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Flow Injection Monitoring and Analysis of Mixtures of Hydrazine Compounds Using Filter-Supported Bilayer Lipid Membranes with Incorporated DNA

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This work describes a technique for the rapid and sensitive electrochemical flow injection monitoring and analysis of mixtures of hydrazine compounds using stabilized systems of filter-supported bilayer lipid membranes (BLMs) composed of egg phosphatidylcholine (egg PC) with incorporated DNA. Injections of hydrazines were made into flowing streams of a carrier electrolyte solution, and a transient current signal with a duration of seconds reproducibly appeared in less than one min after exposure of the DNA-modified lipid membranes to the hydrazines. The magnitude of this signal was linearly related to the concentration of hydrazines, which could be determined at sub-micromolar levels. Repetitive cycles of injection of hydrazines have shown no signal degradation during each cycle (30 sequential injections). The time of appearance of the transient response was different for each hydrazine and increased in the order of hydrazine, methylhydrazine or dimethylhydrazine, and phenylhydrazine. The difference in time of response has allowed selective detection and analysis of these hydrazines in mixtures.

Hydrazine, methylhydrazine, dimethylhydrazine, and phenylhydrazine are high-energy propellants used in large quantities for the space shuttle program and for other aerospace operations. They are also involved in a number of commercial applications in industry, agriculture, explosives, the plastics industry as blowing agents, and other fields (e.g., they are used as antioxidants, preservatives, welding fluxes, and oxygen scavengers).[1,2] The large volumes of hydrazine being used in the public and private sectors have generated concern for the health and safety of persons in close contact with these chemicals. The toxicological problems associated with the inhalation or digestion of the hydrazines have been monitored in laboratory animals, and they include damage to internal organs, creation of blood abnormalities, irreversible deterioration of the nervous system, and documented carcinogenic effects in humans.[3,4] Therefore, the threshold limit values (TLVs) for hydrazine compounds were lowered to the order of 10 ppb in air.[5] Compliance with these standards will necessitate the development of new sensors for detecting these low levels of hydrazine in the workplace, i.e., in air and wastewater.

Several different analytical methods have been used for the detection of hydrazines. Examples include coulometry, potentiometry, titration, colorimetry, dosimetry, fluorescence, mass spectroscopy, ion-mobility spectroscopy, gas chromatography, and liquid chromatography.[6] Each of these techniques has merits in specific applications; however, none has achieved the sensitivity, selectivity, and real time monitoring capability necessary for determining the new TLVs for hydrazines in the workplace. Recently, a sensitive DNA biosensor for the rapid detection of hydrazines appeared in the literature;[7] this DNA biosensor, however, lacks selectivity and is not able to discriminate between hydrazine compounds.

The analytical utility of metal supported bilayer lipid membranes (s-BLMs) which contained DNA was recently described in the literature, and the results showed that such sensors could electrochemically monitor hydrazines;[8] interactions of hydrazines with s-BLMs could be detected by observation of ion current signal increases within a few seconds after exposure of the membrane to hydrazines. sBLMs with incorporated DNA systems could be the basis for the construction of one-time use biosensors for direct monitoring of such environmental pollutants. A technique for the preparation of stabilized BLMs for uses in flow-through experiments has recently been reported.[9] Ultrafiltration membranes such as glass fiber and polycarbonate were found to act as supports to

