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Binding of chloroquine to glass

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Summary

Buffered chloroquine diphosphate solutions of varying pH and concentration were stored in soda or borosilicate glass. Storage in soda glass (test tubes, glass wool) showed a decrease in original drug concentration of up to 60% and 97%, respectively. Borosilicate glass did not show any binding. The highest binding recorded was at physiological pH and at low concentration (7.8 $ng \cdot ml^{-1}$). It is important for laboratory workers to realize that significant reductions in chloroquine concentrations may occur under such conditions.

Introduction

Chloroquine has recently been shown to bind to glass but not to certain plastics (Geary et al., 1983). This binding is most important in laboratory studies where significant reductions in chloroquine concentrations may occur when the drug is prepared or stored in laboratory glassware. Reductions in chloroquine concentrations of this type may alter the interpretation of chloroquine sensitivity test data and could lead to inaccurate reports of malaria resistance to chloroquine. The 'sorption' of chloroquine to glass may also seriously upset the results obtained in pharmacokinetic studies when glassware is used in drug analysis.

The aims of the present study were therefore two-fold: (1) to examine the rate and extent of binding of chloroquine to different grades of glass; and (2) to examine the binding of chloroquine to glass at different concentrations and under different pH conditions.

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Materials and Methods

Two sources of soda glass were used, namely glass wool and soda glass test tubes. Borosilicate glass was available only as test tubes. Chloroquine in all cases was used in the form of the diphosphate salt.

(a) Investigation of chloroquine binding to glass wool

The method involved storing buffered chloroquine solutions of varying concentrations in polypropylene syringes each containing 2 g of glass wool (which was first wetted with deionized water). Polypropylene syringes were previously exposed to chloroquine (400 $\mu g \cdot ml^{-1}$) and washed thoroughly with deionized water. This pre-exposure was shown to saturate binding sites on the polypropylene and prevent further chloroquine sorption. During storage all syringes were covered with aluminium foil since chloroquine decomposition is accelerated by light.

Three borate buffers were used (pH 9.5, 7.4 and 4.0) to investigate whether the binding of chloroquine to glass was pH-dependent. Ten different concentrations of the drug were used at each of the pH values namely 4 $\mu g \cdot ml^{-1}$ decreasing by two-fold serial dilutions to 0.0078 $\mu g \cdot ml^{-1}$, to determine whether chloroquine binding was concentration-dependent. A volume of 30 ml of the drug was required to cover the glass wool. Chloroquine concentration was monitored at 1, 2, 6, 12 and 24 h after addition to the glass wool. Chloroquine was assayed spectrofluorometrically (Perkin-Elmer LS-5 excitation $\lambda = 335$ nm; emission $\lambda = 400$ nm) the pH of all samples being adjusted to pH 9.5 before analysis (Vogel and Konig, 1975). Ten replicates were made of each assay.

(b) Investigation of chloroquine binding to soda glass test tubes and borosilicate glass test tubes

Buffered chloroquine solutions (10 ml) were placed in either soda glass or borosilicate glass test tubes which had similar internal surface areas. The test tubes were wrapped in aluminium foil. The buffer pH values and drug concentrations were the same as those used in the experiments with glass wool. Chloroquine concentrations were monitored over a 24-h period. All experiments were repeated 10 times.

Results and Discussion

In spite of the long use of chloroquine there is little published information on the behaviour of chloroquine in solution. The present study confirms some earlier observations that chloroquine binds to glass (Yayon and Ginsburg, 1980; Geary et al. 1983) and characterizes the binding of chloroquine to a variety of glass materials. In the present study, chloroquine binding to glass wool remained approximately constant over the 24 h observation period after a binding equilibrium was reached at 1 h (Table 1). The binding was, however, concentration- and pH-dependent (Table 2). In general as the concentration increased the percentage binding of chloroquine

TABLE 1
THE INFLUENCE OF EXPOSURE TIME ON CHLOROQUINE BINDING TO GLASS WOOL AT pH 9.5

Time (h):	1	2	6	12	24
Concentration* ($\mu g \cdot ml^{-1}$)	Percentage binding of chloroquine ± S.E.				
4	48.38 ± 0.65	46.50 ± 1.25	48.38 ± 1.10	48.75 ± 0.49	50.00 ± 0.42
2	68.02 ± 2.69	67.00 ± 0.63	68.55 ± 0.92	67.68 ± 0.57	68.86 ± 0.56
1	73.05 ± 0.83	73.57 ± 0.53	72.98 ± 0.80	73.82 ± 0.53	72.52 ± 0.46
0.5	76.98 ± 1.42	74.14 ± 2.79	74.19 ± 1.51	78.34 ± 1.65	78.14 ± 1.77
0.25	81.83 ± 0.39	81.00 ± 1.13	82.01 ± 0.94	81.53 ± 0.47	82.12 ± 0.69
0.125	86.63 ± 2.38	85.23 ± 1.85	86.94 ± 0.86	90.08 ± 1.08	90.15 ± 1.16
0.0625	92.74 ± 0.91	90.34 ± 0.36	89.00 ± 1.17	90.81 ± 1.19	91.51 ± 0.81
0.03125	91.63 ± 0.35	92.68 ± 0.35	90.30 ± 0.63	92.76 ± 1.30	91.31 ± 0.26
0.0156	90.76 ± 0.01	92.13 ± 0.73	91.67 ± 0.22	90.87 ± 0.87	91.10 ± 1.21
0.0078	93.54 ± 0.85	92.82 ± 0.52	93.22 ± 0.64	92.53 ± 0.53	93.88 ± 0.53

