Quantitative Analysis of Sodium Sulfacetamide Solutions in the Presence of Hydrolysis Products, Sulfanilamide and Sodium Acetate

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Abstract □ The official assay for sodium sulfacetamide, the diazotization method, does not differentiate between sulfacetamide and its hydrolytic product, sulfanilamide. A spectrophotometric method involving the formation of ferric acethydroxamate was developed for utilization in studies of the rate of hydrolytic decomposition of 30% buffered and unbuffered solutions of sodium sulfacetamide at several elevated temperatures. The method provided a relatively simple and rapid means by which sodium sulfacetamide is quantitatively determined in the presence of its hydrolysis products, sulfanilamide and sodium acetate.

Keyphrases □ Sulfacetamide solutions—analysis □ Hydrolysis products, presence—sulfacetamide determination □ Colorimetric analysis—spectrophotometer

Sodium sulfacetamide ophthalmic solution USP (1) is a sterile, 30% aqueous solution. On storage, aqueous solutions of sodium sulfacetamide undergo hydrolysis to sulfanilamide and sodium acetate (2). The low solubility of the sulfanilamide (0.75 g./100 ml. of water at 25°) causes the deposition of needle-like crystals in an ophthalmic solution and could conceivably cause serious damage to eye tissue.

The analytical procedure presently official in *The United States Pharmacopeia* is based on the diazotization of the primary amino group attached to the aromatic ring. Since this functional group is present in both sulfanilamide and sulfacetamide, this method of analysis cannot be used to differentiate between the two sulfonamides.

Sulfonamides have been analyzed volumetrically by diazotization (3), bromination (4), and nonaqueous titration with perchloric acid (5). The acidic hydrogen has been titrated argentimetrically (6) and acidimetrically (6) with alkali metal alkoxides in nonaqueous media. Colorimetric methods, particularly those based on diazotization and subsequent coupling (7), have also been developed. Analytical methods involving UV and IR spectrophotometry have also been reported. Several gravimetric and titrimetric methods based on the acidic properties of the sulfonamido group have been developed for the quantitative determination of sulfonamides. Neither the cited procedures nor the official method allow for the direct quantitative analysis of sulfacetamide in the presence of its principle degradation product, sulfanilamide.

Paper chromatographic techniques may be employed for the separation of sulfacetamide-sulfanilamide mixtures. While these techniques are relatively simple and rapid when used qualitatively, they are very time consuming quantitative methods. Gruber and Klein (8) reported the application of TLC to the separation of sulfacetamide from its hydrolysis product, sulfanilamide.

The most common method for the analysis of sulfonamides is the colorimetric method of Bratton and Marshall (9). The procedure involves the diazotization of the sulfonamide with sodium nitrite in dilute acid, followed by decomposition of the excess nitrite with sulfamic acid and coupling of the diazo compound with *N*-(1-naphthyl)-ethylenediamine. The important disadvantage of this method and other colorimetric methods reported in the literature (10–14) is that the absorption frequencies and molar absorbance coefficients produced with sulfanilamide and sulfacetamide are identical. Therefore, these methods are not differential.

In an attempt to find a specific analysis that would be both quantitative and differential between the two sulfonamides, a method introduced by Feigl *et al.* (15) was selected. By this method, carboxylic acid derivatives (acid anhydrides, acid halides, and esters) are converted to corresponding hydroxamic acid salts by allowing them to react with hydroxylamine hydrochloride in an alkaline media. The hydoxamic acid salt is then allowed to react with ferric chloride in the presence of dilute acids to produce a red-violet colored ferric hydroxamate.

