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An analytical study of drug adsorption at mercury electrodes using square wave polarography

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Summary

Selected 1,4-benzodiazepines and steroids have been studied by square wave polarography at growing Hg drops and square wave (SW) voltammetry at the static mercury drop electrode after application of a rapid and automated sample-handling approach. The variation of the resulting peak currents of these drugs with time of measurement during the lifetime of the drop in square wave polarography yields information on their adsorption at mercury electrodes which can be put to analytical advantage in adsorptive stripping voltammetric methods using the static mercury drop electrode. Rapid scan polarography can also be used to monitor the particularly rapid hydrolysis of flurazepam (I) in aqueous solutions of $HClO_4$.

Introduction

The technique of square wave polarography was first suggested by Barker as early as 1957, but was not reconsidered until Ramaley and Krause in 1969 published two papers (Krause and Ramaley, 1969; Ramaley and Krause, 1969), one on the theory of the technique at the hanging mercury drop electrode and one on its application to the Fe(III)/Fe(II) oxalate redox system. Christie et al. (1977) presented a theoretical treatment for square wave polarography at the dropping mercury electrode and verified their proposed theory experimentally using ferric oxalate and Cd(II) in HCl supporting electrolyte (Turner et al., 1977). O'Dea et al. (1981) have published a paper on the theory of square wave voltammetry as it applies to chemical systems complicated by solution or electrode kinetics and Zeng and Osteryoung have derived in 1986 a theoretical expression for square wave voltammetry in the case of a pseudo-first-order catalytic process. The latter authors have tested the validity of the theory by determination of the rate constants for Ti(III)/NH_2OH, Ti(III)/ClO_3^- and Fe(II) NH_2OH redox catalytic systems. Kounaves and O'Dea (1986) have recently derived the response for the application of square wave voltammetry to a reversible system at the mercury film electrode. To date, the technique of SWP or SWV has seen little application in organic analysis.

The instrument design for the One-Drop Square Wave Analyser, partly used in these studies, has been published elsewhere (Yarnitzky et al., 1980). The instrument resembles a differential pulse polarograph in that it consists of a voltage generator, a potentiostat and a signal processor. The

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main difference is that a much faster scan rate is used i.e. 50–100 $mV \cdot s^{-1}$ compared to the DPP with a usual range of 1–5 $mV \cdot s^{-1}$. The square wave generator produces a square wave superimposed on a staircase rather than a series of pulses applied to the mercury drops in DPP. With the fall of the mercury drop the generator is set to a constant initial potential for a delay time, t_d, during which a new drop is formed. The scan is then initiated and the current is measured and integrated before the polarity of the square wave is altered. The differences between successive measurements, i.e. before application of and at the end of application of the square waveforms, are stored in the sample-and-hold circuitry and then displayed in the resulting I–V curve.

The time-consuming step in a square wave polarographic analysis is therefore the deaeration time. Yarnitzky and Ouziel (1976) invented an automated sample-handling approach which can be controlled by the polarographic analyser (Yarnitzky, 1985) in order to lessen significantly the deaeration of the sample and automate the whole electroanalytical procedure.

EG&G have taken commercial advantage of this development and have produced the model 309 Automatic Voltammetric Electrode controlled by the EG&G PAR 384B Analyser used in these studies. The static mercury drop electrode (SMDE) is used as indicator electrode.

This paper is concerned with a study of the 1,4-benzodiazepines, flurazepam (I) and chlordiazepoxide (II), the steroids, testosterone (III), oestrone (IV) and oestradiol (V), at mercury electrodes using the techniques of automated sample-handling–square wave polarography/voltammetry, with particular reference to the adsorption of these drugs and the resulting analytical applications by adsorptive stripping. Such information on drug adsorption at a charged mercury/solution interface would be expected to be of value in the study of the interaction of drugs at cell membranes.

