Journal of Chromatography, 321 (1985) 375–384
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17,408

OXYGEN EFFECTS IN AMPEROMETRIC LIQUID CHROMATOGRAPHIC DETECTION OF OMEPRAZOLE AT A MERCURY ELECTRODE

BJÖRN PERSSON*,* and STIG WENDSJÖ**
*Analytical Chemistry, AB Hässle****, *S-431 83 Mölndal (Sweden)*
(Received October 30th, 1984)

SUMMARY

The determination of omeprazole, a reducible benzimidazolyl pyridyl methyl sulphoxide, was investigated in a reversed-phase liquid chromatographic system by electrochemical detection at a static mercury drop electrode. The mobile phase was composed of acetonitrile and phosphate buffer (pH 7.6).

In the presence of oxygen dissolved in the mobile phase, omeprazole could be indirectly detected at potentials between +0.2 and −0.8 V *vs.* Ag/AgCl, where the compound is not electroactive. The resulting chromatographic peak is explained by the interference of omeprazole with the electrochemical reduction of oxygen residuals in the mobile phase. The adsorption of omeprazole at the mercury electrode is suggested as a prerequisite for the appearance of the peak, as the effect was not observed for compounds with limited adsorption properties.

In a thoroughly deoxygenated system, the oxygen level was decreased to about 0.2% of the air-saturated value. Detection at −1.2 V, where omeprazole is reducible, resulted in a detection limit of 5 pmol (2 ng).

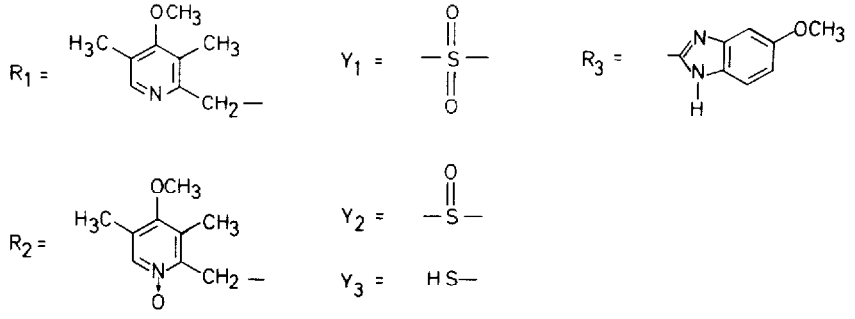
INTRODUCTION

A general aim in modern analysis is detection of smaller amounts with higher selectivity. This situation is typically encountered in liquid chromatography (LC) with electrochemical detection. Enhanced selectivity is achieved by an appropriate choice of the chromatographic separation conditions, type of sensor electrode, detection potential and detection mode. Reducible compounds are conveniently monitored by LC detectors based on the mercury drop electrode, often called polarographic detectors. The use of the mercury electrode as a detector for chromatographic effluents was introduced in the early 1950s[1,2], and has increased in the last few years, particularly after the introduction of commercially available instrumentation. Literature on this subject has been reviewed[3,4].

* Present address: Analytical Chemistry, AB Draco, P.O. Box 34, S-221 00 Lund, Sweden.
** Present address: Department of Analytical Chemistry, University of Uppsala, P.O. Box 531, S-751 21 Uppsala, Sweden.
*** Subsidiary of AB Astra.

0021-9673/85/$03.30

This investigation was focused on the role of oxygen dissolved in the mobile phase as the largest contributor to the background current in reductive amperometric detection. This subject has been treated extensively by other investigators[4–9]. Our primary interest in examining more closely the effects of oxygen arose from chromatographic work with omeprazole (I) and some structurally related derivatives (II–V), applying amperometric detection at a mercury electrode. Omeprazole, a candidate drug for inhibition of gastric secretion, has a half-wave potential at about −1 V in neutral solutions. The attempt to monitor selectively the thiol (V) at 0 V in the presence of the other four derivatives resulted unexpectedly in anodic peaks for all compounds, despite the fact that only the thiol was electroactive at the applied detector potential. Preliminary experiments indicated that the amount of oxygen dissolved in the mobile phase had a strong influence on the heights of these peaks. A systematic study of this phenomenon was started with omeprazole as a model compound. As the use of the mercury drop detector is often associated with unsatisfactory exclusion of atmospheric oxygen from the mobile phase, it was believed that the results of this work could be of general interest. Reference is also made to the detection of omeprazole at −1.2 V on the limiting current plateau in a thoroughly deoxygenated system.



