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THE EFFECT OF PULSES AND OTHER EXPERIMENTAL PARAMETERS ON KALOUSEK
POLAROGRAPHIC WAVES OF CHLORHEXIDINE

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Summary

Pulse amplitude, frequency, drop time, concentration, buffer strength, ionic strength, pH and temperature effects on the morphology of chlorhexidine waves have been studied. It has been proved that the irreversibility of the waves decreases the height of polarograms with respect to the theoretical polarograms which should correspond in a reversible process. The cathodic and anodic values of the transfer coefficients are explained by means of an electrodic model in which the protonation steps are alternated with electronic transfer steps in the reduction and reoxidation of four azomethinic groups of the adsorbed protonated chlorhexidine.

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INTRODUCTION

Chlorhexidine (N,4 chlorophenil, N'hexil imido dicarbonimidic diamide) is a bisbiguanide that acts as bactericide which presents a hexamethylen $-(CH_2)_6-$ radical between two biguanide groups^(1,2). The flexibility and length of a hexamethylen radical, permit the molecule to fold and unfold with relative facility, changing its structure when the pH, temperature and composition of the medium in which it is dissolved are modified. This structural change is produced on a pH next to the physiological pH and was detected by UV spectroscopy and polarography^(3,4). On the other hand, Kalousek polarography is receiving attention in our laboratory as a reliable tool for characterizing electrode processes, due to its advantages over other polarographic techniques in sensitivity for reversible process, rapidity for determining this reversibility and reoxidation of electroactive species. The aim of this work is, firstly, to apply the Kalousek methods to the study of chlorhexidine which gives irreversible process by other polarographic methods.

EXPERIMENTAL

Chlorhexidine dihydrochloride from ICI Farma S.A. was used.

A Metrohm Polarecord E-506, with an Ag/AgCl, KCl (saturated) reference electrode, was used for the dc, K_1 , K_2 , and K_4 controlled

drop time techniques. The pH measurements were carried out by means of a pH-meter Radiometer PHM-62. The samples contain 8% DMF. The pH was controlled by Britton-Robinson buffer media.

The amplitude of Kalousek polarography was determined by the initial and final voltages of the step (E_{step} , $E_{\text{step}} + \Delta E$). For methods K_1 and K_2 the pulse base voltage (E_{pb}) was adjusted. For methods K_3 and K_4 the square-wave amplitude was fixed at 50 mV. In K_1 and K_3 , measurements were taken at the base of the pulses (anodic waves), while in K_2 and K_4 they were taken at the peaks of the pulses (cathodic waves). All other experimental conditions are described previously⁽⁷⁾.

RESULTS AND DISCUSSION

The chlorhexidine provides well-defined K_1 anodic waves, while by means of K_3 polarography only the dc component was recorded. Near to the neutral pH, K_2 cathodic waves present a slight depression in the diffusive zone. A cathodic peak superimposed to the dc component in K_4 polarography was recorded.

The morphology of K_1 and K_2 waves of chlorhexidine was affected by the applied pulse amplitude (Fig.1). K_1 waves present greater heights for base potentials corresponding to the dc diffusive zone. On the contrary, K_2 waves show higher current intensities for E_{pb} potentials corresponding to the dc residual zone (Fig.2). A qualitative relation between $E_{1/2}$ and pulse amplitude could not be established clearly (Table I) for the K_1 technique. For the K_2 technique, the peak potential remains constant for E_{pb} values

corresponding to the diffusive zone, becoming more negative as more cathodic E_{pb} values were applied.

The pulse frequency applied in K_1 and K_2 polarography affected the anodic and cathodic current intensities equally, although this influence is lesser than the one established for reversible

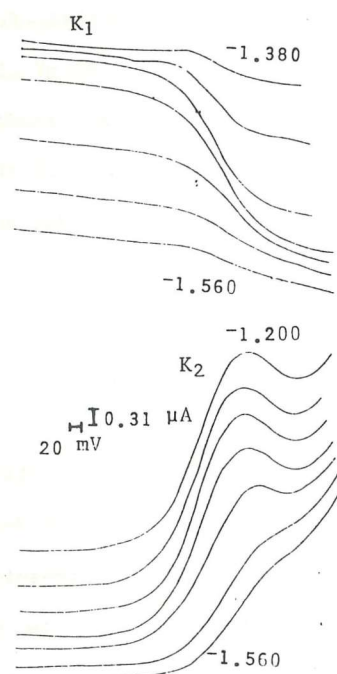


Fig.1.- Influence of the applied base potential on K_1 and K_2 waves shape of chlorhexidine.