Experimental

Apparatus and techniques

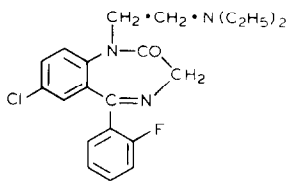
Rapid-scan square wave polarography at single mercury drops was performed by an instrument designed and built at the Technion, Haifa. Samples were deaerated and delivered for analysis by the aforementioned automated sample-handling approach of Yarnitzky and Ouziel (1976), controlled by the square wave polarograph. Rapid scan square voltammetry at the SMDE was carried out using an EG&G PAR 384B Polarographic Analyser and the model 309 Automatic Voltammetric Electrode. All potentials are measured with reference to an Ag/AgCl electrode.

Reagents

Stock solutions of ca. 10^{-3} M of the drugs selected for this study were made up weekly in triple-distilled water and stored in the dark and under refrigeration to minimise decomposition.

Results and Discussion

(1) Flurazepam (I)



I

The 1,4-benzodiazepine tranquilliser, flurazepam (I), gives rise to well-defined square wave polarographic peaks corresponding to reduction of the C=N group over a wide pH range e.g. in 0.1 M $HClO_4$, 0.1 M acetate buffer, 0.1 M phosphate buffer, 0.1 M borate buffer and 0.1 M NaOH. At a concentration of 0.47×10^{-4} M half-peak widths are 45 mV, 40 mV, 40 mV, 45 mV and 45 mV, respectively. Peak heights were stable with time in the latter 4 electrolytes but rapid decomposition of I occurred in 0.1 M $HClO_4$, a reaction which could be monitored using the rapid scan technique in situ and is discussed later.

Peak height is linearly related to concentration in 0.1 M acetate buffer in the range 10^{-7}–10^{-5} M using the square wave settings of 50 $mV \cdot s^{-1}$ scan speed, 20 ms pulse width and a pulse amplitude of 50 mV. The peak potential is −0.76 V using an initial potential of −0.5 V. Above a concentration

of ca. 10^{-5} M the i_p vs C plot undergoes a negative deviation from linearity due to saturation of the electrode surface with adsorbed I. This can be understood as follows. While the electrode potential is held constant at -0.50 V at the start of the drop's life, I is adsorbed and the surface concentration is given by Γ_0, as in the Koryta equation (1953):

$$\Gamma_0 = 0.739 \cdot D_0^{1/2} \cdot C^* \cdot t^{1/2} \quad (1)$$

where C^* is the bulk concentration of I, D_0 is the diffusion coefficient and t is the time from the beginning of the drop's life.

Eqn. 1 applies when all molecules arriving at the electrode surface are adsorbed – thus the bulk concentration must be below that at which the surface saturates in the time in question. Under this condition, flurazepam (I) continuously diffuses to the DME from the beginning of the drop's life. The concentration of I near the electrode is low in comparison to the increasing surface concentration with the drop acting as a preconcentration site. When the sweep is applied and reduction occurs, most of the current is supplied by adsorbed I with little contribution from diffusion. At surface saturation, the concentration of I near to the surface is no longer negligible and diffusion begins to contribute to the reduction current until at higher concentrations the reaction appears to be controlled by diffusion.

The fractional surface coverage is given by:

$$\Theta = A_m \cdot N \cdot \Gamma_0 \quad (2)$$

in which A_m is the area occupied by a molecule of adsorbed I and N is Avogadros number. On substitution of Eqn. 1 into Eqn. 2.

$$\Theta = 0.739 \cdot A_m \cdot N \cdot D_0^{1/2} \cdot C^* \cdot t^{1/2} \quad (3)$$

For a system in which the reactant is strongly adsorbed and when this provides the major contribution to the observed current, Ramaley et al. (1981) have illustrated that for the adsorption of the picolinic acid complexes of Cd(II) and Pb(II)

$$i_p = K \cdot A_p \cdot \Gamma_0 \quad (4)$$

where A_p is the electrode area and K is a proportionality constant.

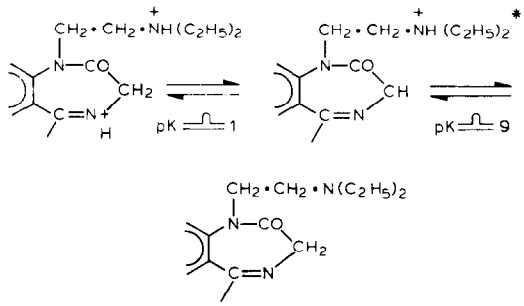
Given that t_p is the time during the drop life at which i_p is measured, then Eqn. 4 can be modified to Eqn. 5:

$$i_p = K_1 \cdot t_p^{2/3} \cdot t_p^{1/2} = K_1 \cdot t_p^{7/6} \quad (5)$$

where K_1 is another proportionality constant and peak current is assumed proportional to both electrode area and surface concentration. A dependence of peak current on $t_p^{7/6}$ is expected under adsorption conditions and on $t_p^{2/3}$ under diffusion-controlled conditions.

