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Note

New electrochemical detector for high-performance liquid chromatography

The differential amperometric detector

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A common problem with electrochemical detectors in high-performance liquid chromatography (HPLC) is the high background current caused by traces of electroactive impurities in the eluent. The main sources of these impurities are dissolved oxygen and traces of metal dissolved from the stainless-steel column, tubing and pump[1–3], and from the needle of the syringe[4] with which the sample is introduced into the system. Also, the pH and the electrode potential can significantly affect the background current[5]. A high background current can cause problems when measuring small changes caused by electroactive substances; especially when it concerns reducible substances because reduction of dissolved oxygen, trace-metal ions and hydrogen ions can be difficult to eliminate[6]. To eliminate trace-metal ions in the eluent, Felice *et al.*[2] advise the use of glass or PTFE-lined tubing and glass columns; other investigators[7] have also used glass columns in combination with electrochemical detection. Nevertheless, an off-set circuit to compensate for the background current is necessary. To overcome the problem of the high-background current caused by the electrode potential, the pH of the eluent and traces of electroactive impurities in the eluent, we have developed a differential amperometric detector.

The principle of this detector is the same as that of a differential refractometer detector or a UV detector with sample and reference cells. These detectors do not measure absolute changes in refractive index or UV absorption in the column effluent, but measure the difference in these properties between the influent and effluent of the column.

DETECTOR DESIGN

In principle, the differential amperometric detector consists of two identical amperometric detectors coupled by a differential amplifier. Four electrodes are connected at the detector, namely, two working electrodes (one for the column influent and the other for the effluent), a reference electrode and an auxiliary electrode. The potential of the two working electrodes relative to the reference electrode is always the same and can be adjusted to any value between +3 and −3 V. The working

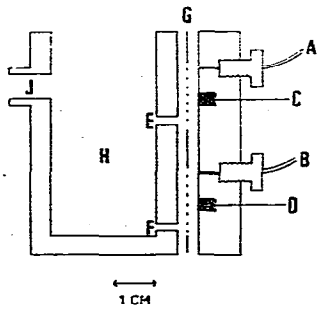

Fig. 1. Thin-layer flow-cell of the differential amperometric detector. A = Inlet sample cell; B = inlet reference cell; C = vitreous-carbon working electrode (sample cell); D = vitreous-carbon working electrode (reference cell); E = sample-cell outlet; F = reference-cell outlet; G = spacer (100 μm thick); H = compartment for reference and auxiliary electrodes; J = overflow to waste.

potential depends on the substances to be detected and on the potential at which the supporting electrolyte begins to decompose.

The flow-cell of the detector (see Fig. 1) comprises two working electrodes in two identical thin-layer cells, which are in contact with a compartment containing the reference and auxiliary electrodes. In this cell, the distance between working electrode and reference electrode is small, so that the uncompensated resistance is also small ($\pm$ 30 kΩ). Fig. 2 shows a schemetic diagram of the detector in combination with the HPLC equipment. Two liquid streams start from the eluent vessel. One stream is pumped by a high-pressure pump through a manometer, a damping coil, an injection valve and the column to the sample cell of the differential detector. The other stream is pumped by a low-pressure pump through a manometer, a damping coil and a length of stainless-steel tubing (equal to that through which the first stream has passed) to the reference cell. The difference in electrochemical activity between the sample and the reference cells is measured and registered on the recorder chart. In this way, the signal is continuously compensated for the background current.

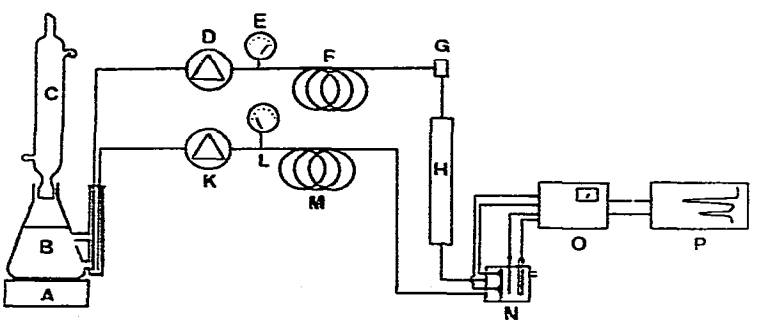

Fig. 2. Detector in combination with HPLC equipment. A = Heater; B = bulk mobile phase; C = reflux cooler; D = high-pressure pump; E = manometer; F = damping coil; G = sample-injection system; H = column; K = low-pressure pump; L = manometer; M = damping coil; N = detector flow-cell; O = detector electronics; P = recorder.

CHEMICALS AND INSTRUMENTATION

As test compound, we used L-ascorbic acid, because it is easily oxidised. In order to minimise oxidation by air, the solvent for the samples was de-aerated by passage of a stream of nitrogen, and each sample was freshly prepared. An aqueous solution of 0.1 *M* acetic acid adjusted to pH 4.5 with concentrated sodium hydroxide solution was used as mobile phase, and this was de-gassed by continuous refluxing.

The liquid chromatograph was constructed from individual components. The pumps were a high-pressure Milton Roy duplex mini-pump and a low-pressure FMI lab pump. The sample-injection system consisted of a Rheodyne Model 70-10 valve valve with a 20-μl sample loop. A CDM2 radiometer conductivity meter was also used.

