ATROPINE AND QUININE IONIC ASSOCIATES WITH SOME ACID DYES

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The formation of ionic associates of atropine and quinine with bromothymol blue, metanil yellow and cresol red was studied by extraction spectrophotometry. In aqueous solutions, formation of ionic associates was only observed for quinine with bromothymol blue; ionic associates of both dyes with all of the three dyes could be, however, studied by their extraction into chloroform. The conditional extraction constants were calculated for the equilibria involved. The ability of atropine, quinine and bromothymol blue to be extracted into chloroform was examined in dependence on pH and ionic strength of the aqueous phase. The $pH_{1/2}$ value corresponding to 50% extraction recovery decreases with increasing ionic strength for quinine whereas for atropine the extraction recovery is only slightly affected by a higher ionic strength and for bromothymol blue the $pH_{1/2}$ ($E = 50\%$) value increases with increasing ionic strength.

Extraction photometric methods can be applied to the determination of organic bases, namely so that a suitable acid dye is added and the forming ionic associates are extracted into an organic solvent and determined.

Modin and Schill[1] were able to demonstrate that hydrophilic phthalic acid anions can be extracted into chloroform as associates with long-chain alkylammonium cations. Motomizu and coworkers[2] examined the extraction of ionic associates with *p*-rosaniline derivatives, assuming that possessing a low stability in water, the associates are extracted into organic solvents.

Stable associates with some dyes in aqueous solutions are formed by tensides. The acid-base properties of systems of Chromazorul S or Eriochromazurol B with Septonex[R] were studied and the conditional stability constants determined by Burešová and coworkers[3]. Havel and coworkers[4] described the properties of bromocresol green and its ability to form associates with tensides in aqueous solutions. If the concentration of the ionic associate formed was too low to lend itself to isolation from aqueous solution, extraction with chloroform or some other lipophilic organic solvent was used.

Atropine and quinine were determined[5,6] as well-extractable ionic associates with acid dyes. Interfering effects from co-extracted components were eliminated by using the method of constant analyte additions.

In the present work, conditions are sought for quininium chloride and atropinium sulphate to form associates with bromothymol blue, metanil yellow and cresol red. The effect of ionic strength (KCl) on the extractability of the bases and dyes into chloroform is also investigated.

EXPERIMENTAL

Chemicals and Apparatus

The purity of quininium chloride and atropinium sulphate (Spofa, Prague) was checked by titration with sodium tetraphenylborate using a potassium ion selective electrode[7]. The preparations of the two bases contained 97·64% and 99·98% active component, respectively. Working solutions were obtained by diluting their stock solutions, $c = 1$ mmol l^{-1}. The purity of bromothymol blue (3,3'-dibromothymolsulphophthalein), metanil yellow (sodium diphenylaminobenzene-3-sulphonate) and cresol red (*o*-cresolsulphophthalein) was checked by thin layer chromatography on commercial Silufol plates for UV 20 × 20 cm (Kavalier, Sázava) and spectrophotometrically[8]. The preparations were applied to the start in amounts of 10—15 μg in 1% methanolic solutions, and the plates were developed with a butyl acetate–pyridine–water 2 : 2 : 1 mixture. Bromothymol blue and metanil yellow gave each a single spot with R_F 0·5 and 0·55, respectively, their purity was 96·7% and 98·3%, respectively. Cresol red, in a purity of 88·2%, gave a spot with R_F 0·54 and a minor spot with R_F 0·78.

The concentration of the dye solutions was 1 mmol l^{-1}. The pH was held constant over the region of pH 1·1—8·0 by means of citrate and phosphate buffers according to Sörensen and Walbum[9] and adjusted with 0·1M-KOH or HCl using an MW 870 precision pH-meter (Präcitronic, Dresden, G.D.R.) equipped with a GA 50N glass electrode and an SE 20N calomel electrode. The other chemicals were of reagent grade or better purity (Lachema, Brno). Chloroform was extracted with water, dried with anhydrous calcium chloride and distilled at 60·5 to 61·5°C.

The spectra were recorded and determinations performed with a Specord UV-VIS double-beam spectrophotometer and a Spekol 11 spectrocolorimeter (both Carl Zeiss, Jena, G.D.R.). The results were processed using an ADT 4 500 minicomputer (ZPA, Čakovice) by means of our own programs adapted according to Eckschlager et al.[10].

