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EVALUATION OF A FLOW-THROUGH POLAROGRAPHIC DETECTOR FOR THE DETERMINATION OF REDOX COMPOUNDS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The utility of a polarographic detector, manufactured in this Institute, in the determination of redox substances eluted from high-performance liquid chromatographic columns has been studied using the direct current polarographic mode of operation. The conditions for separation and detection of nitroanilines, nitrophenols, chloronitrobenzenes, nitroalkanes, nitrofuran derivatives, nitronaphthalenes and *p*-methoxyazobenzenes on RP-18 or clathrate columns have been elucidated. The reproducibility, detectability and limits of application of this method are discussed.

INTRODUCTION

Polarographic detection was first employed for column liquid chromatography nearly 30 years ago[1]. Further development of this method, "chromatopolarography", yielded new procedures for determination of many different organic mixtures[2]. Recently, in the course of development of high-performance liquid chromatography (HPLC) a detector having a fixed, precisely defined geometry giving very reproducible results, FTPD-101, has been elaborated[3] and manufactured in the workshops of this Institute for routine studies. In this paper the evaluation of the detector for determination of redox substances in the direct current (d.c.) polarographic mode of operation is presented.

The application of d.c. polarographic detection to liquid chromatography is limited not only by the nature of the substances to be detected, but also by the separation systems. It can be used only for so-called redox substances and can be performed only in polar solvents, when an inert "background" electrolyte is present in the percolating solution to control the diffusion mass transport of a redox substance to the electrode surface; moreover, a constant pH value in the solution must often be maintained.

It seemed promising to apply this detection method to straight-phase gel permeation, ion-exchange and especially clathrate and reversed-phase chromatography. Applications of the FTPD-101 detector to the last two kinds of chromatography are exemplified in this paper.

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EXPERIMENTAL

The solvents and all the reagents were p.a. grade. An HPLC apparatus Type 302 (Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland) equipped with a 5-μl high-pressure injection valve was used in chromatographic experiments. For glass tube columns in the clathrate systems another glass pumping apparatus was used. Commercially available instruments were employed for the polarographic recording, *i.e.*, OH-102 (Radelkis, Budapest, Hungary), LP-7 (Laboratorni Přistroje, Prague, Czechoslovakia) and PLP 321 XY polarographs (Cobrabid, Warsaw, Poland).

Separations were performed with stainless-steel columns (250 × 4 mm I.D.), slurry packed at 435 kg m^{-2} using the "balanced weight" technique with 10-μm LiChrosorb RP-18 (E. Merck, Darmstadt, G.F.R.), and glass columns (60 × 4 mm I.D.) dry or slurry (at 2 kg m^{-2}) packed with clathrate (particle size 0.2–10 μm). which was prepared from the basic clathrate of formula β-[Ni(NCS)$_2$(4-methylpyridine)$_4$]·0.7 (4-methylpyridine) (ref. 4); the slurry consisted of an aqueous solution containing NH_4SCN and 4-methylpyridine.

Mobile phase solutions for RP-18 columns were deaerated with argon; those for clathrate columns were not deaerated. The sample concentrations varied in the range 0.5–50 m*M*. The flow-rate from RP-18 and clathrate columns was 0.5 ml/min and 10 ml/h, respectively, unless otherwise stated. All mixtures to be separated were prepared from pure components.

RESULTS AND DISCUSSION

Separations on RP columns

Figs. 1–4 show the analyses of nitrophenols, chloronitrobenzenes, nitrofuran derivatives and homologous nitroalkanes, with the FTPD-101 detector. This detector is sensitive to oxygen dissolved in the injected sample, oxygen peaks being recorded on the chromatograms (Figs. 1 and 2). If the presence of such peaks is disadvantageous, the sample must be deaerated before injection, as in the case of the experiments shown in Figs. 3 and 4.

