Colorimetric Determination of Phenylephrine Using 4-Aminoantipyrine

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Two colorimetric methods are described for phenylephrine hydrochloride using potassium ferricyanide and 4-aminoantipyrine as reagents. Using 2 per cent sodium borate as the medium, a sufficiently stable color is obtained. This method is suitable for the analysis of phenylephrine in common tablet formulations without prior separation. A second procedure using tris(hydroxymethyl)aminomethane (Tham) buffer, pH 9, and isopropyl alcohol as medium gives a color stable for over 1 hour.

THE COLOR REACTION of 4-aminoantipyrine with phenols has been extensively investigated (1–11). Application of this reagent to the analysis of phenylephrine has been reported by Hiskey and Levin (12). Their procedure consisted of adding to the sample, solutions of potassium ferricyanide, 4-aminoantipyrine, and sodium bicarbonate in the above order and measuring the absorbance 15 minutes after addition of reagents. The waiting period was necessary to allow for the dissipation of an interfering color. The reagent blank had a high initial absorbance which dropped to about 0.06–0.07 (1 cm. cells) after 15 minutes. The color of the sample also faded rapidly during the first 15 minutes and at a less rapid rate thereafter.

Investigation of the reaction of 4-aminoantipyrine and phenylephrine was undertaken because of the specificity of the reaction and the possibility of application in complex mixtures. Critical factors found by literature review and investigation were pH, concentration of reagents, order of addition of reagents, and polarity.

A rapid and convenient procedure was developed utilizing a 2% buffer solution of sodium borate. The interfering red color reported by Hiskey and Levin (12) could be avoided by the addition of 4-aminoantipyrine after dilution of the phenylephrine-potassium ferricyanide reaction mixture with the buffer solution. An additional advantage of the revised procedure was that over a wide range neither the concentration nor the proportion of the two reagents were critical factors. The color obtained was fairly stable, and the absorbance due to the reagent blank was low (Fig. 1). Accurate and reproducible results were obtainable, and the color reaction was found to obey Beer's law in the range of 1–10 mcg./ml.

Studies relative to the increase of the stability of the color indicated that polarity of the medium was a factor. Water-miscible organic solvents were tried in combination with different buffer solutions to improve color stability. It was found that isopropyl alcohol when used with Tham buffer pH 9 had a stabilizing effect. The procedure consisted of the addition of the buffer solution and isopropyl alcohol to the mixture of phenylephrine and potassium ferricyanide, followed by 4-aminoantipyrine and more of the buffer solution to a definite volume. Again, the order of addition of reagents was important, as a more intense but less stable color was obtained if the 4-aminoantipyrine was added prior to the addition of isopropyl alcohol.

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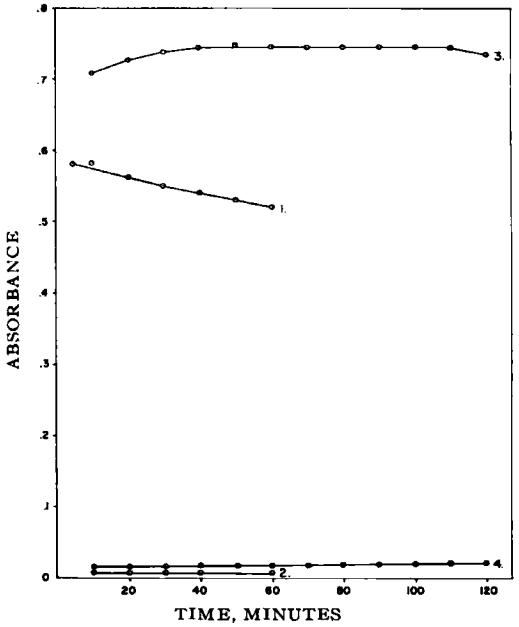

Fig. 1.—Phenylephrine–4-aminoantipyrine color reaction. 1, two per cent sodium borate medium; 2, blank for 1; 3, Tham buffer pH 9 —isopropyl alcohol medium; 4, blank for 3.

The color reaction was reproducible, obeyed Beer's law at concentrations down to 1 mcg./ml., and gave a low blank value. It was 25% more sensitive than the suggested procedure using borate buffer.

Application of the reactions in the two buffer media described was studied with a number of tablet formulations containing phenylephrine. Common tablet excipients and ingredients such as aspirin, ascorbic acid, phenacetin, caffeine, and chlortrimeton did not interfere in the borate buffer system when assaying a filtered aqueous extract of ground tablets for their phenylephrine content. When Tham buffer was utilized, cloudy solutions were frequently encountered on addition of isopropyl alcohol, and this system could not be used without prior separation of phenylephrine from interfering substances.