Ionic Associates of Quinine with Bromothymol Blue in Aqueous Solutions

Quininium chloride solution (0·2 ml; 0·1—0·3 mmol l^{-1}) and bromothymol blue solution (0·2 ml; 0·365 mmol l^{-1}) were mixed and diluted to 2·5 ml with buffer pH 5·0—8·0 in pH steps of 0·2. In blank experiments, quininium chloride solution was replaced by water. Absorbance spectra were recorded for various pH and the absorbance differences ΔA at 433 and 615 nm were plotted in dependence on pH.

Extraction of Ionic Associates into Chloroform

Absorbances at the absorption maximum wavelength were measured for chloroform solutions of the extracts in dependence on the pH of the aqueous phase over the region of pH 1·0—8·0 in pH steps of 0·1. Pipetted were 0·1 ml of aqueous solution of dye and 0·1 ml of solution of base (each in a concentration of 1 mmol l^{-1}), the mixture was diluted to 2·0 ml with buffer solution, and the whole was extracted with 2·0 ml of chloroform for 45 min; thereafter, the phases were allowed to separate and the absorbance of the organic phase was measured.

The molar absorptivities ε of the associates, calculated assuming their quantitative extraction into chloroform, were determined using excess base in the aqueous solution. Buffered solution of the dye (0·05 mmol l^{-1}) was pipetted in amounts of 0·1—1·0 (step 0·1 ml), diluted to 1·0 ml with buffer solution, and 1 ml of aqueous solution of base was added (c_B = 10 mmol l^{-1}). The aqueous phase was extracted with 2·0 ml of chloroform. The extraction recovery was checked by determining the alkaline form of the dye remaining in the aqueous phase, viz. by absorbance measurement at λ_{max} of this form. The molar absorptivities were obtained as the slopes of the $A = f(c_L)$ dependences (c_L is the concentration of dye). In the other cases, where the associates did not extract quantitatively, the slopes of the dependences of absorbance on concentration gave the conditional absorptivities ε' which included the extraction recovery.

Effect of Ionic Strength on the Extraction of Bases or Dyes into Chloroform

The log $D = f$(pH) dependences (D is the distribution ratio) were measured with solutions of the dyes or bases in concentrations of 0·1—50 mmol l^{-1} adjusting the pH with 0·01M-HCl or NaOH and the ionic strength with aqueous 1M-KCl. The aqueous solutions prepared were extracted with chloroform (3 ml of each phase), and the absorbances of both the extracted and un-extractable aqueous phases were measured at 303 nm for quinine and at 258 nm for atropine. For the measurement, the dye solution before and after extraction were diluted with 0·1M-H_2SO_4 (metanil yellow) or 0·1M-NaOH (cresol red, bromothymol blue). Absorbances of the acid and alkaline solutions were measured at 525 and 615 nm, respectively.

RESULTS AND DISCUSSION

In aqueous solutions, quinine (c_B = 5 μmol l^{-1}) forms ionic associates with bromothymol blue; this manifests itself by a yellow-green colour, as compared to the green colour of the blank solution of the dye. Fig. 1 demonstrates absorbance lowering in solutions of the associate at pH 6·0, Fig. 2 shows the differences between the absorbances of blank solutions and solutions with the associate at 433 and 615 nm in dependence on pH for various quinine concentrations.

Fig. 1 shows that the absorbance decreases in both peaks of the curve of the dye, this decrease being dependent on the concentration of quinine (Fig. 2). The remaining two dyes in aqueous solutions do not form ionic associates with quinine in detectable quantities. Atropine forms no associates stable in aqueous solutions with the three dyes used.

Quinine and atropine ionic associates could be obtained by extraction into chloroform. It can be assumed that in aqueous solutions, protonation of the bases occurs, giving rise to cations BH_n^{n+}:

$$B + n\,H^+ \rightleftharpoons BH_n^{n+} \qquad (A)$$

characterized by protonation constant

$$\beta_{Hn} = [BH_n^{n+}]/([B]\,[H^+]^n)\,. \qquad (1)$$

The dye (bromothymol blue, metanil yellow) H_nL dissociates:

$$H_nL \rightleftharpoons L^{n-} + n\,H^+ \qquad (B)$$

with

$$\mathscr{K}_{ai} = [H^+]^n\,[L^{n-}]/[H_nL]\,. \qquad (2)$$

For the formation of the ionic associate, a particular protonated base species BH_i^{i+} with a protonation constant β_{Hi} can be considered.