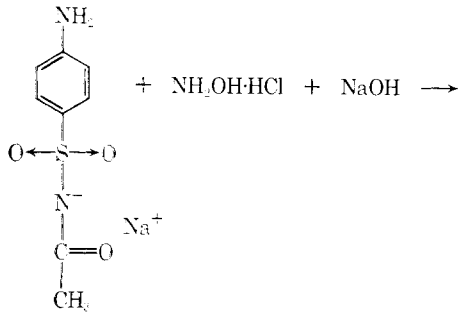
Soloway and Lipschitz (16) reported the conversion of amides to hydroxamic acid salts with hydroxylamine. However, the reaction proceeded more slowly than the reaction with acid chlorides, acid anhydrides, and esters. Bergman (17), using the procedures outlined by Hestrin (18), found that amides react more readily with hydroxylamine hydrochloride if the reaction is allowed to occur at an elevated temperature. He studied several amides and found that the optimum temperature and duration of heating differed with each compound studied. Bergman also noted that a linear relationship

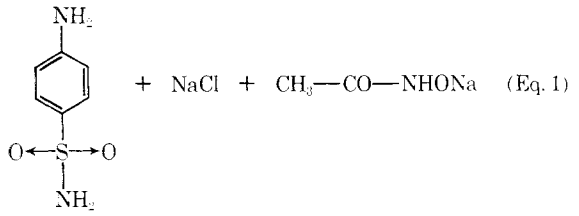
Table I—Effect of Temperature on Color Produced

Sample Number	Volume	Absorbance	
		At Room Temp.	After Heating
1 & 2	0.00 ml.	0.000	0.000
3 & 4	0.05 ml.	0.244	0.164
5 & 6	0.10 ml.	0.611	0.347
7 & 8	0.15 ml.	0.928	0.444
9 & 10	0.20 ml.	1.046	0.699
11 & 12	0.25 ml.	0.979	0.839

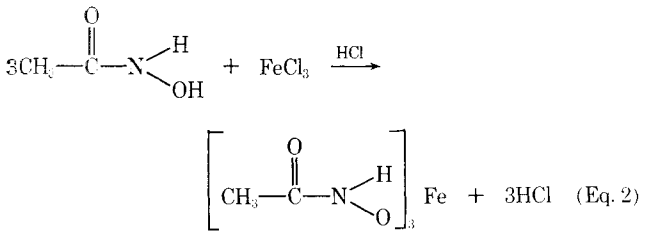
existed between the concentration of the amide and the "extinction" (absorbance) of color.

Although sodium sulfacetamide was not mentioned in any of these studies, it is an amide and could conceivably react with hydroxylamine hydrochloride in alkaline medium to form an acethydroxamic acid salt as illustrated by the following equation:





The acethydroxamic acid salt produced could then react with ferric chloride in acid medium to produce the colored ferric hydroxamate according to the equation:



EXPERIMENTAL

Apparatus—Colorimeter-spectrophotometer (Spectronic 20, Bausch & Lomb, Inc.); 1 set (12) of standard, matched test tube cells, i.d. = 11.67 mm., (Fisher Scientific Co.); microsyringe, 10 μl. (Hamilton Co.); unitized constant-temperature oil baths and an air oven (Fisher Scientific Co.).

Reagents—Sodium sulfacetamide (Ruger and Co.; K & K Laboratories); sulfanilamide (Eastman Organic Chemicals); sodium acetate, sodium acid phosphate, and disodium phosphate (Fisher Scientific Co.); sodium hydroxide (Baker and Adamson); hydroxylamine hydrochloride, A.R. and ferric chloride (Mallinckrodt Chemical Works), and distilled water.

Solutions—Hydroxylamine hydrochloride, 2 *M;* sodium hydroxide, 3.5 *M;* hydrochloric acid solution, 3.9 *M;* ferric chloride solution, 0.37 *M* in 0.1 *M* hydrochloric acid; sodium sulfacetamide solution, 30%.

Studies of the proposed method of analysis as applied to solutions containing sodium sulfacetamide, sulfanilamide, and sodium acetate indicated that color was produced exclusively with sodium sulfacetamide. The effects of temperature, quantity of reagents, and reaction time on optimum color formation were determined.

