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Square wave polarographic and voltammetric analysis of selected electroreducible drugs

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Summary

The square wave polarographic and voltammetric behaviour of the antibiotics chloramphenicol (**I**), metronidazole (**II**), oxytetracycline (**III**), cephalothin (**IV**) and trimethoprim (**V**), together with the tranquilliser chlordiazepoxide (**VI**), has been studied at single growing and stationary mercury drop electrodes. Single drop square wave polarography was used to investigate the contribution of adsorption by interpretation of $i_p - t_p$ relationships. A study of the variation of peak current with solution variables such as pH, constitution of supporting electrolyte, concentration of electroactive drug and instrument variables such as square wave frequency, scan speed and accumulation time for those molecules amenable to adsorptive stripping voltammetry has led to optimisation of the reduction signal for analytical purposes using square wave voltammetry at the stationary mercury drop electrode. Detection limits have been found for the drugs in question and are generally of the order of 10^{-8}–10^{-7} M. The use of the automated cell together with rapid-scan square wave voltammetry for the determination of these drugs singly, in formulations and in mixtures with each other was investigated.

Introduction

Until the advent of the automated polarographic/voltammetric cell invented by Yarnitzky (1985) and manufactured by Jordan Valley Applied Radiation Ltd (Model 309 AVE PARC EG&G), electroanalytical techniques such as differential pulse polarography and fast scan square wave voltammetry have suffered in terms of slow sample handling, i.e., the steps of rejection of old sample including some mercury into waste reservoir, washing of cell, introduction of new sample and deaeration prior to electroanalysis. In 1985, EG&G PARC introduced the Model 309 automatic voltammetric electrode which uses the unique rapid deaeration technique of nebulisation. This novel sample handling approach in conjunction with the rapid scan-square wave voltammetric mode (Christie et al., 1977) of the 384B polarographic analyzer can reduce total analyses times per sample to 2 min or so. The overall technique is controlled by the polarographic analyzer and is fully automated from introduction of the sample to calculation of the result.

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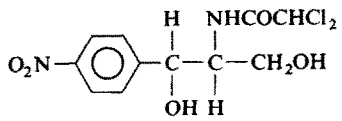
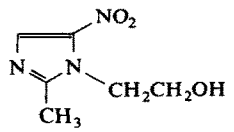
The drugs **I–VI** have been studied by various polarographic techniques, e.g., Siegerman (1983) has reviewed the polarographic behaviour and analysis of antibiotics such as chloramphenicol (**I**), metronidazole (**II**), oxytetracycline (**III**), cephalothin (**IV**) and trimethoprim (**V**) and chlordiazepoxide (**VI**) has been mentioned in many polarographic texts (e.g. see Brooks (1979)) over the years. All the molecules **I–VI** give rise to polarographic reduction waves under various solution conditions (Table 1) and these have been utilised in both formulation analysis and biological fluid analysis. The sensitivity of differential pulse polarography has been of particular use in this latter area of application. As yet, they have not been examined by rapid-scan square wave polarography and voltammetry at mercury drop electrodes.



I



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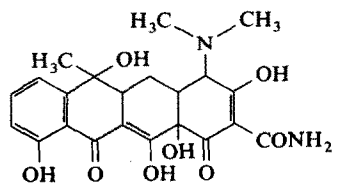

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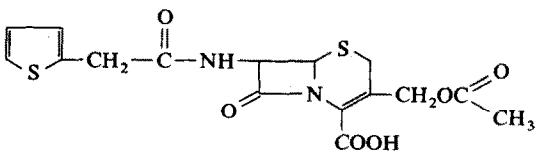

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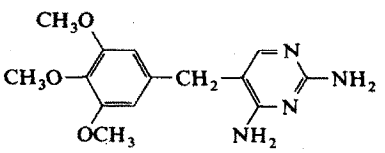
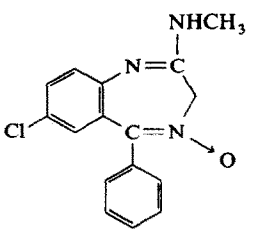


V



VI

Experimental

Apparatus

Rapid-scan square wave polarography at a dropping mercury electrode was performed using an instrument designed and constructed in the Technion, Haifa (Yarnitzky and Ouziel, 1976). Rapid scan square wave voltammetry at the stationary mercury drop electrode (s.m.d.e.) was carried out using an EG&G PARC 384B polarographic analyzer which controlled the operation of a model 309 automatic voltammetric electrode for rapid and automated sample handling. All potentials are measured with reference to an Ag/AgCl electrode. A step level of 2 mV was used in all square wave experiments.

