TABLE V.—COMPOSITION OF ISOMER MIXTURE Z

Sample	% U	% V
Mixture Z	36 ± 2	62 ± 3
Mixture Z[a]	37 ± 1	61 ± 2
Mixture Z[b]	40 ± 1	59 ± 1
Z[a]	14.0 ± 1.9	29.6 ± 2.4

[a] Separated by TLC from norbormide.

TABLE VI.—MOLAR FLUORESCENCE VALUES OF THE NORBORMIDE ISOMERS

Sample	Molar Fluorescence (Arbitrary Fluorescent Value Divided by the *M* Concn.)
Isomer R	3.33×10^9
Isomer S	2.49×10^9
Isomer U	5.85×10^9
Isomer V	6.51×10^9
Isomer W	2.40×10^9
Isomer X	3.47×10^9
Isomer Y	2.74×10^9
Isomer mixture Z	6.27×10^9
McN-1392	0.82×10^9

the 7:1 mixture of 0.1 *M* hydrochloric acid–ethanol, 25 ml. of methanol was added and the sample shaken for 1 hr. on a mechanical shaker. The sample was centrifuged and exactly 20 ml. of the clear solution was evaporated to dryness without the aid of heat. The residue was quantitatively taken up in methanol and streaked on the silica plates. The silica plate was developed as previously described.

Paper chromatography was used to determine the sharpness of separation of U and V. If the silica plates were not kept at 25° and 50% relative humidity there was from 5 to 10% tailing of the front running band V into the slower band U. The data given for Z separated from the other isomers by TLC represents separations from 5 different lots of norbormide. The standard deviations are also given in the table. Isomer mixture Z, obtained by recrystallization techniques, was found to contain U and V in the ratio of about 4:6 while, mixture Z separated from samples of norbormide by TLC contained them in the ratio of about 1:2.

Fluorescence Assay.—The norbormide isomers were also assayed by measurement of their fluorescence in acid. All the isomers have an activation maximum at 320 ± 5 mμ and a fluorescence maximum at 460 ±5 mμ in a 1:7 ethanol–0.1 *M* hydrochloric acid solvent. When arbitrary fluorescence units were plotted against concentration, a straight line relationship was found for each isomer in the concentration range of 1 to 10 mcg./ml. The method of assay is the same used for the spectrophotometric assay except that the sample dilution is increased. Standards containing approximately the same amount of silica as the samples are taken through the sample extraction procedure with 1:7 ethanol–0.1 *M* hydrochloric acid. Because of the care needed to avoid traces of impurities anywhere in the fluorometric assay, the spectrophotometric assay is preferred. Good agreement has been observed between the fluorescence and spectrophotometric assays for samples assayed by both methods. The molar fluoresence, arbitrary fluorescence units divided by the molar concentration, is given in Table VI for the major isomers of norbormide.

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Ion-Pair Extraction of Pharmaceutical Amines II

Extraction Profile of Chlorpheniramine

By TAKERU HIGUCHI and K. KATO

The extractability of chlorpheniramine in its ion-pair form has been investigated under several conditions to determine the suitability of the process for separation and isolation of the drug in analytical samples. Chlorpheniramine, chosen as an example of a drug having two basic centers per molecule, exists in aqueous solution as a mixture of uncharged, singly charged, and doubly charged species. Data are presented to show that the drug can be extracted as the chloride, bromide, maleate, trichloroacetate, picrate, etc. The extraction-pH profiles of both the picrate and the bromide correspond closely with the theoretical relationship. As with the monoacidic amines, extraction into the organic phase requires the presence of proton donating species. Experimental data suggest, for example, that the extracted species is coordinated with 5 molecules of chloroform.

THE EFFECTS of various anions and the dependence on solvating agents present in the organic phase on the formation of ion pairs extractable into lipoidal solvents were previously presented (1), with dextromethorphan as an example of a monoprotic amine. The current work deals with the behavior of a diprotic organic base, chlorpheniramine.

It has been found that, in addition to showing a marked dependency on the masking or complex-

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ing agent in the organic phase, the extent of ion-pair formation by the chlorpheniraminium cation is a function of the drug species in solution. The studies indicate that the singly protonated form of the drug can give rise to ion pairs, but none were detected with the doubly protonated form. This species specificity would allow separation of chlorpheniramine from monoacidic organic bases simply by ion-pair extraction at two appropriate pH values.

