(15) J. R. Weeks and J. D. Davis, *J. Appl. Physiol.*, **19**, 540 (1964).
(16) J. J. Ashley and G. Levy, *Res. Commun. Chem. Pathol. Pharmacol.*, **4**, 297 (1972).
(17) A. C. Bratton and E. K. Marshall, *J. Biol. Chem.*, **128**, 537 (1939).
(18) J. Rieder, *Chemotherapy*, **17**, 1 (1972).
(19) C. M. Metzler, "NONLIN, a Computer Program for Parameter Estimation in Nonlinear Situations," The Upjohn Co., Kalamazoo, Mich., 1969.
(20) M. Gibaldi and D. Perrier, "Pharmacokinetics," Dekker, New York, N.Y., 1975.
(21) R. A. Roth, Jr., and R. J. Rubin, *Drug Metab. Disp.*, **4**, 460 (1976).
(22) C.-M. Lai, A. Yacobi, and G. Levy, *J. Pharmacol. Exp. Ther.*, **199**, 74 (1976).
(23) R. Hori, K. Sunayashiki, and A. Kamiya, *J. Pharm. Sci.*, **65**, 463 (1976).
(24) M. Gibaldi, G. Levy, and P. J. McNamara, *Clin. Pharmacol. Ther.*, **24**, 1 (1978).
(25) J. R. Gillette, *Ann. N.Y. Acad. Sci.*, **179**, 43 (1971).
(26) M. Gibaldi and P. J. McNamara, *J. Pharm. Sci.*, **66**, 1211 (1977).
(27) G. Levy, C.-M. Lai, and A. Yacobi, *ibid.*, **67**, 229 (1978).
(28) C.-M. Lai and G. Levy, *ibid.*, **67**, 1492 (1978).
(29) P. J. McNamara, G. Levy, and M. Gibaldi, *J. Pharmacokinet. Biopharm.*, in press.

ACKNOWLEDGMENTS

Supported in part by Grant GM-20852 from the National Institute of General Medical Sciences, National Institutes of Health.

Analysis of Hydrophobic Amine Antimalarials in Biological Fluids with the Plastic Ion-Selective Electrode

DOUGLAS W. MENDENHALL, TAKERU HIGUCHI, and LARRY A. STERNSON ˣ

Received September 11, 1978, from the *Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66045.* Accepted for publication December 5, 1978.

Abstract □ Plastic ion-selective electrode analysis of the hydrophobic amine antimalarial mefloquine in blood samples was investigated. The direct electrode response in plasma samples provided poor drug sensitivity due to high mefloquine protein binding. The drug was analyzed in whole blood by initial extraction into ether as its trichloroacetate ion-pair. Mefloquine was monitored in whole blood extracts with the electrode to moderately low levels (to 0.4 μg/ml). Rapid blood mefloquine level monitoring by this electrode was demonstrated in a bioavailability study. Mefloquine alkylation with various alkyl halides produced derivatives detectable by the electrode at much lower levels (up to two orders of magnitude) than the parent. A kinetic study of this alkylation reaction revealed that an alkyl amine base was necessary to scavenge the acid produced during reaction and to allow the reaction to go to completion. At room temperature, with benzyl bromide as the reagent, reaction was 99% complete in 30 min and mefloquine could be detected to $\sim 10^{-8}$ *M*, a 100-fold improvement in sensitivity over electrode monitoring of underivatized mefloquine.

Keyphrases □ Electrodes, plastic ion selective—analysis, mefloquine and derivatives in blood, dogs, hydrophobic amines □ Mefloquine—analysis, in blood, plastic ion-selective electrodes, derivatives □ Hydrophobic amines—mefloquine, analysis in blood, plastic ion-selective electrodes □ Antimalarials—mefloquine, analysis in blood, plastic ion-selective electrodes

Recent interest in routine drug level monitoring in biological samples (*e.g.*, blood, plasma, and urine) for determining pharmacokinetic parameters and individualizing dosage regimens had produced a need for analytical methods that can be practically applied in the clinical laboratory. These methods should be rapid, simple, and sensitive, should require minimal sample preparation, and should employ inexpensive instrumentation.

One such method recently was reported (1) from these laboratories for urine methadone analysis. A plastic ion-selective electrode was used that detects hydrophobic cations in aqueous solutions (2, 3). This report describes further studies quantitating hydrophobic amines to low levels in whole blood samples. Amines could be derivatized for detection by the plastic ion-selective electrode, increasing sensitivity up to two orders of magnitude.

The model compounds selected for these studies were the quinolinemethanol antimalarial series mefloquine (I), II, and III, presently under investigation for the treatment of persistent disease caused by *Plasmodia* strains. Problems associated with the blood concentration determination of such hydrophobic amines include glass adsorption, protein binding, and poor sensitivity by conventional detection methods [*e.g.*, high-performance liquid chromatography (HPLC) using UV detection].

