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Investigation of chloroquine binding to plastic materials

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Summary

Chloroquine diphosphate was sorbed (from buffer solutions of varying pH and chloroquine concentration) by cellulose propionate, ethylvinyl acetate, methacrylate butadiene styrene, polyethylene, polypropylene and polyvinyl chloride, but not by polystyrene. Passing chloroquine solutions through cellulose acetate filters once, resulted in a high loss of chloroquine (up to 85%; 16 $ng \cdot ml^{-1}$, pH 9.5). Investigation of the effect of exposure time on chloroquine binding to polyvinyl chloride indicated that 12 h was sufficient to reach the equilibrium state. The kinetics of sorption of the drug could be described in terms of a diffusion-controlled absorption process with adsorption playing a minor role in the overall loss of chloroquine. The rate and extent of sorption of chloroquine showed a dependence on pH which could be interpreted in terms of ionization of the drug, i.e. the unionized form was preferentially sorbed. The present findings indicate that care must be taken to avoid contact with the plastic materials outlined during laboratory quantitation of chloroquine, e.g. in kinetic studies and also during malarial sensitivity testing.

Introduction

Currently, there is much concern about the world-wide development and spread of chloroquine-resistant *P. falciparum* malaria (see, for example, Wagner, 1980; Bruce-Chwatt, 1982; Harinasuta et al., 1983; Wyler, 1983; Mutambu et al., 1986). Proof of the development of such resistance depends on the use of an in vitro continuous culture technique of the parasite established by Trager and Jensen in 1976. Modifications of this basic technique have since been developed to improve the culture environment and the yield of parasite (Jensen and Trager, 1977; Jensen et al., 1979; Trager, 1979). These later studies have improved the in vitro testing of *P. falciparum* for chloroquine sensitivity (or resistance) and have also enabled studies to be made on the mechanism(s) of the resistance development.

In previous work (Yahya et al., 1985) we have shown that chloroquine binds significantly to soda-glass and this may well adversely influence the results of in vitro tests of chloroquine sensitivity of the malaria parasite if soda-glass vials or pipettes are used during such testing. The fact that chloroquine is used in low concentrations during such tests (e.g. 0.1 ng/25 μl; Rieckmann et al., 1978) complicates the situation since percentage binding is increased at low concentration.

Apart from glass apparatus, plastic microtiter plates and pipettes are also commonly used in sensitivity testing techniques (Kouznetsov et al.,

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1980; Nguyen-Dinh and Trager, 1980; Gajanana et al., 1982; Onori et al., 1982; Geary et al., 1983; Geary and Jensen, 1983; Jensen et al., 1983; Spencer et al., 1983), and it is possible that chloroquine may also bind to some of these materials.

It should also be borne in mind that low concentrations of chloroquine will also be encountered in drug analyses of biological samples during pharmacokinetic studies and that chloroquine may be bound to plastic syringes during sampling or to plastic pipettes used to transfer samples during assay procedures. Binding to membrane filters used to clarify and/or sterilize chloroquine solutions prior to use or analysis could also give rise to erroneous experimental results in drug sensitivity and pharmacokinetic studies.

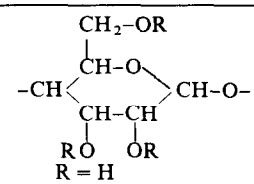
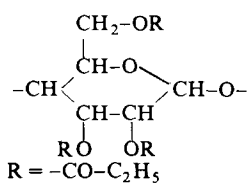
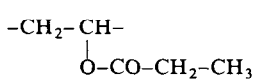
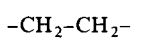
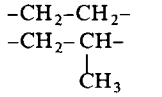
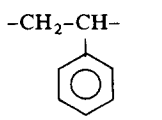
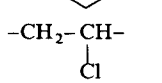
There were three objectives in the present study.

(i) To investigate the binding of chloroquine to a range of plastic materials having different physicochemical characteristics (Table 1; cellulose propionate, ethylvinyl acetate, methacrylate butadiene styrene, polyethylene, polypropylene, polystyrene and polyvinyl chloride) using a range of concentrations and pH values.

(ii) To determine more closely the effect of exposure time on the binding of chloroquine to one model plastic material – polyvinyl chloride.