$T = 298K$; $c = 4 \times 10^{-4}M$;
 $pH = 6.2$; $I = 0.5M$; $t = 0.6s$;
 $m = 0.88 \text{ mg s}^{-1}$; $f_k = 75 \text{ Hz}$.

processes, since slopes ($\Delta \ln i_{1,p} / \Delta \ln f_k$) are approximately 1/6 (Table II). K_A wave intensities are not dependent on f_k , since the smaller part of recorder belongs to the dc component.

The drop time variation, from 0.4 to 3 s, affects the height of K_1 and K_2 records of chlorhexidine more than dc and K_A polarograms. Slopes ($\Delta \ln i_{1,p} / \Delta \ln t$) present values equal to 0.21 and 0.18 for dc and K_A which correspond to processes controlled by diffusion, although they are slightly higher than the exponent of the drop time expressed in Ilkovic's equation. For K_1 and K_2 registers, slopes equal to 0.33 and 0.31 are obtained and are lower than the ones shown in Ruzic's equations⁽²⁾.

Table I.- Influence of the base potential on half wave peaks of K_1 waves.

$T = 298K$; $c = 4 \times 10^{-4}M$; $pH = 6.2$; $I = 0.5M$; $t = 0.6s$;
 $m = 0.88 \text{ mg s}^{-1}$; $f_k = 75 \text{ Hz}$. Half-wave or peak potentials are little affected by drop time variations (Table III).

$E_{pb}(mV)$	-1300	-1410	-1440	-1470	-1500	-1530	-1560
$E_{1/2}(mV)$	-1452	-1448	-1444	-1458	-1476	-1490	-1477

Table II.- Influence of the frequency.

$T = 298K$; $c = 4 \times 10^{-4}M$;
 $pH = 6.3$; $I = 0.5M$; $t = 0.6s$;
 $m = 0.88 \text{ mg s}^{-1}$; $E_{pb}(K_1) = -1400 \text{ mV}$; $E_{pb}(K_2) = -1200 \text{ mV}$.

	$\ln i_{1,p}(\mu A) = a + b \cdot \ln f_k(\text{Hz})$		
	a	b	r
K_1	0.14	0.14	0.98
K_2	0.58	0.16	0.99

Table III.- Drop time effect.

$T = 298K$; $c = 3.7 \times 10^{-4}M$;
 $pH = 5.9$; $m = 0.88 \text{ mg s}^{-1}$;
 $I = 0.5M$; $f_k = 75 \text{ Hz}$; $E_{pb}(K_1) = -1440 \text{ mV}$; $E_{pb}(K_2) = 1200 \text{ mV}$.

	$\ln i_{1,p}(\mu A) = a + b \cdot \ln t(s)$			$E_{1/2,p}(mV) = a' + b' \cdot \ln t(s)$		
	a	b	r	a'	b'	r'
dc	0.76	0.18	0.99	-1402	12.3	0.99
K_1	0.97	0.33	0.98	-1445	5.7	0.98
$K_2(I_{max})$	1.46	0.25	0.99	-1485	0.0	-
$K_2(I_{min})$	1.34	0.31	0.99	-1435	0.0	-
K_A	1.21	0.21	0.98	-1435	0.0	-

$\ln i_{1,p}$ values vary linearly with $\ln h_{corr}$. Analogous slopes were obtained for all techniques, and having values slightly higher than 2/3, due to the effect that the charge current produces.

On the pH interval between $3.5 < pH < 8.0$ dc, K_2 and K_A wave heights corresponding to chlorhexidine, increase linearly with its concentration, while the graphical plot of $i_1(K_1)$ vs $c(M)$ is separated from the straight line in the range $1 \times 10^{-4}M < C < 5 \times$

10^{-4}M (Fig. 3). The half-wave or peak potentials vary with the concentration. On the other hand, the influence of c on $E_{1/2,p}$

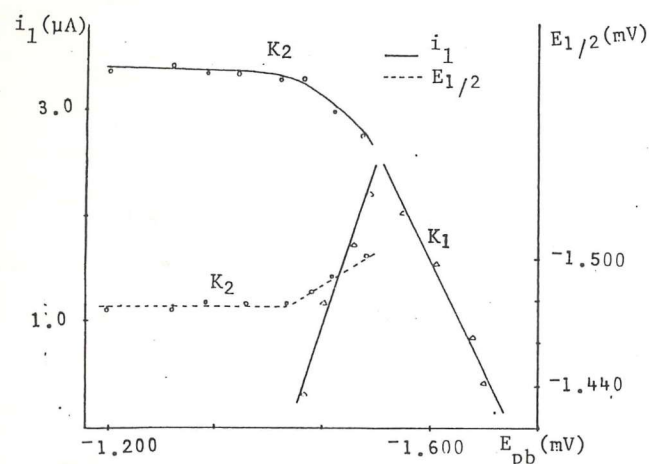


Fig.2.- Influence of the base potential on i_1 and $E_{1/2}$ of K_1 and K_2 chlorhexidine waves.