EXPERIMENTAL

Materials—Mefloquine, II, and III were used as obtained[1]. The free mefloquine base was precipitated from an alkaline salt solution and recrystallized from water–ethanol to a constant melting point (174–175°). Dodecyl, octyl, and hexyl iodides and tetrabutylammonium bromide were used as obtained from commercial sources. Benzyl bromide was freshly distilled (55–56°/0.03 mm Hg) under nitrogen and stored protected from light under nitrogen until used; at the first sign of a yellow color, fresh reagent was distilled. Methanol and acetonitrile were reagent grade and were distilled in glass prior to use. Electrodes were fabricated as described previously (1).

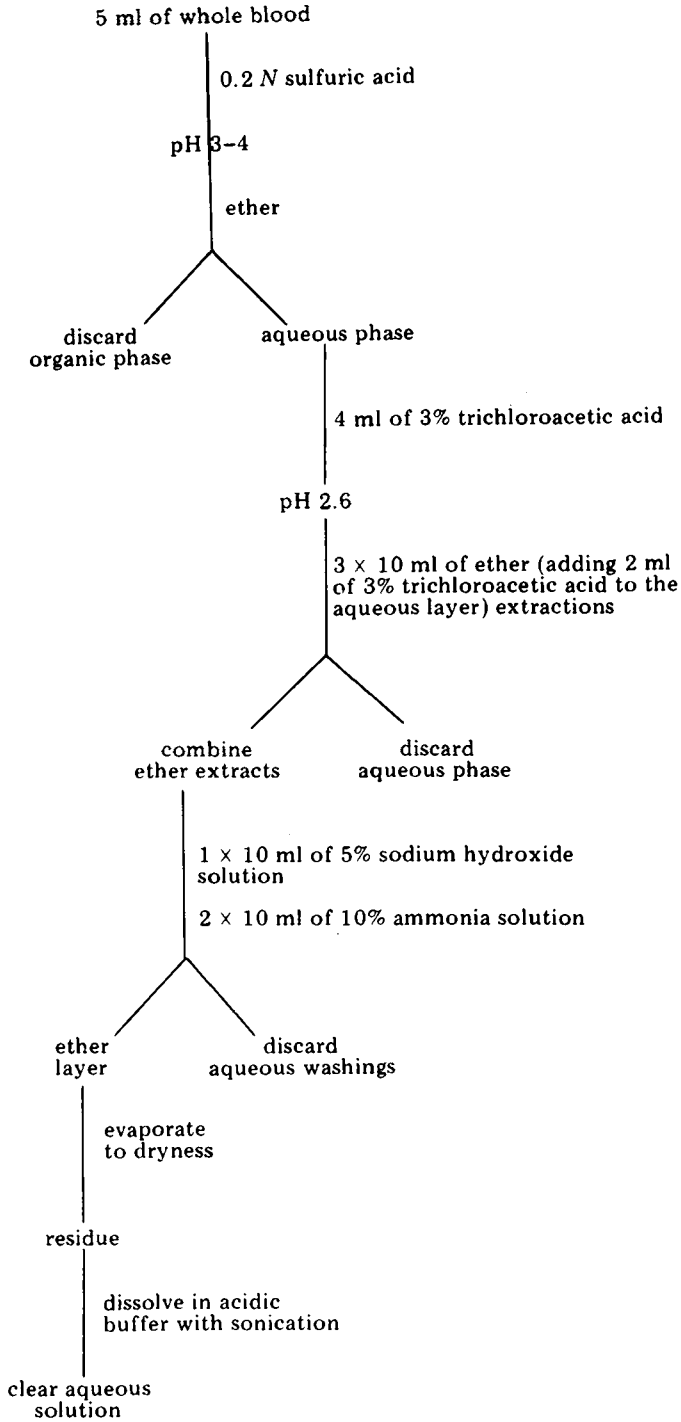
Fresh whole blood was obtained from beagle dogs and maintained under refrigeration until used, usually within 48 hr. Anticoagulants (usually sodium citrate) were added at collection. Plasma was prepared from whole blood by centrifugation at 5000 rpm for 40 min and collection of the clear supernatant fraction.

Electrodes—Coated wire electrodes were prepared as previously described (1). All electrodes were checked for response to tetrabutylammonium ion before use, and a minimum −56-mv/decade slope (*versus* theoretical −59 mv) was required for electrode acceptability.

Apparatus—All potentiometric measurements were made with a

[1] Through INTERx Research Corp., Lawrence, Kans., from Walter Reed Army Research Institute.

