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Rapid flow injection electrochemical detection of 3,3',4,4' tetrachlorobiphenyl using stabilized lipid membranes with incorporated sheep antibody

Research Article

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Abstract: An electrochemical biosensor based on a supported polymerized lipid film with incorporated sheep anti-3,3',4,4' tetrachlorobiphenyl (PCB congener 77) antibody using flow injection analysis was developed. The polymerized lipid film contained 85% (w/w) dipalmitoylphosphatidylcholine (DPPC) and 15% (w/w) dipalmitoylphosphatidic acid (DPPA), methacrylic acid, ethylene glycol dimethacrylate, AIBN and sheep anti-congener 77 antiserum. Congener 77 was injected into flowing carrier electrolyte and the flow stopped to detect the antigen. These membranes gave only a single transient proportional to log [congener 77] from 10^{-8} to 10^{-5} M, with a detection limit of *ca*. 10^{-8} M. A membrane containing 35% (w/w) DPPA was used to examine regeneration. The maximum number of cycles was about 5.

Keywords: *Congener 77 • Stabilized lipid films • Biosensor • Flow injection • Antibody regeneration*

1. Introduction

Polychlorinated biphenyls (PCBs) constitute a well-known environmental problem [1]. Commercial formulations (*e.g.* Aroclor manufactured by Monsanto, USA; Clophen by Bayer, Germany; and Phenoclor by Caffaro, Italy) contain complex mixtures of the 209 congeners. Each product is identified by the chlorine percentage (*e.g.* Aroclor 1242 contains 42% chlorine). Arochlor 1242 is a mixture of monochloro-BPs (0.08%), dichloro-BPs (14.5%), trichloro-BPs (42.8%), tetrachloro-BPs (33.5%), pentachloro-BPs (6.6%), hexachloro-BPs (1.7%), heptachloro-BPs (0.1%),) and octachloro-BPs (0.01%) [2]. Congener 77 (3,3',4,4'-tetrachlorobiphenyl) is one of its main components.

Although their use has been banned in industrialized countries since the late 1970s [3], their environmental half-life of 10–20 years (especially highly substituted congeners) makes PCBs one of the most widespread environmental pollutants [4]. PCBs' aquatic toxicity is well known and they are suspected carcinogens. They cause liver injury in monkeys. PCBs also cause eye irritation and show slight inhalation toxicity.

Human and wildlife health risks due to non-*ortho*-substituted PCB exposure are expressed by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQ) using

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toxic equivalency factors (TEF). For comparison, 3,3',4,4'-tetrachlorobiphenyl (PCB 77), 3,3',4,4',5-pentachlorobiphenyl (PCB 126) and 3,3',4,4',5,5'-hexachlorobiphenyl (PCB 169) are the most toxic congeners; their TEF-values are 0.0001, 0.1 and 0.01, respectively [5].

The most common technique in the literature to analyse PCBs is GC (gas chromatography) coupled with mass spectrometry (GC/MS) [6], immunoassays provide an alternative inexpensive, rapid sensitive and specific route for both in the field and laboratory detection method. Among these methods, Enzyme-linked immunosorbent assays (ELISA) are the most widely used [7,8]. Recently, a number of immunosensors appeared in the literature. A specific antibody is immobilized at the sensor surface and used as the recognition element [9]. Among these biosensing devices electrochemical immunosensors have gained most of the attention; these devices are based on an enzyme-labelled reagent that generates an electroactive product which is detected [10,11]. Examples of these biosensors have been reported by, Laschi and Mascini [10] which immobilized an antibody onto screen-printed carbon electrodes (SPCEs) and were able to detect 14 μg mL^{-1} of Aroclor 1248. Soo Kim *et al.* [12] also have developed an indirect competitive PCB immunoassay with a detection limit of 25 ng mL^{-1} Aroclor 1254. The significant signal amplification was the main advantage of these methods.

The PCB congener 77 hapten was mimicked using a *para*-substituted spacer arm to retain conformational coplanarity, giving antibodies highly specificy for coplanar congeners [2]. Direct competitive ELISAs showed good assay stability and high specificity towards congener 77 [2].