I : $R_1 - Y_2 - R_3$ (omeprazole)

II : $R_1 - Y_1 - R_3$

III : $R_2 - Y_2 - R_3$

IV : $R_2 - Y_1 - R_3$

V : $Y_3 - R_3$

The polarographic reduction of O_2 proceeds through H_2O_2 (HO_2^-) to H_2O (OH^-) in two reduction steps around 0 and −1 V, respectively, in acidic (neutral and alkaline) solutions[10]. The first reduction step, which itself is a multi-step process, is important here. The reduction of O_2 to H_2O_2 proceeds from reversibility in alkaline solutions towards irreversibility in neutral to acidic solutions[11,12].

EXPERIMENTAL

Equipment

The chromatographic system consisted of an Altex Model 100 A dual piston

pump, a Touzar TM Atignon Model Amortisseur pulse damper, a Rheodyne Model 7010 injector with a 20-μl sample loop, a Brownlee Labs. Spheri-5 RP-8 guard column (30 × 4.6 mm I.D.), and either a home-packed column (150 × 4 mm I.D.) filled with 5- or 7-μm LiChrosorb RP-8 (Merck) or a purchased Hibar 5-μm LiChrosorb RP-8 (125 × 4.6 mm I.D.) column (Merck). 316 Stainless steel (0.020 in. I.D.) was used for all connective tubing exposed to atmospheric oxygen, except in the initial experiments, where Teflon tubing was used in some connections.

The detector was a PARC Model 310 mercury drop detector (EG&G Princeton Applied Research), which was placed completely in a plastic glove-bag (Instruments for Research and Industry) filled with nitrogen. The Ag/AgCl (saturated KCl) reference electrode was −47 mV with respect to a saturated calomel electrode. A platinum wire served as the counter electrode. A PAR 174A polarographic analyser (Princeton Applied Research) served as a controller. The chromatograms were registered on an x–t recorder (W + W Recorder 1107) and the polarograms were recorded on a Hewlett-Packard 7044A x–y recorder.

Chemicals

Acetonitrile of chromatographic quality (LiChrosolv; Merck) served as an organic modifier in the mobile phase. The buffer components, $NaH_2PO_4 \cdot H_2O$ and $Na_2HPO_4 \cdot 2H_2O$, were of analytical-reagent grade (Merck). Water was obtained from an ion-exchange system (Elga). Suprapur mercury (Merck) was used in the static mercury drop electrode.

Omeprazole (I) and its derivatives (II–V) were synthesized in our laboratories and had chemical compositions, IR spectra and ^{1}H NMR spectra consistent with the given structures. Omeprazole had a purity of >99%. Nitrogen (AGA Gas) was used as an inert gas for the deoxygenation purposes and contained <5 ppm of oxygen, according to the specification.

Operating conditions

The mobile phase was prepared by adding appropriate amounts of acetonitrile to an aqueous pohosphate buffer (pH 7.6, μ = 0.025) that had been pre-filtered through a 0.45-μm Millipore membrane filter. The initial measurements on the five-component sample were performed with a mobile phase containing 27.5% (v/v) acetonitrile and the systematic study was carried out with 32.5% acetonitrile in the mobile phase. Sample substances were dissolved in mobile phase prior to injection on to the column.

The flow-rate was 1 ml min^{-1}, which resulted in a retention time of about 5 min for omeprazole in 32.5% acetonitrile. Hydrodynamic polarograms of the mobile phase were recorded in the flow cell at the same flow-rate as used in the chromatographic work. All measurements were made with a "small" drop size setting and with a 1-sec drop time in the sampled d.c. mode. Throughout this work, the time constant setting on the PAR 174A was 0 sec, which, depending on the electronics of the instrument, resulted in a time constant of 16.7 msec. Normal polarographic measurements were carried out with the PARC Model 310 detector after removal of the attached LC adapter from the capillary. All experiments were performed at ambient temperature, normally around 21°C.

