Renewable Perfluorosulfonated Ionomer Carbon Paste Electrode for Competitive Homogeneous Electrochemical Immunoassays Using a Redox Cationic Labeled Hapten

ARTICLE *in* ANALYTICAL CHEMISTRY · APRIL 1996

Impact Factor: 5.64 · DOI: 10.1021/ac950510p · Source: PubMed

3 AUTHORS, INCLUDING:

Benoît Limoges
Paris Diderot University
118 PUBLICATIONS **2,326** CITATIONS

SEE PROFILE

Anal. Chem. **1996,** *68,* 930−935

Renewable Perfluorosulfonated Ionomer Carbon Paste Electrode for Competitive Homogeneous Electrochemical Immunoassays Using a Redox Cationic Labeled Hapten

Stéphane Rapicault, Benoît Limoges, and Chantal Degrand*

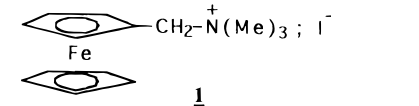
Laboratoire d'Electrochimie Organique, Thermodynamique et Electrochimie en Solution, URA 434, Université Blaise Pascal de Clermont-Ferrand, 24 Avenue des Landais, 63 177 Aubière, France

A Nafion-loaded carbon paste electrode (CPE) was prepared and its square wave voltammetric response at a fresh surface was optimized using (ferrocenylmethyl)-trimethylammonium iodide (1) as a model of a cation that accumulates irreversibly. Low current responses could be obtained reproducibly without preconditioning at a CPE loaded with 1-octanol and neutralized Nafion. Use of the former made the method more sensitive, and use of the latter gave lower background currents and stable analytical response over a long period of Nafion-loaded CPE use. Under optimized conditions and using a 5 min accumulation period, the modified electrode gave a linear current response over the range of 5 nM to 1.5 μM 1, a detection limit of 5 nM 1 and a relative standard deviation of 5% for 0.5 μM 1. Using these optimized conditions, the competitive homogeneous immunoassay of phenytoin as a model hapten was carried out with cobaltocenium-labeled phenytoin as tracer. Clinical serum samples (5 μL) of phenytoin at therapeutic concentrations (20−80 μM) could be analyzed using this method.

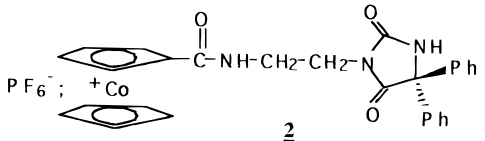
We have recently developed an immunoassay technique with electrochemical detection using a Nafion-coated glassy carbon electrode (GCE) and an antigen labeled with a redox cation.[1−3] This technique can be used to analyze nanomolar concentrations of haptens (drugs, pesticides, etc.). Since the immunoassay is conducted in homogeneous media, it is not necessary to separate free antigen from antibody-bound labeled antigen. Moreover, immunoassay of several analytes is also possible by using haptens labeled by different redox cations. Despite these advantages, this new method has the limitation that the electrode can only be used once. Indeed, an accumulation step precedes electrochemical detection during which time the hydrophobic labeled antigen irreversibly accumulates within the Nafion film, and so the electrode cannot be reused.

A possible solution to this problem is a single-use, low-cost sensor. A modified carbon paste electrode (CPE) could be a good choice for such a sensor since fresh modified electrode surfaces can be generated in a rapid and quantitatively reproducible fashion.[4−6] Gao et al. employed CPE containing Nafion and a complexing reagent as modifiers for the determination of trace amounts of metal ions.[7−10] These ions could be easily expelled from the modified electrode surface by a chemical renewal procedure, and so the use of a single electrode in repetitive analytical determinations was possible. The freshly prepared modified electrode required an initial conditioning of its surface to ensure reproducible responses between each preconcentration/determination/renewal cycle.[9]

In this paper, we describe the preparation of a new Nafion-loaded CPE which ensures low background currents, reproducible and sensitive responses at its fresh surface for irreversibly accumulated hydrophobic cations without the need for preconditioning. The sensor response was studied by cyclic voltammetry (CV) or square wave voltammetry (SWV) using the model cation **1**, which is reversibly oxidized to the corresponding ferrocenium



1



2

salt at 0.2 V vs Ag/AgCl (Cl^-, 50 mM). It was found that the optimum experimental conditions for analysis required the incorporation of neutralized Nafion and 1-octanol within the graphite paste. The optimized conditions were applied to the competitive homogeneous immunoassay of phenytoin [5,5-diphenylhydantoin (DPH)], using cobaltocenium-labeled phenytoin **2** (DPH-Cc^+) as tracer. The cobaltocenium label is reversibly reduced to cobaltocene at −1.1 V. It is shown that the Nafion-loaded CPE competes favorably with the Nafion-coated GCE in phenytoin immunoassays.

