Journal of Chromatography, 541 (1991) 257–264
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 914

Sensitivity of electrokinetic detection of acidic aromatic nitro derivatives using column as a detector

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(First received January 25th, 1990; revised manuscript received October 8th, 1990)

ABSTRACT

A stainless-steel analytical column of 2 mm I.D. served as an electrokinetic detector. The minimum detectable amounts of nitrophenols eluted from both unmodified and chemically modified silica gel with *n*-heptane–acetone (90:10) acidified with 10 ppm of nitric acid were of the order of 10^{-9}–10^{-11} mol. The minimum detectable quantity of dipicrylamine, $1.8 \cdot 10^{-13}$ mol, represents a detection sensitivity one order of magnitude higher than the highest possible using photometric detection.

INTRODUCTION

Electrokinetic detection is considerably selective towards ionizing solutes [1,2] and suitable even for columns of small dimensions [3]. The technique is based on the measurement of the charge acquired by the liquid flowing along the solid surface.

Charging of the liquid results from the tendency for any phase (in a physicochemical sense) to accept the charge. This tendency depends only on the composition [4]. If two phases of different chemical composition come into contact, their charges and also those originating from the contact between the two phases will redistribute. As a result, the positive charge is in excess in one phase and the negative charge is in excess in the other. Redistributed charges of opposite polarities attract one another. They are therefore localized at the plane of contact of the phases and form an electric double layer.

The model of the electric double layer at the solid–liquid boundary (Fig. 1) assumes that the charge acquired by the liquid occurs within a certain distance from the solid surface [4]. The thickness of the electric double layer is usually 5–200 nm in aqueous solutions of electrolytes [4]. In low-polarity mixtures of organic solvents it can even be more than 10 μm [5]. If the liquid is made to move by the pressure gradient, it carries part of the charge scattered in the liquid [4]. The charge carried can be trapped on the electrode and then measured as the streaming current, I_s, and sometimes also as

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0021-9673/91/$03.50

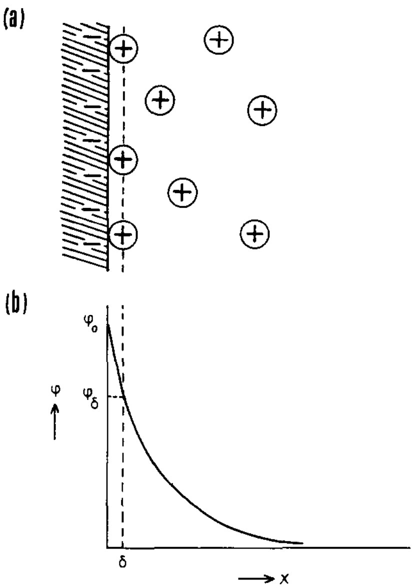

Fig. 1. Stern's model of electric double layer at a solid–liquid interface. (a) Distribution of charges on the solid surface and in the liquid. (b) Course of the potential created by the solid-phase charge in the liquid. δ = Thickness of the compact layer of electric double layer; ϕ_0 = surface potential of the solid-phase; ϕ_δ = potential at the boundary between the compact and the diffuse layers of the electric double layer.

the streaming potential [4,6]. Generation of responses by solute zones results from the dependence of the redistributed charge on the flowing liquid composition.

Minimum detectable quantities (MDQ) of ionizable solutes of the order of 10^{-9}–10^{-11} are typical of reversed-phase columns of I.D. 4–4.6 mm [1,2]. An MDQ one order of magnitude lower was reported in a single instance [7]. Post-column electrokinetic detectors were used. The measured signal was generate either in an empty or a filled capillary. The disadvantage of these detectors, *viz.*, a high pressure drop, can be eliminated if the chromatographic column is used as a source of the measured signal [3]. If the column was operated as a detector, carboxylic acids were detected in a reversed-phase system with a sensitivity [8] comparable to that obtained with detectors behind the column [1,2]. The possibility to reaching sufficiently high sensitivities in normal-phase systems with the column as a detector was therefore tested in this work.