Reagents

Stock solutions (approx. 2×10^{-3} M) of the drugs **I–VI** are made up in either triply distilled water (for the sodium salt of cephalothin, the hydrochloride salts of oxytetracycline, chlordiazepoxide) or absolute ethanol and then diluted accordingly with the appropriate supporting electrolytes. The stock solutions were stored in the dark and under refrigeration to minimise decomposition. All buffer and supporting electrolytes were made up from Analar chemicals with triply distilled water.

Results and Discussion

Chloramphenicol (I)

Chloramphenicol gave rise to a well-defined square wave polarographic peak at -0.16 V in

TABLE 1

Polarographic reduction behaviour of I–VI

Molecule	Supporting electrolyte	E_p (V vs s.c.e.) (using d.p.p.)	Concentration range for calibration plot
I	0.1 M acetate buffer, pH 4	−0.27	0.48–0.96 ppm
II	phosphate buffer, pH 7	−0.625	0.1 μg ml^{-1} [a]
III	10% MeOH-90% phosphate buffer, pH 4.1	−1.65 [b]	16 ppm [a]
IV	1 N H_2SO_4	−1.10 [c]	1–30 ppm
V	0.1 N H_2SO_4	−1.07	–
VI	0.1 N H_2SO_4	−0.28, −0.60, −1.13	–

[a] Limit of detection.
[b] Versus Hg pool using a.c. polarography.
[c] Using single-sweep polarography.

4×10^{-3} M $HClO_4$ when investigated at a concentration of 2×10^{-6} M. Variation of the height of this peak (i_p) with the drop life t_p at which i_p is measured is shown in Fig. 1a. A gradient of 0.85 suggests that at a concentration of 2×10^{-6} M (**I**) the process is controlled by both diffusion and adsorption, since Ramaley et al. (1981) have shown $i_p = K_1 \cdot t_p^{2/3}$ for a square wave diffusion-controlled process and $i_p = K_2 \cdot t_p^{7/6}$ for an adsorption-controlled process. **I** was then investigated by square wave voltammetry at the s.m.d.e. in 10^{-3} M acetate buffer using a concentration of 2×10^{-5} M and a frequency of 25 Hz. The response due to reduction of the $-NO_2$ group in **I** at −0.33 V was relatively small (i.e. of the order of 80 nA) using these operating conditions. The peak height could be increased by a factor of 2 and the shape considerably enhanced with a deposition time of 30 s at $E_{initial} = -0.25$ V. No further increase in height occurred for deposition times in excess of 30 s. A concentration of 2×10^{-6} M **I** under these operating conditions with zero deposition time at initial potential was not detectable. The substance could just be detected at this concentration with deposition times in excess of 60 s and up to 180 s.

In an attempt to lower the detection limit for chloramphenicol, alternative supporting electrolytes were tried. The use of 0.081 M $HClO_4$ gave rise to a significantly larger current ($i_p = 70$ nA) for a concentration of 2×10^{-6} M **I** at a frequency of 25 Hz. This well defined peak at −0.22 V did not undergo current enhancement by adsorptive accumulation at 0 V using times up to 20 s. A detection limit of 3×10^{-7} M for **I** was found in 0.081 M $HClO_4$ which corresponds to 0.1 ppm, identical to Siegerman's (1983) for the d.p.p. detection limit for **I** in 0.1 M acetate buffer. This limit of detection can be lowered to 1×10^{-7} M (30 ppb) using a frequency of 5 Hz and 4×10^{-3} M $HClO_4$ as supporting electrolyte.

