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A study of interactions of chloroquine with ethanol, sugars and glycerol using ultraviolet spectrophotometry

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Summary

The percentage of unprotonated aromatic amino grouping (pK_a 8.4) in the chloroquine molecule has been determined spectrophotometrically from measurements at 329 nm and 343 nm in suitable buffer solutions from pH 2.20 to 9.00. It is shown that ethanol, glycerol, lactose and dextrose can reduce the extent of protonation of chloroquine over this pH range, and could therefore possibly promote a more rapid absorption of chloroquine from the gastrointestinal tract. Complexation effects in the presence of these compounds cause chloroquine spectral shifts even in 0.01 M hydrochloric acid and in 0.01 M sodium hydroxide solutions. Errors can be minimised in the direct spectrophotometric assay of chloroquine by making measurements at 343 nm in 0.01 M hydrochloric acid. The ionisation of many compounds used in medicine that are weak acids or bases could be subject to effects similar to those shown by chloroquine.

Introduction

Chloroquine (I) has been used extensively for several decades as a suppressant in the prophylaxis and treatment of clinical attacks of malaria, which is probably the most widespread disease to afflict mankind. It is used also in the treatment of rheumatoid arthritis and similar collagen diseases, and for amoebic hepatitis. Although it can be administered by injection for emergency treatment, it is normally given orally as tablets or syrup. At present, a dose of 300 mg of chloroquine base weekly is recommended for the prophylaxis of malaria. It is clearly important that effective blood concentrations should be maintained, yet the pharmacokinetics of the drug are complex, and not completely understood. It is known (Frisk-Holmberg et al., 1984) that the drug is rapidly absorbed from the gastrointestinal tract after administration to fasting subjects ($t_{1/2abs}$ = 1.1 h; absorption rate constant, $K_a = 15$ days^{-1}). In addition, Gustafsson et al. (1983) have found that the bioavailability after an oral dose of chloroquine is almost complete (78–89%). The time taken to reach maximum plasma concentration (t_{max}) can vary from 1 to 6 h, and it appears to be independent of dose.

Most cells are selectively permeable to molecules in the unionised form, but the rapid uptake

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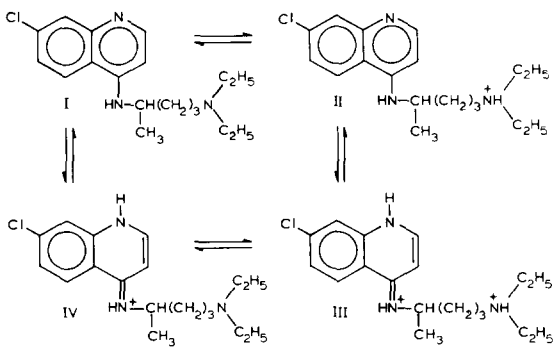

Formulas I–IV

of chloroquine suggests that it is largely absorbed as the dication (**III**), although it is feasible that there could be some absorption from the ileum as the monocation (**II**).

The dissociation constants of the drug are $pK_{a_1} = 10.8$ and $pK_{a_2} = 8.4$ (20 °C) (The Pharmaceutical Codex, 1979). This means that in the stomach (pH 1–3.5) and duodenum (pH 5–6) it must be present almost entirely as the dication (**III**). In the lower ileum (pH 8), it will exist as a mixture of approximately 71.5% of the dication and 28.3% of the monocation. The singly charged cation would be expected to correspond to the structure **II**, although Rosenberg and Schulman (1978) have postulated that because the difference between the two pK_a values is small, it is probable that some dissociation occurs from the alkylamino side-chain grouping of the dication, producing a second monocation (**IV**) tautomeric with the first.

Schulman and Young (1974) have shown that ionisation of the aromatic amino grouping influences the ultraviolet absorption and fluorescence spectra of chloroquine, but that the alkylamino side-chain grouping, which is far from the nucleus, has no effect upon spectra. This paper reports a spectroscopic study of the effects of alcohol, glycerol and some sugars on chloroquine at different pH values, and discusses the likely significance of these effects on drug uptake from the gastrointestinal tract. It also discusses the feasibility of determining chloroquine in the presence of these substances by a direct spectrophotometric procedure.

Materials and Methods

Reagents

Analytical-reagent grade or spectroscopic-reagent grade chemicals were used, unless otherwise specified.

Chloroquine phosphate, Sigma Chemical Co.