(1) Limoges, B.; Degrand, C.; Brossier, P.; Blankespoor, R. L. *Anal. Chem.* **1993**, *65*, 1054.
(2) Le Gal La Salle, A.; Limoges, B.; Anizon, J. Y.; Degrand, C.; Brossier, P. *J. Electroanal. Chem.* **1993**, *350*, 329.
(3) Le Gal La Salle, A.; Limoges, B.; Rapicault, S.; Degrand, C.; Brossier, P. *Anal. Chim. Acta* **1995**, *311*, 301.
(4) For a review on chemically modified CPE, see: Kalcher, K. *Electroanalysis* **1990**, *2*, 419.
(5) Digua, K.; Kauffmann, J. M.; Delplancke, J. L. *Electroanalysis* **1994**, *6*, 451.
(6) Digua, K.; Kauffmann, J. M.; Khodari, M. *Electroanalysis* **1994**, *6*, 459.
(7) Gao, Z.; Li, P.; Wang, G.; Zhao, Z. *Anal. Chim. Acta* **1990**, *241*, 137.
(8) Gao, Z.; Wang, G.; Li, P.; Zhao, Z. *Anal. Chem.* **1991**, *63*, 953.
(9) Gao, Z.; Li, P.; Zhao, Z. *Talanta* **1991**, *38*, 1177.
(10) Gao, Z.; Li, P.; Zhao, Z. *Fresenius J. Anal. Chem.* **1991**, *339*, 137.

0003-2700/96/0368-0930$12.00/0

EXPERIMENTAL SECTION

Reagents and Equipment. A 5% (wt) solution of Nafion (EW 1100) and phenytoin were purchased from Aldrich. (Ferrocenylmethyl)trimethylammonium iodide (**1**) was obtained from Lancaster. Graphite powders of average diameter 100 and 1−2 μm were supplied by Johnson Matthey (ref 730181) and Aldrich (ref 28, 286-3), respectively. Unless otherwise stated, the 100 μm diameter powder was utilized, because it gave lower background currents and higher currents in CV and SWV. Glassy carbon (GC) rods (3 mm diameter) were obtained from Carbone Lorraine. Cobaltocenium-labeled phenytoin **2** was prepared as described in ref 2. Normal serum was taken from a rabbit and polyclonal phenytoin antiserum (frozen at −20 °C) was a gift from the Unité d'Immunoanalyse, Faculté de Pharmacie, Dijon, France. Clinical serum samples containing phenytoin were provided by Hôpital de la Chartreuse, Dijon, France. Stock solutions of phenytoin (1 mM), **1** (10 mM), and **2** (0.8 mM) in ethanol were stored at 0−4 °C. To prepare neutralized Nafion, the acidic hydrogens of the sulfonate polymer were replaced by Li^+ (1 equiv) by addition of an ethanolic solution containing 0.14 M LiOH in the Nafion solution (it was found by titration that the concentration of the sulfonic functions present in the commercial Nafion solution was 33.6 mM). Phosphate buffer (PB; 4.35 mM NaH_2PO_4, 15.1 mM Na_2HPO_4, and 50 mM NaCl, pH 7.4) was used in all experiments. The solutions were not deaerated unless stated otherwise. All reagents were analytical grade, and water was deionized and doubly distilled.

The electrochemical apparatus and cell were described previously.[2] An Ag/AgCl (50 mM NaCl) electrode was used as reference.

Nafion-Loaded CPE Preparation. The modified carbon paste was prepared by mixing the graphite powder with an alcohol solution containing the required amount of Nafion. After the slurry was sonicated for 15 min, the solvent was removed under vacuum. Silicon oil was added to the residue, and thorough mixing was accomplished using a mortar and pestle.