Adsorptive accumulation at 0 V for 120 s had no effect.

Metronidazole (II)

Metronidazole (**II**) gives rise to a well defined peak at −0.3 V in 0.081 M $HClO_4$ (frequency 25 Hz) at a concentration of 2×10^{-5} M. This peak can be followed down to a limit of detection of 10^{-6} M (0.17 ppm). Attempts at adsorptive accumulation at this latter concentration at 0 V for time periods 30–60 s were unsuccessful. This detection limit can be lowered to 10^{-7} M (17 ppb) using a frequency of 5 Hz and 4×10^{-3} M $HClO_4$ as supporting electrolyte. Attempted deposition of **II** on the surface of the s.m.d.e. at potentials in the range 0 V to −0.15 V and for times up to 300 s were unsuccessful. Resolution of a mixture which was 2×10^{-7} M in **I** and **II** in 4×10^{-3} M $HClO_4$ (frequency 5 Hz) was also unsuccessful. Metronidazole (**II**) also gives rise to well defined peaks in the potential region −0.7 to −0.8 V in 0.1 M borate buffer, 0.1 M NaOH and 0.1 M Et_4NOH on application of rapid-scan

square wave voltammetry at a frequency of 25 Hz. Detection limits were of the order of 2×10^{-6} M, i.e., 0.34 ppm in these electrolytes.

Oxytetracycline (III)

Oxytetracycline (**III**) gave rise to a well defined square wave polarographic peak at -0.94 V in 4×10^{-3} M $HClO_4$ when investigated at a concentration of 1.4×10^{-6} M **III**. A plot of log i_p vs log t_p (Fig. 1) gave a gradient of 1.08 and illustrates that the process of electroreduction is strongly governed by adsorption, a fact which is borne out by adsorptive stripping measurements at the s.m.d.e. as are now described.

Oxytetracycline gives a well defined three-peak pattern in 10^{-3} M acetate buffer when subjected to square wave voltammetry at 25 Hz and at a concentration 1.4×10^{-5} M. Peaks are located at -1.08 V (300 nA), -1.2 V (125 nA) and -1.25 V (12 nA). Only the first peak is clearly visible at one order of magnitude of concentration lower, i.e., at 1.4×10^{-6} M using a frequency of 25 Hz. Peak enhancement at this concentration does occur with accumulation times up to 20 s at the accumulation potential -0.8 V. Beyond 20 s there is a change in the relative heights of the first two peaks but no increase in overall peak height as one would expect with full surface coverage. Overall peak enhancement is also observable at a concentration of 1.4×10^{-7} M **III** with deposition times up to 120 s. The concentration of the supporting electrolyte in this case appears to have a pronounced effect on adsorptive accumulation of oxytetracycline at the s.m.d.e. in that accumulation is not observed at a 1.4×10^{-7} M concentration of **III** when the concentration of the acetate buffer is raised to 10^{-1} M.

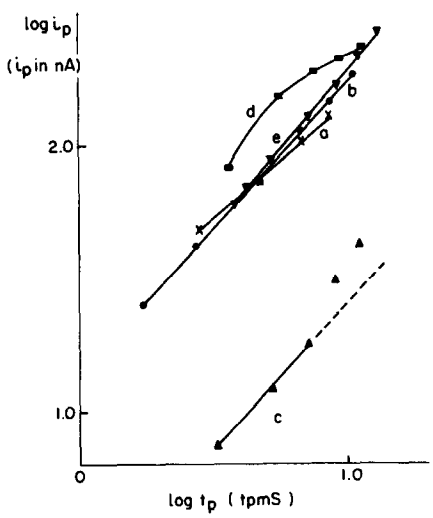


Fig. 1. Variation of log i_p vs log t_p in square wave polarographic analysis of (a) 2×10^{-6} M chloramphenicol (**I**) (× ——— ×); (b) 1.4×10^{-6} M oxytetracycline (**III**) (● ——— ●); (c) 1.7×10^{-6} M cephalothin (**IV**) (▲ ——— ▲); (d) 2.5×10^{-6} M trimethroprim (**V**) (■ ——— ■); (e) 1.5×10^{-6} M chlordiazepoxide (**VI**) (▼ ——— ▼); all in 4×10^{-3} M $HClO_4$; pulse width, 20 ms; pulse amplitude, 50 mV; scan rate, 200 mV s^{-1} (except (e) 50 mV s^{-1}).