Chloroquine phosphate stock standard solution, approximately 0.15% m/V in de-ionised water. Accurately prepared.

Buffer solutions were prepared as follows: *pH 2.20*: 0.2 M potassium chloride–hydrochloric acid; *pH 4.95*: 0.1 M sodium acetate – acetic acid; *pH 6.98–9.00*: 0.1 M tris(hydroxymethyl)aminomethane–hydrochloric acid; *pH 7.40*, 0.1 M disodium hydrogen phosphate–sodium dihydrogen phosphate.

Absolute ethanol.

Glycerol B.P.

Lactose, BDH Chemicals, laboratory-reagent grade.

Lactose solution, 10.0% m/V in distilled water.

Anhydrous dextrose, BDH Chemicals, GPR grade.

Anhydrous dextrose solution, 40.0% m/V in distilled water.

Apparatus

Ultraviolet absorption spectra and absorbance measurements were determined using a Kontron Uvikon spectrophotometer attached to a Kontron Plotter 800. All measurements were made using 1-cm silica cells. A Jenway PHM6 pH meter was used in the adjustment of pH values.

Procedure

Two ml aliquots of the stock standard solution of chloroquine phosphate (0.1562% m/V) were diluted to 25.0 ml in buffer solution, in 1×10^{-5} M, 0.01 M and 0.1 M hydrochloric acid and in 0.01 M and 0.1 M sodium hydroxide solution. 0.5 ml and 1.0 ml aliquots of each dilution in buffer solution were mixed with 0, 1.0, 2.0, 3.0 and 4.0 ml of ethanol, glycerol, 10.0% m/V lactose solution and 40.0% m/V dextrose solution, and made up to 5.0 ml where necessary using the same buffer solution. Similarly, 0.5 ml and 1.0 ml aliquots of each dilution in 1×10^{-5} M, 0.01 M and 0.1 M hydrochloric acid, and in 0.01 M and 0.1 M

sodium hydroxide were mixed with 0, 1.0, 2.0, 3.0 and 4.0 ml of 1×10^{-5} M, 0.01 M and 0.1 M ethanolic hydrochloric acid and 0.01 M and 0.1 M ethanolic sodium hydroxide and made up to 5.0 ml where necessary with the same aqueous acid or aqueous alkali solution.

Two ml aliquots of the stock standard solution of chloroquine phosphate were diluted to 25.0 ml in 0.0543 M hydrochloric acid and 0.0543 M sodium hydroxide solution. 1.0 ml of each dilution was mixed with 0, 1.0, 2.0, 3.0 and 4.0 ml of glycerol, 10.0% m/V lactose solution and 40.0% m/V dextrose solution, and made up to 5.0 ml where necessary with distilled water, so that hydrochloric acid and sodium hydroxide concentrations were approximately 0.01 M.

Ultraviolet absorption spectra were recorded over the range 240–360 nm, and absorbance measurements at 329 nm and 343 nm were made against the corresponding reagent blank solutions.

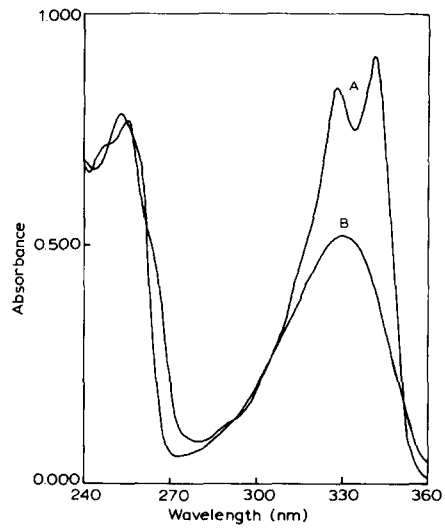

Fig. 1. Ultraviolet absorption spectra for 2.50 mg% m/V chloroquine phosphate in (A) 0.01 M hydrochloric acid and (B) 0.01 M sodium hydroxide.

Results and Discussion

Effects on ionisation

In acid solution, the ultraviolet absorption spectrum of chloroquine (Fig. 1) shows a single peak at 256 nm and a doublet with maxima at 329 and 343 nm. In alkaline solution, the last peak disappears, leaving the two peaks at 254 nm and 330 nm. The transition is dependent upon the ionization state of the heterocyclic ring system as represented by the chloroquine structures **II** and **III** (Rosenberg and Schulman, 1978).