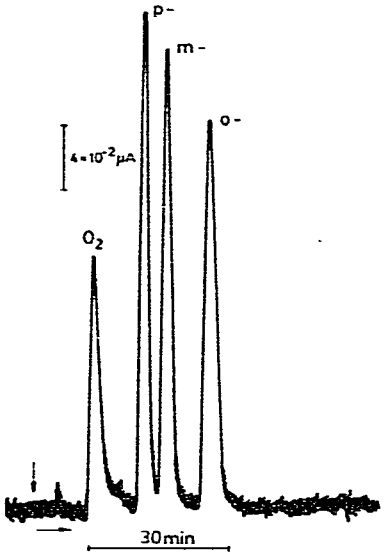
Separations on clathrate columns

For separation of isomers, the clathrate sorbents[5] formed by some Werner-type complexes were employed. Their main representatives are of the formula β-[Ni(NCS)$_2$(4-methylpyridine)$_4$]·G, where G is a mixture of a pyridine base, *e.g.*, 4-methylpyridine and an aliphatic organic solvent. The mobile phases used with the clathrate systems are aqueous solutions containing NCS^- ions, 4-methylpyridine and also other aliphatic and aromatic components, used as modifiers of the chromatographic properties of the sorbent.

It should be noted that the polarographic detector is especially suited for clathrate systems, in contrast to the UV detector which is useless if the mobile phase contains pyridine bases and thiocyanates.

Figs. 5–8 show chromatograms of isomeric nitropropanes, nitronaphthalenes, *p*-methoxyazobenzenes and dinitrobenzenes recorded with the FTPD-101 detector.

In contrast to the RP-18 columns, the high performance of the clathrate col-



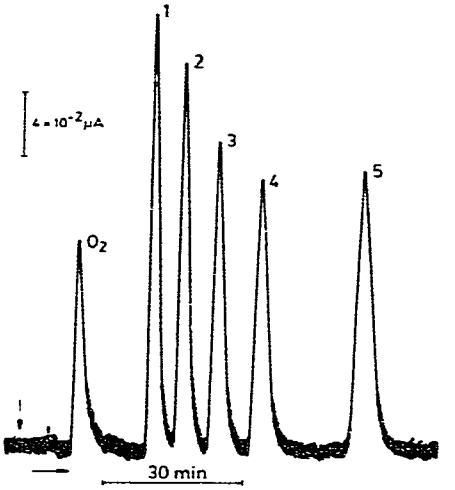
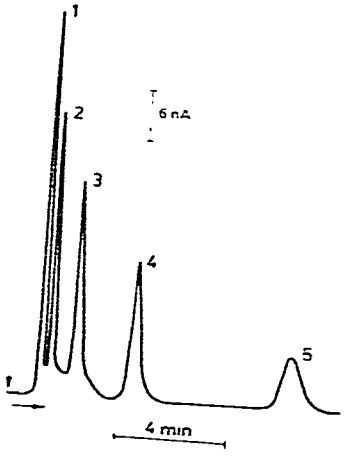


Fig. 1. Elution curve of a mixture of 3.0 m*M* *o*-, *m*- and *p*-nitrophenols. Sample size: 5 μl. Mobile phase: methanol–1/15 *M* phosphate buffer, pH 6.0 (40:60). Detection potential: −1.2 V *vs.* mercury pool anode.

Fig. 2. Elution curve of a mixture of 5.0 m*M* 1-chloro-2,4-dinitrobenzene (1), *o*-chloronitrobenzene (2), *p*-chloronitrobenzene (3), *m*-chloronitrobenzene (4) and 1,4-dichloro-5-nitrobenzene (5). Sample size: 5 μl. Mobile phase: methanol–1/15 *M* phosphate buffer, pH 6.0 (50:50). Detection potential as in Fig. 1.



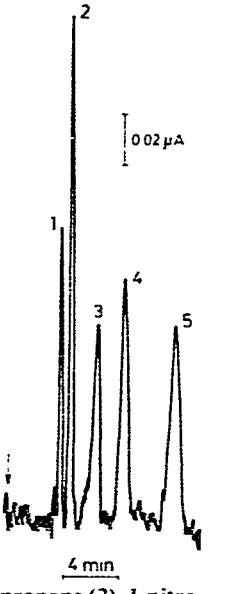


Fig. 3. Elution curve of a mixture of 1.0 m*M* nitromethane (1), nitroethane (2), 1-nitropropane (3), 1-nitro-*n*-butane (4) and 1-nitro-*n*-pentane (5). Sample size and mobile phase as in Fig. 1. The sample was deaerated with argon. Detection potential: −1.2 V *vs.* Ag/AgCl. Flow-rate: 2 ml/min.

