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In Situ Antigen Immobilization for Stable Organic-Phase Immunoelectrodes

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A new method based on enzymatic single-step in situ synthesis of hapten−carrier conjugates on electrodes is described yielding stable, reproducible, and reusable organic-phase immunoelectrodes (OPIEs). The electrodes developed were tailored for analyte detection in organic solvents and allow for the analysis of soil extracts without further sample processing and cleanup. Catalyzed by transglutaminase from a variant of *Streptoverticillium mobaraense*, the reaction proceeds in aqueous solution with and without addition of organic media in only 1.5 hours. In this study, the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) was chosen as model compound and chemically amino-functionalized prior to its enzymatic immobilization. The high reproducibility of the immobilization procedure allowed for batch calibration of the immunoelectrodes. Moreover, pure methanol or treatment with diluted sulfuric acid used for regeneration studies did not disturb the hapten layer. The OPIE consists of screen-printed carbon electrodes, monoclonal anti-2,4-D antibodies, and the immunochemical recognition reaction and was optimized with regard to a high stability in organic media. For electrochemical detection, horseradish peroxidase was used as enzyme label together with H_2O_2 as substrate and hexacyanoferrate(II)/(III) as mediator. The OPIE showed high stability upon storage over 93 days. Response times of 17 s (t_{95}) were found to be advantageous compared to those of other biosensors. Including the immunochemical reactions, the complete assay takes 30 min. A calibration curve for 2,4-D in 30% methanol/buffer obtained with 70 electrodes within 4 weeks revealed a detection limit of 9 mg/L, a sensitivity of 1.3 nA L mg^{-1} cm^{-2}, and a repeatability of 6.8%. Although we calculated a lowered repeatability for reused electrodes of 13.4% and a slightly decreased sensitivity of 0.9 nA L mg^{-1} cm^{-2}, multiple-used OPIEs could also be applied for calibration.

Due to an enormous release of chemicals into the environment, there is an increasing demand for simple, rapid, and inexpensive analytical tools for the determination of a wide variety of compounds. In this respect, immunochemical tests gained in importance for their time- and cost-efficient screening potential.[1−5] An aqueous environment is traditionally believed to be ideal for analytical procedures involving biomolecules. However, when application is confined to aqueous media, numerous analytical determinations are hampered due to the poor water solubility of many organic analytes. Moreover, such an analyte is usually recovered from sample matrices by organic solvent extraction. An analytical system capable of measuring directly in the extract would be particularly well suited for the rapid analysis of soil extracts without further sample processing and clean-up. Although fast analysis in organic environments elicits increasing interest, only a few papers describe immunochemical tests in organic systems.[6−8]

In immunochemical test formats, either the antigen or the antibody is immobilized on a solid matrix. Several techniques, such as adsorption, entrapment, covalent attachment, or cross-linking, have been described to immobilize biologically active compounds.[9,10] In order to ensure accessibility for the antibodies, immobilization of low-molecular-weight substances (haptens) is realized in most cases in two steps. First, the analyte is covalently bound to a macromolecule as a carrier. Second, the hapten−carrier conjugate is immobilized on the selected surface, e.g., by simple adsorption. This procedure is, for example, widely used in ELISA formats. In certain cases, however, hapten conjugates are not needed, as haptens can also be attached directly to activated surfaces.

The conjugation procedure strongly depends on the availability of functional groups, particularly those of the hapten, and has to

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be selected carefully.[11] The chemical synthesis of these hapten−protein conjugates, however, is often laborious and hard to reproduce. Hence, it is difficult to evaluate such assays. Once a conjugate is used up, the assay conditions for a conjugate from a new batch need to be optimized again. Here we describe a conjugation and immobilization procedure that needs to be optimized only once and that offers new and advantageous features.