TABLE 1

COMPARISON OF THE PHYSICAL AND CHEMICAL PROPERTIES OF DIFFERENT TYPES OF THERMOPLASTICS MATERIALS USED IN THE PRESENT STUDY

Chemical name	Plastic type	Repeating unit of the polymeric structure of the plastic	Thickness (μm)	Density (g/cm^3)
Cellulose acetate	Amorphous thermoplastic		150	0.287
Cellulose propionate	Amorphous thermoplastic		750	0.755
Ethylvinyl acetate	Amorphous thermoplastic		370	0.856
Methacrylate butadiene styrene	Amorphous thermoplastic	Co-polymer	450	0.918
Polyethylene	Partially crystalline thermoplastic		40	0.684
Polypropylene	Partially crystalline thermoplastic		1 550	0.699
Polystyrene	Crystalline thermoplastic		840	1.084
Polyvinylchloride	Amorphous thermoplastic		350	1.069

(iii) To characterise the influence of pH on the binding of chloroquine to cellulose acetate membrane filters.

Materials and Methods

In all experiments chloroquine was used in the form of chloroquine diphosphate and all concentrations quoted in the text refer to this salt. New plastics materials were used in all studies and they were discarded after each experiment.

Chloroquine binding to polystyrene

Fifty ml samples of borate-buffered chloroquine solutions (pH 9.5, 7.4 or 4.0) of varying concentrations were placed in petri dishes (surface area 83.41 cm^2) made from polystyrene (Sterilin Laboratories, Middlesex, U.K.). During experimentation, the petri dishes were covered with aluminium foil to protect them from light as chloroquine decomposition is said to be accelerated by light.

Six different concentrations of the drug were used at each of the pH values namely 128, 64, 32, 16, 8, and 4 $ng \cdot ml^{-1}$. The choice of the six concentrations and the exposure time (24 h) used in this study were based on those used in our earlier in vitro chloroquine binding studies (Yahya et al., 1985). Ten individual containers were used for each of the experimental conditions.

Chloroquine in this section, and in all further experiments, was assayed spectrofluorometrically (Perkin-Elmer luminescence spectrophotometer model LS-5; excitation $\lambda = 335$ nm, emission $\lambda = 400$ nm). The pH of all samples was adjusted to 9.5 prior to analysis (Vogel and Konig, 1975).

In all cases test data were compared with control values which were obtained by storing the same chloroquine solution for 24 h in borosilicate test tubes covered with aluminium foil. Earlier work (Yahya et al., 1985) has shown that chloroquine is not bound to borosilicate glass.

Chloroquine binding to cellulose propionate, ethylvinyl acetate, methacrylate butadiene styrene, polyethylene, polypropylene and polyvinyl chloride

Buffered chloroquine solutions (10 ml) were placed in identical borosilicate glass tubes containing a sheet of cellulose propionate (Avon Medicals, Redditch, U.K.), ethylvinylacetate (Kabi Vitrum, Middlesex, U.K.), methacrylate butadiene styrene (Avon Medicals, Redditch, U.K.) polyethylene (Sterilin, Middlesex, U.K.), polypropylene (Becton, Dickinson and Co. Ireland), or polyvinyl chloride (Galen Pharmaceutical Laboratories, N. Ireland). The surface area of the sheets used was the same in all the experiments (49 cm^2). The borosilicate containers were again covered completely with aluminium foil. The buffer pH values and the drug concentrations used were the same as those outlined for previous experiments with the petri dishes.

Chloroquine solutions were stored in contact with the plastics for 24 h and were then analysed for chloroquine content. Each experiment was repeated 10 times and results were compared with those of control solutions stored in the absence of the plastics.

Effect of contact time on chloroquine binding to polyvinyl chloride

Buffered chloroquine solutions (10 ml; pH 9.5; 8 $ng \cdot ml^{-1}$) were placed in identical borosilicate glass containers each containing a sheet of polyvinylchloride (49 cm^2). The containers were protected from light. Chloroquine solutions were stored in contact with the plastic for different time periods (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 24 h); each experiment was repeated 10 times.

Chloroquine binding to cellulose acetate membrane filters

Buffered chloroquine solutions (20 ml, 16 $ng \cdot ml^{-1}$) were passed once through cellulose acetate membrane filters obtained from two commerical sources (Amicon, U.S.A. and Molsheim, France). All filters used had a pore size of 0.5 μm and were 4.52 cm^2 in surface area. The filters were fitted into a disc filter holder (made from polypropylene with a silicone gasket; Swinnex 25, Millipore, Middlesex, U.K.) and connected to polypropylene syringes. The syringes and the filter holder were previously exposed to concentrated solutions of 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. The syringes were protected from light by aluminium foil during the passage of chloroquine solutions through the filters, over a period of approximately 40 s. The chloroquine solution pHs were the same as those used in the previous experiments (i.e. pHs 9.5, 7.4 and 4).

