Colorimetric Determination of Phenylephrine Using 4-Aminoantipyrine

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A study of the coupling reaction between 4-aminoantipyrine and phenylephrine in alkaline ferricyanide solutions is reported herein. A number of important variables were studied to determine their effect on the kinetics and extent of the reaction. A procedure for the determination of phenylephrine in some pharmaceutical preparations is also included.

RECENTLY it became desirable to have a simple colorimetric determination for phenylephrine in the presence of a mixture of nitrogenous bases such as pyrilamine, dihydrocodeinone, etc., that interfered with the usual bromination method (1) and the coupling reaction with diazotized *p*-nitroaniline (2).

Since phenylephrine is a phenol with the *para*-position available for coupling with 4-aminoantipyrine, it was decided to try to apply the reaction in this instance.

The utility of this reaction for analytical purposes has been extensively investigated (3–8) since its discovery by Emerson (9, 10) in 1938. Recently Johnson and Savidge (11) reviewed its applicability to a wide range of pharmaceutical products.

EXPERIMENTAL

In our preliminary work we used the conditions outlined by Johnson and Savidge for phenols giving 4-aminoantipyrine dyes insoluble in chloroform. The first results obtained were not encouraging, since the color development was very weak and not reproducible. By changing the order of addition so that the phenylephrine, the 4-aminoantipyrine, and the ferricyanide were first combined and then diluted to volume with buffer, a set of conditions satisfactory for the assay were found.

Reagents.—4-Aminoantipyrine, mol. wt. 203.25, purchased from Eastman Organic Chemicals and used without further purification. Solutions (0.025 *M*) were prepared daily.

Potassium ferricyanide C. P., 0.010607 *M* (2 Gm./100 ml.) prepared daily.

Phenylephrine hydrochloride solution, 200 mg./100 ml. in water. Sodium bicarbonate buffer, 0.01 *M*.

Preliminary Observation.—A preliminary measurement of the absorption spectrum of the coupled phenylephrine was effected and compared with that of the reagent blanks to establish whether any interference was to be expected. The procedure employed consisted of adding 3 ml. of the phenylephrine standard to a 100-ml. volumetric flask, followed, in turn, by 1 ml. of the ferricyanide solution and then by 2 ml. of the aminoantipyrine solution. The solution was then brought to volume immediately with 2.5×10^{-3} *M* sodium carbonate. The reagent blank was prepared similarly, except that the phenylephrine was omitted.

It was observed that as the aminoantipyrine was admitted to the solution, an intense red color was formed, which in the case of the reagent blanks was discharged shortly after the addition of the carbonate solution. When the phenylephrine was present the red color diminished in intensity as the carbonate was added but did not fade completely.

In Fig. 1, the plotted absorption spectra of these two solutions, using water as a reference, show that the solution containing phenylephrine (*A*) has a well defined peak at 500 mμ, at which wavelength the reagent blank (*B*) has practically zero absorbance. The residual spectrum in the reagent blank was shown to be due to excess ferricyanide and not to any reaction by-product.

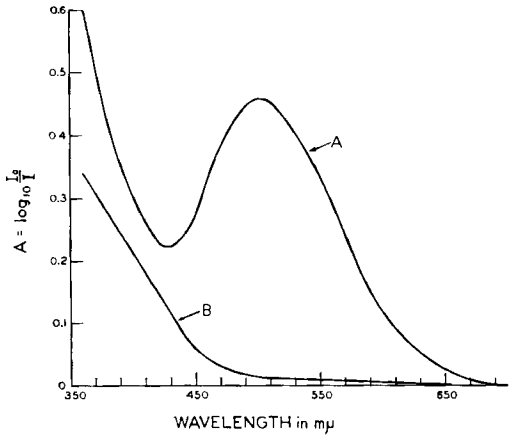

Fig. 1.—Spectra of phenylephrine (*A*) and reagent blank (*B*) under one set of reaction conditions.

Absorption Spectra of Reaction Intermediates.—The appearance of an intense red color when the reagent blank was prepared suggested further investigation of this process. Accordingly, 5 ml. of the potassium ferricyanide was mixed with the 4-aminoantipyrine reagent solution in a 100-ml. volumetric flask. As soon as the red color had formed the solution was diluted to volume with 0.01 *M* sodium bicarbonate solution. This diluted solution was then transferred to an optical cell placed in the sample side of the DK-2 spectrophotometer. As

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reference, 0.01 *M* sodium bicarbonate solution was used. The spectra were determined at various time intervals after mixing. It was observed that the red substance had an absorption maximum at 525 mμ in contrast with the 500 mμ maximum observed for the phenylephrine dye.