The two working electrodes of the detector were of vitreous carbon (Tokai, GC-30S); a saturated calomel electrode reference electrode was used, and a platinum wire formed the auxiliary electrode.

RESULTS AND DISCUSSION

The electronic noise of the detector, measured with a simple electronic dummy cell instead of the differential flow-cell, was *ca.* 0.03 nA; no base-line drift was observed.

At present, the detector has only been tested in liquid streams without a chromatographic column. Fig. 3 shows the response produced by 5-ng samples of L-asorbic acid in 20-μl portions. The detection limit (*ca.* 1 ng of ascorbic acid per 20-μl sample) is limited by pulsation in the flow-rate caused by the reciprocating pump and not by lack of sensitivity of the detector. It appears that, in a chromatographic system with an anion-exchange column (and hence much better pulse-damping) the detection limit is still *ca.* 1 ng despite dilution of the sample through the column.

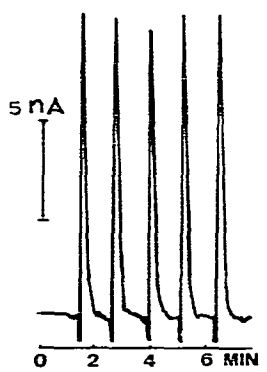

Fig. 3. Response peaks of the detector to 20-μl samples containing 5 ng of L-ascorbic acid. Working-electrode potential, 900 mV (*vs.* the S.C.E.).

Mixing of the mobile phase with an organic solvent (*e.g.*, ethanol) produces a lower detector response, but does not affect the detection limit or the linear range of detector response (as far as could be measured) (see Fig. 4). The maximum detector response is 1000 nA, and that is inadequate for measuring the upper part of the linear-response range. The relative standard deviation for samples in the lower-

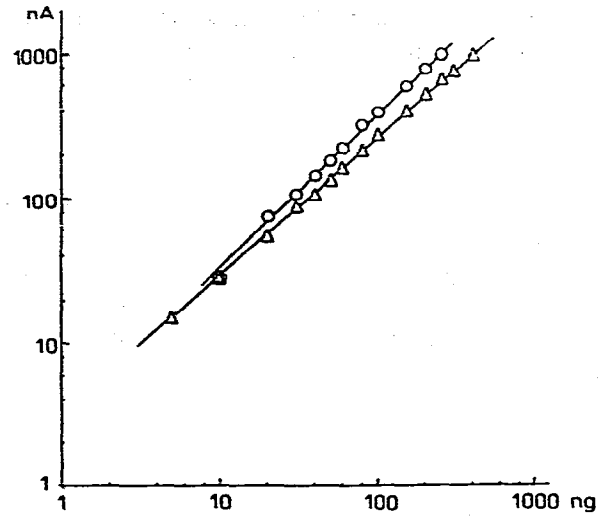

Fig. 4. Detector response to 20-μl samples of L-ascorbic acid solution. Working-electrode potential, 900 mV (*vs.* the S.C.E.); mobile-phase flow-rate, 0.5 ml/min. ○–○–○, aqueous 0.1 *M* acetate buffer (pH 4.5); △–△–△, ethanol–aqueous 0.1 *M* acetate buffer pH 4.5 (2:3).

response range is *ca.* 3% for 5-ng samples, *ca.* 1.5–2% for 100-ng samples and less than 1% for samples of 300 ng or more.

The impedance of the mobile phase, and thus the uncompensated resistance between the reference electrode and the working electrodes, increases significantly when the aqueous mobile phase is mixed with an organic solvent. This can cause deviations in the linear response of the detector, especially at higher concentrations (see Fig. 5). In order to avoid such deviations, the oxidation (or reduction) potential

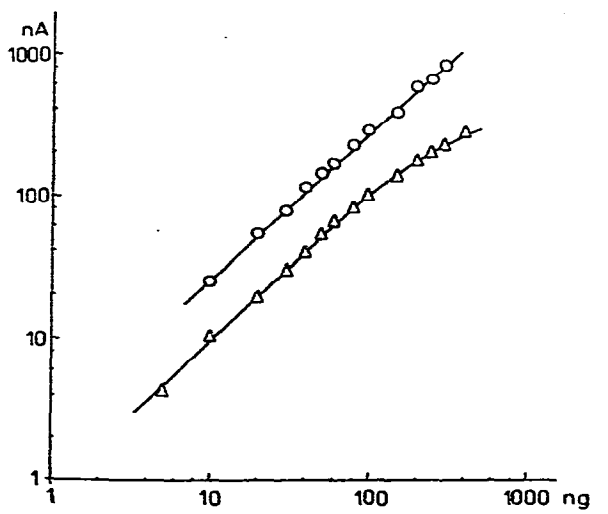

Fig. 5. Detector response with the working-electrode potential at 500 mV (*vs.* the S.C.E.); other conditions as in Fig. 4.

of the working electrodes should be chosen (if possible) to be somewhat higher than is absolutely necessary; the deviation (caused by the *iR* drop) then manifests itself later (compare Figs. 4 and 5).

CONCLUSIONS

The differential amperometric detector seems most promising. With a better pulse-damping device, or a pulse-free pumping system, it should be possible to lower the detection limit to the picogram range. The automatic compensation of the background current by means of the differential measurements is satisfactory; more detailed information about the detector and its application in reversed-phase and ion-exchange chromatography will be given in later papers.

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