The percentage of chloroquine unionised with respect to this ring system was determined spectrophotometrically in different aqueous buffer solutions of pH 4.95, 6.98, 7.40, 8.00 and 9.00. Absorbance measurements were made at 329 and 343 nm for 1.25 and 2.50 mg% m/V solutions of chloroquine phosphate in the various buffer solutions, and in 0.01 M sodium hydroxide (A_{min}) and 0.01 M hydrochloric acid (A_{max}), for substitution in the equation (Donbrow, 1967):

$$\text{Percentage unionised (\%B)} = \frac{(A_{max} - A_{measured})}{(A_{max} - A_{min})} \times 100$$

Table 1 shows that over the pH range 4.95–8.00 the values obtained at the two wavelengths were on average within 1.7% of the values calculated by means of the pK_a constant. Within this pH range, there were clearly defined maxima at 329 nm and 343 nm in the respective buffer solutions. At pH 9.00 there was a greater discrepancy between the results determined at 329 nm and 343 nm, which showed average deviations of 3.9% and 15.4%, respectively, from the calculated values. The greater deviation measured at 343 nm was associated with the absence of an absorption peak at this wavelength in the pH 9.00 buffer.

The same procedure was followed for measurements in the presence of ethanol. It was, however, observed that the peak maxima which occur at 329 nm and 343 nm in 0.01 M hydrochloric acid gradually undergo a bathochromic shift with increasing amounts of ethanol (Fig. 2), until in 0.01 M ethanolic hydrochloric acid they occur at 331 nm and 345 nm. Slight adjustments were made to the A_{max}-values (measured at 329 nm and 343 nm) corresponding to each ethanol concentration to compensate for this solvent effect, which is probably caused by changes in hydrogen bonding. The wavelength of the 330 nm maximum in 0.01 M sodium hydroxide is unaffected by the addition of

TABLE 1

Percentages of chloroquine unionised with respect to the aromatic ring system (pK_a 8.4) determined spectrophotometrically in aqueous buffers

Measurements were made using: (a) 0.00125% m/V and (b) 0.00250% m/V chloroquine phosphate solutions.

pH	Percentage unionised			
	Found, $pK_a = 8.4$		Calculated [a]	
	At 329 nm	At 343 nm	$pK_a = 8.4$	$pK_a = 10.8$
4.95	(a) –	–	0.0	0.0
	(b) 0	0		
6.98	(a) 2.6	2.8	3.7	0.0
	(b) 1.9	2.0		
7.40	(a) 8.4	8.8	9.1	0.0
	(b) 9.6	7.9		
8.00	(a) 29.7	30.5	28.5	0.2
	(b) 31.8	33.9		
9.00	(a) 85.8	96.0	79.9	1.6
	(b) 81.8	94.5		

[a] Using equation: $pK_a - pH = \log \frac{100 - \%B}{\%B}$

ethanol with respect to position, but a gradual hyperchromic effect is observed (Fig. 3) which reaches a maximum when the ethanol concentration is 80% v/v or above. The relevant changes in absorbance at 329 nm and 343 nm were therefore also taken into account when substituting for the A_{min}-values.

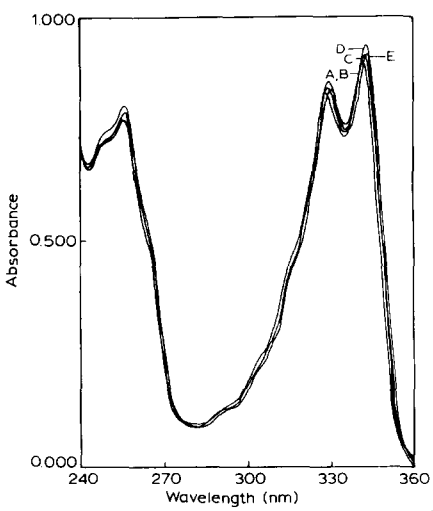

Fig. 2. Ultraviolet absorption spectra for 2.50 mg% m/V chloroquine phosphate in 0.01 M hydrochloric acid containing ethanol (A) 0% v/v, (B) 20% v/v, (C) 40% v/v, (D) 60% v/v, and (E) 80% v/v.