In Fig. 2 the absorbance at 525 mμ of the solution is plotted as a function of time. It is seen that in fifty minutes the red compound has completely decomposed.

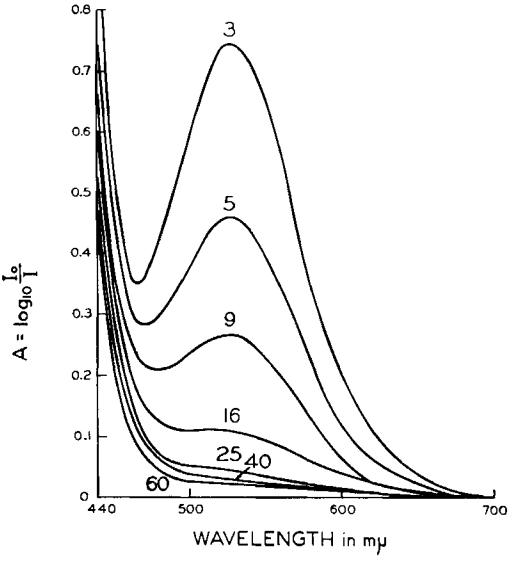

Fig. 2.—Spectrum of the reagent blank as a function of time in minutes.

A similar study was performed incorporating phenylephrine into the solution. This was done by taking 3 ml. of the phenylephrine standard and adding to it, in turn, 5 ml. of the ferricyanide and 5 ml. of the aminoantipyrine reagent. As soon as the red color had formed, the solutions were diluted to volume with 0.01 *M* sodium bicarbonate. Again the spectra were measured in the DK-2 using 0.01 *M* sodium bicarbonate in the reference beam. In Fig. 3, we see that the red color has an absorption peak close to, but not exactly at, 525 mμ. With time, the fading of this absorption peak is quite rapid and it will be noticed that there is a gradual shift away from the 525 mμ region to the 500 mμ region. After this had been accomplished the rate of fading with time diminished appreciably and there was no further spectral shift associated with that fading.

These data are somewhat better understood if one plots the peak absorption in the 500–525 mμ region for both of these experiments as a function of time. When this is done, the rapid decline to background when phenylephrine is absent is noteworthy. When phenylephrine is present the decrease in absorbance is exceedingly rapid for the first fifteen minutes, after which the slope of the curve changes abruptly to a much lower rate.

It appears that in the absence of phenylephrine the 4-aminoantipyrine reacts to form a red intermediate complex or free radical which decomposed to a colorless substance in a relatively short period of

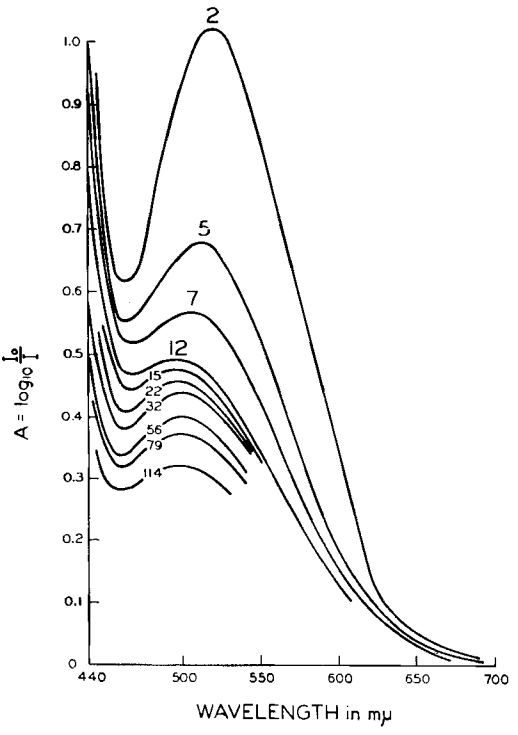

Fig. 3.—Spectrum of reagent and phenylephrine as a function of time.

time. However, when phenylephrine is present this intermediate is able to interact with the phenylephrine to form a much more stable reaction product. The antipyrine phenylephrine moiety, while not completely stable under the conditions of this reaction, nevertheless has a rate of decomposition sufficiently slow to enable us to make a practical use of it in this method of assay. If a period of time of about fifteen to thirty minutes is allowed to elapse before the readings are taken, the red intermediate of aminoantipyrine will have completely disappeared, leaving a red color due only to the coupled phenylephrine.