Deoxygenation

Except for the initial experiments (Figs. 1 and 2), the exclusion of dissolved oxygen from the chromatographic system followed the procedure of Bratin and Kissinger[7], with the exception that the eluent reservoir was not heated. After a few minutes of intense nitrogen purging, a gentle flow of nitrogen was maintained overnight through the eluent (1 l). The plastic bag enclosing the detector was filled with nitrogen and the solution in the detector cell was thoroughly purged with the inert gas for 15 min prior to use. In order to prevent oxygen from re-entering the LC system during the chromatographic work, a gentle flow of nitrogen was maintained through the eluent in the reservoir, to the plastic bag and to the space above the detector cell solution. The system was equilibrated with mobile phase for about 15–30 min until a stable baseline was achieved.

The sample solutions were deoxygenated by purging with nitrogen for 15 min prior to injections.

Oxygenation of the mobile phase

Constant concentrations of dissolved oxygen in the mobile phase were achieved by inserting Teflon tubing of appropriate length (0.8 mm I.D. and 1.6 mm O.D.) in the connection between the eluent reservoir and the pump inlet.

RESULTS

Initial results

Detection at −1.3 V of a mixture containing the thiol (V), which is oxidized in two steps with anodic half-wave potentials at −0.2 and −0.5 V, and the four reducible omeprazole derivatives (I–IV), all having cathodic half-wave potentials around −1.1 V, resulted in a chromatogram with peaks for all the reducible compounds and for oxygen (Fig. 1). When the detector potential was adjusted to 0 V,

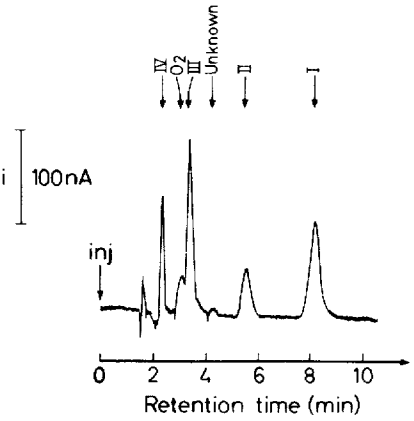


Fig. 1. Chromatogram of 25 μg of omeprazole (I), 25 μg of II, 20 μg of III, 15 μg of IV and 50 μg of V. Column, LiChrosorb RP-8, 7 μm; mobile phase, 27.5% (v/v) acetonitrile, pH 7.6; flow-rate, 1 ml min^{-1}; detection potential, −1.3 V *vs.* Ag/AgCl; background current, 2 μA, equal to 20% of the air-saturated value.

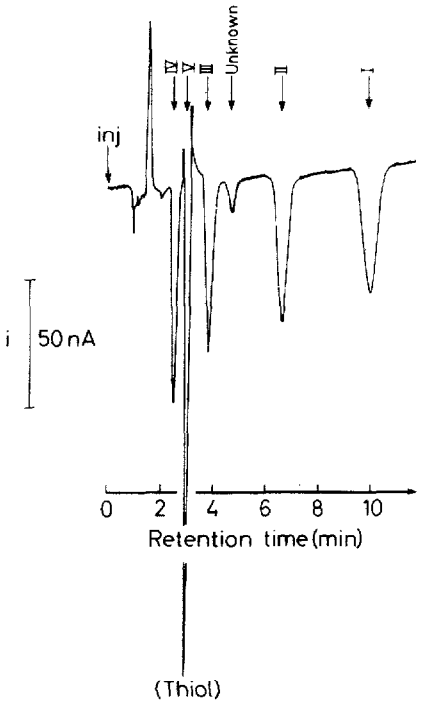


Fig. 2. Detection at 0 V *vs.* Ag/AgCl. Other experimental conditions as in Fig. 1.

however, the same mixture gave a chromatogram with peaks for all components (I–V), although only the thiol was electroactive at the applied potential (Fig. 2). Losses of acetonitrile by the nitrogen purging in these initial experiments prolonged the retention times in subsequent runs (compare Figs. 1 and 2).