$$[BH_i^{i+}] = x_i\,c_B \qquad (3)$$

or

$$[BH_i^{i+}] = \beta_{Hi}[B]\,[H^+]^i\,. \qquad (4)$$

The concentration of the base c_B is

$$c_B = [B] + \beta_{H1}[B]\,[H^+] + \beta_{H2}[B]\,[H^+]^2 + \ldots + \beta_{Hn}[B]\,[H^+]^n\,, \qquad (5a)$$

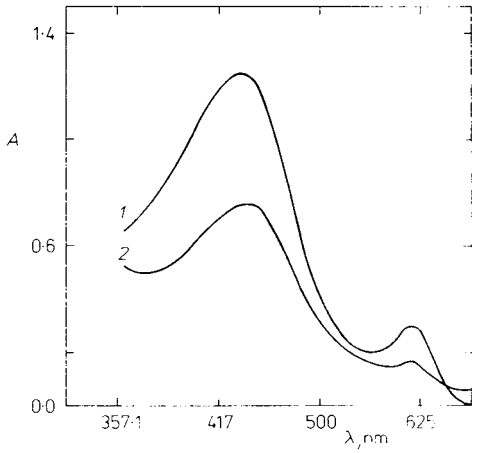

FIG. 1

Absorption spectra of *1* bromothymol blue and *2* its ionic associate with quinine in aqueous solutions

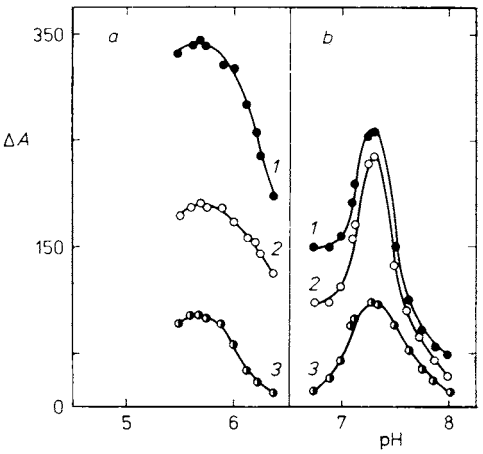

FIG. 2

Absorbance difference *a* at 433 nm and *b* at 615 nm for aqueous solutions of bromothymol blue and its ionic associates with quinine in dependence on pH; c_{dye} = = 365 μmol l^{-1}, c_{base}(μmol l^{-1}): *1* 250, *2* 167, *3* 8·3

or

$$c_B = [B] + (1 + \sum_{i=1}^{n} \beta_{Hi}[H^+]^i) \tag{5b}$$

Similarly for a particular dye species H_iL (charges are omitted),

$$[H_iL] = x_i c_L \tag{6}$$

or

$$[H_iL] = \mathscr{K}_{ai}^{-1}[L^{n-}][H^+]^i . \tag{7}$$

The concentration of the dye c_L can be expressed as

$$c_L = [L^{n-}] + \mathscr{K}_{a1}^{-1}[L^{n-}][H^+] + \mathscr{K}_{a2}^{-1}[L^{n-}][H^+]^2 + \ldots + \mathscr{K}_{an}^{-1}[L^{n-}][H^+]^n , \tag{8a}$$

or

$$c_L = [L^{n-}] + (1 + \sum_{i=1}^{n} \mathscr{K}_{ai}^{-1}[H^+]^i) . \tag{8b}$$

Interaction of cation BH_n^{n+} with anion L^{n-} gives rise to the ionic associate $\{BH_n^{n+} . L^{n-}\}$, which is extracted into the organic phase:

$$BH_n^{n+} + L^{n-} \rightleftharpoons \{BH_n^{n+} . L^{n-}\}_{org} \tag{C}$$

with the extraction constant

$$K_{ex} = \{BH_n^{n+} . L^{n-}\}_{org}/([BH_n^{n+}][L^{n-}]) \tag{9}$$

accounting for the protolysis, distribution as well as the resultant extraction equilibria. Its value should be actually expressed in activity terms. Instead, we introduce the conditional extraction constant K'_{ex} in terms of the distribution ratio D, which virtually represents the concentration ratio of the ionic associate in the chloroform extract to the protonated base BH_n^{n+} in the aqueous phase.