Fig. 4. Elution curve of a mixture of 0.3 m*M* 5-nitrofuran-2-carboxylic acid (1), β-(5-nitrofuryl-2)acrylic acid (2), 5-nitro-2-furaldehyde (3), N-[β-(5-nitrofuryl-2)acrylidene]-1-aminohydantoin (furagin, 4) and β-(5-nitrofuryl-2)acrolein (5). Sample size: 10 μl. Mobile phase: methanol–1/15 *M* phosphate buffer, pH 6.0 (40:60). The sample was deaerated with argon. Detection potential: −1.0 V *vs.* mercury pool anode.

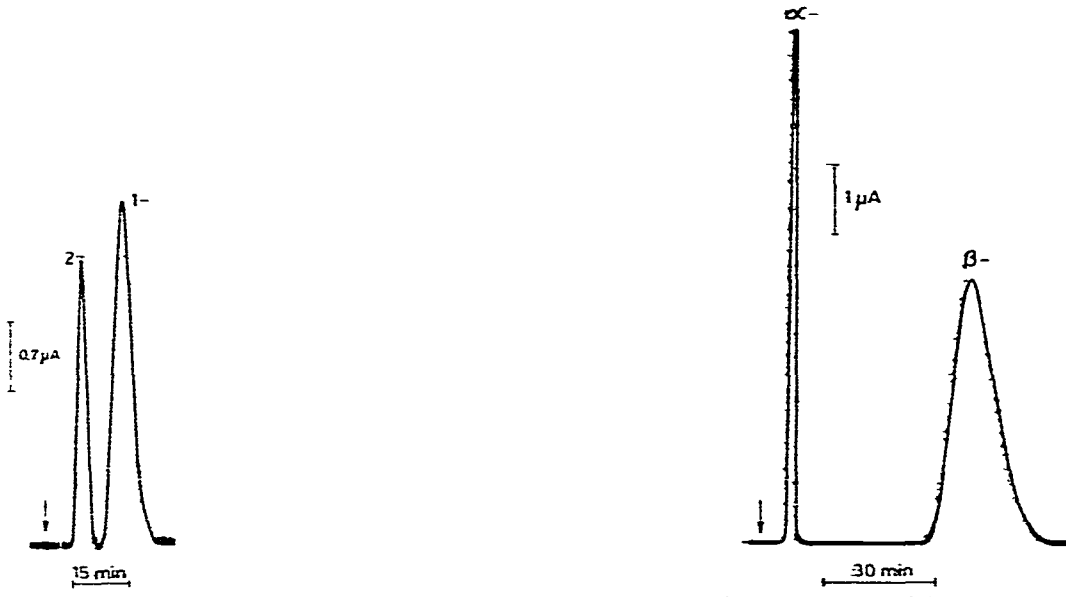


Fig. 5. Elution curve of a mixture of 0.02 *M* 2-nitropropane (1) and 1-nitropropane (2). Mobile phase: 0.1 *M* NH_4SCN, 0.1 *M* 4-methylpyridine in water. Sample size: 10 μl. Detection potential: −1.0 V *vs.* mercury pool anode.

Fig. 6. Elution curve of a mixture of 0.25 m*M* α-nitronaphthalene and 1.0 m*M* β-nitronaphthalene. Mobile phase: 0.2 *M* NH_4SCN + 0.5 *M* methylpyridine in 50% (v/v) aqueous methanol. Sample size: 20 μl. Detection potential: −0.7 V *vs.* mercury pool anode.