Indirect detection

Indirect detection implies detection at electrode potentials where the compound to be detected is not electroactive. Repetitive injections of omeprazole standards at different potential settings revealed that both the peak height and peak polarity varied with detector potential (Fig. 3). An anodic peak was obtained from +0.2 to about 0 V and a cathodic peak from about 0 to −0.8 V. At more negative potentials the faradaic reduction of omeprazole commenced. The highest cathodic peak was observed at −0.2 V, which roughly corresponded to the steepest part of the first polarographic wave of oxygen (Fig. 4). The potential of the highest anodic peak was +0.1 V, which corresponded to a small pre-wave of the first oxygen wave (Fig. 4). The heights of the indirectly detected peaks were related to the amount of oxygen dissolved in the mobile phase, irrespective of peak polarity (Fig. 3). The dependence of peak height on the amount of omeprazole varied with the detector potential and oxygen concentration in a complex manner, as shown in Fig. 5.

By disconnecting the columns and pumping the solution directly to the detector, hydrodynamic polarograms of the air-saturated mobile phase containing different concentrations of omeprazole were recorded (Fig. 4). These polarograms show

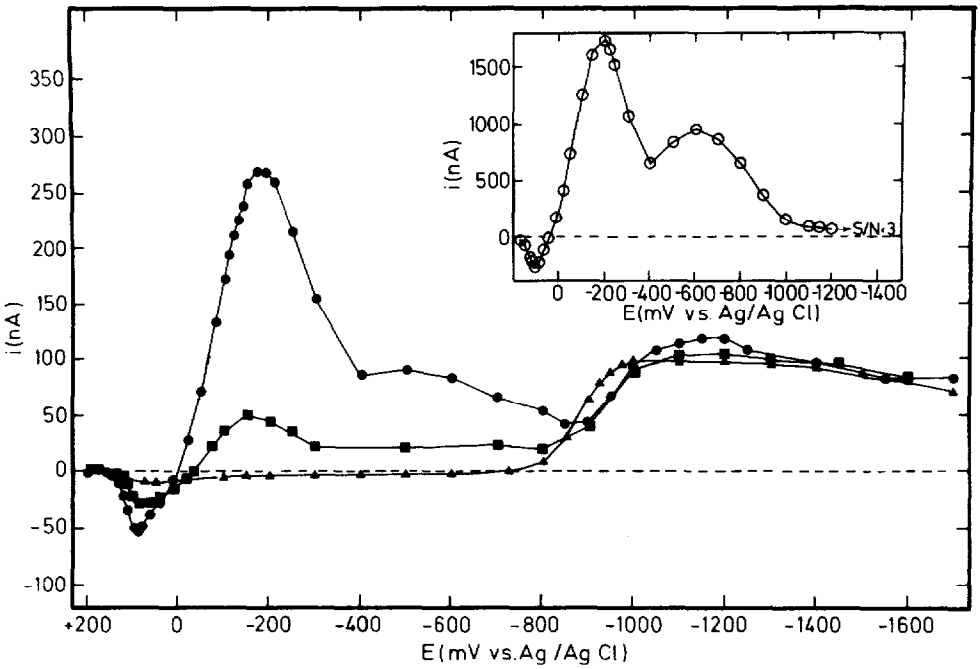


Fig. 3. Hydrodynamic voltammogram of omeprazole in mobile phases with different oxygen concentrations. Column, LiChrosorb RP-8, 5 μm; mobile phase, 32.5% (v/v) acetonitrile, pH 7.6; flow-rate, 1 ml min^{-1}; amount injected, 2.01 nmol (695 ng). Oxygen concentrations are relative to the air-saturated value and determined at −1.0 V *vs.* Ag/AgCl: ▲, 0.2%; ■, 4.5%; ●, 13%; ○, 100% (see inset).

