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Optimization of Nafion-coated electrodes for selective detection in high-performance liquid chromatography

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ABSTRACT

The performance of Nafion-coated electrodes for detection in liquid chromatography was optimized. The effect of the composition of the mobile phase and the film thickness on the selectivity of the detection of catecholamines was investigated. The stability and the response of the coated electrodes were also determined. From the results, general guidelines were formulated for the optimum use of Nafion-coated electrodes in high-performance liquid chromatography. The increase in the selectivity when using the Nafion-coated electrode was demonstrated by analyzing urine samples.

INTRODUCTION

The determination of catecholamines in biological samples has long been a challenge. In the 1970s, the early radioenzymatic assay method [1–4] was gradually replaced with the less laborious high–performance liquid chromatography (HPLC). Among the various detection techniques in HPLC, electrochemical detection became very popular for catecholamine assay owing to its high sensitivity and relatively low cost. Owing to the low concentration of catecholamines in biological samples and the complexity of the matrix, an extra precolumn extraction or clean-up step in the sample preparation is unavoidable [4–7]. However, it has been shown recently that with urine samples precolumn separation can be omitted when the selectivity of the detector is enhanced by modifying the surface of the working electrode [8—10].

Chemically modified electrodes have been at the focus of attention since the early 1970s, when the first electrode surfaces were chemically functionalized [11]. These electrodes, modified with compounds that incorporate electrochemical catalysts, can be used to achieve more sensitive or selective detection owing to the enhanced electron exchange rate [10–12]. Electrodes have also been modified with permselective coatings to achieve protection against high-molecular-weight compounds, or (as in our work) to provide shielding from anions or cations [8–10]. Recent reviews on chemically modified electrodes give a systematic summary of the state of art [11,12]. Modified electrodes for electrochemical detection in flowing streams have also been reviewed recently [13].

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Chemically modified electrodes with permselective, shielding coatings can provide enhanced selectivity and stability in electrochemical detection. Nafion, a polymeric cation exchanger, has proved to be suitable for the preparation of shielded electrodes. Nafion-modified electrodes have been used in various research projects ranging from fundamental studies on transport processes [14–17] through research on the electrocatalytic activity of incorporated particles [18,19] to analytical applications in flowing liquids [20–24]. Nafion coatings on microvoltammetric electrodes have provided sufficient shielding to be able to monitor the concentration of catecholamines *in vivo* [25,26].

Several groups have published results on the detection of catecholamines with Nafion-modified electrodes in flow systems. Matsue *et al.* [20] demonstrated that a detector cell containing indium–tin oxide microelectrode arrays coated with Nafion is suitable for the selective detection of catecholamines. The same group published another paper on catecholamine detection with Nafion-coated glassy carbon electrodes [21].

Wang *et al.* [22] demonstrated the usefulness of Nafion coating on a carbon working electrode for the detection of neurotransmitters in flow streams. A rapid loss of detector activity showed that Nafion did not provide the desired protection. A second, cellulose acetate, film had to be cast on top of the electrode to prevent it from passivation. Ji and Wang [23] found, however, that a single Nafion coating was sufficient to protect an electrode from anions (ascorbic acid) and that the electrode was highly stable even in the presence of bovine serum albumin [23].

Here, we report on the optimization of detection in HPLC using a Nafion-modified glassy carbon electrode. First, the reproducibility of the film preparation and the optimum film thickness were determined. Second, the effect of the composition of the mobile phase was studied; the influence of the methanol concentration on the film stability was clarified, and also the effects of pH and the type and concentration of buffer cations on the selectivity. The response of the coated electrodes was determined from the peak broadening. Finally, the applicability of the electrode under the determined optimum conditions was demonstrated by detecting catecholamines in urine samples.

EXPERIMENTAL

Apparatus and reagents

The HPLC system consisted of a Gilson (Villiers-le-Bel, France) pump, a 150 × 4.6 mm I.D. column packed with 5-μm Hypersil ODS (Shandon Scientific, Astmoor, UK) and an AMOR (Spark Holland, Emmen, Netherlands) detector cell equipped with a glassy carbon working electrode. The detector potential was set at 0.5 V *vs.* an Ag/AgCl reference electrode. Unless stated otherwise, the mobile phase contained $5 \cdot 10^{-3}$ *M* hexanesulphonic acid (HSA), 10^{-4} *M* EDTA and 10% methanol in phosphate buffer (pH 7).