Each value represents the mean ± S.E. of 10 determinations.
* As chloroquine diphosphate.

TABLE 2
THE EFFECT OF CHLOROQUINE CONCENTRATION AND SOLUTION pH ON CHLOROQUINE BINDING TO GLASS WOOL

pH:	9.5	7.4	4
Concentration* ($\mu g \cdot ml^{-1}$)	Percentage binding of chloroquine ± S.E.		
4	50.00 ± 0.42	53.69 ± 0.93	52.19 ± 1.72
2	68.86 ± 0.56	82.99 ± 0.63	77.80 ± 1.64
1	72.52 ± 0.46	85.63 ± 0.92	79.80 ± 0.20
0.5	78.14 ± 1.77	87.76 ± 0.59	83.49 ± 1.17
0.25	82.12 ± 0.69	91.27 ± 0.80	89.77 ± 0.88
0.125	90.15 ± 1.16	95.40 ± 0.14	92.13 ± 0.71
0.0625	91.51 ± 0.81	94.57 ± 0.31	93.32 ± 0.24
0.03125	91.31 ± 0.26	96.07 ± 0.32	93.89 ± 0.22
0.0156	91.10 ± 1.21	96.99 ± 0.43	94.37 ± 0.26
0.0078	93.88 ± 0.53	97.14 ± 0.34	94.50 ± 1.22

Each value represents the mean ± S.E. of 10 determinations after exposure for 24 h.
* As chloroquine diphosphate.

decreased. Percentage binding was almost identical for the low concentrations examined, i.e. up to 0.125 $\mu g \cdot ml^{-1}$ but it decreased as the concentration rose above 0.25 $\mu g \cdot ml^{-1}$. Greatest binding was recorded at physiological pH for all the concentrations examined with the percentage binding decreasing at pH values above and below this (Table 2). This concentration-dependent binding to glass wool may be of particular relevance in chloroquine assay procedures. For example, one published assay technique uses glass wool as a physical filter before subjecting the samples to high-pressure liquid chromatography (Alvan et al., 1982).

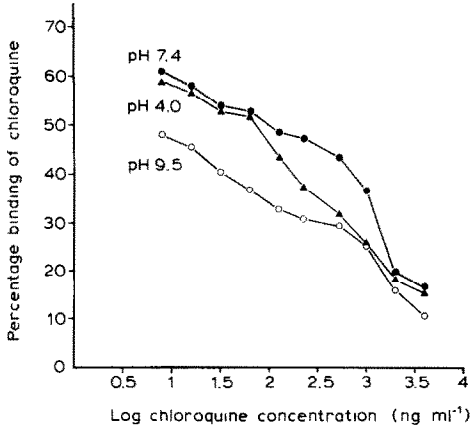

Fig. 1. The effect of chloroquine diphosphate concentration on chloroquine binding to soda glass test tubes. (Each point represents the mean of 10 determinations after storage for 24 h.)

TABLE 3
THE INFLUENCE OF EXPOSURE TIME ON CHLOROQUINE BINDING TO SODA GLASS TEST TUBES AT pH 7.4

Time (h):	1	2	6	12	24
Concentration* ($\mu g \cdot ml^{-1}$)	Percentage binding of chloroquine ± S.E.				
4	15.81 ± 0.72	15.00 ± 0.61	15.12 ± 0.30	16.21 ± 0.58	16.55 ± 0.62
2	20.00 ± 0.74	20.14 ± 0.31	19.07 ± 1.15	19.48 ± 0.29	20.23 ± 0.54
1	34.35 ± 0.13	35.97 ± 0.18	36.79 ± 0.66	36.50 ± 0.66	36.71 ± 0.17
0.5	39.97 ± 0.83	40.75 ± 1.28	41.84 ± 0.33	42.09 ± 1.01	43.71 ± 0.34
0.25	43.01 ± 0.56	45.13 ± 0.42	44.83 ± 0.29	44.23 ± 0.32	46.89 ± 0.44
0.125	45.32 ± 1.62	44.98 ± 1.96	48.17 ± 0.96	48.77 ± 1.44	48.56 ± 0.54
0.0625	51.68 ± 0.38	52.09 ± 0.36	52.97 ± 0.44	53.00 ± 0.29	53.00 ± 0.24
0.03125	54.55 ± 0.16	54.01 ± 0.18	55.02 ± 0.63	54.70 ± 0.66	54.00 ± 0.49
0.0156	56.92 ± 0.13	56.35 ± 1.76	56.87 ± 0.43	58.14 ± 0.66	58.13 ± 0.55
0.0078	55.64 ± 1.19	57.23 ± 2.34	56.14 ± 2.33	57.51 ± 2.10	60.89 ± 1.19

Each value represents the mean ± S.E. of 10 determinations.
* As chloroquine diphosphate.