Assuming that the two bases, quinine and atropine, exist in the aqueous phase in the BH^+ form and the associates are 1 : 1 type, the conditional extraction constant can be written as

$$K'_{ex} = D/[L^-] . \tag{10}$$

This equation can be adapted to

$$K'_{ex} = D/\{c_L x_i - (A - A_{b1})/\varepsilon l)\} , \tag{11}$$

where c_L is the initial concentration of dye, x_i is the mole fraction of the L^- dye species, A is absorbance of solution of dye with base, A_{bl} is absorbance of blank solution, ε is molar absorptivity of ionic associate in aqueous solution and l is optical pathlength. The second term in the denominator accounts for the loss of dye due to the formation of the ionic associate.

Molar absorptivity ε, conditional molar absorptivity ε' and extraction recovery E in percent values are interrelated by

$$E = (\varepsilon'/\varepsilon) \cdot 100 . \qquad (12)$$

For equal volumes of the aqueous and organic phases ($V_{aq} = V_{org}$), the distribution ratio is

$$D = E/(100 - E) = \varepsilon'/(\varepsilon - \varepsilon') . \qquad (13)$$

The extraction recoveries and distribution ratio of the ionic associates of atropine and quinine with the three dyes as well as the molar absorptivities ε and ε' are given in Table I. This table also includes the detection limits[11].

Table I demonstrates that quinine is extracted quantitatively in the presence of bromothymol blue and metanil yellow. The behaviour of atropine approaches that of quinine only in the presence of bromothymol blue. The dependences of the extraction recoveries on the pH of the aqueous phase for the ionic associates are

TABLE I

Characteristics of quinine and atropine associates with bromothymol blue, metanilic yellow and cresol red

Characteristics[a]	Bromothymol blue		Metanil yellow		Cresol red	
	quinine	atropine	quinine	atropine	quinine	atropine
ε, l mol^{-1} cm^{-1}	36 190	18 880	37 600	26 890	12 550	9 680
ε', l mol^{-1} cm^{-1}	35 800	17 200	37 510	7 060	11 360	110
D	92·0	10·2	416·8	0·36	9·6	0·01
E, U	98·9	91·1	99·8	26·2	90·6	1·10
log K'_{ex}	5·9	4·8	6·4	3·9	4·6	0·9
D.l., μmol l^{-1}	1·0	5·2	3·3	7·6	1·7	3·2
pH_{opt}	5·3	5·0	2·0	4·0	5·0	3·0
λ_{max}, nm	415	415	405	405	410	410

[a] ε molar absorptivity of associate; ε' conditional molar absorptivity of associate; D distribution ratio; E extraction recovery; K'_{ex} conditional extraction constant; D.l. detection limit; pH_{opt} optimum pH for associate extraction into chloroform; λ_{max} position of absorption maximum of associate in chloroform solution.

shown in Figs 3 and 4. The maximum extraction of quinine associates with bromothymol blue and cresol red is attained at pH 5; with metanil yellow the extraction is highest at pH 2 and decreases with increasing pH. Atropine with bromothymol blue is extracted similarly as quinine. With metanil yellow the extraction recovery is lower for atropine than for quinine; also with cresol red the atropine associate is extracted markedly less than the quinine associate. The different extractability of the ionic associates of atropine and quninine with the three dyes can be explained in terms of the different polarity and solvation of the compounds involved.

The results indicate that bromothymol blue cannot be employed for the determination of quinine in the presence of atropine, whereas if cresol red is used, this determination will be possible up to a limiting concentration of atropine. Generally, the possibility of determining analyte (a) in the presence of interferent (i) can be expressed as

$$\delta_i = n_i/n_a \tag{14}$$

which is the highest amount-of-substance ratio of interferent to analyte at which the latter can still be determined[12]. For the extraction spectrophotometric determination,

$$\delta_i = V_i A_i \varepsilon'_a / V_a A_a \varepsilon'_i , \tag{15}$$

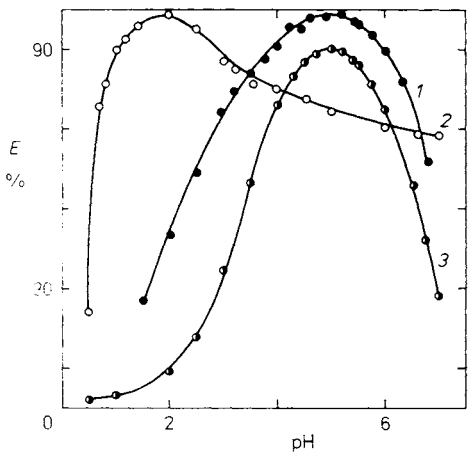

FIG. 3

Dependence of extraction recovery on pH for ionic associates of quinine with *1* bromothymol blue (λ 415 nm), *2* metanil yellow (λ 405 nm), *3* cresol red (λ 410 nm)