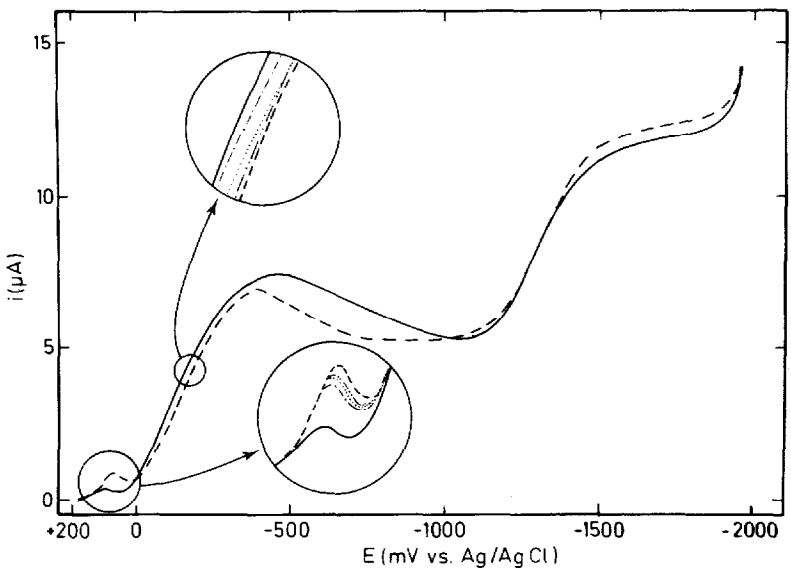


Fig. 4. Hydrodynamic polarogram of the air-saturated mobile phase containing different concentrations of omeprazole. Mobile phase, 32.5% (v/v) acetonitrile, pH 7.6; flow-rate, 1 ml min^{-1}. ------, 0 *M*; -·-·-·-·-, $1.0 \cdot 10^{-7}$ *M*; ·····, $1.0 \cdot 10^{-6}$ *M*; -·-·-·-·-, $3.4 \cdot 10^{-6}$ *M*; ——, $1.0 \cdot 10^{-5}$ *M*.

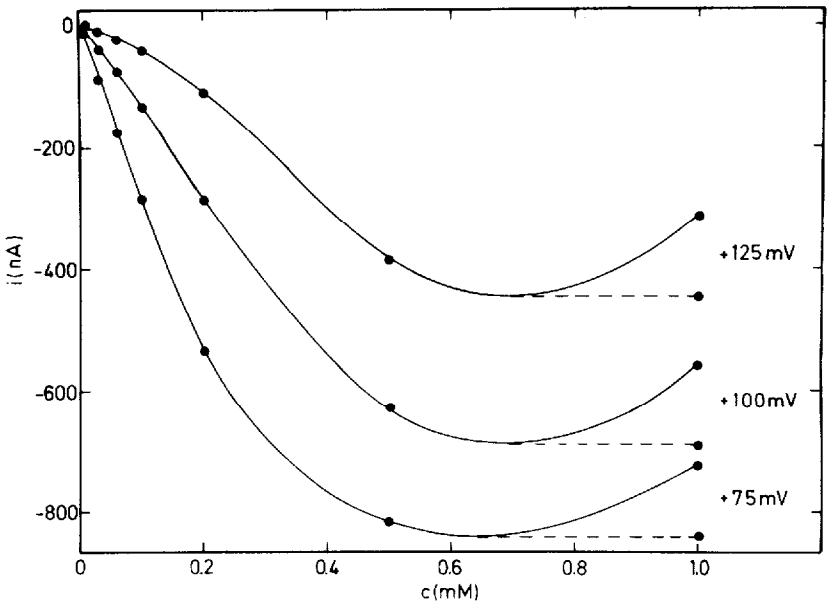


Fig. 5. Dependence of peak current on the concentration of omeprazole at three different potentials on the oxygen pre-wave around +0.1 V *vs.* Ag/AgCl. Mobile phase, 32.5% (v/v) acetonitrile, pH 7.6, saturated with air; flow-rate, 1 ml min^{-1}; volume injected, 20 μl. Solid lines, current measured at the apex of the peak; broken lines, maximum anodic current of the partially inverted peaks.

the two oxygen waves around 0 and −1 V, respectively, and a small oxygen pre-wave at +0.1 V. Fig. 4 also shows that omeprazole has an effect on both the wave height and the reduction potential of the first oxygen wave, and on the height of the prewave around +0.1V. Qualitatively, both the magnitude and the polarity of this difference in current between the two curves (Fig. 4) corresponds to the hydrodynamic voltammogram of omeprazole recorded in an air-saturated mobile phase (Fig. 3).

Polarograms were also taken in static solutions under normal polarographic conditions. In contrast to the results obtained in the flow system (Fig. 4), omeprazole did not cause any enhancement of the current of the first oxygen wave between 0 and −1 V. However, the inhibiting effect of omeprazole was manifested on the small pre-wave around +0.1 V.

Direct detection

Efforts to minimize the background current and the noise included careful exclusion of atmospheric oxygen from the entire flow system, optimization of the pump in order to deliver a pulseless flow, use of a pulse damper and passivation of the system up to the column with 6 *M* nitric acid followed by EDTA in a phosphate buffer solution of pH 8.