In our earlier work we reported the electrochemical transduction of Arochlor 1242 using bilayer lipid membranes (BLMs) composed of egg phosphatidylcholine and dipalmitoyl phosphatidic acid (DPPA) [13]. Antibody-antigen complexation at BLMs caused transient ion current due to membrane surface charge modulation in less than two minutes. Signal increased with bulk antigen concentration, with nanomolar detection limits. The aim of the present work was develop a rapid immunosensor selective to congener 77 using stabilized BLMs for flow injection immunoanalysis.

2. Experimental procedure

2.1. Materials and apparatus

The materials and instruments have been described [13]. One of the Ag/AgCl reference electrodes was in the carrier electrolyte waste and the other in the cylindrical cell. 25 mV DC was applied across the filter membrane between the two and the current monitored with a digital electrometer (Model 614, Keithley Instruments, Cleveland, OH). The entire apparatus was isolated in a grounded Faraday cage.

2.2. Procedure

Stabilized lipid films were formed on a microporous filter glass fiber disk (*ca*. 0.9 cm diameter) by polymerization similar to that previously described [13]. However, polymerization used UV irradiation instead of thermal polymerization. A 5 mg sample of mixed lipid powder containing 85% (w/w) DPPC and 15% (w/w) DPPA was mixed with 0.070 mL of methacrylic acid, 0.8 mL of ethylene glycol dimethacrylate, 8 mg of AIBN and 1.0 mL of acetonitrile. The mixture was bubbled with nitrogen for about 1 min and sonicated for 30 min. This mixture could be stored in the refrigerator. Using a microsyringe, the microfilter surface was covered with 0.15 mL of the mixture and 10 μL of sheep anti-congener 77 antiserum, then irradiated with a deuterium UV lamp. The polymerization was monitored by Raman spectrometry.

The stock congener 77 solution was 1.0 mM in methanol. Dilute aqueous solutions were prepared daily just before use.

The filter disk with the film was placed between two Saran Wrap layers with the filter centered on the 0.32 mm orifice. This was then clamped between the Plexiglass chambers. The carrier electrolyte was 0.1 M KCl - 20 mM pH 7.0 HEPES. Injections (75 μL) of congener 77 solution were made into the carrier, flowing at 1.0 mL min^{-1}. The flow was stopped for less than 2 min until a transient signal was obtained. A 5 minute wash out with carrier regenerated the antibody for repeat injections. All experiments were made at 25±1ºC.

3. Results and discussion

Electrochemical determination of Arochlor 1242 with stabilized polymerized BLMs prepared from mixtures of egg PC and DPPA has been reported [13]. Antibody-antigen complexation caused charging currents due to electrostatic field changes at the membrane surface. The transients occurred as singular or multiple events using films containing 15% or 35% (w/w) DPPA respectively, where each transient lasted for several seconds.

A pH 6.0 - 8.0 electrolyte (free of Ca^{+2}), BLMs containing 15% (w/w) DPPA, and stopped-flow detection was used. Flow was resumed after the transient. Membranes containing 15% (w/w) DPPA produced

