Loss of a Hydrophobic Amine from Solution by Adsorption onto Container Surfaces

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Abstract □ The apparent loss of the hydrophobic amine drug α-[(dibutylamino)methyl]-6,8-dichloro-2-(3′,4′-dichlorophenyl)-4-quinolinemethanol monohydrochloride from solution due to its adsorption onto the surface of its storage container was studied. The drug appeared to be adsorbed only as the free base. Therefore, any perturbations to the solution phase that will help solubilize the drug and thus lower its chemical potential will minimize adsorption. Multilayer drug adsorption to the container surfaces appeared to take place, with some evidence of a highly organized system in the adsorbed phase. Adsorption was minimized when the heterogeneous polar functionalities on glass surfaces were covered by a layer of silicone or methacrylate polymer, which yielded less reactive, more hydrophobic surfaces. Loss was also minimized when the environment was kept acidic (pH ≤ 4.8), the drug was dissolved in a proton-donating solvent (*e.g.*, chloroform), and an ion-pairing agent (*e.g.*, trichloroacetate) was present to solubilize further the monocationic form of the drug in organic media.

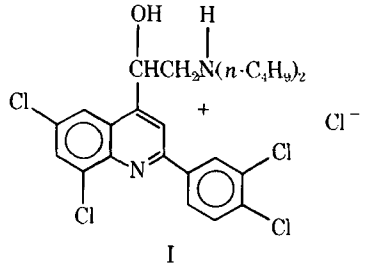
Keyphrases □ α-[(Dibutylamino)methyl]-6,8-dichloro-2-(3′,4′-dichlorophenyl)-4-quinolinemethanol—adsorption onto container surfaces □ Adsorption—α-[(dibutylamino)methyl]-6,8-dichloro-2-(3′,4′-dichlorophenyl)-4-quinolinemethanol onto container surfaces

Quantitative analysis at the submicrogram level poses challenges not encountered in the determination of analytes at higher levels. One problem often encountered, particularly with analysis of hydrophobic drugs, is the apparent loss of compound from solution by adsorption onto container surfaces (1, 2). The experimental antimalarial α-[(dibutylamino)methyl]-6,8-dichloro-2-(3′,4′-dichlorophenyl)-4-quinolinemethanol monohydrochloride (I) and its free base (II) adsorb to container surfaces to a significant and readily measurable extent. The loss of I from solution in hydroxylic solvents by adsorption was studied, and methods to minimize this source of error were investigated.

EXPERIMENTAL

Materials—Compounds I and II were used as received[1]. Glass containers (10-ml volumetric flasks) were siliconized by immersion in a 1% silicone[2] solution for 5 min. The silicone solution was then discarded, and the glassware was rinsed with double-distilled water and dried in an oven at 100° for ~24 hr.

The inner surfaces of volumetric flasks were coated with methacrylate polymer[3] by filling the container with a 10% solution of polymer for ~10 min. The solution was then evaporated, the inner surfaces were rinsed with methanol, and the flasks were dried at 100° for 48 hr in an oven.



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Adsorption Experiments—Solutions were prepared containing 5 ml of 0.02 *M* H_2SO_4 [or 0.02 *M* phosphate buffer (pH 5.8)], an appropriate volume of stock solution of I (10–40 μg/ml in methanol), and sufficient methanol to bring the total volume to 10 ml. The solution was stirred at a constant rate and monitored fluorometrically (λ_{ex} 272 nm and λ_{em} 370 nm) as a function of time. The solutions then were discarded, and the inner surface of the vessel was rinsed with 2-ml portions of a wash solvent for prescribed periods with shaking. Each rinse was assayed fluorometrically for desorbed I.

Equilibrium Desorption of Adsorbed Phase—Methanolic solutions of I were allowed to stir for about 20 days at 25°. The I concentration remaining in solution was determined fluorometrically; intensities were compared with standard curves made at the time of analysis. Drug adsorption to surfaces of the flasks was assumed to be complete when four consecutive concentration measurements were constant. The solutions were stirred for 3 weeks after adsorption was assumed complete.

The total amount of the drug adsorbed was calculated by difference (the total amount added and the amount remaining in solution). The solution was then discarded. Solvent (2 ml) was added to the flasks, and the flasks were shaken in a vertical position for 0.5 hr. The amount of the drug desorbed in 0.5 hr was determined fluorometrically. The solution was discarded, and 2 ml of fresh solvent was added. A number of successive washes was made, until no drug could be detected in the wash solution. The volume of the solvent (2 ml) and the time of shaking the solution (0.5 hr) were kept constant for all washes.

In all cases, volumes were kept constant. When a plastic container or a glass container coated with silicone or methacrylate polymer solutions was used, a Pyrex glass container of the same dimensions was used as a standard for comparison.