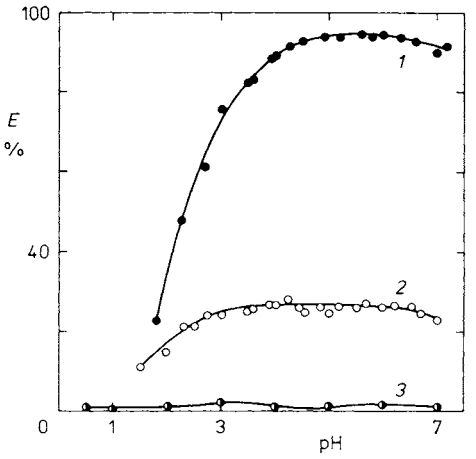

FIG. 4

Dependence of extraction recovery on pH for ionic associates of atropine with *1* bromothymol blue (λ 415 nm), *2* metanil yellow (λ 405 nm), *3* cresol red (λ 410 nm)

TABLE II

Effect of ionic strength (I) on the extraction of the neutral atropine, quinine and bromothymol blue acid-base species

I	a^a	b^a	$s_a{}^b$	$s_b{}^b$	$pH_{1/2}{}^c$	r^d
			Atropine			
0·002	−7·10	0·69	0·002	0·001	10·24	1·000
0·1	−7·50	0·73	0·100	0·010	10·27	0·9986
0·3	−4·98	0·48	0·200	0·020	10·39	0·9853
0·6	−6·30	0·6	0·090	0·008	10·49	0·9992
			Quinine			
0·002	−5·36	0·83	0·480	0·075	6·46	0·9474
0·1	−2·88	0·51	0·220	0·033	5·24	0·9758
0·3	−1·59	0·37	0·530	0·082	4·33	0·8447
0·6	−1·18	0·35	0·350	0·057	3·36	0·9083
			Bromothymol blue			
0·002	5·33	−1·29	0·860	0·210	4·15	0·9623
0·1	4·26	−0·99	0·270	0·070	4·28	0·9829
0·3	3·53	−0·75	0·500	0·120	4·70	0·9105

[a] Parameters in the equation $\log D = a + b\,pH$; [b] standard deviations; [c] for $E = 50\%$; [d] correlation coefficient.

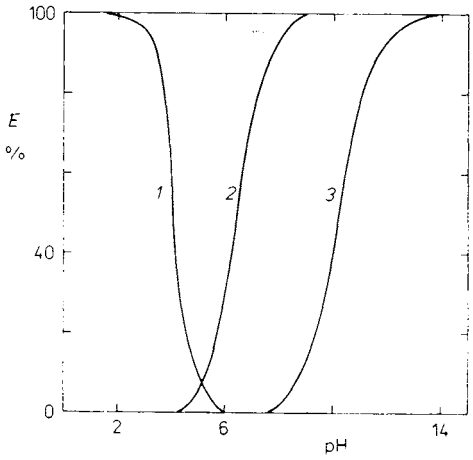

FIG. 5

Dependence of extraction recovery on pH for *1* bromothymol blue, *2* quinine, *3* atropine

where V_i and V_a are the volumes of interferent and analyte solutions in sample, respectively, and A_i and A_a are the absorbances caused by them. The values obtained are $\delta_i = 2$ for bromothymol blue, 5 for metanil yellow and 103 for cresol red.

The dependences of the extraction recoveries on pH for quinine, atropine and bromothymol blue extracted with chloroform from aqueous solutions are shown in Fig. 5, demonstrating that the neutral acid-base species are extracted beyond the pH range where the ionic associates are formed to the highest extent.

The dependences of log D on pH for selected ionic strengths were subjected to linear regression processing by a computer program based on that in ref.[12]; outliers were eliminated by the Grubbs test. The results are given in Table II. The $pH_{1/2}$ values, i.e. pH at which the extraction recovery is 50%, were obtained as the x-intercepts of the log $D = a + b$ pH straight line plots. For quinine, the $pH_{1/2}$ value decreases markedly with increasing ionic strength, whereas for atropine the extraction recovery is not affected appreciably by changes in the ionic strength. The different extractability of the molecular forms of the bases and dyes in relation to changes in the ionic strength is consistent with their ability to form ionic associates.

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