RESULTS AND DISCUSSION

Extraction as a Function of the Anion and Solvating Agent Concentrations. Studies of the partitioning behavior of ion pairs formed from the chlorpheniraminium cation with a number of inorganic and organic anions were carried out as reported previously on dextromethorphan (1). The nature of the partition data in this instance is evident in a typical set of data illustrated in Fig. 1. This particular plot shows the results obtained with varying concentrations of bromide ion in systems containing different concentrations of chloroform as the masking agent in cyclohexane. The pH of the aqueous layer was adjusted to pH 3.5 with citrate buffers. All data are corrected for the amount of the free base which could be extracted in the absence of ion-pair forming anions.

The system can be represented by the following relationship:

$$BH_{aq.}^{+} + X_{aq.}^{-} \overset{K_e}{\rightleftharpoons} [BH^{+}X^{-}]_{org.} + nM \overset{K_c}{\rightleftharpoons} [BH^{+}X^{-}\cdot M_n]_{org.} \quad \text{(Eq. 1)}$$

where $BH_{aq.}^{+}$ represents the monoprotonated chlorpheniraminium cation in the aqueous phase and $X_{aq.}^{-}$ the anion in the aqueous layer, in this case bromide; $[BH^{+}X^{-}]_{org.}$, the ion pair in the organic phase, and $[BH^{+}X^{-}\cdot M_n]_{org.}$, the solvated ion pair; K_e represents the classical extraction constant (2) and K_c, the stability constant for the complex formation.

Although from subsequent data it will be shown that BH^{++}, the doubly protonated chlorpheniraminium cation does not appear to be extractable in ion-pair form for the anionic species studied so far, the expression for the apparent partition coefficient must consider its presence in the aqueous phase, therefore:

$$PC_{app.} = \frac{[BH^{+}X^{-}]_{org.}}{[BH^{+}]_{aq.} + [BH^{++}]_{aq.}} \quad \text{(Eq. 2)}$$

It is readily apparent from Fig. 1 that $PC_{app.}$ is highly dependent on the concentration of chloroform. The data again suggest that the chloroform interacts with the ion pair in the organic layer and apparently renders it more compatible with the organic solvent by masking the anionic ion. As shown earlier at constant bromide ion concentration, $PC_{app.}$ is proportional to $(CHCl_3)^n$, where n is the number of molecules of chloroform reacting or associating with each ion pair, a plot of log $PC_{app.}$ *versus* log $[CHCl_3]$ yielding a straight line with a slope equal to n. Figure 2 shows such a plot for the chlorpheniramine–bromide system at constant bromide concentrations but varying chloroform concentrations. Evaluation of the slopes yields a coordination value of 5 for this system.

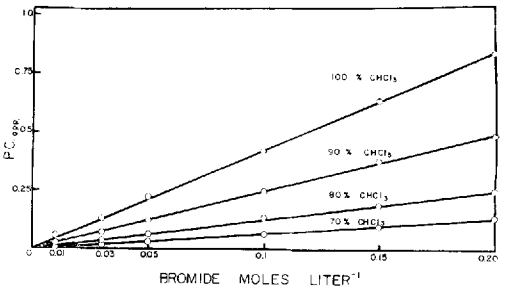

Fig. 1.—The effect of bromide ion on the partitioning of chlorpheniramine at pH 3.5 as a function of chloroform concentration in the organic phase.

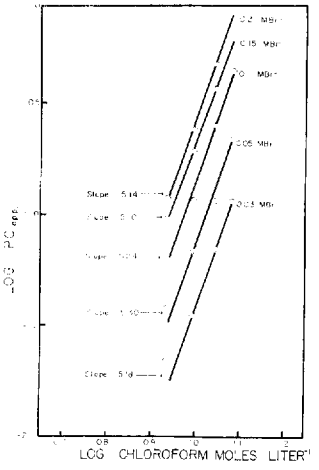

Fig. 2.—The dependency of apparent partition coefficient of chlorpheniramine on the concentration of $CHCl_3$ in the presence of Br at pH 3.5.