$T = 298\text{K}$; $c = 4 \times 10^{-4}\text{M}$; $\text{pH} = 6.2$; $I = 0.5\text{M}$; $t = 0.6\text{s}$; $m = 0.88 \text{ mg s}^{-1}$; $f_k = 75 \text{ Hz}$.

values is different for dc and K_4 waves than for K_1 and K_2 (Table IV). Values of negative slopes show that the process is thermodynamically hampered by the increase in concentration of chlorhexidine.

Anodic and cathodic intensities recorded in the electroodic process of chlorhexidine, in Britton-Robinson buffer media and within interval $3.5 < \text{pH} < 8.0$ are not dependent on the acid concentration (Fig. 4a).

On the contrary, half-wave or peak potentials are slightly affected by acid concentration (Fig. 4b). Slope values $(\Delta E_{1/2,p} / \Delta[\text{acid}])$, for

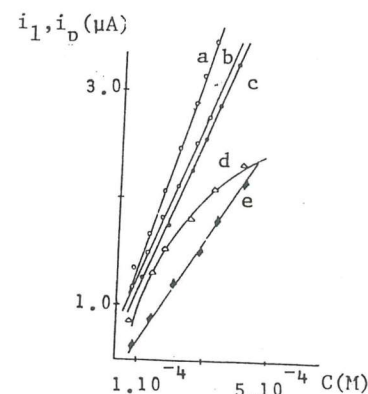


Fig.3.- Chlorhexidine concentration effect.

$T = 298\text{K}$; $\text{pH} = 6.1$; $I = 0.5\text{M}$; $t = 0.6\text{s}$; $m = 0.88 \text{ mg s}^{-1}$; $f_k = 75 \text{ Hz}$; $E_{pb}(K_1) = -1440 \text{ mV}$; $E_{pb}(K_2) = -1200 \text{ mV}$.

a) $K_2 (i_{\text{max}})$; b) $K_2 (i_{\text{min}})$; c) K_4 ; d) K_1 ; e) dc.

cathodic waves of chlorhexidine, can be seen in table V.

Buffer strength hardly effects current intensities and potentials characteristic of cathodic waves, showing that the second protonation of chlorhexidine,

Table IV.-Concentration effect

$T = 298\text{K}$; $\text{pH} = 6.1$; $I = 0.5\text{M}$; $m = 0.88 \text{ mg s}^{-1}$; $t = 0.6\text{s}$; $f_k = 75 \text{ Hz}$; $E_{pb}(K_1) = -1440 \text{ mV}$; $E_{pb}(K_2) = -1200 \text{ mV}$.

	$i_{1,p} (\mu\text{A}) = a + b \cdot c \text{ (M)}$			$E_{1/2,p} (\text{mV}) = a' + b' \cdot c \text{ (M)}$		
	a	b	r	a'	b'	r'
dc	0,18	4798	0,99	-1372	-96000	0,99
$K_2(i_{\text{max}})$	0,51	8900	0,99	-1464	-55000	0,99
$K_2(i_{\text{min}})$	0,49	7636	0,99			
K_4	0,37	7400	0,99	-1392	-104000	0,99

previous to the reduction, does not control the rate of the electroodic process in the interval $3.5 < \text{pH} < 8.0$.

When the ionic strength of solutions is modified by means of adding KCl, it is observed that dc and Kalousek current intensities vary analogously, and are maintained constant within the interval $0.4\text{M} < I < 0.7\text{M}$ (Fig.5). This anomalous behaviour we can explained in base to the removal of DMF adsorbed on *dme* by chloride ions. When

the mercury interfaces is saturated of chloride ions, at $I < 0.8$, decreases the overall rate of the faradaic process because the ionic medium of the bulk solution decreases the diffusion rate of the diprotonated chlorhexidine

The influence of ionic strength on half-wave or peak potentials has not allowed us to establish a functional relation. Thus, while $E_{1/2} (K_1)$ and $E_{1/2} (dc)$ are not totally dependent on the ionic strength, $E_p (K_2)$ and $E_p (K_4)$ values vary slightly with the ionic strength (Fig. 5).

In Britton-Robinson buffer media and within the interval $1.8 < \text{pH} < 9.0$, dc and Kalousek waves of chlorhexidine are affected by the

Table V.- Buffer strength effect.

