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Electrochemical Evidence for Interaction between Chlorpromazine Hydrochloride and Trifluoperazine Hydrochloride and the Flavin Coenzymes

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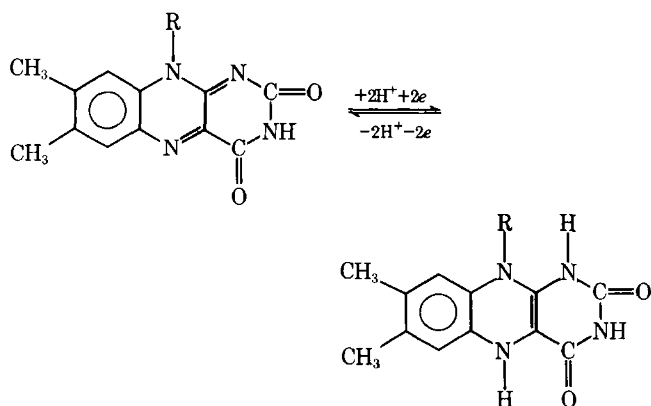
Abstract ☐ Polarographic and chronopotentiometric methods were applied to study the effects of the phenothiazine tranquilizers chlorpromazine hydrochloride and trifluoperazine hydrochloride on the electrochemical behavior of the flavin coenzymes flavin mononucleotide and flavin adenine dinucleotide. The effects of the drugs were measured mainly by decreases in the diffusion currents, i_d, developed in the polarographic experiments and by a similar decrease in the chronopotentiometric constant, $i_0\tau^{1/2}$, in the chronopotentiometric experiments when the coenzymes were reduced in the presence of the added drugs. The observed interference with the redox properties of the coenzymes could conceivably be related to the reported ability of the drugs to inhibit respiration and produce their tranquilizing effect.

Keyphrases ☐ Chlorpromazine hydrochloride—effect on electrochemical behavior of flavin coenzymes, polarographic and chronopotentiometric investigation ☐ Trifluoperazine hydrochloride—effect on electrochemical behavior of flavin coenzymes, polarographic and chronopotentiometric investigation ☐ Flavin coenzymes—electrochemical behavior, effect of chlorpromazine and trifluoperazine hydrochlorides ☐ Electrochemical behavior—flavin coenzymes, effect of chlorpromazine and trifluoperazine hydrochlorides ☐ Polarography—investigation of effect of chlorpromazine and trifluoperazine hydrochlorides on electrochemical behavior of flavin coenzymes ☐ Chronopotentiometry—investigation of effect of chlorpromazine and trifluoperazine hydrochlorides on electrochemical behavior of flavin coenzymes ☐ Tranquilizers—chlorpromazine and trifluoperazine hydrochlorides, effect on electrochemical behavior of flavin coenzymes ☐ Coenzymes, flavin—electrochemical behavior, effect of chlorpromazine and trifluoperazine hydrochlorides

One mechanism proposed to account for the tranquilizing action of the phenothiazine drugs involves the possible interaction between the drugs and certain electron-transferring coenzymes of the respiratory chain. The inhibitory effects of chlorpromazine on respiration were suggested to result from complex formation between chlorpromazine and flavin adenine dinucleotide (1). The studies involved observation of changes in the fluorescent and spectrophotometric properties of the coenzymes upon the addition of the drug.

Similar inhibitory effects were reported in studies of the effects of chlorpromazine upon the mitochondrial systems of both the brain and heart of rats (2, 3). It was



Scheme I

proposed that the drug inhibited electron transport between reduced nicotinamide adenine dinucleotide (NADH) and cytochrome c and must, therefore, exert its effect between NADH and the flavo-proteins, including riboflavin phosphate (flavin mononucleotide) (I) and flavin adenine dinucleotide (II).

In other studies, the electron-donor properties of chlorpromazine were investigated, and the drug was found to be an "extraordinary electron donor" (4). Therefore, the assumption was made that charge-complex formation between the coenzyme, I, and the drug could form the basis for the pharmacological action. When a solution of riboflavin, the parent moiety of the flavins, was frozen in the presence of chlorpromazine, the solution turned greenish brown and lost all fluorescence, an indication of complex formation. In subsequent studies, the phenothiazines did not possess the exceptional electron-donating ability formerly attributed to them. It was proposed that the tranquilizing action of the drugs might be due to the "ability of the S atom to form complexes with the localized electrons" (5).