Results

The binding data for each individual plastic were reproducible with a typical coefficient of variance for 10 determinations being of the order of 0.035.

Chloroquine binding to polystyrene

Polystyrene petri dishes exhibited minimal drug

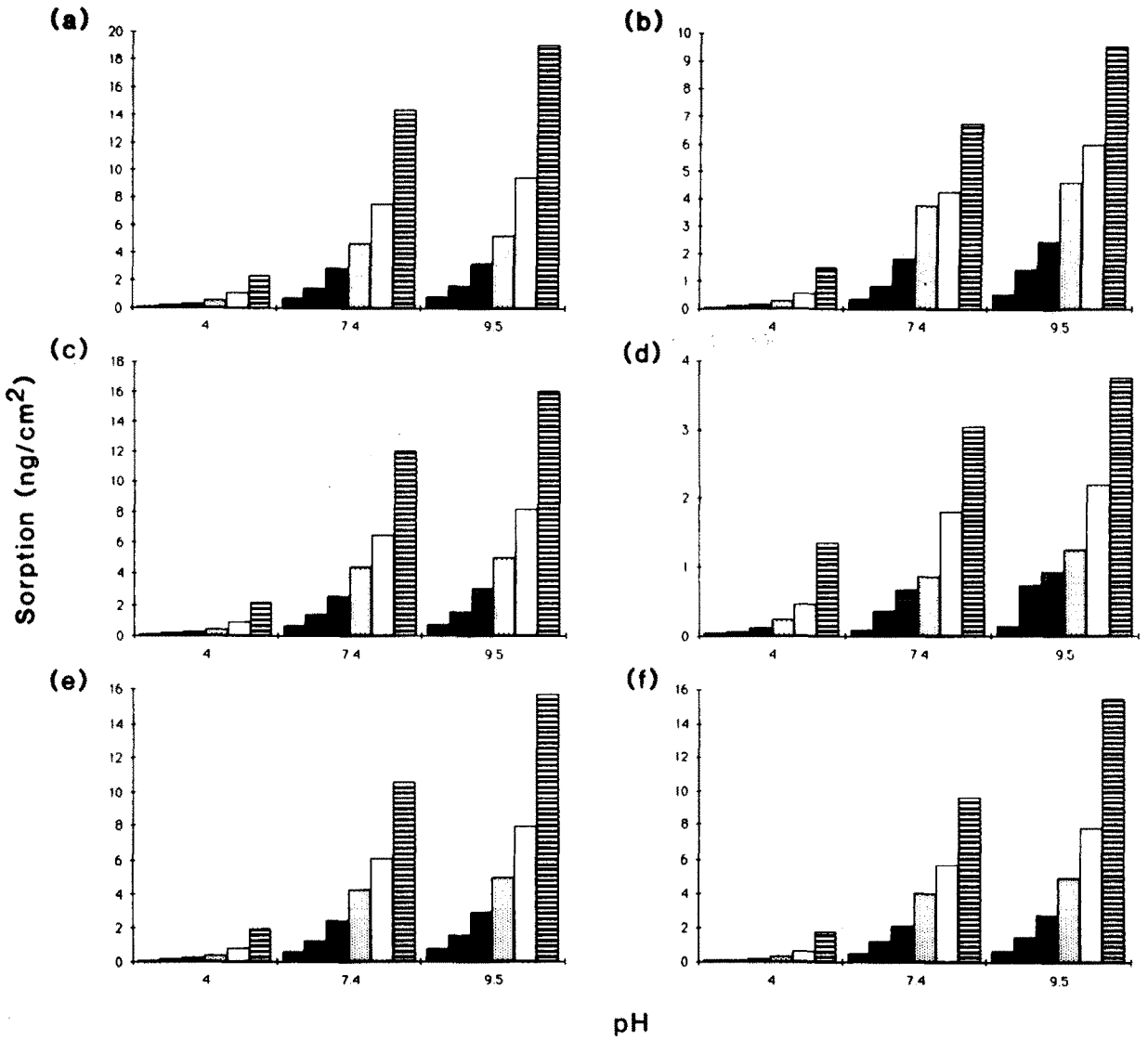


Fig. 1. The effect of solution pH on chloroquine sorption to: (a) cellulose propionate; (b) ethylvinyl acetate; (c) methacrylate butadiene styrene; (d) polyethylene; (e) polypropylene; and (f) polyvinyl chloride. The concentrations investigated were (from right to left): ▤, 128 ng · ml^{-1}; □, 64 ng · ml^{-1}; ▨, 32 ng · ml^{-1}; ▩, 16 ng · ml^{-1}; ▦, 8 ng · ml^{-1}; and ■, 4 ng · ml^{-1} (each value is the mean of 10 determinations after storage for 24 hours) (see Table 1 for reference to thickness of plastic sheets used).