EXPERIMENTAL

Reagents and Equipment.—4-Aminoantipyrine, Eastman Organic Chemicals, 3% in water[1]; potassium ferricyanide, C.P., 4% in water[1]; sodium

[1] Solution stable for at least 1 week if stored in amber reagent bottle.

borate, $Na_2B_4O_7 \cdot 10H_2O$, A.C.S., Fisher Scientific Co., 2% in water; tris(hydroxymethyl)aminomethane (Tham), purified, Fisher Scientific Co., 50 ml. 0.1 *M* solution adjusted to pH 9 with 0.1 *M* HCl and diluted to 100 ml. with water; phenylephrine hydrochloride, Winthrop Laboratories; Beckman DU spectrophotometer; and 1-cm. Corex cells were utilized.

Procedure Using 2% Sodium Borate Solution.—Three milliliters of an aqueous solution containing 150–450 mcg. of phenylephrine hydrochloride was pipeted into a 50-ml. volumetric flask. One milliliter of potassium ferricyanide reagent was added, and the solution was diluted to about 48 ml. with sodium borate solution. One milliliter of 4-aminoantipyrine reagent was added, and the volume was made up with borate buffer and mixed. The absorbance of this solution was determined immediately at 490 mμ against a reagent blank. The concentration of the sample was calculated by comparison with the color developed simultaneously on a standard solution of phenylephrine hydrochloride.

Procedure Using Tham Buffer and Isopropyl Alcohol.—Three milliliters of an aqueous solution containing approximately 150–450 mcg. of phenylephrine was pipeted into a 50-ml. volumetric flask. One milliliter of potassium ferricyanide was added, followed by 15 ml. of the buffer, 15 ml. of isopropyl alcohol, 1 ml. of 4-aminoantipyrine, and more of the buffer to volume. The contents of the flask were mixed after the addition of each reagent. The absorbance of the solution was determined at 490 mμ against a reagent blank 30 minutes after the development of color. The color developed simultaneously on a standard solution of phenylephrine hydrochloride was used to calculate the concentration of the sample.

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Acute Toxicity of Intravenous Sodium Lauryl Sulfate

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Sodium lauryl sulfate (SLS) is an excellent emulsifying agent. An emulsion of methoxyflurane in oil, stable to autoclaving, and employing SLS as an emulsifier was prepared. The acute effects of intravenous SLS on red cells, electrocardiogram, vital organs of rabbits, dogs, and monkeys, and isolated hearts of rabbits and frogs were studied. The dose levels were 10 and 50 mg. per cent in 5 per cent glucose or in 3.5 per cent emulsion of methoxyflurane at administration rates of 6.2 ml./Kg./hr. intravenously. The hemolytic effects of SLS and its effect on the electrocardiogram are negligible. SLS evokes a precipitous transient depressor response in dogs. However, SLS has marked acute effect on lungs, kidneys, and especially liver. The hepatotoxicity of SLS seems to preclude its intravenous use in man.

SODIUM LAURYL SULFATE (SLS) has been used in dentifrices for years. Its pharmacologic and toxicologic properties have been studied by a number of investigators (1–5). Recently the Council on Drugs has directed attention to possible hepatosensitivity associated with erythromycin propionate lauryl sulfate (6).

The authors' work with intravenous anesthetic emulsions (7) prompted an investigation of the suitability of SLS as an emulsifier. SLS in concentrations of 2–5 mg. % proved to be an excellent emulsifier and produced a very stable emulsion that tolerated autoclaving for 19 minutes at 15 lb.

After we had established the usefulness of SLS as an emulsifier for emulsions of volatile anesthetics, we then proceeded to investigate its acute toxicity upon intravenous administration.

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The LD_{50} of SLS upon intravenous administration in rats and mice (kindly supplied to us by K. K. Chen) was found to be 118.2 ± 7.2 mg./Kg.

The properties that were of special interest to us were (*a*) lysis of the red cell membrane, (*b*) its influence on the electric activity of the heart, and (*c*) its acute toxic effects on liver, kidneys, and lungs.

METHODS

Hemolysis.—Two mongrel dogs were anesthetized with 25 mg./Kg. pentobarbital intravenously. The animals were then infused with a solution of 50 mg. % SLS in 5% glucose at a rate of 6.2 ml./Kg./hr. for 1 hour.

Prior to and after infusion, blood samples for hemolysis were taken. Samples were drawn through 17-gauge needles in plastic syringes, bubble free. Plastic centrifuge tubes and a well balanced centrifuge were used to avoid mechanical disrupture of the red cells.