RESULTS AND DISCUSSION

Loss of I from Solution—In early investigations with aqueous solutions of I and II, the drug concentration in solution appeared to decrease rapidly when monitored spectrophotometrically.

When solutions of I [0.08 μg/ml in water–methanol (50:50 v/v), buffered at pH 5.8] were stirred at 430 rpm, 50% of the compound was lost to the surface of each container within 28 min. The stirring rate had a considerable effect on the depletion of the drug from solution. A reduction in the stirring rate from 430 to 315 rpm increased the I half-life in solution from 28 to 168 min. Although these data are insufficient to draw definitive conclusions, the results are consistent with the assumption that mass transfer *via* drug diffusion to the container surface is rate determining in adsorption.

The extent of drug adsorption was dependent on the amount of drug already adsorbed on the container surface. When 0.78 μg of drug was adsorbed to the surface of a 20-ml beaker, 0.03 μg of drug/ml remained in solution; when 2.25 μg of drug was adsorbed to an identical container, the I concentration in solution was 0.05 μg/ml. The I concentration remaining in solution in equilibrium with its adsorbed phase was also dependent on the total amount of the drug adsorbed. These results may be explained by assuming that the chemical potential of the drug in the adsorbed phase may be different when different amounts of the drug are totally adsorbed, as would be the case if the drug were adsorbed in layers on the container surface.

Influence of pH on Adsorption—The pH of the solvent system in which I was dissolved had a pronounced effect on adsorption. Whereas the half-life of I in methanol–water (50:50) buffered at pH 5.8 was approximately 28 min (Fig. 1), adsorption occurred more slowly ($t_{1/2}$ = ~15 hr) in the presence of pH 4.2 buffer (Fig. 2) and was eliminated substantially in the presence of 0.01 *M* H_2SO_4 at the same stirring rate (430 rpm) and at the same initial drug concentration (0.08 μg/ml).

[1] Walter Reed Army Research Institute.
[2] Siliclad, Becton-Dickinson.
[3] Plexiglas.

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Table I—Adsorption of I [a] from Solution [b] to Surface of Various Containers

Time	Percent of Drug Remaining in Solution after Agitation in: Glass Beaker[c]	Polypropylene Beaker[c]	Polyfluoroethylene Beakers A[c]	Polyfluoroethylene Beakers B[d]
5 min	94	96	88	84
15 min	89	95	88	65
30 min	90	94	87	55
45 min	—	—	84	53
60 min	87	94	82	46
3 hr	78	85	81	36
5.5 hr	71	74	—	—
6 hr	65	—	—	—
9 hr	—	59	80	27
10 hr	36	—	—	—

[a] 0.11 μg/ml in water–methanol (1:1). [b] Analyzed fluorometrically at 370 nm (excitation wavelength 272 nm). [c] In the presence of 1×10^{-2} *M* H_2SO_4. [d] In the presence of pH 5.8 phosphate buffer.

At or below pH 4.2, the predominant species in solution is the monocationic form of the drug [$pKa_1^* = 0.5$ and $pKa_2^* = 6.3$ determined in water–methanol (75:25) in this laboratory]. The decreased drug adsorption in relatively acidic solutions may well be due to the predominance of the more polar monocationic form of the drug, which is better solubilized in hydroalcoholic solution than is the free base. The reduced adsorption at lower pH suggests that only the free base, II, adsorbs to container surfaces; *i.e.*, the thermodynamic activity of the free base in hydroalcoholic solution is greater than the activity of the monocation in the same solvent. It is presumed that the acidic environment does not significantly affect the container surface, which would modify adsorption characteristics.

Equilibrium Desorption of Free Base (II)—After a solution containing I or II was equilibrated with its adsorbed phase, addition of a fresh volume of solvent resulted in desorption of the drug until equilibrium was reestablished.

Equilibrium desorption of II, when a range of 10–60 μg was initially adsorbed on glass containers, is shown in Fig. 3. Each point represents a single desorption step with 2 ml of wash solution [methanol–water (1:1)].

The apparent constancy in the amount of drug desorbed as a function of the amount remaining adsorbed suggests multilayer drug adsorption on the surface as opposed to precipitation of the slightly soluble drug onto the surface. It was not possible to desorb the drug with the methanol–water (1:1) solution when less than 5 μg was adsorbed, suggesting that this amount may represent monolayer formation on the specific containers used. Simple calculations, with the assumption that 5 μg of the drug formed a monolayer on the beakers used, indicated the surface area per molecule of I to be about 18–19 $Å^2$. This value is comparable with the reported surface area per molecule for stearic acid in monolayers (about 22 $Å^2$) (3), lending support to the assumption that 5 μg of II adsorbed to the surfaces of these containers may represent a monolayer.

