the reaction mixture of reserpine and nitrous acid was heated at 50° to remove any gas, then cooled in the jacketed mixer to minimize Schlieren before entering the flow cell. Addition of 5 ml. polysorbate reagent to the sodium nitrite reagent facilitated

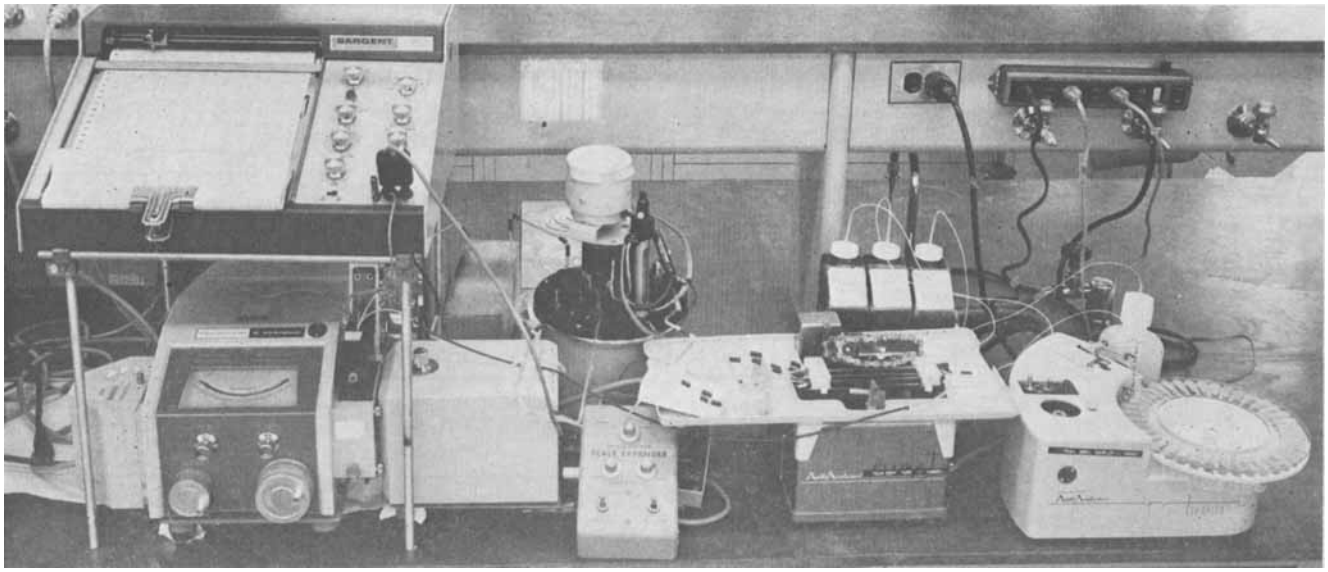

Figure 5—*Colorimetric analytical system assembly.*

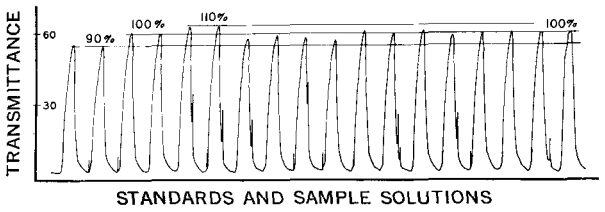

Figure 6—*Analysis of tablets containing 0.5-mg. reserpine.*

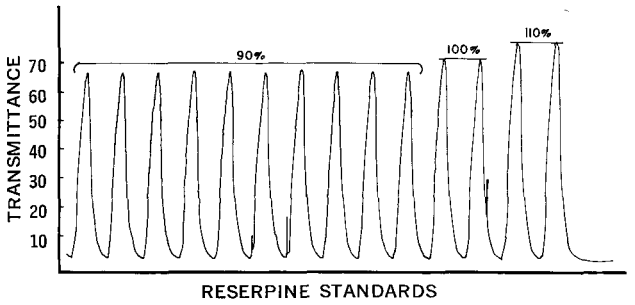

Figure 7—*Absorbance recordings of 0.25-mg. reserpine standards.*

the smooth escape of the gas from the flow cell. Further, on the spectrophotometer a cell adapter was used to avoid trapping the gas bubbles in the sharp bends of the Teflon tubing of the flow cell.

Sensitivity—In the colorimetric determination of 0.25-mg. potency reserpine tablets, the absorbance was low. The scale was expanded twice using the Photovolt Scale Expander in conjunction with the Sargent Recorder on the spectrophotometer. No scale expansion was necessary in the analysis of 0.5-mg. potency tablets (see Fig. 6).