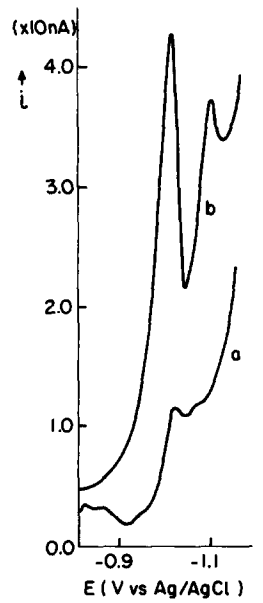


Fig. 2. Effect of deposition time on square wave voltammogram of 1.4×10^{-7} M oxytetracycline (**III**) in 4×10^{-3} M $HClO_4$, at a frequency of 5 Hz. (a) No deposition; (b) 60 s deposition at initial potential -0.8 V.

Higher currents and greater enhancement effects are observable for the electroreduction of oxytetracycline (**III**) in 0.081 M $HClO_4$ supporting electrolyte. An enhancement factor of 5.6 for 1.4×10^{-6} M **III** can be calculated in this supporting electrolyte using a 20 s enrichment time at -0.8 V. A concentration of 1.4×10^{-7} M (**III**) is just detectable in 0.081 M $HClO_4$ (25 Hz) but when adsorptive accumulation at -0.8 V is used for a 60 s period, the peak shape considerably

improves and the current is enhanced by a factor of 14. The same is the case using 4×10^{-3} M $HClO_4$ (5 Hz) except that the enhancement factor is 7. (Fig. 2). These enhancement factors calculated for the main oxytetracycline reduction peak should be compared with the unity factors observed for chloramphenicol (**I**) and metronidazole (**II**) at similar concentrations in the range approx. 2×10^{-7}–2×10^{-6} M, in similar supporting electrolytes and using accumulation potentials several hundred millivolts more positive than the corresponding reduction peaks.

Oxytetracycline (**III**) was therefore found to be detected at 10^{-8}–10^{-7} M concentrations using adsorptive enhancement for 1–2 min followed by square wave voltammetry in either 0.081 M $HClO_4$ (frequency 25 Hz) or 4×10^{-3} M $HClO_4$ (frequency 5 Hz) supporting electrolytes.

Oxytetracycline (**III**) also gave a well defined but relatively small peak in supporting electrolyte 1.5×10^{-2} M Et_4NOH at a frequency of 25 Hz. It was of comparative little analytical value compared to the previously discussed measurements in $HClO_4$ supporting electrolytes.

Cephalothin (IV)

Cephalothin (**IV**) at a concentration of 1.7×10^{-6} M gave rise to a well defined square wave polarographic peak at -1.03 V in 4×10^{-3} M $HClO_4$. A plot of log i_p vs log t_p (Fig. 1c) was linear with slope 1.1, characteristic of an adsorption-controlled process, for t_p values up to 7 s. Divergence from linearity was observed for $t_p > 7$ s which may well be due to the increasing effect of the catalytic hydrogen evolution due to adsorbed cephalothin. Cephalothin can be detected down to 1.5×10^{-7} M using rapid scan square wave voltammetry at 100 Hz in 0.075 M $HClO_4$ supporting electrolyte. Its single reduction peak can be subjected to adsorptive enhancement at a concentration of 5.1×10^{-6} M until the surface is saturated, a process which takes only 5 s at this high concentration. At a significantly lower concentration of 1.7×10^{-7} M, the enhancement effect is observable up to 60 s. Beyond that time, the increasing effect of hydrogen evolution catalysed by adsorbed cephalothin is believed to mask the latter's reduction signal (Peled et al., 1987). An enhancement factor, calculated under the same conditions as for oxytetracycline (**III**), i.e., concentration of 1.7×10^{-6} M, (in 0.081 M $HClO_4$) and 20 s deposition, was found to be identical at 5.6.