When 10–60 μg is adsorbed to the same container, several layers of the drug may be expected to form on the container surface and these layers

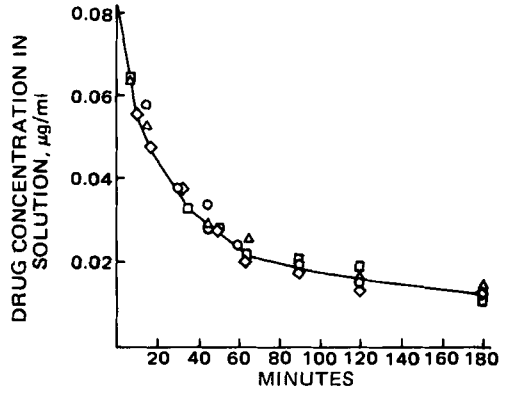

Figure 1—*Adsorption of the drug from solutions (0.08 μg/ml) in water–methanol (1:1) in the presence of pH 5.8 phosphate buffer to surfaces of four identical containers (△, ○, □, and ◇).*

Table II—Adsorption of I from Solution [a] to the Surfaces of 10-ml Volumetric Flasks

Time	Percent of Drug Remaining in Solution[b] in: Uncoated Flasks	Flasks Coated with Methacrylate Solution	Flasks Coated with Silicone Solution
5 min	92	100	99
15 min	—	100	96
35 min	83	100	95
60 min	78	99	94
4 hr	76	97	91
8 hr	—	98	85

[a] 0.098 μg/ml in water–methanol (1:1) in the presence of 1×10^{-2} *M* H_2SO_4. [b] Analyzed fluorometrically at 370 nm (excitation wavelength 272 nm).

may possess a definite order, *i.e.*, layers of decreasing chemical potential in proceeding from the outerlayer to the monolayer. The desorption of such an ordered phase may occur in several steps, with one or more layers desorbing at the same time. The general pattern for desorption in Fig. 3 conforms with the hypothesized ordered layering of II in the adsorptive phase if it is assumed that 5 μg of the drug represents a monolayer and that approximately two layers of drug desorb simultaneously.

The scatter in the data in Fig. 3 may result from the long periods required to reach equilibrium, making the analytical technique rather inaccurate, and the nonhomogeneity of container surfaces, offering sites of different energy to the drug being adsorbed. This situation may result in exposure of different layers to the solvent at the same time during desorption.

Reduction or Prevention of Drug Adsorption to Surfaces of Containers—Drug adsorption to surfaces of different types of containers was studied to see if any reduction or prevention of adsorption to surfaces other than glass occurred. The adsorption of II from solutions [in water–methanol (1:1)] to surfaces of different containers is presented in Tables I and II. Except when otherwise indicated, solutions were made 0.01 *M* in sulfuric acid since an acid environment minimized adsorption.

Vessel composition did not appear to affect adsorption since losses of drug from solution to the surface of polyfluoroethylene beakers (20% loss) and polypropylene beakers (25% loss) were similar to each other and similar to glass beakers (29% loss) of identical surface area when adsorption was studied over 5 hr (Table I). However, the coating of glass vessels with materials that increase surface hydrophobicity did affect the propensity for adsorption. When II was dissolved in methanol–water solution (containing 0.01 *M* H_2SO_4) in 10-ml volumetric flasks coated with a silicone solution (Table II), only 9% of the drug was lost from solution in 4 hr and 15% in 8 hr. Alternatively, when the flasks were coated with a methacrylate polymer solution, less than 2% of drug was adsorbed over 8 hr.

Container surfaces were not examined in microscopic detail, so no attempts were made to explain the effect of the surface on drug adsorption. The effect of environmental pH on adsorption was demonstrated further by the observations that only 20% of drug was lost to the surface of polyfluoroethylene beakers in 10 hr in the presence of 0.01 *M* H_2SO_4 but that 70% was lost when pH 5.8 phosphate buffer was used in place of the acid (Table I). Adsorption of II occurred to all surfaces investigated; however, adsorption occurred to a much lesser extent with methacrylate- and silicone-coated containers.

The desorption of II from glass surfaces into the solution phase was

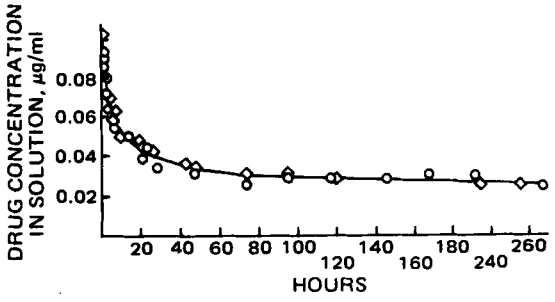

Figure 2—*Adsorption of the drug from solutions (0.105 μg/ml) in water–methanol (1:1) in the presence of pH 4.2 succinate buffer to surfaces of two identical containers (○ and ◇).*