The isoalloxazine moiety of the flavin coenzymes constitutes a reversible redox system (Scheme I). The redox activity of I and II has been extensively studied by several techniques, and their electrochemical behavior has been well established (6–8). The effect of pH on the reduction potentials and the tendency of the compounds to be adsorbed on electrode surfaces have also been well documented (9). Complex formation between I and iron(III) was studied by polarography and chronopotentiometry (10).

Any correlation between the processes involved in intact biological redox systems and those occurring in electrolytic cells must, of necessity, be made with due caution. Nevertheless, it is reasonable to believe that useful information might be gained from the electrochemical behavior of some of these compounds of biological significance. As pointed out previously (11), several points of similarity between the two processes seem to permit an evaluation of the biological processes on the basis of the electrochemical reactions.

The purpose of this study was to utilize electrochemistry to determine whether any interaction between the phenothiazine drugs and the flavin coenzymes would be reflected in changes in the electrochemical behavior of the latter. Such a study would help elucidate the mechanism by which the drugs produce their pronounced tranquilizing effect. Since electron-transfer processes are involved, electrochemical methods could be a direct approach. Furthermore, since the phenothiazines undergo electrochemical oxidation in solutions ranging from pH 1.5 to 9.5 (12, 13), interference due to their electroactivity was not anticipated during electrolytic reductions.

EXPERIMENTAL

Apparatus and Equipment—Polarographic experiments were performed with a polarograph[1] and a suitable recorder. The polarographic H-cell had a conventional dropping mercury electrode as the working electrode and a mercury–mercurous chloride reference electrode. The dropping mercury electrode had an open circuit drop time of about 3.65 sec in 0.1 *M* potassium chloride at a column height of 60 cm and a mercury flow rate of 1.25 mg/sec.

Chronopotentiometric measurements were made with an instrument[2] equipped with a high speed recorder, with chart speeds ranging from 100 to 1 sec/2.54 cm (1 in.). The controlled current was checked by measuring with a potentiometer[3] the voltage drop across a precision resistor in series with the electrolysis cell.

The electrolysis cell consisted of a glass cylinder fitted with a stopcock at the bottom to permit easy drainage. An outer glass jacket connected to a constant-temperature bath provided for the circulation of water to maintain the temperature at 25°. The cover[4] had openings for introducing the electrodes and provided an inlet for the nitrogen used to deaerate the solutions.

A conventional three-electrode assembly was used for the chronopotentiometric measurements. A mercury pool electrode, consisting of about 1 ml of mercury contained in a cup[4] and calculated to have an area of 2.75 cm^2, was the working electrode. A saturated calomel electrode and a platinum electrode (supplied with the chronopotentiometer) were used as the reference and counter electrodes, respectively. The counter electrode was placed in a separate compartment and connected to the main cell by a sintered-glass disk. The solutions to be analyzed were placed in both compartments.

Chemicals and Reagents—All chemicals used as supporting electrolytes and for the preparation of buffer solutions were of reagent grade. The mercury used in preparing the pool electrode and the dropping mercury electrode was triple distilled and of high purity. Prepurified nitrogen was used for degassing the solutions.

Compounds I and II[5] were reportedly of high purity and were used without further purification. Chlorpromazine hydrochloride and trifluoperazine hydrochloride[6] were also of high purity.

Procedure—Solutions to be analyzed were prepared with double-distilled water. In all experiments, solutions of I and II were made 10^{-4} *M* in phosphate or phosphate–citrate buffers to which the appropriate supporting electrolytes were added. In experiments involving the phenothiazines, the analytical amounts of chlorpromazine hydrochloride and trifluoperazine hydrochloride were added to the coenzyme solutions. These solutions were prepared in pH 6.5 phosphate buffer, since the drugs exhibited maximum solubility at this pH and this value was close to the physiological pH range.

Solutions of potassium chloride, potassium nitrate, ammonium chloride, tetrabutylammonium chloride, tetraethylammonium chloride, and tetrabutylammonium bromide were used as supporting electrolytes in the different experiments. To maintain the alkylammonium salt concentrations at 0.1 *M* throughout a series of experiments, potassium chloride was added to adjust the ionic strength where necessary.

All solutions were freshly prepared daily, deoxygenated with prepurified nitrogen, and prewashed by passage through a gas washing bottle containing distilled water. Due to the reported tendency of the phenothiazines to form complexes with elemental oxygen, the degassing periods were extended to between 20 and 30 min for each run for both the polarographic and chronopotentiometric experiments.