0022-3549/79/0600-0746$01.00/0

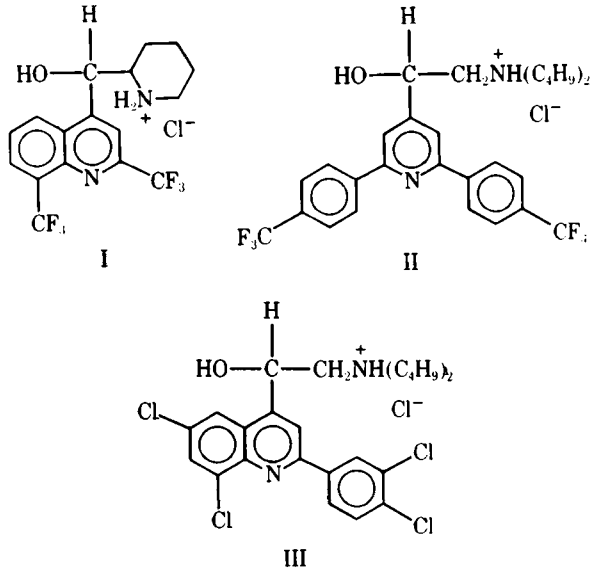


Scheme I—Ion-pair extraction scheme for mefloquine from whole blood.

digital ionanalyzer equipped with a miniature saturated calomel electrode (fiber junction[2]).

HPLC analysis was carried out with a component system[3] fitted with a column containing 10-μm silica with bonded octadecylsilane and an absorbance detector (280-nm phosphor). Detector output was monitored on a 10-mv strip-chart recorder, and peak areas were determined with a polar planimeter.

Plastic Ion-Selective Electrode Determination—The plastic electrode–calomel electrode pair was immersed in a stirred aqueous sample solution, and the potential was measured after the electrode equilibrated for 3 min. Electrodes were removed from the sample solution and washed thoroughly with deionized water before reuse. Analytes were



I II III

quantitated by comparison of the unknown solution potential with a standard curve constructed after plastic electrode analysis of a series of samples containing known analyte amounts prepared in the matrix (*e.g.*, plasma, whole blood, or water) in which the unknown was present.

Direct Plastic Ion-Selective Electrode Determination in Plasma—Plasma samples (1.0 ml) spiked with mefloquine (0.414–414 μg/ml) were adjusted to pH 4–5 with 0.3 ml of 0.1 *N* HCl, and the solution was diluted to 10 ml with water. Potentials were determined with the plastic electrode. Samples containing II were treated similarly; however, the pH was adjusted to 3 prior to electrode monitoring.

Blood Mefloquine Analysis—Fresh whole blood samples containing mefloquine were transferred to 35-ml heavy duty screw-capped culture tubes and extracted by the ion-pair technique (4) (Scheme I). The extracts were evaporated to dryness, and the residue was redissolved in 250 μl of acetone and 4.75 ml of 0.001 *M* phosphate buffer (pH 6.0). Extracted blanks, used as standards, were spiked with known mefloquine amounts before dilution with buffer. The plastic electrode response was determined and transformed to concentration units by comparison of the response with a standard curve constructed by analysis of whole blood samples spiked with known mefloquine amounts (0.414–41.4 μg/ml).

Mefloquine Derivation with Benzyl Bromide—Alkylation kinetics were determined with 2-ml samples containing mefloquine (7×10^{-4} *M*) dissolved in acetonitrile to which was added 200 μl of benzyl bromide. The mixture was analyzed by HPLC. Samples (50 μl) were withdrawn at timed intervals and diluted with methanol (to 1.0 ml), and a 50-μl aliquot was chromatographed. Components were eluted isocratically with methanol–0.012 *M* ammonium dihydrogen phosphate (69:31) pumped through the system at 2 ml/min. Standard methanolic mefloquine (V_r = 9.1 ml) and *N*-benzylmefloquine (V_r = 11.5 ml) solutions were injected periodically to determine if instrument performance was deteriorating.

For characterization purposes, *N*-alkylmefloquines were synthesized by refluxing equimolar alkyl halide and I in acetone (acetonitrile for benzyl bromide) for 48 hr. The mixture was cooled and diluted threefold with ether, and the product was precipitated as the hydrochloride salt by the addition of one volume of 5% hydrochloric acid. The solid was collected and recrystallized from ether–acetone or methanol–water, and its structure was confirmed by elemental analysis.

Animal Studies—Beagle dogs were given mefloquine orally as a 250-mg tablet[4] followed by 100 ml of water by gastric lavage or as an intravenous bolus of 50 mg of mefloquine in 5 ml of 20% propylene glycol–6% dextrose solution into one jugular vein over 2 min. Blood samples (5 ml) taken from the other jugular vein just prior to drug administration and at various times postadministration were processed by ion-pair extraction and were analyzed with the plastic ion-selective electrode.