A transglutaminase produced by a variant of *Streptoverticillium mobaraense* catalyzes an acyl-transfer reaction between the γ-carboxyamide group of peptide- or protein-bound glutaminyl residues and a variety of primary amines.[12,13] This microbial transglutaminase (MTGase) has been used mainly to polymerize or functionalize proteins for food processing.[14,15] Very recently, we started to apply this enzyme for immunoconjugate synthesis,[16] and we describe here the MTGase-catalyzed synthesis of a hapten−carrier conjugate on an electrode surface for application in amperometric organic-phase immunoelectrodes (OPIEs). Conjugate synthesis and immobilization are simultaneous processes in this procedure. Due to the cross-linking properties of MTGase, the protein network generated is very dense, carries the haptens for the immunoassay, and, at the same time, minimizes unspecific binding. The device has been designed to operate directly in organic extract solutions, preferably soil extracts, with the aim to reduce the number of steps required for sample pretreatment and analysis. The feasibility of the technology will be demonstrated taking the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) as a model analyte. The system, however, can easily be transferred to other analytes by changing the biocomponents of the sensor.

EXPERIMENTAL SECTION

Reagents. 2,4-D-specific monoclonal antibodies (clone 24D5) were produced and screened for application in 30% methanol, and anti-2,4-D antibody−horseradish peroxidase conjugates (subsequently called antibody−HRP) were synthesized by Cell Diagnostica (Münster, Germany). The screening was performed in microtiter plates coated with BSA−2,4-D conjugates in the presence of 30% methanol or PBS as control. Bound antibodies were subsequently detected using a goat-anti-mouse−HRP conjugate. Positive hybridomas were recloned and selected for binding in the presence of 5, 10, 20, 30, 40, and 50% methanol. Finally, the clone 24D5 was selected for further experiments.

Peroxidase-labeled goat-anti-mouse antibody was purchased from Dianova (Hamburg, Germany). Microbial transglutaminase was a generous gift from Ajinomoto Co., Inc. (Kawasaki, Japan). Casein sodium salt from bovine milk was purchased from Sigma (Deisenhofen, Germany), and 2,4-dichlorophenoxyacetic acid was obtained from Promochem (Wesel, Germany). All other chemicals were supplied by Fluka (Buch, Switzerland), Sigma, and Merck (Darmstadt, Germany) in the highest quality available.

Synthesis of *N*-(2-Aminoethyl)-2,4-dichlorophenoxy Acetamide. This 2,4-D derivative was prepared from 2,4-dichlorophenoxyacetic acid via its methyl ester with 1,2-diaminoethane according to a procedure described by Josten et al.[16]

Electrode System. A screen-printed three-electrode device arranged on one strip was used comprising a carbon counter electrode, a carbon working electrode (geometric area 0.24 cm^2), and a Ag/AgCl reference electrode. The electrode system was developed and optimized for application in the organic phase at Cranfield Biotechnology Centre (Cranfield, England).[17]

Determination of Hydrophobicity via Contact Angles. Contact angles of the electrodes as a parameter for hydrophobicity were determined with a contact angle measurement device (Krüss G1/G23, Hamburg, Germany). The electrodes were rinsed with water and subsequently dried at 120 °C. After a water droplet of 5 scale units was placed on the electrode surface, the contact angle was statically determined in duplicate on both sides of the droplet at 26 °C. Each determination was repeated three times.

Electrode Pretreatment. In order to remove impurities on the surface due to fabrication and storage, electrodes were pretreated prior to hapten immobilization. The electrodes were rinsed with water and shaken in 50% (v/v) methanol/water for 1.5 h at room temperature (shaker MTS4, 100 rpm, IKA Labortechnik, Staufen, Germany). After additional washing with water, the electrodes were dried at 35 °C within 10 min. The water used for the pretreatment was Seralpur PRO 90 CN-purified (Seral, Rausbeck-Baumbach, Germany).

Immobilization of Hapten. An aqueous solution of 0.83 mg/mL *N*-(2-aminoethyl)-2,4-dichlorophenoxy acetamide (2,4-D derivative), 0.38 mg/mL MTGase, and 0.8 mg/mL casein in carbonate buffer (0.1 M, pH 9.6) containing 24% (v/v) methanol was prepared. The area of the working electrode was covered with 15 μL of this immobilization cocktail for 1.5 h. The electrode was rinsed with water, washed with phosphate buffer (0.1 M, pH 7.4) containing 0.05% Tween-20 (Pb-Tween), and rinsed with water again. After drying at 35 °C for 10 min, the hapten-coated electrode was ready for use or was stored at 4 °C until use.