Typical values of the background current in the thoroughly deoxygenated system were 10 nA at −1.0 V and 20 nA at −1.2 V. Compared with air-saturated conditions, these values correspond to a residue of about 0.2% oxygen.

The noise level, measured as the average peak-to-peak difference of the per-

turbations of the signal, was about 80 pA at −1.0 V and about 160 pA at −1.2 V, which corresponds to 0.8% of the averaged signal.

With a thoroughly deoxygenated mobile phase, omeprazole gave a sigmoidal hydrodynamic voltammogram (Fig. 3). In order to achieve the most favourable sensitivity and detection limit, the detector potential was chosen as −1.2 V, corresponding to the beginning of the limiting current plateau.

The chromatograms in Figs. 1 and 2 and the hydrodynamic voltammogram in Fig. 3 were achieved with a column partly degraded in performance. Using a new Hibar column, a sensitivity of 90 A mol^{-1} was achieved for the detection of omeprazole, which is reduced by four electrons per molecule. This corresponds to a detection limit of 5 pmol, equivalent to 2 ng. The detection limit is defined here as three times the ratio between the noise and the sensitivity. The peak current was proportional to the amount of omeprazole injected in the investigated range from 0.7 ng, *i.e.*, below the operationally defined detection limit, to 700 ng, with a regression coefficient (r^2) equal to >0.99999. The precision was 1.0% when calculated from the standard deviation of repeated injections of a 2.01 nmol (695 ng) sample.

DISCUSSION

Indirect anodic peak

The small pre-wave around +0.1 V on the first oxygen wave appeared both in static solutions and in hydrodynamic systems. The wave height depended on the concentration of oxygen dissolved in the solution. The prewave is believed to be the results of an acetonitrile-assisted reduction of oxygen. The influence of omeprazole on this acetonitrile-induced oxygen reduction at the mercury electrode is explained as an inhibition effect resulting from the adsorption of omeprazole at the electrode

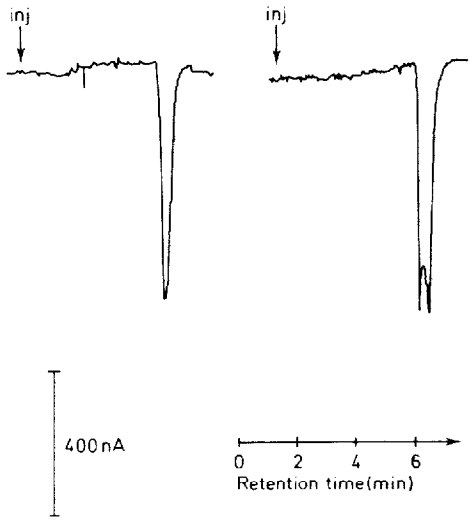


Fig. 6. Chromatogram of omeprazole applying indirect detection at +0.1 V *vs.* Ag/AgCl. Mobile phase, 32.5% (v/v) acetonitrile, pH 7.60, saturated with air; flow-rate, 1 ml min^{-1}; volume injected, 20 µl; concentration of omeprazole in the injected sample, 0.5 m*M* (left) and 1.0 m*M* (right, "partially inverted peak").

surface. It has been demonstrated elsewhere that structural analogues are strongly adsorbed at the mercury electrode[13,14]. The adsorption of omeprazole from a solution of the same compoosition as the mobile phase has been confirmed by normal pulse polarography in connection with this work. As the height of the anodic peak increased towards a maximum value with increasing amounts of substance injected (Fig. 5), it is assumed that maximum coverage of the electrode surface was reached by omeprazole at the higher amounts.

A peculiar shape of the anodic peak was observed for amounts injected exceeding that required to reach the maximum anodic value (Fig. 6). Here, the anodic current at the apex of the peak decreased below the maximum anodic value. This behaviour is believed to be the result of two counteracting effects, the relative importance of which depends on the concentration of omeprazole along the sample band eluted from the column. One is the inhibiting effect of omeprazole on the acetonitrile-induced oxygen reduction which dominates at detector concentrations below about 0.1 m*M*, corresponding to approximately 1 m*M* in the injected sample solution. The other effect, discussed in the following section, results in a cathodic current that is proportional to detector concentrations of omeprazole in a wider concentration range and becomes dominant at higher concentrations.