The adopted method entailed a modification of the procedure originally suggested by Feigl (15) and Lipschitz (16). Accordingly, solutions of known amounts (3.0–6.6 mg.) of sodium sulfacetamide were allowed to react with hydroxylamine hydrochloride and sodium hydroxide at room temperature and at an elevated temperature (80°). The effect of heating time at the elevated temperature was also determined. The hydrogen ion concentration of each solution was adjusted to a pH of 1.2 with hydrochloric acid. Ferric chloride solution was then added followed by dilution to constant volume (25 ml.). The absorbance of each solution was determined spectrophotometrically at a wavelength of 540 mμ. The absorbance was then plotted as a function of the concentration of sodium sulfacetamide. Straight line relationships were observed up to approximately 450 mg. sodium sulfacetamide per aliquot indicating adherence to Beer's law over the range of concentrations to this limit.

DISCUSSION

The initial reaction between sodium sulfacetamide and hydroxylamine hydrochloride (after subsequent acidification and addition of ferric chloride) produced optimal color formation when subjected to a temperature of 80° for a period of 45 min. Minimum amounts of reagents necessary for maximum color production in systems containing 6.6 mg., or less, of sodium sulfacetamide were determined.

In the current investigation, sulfanilamide and sodium acetate were the only observable hydrolytic products. This fact was reported by Fletcher and Norton (2) and confirmed in this laboratory by separation and isolation of the hydrolytic products. The melting point of the isolated sulfanilamide was identical with the literature value and a mixed melting point determination with an authentic sample remained undepressed. Other products, if present, were in quantities too minute to be detected by the procedures employed.

Sodium sulfacetamide USP, obtained from different sources, produced minor variations in the Beer-Lambert curves obtained in this study. Furthermore, lot to lot variation in sodium sulfacetamide powder received from the same source produced similar variations in the Beer-Lambert curves obtained. The reproducibility of each value on the several curves was excellent. These observations suggested that slight differences in the purity of the dry powder existed from lot to lot and supplier to supplier although each sample met the specifications of the USP.

Effect of Temperature, Applied after the Addition of Hydroxylamine Hydrochloride, upon Absorbance—A series of duplicate test tubes containing 0.00, 0.05, 0.10, 0.15, 0.20, and 0.25 ml. of 30% sodium sulfacetamide solution were prepared. To each sample, 1.0 ml. of 2 *M* hydroxylamine hydrochloride and 1.0 ml. of 3.5 *M* sodium hydroxide were added. One set of tubes was allowed to remain at room temperature for 5 min. The other set of tubes was placed in a water bath at a temperature of 80 ± 1° for 5 min. Each sample was acidified to a pH of 1.2 ± 0.2 with 1.0 ml. of hydrochloric acid solution (3.9 *M*). One milliliter of 0.37 *M* ferric chloride solution was then added. After addition of 5.0 ml. of distilled water to each sample, the samples were transferred to cells and the absorbance was determined (see Table I).

Effect of Heat for Varying Lengths of Time after Addition of Hydroxylamine upon Absorbance—A sample of 0.01 ml. of a 30% sodium sulfacetamide solution was placed into each of nine test tubes. Hydroxylamine hydrochloride and sodium hydroxide solutions were added as described above. All samples were then placed into a constant temperature bath. Each sample was allowed to react, at 80°, for 10, 20, 25, 30, 35, 40, 45, 50, and 60 min., respectively. As each sample was removed and cooled, hydrochloric acid and ferric chloride solutions were added as described and diluted with 5.0 ml. of distilled water. Blanks, corresponding to each time interval were also prepared. The absorbances were then determined at a wavelength of 540 mμ (see Table II).

Effect of Pooled Quantities of Reagents upon Absorbance—In order to facilitate the accurate determination of absorbance a 6.0-mg. sample was employed. One milliliter of a 30% solution of sodium sulfacetamide was placed into a 50-ml. volumetric flask, and the sample was diluted with sufficient distilled water to produce 50 ml. of solution. One milliliter of this 1:50 dilution (containing 6.0 mg. of sodium sulfacetamide) was placed into each of the five test tubes. Five other test tubes were prepared as blank samples. One sample and one blank were treated with 1.0, 2.0, 3.0, 4.0, and 5.0 ml., respectively, of each of the reagents. The analysis was performed as previously described. Prior to the determination of the absorbance, the samples were transferred to a 25-ml. volumetric flask and brought to volume with distilled water. The results are presented in Table III.