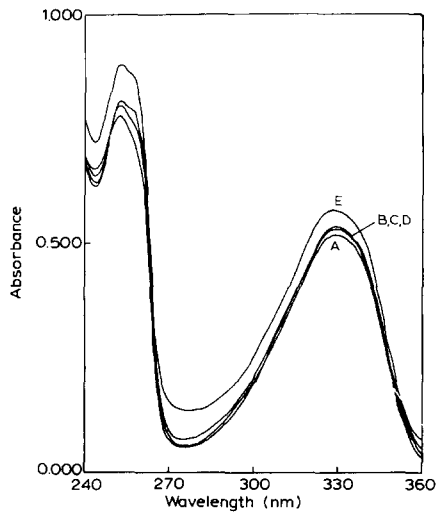

Fig. 3. Ultraviolet absorption spectra for 2.50 mg% m/V chloroquine phosphate in 0.01 M sodium hydroxide containing ethanol (A) 0% v/v, (B) 20% v/v, (C) 40% v/v, (D) 60% v/v, and (E) 80% v/v.

Table 1 shows that in aqueous buffer solutions chloroquine is almost completely ionised as the dibasic salt up to about pH 7. As the pH increases above this value, the more weakly basic aromatic ring system (pK_a 8.4) is gradually converted to the free base, but the side chain alkylamino grouping remains largely ionised up to pH 9.0.

Typical spectra showing the effects of ethanol addition to chloroquine in Tris buffer are shown in Figs. 4 and 5. Graphs (Fig. 6) show that over the range pH 6.98–9.00, an increase in ethanol concentration causes a corresponding linear increase in the percentage of unprotonated aromatic amine. Regression equations determined for the graphs were: *pH 6.98*, $y = -0.16 + 0.549x$, $r^2 = 98.5\%$; *pH 7.40*,, $y = 5.62 + 0.720x$, $r^2 = 98.3\%$; *pH 8.00*, $y = 29.4 + 0.837x$, $r^2 = 99.1\%$; pH 9.00, $y = 84.5 + 0.237x$, $r^2 = 96.9\%$ (where $y = \%$ unprotonated aromatic amine; $x = \%$ v/v ethanol).

The effect of ethanol upon ionisation depends not only upon the pH of the aqueous system, but also upon the effectiveness of the buffer system

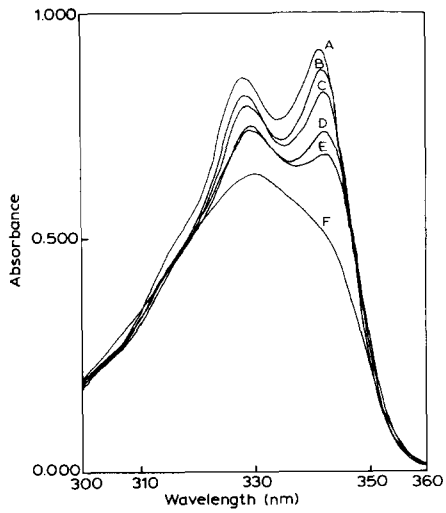

Fig. 4. Ultraviolet absorption spectra for 2.50 mg% m/V chloroquine phosphate in pH 6.98 0.1 M Tris buffer containing ethanol (A) 0% v/v, (B) 20% v/v, (C) 40% v/v, (D) 60% v/v, (E) 80% v/v, and (F) in 99% v/v ethanol.

employed. Fig. 7 shows that 0.1 M phosphate buffer is considerably less effective than 0.1 M Tris buffer at pH 7.40 in maintaining the ionisation state of chloroquine. (It was not possible to use more than 50% v/v ethanol with the phosphate buffer without causing precipitation of the

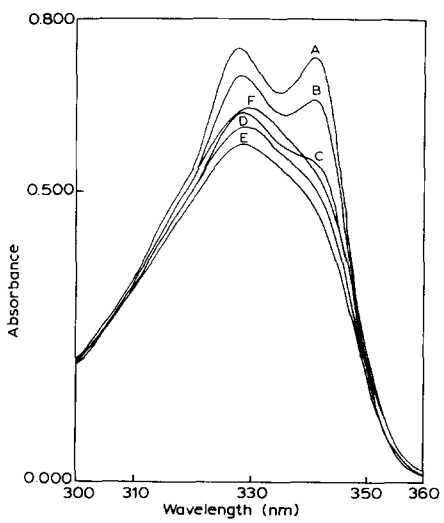

Fig. 5. Ultraviolet absorption spectra for 2.50 mg% m/V chloroquine phosphate in pH 8.00 0.1 M Tris buffer containing ethanol (A) 0% v/v, (B) 20% v/v, (C) 40% v/v, (D) 60% v/v, (E) 80% v/v, and (F) in 99% v/v ethanol.