Optimum experimental data were obtained using an alcohol slurry prepared from 125 mg of graphite powder (average diameter, 100 μm), 500 μL of Aldrich Nafion solution, 120 μL of an ethanolic solution containing LiOH (0.14 M), 80 μL of 1-octanol, and ~3 mL of ethanol. After evaporation under vacuum, 104 mg of silicon oil was added. A plastic tip with an internal diameter of 3 mm was filled with the modified carbon paste, which was then pressed into the holder as described in ref 11. The surface of the CPE was polished on a Teflon sheet until a smooth surface was achieved. This disk was used with a Tacussel rotator as a working rotating-disk electrode.[2] Unmodified carbon paste was prepared by hand-mixing 125 mg of graphite powder with 104 mg of silicon oil.

Accumulation and Detection Procedures. For the accumulation procedure, the rotating modified CPE was exposed to the solution at 600 rpm under open electrical circuit (for 5 min unless otherwise stated) before CV or SWV experiments were performed. SWV parameters are given in ref 1. Between each measurement, a small amount of paste was pushed out of the tip and cut with a razor blade, and then the resulting new electrode surface was polished.

Influence of Serum Matrix. The sample (1 mL) was prepared in a vial. To 135 μL of DPH-Cc^+ (2 μM in PB) were added x μL ($x = 0-50$) of rabbit normal serum (RNS), 10 μL of ethanol, and (855 − x) μL of PB. After thorough mixing, the sample was incubated for 1 h at 37 °C and then assayed using the above accumulation and detection procedures.

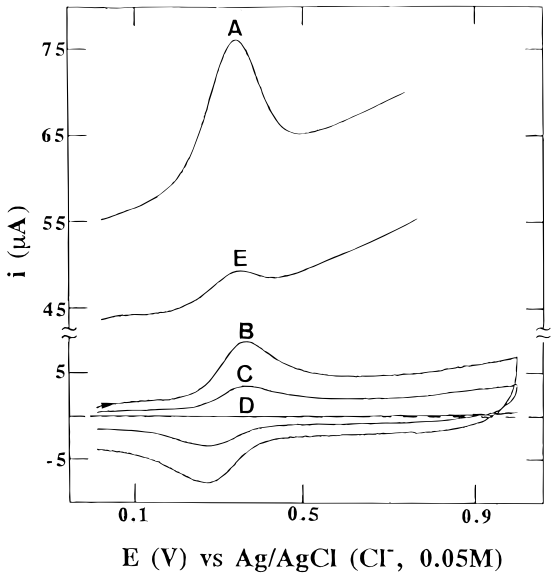


Figure 1. SWV curves (A, E) and CV curves (B−D; scan rate, 100 mV/s), on a CPE loaded with (w/w) 11.6 (A, B, E), 5 (C), and 0% (D) of nonneutralized Nafion and immersed in blank PB solution. The immersion time was 30 s (A−D) and 5 min (E), under 600 rpm.

Phenytoin Antiserum Calibration Curve. To 135 μL of PB containing DPH-Cc^+ (2 μM) were added 815 μL of PB, 10 μL of ethanol, y μL ($y = 0-20$) of phenytoin antiserum, and (40 − y) μL of RNS. After thorough mixing, the solution was incubated at 37 °C for 1 h, and the accumulation and detection procedures were followed.

Phenytoin Calibration Curve. To 135 μL of DPH-Cc^+ (2 μM in PB) were added z μL ($z = 0-100$) of DPH (10 μM in PB), (815 − z) μL of PB, 32 μL of RNS, 10 μL of ethanol, and 8 μL of phenytoin antiserum. The contents were incubated at 37 °C for 1 h. The solution was then assayed using the accumulation and detection procedures.

Assay of a Clinical Serum Sample. To 135 μL of DPH-Cc^+ (2 μM in PB) were added 815 μL of PB, 10 μL of ethanol, 27 μL of RNS, 8 μL of phenytoin antiserum, and 5 μL of human serum containing DPH. After thorough mixing, the contents were incubated for 1 h at 37 °C and then assayed.