EXPERIMENTAL

The design used for the sensing of the streaming current from the metallic column jacket, the measurement procedure and the apparatus used were described previously [8]. The streaming current was measured with a Model 427 current amplifier (Keithley Instruments, Cleveland, OH, U.S.A.). If not stated otherwise, 150 × 2 mm I.D. columns were used. Silasorb 300 (mean particle diameter d_p = 12.4 μm, specific surface area S = 350 m^2/g, specific pore volume v_p = 0.79 ml/g) supplied by Lachema (Brno, Czechoslovakia) served as a stationary phase. Methyl (Silasorb C_2), octyl

TABLE I

INJECTED SOLUTES

Solute	pK_a[a]	Abbreviation
3-Nitrophenol	8.29	3-NP
4-Nitrophenol	7.15	4-NP
2,4-Dinitrophenol	4.08	2,4-DNP
2,5-Dinitrophenol	5.15	2,5-DNP
Picric acid	0.80	HPi
Dipicrylamine	5.42[b]	DPA

[a] Ref. 9.
[b] Ref. 10.

(Silasorb C_8) and cyanopropyl (Silasorb Nitrile) derivatives were prepared from Silasorb 300 by common derivatization procedures. A methyl derivative (Silasorb 600 C_2) was also prepared from Silasorb 600 (Lachema) (d_p = 12.4 μm, S = 460 m^2/g, v_p = 0.80 ml/g).

The filled columns were flushed with 200 ml of ethanol (flow-rate 2 ml/min), 40 ml of distilled water (1 ml/min), 100 ml of 20 mM nitric acid (0.2 ml/min), 25 ml of distilled water (1 ml/min) and 40 ml of ethanol (1 ml/min). Prior to the measurement each column had been being flushed with the mobile phase (0.5 ml/min) until the streaming current generated by the mobile phase (background current), I_s, was constant for at least 1 h.

n-Heptane, acetone and nitric acid used for the preparation of the mobile phases were of analytical-reagent grade. Solutes of analytical-reagent grade (Table I) were injected by a microsyringe as solutions in acetone. pK_a values are valid for aqueous solutions at 25°C.

RESULTS AND DISCUSSION

The solute response is expressed as the change of the background current, ΔI_s, at the zone maximum. In reversed-phase systems the detection sensitivity increases with the background current, I_s, [1] and solute pK_a [1,7]. Chemical derivatization of silica gel increases the acidity of residual silanols [11] and hence also I_s. The influence of the derivatization appeared very marked with the n-heptane–acetone (90:10) mobile phase [5] and this mobile phase was therefore chosen for the present study. The acidity of nitrophenol depends on the number and position of nitro groups in the molecule (Table I). Dipicrylamine was used as a nitro compound of another type.

With n-heptane–acetone (90:10) mobile phase all nitrophenols were eluted from the column filled with Silasorb 300. However, the zones of more acidic solutes, especially picric acid, were asymmetric. The addition of 10 ppm of nitric acid suppressed zone asymmetry (Fig. 2). The elution time of dipicrylamine was longer than 2 h.

The electrokinetic detection has character of mass detection in the experimental arrangement used [12]. The charge generated by unit mass of the solute (the charge yield) is therefore an exact theoretical measure of the detection sensitivity. Charge