$T = 298\text{K}$; $c = 4 \times 10^{-4}\text{M}$;
 $\text{pH} = 6.0$; $I = 0.5\text{M}$; $m = 0.88$
 mg s^{-1} ; $t = 0.6\text{s}$; $f_k = 75\text{ Hz}$;
 $E_{pb}(K_1) = -1560\text{ mV}$; $E_{pb}(K_2) = -1200\text{ mV}$.

	$E_{1/2,p}(\text{mV}) = a + b[\text{Acid}]/(M)$		
	a	b	r
dc	-1420	123	0.99
K_1	-1514	290	0.99
K_2	-1502	135	0.99
K_4	-1447	135	0.99

pH of the medium (Fig. 6). At acid or basic pH, K_2 waves present a sigmoidal shape, which show that the adsorption effect in the diffusive zone has disappeared, due to the structural change that

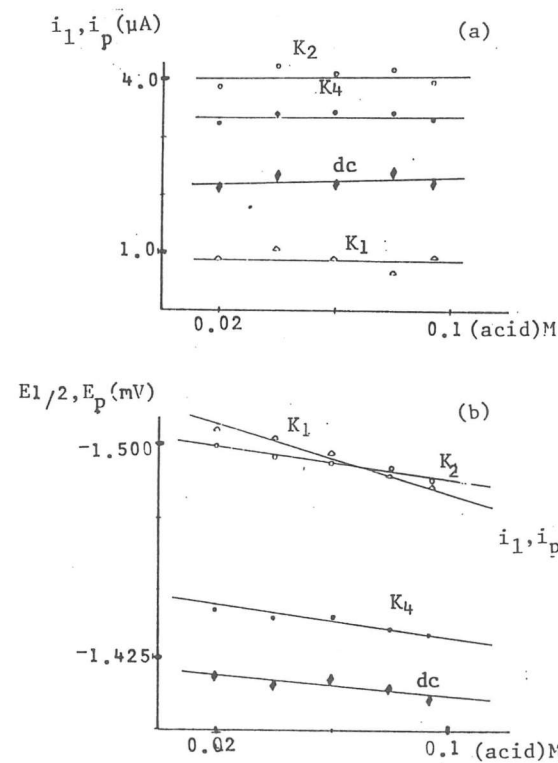


Fig.4.- Buffer strength effect on (a) $i_{1,p}$ and (b) $E_{1/2,p}$ of chlorhexidine waves.

$T = 298\text{K}$; $c = 4 \times 10^{-4}\text{M}$; $\text{pH} = 6.0$; $I = 0.5\text{M}$; $m = 0.88\text{ mg s}^{-1}$; $t = 0.6\text{s}$; $f_k = 75\text{ Hz}$; $E_{pb}(K_1) = -1560\text{ mV}$; $E_{pb}(K_2) = -1200\text{ mV}$.

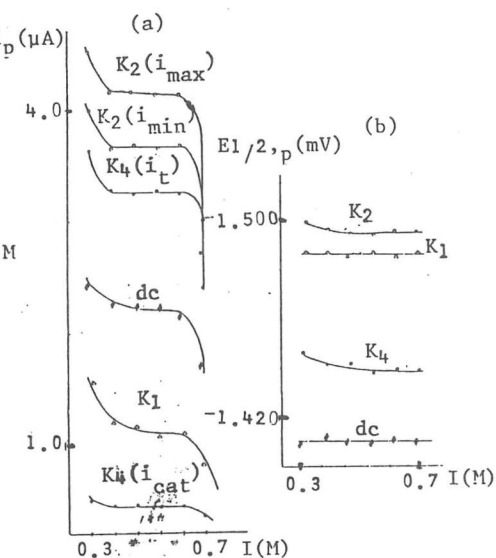


Fig.5.- Ionic strength effect on (a) $i_{1,p}$ and (b) $E_{1/2,p}$.

$T = 298\text{K}$; $c = 4 \times 10^{-4}\text{M}$; $\text{pH} = 6.1$; $m = 0.88\text{ mg s}^{-1}$; $t = 0.6\text{s}$; $f_k = 75\text{ Hz}$; $E_{pb}(K_1) = -1560\text{ mV}$; $E_{pb}(K_2) = -1200\text{ mV}$.

chlorhexidine undergoes when it is reduced at pH next to the neutral pH.

In acid media ($1.8 < \text{pH} < 2.4$), limiting intensity values of the waves decrease in parabolic shape with the increase of pH, although, this decrease is more pronounced in the zone of pH between $1.8 < \text{pH} < 2.2$ due to the superposition of the hydrogenion discharge present in the medium (Fig. 7). The variation of $E_{1/2}(K_1)$ can be considered pH is dependent (Table VI), which means that the reoxidation process of the amined product proceeding the reduction of the biguanide, is not affected by previous protonation steps.