RESULTS AND DISCUSSION

Solutions containing mefloquine, II, or III prepared in dilute aqueous acid (10^{-4} *N* H_2SO_4) could be quantified by direct measurement with

[2] Corning 476017.
[3] μBondapak C18, Waters Associates, Milford, Mass.
[4] Lot B-512, INTERx Research Corp., Lawrence, Kans.

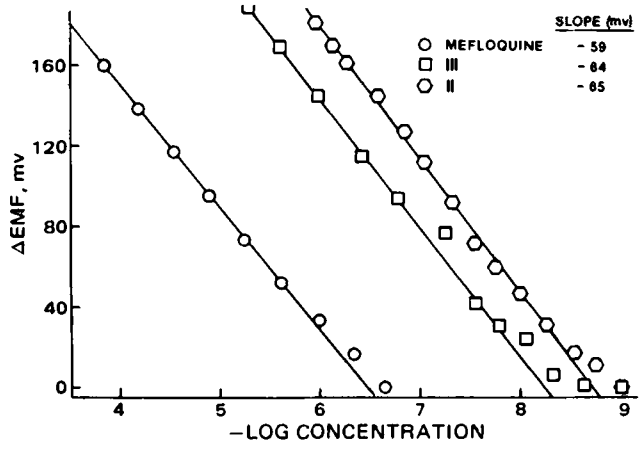


Figure 1—*Plastic ion selective response to hydrophobic antimalarials. Key: ○, mefloquine in 10^{-4} N H_2SO_4; ○, II in 10^{-3} N HCl containing 0.1% methanol; and □, III in 10^{-3} N HCl containing 1% methanol.*

the plastic ion-selective electrode. The potential response was linearly related to analyte concentration over three orders of magnitude (Fig. 1). In this simple aqueous matrix, measurements could be made with ±4% accuracy and ±2% precision over the linear concentration range.

Electrodes were thoroughly washed with deionized water (*e.g.*, 5 min) after removal from sample solutions to achieve this accuracy and precision. After determinations in more concentrated solutions, electrodes were immersed in stirred deionized water for 3 hr and air dried before reuse on a dilute sample. Failure to take such precautions reduced accuracy and precision and produced meaningless results.

The electrode response increased with the analyte hydrophobicity. For the more hydrophobic drugs (II and III), sensitivity approaching 10^{-9} *M* could be achieved; mefloquine could be quantified to 10^{-7} *M*. The Nernst equation predicts a change of 59 mv/decade in the activity of a species to which the electrode responds (5). A near-Nernstian linear response was observed in aqueous acid. The slightly super-Nernstian slopes (>59 mv) seen with the more hydrophobic compounds may arise from alterations in the membrane caused by adsorbed analyte, producing a more favorable environment for subsequent analyte partitioning into the membrane phase, an explanation consistent with the proposed mechanism for the plastic ion-selective electrode (3).

Direct Determination of Plasma Antimalarials—Compared with aqueous solutions, a loss of two orders of magnitude in sensitivity was observed for plastic ion-selective electrode response to mefloquine and II in buffered plasma. In both cases, linear responses were observed, although slopes were sub-Nernstian (I, −49 mv; and II, −53 mv). This loss in sensitivity is accounted for by the extensive protein binding observed with hydrophobic molecules (6), which reduces the free drug activity in solution. Mefloquine is 98% protein bound in plasma (7).

Attempts to dislodge mefloquine from protein binding sites and thereby to allow detection at lower levels by pH manipulation or heat denaturation were unsuccessful. Sample matrix effects severely hampered

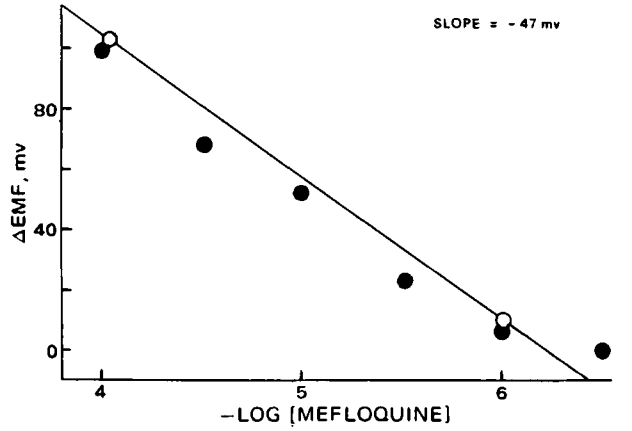


Figure 2—*Plastic ion-selective electrode determination of mefloquine in ion-pair extracts of whole blood dissolved in 5% acetone–pH 6 buffer spiked before (●) and after (○) extraction. Potential was measured versus a saturated calomel electrode.*