TABLE 2

THE EFFECT OF CHLOROQUINE CONCENTRATION * AND SOLUTION pH ON CHLOROQUINE SORPTION TO POLYSTYRENE

Conc.	Sorption ($ng \cdot cm^{-2}$) ± S.E.		
($ng \cdot ml^{-1}$) pH:	4	7.4	9.5
4	0.04 ± 0.004	0.04 ± 0.002	0.05 ± 0.010
8	0.05 ± 0.004	0.11 ± 0.004	0.14 ± 0.004
16	0.09 ± 0.005	0.26 ± 0.008	0.31 ± 0.002
32	0.21 ± 0.015	0.48 ± 0.013	0.59 ± 0.004
64	0.38 ± 0.013	1.38 ± 0.017	1.47 ± 0.008
128	1.12 ± 0.013	2.52 ± 0.028	2.70 ± 0.188

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

binding to their surfaces. Data supporting this result are presented in Table 2. The maximum binding was recorded at pH 9.5 and binding increased with increasing concentration of chloroquine.

Chloroquine binding to cellulose propionate, ethylvinyl acetate, methacrylate butadiene styrene, polyethylene, polypropylene and polyvinyl chloride

The effect of solution pH on chloroquine binding to these plastics is shown on Fig. 1. These data show that the highest sorption recorded was at pH 9.5 and became lower at pH 7.4. There was only minimal binding at pH 4 for all of the six plastics

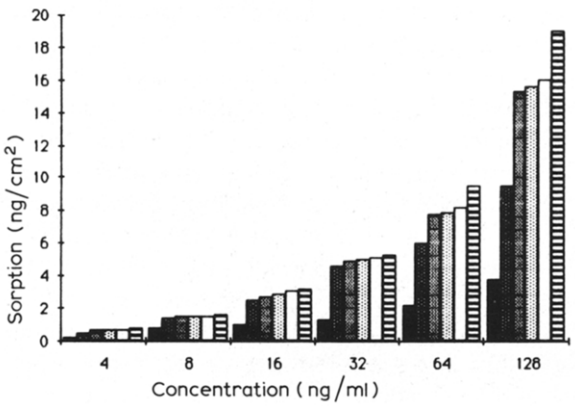


Fig. 2. The effect of chloroquine concentration on chloroquine sorption to: ■, polyethylene; ▦, ethylvinyl acetate; ▩, polyvinyl chloride; ▤, polypropylene; □, methacrylate butadiene styrene; and ▬, cellulose propionate (pH 9.5). Each value is the mean of 10 determinations after storage for 24 h; see Table 1 for reference to thickness of plastic sheets used.

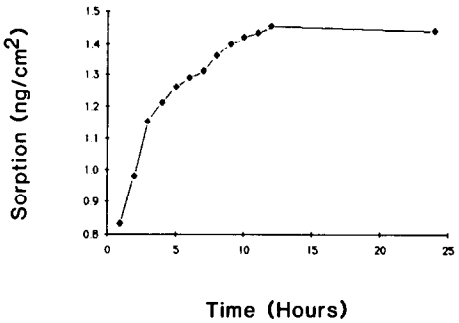


Fig. 3. The effect of time on chloroquine sorption to polyvinyl chloride (pH 9.5, 8 $ng \cdot ml^{-1}$). Each value is the mean of 10 determinations.

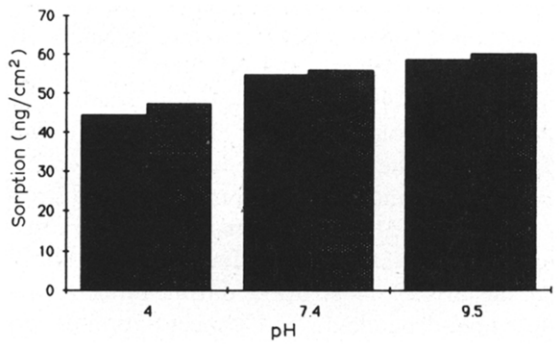


Fig. 4. The effect of solution pH on chloroquine binding to cellulose acetate membrane filters. ■, Molsheim; ▦, Amicon; concentration 16 $ng \cdot ml^{-1}$. Each value is the mean of 10 determinations.

examined. The data for chloroquine binding to the various plastics at pH 9.5 are compared on Fig. 2. These data clearly demonstrate that as the concentration increased the binding per cm^2 increased.