Quantification of Immobilized Hapten. A solution of 7 μg/mL monoclonal anti-2,4-D antibody and 8 μg/mL peroxidase-labeled goat-anti-mouse antibody in Pb-Tween was prepared. After preincubation for 45 min, 10 μL of this solution was pipetted onto the coated working electrode area. The antibodies were allowed to react with the immobilized hapten for 1 h in a humid box before the immunochemical reaction was stopped by washing with water, Pb-Tween, and water again. Upon substrate addition, retained enzyme-labeled antibodies were subsequently determined amperometrically.

Immunochemical Assay Procedure. The hapten-coated electrodes were used for 2,4-D determination according to the following protocol: A solution of 37.5% (v/v) methanol in phosphate buffer (0.1 M, pH 7.4) was prepared. For calibration, concentrations of 2,4-D from 0 to 400 mg/L were added to this mixture. Then, peroxidase-labeled anti-2,4-D antibodies (antibody-HRP) were added (final concentration, 100 μg/mL) and preincubated for 15 min. From this incubation mixture, 8 μL was placed on the hapten-coated working electrode area and allowed to react for 15 min, followed by washes with water, Pb-Tween, and water

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Table 1. In Situ Synthesis of 2,4-D−Casein Conjugates on the Electrode Surface[a]

experiment	current (nA/cm^2)
complete immobilization cocktail containing casein, MTGase, and 2,4-D derivative	510 ± 74
control experiments without MTGase	129 ± 16
control experiments without 2,4-D derivative	58 ± 9

[a] Immobilized hapten was detected with a cocktail containing 2,4-D-specific antibody and HRP-labeled secondary antibody in Pb-Tween (mean ± range, $n = 3$).

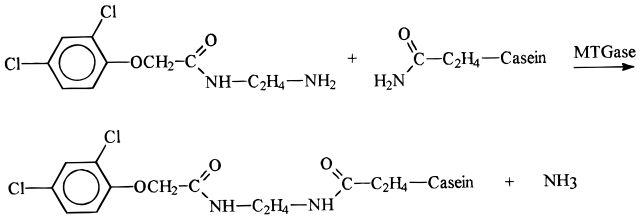
again. The electrodes were stored in 0.1 M phosphate buffer (pH 7.4) containing 0.1 M potassium chloride until measurement.

Amperometric Measurements. Measurements were performed with a software-controlled (FIABOLO, ICB Münster, Germany) amperometric detector (PED 300, Biometra, Göttingen, Germany) using the screen-printed three-electrode device. The experiments were carried out in 0.1 M phosphate buffer (pH 7.4) containing 0.1 M potassium chloride as a supporting electrolyte, 4 mmol/L hexacyanoferrate(II) as a mediator, and 50 μmol/L hydrogen peroxide as the substrate. The potential was adjusted to −20 mV, and signals were determined after reaching a stable current level.

Regeneration of Immunoelectrodes. The immunoelectrodes were treated after each electrochemical measurement with 2 M diluted sulfuric acid for 15 min, consecutively washed with water, Pb-Tween, and water, and finally dried at 35 °C. The electrodes were then ready to be incubated again with antibody−HRP solution according to the protocol described above.

RESULTS

Enzymatic-Catalyzed Antigen Immobilization. For single-step in situ synthesis of hapten−carrier conjugates on the electrode surface, a cocktail of the amino-functionalized 2,4-D as acyl acceptor, casein as acyl donor, and MTGase as catalyst was dropped on the surface of the electrode, thereby immobilizing the derivatized herbicide according to the following reaction:



Bovine casein was chosen as a hapten−carrier molecule, as literature describes this protein as excellent substrate for MTGase.[18] It can be assumed that, due to cross-linking, the conjugate forms an extensive molecule consisting of 2,4-D covalently attached to casein and intra- and intermolecular cross-links between glutamine and lysine residues of the casein carrier protein. In two control experiments, either MTGase or 2,4-D derivative was left out in the immobilization cocktail (Table 1). Only in the presence of enzyme and hapten were high signals obtained. The signal of the complete reaction cocktail can thus only be attributed to MTGase-catalyzed coupling of 2,4-D and casein. These data demonstrate the feasibility of the procedure.