Cephalothin (**IV**) was barely detectable at 10^{-6}–10^{-5} M concentrations using less acidic supporting electrolytes and consequently further study was not deemed to be of any analytical advantage.

Keflin (registered by Eli Lilly & Co., Indianapolis, U.S.A.), is manufactured in ampoules which contain 1 g cephalothin and 30 mg $NaHCO_3$. It can be assayed by the following procedure. A sample (approx. 30 mg) of the ampoule contents is accurately weighed and made up with freshly distilled water to 100 ml in a volumetric flask. Complete dissolution occurs within seconds to give a solution which is about 0.7×10^{-3} M in **IV**. This solution must then be diluted of the order of 100 times so that the final solution of concentration below 10^{-5} M gives a peak current which is on the linear non-surface saturation section of the i_p vs concentration graph. Twelve aliquots of this final solution per 30 ml could be subjected to the automated cell and rapid scan square wave voltammetric approach described and used in this article. This timing includes two rinse washes between all samples. The 384B polarographic analyser controlled all the mechanical and electrochemical operations in these analyses and printed out in addition to the voltammograms the concentrations (in ppb) of **IV** in the respective samples when compared to a reference standard sample of **IV**. A coefficient of variation of 2% was found.

Trimethoprim (V)

Trimethoprim gives a square wave polarographic peak at -1.14 V in 4×10^{-3} M $HClO_4$. Although it will be shown later in this section that the molecule is amenable to adsorptive stripping, the process is doubtless more complicated, since a plot of log i_p vs log t_p is non-linear (Fig. 1d).

Trimethoprim gives a well defined square wave voltammetric peak at the s.m.d.e. in $HClO_4$ supporting electrolytes. For example, in 0.081 M

$HClO_4$, a peak is given at -1.17 V. The peak at -1.17 V can be enhanced by adsorptive accumulation at -0.80 V when the concentration of (V) is 2×10^{-6} M. 20 s accumulation at this potential gives an enhancement factor of 3.74. This peak also undergoes enhancement at a 2×10^{-7} M concentration but due to the proximity of the hydrogen evolution due to the supporting electrolyte, it lacks proper peak definition and merges into it at accumulation times of 60 s or more. More reliable detection of **V** at 10^{-7} M concentrations can be achieved in 0.004 M $HClO_4$ (5 Hz).

Trimethoprim also gives well defined square wave voltammetric peaks in other supporting electrolytes such as 10^{-3} M acetate buffer (-1.46 V) and 10^{-1} M acetate buffer (-1.40 V).

The formulation Resprim Forte (manufactured by Teva Pharmaceutical Industries, P.O. Box 1423, Tel-Aviv for Ikapharm) which contains 160 mg trimethoprim and 800 mg sulphamethoxazole can be assayed very rapidly for its trimethoprim content by this technique of automated cell-rapid scan square wave voltammetry. A tablet is crushed and shaken vigorously with 25 ml Analar methanol for 5 min, made up to a volume of 100 ml with the same solvent and then an appropriate aliquot diluted with $HClO_4$ supporting electrolyte (1.5, 0.08 or 0.004 M concentrations of $HClO_4$ can all be used with frequencies of 100, 25 and 5 Hz, respectively). The samples throughput is as high as 24 samples per h.