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[1] Sargent model XV.
[2] Electroscan 30 electroanalytical system, Beckman Instruments, Inc.
[3] Leeds & Northrup 7553-5 type K-3 Universal potentiometer.
[4] Lined with Teflon (du Pont).
[5] Nutritional Biochemicals Corp., Cleveland, Ohio.
[6] Courtesy of SmithKline Corp., Philadelphia, Pa.

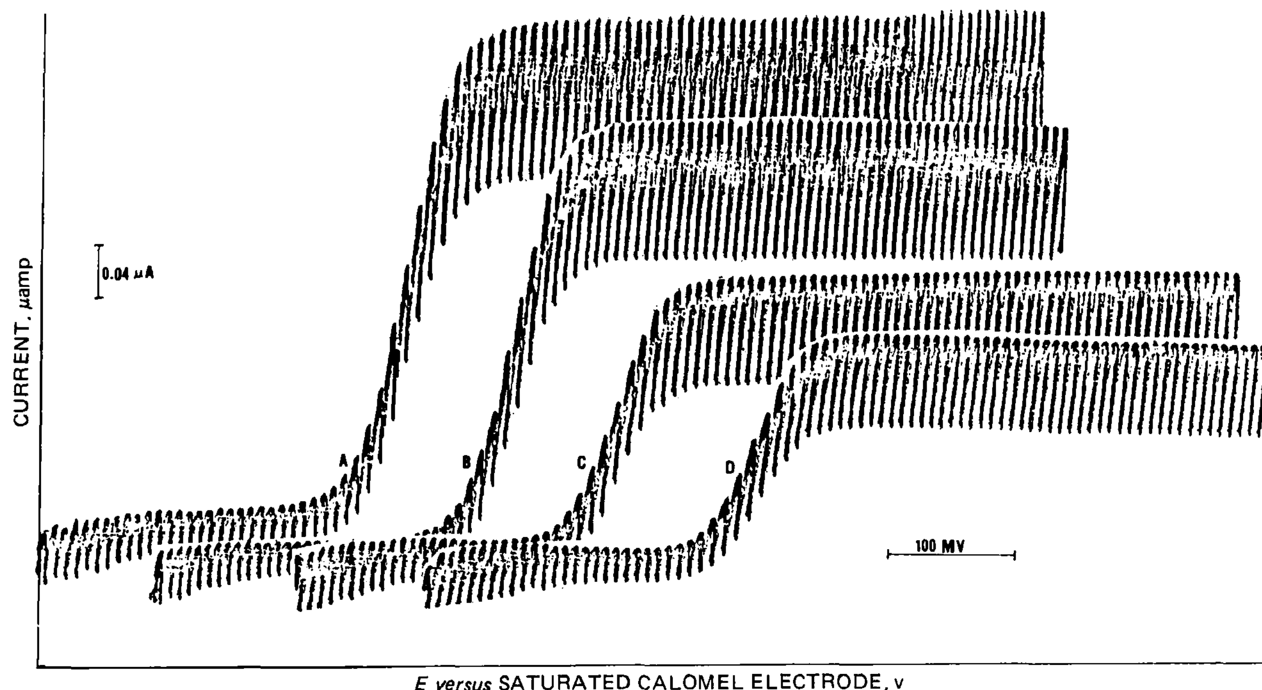

Figure 1—*Effect of chlorpromazine hydrochloride on polarography of 10^{-4}* M *II. Key: A, no chlorpromazine; B, with 10^{-2}* M *chlorpromazine; C, with 10^{-1}* M *chlorpromazine; and D, with 2×10^{-1}* M *chlorpromazine. All waves start at −0.15 v.*

As an added precaution, the coenzyme solutions were degassed prior to drug addition and then the additional degassing process was continued. Furthermore, because of the possibility of adsorption of the coenzymes onto the mercury electrode, the solutions were constantly agitated between runs and sufficient time was allowed for the mercury pool potential to return to its initial value after each run. To avoid photolytic decomposition of the coenzymes, all preparations and experiments were carried out with minimum light.

RESULTS AND DISCUSSION

Polarographic Behavior of Coenzymes—Polarographic reduction of I and II at the dropping mercury electrode gave results similar to those reported previously (6–13). Both compounds showed single well-defined polarographic waves in solutions buffered to pH 6–8 with all supporting electrolytes at 25°. The half-wave potentials, $E_{1/2}$, for both compounds at pH 6.5 were observed between −0.42 and −0.45 v *versus* the saturated calomel electrode. The pH dependence of the reductions was evidenced by an increase in the $E_{1/2}$ values as the pH increased from 6.0 to 8.0, ranging from about −0.42 to −0.50 v for I and from −0.40 to −0.52 v for II. At pH 2.2, the polarograms exhibited two reduction waves, thus supporting the evidence for an adsorption-controlled prewave and a diffusion-controlled main wave in acidic solutions. The prewave appeared between −0.05 and −0.10 v, while the primary wave was observed between −0.15 and −0.20 v for both compounds.