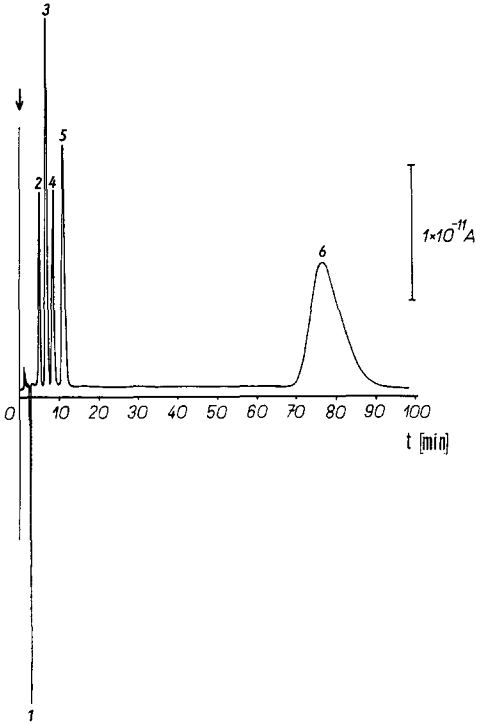

Fig. 2. Chromatogram of a mixture of phenol nitro derivatives. Column, 150 × 2 mm I.D.; stationary phase, Silasorb 300; mobile phase, *n*-heptane–acetone with 10 ppm of HNO_3; flow-rate, 0.2 ml/min; noise, $1 \cdot 10^{-13}$ A. Peaks: 1 = acetone (1 μl); 2 = 2,5-DNP ($4.0 \cdot 10^{-7}$ mol); 3 = 2,4-DNP ($3.0 \cdot 10^{-7}$ mol); 4 = 3-NP ($4.0 \cdot 10^{-7}$ mol); 5 = 4-NP ($2.0 \cdot 10^{-7}$ mol); 6 = HPi ($3.0 \cdot 10^{-7}$ mol).

yields obtained from the chromatogram in Fig. 2 and re-calculated for 1 mol (molar charge yields, *Q*) are summarized in Table II. With a common assumption that only univalent ions exist in low-polarity liquids [13], *Q* can theoretically reach 96 500 C/mol. The calculated *Q* values are 4–7 orders of magnitude lower.

TABLE II

MOLAR CHARGE YIELDS (*Q*) AND MINIMUM DETECTABLE QUANTITIES (MDQ) OF AROMATIC NITRO DERIVATIVES IN A SEPARATION SYSTEM OF SILASORB 300 WITH *n*-HEPTANE–ACETONE (90:10) ACIDIFIED WITH 10 ppm OF NITRIC ACID

The values were calculated from the chromatogram in Fig. 2.

Solute	*Q* (C/mol)	MDQ (mol)
3-Nitrophenol	$1.8 \cdot 10^{-3}$	$5.8 \cdot 10^{-9}$
4-Nitrophenol	$5.4 \cdot 10^{-3}$	$2.5 \cdot 10^{-9}$
2,4-Dinitrophenol	$3.9 \cdot 10^{-3}$	$2.2 \cdot 10^{-9}$
2,5-Dinitrophenol	$1.5 \cdot 10^{-3}$	$5.3 \cdot 10^{-9}$
Picric acid	1.6	$6.5 \cdot 10^{-11}$

TABLE III

INFLUENCE OF DIFFERENT ORGANIC GROUPS MODIFYING SILICA GEL SURFACE ON BACKGROUND CURRENT (I_s), CAPACITY FACTORS (k'), MOLAR CHARGE YIELDS (Q) AND MINIMUM DETECTABLE QUANTITIES (MDQ) OF SELECTED SOLUTES IN *n*-HEPTANE–ACETONE (90:10) MOBILE PHASE ACIDIFIED WITH 10 ppm OF NITRIC ACID

For other conditions of measurements, see Fig. 2.

Stationary phase	I_s (pA)	4-NP			HPi		
		k'	$Q \cdot 10^3$ (C/mol)	MDQ $\cdot 10^{10}$ (mol)	k'	Q (C/mol)	MDQ $\cdot 10^{10}$ (mol)
Silasorb 300	15	2.9	5.4	25	27.6	1.6	0.7
Silasorb C_2	15	–	–	–	5.1	2.0	1.1
Silasorb C_8	300	–	–	–	1.7	3.3	3.2
Silasorb Nitrile	90	0.9	3.2	200	7.3	0.9	1.9
Silasorb 600 C_2	25	1.3	2.3	64	8.8	1.9	0.5