To analytical effects, it is performed that $i_1 = i_2$ within the interval $3.5 < \text{pH} < 8.0$ since the process is controlled by diffusion

Table VI.- pH effect For dc, K_2 and K_4 cathodic ($1.8 < \text{pH} < 2.4$) on $E_{1/2}(K_1)$.

$T = 298\text{K}$; $c = 4 \times 10^{-4}\text{M}$; $m = 0.88 \text{ mg s}^{-1}$; $t = 0.6\text{s}$; and 40 mV respectively, are $f_k = 75 \text{ Hz}$; $E_{pb}(K_1) = -1360 \text{ mV}$

pH	$E_{1/2}(\text{mV})$
1.84	-1256
2.00	-1252
2.15	-1248
2.30	-1256
2.37	-1250
2.38	-1254

obtained. From these values and the calculated α_c values we deduce that before the first electron transfer occurs, the hydrogen ions react with a molecule of chlorhexidine.

In basic medium ($7.5 < \text{pH} <$

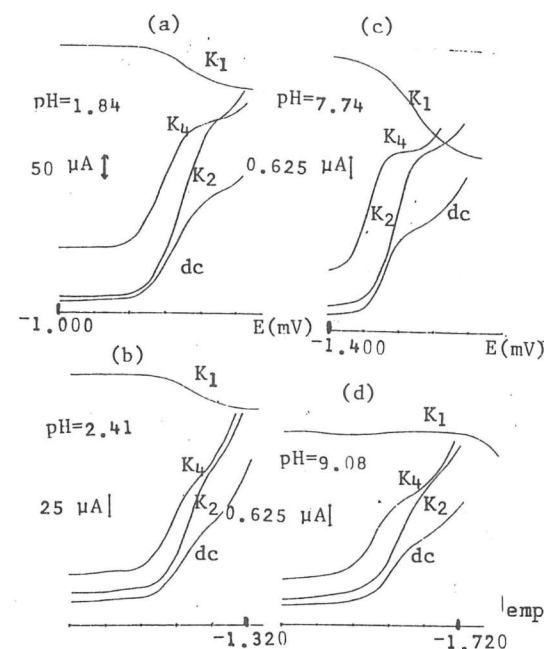


Fig.6.- shape of chlorhexidine waves at different pH.

$T = 298\text{K}$; $c = 4 \times 10^{-4}\text{M}$; $I = 0.5\text{M}$; $m = 0.88 \text{ mg s}^{-1}$; $t = 0.6\text{s}$; $f_k = 75 \text{ Hz}$; $E_{pb}(K_1) = -1360 \text{ mV}$ (a, b); $E_{pb}(K_1) = -1720 \text{ mV}$ (c, d); $E_{pb}(K_2) = -1100 \text{ mV}$ (a, b); $E_{pb}(K_2) = -1400 \text{ mV}$ (c, d).

In basic medium ($7.5 < \text{pH} < 9.0$), cathodic current intensity values, $i_1(K_2)$ and $i_1(K_4)$, decrease lineally with the increase of pH, producing a slope change with respect to the $\text{pH} = 8.5$ (Fig.8), probably due to the solubility change of chlorhexidine at these pH.

It is necessary to emphasize that slopes ($-\Delta i_1 / \Delta \text{pH}$) for cathodic waves, dc, K_2 and K_4 ,

present small values when $\text{pH} < 8.5$, that is, limiting intensities at these pH are little affected by the pH. On the contrary, at $\text{pH} > 8.5$ slopes, ($\Delta i_1 / \Delta \text{pH}$) are obtained for dc K_2 and K_4 equal to 0.97, 1.56, and 1.47 μA respectively, which point toward a bigger dependence of cathodic waves on the pH (Table VII), since the second protonation is the controlling step of the process.

Half-wave potentials become more negative with the increase of pH (Fig.8). Slopes ($-\Delta E_{1/2} / \Delta \text{pH}$) present

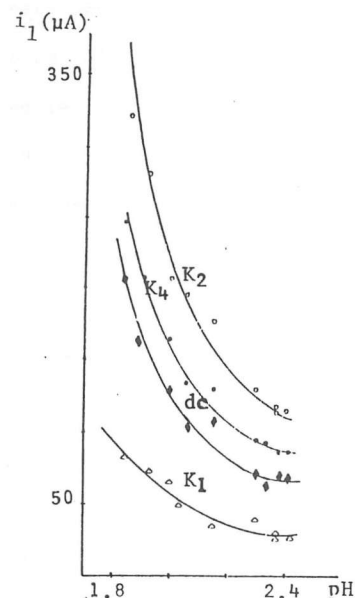


Fig.7.- pH effect on i_1 of chlorhexidine waves..