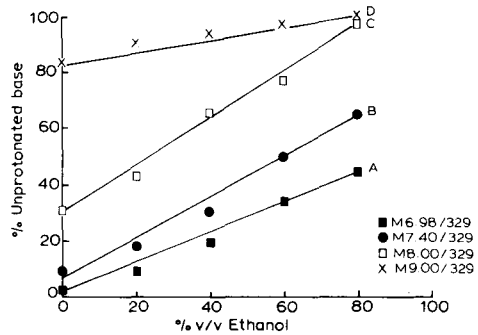

Fig. 6. Graphs showing the relationship between ethanol concentration in 0.1 M Tris buffer (A) pH 6.98, (B) pH 7.40, (C) pH 8.00, and (D) pH 9.00, and the mean percentage of unionised chloroquine base (pK_a 8.4) determined spectrophotometrically at 329 nm using 1.25 and 2.50 mg% m/V chloroquine phosphate concentrations.

buffer salt.) Similarly (Fig. 8), pH 4.95 acetate buffer is more effective than 1×10^{-5} M ethanolic hydrochloric acid, although the graphs show poor linearity in both acid solutions. The overall mean results are given in Table 2.

It is difficult to predict the influence of ethanol upon chloroquine absorption from the gastrointestinal tract. After oral ingestion, high concentrations of ethanol, similar to those of alcoholic beverages, are said to be reached in the stomach and jejunum (Halsted et al., 1973). Spirits, such as brandy, whisky and rum may contain 40–50% v/v, or more, of alcohol (Martindale, 1982). These could promote the formation of a high proportion

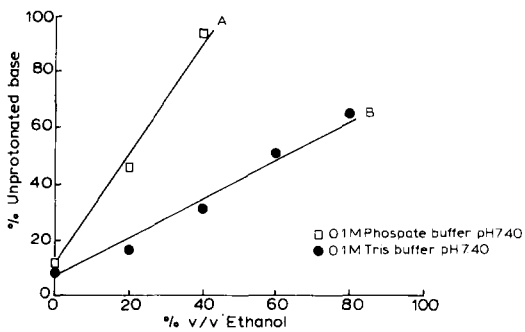

Fig. 7. Graphs showing the relationship between ethanol concentration and the mean percentage of unprotonated chloroquine base (pK_a 8.4) at pH 7.40 in (A) 0.1 M phosphate buffer, and (B) 0.1 M Tris buffer, using a concentration of 1.25 mg% m/V chloroquine phosphate.

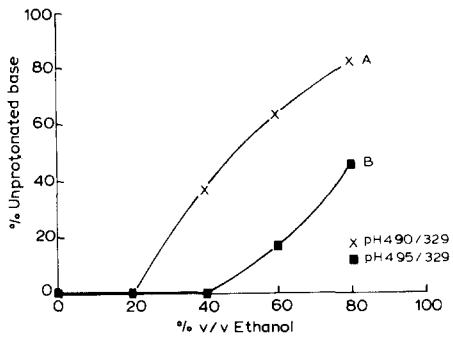

Fig. 8. Graphs showing the relationship between ethanol concentration and the mean percentage of unprotonated chloroquine base (pK_a 8.4) in (A) 1×10^{-5} M ethanolic hydrochloric acid, and (B) pH 4.95 acetate buffer using a concentration of 2.50 mg% m/V chloroquine phosphate.

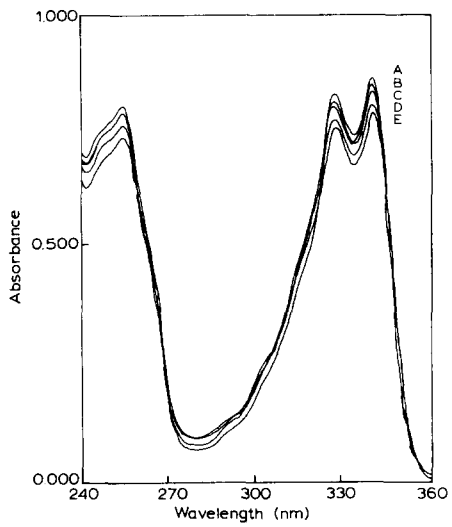

Fig. 9. Ultraviolet absorption spectra for 2.50 mg% m/V chloroquine phosphate in pH 7.40 0.1 M Tris buffer in the presence of lactose: (A) 0% m/V, (B) 2% m/V, (C) 4% m/V, (D) 6% m/V, and (E) 8% m/V.

of unprotonated aromatic amine, approaching that obtained in vitro with 1×10^{-5} M hydrochloric acid, where, in the presence of 40% v/v ethanol, 41.0% of the amine is in the non-protonated form. The transformation might be expected to favour a more than usually rapid absorption of chloroquine into the bloodstream.