Chlordiazepoxide (VI)

The square wave polarographic investigation of 1.5×10^{-6} M chlordiazepoxide (**VI**) at single growing mercury drops gave a well defined peak at -0.61 V in 4×10^{-3} M $HClO_4$ corresponding to the C = N reduction. The other reductions corresponding to N → O and N = C reductions were significantly smaller and ill-defined. A plot of log i_p vs log t_p was linear with slope 1.14 characteristic of an adsorption-controlled process, as is further demonstrated in the adsorptive stripping results at the s.m.d.e. **VI** gives rise to well defined square wave voltammograms in acidic supporting electrolytes such as $HClO_4$ and acetate buffer; e.g., in 0.1 M acetate buffer a concentration of 1.5×10^{-5} M **VI** gives three peaks at -0.52, -0.84 and -1.25 V with a peak height ratio of 3.75 : 10.7 : 9.00. The middle peak corresponding to reduction of the 4,5-azomethine functional group in **VI** is not only the largest peak but also the sharpest and best defined (half peak width = 55 mV) for analytical purposes. **VI** will undergo adsorptive enhancement in 0.1 M acetate buffer at concentrations where less than complete monolayer surface adsorption exists. This occurs at 10^{-5} M for **VI** and many other electroactive molecules of similar molecular weight. At a concentration of 1.5×10^{-7} M using a frequency of 25 Hz, the optimum deposition potential was found to be -0.70 V from the results listed in Table 2.

TABLE 2

The relationship between deposition potential and peak height for 1.5×10^{-7} M chlordiazepoxide in 0.1 M acetate buffer using 60 s deposition at initial potential

Initial/deposition potential (V)	Peak height [a] (arbitrary units)
−0.20	2.0
−0.30	2.0
−0.40	3.2
−0.50	8.5
−0.70	10
−0.80	less definition of peak

[a] The peak height refers to the 4,5-azomethine reduction process.

Using this optimum deposition potential, peak height increases with deposition time over a 60 s period for **VI**. An enhancement factor of 5.0 was calculated over 60 s deposition period.

A formulation containing 10 mg chlordiazepoxide (Servium) could be rapidly determined by this technique of automated cell-rapid scan square wave voltammetry at the s.m.d.e. by grinding the aforesaid formulation, dissolution of the active constituent in absolute ethanol followed by dilution of an aliquot of the supernatant with 0.1 M acetate buffer (to a concentration of below 10^{-5} M) prior to application of the above-mentioned technique. As with cephalothin (**IV**) and trimethoprim (**V**) formulations, 24 samples of the

final solution ready for voltammetric determination could be assayed per h.

Determination of mixtures of drugs I–VI

The reduction potentials of oxytetracycline (**III**), cephalothin (**IV**) and trimethoprim (**V**) are all contained in the same relatively small potential region, i.e., between -1.0 V and decay of the supporting electrolyte in acidic supporting electrolytes such as $HClO_4$. In order to resolve this mixture satisfactorily, 4×10^{-3} M $HClO_4$ (5 Hz frequency) was chosen as the supporting electrolyte since oxytetracycline (III) at low concentrations of the order of 10^{-6} M gave only one well defined peak and since all three molecules could be subjected to adsorptive enhancement under these conditions.

The addition of 2.5×10^{-6} M trimethoprim (**V**) and 1.7×10^{-6} M cephalothin (**IV**) to a solution of 1.4×10^{-6} M oxytetracycline (**III**) followed by the addition of 2×10^{-6} M concentrations of chloramphenicol (**I**) and metronidazole (**II**) and subsequent adsorptive preconcentration experiments is shown in Fig. 3. The peak height and shape of oxytetracycline (**III**) are unaffected by the addition of comparable concentrations of cephalothin (**IV**) and trimethoprim (**V**) (Fig. 3b). Deposition for up to 20 s at -0.80 V slightly diminishes the first oxytetracycline reduction peak and increases the height of the second oxytetracycline peak at the same potential as the cephalothin reduction at -1.08 V. The trimethoprim peak approximately doubles in height during this 20 s period — this could be compared to an enhancement factor of 3.74 when trimethoprim is present on its own. Addition of 2×10^{-6} M concentrations of non-adsorbing chloramphenicol (**I**) and metronidazole (**II**) to this mixture gives the voltammogram shown in Fig. 3e. Optimum separation of (**III**), (**IV**) and (**V**) is therefore best carried out with no adsorptive accumulation with an initial potential of -0.80 V (as in Fig. 3b). **I** and **II** cannot be separated under these voltammetric conditions and will be determined as a composite peak (Fig. 3e). Dilution of this solution which is approx. 2×10^{-6} M in drugs **I–V** results in detection of the composite peak of **I** and **II**, oxytetracycline (**III**) and