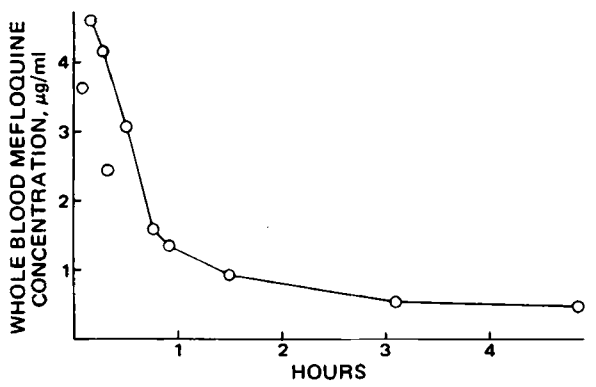


Figure 3—*Blood mefloquine level versus time profile after intravenous administration of 50 mg of the drug (as the hydrochloride) to a beagle dog.*

the assay accuracy (±25%) and precision when carried out directly in biological fluid. Therefore, methods were necessary to remove the drug from the biological matrix prior to plastic ion-selective electrode monitoring.

Blood Mefloquine Analysis—The drug was separated from blood components by extraction. Direct extraction from neutral or basic solution was ineffective because of drug loss due to adsorption to glass surfaces; interference from endogenous amines coextracted from blood, especially when derivatization with alkyl halides was indicated; and incomplete dissolution into aqueous acid after evaporation of the extracts to dryness, apparently due to lipid material coextracted with the drug. Therefore, a more selective, ion-pair extraction was used for mefloquine isolation (Scheme I).

Nakagawa *et al.* (4) showed that mefloquine was extracted into ether from blood acidified to pH 3 as a 1:1 ion-pair with trichloroacetate, giving excellent recoveries (88–100%) over a broad concentration range (1–1000 ng/ml). Initial extraction of acidified blood with ether in the absence of an ion-pairing agent removed acidic compounds, lipids, and other neutral materials that may otherwise have interfered with subsequent drug detection. The extraction scheme (Scheme I) utilized ether, an electron-donating species, as the extractant instead of the proton-donating solvent (*e.g.*, chloroform) normally indicated for solvation (extraction) of a large cation–small anion ion-pair (8). This procedure minimized extraction of potentially interfering, less hydrophobic amines while maintaining efficient analyte recovery (9).

The extracted drug was reconstituted in aqueous buffer (pH 6.0), and the concentration was determined using the plastic electrode. The plastic electrode potential *versus* the log of the spiked blood sample-concentration curve (Fig. 2) indicated a linear response to 10^{-6} *M* drug (0.4 μg/ml free base), the same limit found in aqueous systems. Extractive mefloquine removal from biological fluid returned the analyte to a more uniform matrix. Measurements could be made with ±8% accuracy and ±6% precision. These statistics also reflect the sample to sample variation (±5%) in mefloquine extraction efficiency from a biological sample.

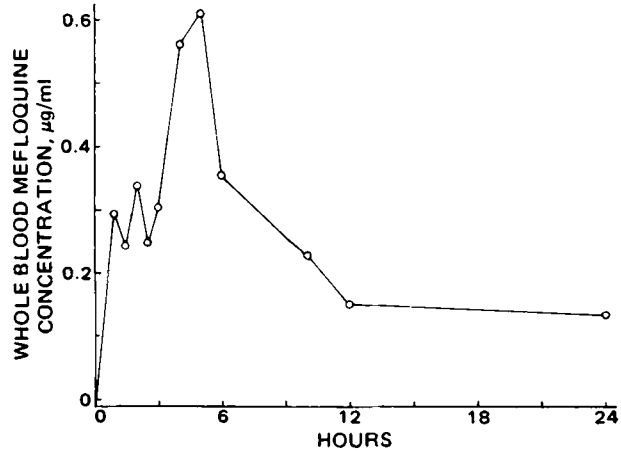


Figure 4—*Blood mefloquine level versus time profile after oral administration of 250-mg mefloquine hydrochloride tablet to a beagle dog.*