The interaction between chloroquine and ethanol has much broader implications, in that the majority of drugs used in medicine are weak bases or weak acids, and likewise could be subject to similar effects from alcohol. Reviews (Sato et al., 1985; Mezey, 1985) show that studies concerning the influence of ethanol on drug absorption have been very limited, and that there is a need for more information, particularly in the light of the present study. Ethanol has a lower dielectric constant and a lower autoprotolysis constant than water, and will lower the dissociation constant for most uncharged acids. The protonation of a weak base such as chloroquine is probably reduced by ethanol due to an effect on the buffer pH. Ethanol would equally be expected to reduce the tendency

TABLE 2

The effect of ethanol concentration upon the percentage of free chloroquine base (pK_a 8.4) in different buffer systems determined spectrophotometrically

Means of determinations at 329 nm and 343 nm using 1.25 and 2.50 mg% m/V chloroquine phosphate. At pH 9.00 determinations at 329 nm are shown.

Buffer system	pH	% free base (pK_a 8.4)				
		0% v/v Ethanol	20% v/v Ethanol	40% v/v Ethanol	60% v/v Ethanol	80% v/v Ethanol
0.2 M KCl–HCl	2.20	0.0	0.0	0.0	0.0	0.0
1×10^{-5} M HCl	4.90	0.0	2.3	41.0	69.0	88.1
0.1 M acetate	4.95	0.0	0.0	0.0	14.9	52.3
0.1 M Tris	6.98	2.3	7.6	19.7	34.0	46.0
0.1 M Tris	7.40	8.7	17.5	32.2	49.8	66.8
0.1 M phosphate	7.40	11.8	47.4	97.0	–	–
0.1 M Tris	8.00	31.5	45.0	68.6	80.0	99.5
0.1 M Tris	9.00	83.8	90.7	93.9	96.8	104.4

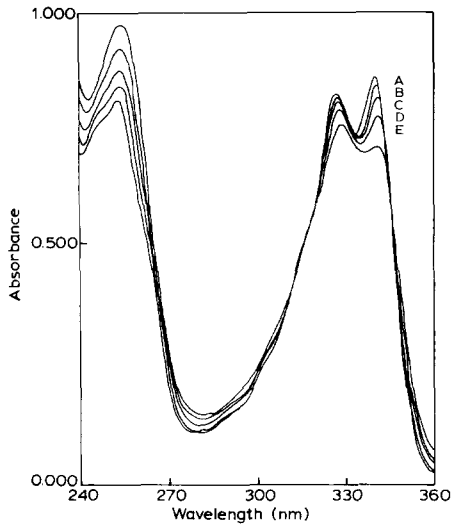

Fig. 10. Ultraviolet absorption spectra for 2.50 mg% m/V chloroquine phosphate in pH 7.40 0.1 M Tris buffer in the presence of glycerol: (A) 0% v/v, (B) 20% v/v, (C) 40% v/v, (D) 60% v/v, and (E) 80% v/v.

of a weakly acidic drug to dissociate. The relationship between drug pK_a and the effect of ethanol upon ionisation needs further study.

Effects on chloroquine, similar to those obtained with ethanol, were also produced by dextrose, lactose and glycerol. Typical ultraviolet absorption spectra showing the effects of different concentrations of lactose and glycerol on chloroquine in Tris buffer pH 7.40 are shown in Figs. 9 and 10. Spectra obtained in the presence of dextrose were similar to those obtained with lactose, although lactose produced the same effect as dextrose in lower concentrations (Fig. 11). Thus, the percentage of unprotonated amine (theoretically 9.1% at pH 7.4) was approximately doubled in the presence of 8% m/V (0.23 M) lactose, 24% m/V (1.33 M) dextrose or 20% v/v ethanol. With glycerol, the calculated percentages of unprotonated amine were not in agreement when absorbances were measured at 329 nm and 343 nm. For example, at pH 7.40 in Tris buffer in the presence of 60% v/v glycerol, the percentage of unprotonated amine was 4% determined at 329 nm compared with 25% determined at 343 nm. The readings at 343 nm were probably more reliable, since graphs (Fig. 12) showing the relationship

