Spectrophotometric Determination of Diphenhydramine Hydrochloride Using Carmoisine by Solvent Extraction

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Summary: Carmoisine (chromotrope FB, C.I. 14720, acid red 14) has been used for the determination of diphenhydramine.

Introduction

Diphenhydramine is generally determined spectrophotometrically [1] by measuring the absorbance of its acidic aqueous solution at 252 nm. Diphenhydramine was also determined spectrophotometrically at λ = 440 nm after treating its solution with cobalt nitrate and potassium thicyanate in acid media followed by extraction of the complex obtained [2]. It was found that many basic compounds combine with a number of acid dyes at certain pH to form additive products which are distinguished by their solubility in organic solvents [3,4]. This reaction has been utilised by many workers for the determination of a large number of basic compounds [5]. Amaranth ($C_{20}H_{11}N_2Na_3O_{10}S_3$) is an example used for the determination of chlorpromazine at pH around 2.5 [6] where the coloured product was measured at 520 nm.

The present work was directed to study the possible use of acid red 14 (carmoisine) for the quantitative determination of diphenhydramine. The alkalolid-carmoisine complex was also prepared as solid complex, analysed and its vibronic acid electronic spectra were identified. The factors affecting the complex formation and extraction of this complex was also studied and its extraction constant was evaluated. The effect of interfering ions was investigated.

Results and Discussion

Preliminary studies showed that both carmoisine and diphenhydramine are insoluble in chloroform. Attempts were made to test the solubility of diphenhydramine-carmoisine complex with different water immiscible organic solvents. Unfortunately benzene, diethyl carbonate, benzyl alcohol, 1,2-dichlorobenzene, toluene, benzyl cyanide, 1,2-dichloroethane, petroleum ether (40-60°C) and nitrobenzene failed to extract either carmoisine or the complex. Methylene chloride and chloroform were about equally efficient in extraction of the complex. Chloroform was preferred on the basis that its boiling point is higher than methylene chloride. Aqueous carmoisine solution displays a maximum absorption peak at 510 nm, and 520 nm on complexation (in $CHCl_3$). This shift may be attributed to the electron donor property of oxygen atom of the SO_3 group of carmoisine.

The effect of pH on the electronic spectra of both carmoisine and the complex were studied. For carmoisine, 5 x 10^{-4}M solution was adjusted to pH's = 2-11 using few drops of dilute HCl and NaOH solutions. These solutions were diluted to 10 ml with bidistilled water and the absorbance was measured against water as a blank. Spectral absorbances (Fig. 1 a) show two absorption bands at λ_{max} 315 and 510 nm. The absorbance at 315 nm increased as the pH increased from 2 to 5 then decreased up to pH 10. This is followed by another increased up to pH 11 with a bathochromic shift from 315 to 327 nm.

The second absorption band at 510 nm showed the same trend but with hypsochromic shift from 510 to 502 nm bove pH 2. For diphenhydramine-carmoisine complex, the pH's of aliquots of the alkaloid solution (10^{-3}M) were adjusted to 2-11 using dilute HCl. Carmoisine solution (10^{-3}M) was allowed to react with the alkaloid. The formed complex was then extracted with $CHCl_3$ by shaking for 20 min in a thermostated shaker at 25°C. The chloroform layer was transferred to 25 ml measuring flask and completed to the mark. The absorbance was measured at 520 nm. The data obtained are represented in Fig. 1b. From this figure it could be concluded that the color of the extracted complex reaches its maxima at pH 2.3 then the absorbance began to decrease as the pH increased.