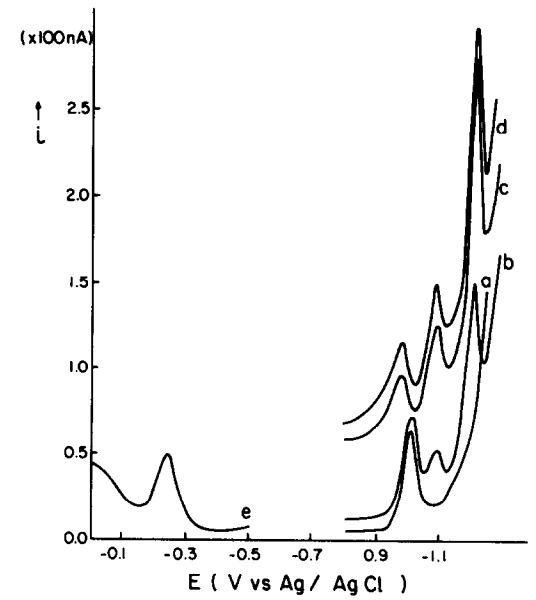


Fig. 3. Square wave voltammograms of mixtures of drugs (**I**)–(**V**) in 4×10^{-3} M $HClO_4$; operating frequency, 5 Hz. (a): 1.4×10^{-6} M oxytetracycline; (b): (a) + 2.5×10^{-6} M trimethoprim + 1.7×10^{-6} M cephalothin; (c): (b) + 10 s deposition at -0.8 V; (d): (b) + 20 s deposition at -0.8 V; (e): (b) + 2×10^{-6} M chloramphenicol + 2×10^{-6} M metronidazole, 0 s deposition.

trimethoprim (**V**) with only an ill-defined shoulder appearing for cephalothin (**IV**). The peaks of **III** and **V** can be enhanced and their definition improved by deposition at -0.80 V for up to 60 s. Beyond this time period, the improvement in peak definition is lost.

Resolution of this five-component mixture was also attempted in 10^{-1} M acetate buffer supporting electrolyte using a frequency of 25 Hz. A solution which was 2×10^{-6} M in **I** and **II**, 1.4×10^{-6} M in oxytetracycline (**III**), 1.7×10^{-6} M in cephalothin (**IV**) and 2.5×10^{-6} M in trimethoprim (**V**) again gave a composite peak for **I** and **II**, three separate peaks for oxytetracycline at -1.07, -1.18 and -1.27 V and a peak for trimethoprim at -1.40 V. Cephalothin does not give an appreciable signal at this pH. The composite peak of **I** and **II** appeared to be partially resolved by deposition at 0 V for time periods up to 60 s but this did not involve current enhancement of the overall peak. 10-fold dilution of this approx. 2×10^{-6} M solution of **I–V** resulted in

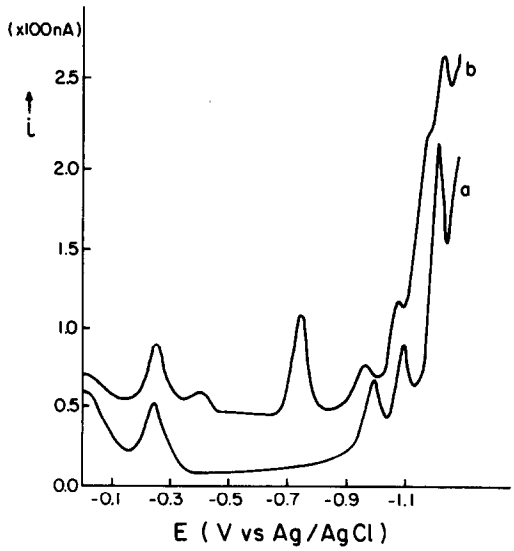


Fig. 4. Square wave voltammetry of mixtures of drugs (I)–(VI) in 5×10^{-3} M $HClO_4$; operating frequency, 5 Hz; no deposition time at initial potential 0 V. (a) 1.7×10^{-6} M cephalothin, 2.5×10^{-6} M trimethoprim, 1.4×10^{-6} M oxytetracycline, 2×10^{-6} M chloramphenicol; 2×10^{-6} M metronidazole; (b): (a)+3×10^{-6} M chlordiazepoxide.