All chemicals were of analytical-reagent grade and were used without further purification. Dopamine · HCl (DA), *dl*-norepinephrine (NE), 3,4-dihydroxyphenylacetic acid (DOPAC) and catechol (C) were obtained from Janssen Chimica (Beerse, Belgium) and *dl*-epinephrine (E) and *dl*-dihydroxyphenylalanine (DOPA) from Sigma (St. Louis, MO, USA). Concentrated stock solutions were stored at 4°C.

Nafion solution was obtained from Aldrich (Brussels, Belgium) (5%, w/w, equivalent weight = 1100); dilutions were prepared in methanol. The concentrations of these solutions were determined by titration with sodium hydroxide.

Urine samples were filtered (0.8-μm AA filter), diluted 1:1 with the mobile phase and 10^{-3} mol l^{-1} of ascorbic acid was added. The mobile phase was buffered with 0.02 mol l^{-1} lithium phosphate.

Preparation of Nafion-coated electrodes

The glassy carbon surface of the working electrode was completely covered with 12.5 μl of the dilute Nafion solution. After about 15 min the methanol had evaporated and the modified electrode was ready for use. The thickness of the film was calculated from the mass of Nafion applied to the electrode. After a day's work the Nafion coating was removed from the electrode by wiping it off with a tissue soaked in methanol.

RESULTS

Film preparation and stability

The reproducibility of the film preparation was assessed by measuring the permeability of several 4-μm films. The permeability of the film towards the tested compounds was calculated by comparing the chromatographic peak heights at coated and at uncoated electrodes, measured under otherwise identical conditions. The permeability (P) is defined as the ratio of the responses at a coated and bare electrode; P ranges from 0 (impermeable film) to 1 (completely permeable film).

The results of the reproducibility measurements are given in Table I for norepinephrine, epinephrine and dopamine. The differences among the individual films are reflected in the permeabilities for all three tested compounds.

Nafion-type shielding films can be considered as a selective resistance to mass transport on the electrode surface, providing especially low resistance against certain compounds and high resistance for others. The resistance of the film against mass transport (R) can be defined as

$$R = (1 - P)/P \quad (1)$$

TABLE I

REPRODUCIBILITY OF FILM PREPARATION

Permeability of 4-μm Nafion films for N, E and DA in 0.2 M potassium phosphate buffer at pH 7.

Film No.	Permeability		
	N	E	DA
1	0.079	0.146	0.198
2	0.094	0.166	0.214
3	0.075	0.134	0.169
4	0.083	0.152	0.181
5	0.070	0.136	0.137
Relative standard deviation (%)	11	9	16

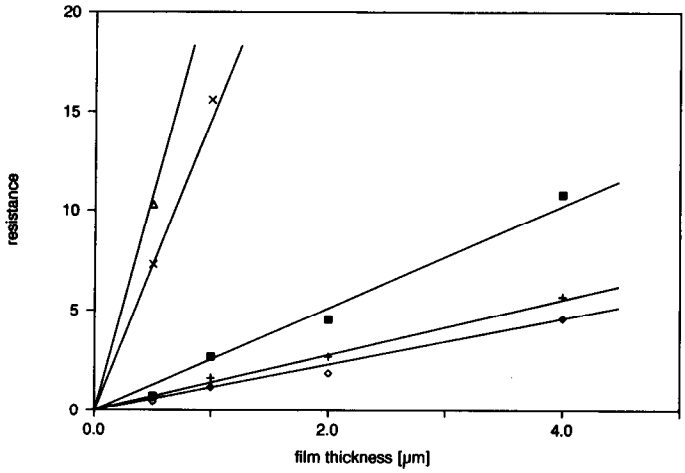

Fig. 1. Effect of the film thickness. Resistance of Nafion films for (■) NE, (+) E, (◇) DA, (△) DOPA and (×) catechol in potassium phosphate buffer at pH 7.

Fig. 1 shows the results of the resistance measurements for NE, E, DA, DOPA and C. The resistance of the Nafion-coated electrode was found to increase linearly with the calculated film thickness. As expected, the slope is low for cationic compounds, indicating a small resistance towards positively charged particles. Neutral and especially anionic species, however, experience higher resistance. The resistance of 4-μm Nafion films was found to be sufficiently low towards the cationic catecholamines (below 10), whereas they provided sufficient shielding towards non-cationic compounds.

Nafion films cast on the electrode do not undergo further chemical reaction (*e.g.*, cross-linking), and remain soluble in alcohol. Therefore, the influence of the methanol content of the mobile phase on the long-term stability of the film was investigated. A 4-μm thick Nafion film was cast on the electrode and the permeability for NE, E and DA was measured immediately after preparation. Then the mobile phase was continuously pumped through the cell, and the permeability was measured again after 2 and 16 h. The decrease in the film thickness was calculated from the increase of the permeability.

