DC Polarographic Reduction of Chloroguanide Hydrochloride

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Abstract □ Chloroguanide hydrochloride, an antimalarial drug, shows one well-defined DC polarographic wave in Britton–Robinson buffered media. In the pH range 3–9 the observed reduction wave is related to the reduction of the two azomethine centers on the monoprotonated biguanide group (BH^+). The effects of pH and other experimental variables on the limiting current and half-wave potentials as well as the reduction mechanism are discussed.

Keyphrases □ Polarography—proguanil hydrochloride, effect of pH □ Proguanil hydrochloride—DC polarographic reduction

The different pharmacological and physical properties of each biguanide derivative depend on the chemical nature of the substituent radicals joined to the biguanide group. Nevertheless, in addition to its chemical properties, the specific applications for biguanides also depend on the solubility and surface-active characteristics (1–4).

The present study concerns results which have been obtained with the antimalarial (5) and antirheumatic (6–10) substance, chloroguanide hydrochloride [1-(*p*-chlorophenyl)-5-isopropylbiguanide hydrochloride] in Britton–Robinson media.

The general difficulties encountered in reducing the biguanide group of other monoprotonated biguanides on the dropping mercury electrode (11, 12) are associated with the great delocalization of its π-electronic system, as is the case of guanidine derivatives (13) since the π electrons are delocalized over the entire area of this monoprotonated or basic group. Under these circumstances, the particular behavior of chloroguanide should be associated with the configuration of its biguanide group. The X-ray studies have demonstrated, for this monohydrochloride in solid form, ~60° angle between the two "guanidine planes" (14), whereas this angle is substantially lower for other biguanides (15, 16). This leads us to speculate that chloroguanide shows a polarographic wave, like chlorhexidine (17–19), that is independent of the guanidine planes. The angle is larger than that in other cationic biguanides that do not show polarographic waves having the characteristics (12, 20) described in this work.

EXPERIMENTAL SECTION

The polarographic waves were obtained with a polarograph[1]. A digital pH meter[2] with a combined glass-reference electrode was utilized which enabled us to attain a pH precision of 0.01 pH unit. Thermostabilization was achieved with a ±0.1° C precision, using a cryostat[3] and a thermostat[4]. In the three-electrode coulometric cell, the working cathode was a mercury pool, the auxiliary electrode was platinum, and the reference electrode was Ag/AgCl, $KCl_{(s)}$.

The chloroguanide hydrochloride was used as supplied[5], mp 238 ± 2° C, and showed one spot on TLC. The UV and IR spectra were obtained with spectrophotometers[6]. Nonaqueous tritration in acetic media (21) was used to confirm the purity. The chemicals used to prepare the buffered solutions were analytical reagent grade; water was distilled and the mercury was double-distilled.

The polarographic measurements were carried out on freshly prepared samples in a total volume of 25 mL. Purified N_2 was passed through all sample solutions (7 min) for deaeration. Other polarographic conditions were: mercury flow, m = 0.88 ± 0.02 mg·s^{-1}; controlled drop time, τ = 0.6 s; depolarizer concentration (chloroguanide hydrochloride), $C = 4 \times 10^{-4}$ M; temperature, T = 298 K; and Britton–Robinson buffer mixture, I = 0.5 M. The diffusion current (i_d) was measured as shown in Fig. 1.

RESULTS AND DISCUSSION

Chloroguanide is electroactive in Britton–Robinson buffered solutions over the pH range studied, 3–9. The polargrams show one reduction wave (Fig. 1).

The drop time effect on the cathodic waves (Table I) is shown by slopes $\Delta E_{1/2}/\Delta \ln \tau = 22 \pm 2$ mV and $\Delta \ln i_l/\Delta \ln \tau = 0.18 \pm 0.02$, two characteristics of the diffusion-controlled processes (22). Also, the effects of both the mercury pressure and chloroguanide concentration on the diffusion current of the wave shows that this current appears to be diffusion controlled at every pH examined. The limiting current intensity remains essentially constant at pH 3.5–6.5 and decreases at pH > 6.5 (Fig. 2). The decreasing current intensity has the form of a dissociation curve with an apparent polarographic pK_s = 8.3 [pK_s = pH, where $i_l = i_d/2$ (23)]. To verify the pK_a value, a spectrophotometric study (24) of this compound was performed: pK_{a1} = 2.3 ± 0.1 and pK_{a2} = 10.4 ± 0.2. The pK_s value is apparently affected by the double layer structure. Due to this change, the overall absorptivities of the protonated and unprotonated forms are different. From the dependence of the half-wave potentials of quasi-diffusion kinetic surface waves on pH (Fig. 2), the K_a values cannot be determined directly, as was possible for quasi-diffusion kinetic volume waves (25).