The diffusion currents for the I systems were significantly higher than those for the II systems of the same composition. When the tetraalkylammonium salts were used as supporting electrolytes, the diffusion currents for both compounds were somewhat lower than those observed with the other electrolytes such as potassium chloride, potassium nitrate, and ammonium chloride.

Chronopotentiometric Behavior of Coenzymes—Chronopotentiograms obtained upon reduction of the coenzymes at the mercury electrode showed single well-defined waves in solutions buffered to pH 6–8 at 25°. The apparent conformity of the systems to the diffusion control model was reflected in the constancy of the $i_0\tau^{1/2}$ values at the applied current densities, with transition times ranging from above 20 to below 0.5 sec. Good reproducibility was obtained when the solutions were degassed for 20–30 min and sufficient time was allowed for the system to return to its equilibrium potential after each run. As with the polarographic experiments, the observed $i_0\tau^{1/2}$ values decreased with the tetraalkylammonium salts as compared with those obtained with potassium chloride and other ionic electrolytes.

The pH dependence of the reductions also was apparent from the chronopotentiometric results. The $E\tau/4$ values (corresponding roughly to the $E_{1/2}$ values) for I ranged from about −0.40 to −0.48 v *versus* the saturated calomel electrode for solutions buffered between pH 6.0 and 8.0. The values for similar II systems ranged from −0.39 to −0.47 v. At pH 2.2, the potential for both reductions was about −0.19 v.

Reversible chronopotentiometric waves were observed for both compounds. At pH 6.5, the ratio of the forward cathodic to the reverse anodic transition time, τ_f/τ_r, was about 3, in accordance with the value expected for a completely reversible system. This relationship between the forward and reverse transition times supported the absence of any kinetic complications resulting from coenzyme adsorption onto the electrode surface. By careful control of experimental conditions, it was possible to get good reproducibility and no interference due to adsorption.

Effect of Chlorpromazine Hydrochloride and Trifluoperazine Hydrochloride on Polarography of I and II—The pronounced

Table I—Polarographic Data Showing Effects of Drugs on I Reduction

System[a]	i_d, μamp	$E_{1/2}$, v
10^{-4} *M* I	0.422	0.44
10^{-4} *M* I plus 10^{-3} *M* chlorpromazine[b]	0.412	0.44
10^{-4} *M* I plus 5×10^{-3} *M* chlorpromazine	0.400	0.44
10^{-4} *M* I plus 10^{-2} *M* chlorpromazine	0.345	0.44
10^{-4} *M* I plus 5×10^{-2} *M* chlorpromazine	0.241	0.42
10^{-4} *M* I plus 10^{-1} *M* chlorpromazine	0.205	0.40
10^{-4} *M* I plus 10^{-3} *M* trifluoperazine[c]	0.412	0.44
10^{-4} *M* I plus 5×10^{-3} *M* trifluoperazine	0.375	0.42
10^{-4} *M* I plus 10^{-2} *M* trifluoperazine	0.341	0.41
10^{-4} *M* I plus 5×10^{-2} *M* trifluoperazine	0.235	0.25
10^{-4} *M* I plus 10^{-1} *M* trifluoperazine	0.203	0.23

[a] Tetraethylammonium chloride was the supporting electrolyte. [b] Hydrochloride salt. [c] Dihydrochloride salt.

Table II—Polarographic Data Showing Effects of Drugs on II Reduction

System[a]	i_d, μamp	$E_{1/2}$, v
10^{-4} *M* II	0.304	0.43
10^{-4} *M* II plus 10^{-3} *M* chlorpromazine	0.310	0.43
10^{-4} *M* II plus 5 × 10^{-3} *M* chlorpromazine	0.299	0.43
10^{-4} *M* II plus 10^{-2} *M* chlorpromazine	0.268	0.43
10^{-4} *M* II plus 5 × 10^{-2} *M* chlorpromazine	0.196	0.41
10^{-4} *M* II plus 10^{-1} *M* chlorpromazine	0.165	0.40
10^{-4} *M* II plus 10^{-3} *M* trifluoperazine	0.296	0.44
10^{-4} *M* II plus 5 × 10^{-3} *M* trifluoperazine	0.294	0.43
10^{-4} *M* II plus 10^{-2} *M* trifluoperazine	0.271	0.42
10^{-4} *M* II plus 5 × 10^{-2} *M* trifluoperazine	0.188	0.25
10^{-4} *M* II plus 10^{-1} *M* trifluoperazine	0.156	0.22

[a]Tetraethylammonium chloride was the supporting electrolyte.