The stability of the film was evaluated at four methanol concentrations, ranging from 5% to 30%. As shown in Table II, the film was stable in 5% methanol. When

TABLE II

INFLUENCE OF METHANOL CONTENT OF MOBILE PHASE ON FILM STABILITY

Methanol content (%, v/v)	Loss of film after 2 h (%)	Loss of film after 16 h (%)
5	0	1
10	2	3
20	5	13
30	10	–

using mobile phases of 10–20% methanol content the film thickness should be checked after a few hours' work. Higher methanol contents cannot be applied without considerable loss of the Nafion film.

Influence of pH

The influence of the pH of the mobile phase on the permeability of the Nafion film was studied on acidic, neutral and basic catechols (Fig. 2). In the pH range studied, the permeability of the film for basic catecholamines did not change significantly with the pH (Fig. 2a); DA, E and NE are cationic in this pH range. The permeability for neutral species (catechol in Fig. 2b) shows almost no change with the pH, because their transport in the film is not an ionic process and it is not influenced by the pH.

The pH can be a determining factor, however, in the permeability of the film

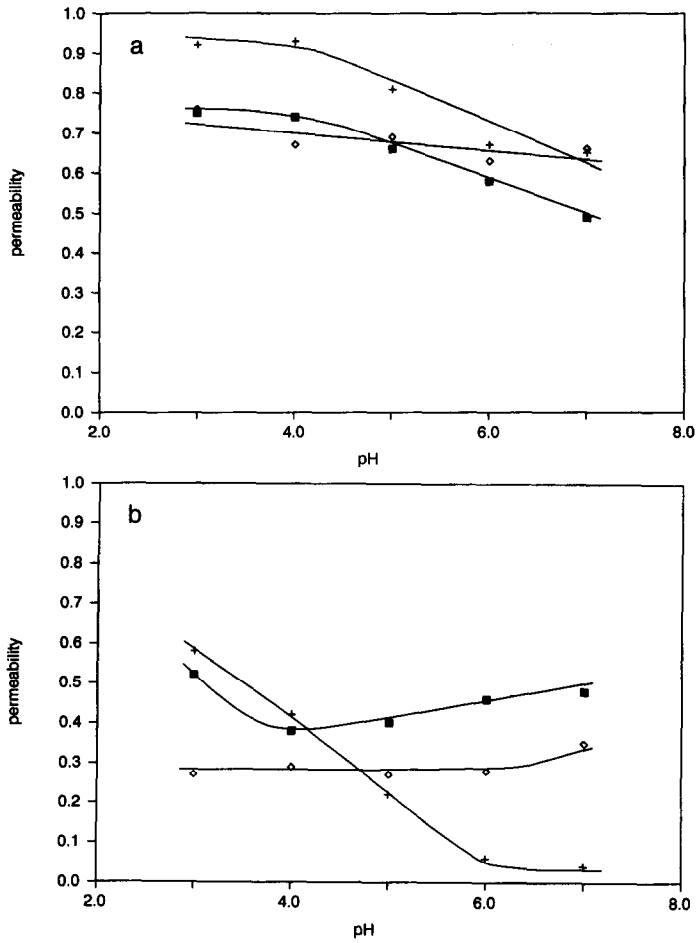

Fig. 2. Effect of the pH on the permeability of the Nafion film. (a) 4-μm Nafion film: (■) NE; (+) E; (◇) DA. (b) 0.2-μm Nafion film: (◇) catechol; (■) DOPA; (+) DOPAC.

towards acidic and zwitterionic analytes. The weak acid DOPAC is transported more rapidly through the film below pH 4, as a neutral molecule, than above pH 5 in its anionic form. The permeability is clearly higher for the amino acid DOPA at pH 3, when it is partly cationic, than at higher pH values, when it is in the zwitterionic form. Hence the detection of DOPA is possible at a Nafion-coated electrode below pH 4. The conclusion from the pH measurement is that a suitable choice of pH is essential if Nafion-coated electrodes are used for electrochemical detection.

Influence of buffer cations

As demonstrated in a previous study [24], the resistance of Nafion films towards cationic analytes is influenced by all cations present in the system. The competitive effect of the buffer cations is determined by two factors: the affinity of the ion exchanger towards the various cations and the cation concentration in the solution.