RESULTS AND DISCUSSION

Electrochemical Response at the Fresh Surface of a Nafion-Loaded CPE in Blank Phosphate Buffer. Initial experiments were conducted with a CPE composed of graphite powder, silicon oil as pasting liquid, and Nafion in amounts similar to those employed by Gao et al.[7−10] and immersed in a PB solution (pH 7.4) in the absence of **1**. Figure 1A−D shows the SWV and CV curves obtained at a freshly prepared surface for a stationary CPE containing 11.6 (A, B), 5 (C), and 0% (D) Nafion. In each case, the electrode was immersed in the solution for only 30 s before the voltammograms were recorded. The CVs reveal a large increase in background current with increasing amounts of Nafion and an unexpected redox couple at positive potentials (~0.35 V). Both CV or SWV peak currents and background currents decrease progressively with continuous cycling or with prolonged immer-

(11) Motta, N.; Guadalupe, A. R. *Anal. Chem.* **1994**, *66*, 566.

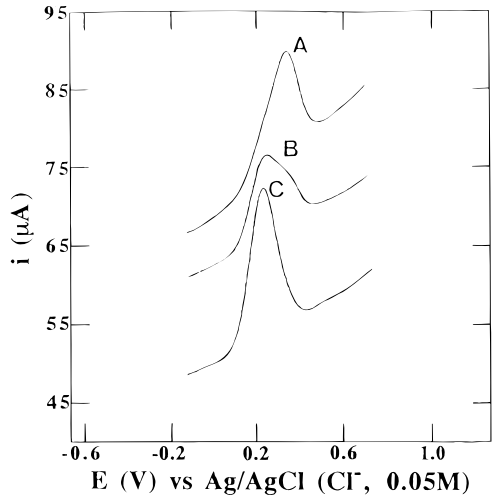


Figure 2. SWV curves on a CPE loaded with 11.6% nonneutralized Nafion and immersed in a PB solution of 1 μM **1** for 30 s (A), 2 min (B), and 5 min (C) under 600 rpm.

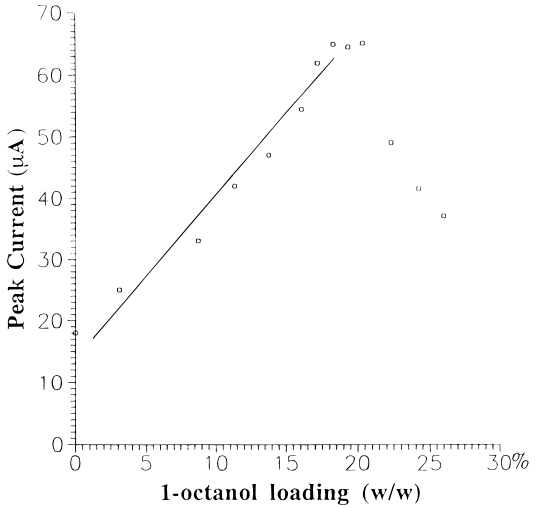


Figure 3. Effect of 1-octanol in carbon paste with 8.8% neutralized Nafion loading on the SWV peak current of 1 μM **1** in PB solution. Preconcentration time, 5 min under 600 rpm.

sion (compare the SWV curves A and E in Figure 1, which were recorded after immersion times of 30 s and 5 min, respectively). Unexpected peaks were also obtained from a thick film that was prepared by syringing a few microliters of the commercial Nafion solution onto a GC disk electrode. A similar anodic peak was previously observed when a CPE was immersed into a sulfuric acid medium containing methanol, and this peak has been attributed to the penetration of methanol into interior of the paste.[12] In the case of the Nafion-loaded CPE, the redox couple could be related to traces of nonvolatile alcohols in the strongly acidic Nafion polymer, which is able to protonate methanol.[13] These alcohols can be expelled from the CPE or from the Nafion film-coated electrode cycling the potential repeatedly or by immersion in pure PB.

Optimum Experimental Conditions for Electroanalysis of 1. The unexpected anodic signal observed at 0.35 V interfered with the peak corresponding to the anodic oxidation of **1** at 0.23 V, as shown by the SWV curves in Figure 2 performed in the presence of **1** (1 μM) and after increasing immersion periods. The impurity oxidation peak at 0.35 V was gradually replaced by the ferrocene oxidation peak at 0.23 V. Since the presence of the oxidation peak at 0.35 V is undesirable, attempts were made to eliminate it or reduce its current. It was found that replacing the acidic hydrogens of the sulfonate polymer with Li^+ was beneficial in two ways: the background current decreased and the current of the impurity peak dropped significantly. For example, the latter was only 40% of the value obtained with nonneutralized Nafion when the immersion period was 30 s and the peak was no longer visible when the immersion period was 5 min. On the other hand, the anodic peak current of the model cation **1** was not significantly modified with neutralized Nafion, and so neutralized Nafion is preferable.