$T = 298K$; $c = 4 \times 10^{-4}M$; m = inferred that either the reduction of $=0.88 \text{ mg s}^{-1}$; $t = 0.6s$; $f_k = 75 \text{ Hz}$; $E_{pb}(K_1) = -1360 \text{ mV}$; chlorhexidine or the reoxidation of $E_{pb}(K_2) = -1100 \text{ mV}$.

cathodic waves which are approximately 78 mV, while for anodic waves they are superior, approximately 119 mV per unity of pH. At $pH > 8.5$ V, waves are barely perceptible due to the fact that at these pH the diprotonated biguanide groups practically are not in the solution ($pK_{m1} = 2.35$), and the reduction rate of the monoprotanated biguanide group is controlled by the previous protonation step.

Wave heights are adjusted to Arrhenius expressions within temperatures of between $10^\circ C$ and $50^\circ C$ (Table VII). From activation energy

values obtained for electroodic processes associated to waves, it is inferred that either the reduction of the corresponding amined product, are processes controlled by diffusion.

$E_{1/2}(K_1)$ and $E_{pb}(K_2)$ potentials are not dependent on temperature, while on the contrary, for dc and K_4 waves, slope values $(\Delta E_{1/2,p}/\Delta E)$

waves, slope values $(\Delta E_{1/2,p}/\Delta E)$ equal to $1.5 \text{ mV}/^\circ C$ are obtained for temperatures lower than $25^\circ C$ and remain constant for temperatures higher than that.

Table VII.- pH effect ($8.5 < pH < 9.0$).

$T = 298K$; $c = 4 \times 10^{-4}M$; $I = 0.5M$; $m = 0.88 \text{ mg s}^{-1}$; $t = 0.6s$; $f_k = 75 \text{ Hz}$; $E_{pb}(K_1) = -1720 \text{ mV}$; $E_{pb}(K_2) = -1400 \text{ mV}$.

For all registers obtained from chlorhexidine solutions in buffer

		$i_1 (\mu A) = a + b \cdot pH$			$E_{1/2}(mV) = a' + b' \cdot pH$		
		a	b	r	a'	b'	r'
dc	(pH<8,5)	2,17	-0,02	0,98	-906	-78	0,98
	(pH>8,5)	10,18	-0,97	0,99			
K_1	(pH<8,5)	6,75	-0,37	0,98	-645	-119	0,98
	(pH>8,5)	16,82	-1,56	0,99			
K_2	(pH<8,5)	8,21	0,65	0,98	-918	-79	0,98
	(pH>8,5)	16,82	-1,56	0,99			
K_4	(pH<8,5)	8,21	0,65	0,98	-803	-77	0,98
	(pH>8,5)	15,05	-1,47	0,98			

media next to the physiological pH, only semilogarithmic relations are applied⁽¹⁰⁻¹¹⁾ to those with waves having a sigmoidale shape. That is, dc, K_1 and K_2 registers. In all tested experimental conditions, $\alpha_c \approx 1.4$ and $\alpha_m \approx 1.0$ values are obtained (Table IX).

From the monoelectronic transfer model for electroodics processes^(12,13), it is inferred:

$$\alpha_a = v\{(n-j_a) + 1/2 + W'\} \quad \alpha_c = v\{(j_c-1) + 1/2 + W'\}$$

in which for all tested experimental conditions: $v=4$ electroactive groups per mole of chlorhexidine and $n_1 = 2$ electrons per electroactive group. These values show that the determining step (for the potential wave) of the reduction processes is the transfer of the first electron, while in the reoxidation process it is the transfer of the second electron.

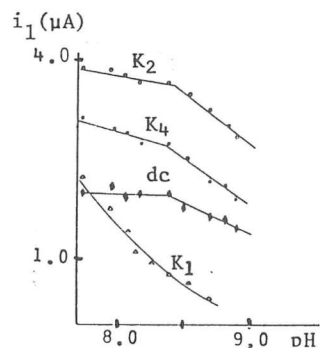
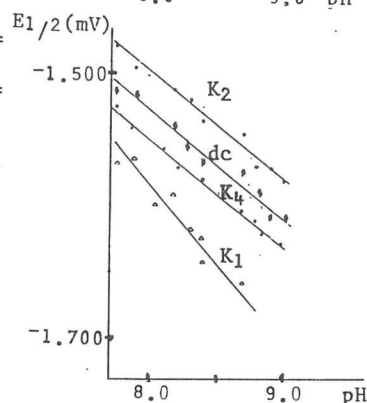


Fig.8.- pH effect on (a) i_1 and (b) $E_{1/2}$ of chlorhexidine waves.