The dependence of i_p on t_p was plotted as a log–log plot and the gradients, intercepts and correlation coefficients computed by application of linear regression analysis using a Sharp PC-1211 and programme P4-B-12 (Table 1).

As the concentration decreases and as one moves from diffusion control (> ca. 10^{-5} M) to adsorption control (< ca. 10^{-5} M) the gradients increase for acetate, phosphate and borate as is expected from the above discussion. It is noticeable that surface saturation appears to be reached at progressively higher concentrations as the pH increases as is evidenced by gradients of 0.6762, 0.7679, 0.9134 and 0.9374 for a concentration of 0.47×10^{-4} M I in the polarographic cell. This can be understood in terms of the changes in the overall charge of the flurazepam molecule as the pH is increased as postulated by Smyth and Groves (1981).



Scheme A.

* The protonated tertiary amine ion pairs with Ac^- from the supporting electrolyte when the latter is ionised as is evidenced by solvent extraction studies (Smyth and Groves, 1981). This ion pairing will cease to function at pH > pK_2 when the tertiary amine loses its positive charge.

TABLE 1

Gradients, intercepts and correlation coefficients of plots of log i_p vs log t_p for flurazepam (I)

Supporting electrolyte	Concentration	Gradient	y intercept	Correlation coeff.
0.1 M acetate buffer	$0.47 \cdot 10^{-4}$ M	0.6762	2.2916	0.9981
	$9.4 \cdot 10^{-6}$ M	1.0285	1.3811	0.9995
	$1.9 \cdot 10^{-6}$ M	1.1389	0.4728	0.9996
0.1 M phosphate buffer	$0.47 \cdot 10^{-4}$ M	0.7679	2.1887	0.9961
	$0.47 \cdot 10^{-6}$ M	1.1251	0.0373	0.9966
0.1 M borate buffer	$0.47 \cdot 10^{-4}$ M	0.9134	2.0365	0.9995
	$0.47 \cdot 10^{-6}$ M	1.5869 *	−0.6207 *	0.8921 *
0.1 M sodium hydroxide	$0.47 \cdot 10^{-4}$ M	1.3099 [a]	1.4184 [a]	0.9672 [a]
		0.9374 [b]	1.7428 [b]	0.9756 [b]

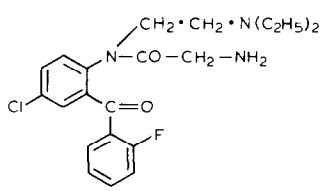
* i_p measurements difficult due to ill-defined peaks.
[a] Considering all data in the range $t_p = 5$–15 s (+ deviation from linearity for log t_p values > 1.10).
[b] Considering data in the range $t_p = 5$–11 s.

The effect of charge repulsion between flurazepam molecules adsorbed on the surface of the mercury drop will therefore tend to decrease their surface concentration for a particular bulk concentration, C^* possibly causing reorientations in the adsorbed state as well. Hence the bulk concentration which gives rise to monolayer surface saturation is reached at a lower value when the molecule is charged as compared to when it is uncharged.

From an analytical viewpoint the square wave polarographic response is both well defined and of similar height in acetate, phosphate and borate buffers. Detection limits of 10^{-7} M are achieved in all cases when a delay time of $t_d = 1$ s is used. The One-Drop Square Polarographic Analyser has delay time settings of 1, 2, 4, 6, 8 and 10 s. When a t_d of 10 s is used, the detection limit can be lowered to ca. 4×10^{-8} M. The use of this adsorption preconcentration procedure could then be applied to square wave voltammetry at the SMDE to yield very low detection limits in the region 10^{-8}–10^{-10} M using preconcentration times of up to 10–15 min. This has obvious implications in the assay of I in biological matrices.