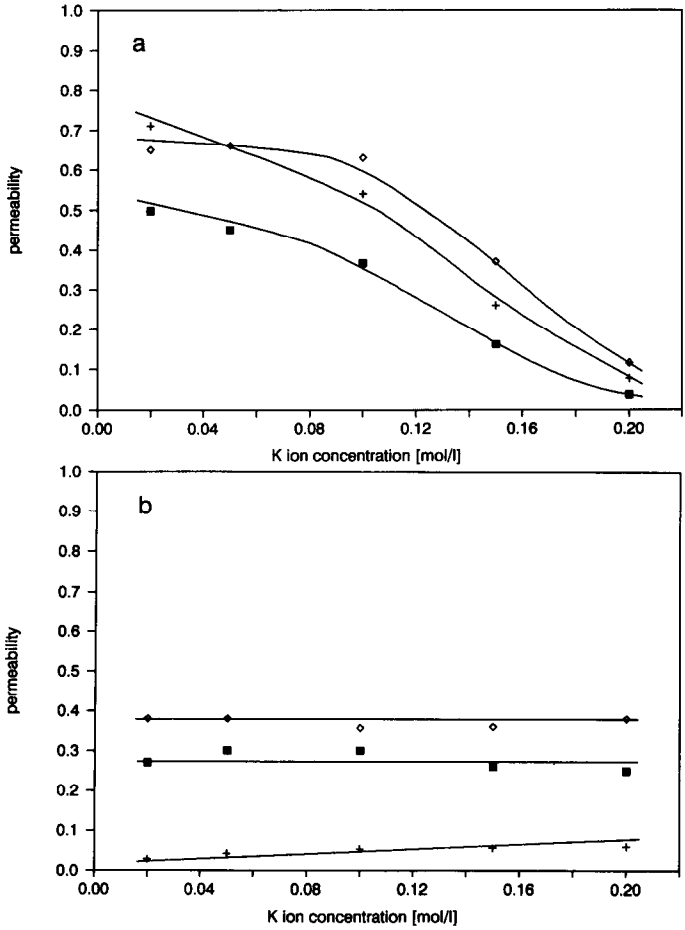

Fig. 3. Influence of buffer cation concentration on the permeability of Nafion films in potassium phosphate buffer at pH 7. (a) Film thickness 4 μm; (■) NE; (+) E; (◇) DA. (b) Film thickness 0.2 μm; (■) DOPA; (◇) catechol; (+) DOPAC.

The effect of the cation concentration was studied with potassium phosphate buffers at pH 7. The results are shown in Fig. 3. The permeability of the film for the cationic compounds decreases at high buffer concentrations, indicating that the majority of the exchange sites will be occupied by the buffer cations (Fig. 3a).

Fig. 3b shows the measured permeability values for the neutral analytes (DOPA and catechol). The only means of transport in the film for these particles is diffusion. As diffusion is not influenced by the ionic state of the film, the buffer concentration has no influence on the permeability towards neutral compounds. The permeability for DOPAC, however, shows some increase with increasing cation concentration. This may be an effect of a reduced zeta potential of the ionic groups in the Nafion film, which causes a decrease in the repulsion against the anionic DOPAC particles.

The ion-exchange characteristics of the Nafion-modified electrode were determined by studying the competition of epinephrine and three different buffer cations (Li^+, Na^+ and K^+). Fig. 4 shows the permeability of a 4-μm Nafion layer for epinephrine at different buffer concentrations. The permeability is highest in the lithium buffer and lower in the sodium buffer. With increasing buffer concentration the permeability decreases at approximately the same rate in both lithium and sodium buffers. The most drastic drop in the permeability of the film is observed with the potassium buffer, indicating that the affinity of the film is the strongest towards potassium ions.

Response of coated electrodes

The use of chemically modified electrodes for detection in HPLC can result in increased peak broadening owing to retarded diffusion of the analytes in the film. A recent theoretical study was devoted to the description of the response of film-coated electrodes, including computer simulations of the transport processes [27].

The response of a coated electrode is determined by the diffusion coefficient of the analyte in the film and by its distribution between the film and the solution. The contribution of the film coating to the peak broadening (σ_{film}) is theoretically

$$\sigma^2_{film} = \lambda(f^4/D_f^2) \tag{2}$$

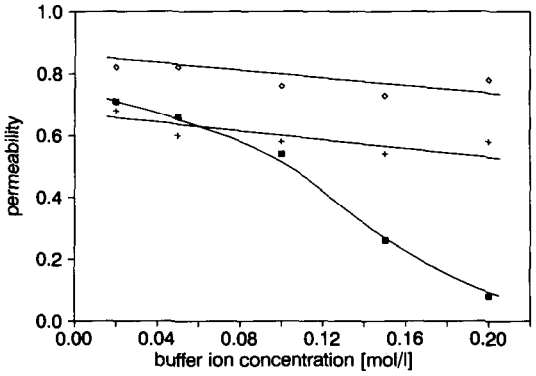

Fig. 4. Permeability of the Nafion-coated electrode for epinephrine in (■) potassium, (+) sodium and (◇) lithium phosphate buffers. Film thickness, 4 μm.