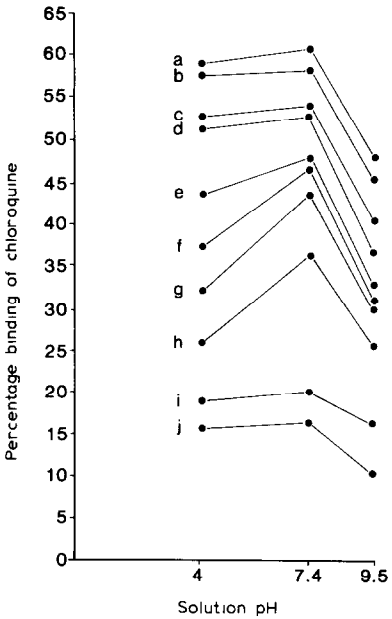

Fig. 2. The effect of solution pH on chloroquine binding to soda glass test tubes. a–j represent serial two-fold dilutions of chloroquine diphosphate from 4 $\mu g \cdot ml^{-1}$ (a) to 0.0078 $\mu g \cdot ml^{-1}$ (j). (Each point is the mean of 10 determinations after storage for 24 h.)

TABLE 4
THE EFFECT OF CHLOROQUINE CONCENTRATION AND SOLUTION pH ON CHLOROQUINE BINDING TO BOROSILICATE TEST TUBES

pH:	9.5	7.4	4
Concentration* ($\mu g \cdot ml^{-1}$)	Percentage binding of chloroquine ± S.E.		
4	1.25 ± 0.24	3.43 ± 0.49	2.72 ± 0.45
2	0.83 ± 0.01	1.94 ± 0.25	1.76 ± 0.29
1	2.66 ± 0.12	3.01 ± 0.21	1.66 ± 0.14
0.5	0.77 ± 0.11	2.48 ± 0.29	4.02 ± 0.12
0.25	3.56 ± 0.11	1.71 ± 0.17	2.91 ± 0.10
0.125	3.20 ± 0.52	4.98 ± 0.65	2.01 ± 0.14
0.0625	2.98 ± 0.28	1.80 ± 0.24	3.01 ± 0.26
0.03125	1.72 ± 0.10	2.69 ± 0.06	2.00 ± 0.03
0.0156	2.76 ± 0.03	3.00 ± 0.29	2.51 ± 0.13
0.0078	2.80 ± 0.28	3.28 ± 0.49	3.66 ± 0.54

Each value represents the mean ± S.E. of 10 determinations after storage for 24 h.
* As chloroquine diphosphate.

TABLE 5
MAJOR CHEMICAL CONSTITUENTS OF SODA GLASS AND BOROSILICATE GLASS

Chemical constituents: Glass type	SiO_2	Al_2O_3	Na_2O	K_2O	B_2O_3	Ca + MgO
Borosilicate	80.0	2.0	4.0	0.5	13.0	–
Soda lime	72.0	2.0	15.0	15.0	–	11

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The time-dependence of chloroquine's binding to soda glass test tubes is shown in (Table 3). Increasing the exposure time to over 1 h did not increase the binding of chloroquine. As with glass wool increasing the concentration decreased the percentage binding of the drug (Fig. 1). Again the greatest binding was recorded at pH 7.4, lower binding occurring at pH values 4 and 9.5 (Fig. 2). Based on these data, pH 9.5 would seem to be the most suitable pH for the preparation or storage of the drug in soda glass if adsorption is to be minimized, i.e. storage should be carried out at high concentration and at high pH.

In contrast there was no appreciable binding of chloroquine to borosilicate glass test tubes (Table 4). This glass is characterized by having optimum resistance to thermal shock and chemical attack; differences in the major chemical constituents of borosilicate glass and soda glass are shown in Table 5. The non-binding of chloroquine to borosilicate glass is therefore likely to be due to the physical and chemical differences in the two glass types.

In conclusion, since chloroquine is bound only to soda glass and not to borosilicate glass, the latter should be used when assaying chloroquine or carrying out sensitivity tests for malaria in order to avoid loss of chloroquine from solution. This is particularly important at low chloroquine concentration and physiological pH.

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