Indirect cathodic peak

The catodic peak obtained for omeprazole in the potential range 0 to −0.8 V where the compound is not electroactive (Fig. 3) agrees qualitatively with the hydrodynamic polarogram in Fig. 4. The presence of omeporazole induces, according to Fig. 4, both a small shift (about 25 mV) of the oxygen wave towards more positive potentials and an enhancement of the limiting current of this wave. Neither of these effects appeared when the corresponding polarographic measurements were performed in static solutions. If only the results from the flow system were considered, the influence of omeprazole on the oxygen wave might have been explained as an acceleration of the rate of the oxygen reduction, *i.e.*, a catalytic effect. However, a decrease in overpotential ought to cause a shift of the corresponding polarographic wave, no matter whether the measurements are performed in a flow system or in a static solution, contrary to our results. At present, no acceptable explanation can be suggested for the observed phenomenon.

Direct detection

The values achieved for the sensitivity and the detection limit illustrate that amperometric detection of reducible compounds at a mercury electrode gives detection limits in about the same range as UV detection of aromatic compounds. For example, the present LC system equipped with a variable-wavelength detector gives a detection limit for omeprazole of about 3 pmol (1 ng) at 280 nm. For the amperometric detector, a further reduction of the noise level and hence detection limit can be achieved by use of the hanging mercury drop electrode (HMDE) mode instead of the dropping mercury electrode (DME) mode applied in this work[8,15].

CONCLUSION

The adsorption of omeprazole (I) and the structurally related derivatives (II–

IV) at the surface of the mercury electrode seems to be a prerequisite for the appearance of the so-called indirect anodic peak. Compounds with only limited adsorption properties, benzaldehyde and phenol, were also investigated but could not be detected indirectly.

Although the sensitivity for omeprazole in the indirect detection mode, particularly in mobile phases containing higher oxygen concentrations, is higher than that with direct detection (Fig. 3), its utility for analytical purposes is doubtful. The increased noise level that results from the increased oxygen concentration, and the small degree of proportionality between peak height and amount injected, which varies with oxygen concentration and electrode potential, render the direct detection mode more advantageous.

REFERENCES

1 B. Drake, *Acta Chem. Scand.*, 54 (1950) 554–555.
2 W. Kemula, *Roczn. Chem.*, 26 (1952) 281–287.
3 R. J. Rucki, *Talanta*, 27 (1980) 147–156.
4 K. Bratin and P. T. Kissinger, *J. Liq. Chromatogr.*, 4 (Suppl. 2) (1981) 321–357.
5 W. A. MacCrehan and R. A. Durst, *Anal. Chem.*, 50 (1978) 2108–2112.
6 H. B. Hanekamp, W. H. Voogt, P. Bos and R. W. Frei, *Anal. Chim. Acta*, 118 (1980) 81–86.
7 K. Bratin and P. T. Kissinger, *Talanta*, 28 (1982) 365–370.
8 J. J. van der Lee, H. B. J. van der Lee-Rijsbergen, U. R. Tjaden and W. P. van Bennekom, *Anal. Chim. Acta*, 149 (1983) 29–38.
9 J. Y. Lewis, J. P. Zodda, E. Deutsch and W. R. Heineman, *Anal. Chem.*, 55 (1983) 708–713.
10 V. S. Bagotskii, L. N. Nekrasov and N. A. Shumilova, *Russ. Chem. Rev.*, 34 (1965) 717–730; *Usp. Khim.*, 34 (1965) 1697–1721.
11 C. J. van Velzen, M. Sluyters-Rehbach, A. G. Remijnse, G. J. Brug and J. H. Sluyters, *J. Electroanal. Chem.*, 134 (1982) 87–100.
12 C. J. van Velzen, A. G. Remijnse, M. Sluyters-Rehbach and J. H. Sluyters, *J. Electroanal. Chem.*, 142 (1982) 229–242.
13 B.-L. Johansson and B. Persson, *Anal. Chim. Acta*, 102 (1978) 121–131.
14 B.-L. Johansson and S. Wendsjö, *J. Electroanal. Chem.*, 147 (1983) 123–138.
15 J. B. F. Lloyd, *J. Chromatogr.*, 257 (1983) 227–236.