The Role of Concentration Ratios.—A study was made next of the effect of the ferricyanide and the 4-aminoantipyrine concentrations on the absorbance. In general these data were taken by combining the indicated amounts of the reagent solutions in a 100-ml. flask, after which the solution was diluted to volume with 0.01 *M* bicarbonate. The solutions were allowed to stand for fifteen minutes, after which the absorbance of the solution was measured at 500 mμ on a Beckman DU spectrophotometer using the 0.01 *M* bicarbonate solution as a reference standard. The data so obtained are presented in Table I.

These data reveal that both the ferricyanide and the aminoantipyrine concentrations are important variables affecting the color intensity. From the data, where 0.5 and 1 ml. of ferricyanide were used with three concentrations levels of aminoantipyrine, it appears that with too great a ratio of aminoantipyrine to ferricyanide, most of the oxidant is used up in forming the unstable red complex of the aminoantipyrine and little is available for the coupling

TABLE I.—EFFECT OF REAGENT CONCENTRATION[a] ON ABSORBANCE

4-Amino-antipyrine Soln., ml.	Ferricyanide Soln., ml. →	0.5	1.0	1.5	2.0	3.0	5.0
1.0		0.350	0.532	0.533	0.472	0.421	...
2.0		0.283	0.512	0.670	0.725	0.699	0.675
3.0		0.175	0.430	0.570	0.695	0.771	0.780
5.0		...	...	...	0.604	0.758	0.875

[a] Phenylephrine concentration 1.20 mg./100 ml. (5.9×10^{-5} *M*).

TABLE II.—TABULATION OF DATA

Phenylephrine mg./100 ml.	0.1 *M* HCO_3^-		0.01 *M* HCO_3^-		Hycomine Syrup	
	A	*A*(1 %, 1 cm.)	*A*	*A*(1 %, 1 cm.)	*A*	*A*(1 %, 1 cm.)
0.03	...	..	0.014	466	...	..
0.05	0.022	440	...	..	...	..
0.06	...	..	...	..	0.037	616
0.15	...	..	0.100	666	...	..
0.20	0.077	385	...	..	0.139	695
0.30	...	..	0.206	687	...	..
0.40	0.154	385	...	..	0.276	690
0.60	0.238	397	0.408	680	0.411	685
0.80	0.301	376	...	..	0.540	675
0.90	...	..	0.602	669	...	..
1.0	0.392	392	...	..	...	..
1.20	0.468	389	0.797	664	0.779	650

reaction. Hence, with increasing amounts of aminoantipyrine, less color is produced by the phenylephrine.

If the concentration of aminoantipyrine is held constant at 2 ml. while the ferricyanide is increased, the degree of coupling is increased, reaching a maximum when equal number of milliliters of reagents are added, corresponding to a ratio of about 2.5 moles of ferricyanide to one of aminoantipyrine. In general, when this ratio is obtained at any other concentration level a maximum amount of coupled phenylephrine is achieved. This is illustrated by the values when 1:1, 2:2, and 3:3 ml. are taken of the two reagents. As the reagents are increased maintaining the ratio of 2.5, leveling off of intensity occurs when the 5:5-ml. level is approached. This appears to be a practical working concentration with the reagent blank having an appreciable absorbance, i.e., 0.06–0.07, and with *A* (1 cm., 1%) for the phenylephrine being about 700. Consequently, these were the conditions that were finally adopted for this assay.

Test of the Procedure.—A test of the procedure was made by measuring the conformity of the reaction to the Bouger-Beer absorption law for both standard solutions and a pharmaceutical preparation. The pharmaceutical was a sugar-sorbo base syrup containing in addition to phenylephrine, the lower esters of *p*-hydroxy benzoic acid, pyrilamine maleate, dihydrocodeinone bitartrate, and homatropine methyl bromide.

The following procedure was employed: Transfer a sample containing 0.03 to 1.20 mg. of phenylephrine to a 100-ml. volumetric flask, add 5 ml. of ferricyanide solution followed by 5 ml. of 4-aminoantipyrine solution. Dilute the solutions to mark with 0.01 *M* bicarbonate buffer and allow to stand for fifteen minutes, then read the absorbance at 500 mμ against a reagent blank.

In Table II, some of the data are tabulated, including a set of results obtained when 0.1 *M* bicarbonate was substituted for the 0.01 *M* buffer.

In all three sets of data it will be observed that agreement with the absorption law is within a few per cent when the absorbance is 0.2 or greater. The pronounced effect of the buffer concentration is apparent from a comparison of the first two sets of data. At the higher concentration of buffer the sensitivity of the reaction is only about 60% of that for the lower concentration.

The precision and reproducibility in all cases is adequate for control operations. In addition, the phenylephrine determination can be made directly upon the sample without any separative steps. This contributes substantially to the simplicity of this procedure.

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