In our experiments, we used screen-printed electrodes based on a carbon printing material. The determination of contact angles of an aqueous droplet on the electrode surface provides a measure of wettability and, hence, gives an estimation of the hydrophobic nature of the surface.[19] Hydrophilic surfaces provide contact angles approaching 0°, whereas a hydrophobic surface leads to nearly hemispherical droplets with contact angles around 90°. Thus, a contact angle increase indicates a more hydrophobic surface. We found in our experiments contact angles of water droplets on the screen-printed electrode surface of approximately 112° ± 10°, which showed, however, that the wettability of this electrode area was not sufficient to achieve a reproducible immobilization. Due to the hydrophobic nature of the surface, it was almost impossible to cover the surface homogeneously with an aqueous solution. Actually, a droplet of the immobilization cocktail was repelled by the electrode surface with a consequence of low reproducibility (data not shown). To overcome the high surface tension, we added methanol to the immobilization cocktail. Insufficient reproducibility was also observed with cocktails containing over 40% methanol as the droplet spread out onto the insulating layer. A homogeneous distribution of the cocktail was achieved, however, with cocktails containing 20−30% methanol. As the signal intensities decreased slightly with increasing amount of organic solvent, a concentration of 24% methanol was finally found to be optimal to cover the electrode surface homogeneously and to yield reproducible signal intensities.

Since the application of conjugates conventionally prepared creates problems in the reproducibility of the analytical tool, the new enzymatic method certainly enhances reproducibility. Haptens were immobilized on different electrodes from one batch on different days within 4 weeks according to the same protocol. The immobilized haptens were titrated with different amounts of antibody−HRP in order to select a working concentration of this conjugate. In addition, we immobilized casein without hapten as a negative control to study the non-specific binding of antibody−HRP conjugates. As can be seen in Figure 1, an excellent reproducibility was obtained within one set of measurements as well as between measurements from different days for both the hapten−casein conjugate and the negative control. Surprisingly, the non-specific binding of antibody−HRP conjugate in the control experiment is extraordinarily low. As this assay was carried out without any blocking step, we assume that our procedure led to an extensive covering of the whole electrode area by the hapten−casein conjugate, avoiding nonspecific antibody−HRP adsorption.

The titration of immobilized haptens was performed not only to investigate the influence of non-specific binding but also to select a suitable antibody concentration as a compromise between sufficient signal intensity and nonspecific binding. With this in mind, 100 μg/mL of the antibody−HRP solution was selected for further experiments.

Storage Stability. The hapten-coated electrodes were stable over at least 13 weeks at 4 °C when stored under dry conditions.

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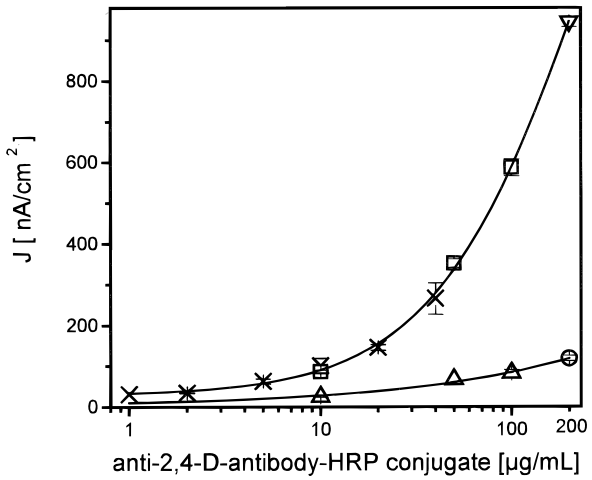

Figure 1. Titration of immobilized 2,4-D−casein on immunoelectrodes with different amounts of antibody−HRP conjugate. Immobilization cocktail containing casein, MTGase, and 2,4-D derivative (□, ×, and ▽) or casein and MTGase as a control (△ and ○). After washing and drying, the coated electrodes were incubated with antibody−HRP according to the immunochemical assay procedure. Each symbol represents a set of electrodes coated and measured on one day. The experiment was done within 4 weeks, thus indicating the repeatability of the immobilization procedure (mean ± range, $n = 2$).