The number of electrons involved in the electroreduction process at different pH values have been estimated by substituting the values of c, τ, m, and the x conductometric coefficient of diffusion, D° [5×10^{-6} cm^2·s^{-1} (26)] in the Ilkovic equation (22). In moderately acidic media the height of the cathodic

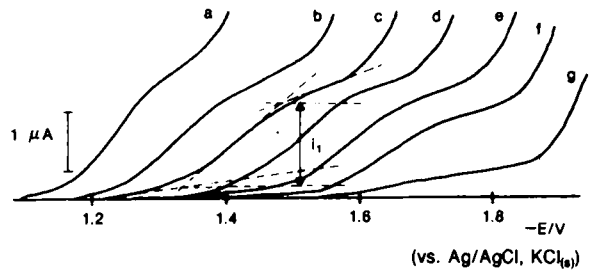

Figure 1—*DC polarograms of chloroguanide in Britton–Robinson buffer at pH 3.01 (a), 3.99 (b), 4.88 (c), 5.75 (d), 6.76 (e), 7.53 (f), and 8.55 (g). Conditions:* $C = 4 \times 10^{-4}$ *M; I = 0.5 M;* T = *298 K; m = 0.88 mg·s*$^{-1}$*;* τ = *0.6 s.*

Table I—Effect of the Drop Time (τ) on the Wave [a]

τ, s	i_l, μA	$-E_{1/2}$, mV
0.4	1.65	1503
0.6	1.75	1489
0.8	1.91	1482
1.0	2.06	1476
1.2	2.10	1470
1.4	2.24	1469
2.0	2.38	1460
3.0	2.54	1449

[a] pH 5.78. Conditions: depolarizer concentration, $C = 4 \cdot 10^{-4}$ M; temperature, T = 298 *K*; mercury flow, m = 0.88 mg·s^{-1}; ionic strength, I = 0.5 M.

[1] Polarecord E-506; Metrohm, S.A.
[2] Radiometer.
[3] Heto 03T623.
[4] Heto 05E623.
[5] Paludrine; I.C.I. Farma S.A.
[6] Model SP-8-11, Pye Unicam was used to obtain UV spectra and a Model 281; Perkin-Elmer was used for IR spectra.

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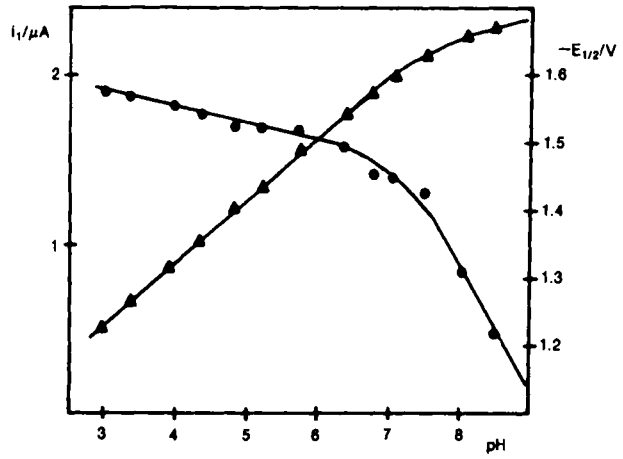


Figure 2—*pH-Dependence of limiting current and half-wave potentials. Key: (●) i_l; (▲) $E_{1/2}$. (Conditions: see Fig. 1).*

wave corresponds to the uptake of 4 electrons. Controlled-potential coulometry with a mercury pool electrode, performed at a controlled-applied potential corresponding to the plateau of the polarographic curve, verified a consumption of 4 electrons per molecule at all values of pH studied.

Plotting $E_{1/2}$ of the wave *versus* pH in the 3-7 range (Fig. 2) gives a straight line of slope, $\Delta E_{1/2}/\Delta pH = 93 \pm 2$ mV. From this slope, the number of H^+ ions (z_{H^+}) in the rate-determining step can be calculated using the following equation (27):

$$z_{H^+} = \frac{\alpha n_a \Delta E_{1/2}}{59 \Delta pH} \qquad \text{(Eq. 1)}$$

By substituting the value of αn_a, obtained from the slope of log $(i/i_l - i)$ *versus* $-E$ plots, the z_{H^+} values are ~1.0 (Table II). The most probable number of electrons (n_a) in the rate-determining step is one electron.