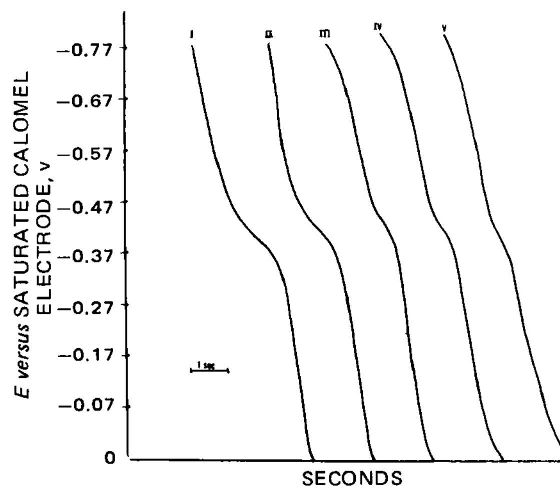

Figure 2—*Effect of chlorpromazine hydrochloride on chronopotentiometry of 10^{-4}* M *II. Key: I, with 10^{-4}* M *chlorpromazine; II, with 10^{-3}* M *chlorpromazine; III, with 10^{-2}* M *chlorpromazine; IV, with 5 × 10^{-2}* M *chlorpromazine; and V, with 10^{-1}* M *chlorpromazine.*

surface activity of the phenothiazines made it impossible to carry out the experiments using ordinary ionic electrolytes. Excessive foaming during the degassing period resulted in loss of solution from the electrolysis cell. For this reason, the tetraalkylammonium salts were used; they were reported (14) to cause marked reduction in the surface activity of the phenothiazines. Several of these cationic agents were used as supporting electrolytes, and the tetraethyl- and the tetrabutylammonium chloride salts were equally effective in reducing surface activity. Figure 1 shows that with various amounts of chlorpromazine hydrochloride added, well-defined polarograms with no apparent distortions due to adsorption were obtained, and it was possible to get adequate reproducibility.

The results shown in Tables I and II indicate that, even at high concentrations of drug, chlorpromazine hydrochloride had little or no effect on the reduction potential for either I or II. On the other hand, with concentrations of trifluoperazine hydrochloride in excess of 10^{-2} *M*, a reduction in the $E_{1/2}$ for both coenzymes was observed. This result was probably due to the high acidity of the drug, resulting in a decrease in the pH of the system from approximately pH 6.5 to 3.0. The effect of pH on the reduction potential of the coenzymes has already been established. However, even at this lower pH, there was no evidence of the adsorption prewave observed earlier when the coenzymes were run in more acidic solutions.

That the diffusion currents for both coenzymes showed a progressive decrease as the concentration of the added drugs increased is clearly indicated in Tables I and II and Fig. 1. With the addition of either drug at 0.10 *M*, the diffusion currents decreased to approximately one-half of the original values obtained in the absence of the drugs. Since the direct proportionality between concentration and i_d had clearly been established in preceding experiments, these results could only be interpreted to mean that the amount of the coenzymes available for electroreduction at the dropping mercury electrode was greatly reduced in the presence of large amounts of the drugs.

Effect of Chlorpromazine Hydrochloride and Trifluoperazine Hydrochloride on Chronopotentiometry of I and II—It was more difficult to obtain reproducible results in the chronopotentiometric experiments than in the polarographic studies. Some distortion of the chronopotentiometric waves was observed unless the systems were degassed for prolonged periods and sufficient time was allowed for equilibration. The tetrabutylammonium salts were most effective in controlling the surface activity of the drugs and in obtaining reproducible results. The chronopotentiometric data of Table III and the potential–time curves of Figs. 2 and 3 clearly show the effect of the added drugs.

From the data, it was once again apparent that chlorpromazine exerted little or no influence on the reduction potential for II even at high drug concentrations. There was, however, clear evidence of a progressive decrease in the calculated $i_0\tau^{1/2}$ values in the presence of increased amounts of the drug (Fig. 2). At the same applied current density, the transition time for the reduction of II showed a decrease as the concentration of added chlorpromazine increased. This finding indicates once again that the amount of II available for reduction at the electrode surface becomes progressively less as the added amount of the drug increases. Although the apparent decrease in the concentration of II observed in the chronopotentiometric experiments was not as pronounced as observed with the polarographic determinations, there was indeed some effect.