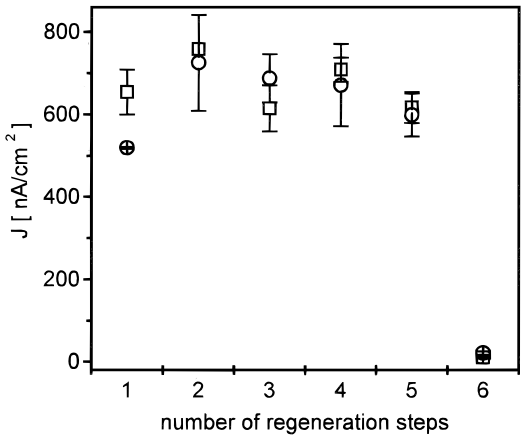

Figure 2. Regeneration of OPIE by washing with diluted sulfuric acid. Each regeneration was followed by a new antibody−HRP incubation step and the electrochemical measurement procedure (steps 1−5). In step 6, the treatment with sulfuric acid was directly followed by the measurement procedure, and the antibody−HRP incubation step was left out. □: Electrodes which were pretreated with sulfuric acid prior to step 1, which is the first incubation with antibody−HRP. ○: Electrodes which were not pretreated prior to step 1. Means ± range, $n = 2$.

Immobilized haptens were quantified electrochemically after incubation with antibody−HRP conjugate according to the immunochemical assay procedure. The current intensities of freshly prepared electrodes and of those after storage for 93 days were 795 ± 88 and 848 ± 96 nA/cm^2, respectively (means ± range, $n = 6$). Moreover, we observed no significant difference between the storage at 4 °C and at room temperature (data not shown). Therefore, a batch of hapten-coated electrodes could be prepared and conveniently stored until use.

Conditioning of the Hapten Layer. Preliminary experiments revealed that exposure of hapten−casein-coated electrodes to methanol affects the signal obtained in the immunosensing procedure. For a systematic investigation, electrodes which were already coated with 2,4-D-casein were treated with mixtures containing increasing methanol concentrations prior the incubation step with antibody−HRP (data not shown). It was found that the hapten layer was stable even in pure solvent and that methanol did not remove the hapten from the electrode surface. Highest signal intensities were obtained with electrodes that were exposed to 40% methanol. These findings can only be attributed to the interaction of the solvent with the hapten layer, but not to antibody binding, as antibody−HRP conjugates were not present in the exposure step. It may be due to a solvent influence on the orientation of 2,4-D immobilized on casein. This may lead to an improved accessibility to the hapten and, consequently, to a higher amount of antibody−HRP bound. The overall effect results in a higher signal intensity.

In a further approach, treatment of the hapten-coated electrodes with sulfuric acid prior to the antibody−HRP incubation step also led to a slight increase in the response. This was indicated by a comparison of electrodes that were washed with sulfuric acid and dried after that prior to the first antibody−HRP incubation and those which were not previously treated (Figure 2, step 1). Analogous to the influence of methanol, this may be attributed to a better accessibility of the hapten.

Regeneration of Immunoelectrodes. The dissociation and subsequent reuse of bound ligands relies on perturbing the antigen-antibody interactions.[20] It can be performed either with chaotropic or acidic buffers or with organic mixtures.[21] In the present study, we removed bound antibodies from the hapten-coated electrodes by treatment with diluted sulfuric acid (Figure 2). Each regeneration was followed by a new antibody−HRP incubation step and the electrochemical measurement procedure. The sixth regeneration with sulfuric acid was followed by the measurement procedure without prior antibody−HRP incubation. The low background found indicated that antibody−HRP conjugates were successfully removed from the electrode surface. As the treatment with sulfuric acid did not disturb the hapten−casein layer on these electrodes, the immunoelectrodes were reusable for at least six times without significant loss of activity. This operational stability enables the application of the electrodes in multiple-use sensing.