Table II—Effect of Reaction Time on Color Produced.

Sample Number[a]	Time Heated, min.	Absorbance
1	10	0.222
2	20	0.328
3	25	0.527
4	30	0.530
5	35	0.666
6	40	0.692
7	45	0.699
8	50	0.692
9	60	0.703

A blank for each sample gave 0.000 absorbance.

Effect of Various Concentrations of Sodium Sulfacetamide on Absorbance—Samples of 0.0, 12.0, 14.0, 16.0, 18.0, 20.0 μl. of a 30% solution of sodium sulfacetamide, containing 0.0, 3.6, 4.2, 4.8, 5.4, and 6.0 mg. of sodium sulfacetamide, respectively, were placed in six test tubes. Two milliliters each of sodium hydroxide (3.5 *M*) and hydroxylamine hydrochloride (2 *M*) was added to the sample and the reaction was allowed to proceed in a constant temperature oil bath at 80° for 45 min. After cooling each sample to room temperature, 3.0 ml. of hydrochloric acid (4.12 *M*) was added to acidify the sample. Two milliliters of ferric chloride solution (0.37 *M* in 0.1 *N* HCl) was then added. The mixture was diluted to 25 ml., and the absorbance was determined at 540 mμ. The absorbance was plotted as a function of the concentration each point representing the average of ten analyses. The resultant was a straight line passing through the origin and having a slope of 0.0886 *A*/mg. Each analysis was performed on a freshly prepared 30% solution of sodium sulfacetamide produced from USP quality crystals, dried at 105° for 4 hr. prior to weighing. Reproducibility of individual values was within the limitation of the spectrophotometer employed.

This series of analyses was repeated upon a 30% solution of sodium sulfacetamide adjusted to a pH of 7.4 with a disodium phosphate-sodium acid phosphate buffer (1.0 *M* total phosphate). Ten analyses were performed upon each of the above concentrations under identical conditions of time and temperature. The observations were identical to those obtained with the unbuffered solutions.

CONCLUSIONS

The procedure necessary for optimum and reproducible color formation of the sodium sulfacetamide solutions employed in this study consisted of the addition of 2.0 ml. each of sodium hydroxide (3.5 *M*) and hydroxylamine hydrochloride (2.0 *M*) to each sample solution. Each reaction was allowed to proceed for 45 min. at 80 ± 0.5°. Subsequently, 3.0 ml. of hydrochloric acid (3.5 *M*) was added to acidify each sample to a pH of 1.2 ± 0.02. Two milliliters of ferric chloride solution (0.37 *M*) was then added followed by sufficient distilled water to bring the total volume of solution to 25 ml. The absorbance of each sample was then determined at 540 mμ. A straight-line relationship passing through the origin was observed in the plot of absorbance as a function of concentration over a range of 3.0–6.0 mg. of sodium sulfacetamide per 25 ml. of solution, indicating adherence to Beer's law over this range.

SUMMARY

Known methods of analysis were examined and applied to the quantitative determination of sodium sulfacetamide in the presence of sulfanilamide. The official diazotization method does not differentiate between these two sulfonamides. Therefore, a spectrophotometric (colorimetric) method involving the formation of ferric acethydroxamate was developed and employed. The effects of temperature, heating time, quantity of reagents, and concentration upon the development of the color were examined. This method provides a relatively simple and rapid means by which the concentration of sodium sulfacetamide is quantitatively determined in the presence of its hydrolysis products. This method is being employed in this laboratory to follow the hydrolytic degradation of sodium sulfacetamide solutions.

Table III—Effect of Reagent Quantities on Color Production

Sample Number[a]	Volume of Reagents, ml.	Absorbance[b]
1	1	0.313
2	2	0.420
3	3	0.456
4	4	0.435
5	5	0.468

[a] A blank for each sample gave 0.000 absorbance. [b] Average of duplicate experiments.

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ACKNOWLEDGMENTS AND RESULTS

Received May 18, 1966 from *Duquesne University, School of Pharmacy, Pittsburgh, PA 15299*

Accepted for publication July 2, 1969.