Effect of time on chloroquine binding to polyvinyl chloride

Polyvinylchloride was chosen as a model plastic to illustrate the influence of exposure time on chloroquine binding; the results obtained for this part of the study are illustrated on Fig. 3.

These data indicate that the binding of the drug per cm^2 increased with increasing time up to 12 h after which an equilibrium was reached.

Chloroquine binding to cellulose acetate membrane filters

Data on chloroquine binding to the membrane

filters are shown on Fig. 4; as with the other plastic materials, highest binding was recorded at pH 9.5 with lower sorption values being noted at pH 7.4 and 4. Filters manufactured by Amicon gave rise to slightly higher binding than filters made by the Molsheim Co.

Discussion

Seven types of thermoplastics were examined in the present study. Physicochemical properties including permeability (the transmission of fluids through plastics), and interaction of plastics with solvents, differ from one material to another. Transmission of substances is by a sequence of absorption followed by diffusion (Birely and Scott, 1982; Brydson, 1982).

It seems likely from the present results that chloroquine molecules were absorbed and then diffused into the plastics, since sorption per cm^2 increased with increasing concentration. In a study by Kowaluk et al. (1982), the sorption of 45 drugs to plastic infusion sets was studied. It was found that the loss of most drugs during infusion was slow, time-dependent, and concentration-dependent, which indicated a diffusion-controlled absorption process rather than a binding adsorption process. Other studies have also shown that the kinetics of sorption can be described in terms of a diffusion-controlled absorption process, adsorption playing only a minor role in the overall loss of the drug (Yuen, et al., 1979; Roberts, et al., 1980; Illum and Bundgaard, 1982).

The highest binding of the drug found in the present study was (in decreasing order) to cellulose propionate, methacrylate butadiene styrene, polypropylene, polyvinyl chloride, ethylvinyl acetate, and polyethylene. Highest binding was recorded at pH 9.5 with lower binding at pH 7.4 and least binding at pH 4. A possible explanation of this result is that chloroquine is present in the non-ionized form at the alkaline pH (pH 9.5) becoming increasingly ionized as the pH decreases. In an earlier study by Kowaluk et al., (1981), the main physicochemical determinant controlling drug sorption appeared to be the extent of ionization and lipid solubility of 46 drugs stored in polyvinylchloride bags. Illum and Bundgaard (1982) also showed that the rate and extent of sorption of warfarin was dependent on pH; this could be interpreted in terms of ionization of the drug, i.e. only the unionized form was sorbed.

Investigation of the effect of exposure time on chloroquine absorption to polyvinyl chloride indicated that 12 h was sufficient to reach the equilibrium state.

The difference in the absorption of the drug to these plastics may be attributed to the difference in their physicochemical properties and to the amount of additives that are used during their manufacture, e.g. fillers, plasticisers, softeners, antioxidants, ultraviolet absorbers, and flame retarders. It should also be borne in mind that the materials available for our binding studies were of differing thickness and density and that the same plastic materials from differing sources may vary in their binding capacity for chloroquine. Chloroquine bound extensively to cellulose acetate membrane filters. Passing chloroquine solutions through these filters once resulted in a high loss of drug (up to 60.40 $ng \cdot cm^{-2}$ at pH 9.5 and at a concentration of 16 $ng \cdot ml^{-1}$). The results also indicated a pH-dependent extent of drug absorption to these filters (Fig. 4). Sorption was immediate since passage of the solutions through the filters took only 40 s.

In conclusion, it is recommended that apparatus made from polystyrene plastics should be used in the in vitro sensitivity testing of *P. falciparum* to chloroquine since binding to this plastic is minimal. Apparatus and equipment made from the other plastics tested should be avoided in chloroquine sensitivity testing and assay procedures; alternatively their binding of chloroquine should be quantified and taken into consideration. This is especially important with the use of disposable plastic pipette tips, test-tubes and beakers. Sterilization and/or clarification of chloroquine solutions using cellulose acetate membrane filters should be avoided.

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