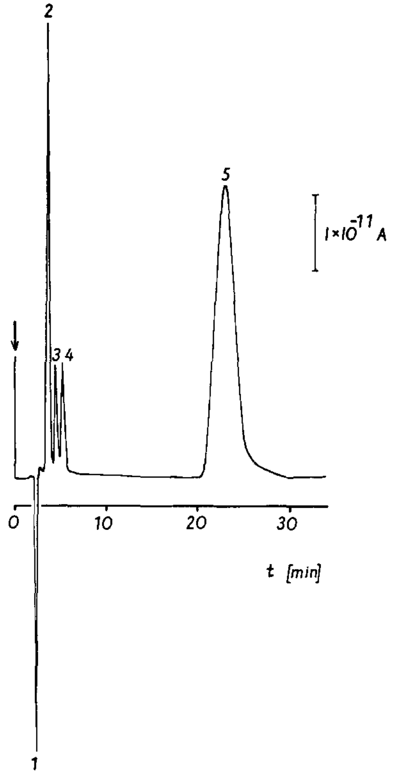

Fig. 3. Chromatogram of a mixture of phenol nitro derivatives. Stationary phase, Silasorb 600 C_2. For other conditions, see Fig. 1. Noise, $3 \cdot 10^{-13}$ A. Peaks: 1 = acetone (1 μl); 2 = 2,4-DNP ($3.0 \cdot 10^{-7}$ mol); 3 = 3-NP ($2.0 \cdot 10^{-7}$ mol); 4 = 4-NP ($1.5 \cdot 10^{-7}$ mol); 5 = HPi ($3.0 \cdot 10^{-9}$ mol).

The practical measure of detection sensitivity is the MDQ. The MDQs in Table II were calculated as the minimum quantities of solute causing a response equal to twice the background current noise (peak-to-peak).

The background current is strongly dependent on the groups bonded to silica gel [5]. The influence of bonded groups on both Q and MDQ was therefore investigated. However, neither the expected growth of the molar charge yield nor an increase in MDQ with increasing I_s was found (Table III). Zones of more acidic solutes and picric acid in particular were highly asymmetric on Silasorb C_2 and Silasorb Nitrile. A reference experiment with Silasorb 600 C_2 showed (Fig. 3) that bad peak shapes on both Silasorb C_2 and probably Silasorb Nitrile cannot be ascribed to bonding of organic groups unambiguously. Dipicrylamine was detected with a higher sensitivity than nitrophenols with elution from any modified silica gel. The highest detection sensitivity for dipicrylamine was observed with Silasorb C_2 (Fig. 4).

The MDQ of dipicrylamine ($k' = 8.8$) calculated from Fig. 4, $1.8 \cdot 10^{-13}$ mol, is one order of magnitude lower than the lowest value reported for a post-column electrokinetic detector (Table IV). In this experiment [7], sulphuric acid was eluted in the column exclusion volume with water as the mobile phase. The best results for nitrophenols are comparable to the highest sensitivities measured using reversed-phase systems with post-column electrokinetic detectors (Table IV).

The composition of the mobile phase was not optimized from the viewpoint of the detection of individual compounds. The nitric acid concentration was chosen tentatively to ensure the symmetry of the zone of the most acidic solute, picric acid, on elution from Silasorb 300. The responses of all the solutes decreased about five-fold with increase in nitric acid concentration to 20 ppm. During the elution from the column filled with Silasorb 300 modified by octadecyl groups, the solute responses increased more than ten-fold after the replacement of acetone mobile phase with acetonitrile [8]. It can therefore be expected that the sensitivity of the electrokinetic detection can be increased further by optimizing the chromatographic system composition from the viewpoints of both separation and electrokinetic detection. Comparison of Q values summarized in Tables II and III with the theoretical value $Q = 96\ 500$ C/mol supports such an expectation.

To compare the sensitivity of the electrokinetic detection that has so far been achieved with that of photometric detection let us consider a good UV spectrophoto-

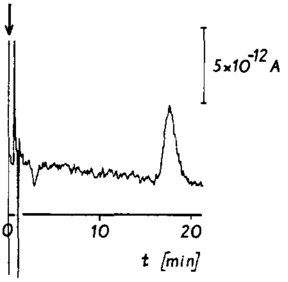

Fig. 4. Chromatogram of DPA. Stationary phase, Silasorb 600 C_2; mobile phase flow-rate, 0.5 ml/min; amount of solute injected, $6.9 \cdot 10^{-13}$ mol in 1 μl of acetone. For other conditions, see Fig. 1. Noise, $8 \cdot 10^{-13}$ A.