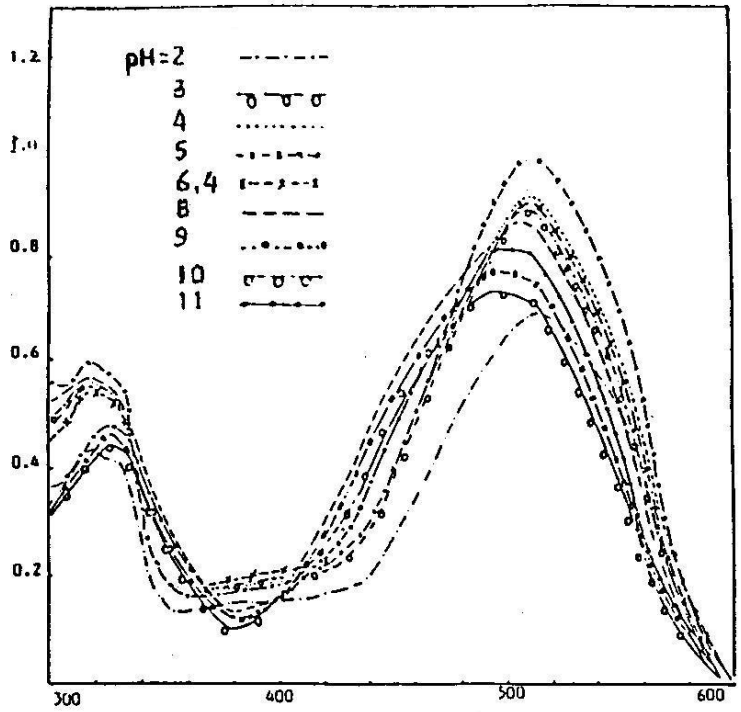


Fig. (1a): Effect of pH on the absorption spectra of $5x10^{-5}$M carmoisine.

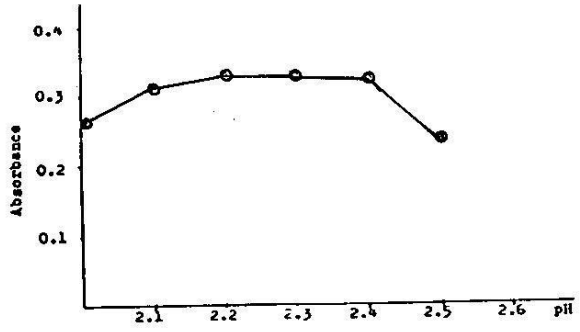


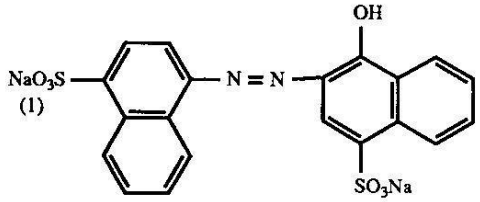
Fig.(1b): Effect of pH on the absorbance spectra at 520 nm.

This decrease can be related to the deprotonation of the tertiary amine centre of the alkaloid. As there are two sulphonate groups (1) and (2) and an -N=N group in carmoisine molecule, the -N=N- group affect only ortho- and para- position. Thus, SO_3H (1) induced positively charges, i.e. increase its acidity where it is responsible for the protonation of tertiary nitrogen in alkaloid during complex formation.

The effect of carmoisine concentration (mole ratio) was studied. A 1.5 ml of 10^{-3}M of diphenhydramine hydrochloride was adjusted at pH 2.3 and allowed to react with (0.5, 1.5, 3.0, 4.5 and 5.0 ml) of 10^{-3}M of carmoisine solution. The mixtures were shaken for 20 min. in a thermostated shaker at temperature of 25°C with 3 x 5 ml $CHCl_3$. The $CHCl_3$ layer was then separated and completed to 25 ml using $CHCl_3$ and the absorbance was measured at 520 nm. The data are represented graphically (Fig. 2). The figure reveal that carmoisine reacts with diphenhydramine with equimolar concentration.

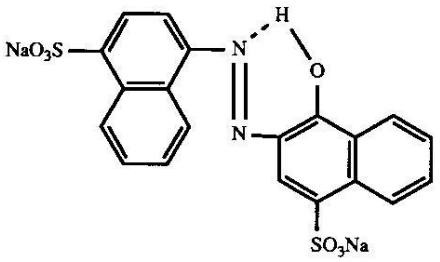
Infrared of solid complex

Carmoisine or chromotrop, FB C.I. 14720 (Acid red 14) has the structural formula:



(2)

The IR spectrum of carmoisine shows a very broad band due to the stretching vibration of the -OH group at 3450 cm^{-1}. The broad band may be usually attributed to the fact that -OH group may be involved in intermolecular hydrogen bonding as follows:



The bands at 1600-1400 cm^{-1} are of interest sinced these bands may be due $\gamma_{N=N}$, $\gamma_{S=O(asym)}$, $\gamma_{OH(bending)}$ and γ_{S-O} respectively. Bands 1400-1000 cm^{-1} region are due to the C-N stretching vibration, O-H and C-H in plane deformation modes, phenyl-O stretching band of the phenolic system, stretching $-SO_3H$ bands and various C-C stretching modes. According to Schreiber [9], the sulphonic acid absorbs at 1182 cm^{-1}.