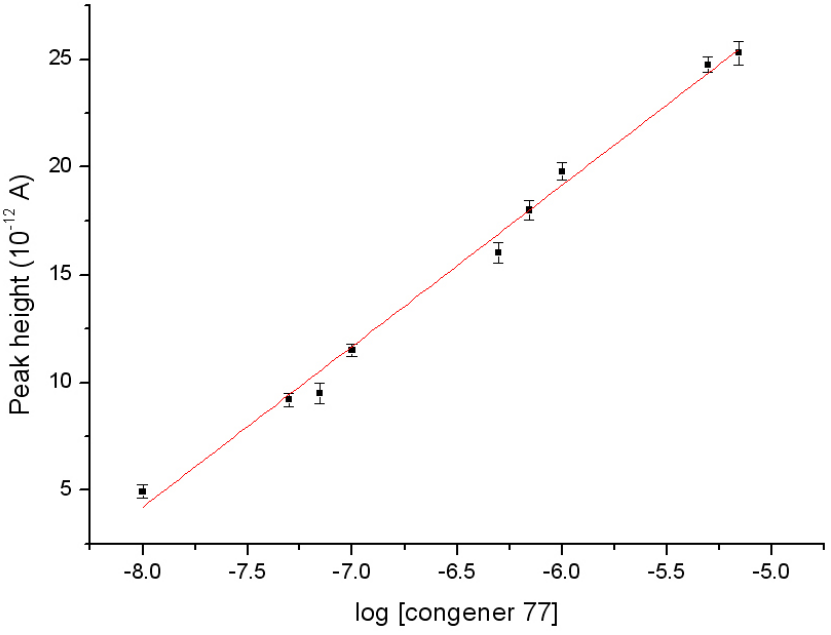

Figure 1. Signal calibration.

a single transient proportional to log [Aroclor 1242]. Continuous flow injection gave no detectable signal.

Similar conditions were used in this work. The 15% DPPA membrane was the only one giving a single transient which was proportional to log [congener 77] in the carrier (Fig. 1: Y=7.46*X+63.9; r^2=0.993). Control experiments using antigen or thyroxin (T4) demonstrated that the signals were due to selective interactions.

The mechanism of signal generation from antisheep- Arochlor 1242 complexation at BLMs has been described [13]. The process may occur in two steps; immunoreaction provides complexes at the surface which may then aggregate and perturb the membrane electrostatically. Aggregation may be relatively independent of the complexation rate. At constant temperature the aggregation rate should be relatively constant, and is expected to be slow as it is based on movement across the membrane surface. Aggregates become larger at higher antigen concentrations; consequently the signal magnitude increases. Provided that the ion current is driven by electrostatic changes at the membrane surface, the ion current magnitude should be logarithmically related to the antigen concentration as given by the Nernst equation.

A biosensor in principle should be regenerable and capable of multiple analyses. We have previously indicated an approach to regeneration of the binding sites is by infinite dilution, *i.e.*, by continuous flow so that dissociated antigen is continuously removed. The rate of dissociation determines the time of regeneration. For a low or moderate affinity interactions, the antibody can be regenerated within a reasonable time.

The time required was estimated using BLMs containing 35% DPPA in the presence of calcium ions. Multiple transients appear over an hour, providing a series of transients that could be used in a stopped-flow mode to determine whether the antibody-antigen complex was present. The results were similar to these previously reported. The time between antigen injection and the first signal was about 3 minutes. Carrier flow was re-initiated 3 minutes after the first signal, so that loss of the antibody-antigen complex could be observed as a reduction of the transient frequency and magnitude.

Approximately 5 minutes were required to eliminate the transients when using 1 pM of congener 77. Similar times were previously found using Arochlor 1242 [13].

About five repeated injections could be performed with retention of calibration. The variability was 3% for the first injection and 7% for the fifth (N = 5). The calibration plot drifts for further injections and the accuracy decreases. Signal reduction was probably due to antibody removal from the membrane by the flowing solution.

The cross-reactivity to other components of Arochlor 1242, *i.e.*, dichloro-BPs, trichloro-BPs, pentachloro-BPs and hexachloro-BPs was evaluated. Congener 126 and trichloro-BPs show a maximum 6% interference; less than 5% interference was observed from the rest of the PCBs. Similar results were obtained by Centi *et al.* [14].

4. Application to real samples

The sensor was tested on extracts of marine sediments and soils. These differed in the spiking step. Marine sediments were extracted by Soxhlet, so the extracts were spiked with PCB mixtures. Soil samples were spiked before extraction, as is common [15,16].

Marine sediment extracts were spiked with congener 77 (0.1 - 10 μM). Recovery was 94-105%. The 95 to 108% recovery from spiked soil samples demonstrated the effectiveness of the sonication method.

5. Conclusions

Using flow injection analysis stabilized polymerized lipid films can rapidly and selectively detect PCB congener 77 in real samples of marine sediment and soil. Stabilized BLMs with incorporated antibody are selective towards congener 77 and can be used for 5 analysis cycles. The method offers fast response times (minutes) and detection of this PCB at nM levels.

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