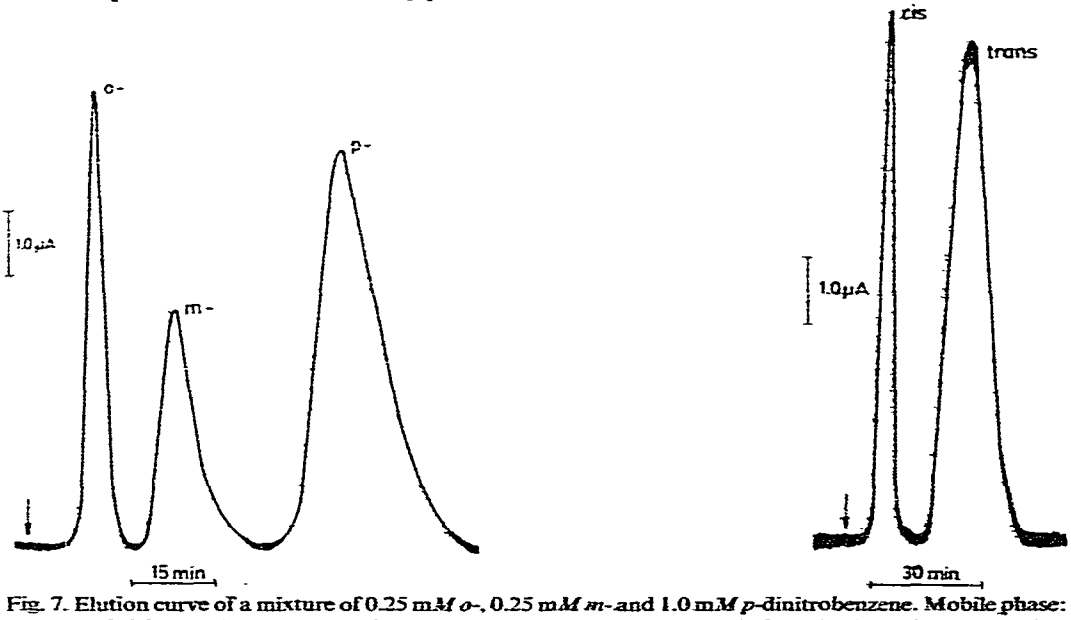


Fig. 7. Elution curve of a mixture of 0.25 m*M* *o*-, 0.25 m*M* *m*- and 1.0 m*M* *p*-dinitrobenzene. Mobile phase: 0.1 *M* NH_4SCN + 0.2 *M* 4-methylpyridine in 50% aqueous methanol. Sample size: 20 μl. Detection potential: −0.8 V *vs.* mercury pool anode.

Fig. 8. Elution curve of a mixture of *cis* and *trans* *p*-methoxyazobenzenes. Initial concentration of *trans* azobenzene: 10 m*M*. The *cis*-isomer was generated by exposure of this solution to light. Sample size: 15 μl. Mobile phase: 0.2 *M* NH_4SCN + 2.2 *M* 4-methylpyridine in 58% aqueous methanol. Detection potential: −0.7 V *vs.* mercury pool anode.

umns is not due to high efficiency but to the remarkable selectivity of the sorbent of relatively moderate efficiency. Therefore the separation of many mixtures of isomers can be performed using clathrate columns only a few centimetres long. The recording of the chromatograms is reliable if the detection volume is small, as in the case of the FTPD-101 detector[6].

Linearity, reproducibility and detectability

For all investigated mixtures the linear dynamic concentration range of the detector extends over at least three decades down to *ca.* $5 \cdot 10^{-4}$ *M* for a sample volume of 5 μl (Fig. 9a, b). From the slope of the dependences in Fig. 9b and a the sensitivity of the detector and detector response index were calculated as $k = 5.2 \cdot 10^{-5}$ C 1 mol^{-1} and $n = 0.96$ (correlation coefficient 0.999), respectively.