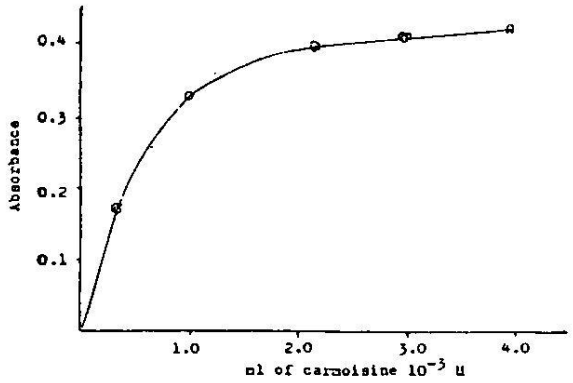


Fig. 2: Effect of mole ratio on the absorbance spectra at 520 nm.

The shaking time of the reactants was studied at 5, 10, 15, 20, 25 and 30 min. where the pH of the alkaloid (10^{-3}M) was adjusted to 2.3 and allowed to react with caromoisine solution (10^{-3}M). The reactants were shaken with $CHCl_3$ for different time intervals up to 30 min. in a thermostated shaker at 25°C. The organic layer was separated and completed to 25 ml with $CHCl_3$, and the absorbance was measured at 520 nm against $CHCl_3$. The absorbance of the extracted coloured complex reach a constant value after 20 min.

The effect of temperature was considered by adjusting the pH of the alkaloid solution (10^{-3}M) to 2.3 and then allowed to react with equal amount of (10^{-3}M) carmoisine solution. The solution mixture was shaken with $CHCl_3$ for 20 min. in a thermostated shaker adjusted at temperature of 10, 20, 30, 40°C. The organic layer was then separated and the absorbance was measured at 520 nm. It was noticed that the $CHCl_3$ extract at 40°C, forms an emulsion with aqueous solution which is difficult to break down. The variations in temperature have no effect on the absorbance at 10-30°C range.

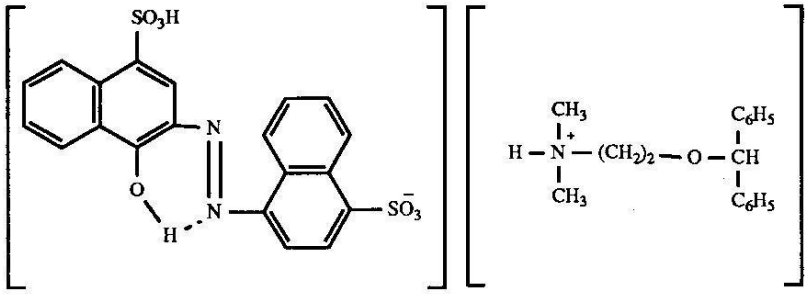
The extraction constant of diphenhydramine-carmoisine complex was obtained spectrophotometrically by the modified method of isomolar series [7,8] and the results showed a mean value of $\log k_E$ = 3.82, indicating good extraction with chloroform.

Diphenhydramine (2-Benzhydryloxy-N,N-dimethylethylamine) is an isomer of phenyltoloxamine. Its IR [1] spectrum shows characteristic peaks at 713, 754, 991, 1017, 1103 and 1180 $cm.^{-1}$ The IR spectrum of diphenhydramine-carmoisine complex shows a significant difference in the region 2200-2800 $cm.^{-1}$ The wide band in this region in the alkaloid spectrum characteristic [10] of R_3NH^+ Cl^-, shifted to higher frequencies in the alkaloid- carmoisine complex spectrum. Its intensity decreases due to overlap of γ_{-NH+} - of the alkaloid and $\gamma_{S=O}$ of the carmoisine during complexation. The interpretation of the IR spectrum and microanalysis is given in Table 1.