The addition of dimethylformamide to chloroguanide solutions decreases the absolute value of $E_{1/2}$ (Table III). This shows the strong influence exerted by the Helmholtz double layer around the electrode on the cathodic mechanism. The addition of gelatin in amounts $<6 \times 10^{-3}$ % by weight prevents registration of these waves. The electrocapillary curves (τ *versus* E) shows a slight adsorption of the monoprotonated chloroguanide on the dropping mercury electrode (DME) at the wave potentials (18). The wave has a surface character.

The ionic strength decreases the i_l values and causes displacement of the $E_{1/2}$ to more negative values (Table IV), whereas the effect of the decreased buffering capacity (Table V) is lower than that produced by variation of the ionic strength.

On the other hand, the energies of activation, Q, which are calculated by the following equation (28):

$$i_d = Ae^{-Q/RT} \qquad \text{(Eq. 2)}$$

are higher than typical for diffusion-controlled processes. Therefore, the value at pH 5.34 is $Q = 3.8 \pm 0.1$ kcal·mol^{-1} and at pH 7.11, the value is $Q = 4.1 \pm 0.1$ kcal·mol^{-1}. This slight dependence on pH values coincides with an increased irreversibility when pH is increased (Fig. 1). The $E_{1/2}$ values remain nearly constant at pH 7.11, when T is varied from 0°C to 50°C, which should be related to the fact that the first single electron transfer is not exceedingly slow.

As Hollek (29) has indicated, the adsorption of a depolarizer or a reaction product on the DME is a potential cause for deviation of the $\Delta E_{1/2}/\Delta pH$ value from −60 mV/pH unit. Therefore, although the $\Delta E_{1/2}/\Delta pH$ surface waves

Table II—Polarographic Data for Chloroguanide [a]

pH	i_l, μA	$-E_{1/2}$, mV	$n_a\alpha$	Number of H^+ ions, z_{H^+}
3.01	1.85	1224	0.86	1.33
3.99	1.83	1307	0.65	1.01
4.88	1.69	1400	0.60	0.93
5.75	1.70	1493	0.64	0.99
6.73	1.43	1576	0.79	1.22
7.53	1.31	1630	0.72	1.12
8.55	0.45	1665	0.67	1.04

[a] Conditions: $\tau = 0.6$ s; others as described in Table I.

Table III—Effect on Cathodic Waves of Dimethylformamide in Britton-Robinson Buffer Mixtures [a]

Dimethylformamide, % (v/v)	i_l, μA	$-E_{1/2}$, mV	$n_a\alpha$
0.0	2.07	1538	0.78
0.1	2.05	1539	0.80
0.2	2.05	1539	0.81
0.3	2.07	1538	0.75
1.0	2.05	1531	0.69
2.0	1.98	1519	0.68
4.0	1.71	1488	0.88
8.0	1.80	1489	1.15

[a] Conditions: pH = 6.40; $\tau = 0.6$ s; $C = 5.6 \times 10^{-4}$ M; others as described in Table I.

Table IV—Effect of Ionic Strength on $E_{1/2}$ [a]

I,M	i_l, μA	$-E_{1/2}$, mV	$n_a\alpha$
0.5	2.13	1545	0.75
1.4	2.04	1598	0.79
1.9	1.70	1599	0.80
2.3	1.64	1602	0.78

[a] Conditions: pH = 6.45; $\tau = 0.6$ s; $C = 5.6 \times 10^{-4}$ M; others as described in Table I.

Table V—Effect of Buffering Capacity on Waves [a]

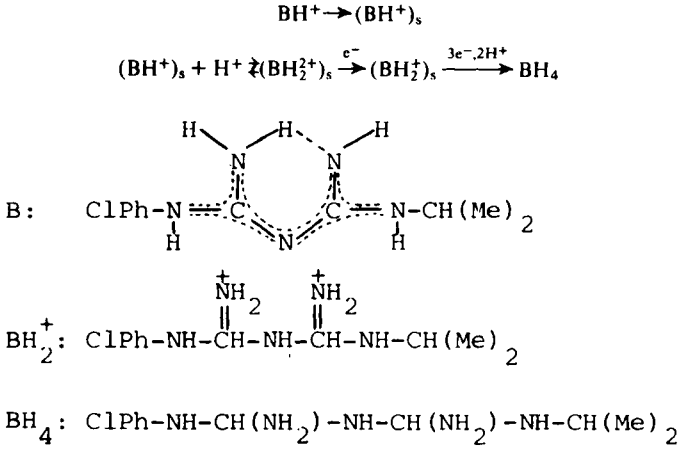
[acids], mol·L^{-1}	i_l, μA	$-E_{1/2}$, mV
0.10	2.13	1545
0.07	2.05	1560
0.05	2.00	1568
0.04	1.95	1569

[a] $\tau = 0.6$ s. Total concentrations: [acetic] = [boric] = [phosphoric]. Conditions: I = 0.5 M (with KCl); others as described in Table I.

are smaller than the slope of their plots on semilogarithmic coordinates, the absolute value of $\Delta E_{1/2}/\Delta pH$ for surface waves is larger than the $\Delta E_{1/2}/\Delta pH$ volume for surface waves. Since the rate of the previous surface reaction is sufficiently high in chloroguanide reduction, the i_l value of the kinetic wave is close to the diffusion intensity. Under these conditions, the wave has a well-defined limiting current plateau, and the wave height is determined by the Ilkovic equation. The wave is a quasi-diffusion surface wave.