Close scrutiny of the chronopotentiograms of Fig. 2 also reveals slight evidence of what would appear to be a prewave at very high concentrations of chlorpromazine. This prewave could possibly be considered as evidence of some drug adsorption onto the mercury

Table III—Chronopotentiometric Data Showing Effects of Chlorpromazine on II Reduction

System Composition[a]	τ Range, sec	Average $i_0\tau^{1/2}$, amp sec$^{1/2}$ cm^{-2} × 10^4 [b]	$E_{\tau/4}$, v
10^{-4} *M* II	13.98–0.63	0.530 ± 0.018	0.42
10^{-4} *M* II plus 10^{-4} *M* chlorpromazine	12.60–0.35	0.505 ± 0.038	0.41
10^{-4} *M* II plus 10^{-3} *M* chlorpromazine	10.24–0.35	0.512 ± 0.029	0.43
10^{-4} *M* II plus 10^{-2} *M* chlorpromazine	7.09–0.26	0.403 ± 0.037	0.42
10^{-4} *M* II plus 10^{-1} *M* chlorpromazine	11.42–0.25	0.303 ± 0.009	0.40
System Composition[c]			
10^{-4} *M* II	16.53–0.79	0.449 ± 0.023	0.44
10^{-4} *M* II plus 10^{-3} *M* chlorpromazine	12.60–0.39	0.434 ± 0.021	0.40
10^{-4} *M* II plus 10^{-2} *M* chlorpromazine	14.17–0.35	0.382 ± 0.029	0.41
10^{-4} *M* II plus 10^{-1} *M* chlorpromazine	10.24–0.98	0.293 ± 0.010	0.41

[a]Tetrabutylammonium chloride was the supporting electrolyte. [b]Uncertainties are based on 95% confidence intervals. [c]Tetrabutylammonium bromide was the supporting electrolyte.

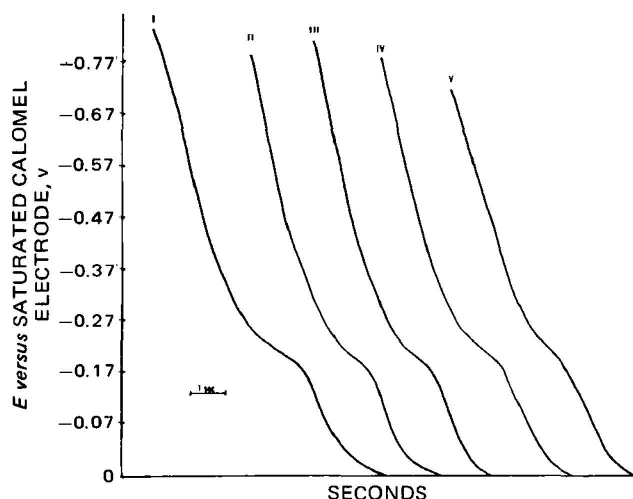

Figure 3—*Effect of 10^{-1}* M *trifluoperazine hydrochloride on chronopotentiometry of II. Current density was increased from I to V.*

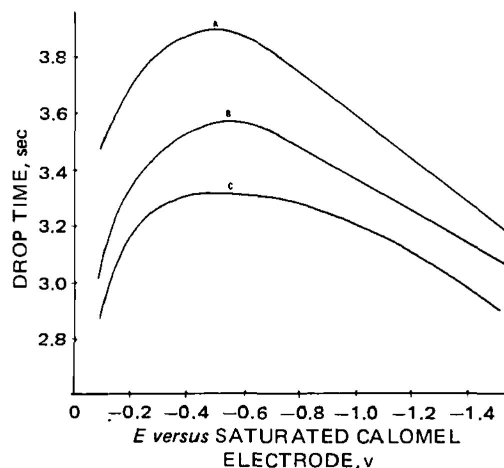

Figure 5—*Effect of trifluoperazine hydrochloride on the electrocapillary curve for II in 0.1* M *tetrabutylammonium bromide and pH 6.5 phosphate buffer. Key: A, 10^{-4}* M *II; B, with 10^{-2}* M *trifluoperazine; and C, with 10^{-1}* M *trifluoperazine.*

electrode surface, thus causing interference with the normal single-wave reduction for II at this pH. However, if adsorption is primarily responsible for the observed decreases both in the transition times in chronopotentiometry and the diffusion currents in polarography, then the evidence for such adsorption should be significantly more pronounced.