TABLE IV

COMPARISON OF THE BEST MDQ VALUES IN THE PRESENT WORK WITH THE BEST FROM REF. 1

Solute	MDQ (mol)	
	Measured	Reported [1]
3-Nitrophenol	$5.8 \cdot 10^{-9}$	$5.7 \cdot 10^{-8}$
4-Nitrophenol	$2.5 \cdot 10^{-9}$	$4.0 \cdot 10^{-9}$
2,4-Dinitrophenol	$2.2 \cdot 10^{-9}$	$3.7 \cdot 10^{-11}$
2,5-Dinitrophenol	$5.3 \cdot 10^{-9}$	–
Picric acid	$5.0 \cdot 10^{-11}$	$2.5 \cdot 10^{-11}$
Dipicrylamine	$1.9 \cdot 10^{-13}$	–
Sulphuric acid	–	$3.5 \cdot 10^{-12}$[a]

[a] From ref. 7.

meter with a noise level of $2 \cdot 10^{-5}$ absorbance and a cell path length of 10 mm. A typical column ($N = 3 \cdot 10^4$–$5 \cdot 10^4$ plates/m) will dilute a sample ($k' = 1$–10) by at least a factor of 10. If the solute has a very high molar absorptivity of about $4 \cdot 10^4$–$5 \cdot 10^4$ (*p*-nitrophenol, picric acid) [14], the lowest possible sample concentration injected on the column is $1 \cdot 10^{-7}$ mol/l. For a 10-μl sample loop injection, such a concentration sensitivity corresponds to an MDQ of $1 \cdot 10^{-12}$ mol. The dipicrylamine MDQ, $1.8 \cdot 10^{-13}$ mol, is one order of magnitude lower than the best value assessed for spectrophotometry. For solutes having very low absorptivity, *e.g.*, 50 (aliphatic carboxylic acids at 200 nm) [14], the minimum sample concentration for spectrophotometry is $1 \cdot 10^{-4}$ mol/l and the sample MDQ is $1 \cdot 10^{-9}$ mol for a 10-ml injection. In accordance with an MDQ of the order of 10^{-10}–10^{-11} mol typical of ionizing solutes, measured in reversed-phase systems with post-column detectors [1,2,7], MDQs of *ca.* $2 \cdot 10^{-10}$ mol were reached for caprylic, palmitic and stearic acids in a reversed-phase system with the column as a detector [8].

MDQs of the order of 10^{-13} mol were reported for a coulometric detector connected behind a 0.5 mm I.D. packed capillary column [15].

An increase in the detection sensitivity with the background current and a simple relationship between the detection sensitivity and the solute pK_a, reported for reversed-phase systems [1,7], were not verified by our experiments with normal-phase systems (Tables I, II and III). However, the dependence of Q on the solute retention was found, in agreement with the earlier observation [16]. Different effects of the solute retention and the background current on the detection sensitivity in normal- and reversed-phase systems can result from different effects of retained solutes on the solid surface charge.

The charge of the solid surface and the charge transported per unit time by the flowing liquid, equal to the streaming current, are directly proportional [4]. Acidic solutes in normal-phase systems can be considered to be retained on the polar silica gel surface at least partly in the form of anions [12]. In this instance the negative charge of silica gel will temporarily increase with increase in the surface solute concentration and hence also with the solute retention. As a result, the detection sensitivity will increase for a retained solute with increase in its retention. In a reversed-phase system the solutes are retained in the form of molecules only [17]. Uncharged molecules retained on bonded chains have no effect on the solid-phase charge. A higher retention of the solute in a reversed-phase system therefore cannot increase the sensitivity of its detection. If the solid-phase charge increases permanently for a certain reason, both the detection sensitivity of any solute and the background current must increase simultaneously.

The explanation of the different influence of the solute pK_a on the detection sensitivity in normal- and reversed-phase systems requires the use of a theory of streaming current generation by a concentration gradient of the liquid. Such a theory has not yet been suggested.

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