The effect of Nafion on the SWV response of **1** in carbon paste was examined using its acidic form, and it was found that the highest SWV peak current for **1** was obtained when the carbon paste contained 5−9% (w/w) Nafion. Carbon pastes containing either lower or higher Nafion loadings were much less effective in accumulating **1**. Similar results were obtained by Gao et al. in the determination of metal ions by differential pulse voltammetry at CPE containing Nafion and a second modifier.[7−10] The current drop at high Nafion loadings is consistent with an increase in the electrode resistance and with mass- and charge-transfer limitations both within the polymer film and at the substrate/film interface.[14,15]

A graphite powder/silicon oil ratio of 1.2 (w/w) was chosen because the consistency and properties of this paste are ideal. A decrease in the silicon oil content resulted in an increase in the residual current, whereas an increase in the silicon oil content gave a paste that was difficult to handle.

Incorporation of an aliphatic alcohol (methanol, ethanol, 1-butanol, 1-hexanol, 1-octanol, etc.) within the carbon paste drastically increases the SWV peak current of the model cation **1**, as shown in Figure 3 with 1-octanol, a high boiling alcohol. The enhancement of current apparently has something to do with the hydrophilic alcoholic function rather than the hydrophobic alkyl chain, since the SWV peak current of **1** was not modified when 1-bromooctane was substituted for 1-octanol. Figure 3 indicates a quasi-linear increase of the electric signal at low octanol concentrations and then a drastic decrease at the highest ones. This effect is not yet fully understood. It was previously observed in this group that increasing the solution ethanol content led to a similar effect on the peak current obtained at a Nafion-coated GCE.[16] Other authors who investigated the accumulation of the hydrophobic $Ru(bpy)_3^{2+}$ cation in a Nafion film found that the presence of alcohol in solution modified the cation partition between the Nafion hydrophobic and hydrophilic regions in which they diffuse slowly and rapidly, respectively.[17,18] Recent experimental evidences have confirmed that alcohols penetrate into the nonpolar regions of Nafion.[19]

Figure 4 shows the relationship between the immersion period (i.e., the preconcentration time) and the SWV response for **1** (0.5

(12) Svancara, I.; Vytras, K.; Renger, F.; Smyth, M. R. *Electrochim. Acta* **1992**, *37*, 1355.
(13) Buzzoni, R.; Bordiga, S.; Ricchiardi, G.; Spoto, G.; Zecchina, A. *J. Phys. Chem.* **1995**, *99*, 11937.
(14) Rubinstein, I.; Bard, A. J. *J. Am. Chem. Soc.* **1981**, *103*, 5007.
(15) White, H. S.; Leddy, J.; Bard, A. J. *J. Am. Chem. Soc.* **1982**, *104*, 4811.
(16) Limoges, B.; Degrand, C.; Brossier, P. *J. Electroanal. Chem.*, in press.
(17) Downey, T. M.; Neiman, T. A. *Anal. Chem.* **1992**, *64*, 261.
(18) Vining, W. J.; Meyer, T. J. *J. Electroanal. Chem.* **1987**, *237*, 191.
(19) Li, H.; Schlick, S. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1993**, *34*, 446.

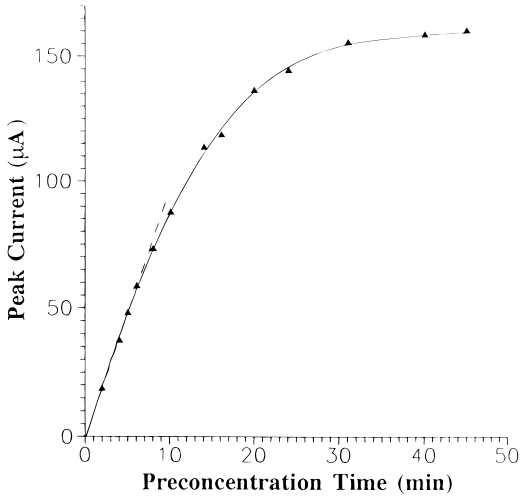


Figure 4. Dependence of the SWV peak current of 0.5 μM **1** in PB on the preconcentration time under 600 rpm. CPE containing 6.9% neutralized Nafion and 20.9% 1-octanol.