Investigation of possible adsorption of the phenothiazines onto the mercury electrode was carried out by preparing electrocapillary curves for the systems in the presence of the drugs at several different concentrations. For clarity, only a few of the electrocapillary curves are shown in Figs. 4 and 5. The curves of Fig. 4 show that neither II nor II with added chlorpromazine appreciably altered the electrocapillary curve, A, for 0.1 *M* tetraethylammonium chloride in pH 6.5 phosphate buffer. There was no obvious shift in the electrocapillary maximum; and although there was slight lowering of the surface tension of the mercury drop with increasing amounts of added chlorpromazine, as reflected in the decrease in drop time, the decrease was so small that the amount of adsorption indicated could not conceivably account for the changes observed in the polarographic and chronopotentiometric experiments. Moreover, the curve for II alone gives no indication of the reportedly strong adsorption of the coenzymes observed in highly acidic solutions.

That adsorption becomes an important factor when trifluoperazine is added to the coenzymes is evident from the electrocapillary curves of Fig. 5. Not only was there an observed decrease in the surface tension of the mercury drop, but the electrocapillary maximum disappeared and the curve flattened out over a span of potentials at 10^{-1} *M* trifluoperazine. This surface activity was observed in earlier chronopotentiometric experiments, and it was considered advisable to increase the tetrabutylammonium salt concentration used as the supporting electrolyte to 0.15 *M* to minimize this effect. Even with this increase, however, it was difficult to obtain reproducible results in the presence of high drug concentrations.

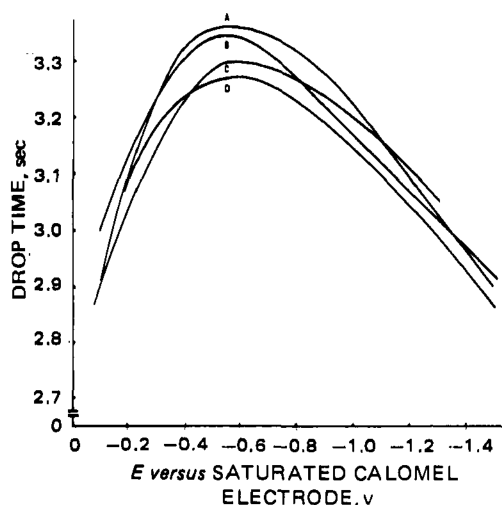

Figure 4—*Effect of II and chlorpromazine hydrochloride on the electrocapillary curve for 0.1* M *tetraethylammonium chloride in pH 6.5 phosphate buffer. Key: A, normal curve; B, with 10^{-4}* M *II; C, with 10^{-4}* M *II and 10^{-2}* M *chlorpromazine; and D, with 10^{-4}* M *II and 10^{-1}* M *chlorpromazine.*

Figure 3 shows some chronopotentiograms obtained for II upon the addition of 10^{-1} *M* trifluoperazine. As with the polarographic experiments, the reduction potential for II was lowered considerably when large amounts of the drug were added. Lowering of the pH was again believed to be the cause of this decrease in the $E_{\tau/4}$ value.

The shape of the chronopotentiograms (Fig. 3) made it virtually impossible to make any meaningful transition time measurements at this high drug concentration. Even at concentrations of trifluoperazine below 10^{-2} *M*, the curves showed some distortions. Had there been a clear indication of more than one chronopotentiometric wave, then perhaps measurements could have been made and some interpretations proposed. The curves were, however, similar to those observed at the higher concentrations, only somewhat less distorted.

Due to the similarity in the electrochemical behavior of I and II as observed in the polarographic experiments, even with the added drugs, it was considered unnecessary to repeat all of the chronopotentiometric experiments on the I systems. It seemed advantageous, however, to verify some results at the highest concentrations of both chlorpromazine and trifluoperazine. Upon the addition of 0.10 *M* chlorpromazine, the $i_0\tau^{1/2}$ value for 10^{-4} *M* I in tetrabutylammonium chloride decreased from 0.564×10^{-4} to 0.294×10^{-4} amp sec$^{1/2}$ cm^{-2}. In the presence of trifluoperazine, a decrease in the reduction potential for I was observed, as were the distortions of the chronopotentiometric waves obtained with II.

Current reversal chronopotentiometry carried out on solutions of the coenzymes containing the drugs resulted in well-defined reversible waves similar to those previously obtained for the coenzymes alone. However, the ratio of the forward to the reverse transition times was in all cases greater than that obtained with the former systems. Instead of being of the order of 3, as was found for the coenzymes alone, the ratio increased to values between 5 and 6, indicating that all reduced material was not being reoxidized at the electrode surface.