detection of **III** and **V**, and partially resolved **I** and **II**.

Fig. 4 shows the attempted resolution of a mixture of these five antibiotics together with chlordiazepoxide (**VI**) at the 2×10^{-6} M concentration level in 4×10^{-3} M $HClO_4$. Using a scan rate of 10 mV s^{-1}, there is a certain amount of adsorptive accumulation during the scan so as a result, not only does chlordiazepoxide interpose its N → O, C=N and  reduction signals in quite a selective fashion between the other reduction signals, but it tends to diminish the oxytetracycline, cephalothin and trimethoprim signals as well. Optimum resolution of a mixture of these antibiotics and, say, a 1,4-benzodiazepine tranquilliser at approx. 10^{-6} M concentration level and using these experimental conditions would therefore best be achieved if that tranquilliser contained only one electroreducible C = N group, e.g., valium and that a minimum time for adsorptive accumulation is allowed for the determination of oxytetracycline and cephalothin. This would in effect mean determining -NO_2-containing antibiotics and the benzodiazepine tranquilliser(s) in one scan 0 → −0.9 V and then determining the other antibiotics such as oxytetracycline, cephalothin and trimethoprim in a second scan from −0.80 → −1.25 V with no recommended accumulation time to optimise selectivity. At concentrations of the order of below 10^{-7} M, adsorptive accumulation may well be necessary to visualise better the reduction signals of **III–VI**. It should be pointed out that many other classes of antibiotics such as penicillins, sulphonamides, erythromycins, neomycins, etc., are not electroreducible under these conditions. This procedure could therefore form a basis for a rapid electroanalytical method for screening in clinical situations with patients who undergo multiple drug therapy.

Conclusions

The technique of automated cell, rapid-scan square wave voltammetry has been found applicable to the -NO_2-containing antibiotics chloramphenicol (**I**) and metronidazole (**II**), to the tetracycline antiobiotic oxytetracycline (**III**), the cephalosporin, cephalothin (**IV**), trimethoprim (**V**) and the tranquilliser chlordiazepoxide (**VI**) in so much as samples in the general concentration range of approx. 10^{-7}–10^{-5} M can be processed at the rate of 24 h^{-1}. Concentrations of less than 10^{-7} M can be determined for molecules such as **III–VI**, since they are amenable to adsorptive enhancement at the s.m.d.e.

Formulations of cephalothin, trimethoprim and chlordiazepoxide can be assayed very conveniently by this technique, since the sample preparation step only requires dissolution of the active constituent of the formulation in an appropriate solvent prior to necessary dilution with supporting electrolyte. Mixtures of the antibiotics **I–V** can be determined at 10^{-6}–10^{-7} M levels in so much as oxytetracycline (**III**), cephalothin (**IV**) and trimethoprim (**V**) can be resolved in 4×10^{-3} M $HClO_4$ supporting electrolyte at an initial potential of −0.8 V and using a square wave operating frequency of 5 Hz. The -NO_2-containing antibiotics **I** and **II** do not interfere under these

conditions and are determined together as a single peak in a separate scan from $0 \rightarrow -0.9$ V. 1,4-Benzodiazepine tranquillisers such as chlordiazepoxide (**VI**) can also be determined by a C=N reduction signal between these two groups of antibiotics, i.e., by scanning from $-0.6 \rightarrow -0.9$ V although another reduction signal of **VI** and possibly its adsorptive accumulation on the s.m.d.e. electrode can interfere with the determination of the mixture of **III–V**.

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