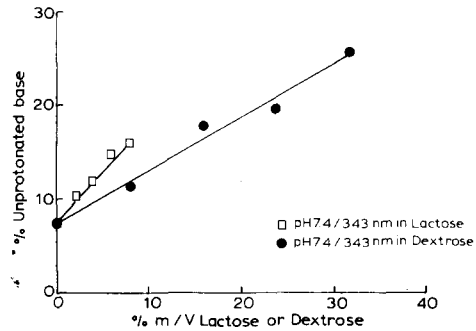

Fig. 11. Graphs showing the relationship between dextrose and lactose concentration in 0.1 M Tris buffer pH 7.40 and the percentage of unprotonated chloroquine base (pK_a 8.4) determined spectrophotometrically at 343 nm using 2.50 mg% m/V chloroquine phosphate.

between glycerol concentration and the percentage of unprotonated chloroquine at different pH values had almost identical slopes. Graphs plotted using values obtained at 329 nm showed quite different slopes at different pH values.

Spectrophotometric assay

The British Pharmacopoeia (1980) in the dissolution test for tablets of chloroquine phosphate or sulphate prescribes spectrophotometric assay measurements at 344 nm in 0.01 M hydrochloric acid, ($A_{1\%,1cm} = 371$ for the phosphate, and 450 for the sulphate). The United States Pharmacopoeia (1980) specifies a direct spectrophotometric

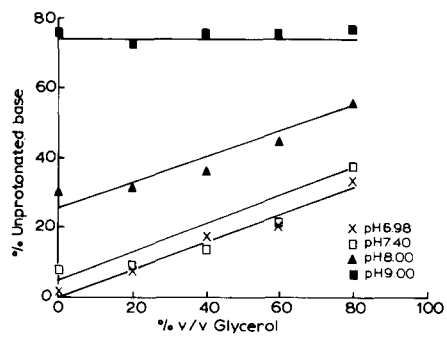

Fig. 12. Graphs showing the relationship between glycerol concentration in 0.1 M Tris buffer (A) pH 6.98, (B) pH 7.40, (C) pH 8.00, and (D) pH 9.00, and the percentage of unprotonated chloroquine base (pK_a 8.4) determined spectrophotometrically at 343 nm using 2.50 mg% m/V chloroquine phosphate.

TABLE 3

Absorbance measurements made using 2.50 mg% m/V solutions of chloroquine phosphate in 0.01 M hydrochloric acid and 0.01 M sodium hydroxide in the presence of some hydroxy compounds

Solvent	Absorbance		
	0.01 M Hydrochloric acid		0.01 M Sodium hydroxide
	At 329 nm	At 343 nm	At 329 nm
No additive	0.833	0.899	0.516
80% v/v ethanol	0.814	0.897	0.580
80% v/v glycerol	0.778	0.864	0.544
32% m/V dextrose	0.804	0.872	0.510
8% m/V lactose	0.807	0.872	0.508
Mean absorbance	0.807	0.881	0.532
Standard deviation	0.020	0.016	0.031
Relative standard deviation (%)	2.5	1.8	5.8

assay at 343 nm in acid solution for injection solutions of chloroquine, but for tablet preparations it requires a preliminary extraction from aqueous solution into chloroform, before evaporation of the chloroform, and assay of the chloroquine at 343 nm in dilute acid.

The present work has shown that hydroxy compounds can interfere with spectrophotometric measurements of chloroquine even in strongly acidic or alkaline solutions due to complexation effects. Absorbance measurements made for 2.50 mg% m/V solutions of chloroquine phosphate in 0.01 M hydrochloric acid and 0.01 M sodium hydroxide in the presence of 80% v/v ethanol, 80% v/v glycerol, 32% m/V dextrose and 8% m/V lactose are shown in Table 3. The amounts of sugars used were limited by their solubilities. The relative standard deviations of readings in 0.01 M sodium hydroxide were high (6%), and were not improved by using 0.1 M sodium hydroxide solution. In 0.01 M hydrochloric acid, the relative standard deviation of readings at 343 nm was approximately 2%, which is not greater than the normal error of spectrophotometric readings. Readings at 343 nm in acid solution have a slight advantage of greater sensitivity over readings at 329 nm.

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