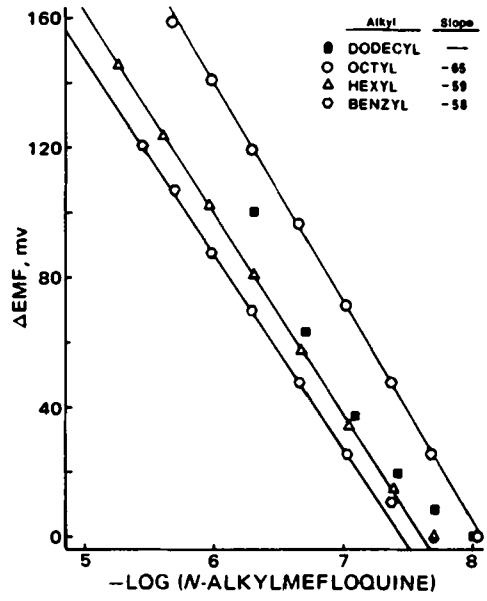


Figure 5—*Plastic ion-selective electrode response to* N-*alkylmefloquines in* 10^{-4} N H_2SO_4 *(dodecyl solutions contained 1% methanol). Potential was measured* versus *a saturated calomel electrode.*

The depressed slope (−47 mv *versus* a theoretical slope of −59 mv) was consistently seen with blood extracts and may have been due to some undefined effect of coextracted blood components on electrode response. Nonetheless, a linear response over the range of interest is adequate as a useful analytical detector. Blank blood samples were alternatively spiked after ion-pair extraction and used to generate a standard response curve (open circles in Fig. 2). An apparent consistent ~85% recovery (as determined by deviations from the theoretical 100% response line in Fig. 2) was indicated by this method, in good agreement with literature results over this concentration range (4).

The use of this method for bioavailability studies evaluating various mefloquine formulations in dogs was demonstrated by analyzing blood samples drawn from two beagles after administration of two experimental formulations. The results (Figs. 3 and 4) indicate adequate sensitivity for formulations having good availability but limited accuracy beyond peak levels for poorly absorbed formulations.

Amine Derivatization for Plastic Ion-Selective Electrode Detection—After hydrophobic amine isolation by a relatively selective procedure such as ion-pair extraction, simple alkylation with a relatively lipophilic alkyl halide prior to plastic ion-selective electrode detection provided a rapid method for dramatically increasing detector sensitivity. Figure 5 illustrates the increased sensitivity limits to various *N*-alkylated mefloquine derivatives, showing up to two orders of magnitude improvement in sensitivity over the parent compound. *N*-Dodecylmefloquine required an organic cosolvent (1% methanol) to achieve dissolution and showed an erratic response to the plastic electrode. The octyl derivative showed the same super-Nernstian slope (−65 mv) previously observed for II and III and may be similarly explained.

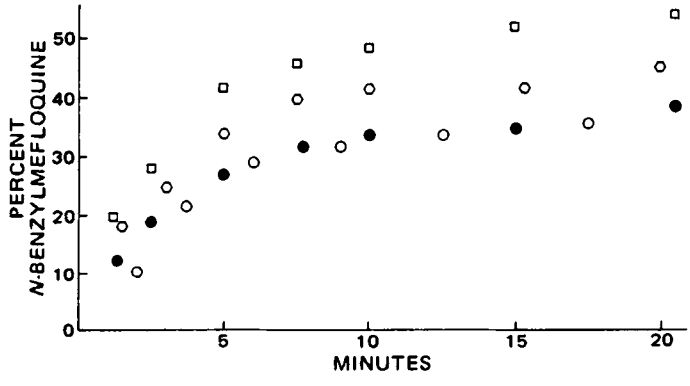


Figure 6—*Effect of added hydrobromic acid and 2,6-di-*tert*-butylpyridine on the percent of mefloquine ([mefloquine]*$_0$ *=* 7×10^{-4} *M) converted to* N*-benzylmefloquine as a function of time. Key ([hydrobromic acid]): □, 0.0; ○,* 3.5×10^{-5} M; ○, 7×10^{-5} M; *and* ●, 7×10^{-5} M *plus* 2.8×10^{-3} M *2,6-di-*tert*-butylpyridine.*

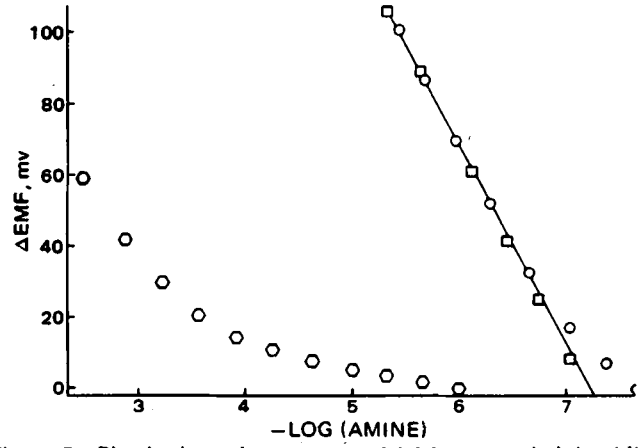


Figure 7—*Plastic electrode response to 2,2,6,6-tetramethylpiperidine (○) and to* N*-benzylmefloquine in the presence (□) and absence (○) of excess benzyl bromide in* 10^{-4} N H_2SO_4. *Potential was measured* versus *a saturated calomel electrode.*