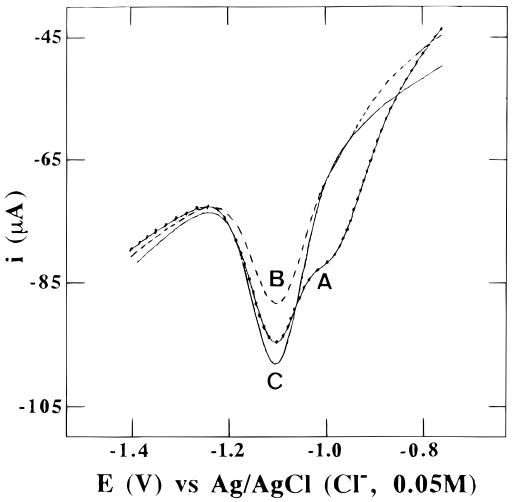


Figure 5. SWV curves on an optimized Nafion-loaded CPE for 0.8 μM DPH-Cc$^+$ in nondeaerated PB (A, C) and deaerated PB (B). For curve C, a potential of −0.96 V was applied for 30 s between the preconcentration period and the electrochemical detection step. Preconcentration time, 5 min under 600 rpm.

μM). Linear behavior is observed at short times, but the plot then becomes hyperbolic, approaching a limiting value which was determined by the equilibrium distribution of **1** between the solution and the Nafion-loaded CPE. A preconcentration time of 5 min, which falls on the linear part of the curve, was employed in this work.

The carbon paste composition was gradually modified to improve the SWV response of **1** (1 μM), and the following optimized composition (w/w) gave the optimun current: 39.4% graphite powder, 20.9% 1-octanol, 32.8% silicon oil, and 6.9% neutralized Nafion. The peak intensity (∼100 μA) was 470 times higher and the peak potential 100 mV less positive than in the absence of Nafion. Under the selected conditions, the SWV signal increased linearly with the ferrocene salt concentration from 5 nM to 1.5 μM and the detection limit was 5 nM (S/N = 2). The relative standard deviation was ∼5% for a series of 15 analyses performed with **1** (0.5 μM) at freshly prepared surfaces of the same modified carbon paste. Series of fresh surfaces, corresponding to five different preparations of the optimized carbon paste over a 6-month period, led to reproducible responses with a relative standard deviation of 5%. The main source of error is probably related to the manual renewal process of the smooth surface. Different portions of the same modified carbon paste (with storage at room temperature) gave reproducible results over at least 1 year with neutralized Nafion (the increase or decrease of peak current was not significantly different from the relative standard deviation) whereas a drastic SWV signal drop was observed with nonneutralized Nafion. For instance the peak current diminution reached 30 and 60% after 5 and 8 months, respectively. Since the initial peak current value could be recovered by adding some 1-octanol, a slow dehydration of this alcohol within the superacidic Nafion bulk[20] is suggested to take place.

In conclusion, addition of 1-octanol improved the SWV response of **1** and substitution of the Nafion protons by Li$^+$ led to lower background currents and stable Nafion-loaded CPE. Hence, reproducible and sensitive responses could be obtained at freshly prepared surfaces with no preconditioning.

(20) Pineri, M.; Aldebert, P.; Gedel, G. *Macromolecules* **1987**, *20*, 1425 and references therein.

Accumulation and Determination of DPH-Cc$^+$. The SWV reduction peak of DPH-Cc$^+$ at ∼−1.1 V was preceded by a significant shoulder which could be suppressed by either bubbling argon during the preconcentration period or applying a potential of −0.96 V for 30 s between the accumulation period and the electrochemical detection step (Figure 5). Hence, it is seems likely that the shoulder results from the reduction of oxygen to hydrogen peroxide. Since this shoulder could be eliminated almost completely in a short time using the electrochemical method, this technique was employed prior to the electrochemical detection step. When no 1-octanol was incorporated into the modified CPE, the peak current of DPH-Cc$^+$ was almost 5 times lower, which is consistent with the results obtained with **1** (Figure 3).