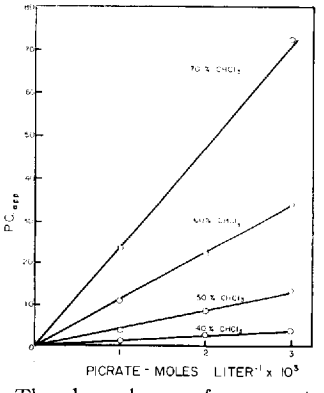

Fig. 3.—The dependency of apparent partition coefficient of chlorpheniramine on picric acid concentration at pH 3.5. The concentration of chlorpheniramine was 5×10^{-5} *M*. The ionic strength was 0.1.

A similar study was carried out using the picrate anion at pH 3.5. The results shown in Fig. 3 are in keeping with those of the bromide interaction, except that the $PC_{app.}$ is substantially greater. The change in the $PC_{app.}$ appears from this and

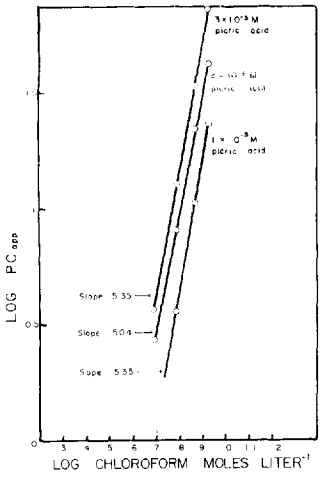

Fig. 4.—The dependency of apparent partition coefficient of chlorpheniramine on the concentration of $CHCl_3$ in the presence of picric acid at pH 3.5.

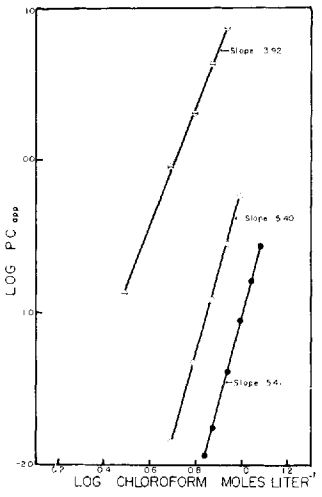

Fig. 5.—The dependency of apparent partition coefficient of chlorpheniramine on $CHCl_3$ concentration in presence of chloride, maleic acid, and trichloroacetic acid at pH 3.5. The concentration of chlorpheniramine was 10^{-3} *M*. Key: ⊖, trichloroacetate, 0.1 *M*; ○, maleate, 0.2 *M*; ●, Cl^-, 0.2 *M*.

TABLE I.—CALCULATED VALUES OF EXTRACTION CONSTANTS FOR CHLORPHENIRAMINE ION PAIRS AT TWO CHLOROFORM CONCENTRATIONS AND DEPENDENCE ON CHLOROFORM CONCENTRATION

$$K_x = \frac{PC_{app.}}{[X^-]} \cdot \left(1 + \frac{[H^+]}{K_a}\right)$$

Anion	1 *M* $CHCl_3$	10 *M* $CHCl_3$	Av. Dependence on $CHCl_3$[a]
Chloride	4.2×10^{-6}	1.9	5.4 = 1
Bromide	1.4×10^{-5}	5.8	5.2 = 1
Maleate	4.4×10^{-5}	15	5.4 = 1
Trichloroacetate	6.7×10^{-2}	550	3.9 = 1
Picrate	7.5×10^{-1}	2.2×10^{4}	5.2 = 1

[a] Slopes of lines such as shown in Figs. 2, 4, and 5.

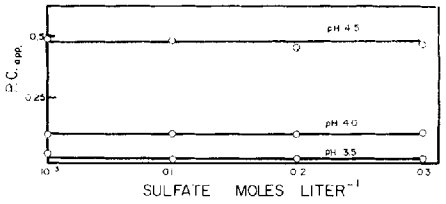

Fig. 6.—The effect of sulfate ion on the partitioning of chlorpheniramine between a pH 3.5 citrate buffer and chloroform.

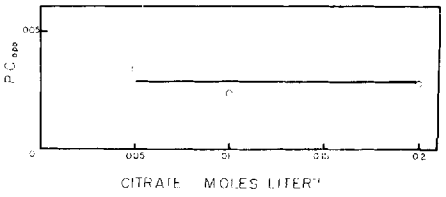

Fig. 7.—The dependency of apparent partition coefficient of chlorpheniramine on citrate buffer concentration as it was extracted by $CHCl_3$ at pH 3.5.