Organic-Phase Immunoelectrodes (OPIEs). A calibration curve was constructed using the data obtained with 70 OPIEs from different batches within 4 weeks (Figure 3). Each value was determined 10 times independently. The detection limits of 2,4-D in solutions containing 30% methanol were 9 and 130 mg/L, with a centerpoint sensitivity of 1.3 ± 0.4 nA L mg^{-1} cm^{-2}; the repeatability was 6.8% over the whole procedure. Only 17 s after substrate addition, the current reached 95% of its final value. In comparison, between calibration curves obtained from experiments in pure buffer and 30% methanol, the calibration curve in the presence of the organic solvent is shifted to higher response values (data not shown). The sensitivity and the lower limit of detection, however, were not affected significantly. The parameters of 2,4-D calibration curves obtained with the OPIEs are

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Table 2. Characteristic Parameters of the 2,4-D Calibration Curves Obtained with OPIEs[a]

electrodes	detection limits[a] (mg/L)	sensitivity[b] (nA L cm^{-2} mg^{-1})	coefficient of regression[c]	repeatability[d] (%)	assay time (min)	response time[e] (s)
single use	9−130	1.3 ± 0.4	>0.98	6.8 ± 0.3	30	17 ± 4
multiple use	10−70	0.9 ± 0.4	>0.99	13.4 ± 0.6	30	18 ± 3

[a] Detection limits were calculated by subtracting or adding the threefold confidence interval of the blank or the saturation value from or to the respective response (single use) or by subtracting/adding the threefold standard deviation (multiple use) and converting the result into the corresponding concentration. [b] Sensitivity is defined as the slope of the calibration curve at the centerpoint. [c] The mean values of the measurements were fitted to a four-parameter logistic equation. [d] Repeatability refers to test variations between electrodes of different batches and independent measurements from day to day (single use) or variation between two electrodes (multiple use) over the whole concentration range. [e] The response time is defined as the time interval over which the signal reaches 95% of its final value. The data are mean values over the total concentration range of seven (single use) or four (multiple use) electrodes.

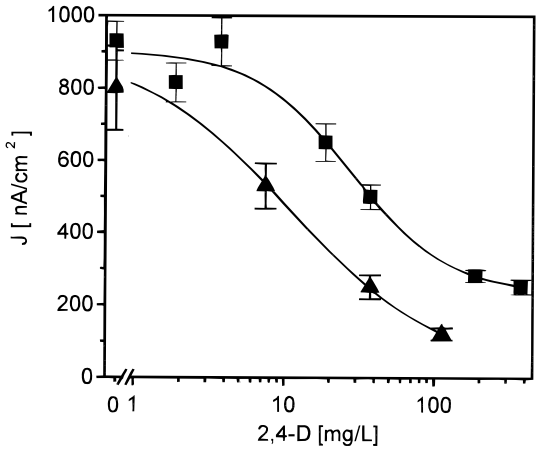

Figure 3. Calibration curves for 2,4-D obtained with OPIE in 30% methanol/buffer. Calibration was performed with 70 single-use immunoelectrodes from different batches on different days (□, mean ± confidence interval with $P = 0.90$, $n = 10$) or two multiple-use immunoelectrodes (▲, mean ± range, $n = 2$).

summarized in Table 2. As shown above, multiple-use application of the OPIE is possible. Thus, a calibration curve for 2,4-D using multiple-use OPIE in 30% methanol/buffer solution was established. A comparison of this calibration curve with the one made with single-use electrodes is depicted in Figure 3, and characteristics are summarized in Table 2. Reduced current responses for the reused electrodes were observed, but response times did not change. The lower detection limit was calculated to 10 mg/L; however, it has to be emphasized that only four concentrations were measured in duplicate. Although we calculated a lowered repeatability for reused electrodes of 13.4% and a slightly decreased sensitivity of 0.9 nA L mg^{-1} cm^{-2}, the calibration is very promising with regard to future studies employing multiple-use OPIEs.