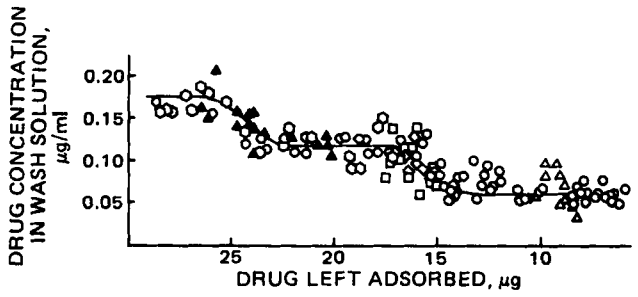

Figure 3—*Equilibrium desorption [into 2 ml of water–methanol (1:1)] of the drug when 60 (○), 40.7 (▲), 30 (○), 17.6 (□), 17.3 (◇), or 10.6 (△) μg was adsorbed initially.*

studied with several organic solvents (Table III). Methanol and ether failed to desorb any detectable quantity of the drug in 30 min. The presence of 20% (v/v) chloroform in methanol facilitated the transfer into the solution phase, resulting in the desorption of 42% of the free base. A relatively efficient transfer (92%) was obtained when chloroform alone was the transfer reagent. Chloroform, a proton-donating solvent, is capable of solubilizing II more efficiently through hydrogen bonding with electron-rich sites (nitrogen and chlorine) on II.

In all cases, the transfer efficiency was increased by the addition of acid to the solvents (Table III). In the presence of 0.01 *M* H_2SO_4 in methanol, a fairly high transfer occurred over the range studied (the 0.2 and 2.0 μg adsorbed initially could be desorbed to an extent of 56 and 88%, respectively). Addition of 33% (v/v) chloroform to that solvent favored the solution phase, making transfer to the solution even more efficient (97%). Acids capable of forming ion-pairs with I, *e.g.*, trichloroacetate, resulted in a 100% transfer of the drug to the solution phase (dichloromethane or chloroform).

General trends observed during the transfer of the adsorbed phase (Table III) suggest that transfer into the solution phase was more favored as the total amount of the free base adsorbed increased. With smaller amounts of adsorbed drug, the adsorbed phase was preferred over the solution phase, except in chloroform solutions. Furthermore, drug transfer from the adsorbed phase into solution (for any solvent system tried) was more efficient when silicone-coated (or methacrylate-coated) containers were used as compared to Pyrex glassware. The Pyrex glass surface is more heterogeneous than the surface of a silicone-coated container. Therefore, adsorption of hydrophobic drug molecules may be stronger onto a glass surface.

In conclusion, the drug appears to adsorb onto container surface in layers possessing a definite energetic order. Minimum adsorption occurred to glassware made hydrophobic by coating with silicone or methacrylate. Desorption was achieved most efficiently with solvent systems that most effectively solvate the drug. The presence of an acid environment converted II to a more polar monocationic species, which was efficiently solvated as an ion-pair in proton-donating solvents (chloroform and dichloromethane), thus eliminating the adsorption of I to container surfaces.

Table III—Transfer of the Drug from the Adsorbed Phase [a] to the Solution Phase

Solvent Used for Transfer	Drug Adsorbed, μg	Drug Transferred, μg	Drug Transferred, %
2 ml of methanol	0.21	0.0	0
	0.21[b]	0.0	0
	2.06	0.0	0
	2.06[b]	0.12	6.0
2 ml of ether	1.92	0.0	0
	1.92[b]	0.0	0
1.6 ml of chloroform and 0.4 ml of methanol	1.09	0.52	48
	1.11[b]	0.47	42
	0.43	0.0	0
	0.43[b]	0.26	61
1.6 ml of chloroform and 0.4 ml of hexane	1.93	1.0	52
	1.93[b]	1.7	88
	0.19	0.0	0
	0.19[b]	0.1	52
2 ml of chloroform	1.28	0.59	45
	1.28[b]	1.18	92
2.0 ml of methanol and 0.03 ml of 3 *M* H_2SO_4	0.24	0.0	0
	0.21[b]	0.18	88
	2.06	0.70	34
	2.06[b]	1.14	55
2.0 ml of methanol, 0.1 ml of 1 *M* H_2SO_4, and 1.0 ml of chloroform	2.38	1.15	48
	0.24	0.13	56
	2.06[b]	2.01	97
	0.21[b]	0.15	75
2.0 ml of chloroform containing 4% CCl_3COOH	0.22[b]	0.22	100
	1.66	1.41	85
	0.22	0.18	82
2.0 ml of dichloromethane containing 4% CCl_3COOH	0.22[b]	0.22	100

[a] The drug was allowed to adsorb onto surfaces of 10-ml volumetric flasks from water–methanol (1:1) solutions. [b] Ten-milliliter volumetric flasks coated with a silicone solution were used.

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