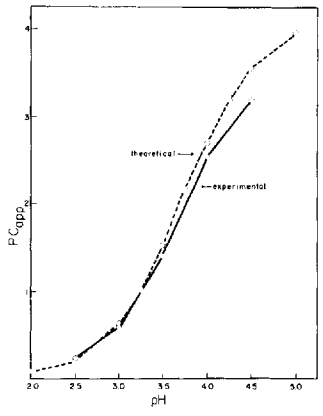

Fig. 8.—pH profile of apparent partition coefficient chlorpheniramine–bromide ion pair. Chlorpheniramine, 10^{-3} *M*; bromide, 0.5 *M*; organic phase, $CHCl_3$; 0.05 *M* citrate buffer.

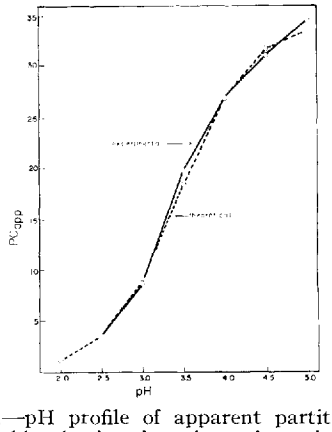

Fig. 9.—pH profile of apparent partition coefficient of chlorpheniramine–picrate ion pair. Chlorpheniramine, 10^{-4} *M*; picrate, 2×10^{-3} *M*; organic phase, 55% $CHCl_3$; in cyclohexane; 0.05 *M* citrate buffer.

other studies to be related to the bulk and hydrophobicity of the anion. In this case, as well as that of the bromide interaction, a plot of log $PC_{app.}$ *versus* $[CHCl_3]$ yielded straight lines as evidenced in Fig. 4 at varying concentrations of picrate anion. From the slopes of the lines it was determined that again 5 molecules of chloroform interacted with each picrate ion pair.

Investigations were also carried out with constant concentrations of chloride, maleate, and trichloroacetate anions under conditions of varying chloroform concentrations. Figure 5 again shows a linear relationship between log $PC_{app.}$ and log $[CHCl_3]$. Table I summarizes the apparent stoichiometry of the interactions of a number of chlorpheniramine–anion ion pairs with chloroform, as well as the extraction constants at 1 and 10 *M* chloroform concentration. Although an expression can be derived for the over-all equilibrium constant as follows:

$$K_e K_c = K_0 = \frac{PC_{app.}}{[X^-][M]^n}\left(1 + \frac{[H^+]}{K_a}\right) \quad \text{(Eq. 3)}$$

where K_0 is the over-all equilibrium constant; K_a is the dissociation constant for $BH^{++} \rightleftharpoons BH^+ + H^+$; in this case 1×10^{-4}, as determined spectrophotometrically.

It is more meaningful to compare the extraction constants at various concentrations of chloroform according to the following equation:

$$K_e = \frac{PC_{app.}}{[X^-]} \cdot \left(1 + \frac{[H^+]}{K_a}\right) \quad \text{(Eq. 4)}$$

Although at this time it is not possible to establish what factors influence the stoichiometry of the complex formed, from this and other work on dextromethorphan, it appears that changes in the anion alter the interaction.

Effect of Polyvalent Anions on the Apparent Partition Coefficient and the Importance of Chlorpheniramine Species in Solution.—As noted earlier, chlorpheniramine exists in aqueous phase in its singly protonated, doubly protonated, or its free base form, the relative concentrations depending on the pH of the system. It was of interest to determine if the sulfate and citrate anions would form ion pairs at different pH values. It was thought that since these anions could exist as doubly charged species they might interact with BH^{++} and form ion pairs.

Figure 6 shows that at a given pH and changing sulfate concentrations the $PC_{app.}$ is essentially independent of $[SO_4^{--}]$ concentration. Below pH 3.0, no chlorpheniramine could be detected in the organic layer. The slight increase in $PC_{app.}$ with pH appears to be extraction of the free base. The effect of citrate anions was determined at pH 3.5 increasing the total citrate concentration from 0.05 mole $L.^{-1}$ to 0.2 mole $L.^{-1}$. The results shown in Fig. 7 show that no ion-pair formation was detected. As sulfate anions show no tendency to form ion pairs, potassium sulfate was used to maintain a constant ionic strength in the studies dealing with bromide, picrate, chloride, maleate, and trichloroacetate anions.