DISCUSSION

Since target analytes with poor water solubility are usually recovered from sample matrices by solvent extraction, fast and simple analysis directly in the soil extract should avoid additional sample pretreatment. The electrode acts as both immunoreagent and electrochemical detector. Thus, to develop an organic-phase immunoelectrode (OPIE), the coated immunoelectrodes, the immunoreaction, and the electrochemical amplification system were optimized with regard to suitability and high stability in organic solvents.

We demonstrated that the enzymatic-catalyzed hapten immobilization is a reproducible immobilization procedure and an advantageous alternative to conventional chemical methods. A once optimized system needs no further optimization, even when a new lot of hapten is used. In contrast to conventional techniques, an additional blocking step is dispensable. The present method can be applied particularly to haptens containing aliphatic amino residues. Almost all low-molecular-weight compounds meeting this requirement are predicted to be bound in a single-step reaction to suitable proteins without any special synthesizing techniques. The reaction proceeds in aqueous solution as well as with additive organics in a few hours only, enabling widespread applications. So-prepared OPIEs obtain a high storage stability. Moreover, the immobilized hapten layer is extraordinarily stable in pure methanol as well as in diluted sulfuric acid, thereby enabling application in organic-phase analysis.

Although a polyclonal antiserum is expected to be more stable than monoclonal antibodies, a destructive effect of 5−10% acetonitrile and even 1% methanol on the ELISA sensitivity using two polyclonal anti-2,4-D antisera has been described recently.[22] Because organic mixtures are used to dissociate antibody-antigen interactions, as mentioned above, this is not surprising. For that reason, we used 2,4-D-specific monoclonal antibodies which were screened and selected to retain affinity even in 30% methanol. As enzyme label, horseradish peroxidase was found to be a suitable in organic-phase immunoprobes. To circumvent problems with electroactive species present in real samples, a mediated amperometric detection system was chosen comprising horseradish peroxidase and the most frequently used mediator, hexacyanoferrate(II). The Fe(III) complex produced by the enzymatic reaction is then reduced at the electrode surface at a very low potential (−20 mV vs screen-printed Ag/AgCl electrode in 0.1 M KCl), thereby yielding a measurable current response. The measurement at this potential prevents interferences of electroactive substances in sample matrices. By using HRP and the mediated detection principle, a modification of the electrode to obtain sufficient response or to reduce the potential applied is not required.[23]

The calibration curve of the OPIE shows a repeatability of 6.8%, which is a considerable improvement as compared to a 2,4-D-specific immunochemical sensor operating in pure aqueous solution, with stated reproducibilities around 15−20%.[24,25] As

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variations of our OPIE within one batch and even between several batches were low, a simple batch calibration can be envisaged for future sensor developments. Inasmuch as response times of biosensors are often in the range of minutes,[26] the behavior of the present immunoelectrode is quite advantageous. Only 17 s after substrate addition, the current reaches 95% of its final value. This short measuring time enables fast data evaluation. The complete immunoassay, however, takes approximately 30 min. Moreover, the determination could be easily performed by reading the steady-state current value instead of analyzing the initial slope, which has led to a main step forward to a convenient and practicable immunoelectrode for on-site screening. It has to be emphasized that the OPIE was not optimized with regard to the lower detection limit but rather with the intention of producing the most highly reproducible system possible. Due to the extraction procedure, the analyte undergoes an enrichment step. Thus, detection limits as low as those in water analysis do not have to be achieved. The aim of our present work was the development of a new technique more than system optimization. A further variation and optimization of assay parameters such as incubation times, pH of solutions, or the amount of reagents in the immobilization procedure should further improve assay characteristics without adversely affecting the device reproducibility.

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For applications where more than one sensor system is required, multiple use of the OPIE is advantageous. Thus, it is promising that we found that exposure of the OPIE to sulfuric acid allows an easy regeneration for multiple use.

The use of OPIEs in environmental analysis provides the possibility to reduce the number of steps required for sample analysis, thereby enabling simple, cost-efficient, and accurate on-site determinations. Further studies will focus on the application of OPIEs with organic solvent extraction of soils and the determination of soil extracts without further sample processing and cleanup. The immunochemical detection in organic phases expands the range of application for a widespread group of hydrophobic molecules not only in environmental analysis but also for food analysis or clinical diagnostics.

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