Precision—The precision of the method was calculated as the variability between values obtained on repeated analysis of a standard. Absorbance recordings on the repetitive continuous sampling of 90% standards, duplicates of 100 and 110% standards of 0.25-mg. potency reserpine tablets is shown in Fig. 7. A relative *SD* of 90% standard replicate analysis was 1.67 %. The peak heights were at the steady state. Recordings of the various concentrations showed minimum carry-over or interaction between peaks, even though the base line was not completely reached during wash cycle. A record of fluorometric analysis is shown in Fig. 8.

Accuracy—The accuracy of the methods was studied by recovery experiments. The results of 0.25-mg. potency tablets are reported in Table I. The same batch of powdered tablets was analyzed manually

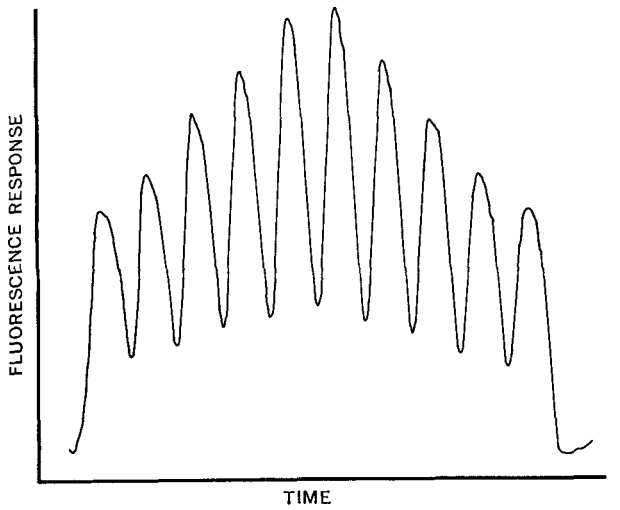

Figure 8—*Reproducibility and the effect of concentration on the fluorometric analysis of reserpine.*

Table I—Recovery of Reserpine from Tablets

Sample No.	Milligram of Reserpine added to 0.25-mg. Tablet; Extracted in 10-ml. Methanol	Calcd., mcg./ml.	Found, mcg./ml.	Recovery, %
1	0.050	30.0	29	96.67
2	0.075	32.5	32.25	99.27
3	0.100	35.0	35.0	100.0
4	0.125	37.5	37.5	100.0
5	0.150	40.0	41.0	102.5

and the results were in agreement with the automated analytical method.

Specificity—There was very little or no interference in the analysis of the tablets. In the colorimetric determination of reserpine in the concentration range of 0.2 mg. and higher the tablets from other manufacturers showed definite interference. To overcome this interference by the excipients and to increase the sensitivity, the fluorometric method was preferred. The fluorometric analysis of 0.25-mg. potency reserpine tablets from other manufacturers showed no interference (see Fig. 9). Table II gives results of reserpine assays from three manufacturers.

SUMMARY

Automated fluorometric and colorimetric methods for the determination of reserpine in tablets have been described.

A simple technique for the extraction of reserpine from the compressed tablets has been developed. The effect of various factors affecting the recordings in colorimetric readings has been discussed. With addition of placebo to the standards the colorimetric method could be applicable to the analysis of any reserpine tablet formulation.

The effect of heat on the colorimetric reaction of reserpine and nitrous acid indicated that increase in temperature proportionately increased both the amount of nitrous acid and nitrogen gas evolved and depth of color developed. A technique of minimizing the effect of this gas on the tracings has been discussed.

The fluorometric determination of reserpine was found to be more sensitive and free from the interferences of the excipients of the tablets.

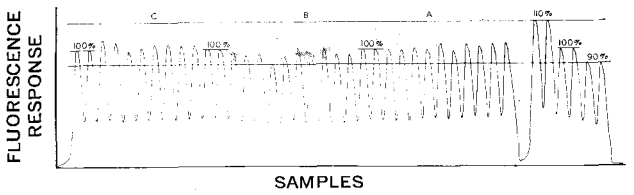

Figure 9—*Fluorometric analysis of 0.25-mg. reserpine tablets from the various manufacturers.*

Table II—Fluorometric Analysis of Ten 0.25-mg. Potency Reserpine Tablets from Various Manufacturers[a]

Manufacturer	Assay, mg./tablet	% Claim	% Variation between Tablets
A	0.2522	100.9	±2.0
B[b]	0.2465	98.6	±1.5
C	0.2495	99.8	±1.5

[a] Average of 10 tablets. [b] Need longer disintegration time.