Table 1: microanalysis data of the diphenhydramine-carmoisine complex.

	C	H	N	S
Calculated:	62.25	4.94	5.88	8.9
Found:	63.00	5.1	6.01	9.01

are in fair agreement with the general formula:



The interfering compounds were tested and the absorbance values were measured at 520 nm with concentration of 10^{-3}M aqueous solutions of both diphenhydramine and the foreign ions (benzamide, amino acids, inorganic cations and other alkaloids) using carmoisine as previously mentioned. The results show that benzamide show little interference at concentration levels equal to diphenbydramine. Sodium chloride, ammonium chloride, potassium chloride, glycine and anthranilic acid did not interfere at concentration levels as high as 10 fold molar excess over concentration of diphenhydramine used.

Some alkaloids such as caffeine, ephedrine hydrochloride, phenylepherine hydrochloride and chlorquine phosphate do not interfere at concentration level of 100 fold molar excess even when they form complexes with carmoisine. This may be due to the fact that the complexes of these alkaloids are not extracted with chloroform. Cinchonine hydrochloride seriously interfere with any concentration level since they form complexes easily extracted with chloroform.

Spectrophotometric determination of diphenhydramine hydrochloride in "Isilin" syrup

(i) Preparation of the sample

Aliquots of 2.7 ml of the Isilin solution (0.270 g diphenhydramine HCl per 100 ml) were introduced to 25 ml volumetric flask and completed to the mark using bidistilled water to give a final solution containing 0.2916 mg/ml.

(ii) Procedure

Aliquots (1.0 - 2.0 ml) of the prepared sample solution were adjusted at pH 2.3 by using dil HCl and treated with 2 ml aliquots of carmoisine solution 10^{-3}M. The constituents were shaken with $CHCl_3$ for 15 min in a thermostated shaker at temperature 25°C.

The organic phase was separated and completed to 25 ml with $CHCl_3$ and the absorbance was measured at 520 nm. The absorbance was compared with the calibration curve. The results obtained are shown in Table 2 with an average recovery of 97.2% and the mean standard deviation of 1.2.

Comparing the suggested method with the official non-aqueous titration method [11] it is clear that the procedure is more simple, accurate and applicable. In addition, the sensitivity of the developed method is within the fraction of mg (Table 2) which is in a range much lower than that reported in B.P. 1988 (0.25 g/20 ml).

Table 2: Results of spectrophotometric determination of diphenhydramine taken in "Isilin" syrup and their recoveries.

Abosrbance at 520 nm	Weight/mg taken	found	Recovery (%)	Standard deviation
0.208	0.292	0.280	95.9	
0.255	0.350	0.338	96.6	
0.301	0.408	0.397	97.3	1.2
0.349	0.467	0.464	99.4	
0.385	0.525	0.507	96.6	
0.431	0.583	0.569	97.6	

Experimental

Reagents

A solution of 10^{-3}M of both diphenylhydramine hydrochloride and carmoisine were prepared by dissolving the appropriate weight in bidistilled water. Alcohol free chloroform has been used during the measurements.

Equipment

The spectral measurements were undertaken using double beam Lambda 3B spectrophotometer (Perkin Elmer). The pH meter model CG (Schott Gerate), a thermostated water bath (Precision) equipped with automatic temperature adjustment were also used. The IR spectra were recorded on Pye Unicam SP 9700 using KBr disc.

Procedure

(a) Preparation of diphenhydramine-carmoisine complex

To a 1.5 ml of diphenhydramine solution (10^{-3}M) 3.0 ml of carmoisine solution (10^{-3}M) was added and pH was adjusted to 2.3. The solution mixture was stirred at 25°C for 20 min. The formed complex was extracted successively for five times with 5 ml $CHCl_3$. The organic extract was collected in 50 ml beaker and evaporated on water bath and solid complex separated.

General procedure for spectrophotometric determination of diphenhydramine

To diphenhydramine solution (0.3-0.8 mg/ml) 3.0 ml of carmoisine solution (10^{-3}M) was added and the pH was adjusted to 2.3. The solution mixture was shaken at 25°C for 20 min. The formed complex was extracted five times with 5 ml $CHCl_3$. The chloroform layer was collected and the absorbance was measured at 520 nm.

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