Since the defined purpose of this study was to determine whether electrochemical methods could provide evidence of the effect of the phenothiazine drugs on the electrochemical behavior of the coenzymes, the results obtained indicate that this purpose has to some extent been achieved. The role of adsorption as a conflicting factor in the case of the highly surface-active trifluoperazine must be recognized, but the evidence for adsorption in the case of chlorpromazine is virtually nonexistent. Therefore, changes in the measured quantities

observed upon the addition of the latter drug to the coenzymes must be attributed to an interaction between the two species.

Whether this interaction provides a basis for the pharmacological activity of the drug is, of course, mere speculation at this stage. Obviously, further and more detailed studies are needed, utilizing other phenothiazines and employing other techniques. The main thrust of the investigation at this point is that electrochemical methods can and do provide a direct and feasible approach to the study of the possible interactions between the drugs and the coenzymes; further studies should lead to the acquisition of some definite knowledge in this area.

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Polymerized Micelles and Their Use as Adjuvants in Immunology

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Abstract ☐ A polymerization process is described for the preparation of hydrophilic micelles containing solubilized drug molecules in a colloidal aqueous system of dissolved monomers. A hydrocarbon medium constitutes the outer phase. After secondary solubilization with the aid of selected surfactants, polymerization of micelles under different conditions takes place. The formulation of drugs including labile proteins in the ultrafine polymer components ("nanoparts") and their isolation are possible without noticeable destruction of the encapsulated molecules. Entrapped tagged material (human ^{125}I-immunoglobulin G) shows a stable fixation in such nanoparts during long-term *in vitro* liberation trials. Nanoparts are visible with the aid of an electron microscope. They are mostly spherical in shape and smaller than 80 nm in diameter. They form real colloidal aqueous solutions. Nanoparts are suitable to embed antigenic material (tetanus toxoid and human immunoglobulin G) for parenteral use. These preparations show intact biological activity and high antibody production in animals.

Keyphrases ☐ Polymerization—hydrophilic micelles containing drug molecules (nanoparts), use as immunological adjuvants ☐ Micelles, hydrophilic—containing drug molecules, polymerization, use as immunological adjuvants ☐ Nanoparts—hydrophilic micelles containing drug molecules, polymerization, use as immunological adjuvants ☐ Adjuvants, immunological—hydrophilic micelles containing drug molecules, polymerization ☐ Immunological adjuvants—hydrophilic micelles containing drug molecules, polymerization

"Nanoparts" are solidified micelles containing drugs; they are spherical particles of nontoxic polymeric material with entrapped bioactive materials. The extremely small diameter of nanoparts, smaller than 80 nm, is measured by electron scanning microscopy. The lower limit for the particle diameter ranges between 20 and 35 nm, as shown by ultrafiltration with special diaphragms of an average pore size between 20 and 50 nm.

These ultrafine capsules or polymer pellets represent a narrow distribution in particle size. The electron scanning photographs show an aggregation of homogeneous, spherical particles of nearly the same shape and size, being small enough to give colloidal aqueous solutions.

According to polarity, dielectric constant, and steric effect of the reactants, a more or less shell-like polymer construction ("nanocapsule") or a compact polymer particle ("nanopellet") can be proposed (Scheme I). The minuteness of the nanoparts and the homogeneity of the final product also enable the formulation of parenteral drug delivery systems.

EXPERIMENTAL

Synthesis—Water-soluble polymerizable monomers and the enclosure material, *e.g.*, the biologically or pharmacologically active agent, are dissolved in water to form a true, or at least a colloidal, solution. This solution is distributed with the aid of surfactants by stirring in a hydrophobic phase (n-hexane) in which the precursors and the drugs are practically insoluble. Minute aqueous micelles, containing the polymerizable monomers, drugs, and possibly other auxiliary agents, are thus solubilized in a relatively large volume of the lipophilic outer phase and form extremely small reaction regions for the ensuing polymerization. The polymerization is induced by known methods (1–7).

The mechanism of micelle polymerization is definitely different from emulsion polymerization. In most cases, mainly in micellar reaction regions, water-insoluble monomers polymerize in water during emulsion polymerization. Here, the radical-containing polymerizing regions may swell to many times their original size. This swelling is due to diffusion of monomers from a stock of monomer droplets in the emulsion into growing latex polymer particles (8, 9). In this process, polymerization is strictly restricted to the micellar regions, since