As mentioned earlier flurazepam (I) hydrolyses very rapidly in 0.1 M $HClO_4$ with its peak at −0.55 V (i_1) decreasing as a new peak i_2 builds up at −0.64 V. Using an initial concentration of I of 0.47×10^{-4} M which gives a current of 650 nA, this rapidly decreases to a constant value of 190 nA with an estimated half-life of 24 min. This short half-life can only be estimated with any degree of accuracy using the rapid-scan technique at successive mercury drops. A delay time of t_d = 10 s is incorporated between scans. The peak i_2 reaches a maximum value of 750 nA after 3 h with a $t_{1/2}$ of 30 min. After about 16 h in situ a third peak, i_3 at −0.76 V, appears and increases at the expense of peak i_2. i_3 reaches a maximum value of 1200 nA after ca. 2 days and the solution becomes yellow with i_2 decreasing to a constant value of 175 nA in the same time period. Also during the period 16 h–6 days i_1 decreases steadily from 190 nA to 50 nA.

The initial degradation of I to give peak i_2 is



Ia

consistent with the formation of the benzophenone (Ia) as suggested as suggested by Smyth and Groves (1982).

The structure of the electroreducible product giving rise to peak i_3 is likely to be a highly conjugated molecule as the result of some intermolecular reaction (Ib).

TABLE 2

Gradients, intercepts and correlation coefficients of log i_p vs log t_p plots for hydrolysis products Ia *and* Ib; *initial concentration of* I *is* 0.47×10^{-4} *M in 0.1 M* $HClO_4$ *(solution A)*

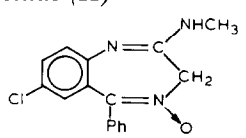
	Gradient		Intercept		Correlation coefficient	
	i_2	i_3	i_2	i_3	i_2	i_3
After 16 h						
Solution A	0.770	1.127	1.992	0.641	0.998	0.994
Solution A diluted by 10	0.991	0.991	0.664	−0.247	0.993	0.993
Solution A diluted by 100	1.718 *	–	−1.090 *	–	0.986	–
After 6 days						
Solution A	–	0.974	–	1.889	–	0.999

* i_p measurements difficult due to ill-defined peaks.

The dependence of log i_p on log t_p was plotted for i_2 and i_3 and the gradients, intercepts on the log i_p axis and correlation coefficients were calculated by linear regression analysis using a Sharp PC 1211 pocket computer using programme P4-B-12 (Table 2).

These results indicate that benzophenone (Ia) shows a transition from adsorption control to diffusion control at a concentration in the range 10^{-4}–10^{-5} M. This molecule is therefore amenable to the technique of adsorptive preconcentration together with rapid-scan square wave voltammetry which in general would suggest that other such derivatives of 1,4-benzodiazepines are also determinable to 10^{-9}–10^{-10} M concentrations after deposition periods of 10–15 min.

(2) Chlordiazepoxide (II)



II

The plot of log i_p vs log t_p for a concentration of 1.5×10^{-6} M II using its well defined C=N peak gave a gradient of 1.14 characteristic of an adsorption controlled process. This is further evidenced by the following results at the SMDE.

In 0.1 M acetate buffer containing 1.5×10^{-7} M II using a deposition potential of −0.7 V, square wave voltammetry operating at a frequency of 25 Hz can be used to follow adsorptive preconcentration of II at the SMDE. An enhancement factor of 5 was calculated over a 60-s deposition period. This suggests that concentrations several orders of magnitude lower than this are accessible using deposition times in excess of 10 min.

(3) The steroids oestrone (IV), testosterone (III) and oestradiol (V)

The steroids oestrone (IV) which contains a reducible C=O group and testosterone (III) which has the reducible C=C–C=O group were studied by square wave polarography at single mercury drops and the results, together with linear regression analysis of log i_p vs log t_p plots are given in Table 3. Molecule III gives a well-defined square wave polarographic peak of half-peak width of 70 mV in 3.6×10^{-3} M $HClO_4$. The slope of its log i_p vs log t_p graph is 0.7043 at a concentration of 0.9×10^{-6} M which suggests non-adsorption under these conditions. However, in borate buffer a transition from adsorption to diffusion control is observed at 3×10^{-5} M (intersection of linear portions of the log i_p vs log C graph). This is borne out by the gradients shown in Table 3. Oestrone gives gradients of 1.1656 and 1.3222 at concentrations of 1.6×10^{-5} M and 1.6×10^{-6} M, respectively, again suggesting adsorption control at these concentrations.