The calibration curve of DPH-Cc$^+$ possesses a linear portion between 30 nM and 1.6 μM and a detection limit of 20 nM (S/N = 2). These results are similar to those using a Nafion-coated GCE (linearity range: 20 nM−1 μM, and same detection limit),[2] but as already mentioned, it was necessary to prepare a series of Nafion-coated GCEs to plot the calibration curve, since DPH-Cc$^+$ accumulated irreversibly.

Competitive Homogeneous Immunoassay of Phenytoin at a Nafion-Loaded CPE. The immunoassay of phenytoin was previously carried out using a Nafion-coated GCE.[2,3] In this technique, a fixed quantity of DPH-Cc$^+$ is in competition with an unknown amount of phenytoin for a limited quantity of phenytoin-antibody binding sites (Ab) when the immunoreaction takes place. The antibody-bound labeled phenytoin (Ab/DPH-Cc$^+$) does not penetrate into the Nafion narrow channels,[21] due to its large size, whereas DPH-Cc$^+$ can freely diffuse and accumulate within Nafion. Its SWV electrochemical response depends on the concentration of DPH in solution, i.e., the higher its concentration, the higher the DPH-Cc$^+$ concentration in solution and, consequently, the higher the peak current, i_p. The DPH concentration is determined from calibration curves.

The effect of the serum matrix constituents (amino acids, proteins, inorganic cations, lipids) on the accumulation and

(21) Hsu, W. Y.; Gierke, T. D. *J. Membr. Sci.* **1983**, *13*, 307.

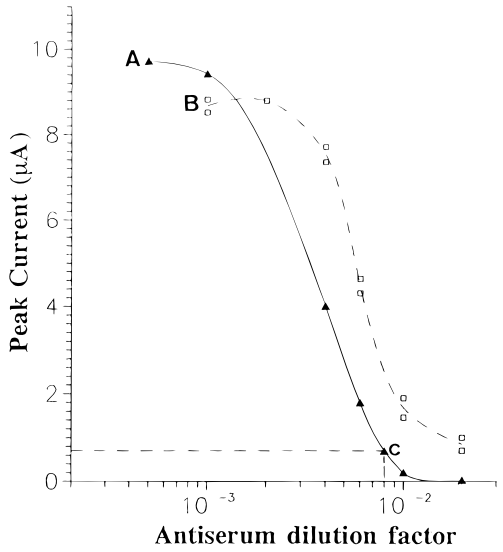


Figure 6. Antiserum titration curves for 0.27 μM DPH-Cc$^+$ obtained at a Nafion-loaded CPE (A) and at a Nafion-coated GCE (B). Incubation time, 1 h at 37 °C. Preconcentration time, 5 min under 600 rpm. Applied potential of −0.96 V for 30 s between preconcentration period and electrochemical detection step.

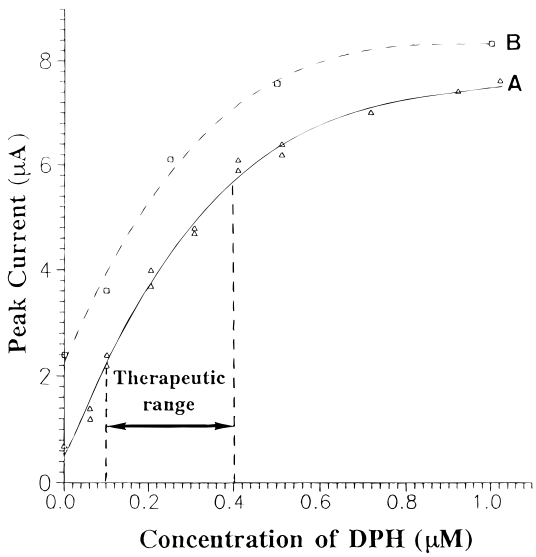


Figure 7. Standard calibration curves for DPH at a Nafion-loaded CPE (A) and at a Nafion-coated GCE (B). Incubation, preconcentration, and detection procedures as for Figure 6. The therapeutic range refers to a clinical serum sample diluted 200 times.