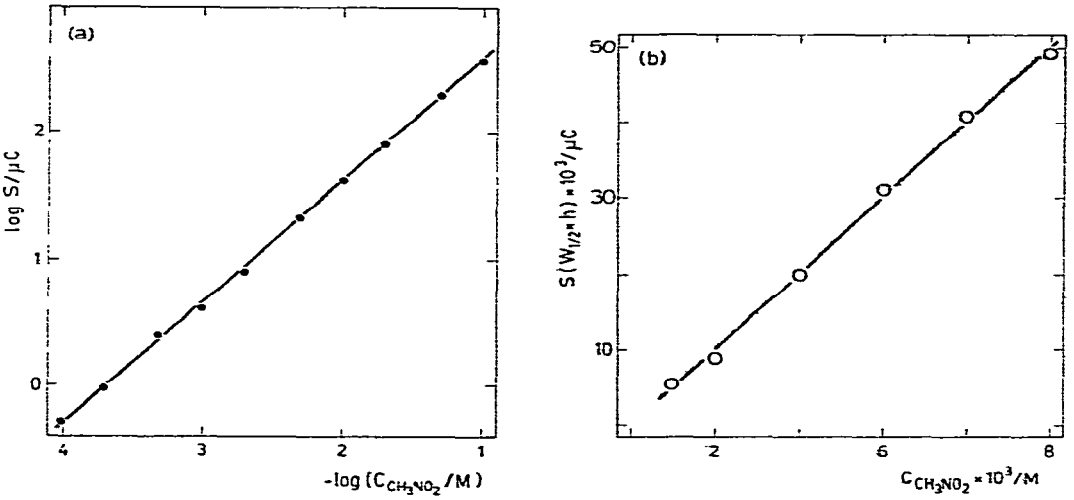


Fig. 9. HPLC calibration curves for nitromethane. Dependences of (a) the logaritĥm of the peak area *S* (μC) on the logarithm of the nitromethane concentration (flow-rate 2 ml min^{-1}) and (b) the peak area on the nitromethane concentration (flow-rate 1 ml min^{-1}). Solid phase: LiChrosorb RP-18, 10 μm. Sample size: 5 μl. Mobile phase as in Fig. 3. $W_{1/2}$ = Half-width; *h* = peak height.

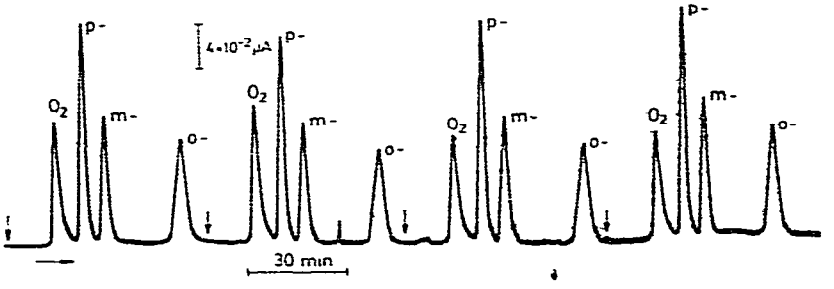


Fig. 10. Four consecutive chromatograms of a mixture of $2 \cdot 10^{-3}$ *M* *o*-, *m*- and *p*-nitroanilines. Sample size: 5 μl. Solid phase: LiChrosorb RP-18, 10 μm (250 × 4 mm I.D.). Mobile phase: methanol + 1/15 *M* phosphate buffer, pH 6.0 (30:70). Detection potential: −1.2 V *vs.* mercury pool anode.

The reproducibility is illustrated by the four consecutive chromatograms of a mixture of *o*-, *m*- and *p*-nitroanilines in Fig. 10. The standard deviation of the peak heights from nine consecutive injections of nitromethane (conditions as in Fig. 3) was 3% and for *p*-nitroaniline (conditions as in Fig. 9) the standard deviation from six consecutive injections was 3.5%. Taking into account that the standard deviation for the injection valve was 2.5% (manufacturer's information), we conclude that the reproducibility achieved with the FTPD-101 detector meets the requirements expected for HPLC determinations. It should be noted that such a reproducibility is possible only if the slope of the dropping mercury electrode, *i.e.*, the position of detector, is kept strictly constant in the course of experiments.

The experiments performed show that the detectability of the majority of the substances is in the range of 10–100 ng. Thus, for aromatic compounds, *e.g.*, nitro compounds, the detectability of the FTPD-101 detector is about one or two orders of magnitude worse than that of commonly used UV detectors, whereas for aliphatic nitro compounds it is at least ten times better.

The applicability of the FTPD-101 detector to the LC of other mixtures is currently being investigated.

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