The biguanide group reduction is made evident by UV spectroscopy, since the chloroguanide absorption $\pi \rightarrow \pi^*$ transition at 232 and 251 nm decreases when chloroguanide is fully reduced, and a single band with one maximum value (~256 nm) is registered. This means a decrease of the electronic delocalization, and a new transition ($n \rightarrow \pi^*$) appears with a maximum absorption value at ~290 nm. This is a typical example of a reduced substance obtained when the two azomethine groups have been transformed into the corresponding amine groups, as Schultz (30) confirmed with the chemical reduction of chloroguanide using a zinc amalgam.

Based on the experimental results, the reduction of the biguanide group is likely to take place as shown in Scheme I:



Scheme I

As stated earlier, this reduction seems to take place through an intermediate $(BH_2^+)_s$ which disappears quickly with the complete reduction of the double bonds at the potentials at which the polarographic wave is detected. The *p*-chlorophenyl substituent in chloroguanide seems to be transformed into a state of reduced electron delocalization with respect to the other biguanides. Perhaps as is the case in the solid state (14), the monoprotonated molecule-cation, (BH^+), which exists at physiological pH in solution, is present as a result of a certain independence of the guanidine groups, as occurs with other efficient antimalarial drugs which show the independent imine groups (31).

The polarographic method can be applied for the analytical determination of chloroguanide at pH 3-7 in buffered media. The plots of i_d *versus* C give linear calibrations at 10^{-5}–5×10^{-3} M concentrations. For example, at pH 5.01: $i_d(\mu A) = (4.25 \times 10^3) \cdot C\ (\text{mol} \cdot \text{L}^{-1})(SD = 5 \times 10^{-3})$.

The assay is rapid and has great sensitivity. The lowest sensitivity limit recorded is 0.05–0.1 $\mu g \cdot mL^{-1}$. At $\tau = 0.6$ s the limit of detection is less favorable than in conventional DC polarography because the ratio of charging current to faradic current increases when drop time decreases (32).

Any substance having a half-wave potential within the range of ±100 mV with respect to the $E_{1/2}$ of chloroguanide produces an overlapping of both waves. Also, the presence of gelatin, Triton X-100, or another strongly absorbable substance on the DME produces interferences since the chloroguanide electroreduction is a heterogeneous process.

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Design of a Slow-Release Capsule Using Laser Drilling

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Abstract □ Conventional hard gelatin capsules were made GI-tract resistant by formalin vapor treatment. The residual formalin content was 80 μg/capsule 24 h after treatment, which decreased with increased storage time. An *in vitro* GI-tract resistance test was performed by exposing the capsules to simulated gastric fluid for 4 h and then to simulated intestinal fluid for 4 h at 37°C. The resistance was further confirmed by *in vivo* X-ray studies in human volunteers. Minute pores were drilled on the hardened shells of the capsules with a carbon dioxide gas laser. This permitted the slow passage of the encapsulated tetracycline hydrochloride when subjected to 0.1 M HCl in *in vitro* dissolution studies. *In vitro* drug release from these capsules followed zero-order kinetics after an initial lag period of 30 min. The factors influencing the *in vitro* release rate of tetracycline hydrochloride from these capsules are discussed.

Keyphrases □ Gelatin capsules–drug release, GI-tract resistance, laser-drilled design □ Laser-drilled capsules–drug release, GI-tract resistance, X-ray studies

The potential application of the laser in the fields of communication, industry, military science, chemistry, biology, and medicine has been well established and well documented (1). The possible use of the laser in designing a controlled-release capsule dosage form has been recently reported (2). Theeuwes *et al.* (3) reported the use of automated laser drilling in making exit pores for indomethacin in the design of an elementary osmotic pump.

Conventional hard-gelatin capsules normally disintegrate rapidly and the encapsulated drug exhibits a cube-root dissolution pattern (4). Exposure to formalin vapors causes the cross-linkage of the gelatin molecule, resulting in an unpre-

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