To optimize reaction conditions for alkylation of nanogram quantities of mefloquine from blood extracts, a brief study of alkylation reaction kinetics was undertaken. Of the alkylated mefloquines, the octyl and benzyl derivatives were the most likely derivatives for plastic electrode detection, the former because of its greater sensitivity and the latter because of its anticipated faster formation rate from I. Only the benzyl bromide–mefloquine reaction kinetics were studied in detail since preliminary experiments indicated that the reaction rate between octyl iodide and the drug was too slow to be useful as a derivatization procedure.

Amine alkylation rates involving bulky reactants are slow (10). Therefore, a large (10,000-fold) alkyl halide excess was used to accelerate the reaction rate since the amine concentration available for the presumed second-order reaction was small. Benzyl bromide (neat) was added to stirred mefloquine free base solutions in acetonitrile; aliquots were periodically removed, the reaction was quenched in methanol, and the mefloquine and *N*-benzylmefloquine concentrations were determined by HPLC. Essentially 100% of the initial mefloquine concentration could be accounted for by the parent and *N*-benzylmefloquine peaks in all experiments, indicating that no unexpected side reactions were occurring; no detectable benzyl bromide loss was observed under the reaction conditions, indicating that the reagent was stable over the reaction period.

Initial mefloquine benzylation studies gave unexpected results. The reaction proceeded rapidly initially, but the rate declined substantially after less than half the mefloquine had been consumed. Furthermore, the data could not be plotted in the expected pseudo-first-order manner. The reaction could be of a higher order than the assumed first-order dependency, which would produce an exaggerated drop in rate as amine was consumed. Variation in initial mefloquine concentration, however, failed to show the concentration dependence that would be expected for such a mechanism.

If the reaction proceeded by an S_N1 mechanism and bromide ion effectively competed with the amine for the reactive benzyl carbonium ion, a depression in rate would be expected as the bromide-ion concentration increased over the reaction course. Addition of relatively high bromide-ion concentrations (two times the initial mefloquine concentration) failed to depress the reaction rate, eliminating this possibility. Addition of hydrogen bromide to the reaction mixture, however, depressed the reaction rate (Fig. 6), indicating that hydrogen bromide produced during the reaction was protonating the secondary amine, effectively reducing the mefloquine concentration available for alkylation and, therefore, the rate. Addition of a sterically hindered (to prevent alkylation) pyridine base (2,6-di-*tert*-butylpyridine) failed to reverse the hydrogen bromide effect (Fig. 6). However, when 2,2,6,6-tetramethylpiperidine was added to the reaction mixture, the expected first-order plot was obtained, indicating that piperidine is a significantly stronger base in acetonitrile than pyridine and, therefore, capable of more effectively trapping the liberated hydrogen bromide.

Under the reaction conditions employed (5 μl of benzyl bromide/50 μl of acetonitrile solution of the amine to be determined, containing 0.001 *M* piperidine base), the pseudo-first-order rate constant (0.146 min^{-1})

predicts that the reaction is essentially (~99%) complete after 30 min at room temperature.

As shown in Fig. 7, 100:1 dilution of this reaction mixture with 10^{-4} N H_2SO_4 allowed direct plastic electrode determination of the alkylated amine without separation from the excess reactants since no interference from benzyl bromide or the piperidine base was seen.

CONCLUSION

Previous reports reviewed the many difficulties in handling and analyzing hydrophobic amines in biological samples, including glass adsorption, protein binding, and poor detector sensitivity (11–15). Coupled with ion-pair extraction, the plastic ion-selective electrode detection method described here offers potential advantages for efficient isolation and quantitation of hydrophobic drugs in whole blood at low levels. Furthermore, the derivatization procedure affords a means of enhancing plastic electrode detector sensitivity up to two orders of magnitude and may, therefore, extend the applicability of the method to other, less hydrophobic primary and secondary amines.

REFERENCES

(1) S. Srianujata, W. R. White, T. Higuchi, and L. A. Sternson, *Anal. Chem.*, **50**, 232 (1978).

(2) T. Higuchi, C. R. Illian, and J. L. Tossounian, *ibid.*, **42**, 1674 (1970).

(3) J. R. Luch, Ph.D. thesis, University of Kansas, Lawrence, Kans., 1976.

(4) T. Nakagawa, T. Higuchi, J. L. Haslam, R. D. Shaffer, and D. W. Mendenhall, *J. Pharm. Sci.*, in press.