$T = 298K$; $c = 4 \times 10^{-4}M$; $I = 0.5M$; $m = 0.88 \text{ mg s}^{-1}$; $t = 0.6s$; $f_k = 75 \text{ Hz}$; $E_{pb}(K_1) = -1720 \text{ mV}$; $E_{pb}(K_2) = -1400 \text{ mV}$.



Ruzic's expressions⁽⁹⁾ have been applied to polarograms of chlorhexidine at $t = 0.6 \text{ s}$ and $f_k = 75 \text{ Hz}$ values. For K_1 , K_2 and K_4 waves, $\alpha_{ox} = 1.0$, $\alpha_{re} = 1.3$ y $\alpha_{ox} = 1.0$ values are obtained. Replacing these values by the overall electron number, and taking into account the value obtained for the dc wave of $i_1(dc) = 1.7 \mu A$, Ruzic's expressions become the following :

$$i_{k_1} = \frac{6,708 + x}{(1 + x)} \cdot 0,85 \mu A \quad (E_{pb} = -1440 \text{ mV})$$

$$i_{k_2} = \frac{6,708 \cdot x}{(1 + x)} \cdot 0,85 \mu A \quad (E_{pb} = -1200 \text{ mV})$$

$$i_{k_4} = \frac{0,075 \cdot x^2 + 6,276 \cdot x}{(1 + x)(1 + 0,075x)} \cdot 0,85 \mu A$$

Table VIII.- Temperature effect on chlorhexidine waves.

$c = 4 \times 10^{-4}M$; $pH = 6.3$;
 $I = 0.5M$; $m = 0.88 \text{ mg s}^{-1}$;
 $t = 0.6s$; $f_k = 75 \text{ Hz}$; $E_{pb}(K_1) = -1320 \text{ mV}$; $E_{pb}(K_2) = -1200 \text{ mV}$.

	$\ln i_{1,p}(\mu A) = a + b \cdot 1/T(K)$			Q (Kcal/mol)	$E_{1/2,p}(mV) = a' + b' \cdot T(^{\circ}C)$		
	a	b	r		a'	b'	r'
(T<25°C)					-1375	1,5	0,98
dc	6,74	-1801	0,99	3,5	-1400	0,0	-
(T>25°C)					-1400	0,0	-
K ₁	4,21	-1035	0,98	2,0	-1438	0,0	-
K ₂	6,28	-1417	0,99	2,8	-1482	0,0	-
(T<25°C)					-1395	1,5	0,98
K ₄	7,23	-1809	0,99	3,5	-1432	0,0	-
(T>25°C)					-1432	0,0	-

Comparing theoretical polarograms

Table IX.- Transfer with those obtained experimentally, coefficients calculated from chlorhexidine waves.

$T = 298K$; $c = 4 \times 10^{-4}M$;
 $I = 0.5M$; $pH = 6.2$;
 $m = 0.88 \text{ mg s}^{-1}$; $t = 0.6s$;
 $f_k = 75 \text{ Hz}$; $E_{pb}(K_1) = -1440 \text{ mV}$
 $(b, c, d, e,)$; $E_{pb}(K_1) = -1360 \text{ mV}$
 (f) ; $E_{pb}(K_1) = -1720 \text{ mV}$ (g);
 $E_{pb}(K_2) = -1100 \text{ mV}$ (f);
 $E_{pb}(K_2) = -1400 \text{ mV}$ (g).

a)	$E_{pb}(mV)$	n_{ac}	n_{aa}	j_c	j_a
K_1	-1410	-	1.4	-	2
	-1470	-	1.4	-	2
	-1530	-	1.5	-	2

b)	$t(s)$	n_{ac}	n_{aa}	j_c	j_a
dc	0.4	1.3	-	1	-
	1.0	1.4	-	1	-
	2.0	1.5	-	1	-
K_1	0.4	-	1.0	-	2
	1.0	-	1.0	-	2
	2.0	-	1.0	-	2

c)	$c(M)$	n_{ac}	n_{aa}	j_c	j_a
dc	$2 \cdot 10^{-4}$	1.3	-	1	-
	$4 \cdot 10^{-4}$	1.4	-	1	-
K_1	$2 \cdot 10^{-4}$	-	0.9	-	2
	$4 \cdot 10^{-4}$	-	1.0	-	2

d)	(Acido)	n_{ac}	n_{aa}	j_c	j_a
dc	0.02	1.2	-	1	-
	0.08	1.4	-	1	-
K_1	0.02	-	0.9	-	2
	0.08	-	1.0	-	2

e)	$I(M)$	n_{ac}	n_{aa}	j_c	j_a
dc	0.3	1.3	-	1	-
	0.7	1.4	-	1	-
K_1	0.3	-	0.9	-	2
	0.7	-	1.0	-	2

a noticeable difference in the morphology of K_2 waves is appreciated, due to the fact that theoretical expressions do not include the adsorption effect of the reduced species on the electrode. Likewise, wave heights are very different in K_1 , K_2 and K_4 registers (Fig. 9).