Table 1. Phenytoin Assays of a Clinical Sample

		assay no.		
		1	2	3
immunoassay at a Nafion-loaded CPE	i_p (μA)	5.4	5.5	5.6
	C^a (μM)	74	77	80
	av	77 ± 3[b]		
immunoassay at a Nafion-coated GCE	C^a (μM)	74 ± 2[c]		
extraction + HPLC	C^a (μM)	75[d]		

[a] Concentration in patient serum. [b] Average ± standard deviation. [c] Average ± standard deviation of two assays. See ref 3. [d] Average of two assays (precision range, 5−10%).

detection of DPH-Cc$^+$ at a Nafion-loaded CPE was examined. Solutions of DPH-Cc$^+$ containing increasing amounts of RNS were incubated 60 min at 37 °C in order to mimic the immunoassay conditions. The peak current decreased to a limiting value corresponding to ~70% of the initial value at RNS contents of >3% (v/v), and so the study was further conducted at constant RNS and/or antiserum contents (4%). A similar signal decrease was previously observed at a Nafion-coated GCE.[2] Two effects can account for the current decrease. First, it is well-known that serum proteins associate with numerous hydrophobic drugs by nonspecific interactions[22] and that the resulting bulky complexes do not accumulate within Nafion.[16] Moreover, the accumulation decreases when the ionic strength is increased, as shown in a detailed study on the influence of the ionic strength on the accumulation of a cobaltocenium-labeled drug in a Nafion film.[16]

As concomitant accumulation of DPH-Cc$^+$ and DPH could occur in the course of the competitive homogeneous immunoassay, it was necessary to examine the influence of DPH on the peak current of DPH-Cc$^+$. i_p did not change significantly when as much as 20 equiv of DPH was added to the buffer solution of DPH-Cc$^+$ (0.27 μM) containing 4% RNS and 1% ethanol.

Incubation of increasing amounts of antiserum at a fixed concentration of DPH-Cc$^+$ (0.27 μM) and at a constant total serum content (4%) (i.e., RNS + antiserum = constant), resulted in the antiserum titration curve shown in Figure 6A. The initial current dropped to almost zero at high antiserum content, confirming that the Ab/DPH-Cc$^+$ complex is unable to penetrate into the Nafion due to its large size. Figure 6B is the antiserum titration curve previously obtained at a Nafion-coated GCE.[2] The two curves have the same shape, but they are slightly shifted, probably due to a difference in the antiserum titrations.

The standard calibration curve in Figure 7A is typical of a competitive immunoassay. It was obtained by adding DPH to a solution of composition corresponding to point C in Figure 6A (antiserum factor dilution of 8×10^{-3}, i.e., 93% inhibition). As the concentration of DPH was increased, the release of DPH-Cc$^+$ from the antibody complex by the competitive binding of DPH took place, and so i_p increased. The calibration curve shows that the therapeutic range of concentrations of DPH, following dilution by factor of 200 (i.e., 1 mL of assay contained 5 μL of patient serum), occurs where the peak current is the most sensitive. These results are equivalent to those obtained at a Nafion-coated GCE[2] (compare with curve B in Figure 7). A clinical sample was assayed three times and reliable results were thus obtained as shown in Table 1, which compares the electrochemical results at a Nafion-loaded CPE with an HPLC determination in a hospital and previous results at a Nafion-coated GCE.[3] The close values indicated in Table 1 confirm the reliability of the new sensor and the feasibility of the technique. At this point, it is necessary to carry out further clinical studies if we want to apply this technique to the evaluation of phenytoin in real samples. It is worth noting that 10 other drugs were present in the clinical sample, including phenobarbital, which is structurally similar to phenytoin, at a concentration of ~150 μM.

In conclusion, the analytical performances of the Nafion-modified CPE and GCE are similar, but the former electrode has

(22) Tillement, J. P.; Horrin, G.; Zini, R.; Urien, S.; Albengres, E.; Barré, J.; Sébille, B. In *Pharmacologie Chimique—Base de la Thérapeutique 1*; Giroud, J. P., Ed.; Expension Scientifique: Paris, 1988; p 1.

the advantage that it can be used for more than one measurement.

ACKNOWLEDGMENT

We acknowledge the Agence Nationale de Valorisation de la Recherche (ANVAR) for financial support of this research. We thank Pr. P. Brossier (Faculté de Pharmacie, Dijon, France) for providing a sample of phenytoin antiserum and Pr. R. L. Blankespoor (Calvin College, Grand Rapids, MI) for useful comments.

Received for review May 25, 1995. Accepted December 3, 1995.[⊗]

AC950510P

[⊗] Abstract published in *Advance ACS Abstracts,* January 15, 1996.