(5) G. Eisenman, in "Advances in Analytical Chemistry and Instrumentation," vol. 4, C. N. Reilly, Ed., Wiley, New York, N.Y., 1965, pp. 213–369.

(6) B. B. Brodie, in "Transport Function of Plasma Proteins," P. Desgrez and P. M. DeTraverse, Eds., Elsevier, Amsterdam, The Netherlands, 1966, pp. 137–145.

(7) J. Y. Mu, Z. H. Israili, and P. G. Dayton, *Drug Metab. Disp.*, **3**, 198 (1976).

(8) T. Higuchi, A. F. Michaelis, T. Tan, and A. Hurwitz, *Anal. Chem.*, **39**, 974 (1967).

(9) D. W. Mendenhall, Ph.D. thesis, University of Kansas, Lawrence, Kans., 1977, pp. 3–34.

(10) J. March, "Advanced Organic Chemistry," McGraw-Hill, New York, N.Y., 1968, pp. 251–375.

(11) B. B. Brodie, in "Handbook of Experimental Pharmacology," vol. 28, part 2, O. Eichler, Series Ed., Springer-Verlag, Berlin, Germany, 1971.

(12) C. R. Jones, in "Methodological Developments in Biochemistry," vol. 5, E. Reid, Ed., North-Holland, Amsterdam, The Netherlands, 1976, pp. 107–113.

(13) E. O. Titus, in "Fundamentals of Drug Metabolism and Drug Disposition," B. N. LaDu, H. G. Mandel, and E. L. Way, Eds., Williams & Wilkins, Baltimore, Md., 1971, pp. 419–436.

(14) G. Schill, in "Methodological Developments in Biochemistry," vol. 5, E. Reid, Ed., North-Holland, Amsterdam, The Netherlands, 1976, pp. 87–102.

(15) K. Thakker, T. Higuchi, and L. A. Sternson, *J. Pharm. Sci.*, **68**, 93 (1979).

ACKNOWLEDGMENTS

Supported in part by NIH Grant CA-09242 from the National Cancer Institute and by grants from the U.S. Army Research and Development Command and INTERx Research Corp.

Colorimetric Acetaminophen Determination in Pharmaceutical Formulations

S. BELAL ˣ, M. ABDEL-HADY ELSAYED *, A. EL-WALIELY, and H. ABDINE

Received April 14, 1978, from the *Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt.* Accepted for publication December 6, 1978.

*Present address: Department of Pharmacy, University of Nigeria, Nsukka, Nigeria.

Abstract □ Different approaches for the colorimetric determination of acetaminophen, based on its coupling with diazotized *o*-nitroaniline, are described. Copper(II) chelation with the coupled compound makes the method highly selective. Sensitivity is increased when the acetaminophen assay is carried out indirectly through the determination of the chelate's copper content. Optimum conditions for performing the different approaches are described. The stoichiometric balance for the reactants in the coupled compound and chelate is determined. The degree of dissociation and the instability constant are computed. The rectilinear relationship between the absorbance of the different products and the concentration of acetaminophen allows for its determination in different pharmaceutical formulations. Compared with the official method, the proposed methods give more accurate results.

Keyphrases □ Acetaminophen—analysis, colorimetry, various pharmaceutical formulations □ Colorimetry—analysis, acetaminophen in various pharmaceutical formulations □ Analgesics—acetaminophen, analysis, colorimetry, various pharmaceutical formulations □ Antipyretics—acetaminophen, analysis, colorimetry, various pharmaceutical formulations

Acetaminophen hydrolysis with subsequent condensation with anisaldehyde (1), *p*-dimethylaminobenzaldehyde (2), *p*-dimethylaminocinnamaldehyde (3), or vanillin (4) or reaction with alkaline 2-naphthol (5) has been used for colorimetric acetaminophen determination in unit-dose formulations. Other methods were based on the reaction with phenol together with hypobromite (6), hypochlorite in the presence of metaarsenite (7), or ferricyanide (8). Nitrosatin (9) and nitration (10, 11) were utilized also for colorimetric determination. Most of these methods are not selective.

The fluorometric method, based on acetaminophen oxidation with alkali ferricyanide (12, 13) or on its hydrolysis followed by reaction with benzylamine (14), is sensitive to many interfering substances present in pharmaceutical formulations.

Acetaminophen was also determined by cerimetric titration (15, 16), gravimetry (17), titration of the hydrolytic product with nitrite (18), and polarography (19). These methods are not sufficiently sensitive.

Acetaminophen, containing the acetamido group $NHCOCH_3$, was determined by hydrolysis, diazotization

0022-3549/79/0600-0750$01.00/0