REFERENCES

(1) C. R. Szalkowski and W. J. Mader, *J. Am. Pharm. Assoc., Sci. Ed.*, **45**, 613(1956).

(2) R. P. Haycock and W. J. Mader, *ibid.*, **46**, 744(1957).

(3) D. Banes, *ibid.*, **46**, 601(1957).

(4) R. P. Haycock, P. B. Sheth, T. Higuchi, W. J. Mader, and G. J. Papariello, *J. Pharm. Sci.*, **55**, 826(1966).

(5) "United States Pharmacopeia," 17th rev., Mack Publishing Co., Easton, Pa., 1965.

(6) "National Formulary," 12th ed., Mack Publishing Co., Easton, Pa., 1965.

(7) W. J. Mader, R. P. Haycock, P. B. Sheth, and R. J. Connolly, *J. Assoc. Offic. Agr. Chemists*, **44**, 13(1961).

(8) W. J. Mader, G. J. Papariello, and P. B. Sheth, *ibid.*, **45**, 589(1962).

(9) K. B. Wrightman and W. W. Holl, *Ann. N. Y. Acad. Sci.*, **130**, 316(1965).

ACKNOWLEDGMENTS AND ADDRESSES

Received April 28, 1969 from *E. R. Squibb & Sons, Inc., New Brunswick, NJ 08903*

Accepted for publication June 13, 1969.

Presented on June 6, 1969 at the Technicon International Congress on Automated Analysis, Chicago, Ill.

NOTES

Hydrophile-Lipophile Balance and Micelle Formation of Nonionic Surfactants

HANS SCHOTT

Abstract □ Comparison of the hydrophile-lipophile balance (HLB) values according to the two prevailing systems was made for two classes of nonionic surfactants, namely, ethylene oxide adducts of *n*-dodecanol and of branched nonylphenol with increasing degrees of polyoxyethylation. The two systems were shown to differ fundamentally because only one treats the HLB values as constitutive and additive. For both HLB systems, simple relationships were found between the HLB values of each class of surfactants and their critical micelle concentrations. These relationships had different forms for the two systems and, within the same system, different numerical values for the two classes of surfactants.

Keyphrases □ Hydrophile-lipophile balance—nonionic surfactants □ Nonionic surfactants, HLB balance—Davies, Griffin methods compared □ CMC-HLB relationships—nonionic surfactants □ Surface activity—CMC-HLB relationship

The hydrophile-lipophile balance (HLB) is a useful index for rating and selecting emulsifying agents. Griffin determined experimentally the HLB values of different surfactants (1) and derived equations which permit one to calculate the HLB value of a surfactant based on its composition (2). Davies assigned HLB group numbers to the various functional groups which make up surfactant molecules, giving positive values to the hydrophilic groups and negative values to the lipophilic ones. The summation of the products of group numbers times group frequencies gives the HLB (3). He also correlated HLB values with coalescence rates of emulsions. The statement (3) that the HLB values calculated from Davies' group numbers are in good agreement with those determined by Griffin is not correct in the case of most surfactants which are ethylene oxide adducts; this is shown below.

Correlation of the empirical HLB values with physicochemical parameters of the surfactants are rare, despite the practical value of the HLB rating system. In one of the few successful studies, HLB values were correlated with the spreading coefficient of the disperse liquid phase of an emulsion on the surface of the continuous liquid phase containing the dissolved emulsifying agent (4).

One of the surfactant properties which should be related to the HLB is the readiness with which the surfactant molecules associate into micelles, namely, the critical micelle concentration (CMC). One would expect such a relationship to exist because the more hydrophilic a surfactant is or the larger its HLB value, the lesser the tendency to form micelles and the higher its CMC. Furthermore, like the HLB, the CMC is a function of composition, at least within a homologous series of surfactants. The obvious limitation of this approach is that it can only be applied to those surfactants whose solubility in water exceeds their CMC. Surfactants used as wetting agents, detergents, solubilizing agents, and o/w emulsifying agents can be included, but not surfactants of low HLB values such as w/o emulsifiers.

HLB AND CMC RELATIONSHIPS

Two homologous series of ethylene oxide adducts, for which surface and micellar properties (CMC, micellar molecular weight and radius) have been studied extensively (5–8), will be used. These