To determine the ion-pair forming ability of the two protonated species of chlorpheniramine, a series of experiments were conducted at constant chlorpheniramine concentrations, constant ionic strength, and varying pH. In one series a constant bromide-ion concentration was maintained and in another a constant picrate. Figures 8 and 9 show similar profiles and would suggest that in both cases the BH^{++} did not form ion pairs, and only BH^+ interacted with the anion to form the extractable species. The theoretical curves in both figures were calculated on this assumption.

Equation 3 can be rearranged to the following form:

$$\frac{1}{PC_{app.}} = \frac{1}{K_0[X^-][M]^n} + \frac{[H^+]}{K_0 K_a [X^-][M]^n} \quad \text{(Eq. 5)}$$

Consequently, a plot of $1/PC_{app.}$ *versus* $[H^+]$ should yield a straight line with a slope of $1/K_0K_a[X^-][M]^n$ and an intercept of $1/K_0[X^-][M]^n$.

Figures 10 and 11 show that both the bromide and picrate data from Figs. 8 and 9 follow the dependency predicted from Eq. 5. The theoretical curve in Figs. 8 and 9 would be calculated from this equation when the appropriate values for n, K_a, and K_0 were utilized.

The K_a for the dissociation of the doubly protonated form was determined spectrophotometrically and found to be 1×10^{-4} at ionic strength of 0.55, but the values obtained from the intercepts and slopes of Figs. 8 and 9 deviated from this value. In the case of the bromide interaction, K_a was found to be 1.84×10^{-4}, and in the picrate system 3.64×10^{-4}.

These differences between the pKa values determined by spectrophotometric determinations and those calculated from Figs. 10 and 11 might possibly be due to interactions resulting in ion-pair formation in the aqueous layer which was not considered in Eq. 1.

From the foregoing and the previous work on dextromethorphan (1), it can be recognized that if chlorpheniramine and dextromethorphan were present in combination in the same system, they would be readily separated by selective ion-pair extraction. At pH 1 in the presence of bromide, dextromethorphan would form ion pairs and could be extracted by the organic phase. Chlorpheniramine being essentially completely in the doubly charged form would not form ion pairs. After separation of dextromethorphan, the pH could be increased to 3.5 and the antihistamine determined.

EXPERIMENTAL

Reagents.—Chlorpheniramine maleate U.S.P.[1] Based upon assay by the U.S.P. nonaqueous titration, it was found to be 99.65% $C_{20}H_{23}ClN_2O_4$.

Chloroform, analytical reagent, was shaken with phosphorus pentoxide and distilled to remove the ethanol. Two per cent (v/v) of *n*-amyl alcohol was added as stabilizer.

All water was glass distilled in the presence of potassium permanganate.

All other reagents were of analytical grade.

Chlorpheniramine Sulfate Stock Solution.—This solution was utilized in the tests determining the apparent partition coefficient in all cases except as described under the maleate system.

Preparation.—Dissolve 3.9 Gm. of chlorpheniramine maleate in 100 ml. of distilled water. Adjust to pH 8–9 and extract with three 50-ml. vol. of

[1] Supplied through the courtesy of the Schering Corp., Bloomfield, N. J.

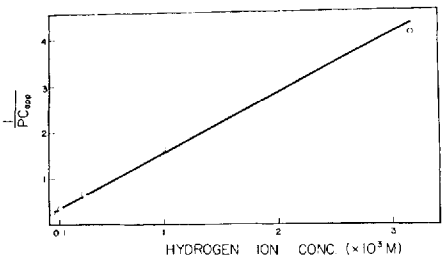

Fig. 10.—Reciprocal apparent partition coefficient of chlorpheniramine in the presence of 0.5 *M* bromide ion as a function of hydrogen-ion concentration.

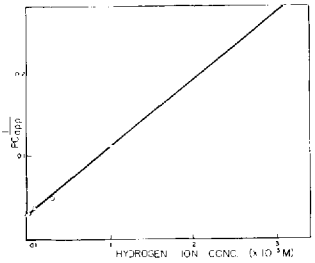

Fig. 11.—Reciprocal apparent partition coefficient of chlorpheniramine in the presence of 2×10^{-3} *M* picrate ion as a function of hydrogen-ion concentration.

cyclohexane. The combined extracts are washed with 50 ml. of distilled water. The cyclohexane is then extracted with three 50-ml. vols. of 0.05 *M* sulfuric acid. The solution is assayed spectrophotometrically and adjusted to a concentration of 5×10^{-2} mole/L. by the addition of distilled water.