CONCLUSIONS

K_1 and K_2 registers, of variable amplitude, are strongly influenced by the base potential value applied. By K_3 polarography only the dc component is recorded since it is an irreversible electrodic process.

K_1 and K_2 wave heights vary with $f^{1/5}$, while K_4 waves are not dependent on f .

Current intensities of K_1 and K_2 waves depend on $t^{1/3}$ and those pertaining to of K_4 waves depend on $t^{1/5}$, which indicates reduction and

reoxidation processes controlled by diffusion.

Only K_2 and K_4 waves of chlorhexidine have analytical validity within the interval $3.5 < pH < 8.0$.

The temperature influence on anodic and cathodic waves, allows us to establish the lineal relation $\ln i_{1,p}$ vs $1/T$ (K). From these slopes we obtain activation energy values, which point toward cathodic and anodic processes controlled by diffusion. The complexity of the reduction mechanism being the cause of the irreversibility of the electrodic process involved.

The poor influence of buffer strength on cathodic waves, indicate that the previous protonation step is not the step that determines the rate of the electrodic process. Paralelly, limiting intensities of anodic registers are not dependent

of acid concentration, which indicate a reoxidation process where

f)	pH	n_{ac}	n_{aa}	j_c	j_a
dc	1.84	1.5	-	1	-
	2.30	1.4	-	1	-
K_1	1.84	-	1.1	-	2
	2.30	-	1.0	-	2
K_2	1.84	1.1	-	1	-
	2.30	1.2	-	1	-
K_4	1.84	1.1	-	1	-
	2.30	1.3	-	1	-

g)	pH	n_{ac}	n_{aa}	j_c	j_a
dc	7.74	1.6	-	1	-
	8.39	1.5	-	1	-
	9.08	1.0	-	1	-
K_1	7.74	-	1.1	-	2
	8.39	-	0.9	-	2
K_2	7.74	1.5	-	1	-
	8.39	1.3	-	1	-
	9.08	0.8	-	1	-
K_4	7.74	1.5	-	1	-
	8.39	1.4	-	1	-
	9.08	1.0	-	1	-

the hydrogenion concentration does not take part in the kinetic law.

From the influence of ionic strength on anodic waves it is deduced that the electroactive species, which is oxidized, does not possess a specific charge.

The pH effect on waves of biguanides, agrees with the reduction mechanism proposed for the monoprotonated biguanide group. The reaction can take place by means of a simultaneous reduction of two protonated azomethinic electroactive groups contained in each diprotonated biguanide group.

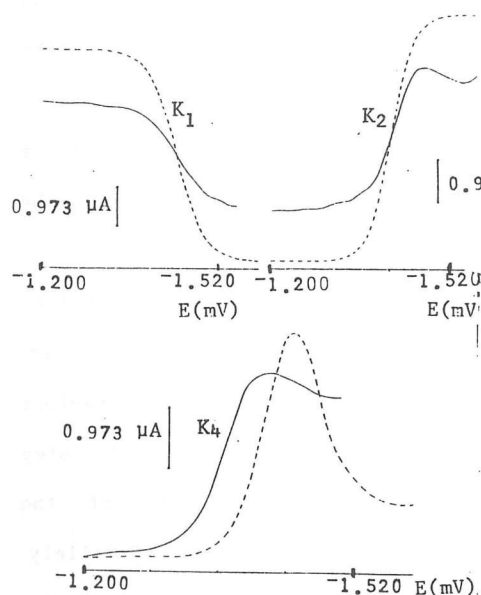


Fig.9.- Theoretical reversible waves (---) and experimental irreversible waves (—) of chlorhexidine.

$T = 298K$; $c = 3.7 \times 10^{-4}M$;
 $pH = 6.7$; $I = 0.5M$; $m = 0.88$
 $mg\ s^{-1}$; $t = 0.6s$; $f_k = 75\ Hz$;
 $E_{pb}(K_1) = -1440\ mV$; $E_{pb}(K_2) = -1200\ mV$.

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