Square wave voltammetry at the SMDE was then applied to oestrone using the automated cell, a supporting electrolyte of 5×10^{-3} M borate and instrumental conditions, frequency 25 Hz, scan-speed 50 mV · s^{-1}. A scan of the potential

TABLE 3

Gradients, intercepts and correlation coefficients of plots of log i_p vs log t_p for oestrone (IV) *and testosterone* (III)

Electrolyte	Concentration	Gradient	Intercept	Correlation coefficient
Testosterone (III)				
3.6×10^{-3} M				
$HClO_4$*	$0.9 \cdot 10^{-6}$	0.7043	0.9872	0.9825
0.1 M borate **	$2.25 \cdot 10^{-6}$ M	1.0257	0.2122	0.8973 (i.d.)
	$0.9 \cdot 10^{-5}$ M	1.1765	0.6718	0.9901
	$0.45 \cdot 10^{-4}$ M	0.5162	1.9415	0.9637
	$0.9 \cdot 10^{-4}$ M	0.7530	1.8697	0.9967
Oestrone (IV)				
5×10^{-3} M borate ***	$1.6 \cdot 10^{-6}$ M	1.3222	0.6598	0.9818
	$1.6 \cdot 10^{-5}$ M	1.1656	1.3952	0.9998

i.d. = peaks ill-defined.
* Scan-speed 100 mV · s^{-1}, pulse width 20 ms, amplitude 50 mV.
** Scan-speed 50 mV · s^{-1}, pulse width 20 ms, amplitude 50 mV.
*** Scan-speed 100 mV · s^{-1}, pulse width 5 ms, amplitude 50 mV.

region −1.1 to −1.9 V of a solution of 1.6×10^{-7} M oestrone (IV) with 60 s deposition at the SMDE prior to scanning gave rise to two peaks, one at −1.38 V (160 nA), and the other at −1.82 V (440 nA). It is probable that the former peak is due to desorption/reorientation at the electrode surface and that the latter peak corresponds to C=O reduction in IV. Adsorptive stripping of IV was attempted in 5×10^{-3} borate buffer using the peak at −1.38 V and the peak height increased linearly with deposition time in the range 30–90 s. At deposition times greater than 90 s the signal became constant as monolayer saturation was achieved. This behaviour is very similar to that observed by Wang et al. (1985) for 2×10^{-7} M testosterone (III) using deposition at −0.8 V followed by differential pulse voltammetric determination. Further confirmation of the more positive wave being non-Faradic in nature came from a similar study of 1.2×10^{-7} M oestradiol (V) which does not contain a keto or $\alpha\beta$-unsaturated keto group. With a deposition potential of −0.8 V, 5×10^{-3} M borate buffer and using a frequency of 25 Hz, scan-speed 50 mV · s^{-1}, there was a linear relationship between peak height (at −1.39 V) and deposition time in the range 30–120 s for the above concentration of V. Again peak height levelled off at times greater than 120 s.

It can therefore be concluded that steroids in general can be determined down to concentrations of ca. 10^{-8} M using the technique of square wave voltammetry at the SMDE after several minutes deposition at a suitable initial potential, e.g. −0.8 V. Wang et al. (1985) have claimed a detection limit of 1.6×10^{-10} M for testosterone using DPP detection at the SMDE after a 15-min accumulation at −0.8 V.

Conclusions

This study of 1,4-benzodiazepine and steroid adsorption at mercury electrodes together with recent similar publications by the authors on the cephalosporin, cephalothin (Peled et al., 1987) and other antibiotics such as oxytetracycline and trimethoprim (Yarnitzky and Smyth, submitted for publication) would suggest that a wide range of molecular structures can adsorb on mercury electrodes which gives rise to lower limits of pulse and square wave voltammetric detection.

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