Procedure of Partition.—*Procedure A.*—This procedure was employed for the partitioning of chlorpheniramine in the presence of bromide, chloride, maleate, and trichloroacetate anions. Solutions were prepared by dissolving acid or potassium salt of anion in the mixture of chlorpheniramine stock solution and citrate buffer (0.05 *M* citric acid, the pH adjusted by addition of 0.05 *M* potassium hydroxide solution). The concentration of chlorpheniramine in the solution was determined spectrophotometrically at the wavelength of absorption maximum using the calibration curve made from chlorpheniramine stock solution. The wavelengths of absorption maximum at each pH were as follows: pH 2.5, 265 mμ; pH 3.0, 264 mμ; pH 3.5, 263 mμ; pH 4.0, 262 mμ; pH 4.5–5.5, 261 mμ. Twenty milliliters of the aqueous solution was placed in a 60-ml. separator, and an equal volume of the organic phase was added. The mixture was allowed to stand for 1 hr. at 25° to allow the temperature to equilibrate. The sample was then shaken for 30 min. Both phases were separated, and the concentration of chlorpheniramine in the aqueous phase was determined. The concentration in the organic phase was calculated by subtracting the concentration in the aqueous phase from the initial concentration. In order to correct for the concentration of free base extracted into the organic phase, the same procedure was carried out for the blank solution which was prepared from chlorpheniramine, buffer, and containing potassium sulfate to adjust the ionic strength. The apparent partition coefficient was calculated from the following equation:

$$\text{apparent partition coefficient, } PC_{app.} = \frac{\text{total concn. in organic phase} - \text{concn. of free base in organic phase}}{\text{concn. in aqueous phase}}$$

The correction for free base concentration was not made for determining the apparent partition coefficient in the studies of the concentration dependency of sulfate and citrate because of the lack of suitable method. In the experiment with maleate ion, the determination of chlorpheniramine was made in the organic phase using the calibration curve made from chlorpheniramine maleate because the absorption of the anion interfered with the determination in the aqueous phase.

Procedure B.—This procedure was used for the partition of chlorpheniramine in the presence of picric acid. Solutions of chlorpheniramine and of picric acid were prepared, respectively, at the same pH using 0.05 *M* citrate buffer. In a 60-ml. separator 10 ml. of the chlorpheniramine solution and 10 ml. of the picric acid solution were placed, and 20 ml. of organic phase was added to this mixture. After both phases were brought to the temperature equilibrium at 25°, they were shaken for 30 min. Both phases were separated, and a 10-ml. aliquot of the organic phase was placed into a second 60-ml. separator, to which 5 ml. of the picric acid solution and 5 ml. of the buffer were added. The remaining organic phase of the first extraction served for determining the absorbance of the first extraction, A_1. The second separator was treated in the same manner as the first, and the organic phase separated was used for determining the absorbance due to the second extraction, A_2. The absorbance was determined at the wavelength of maximum absorption, 342 mμ, using the pure organic phase as the reference. A blank extraction, without chlorpheniramine, was carried out to correct for the free picric acid which was extracted along with the ion pair. A mixture of 10 ml. of the picric acid solution and 10 ml. of the buffer was shaken with 20 ml. of the organic phase in the same manner as cited above. Absorbances, A_1' and A_2', were determined for the blank extraction, and the apparent partition coefficient was calculated according to the following:

$$PC_{app.} = \frac{A_2 - A_2'}{(A_1 - A_1') - (A_2 - A_2')} u$$

where u is volume ratio of both phases, $V_{aq.}/V_{org.}$ The volume ratio, u, was changed according to the magnitude of the apparent partition coefficient. Chlorpheniramine free base shows no absorption in the wavelengths near 342 mμ.

pKa$_1$ of Chlorpheniramine.—Solutions were prepared from chlorpheniramine stock solution, potassium bromide, and 0.05 *M* citrate buffer at varied pH between 1.6 and 6.4. The concentration of chlorpheniramine was 2×10^{-4} *M* and of bromide was 0.5 *M*. The absorbances were determined at the wavelength of 261 mμ, and pKa was calculated according to the method of Flexner (3).

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