TABLE III

PERMEABILITY OF NAFION FILM IN THE MOBILE PHASE USED FOR URINE SAMPLES.

Conditions as in Fig. 5.

Analyte	Permeability[a]	Analyte	Permeability[a]
Epinephrine	0.99 ± 0.02	Catechol	0.35 ± 0.01
Norepinephrine	0.90 ± 0.01	DOPA	0.28 ± 0.005
Dopamine	0.93 ± 0.03	DOPAC	0.05 ± 0.01

[a] Mean ± S.D. (n = 3).

where f is the film thickness, D_f is the diffusion coefficient in the film and λ is a factor that depends on the distribution constant (K_d) of the analyte. The calculated value of this factor ranges from 1/90 at small K_d to 1/6 at high K_d values. In theory, peak broadening can be reduced by increasing the diffusion coefficient or by decreasing the distribution coefficient. The diffusion coefficient cannot be influenced easily, and it also is not advantageous to decrease K_d; in the latter instance the competitive effect of the buffer cation would be increased.

The changes in the peak broadening were studied on analytes having high (NE, E) and low (C) distribution constants in 0.1 M potassium phosphate buffer at pH 7

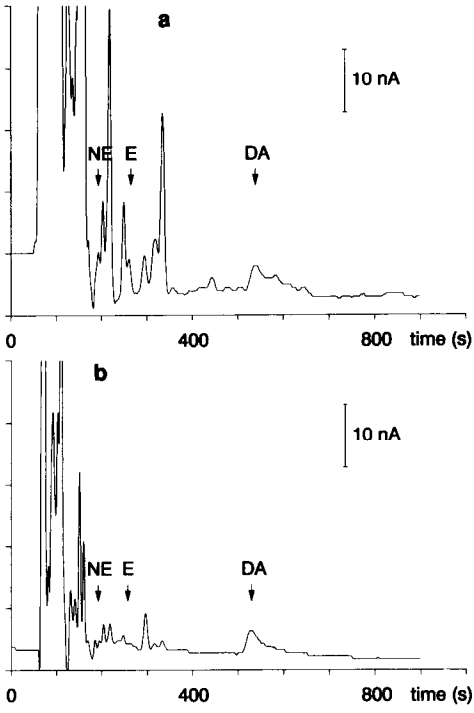

Fig. 5. Chromatograms of a urine sample measured at (a) bare and (b) 4-μm Nafion-coated electrodes. Mobile phase: 0.02 M lithium phosphate (pH 7)–10% methanol–5 · 10^{-3} M HSA–10^{-4} M EDTA.

containing 5% of methanol. The contribution of the film to peak broadening was calculated from

$$\sigma_{film}^2 = \sigma_c^2 - \sigma_b^2 \quad (3)$$

where σ_b and σ_c are halves of the measured peak widths at 0.61 of the peak height at bare and coated electrodes, respectively, under the same chromatographic conditions. The peak broadening measured at a 4-μm Nafion film was 0.5 ± 0.5 for norepinephrine, 1.3 ± 0.6 for epinephrine and 1.1 ± 0.4 s for catechol.

Measurements in urine samples

The permeabilities of the film towards some analytes are given in Table III, determined under the chromatographic conditions used for the analysis of urine samples. As can be seen, under optimum conditions the permeability towards catecholamines approaches 100%, whereas it is below 10% towards negatively charged particles.

Fig. 5 shows the results of measurements in urine samples at (a) bare and (b) Nafion-coated electrodes. In order to achieve optimum selectivity the mobile phase was buffered with 0.02 mol l^{-1} lithium phosphate. Owing to interferences, the peaks of norepinephrine, epinephrine and dopamine cannot be identified at the bare electrode. At coated electrodes the interfering peaks are strongly reduced. Endogenous DA concentrations can be directly determined, and also elevated NE and E concentrations can be detected without sample clean-up.

CONCLUSIONS

From this systematic study to determine the optimum conditions for the use of Nafion-modified electrodes in HPLC, the following guidelines were formulated. The pH should be kept as high as possible in order to convert the weakly acidic interferents into their anionic form, thus preventing them from reaching the electrode. To keep the competitive effect of the buffer cations to the minimum, the buffer concentration must be as low as possible, and lithium or sodium buffers are preferred to potassium buffers. The methanol concentration of the mobile phase is limited to *ca.* 20%. Films of 4 μm can be used without serious deterioration of the peak shape.

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