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(1) *Hydrazine and its Derivatives*: *Preparation*, *Properties*, *Applications*; Schmidt, E. W., Ed.; Wiley: New York, 1984.
(2) Schessl, H. W. *Encyclopedia of Chemical Technology*, 3rd ed.; Othmer, K., Ed.; Wiley: New York, 1980; Vol. 12, p 734.
(3) Hannum, J. E. Recent Developments in the Toxicology of Propellant Hydrazines; CPTR 82-15; Chemical Propulsion Information Agency: Laurel, MD, 1982.
(4) *Criteria for a Recommended Standard-Occupational Exposure to Hydrazines*; Publication No. 78-172; DHEW(NIOSH) National Institute for Occupational Safety and Health: Cincinnati, OH, 1978.
(5) *American Conference on Governmental Industrial Hygienists*: *Documentation of the Threshold Limit Values and Biological Exposure Indices*, 6th ed.; Cincinnati, OH, 1991.
(6) (a) Collins, G. E.; Rose-Pehrsson, S. L. *Analyst* (*Cambridge*,) **1994**, *119*, 1907−1913. (b) Safavi, A.; Baezzat, M. R. *Anal. Chim. Acta* **1998**, *358*, 121−125.
(7) Wang, J.; Chicharro, M.; Rivas, G.; Cai, X.; Dontha, N.; Farias P. A. M.; Shiraishi, H. *Anal. Chem.* **1996**, *68*, 2251−2254.
(8) Siontorou, C. G.; Nikolelis, D. P.; Tarus, B.; Dumbrava, I.; Krull, U. J. *Electroanalysis* **1998**, *10* (10), 691−699.
(9) Nikolelis, D. P.; Siontorou, C. G.; Andreou, V. G.; Krull, U. J. *Electroanalysis* **1995**, *7* (6), 531−536.

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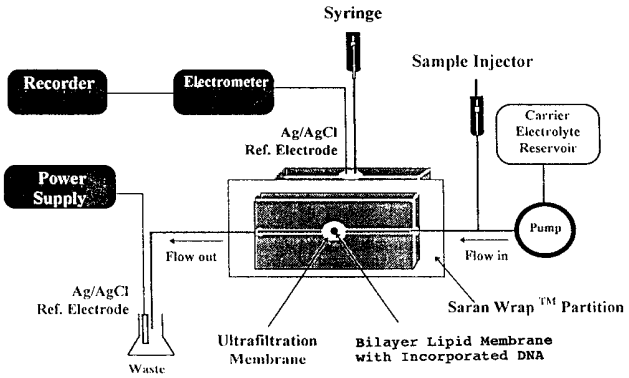

Figure 1. Experimental setup of the apparatus used for the flow monitoring and analysis of mixtures of hydrazine compounds using filter-supported BLMs with incorporated DNA.

enhance the stability of BLMs for flow injection experiments. These microporous filters can act as interfaces separating two solution compartments; one of the compartments is used to cast the lipid membrane on the filter, while a carrier electrolyte concurrently flows through the opposing compartment. The analytical signal in these experiments is based on monitoring changes of current across an electrochemical system which consists of two reference electrodes that are separated by a highly resistive semipermeable membrane (BLM) (Figure 1). The configuration is reminiscent of a glass electrode, with one side of the BLM serving as a chemically selective interface while the other side is exposed to an unchanging reference solution. There are two major distinctions, though, in comparison with a glass electrode. The experiment measures current and not potential, and the ions that carry the current through the BLM originate in the supporting electrolyte solution. These permions are usually small positive cations such as sodium or potassium ions. They travel through the BLM along defect sites in the structure of the membrane and move down a small voltage gradient (approximately 25 mV) that is imposed between the two reference electrodes. The experiment can measure changes of ion current through a BLM as well as surface charging current.[10]

This work describes a modification of filter-supported lipid membranes for the rapid and sensitive electrochemical detection of hydrazines (i.e., hydrazine, methylhydrazine, dimethylhydrazine, and phenylhydrazine). Filter-supported lipid membranes were used as stable and reversible detectors for flow injection analysis (FIA). The modification of the lipid membranes involved incorporation of single-stranded C_{16}-dT_{20}. The first report of the incorporation of C_{16}-dT_{20} into BLMs appeared in 1997.[11] The mechanism of the incorporation of the ssDNA, where this sequence is terminated by a C16 alkyl chain to assist incorporation into the lipid membrane, was also recently explored.[12] The present work provides a technique of an electrochemical detection of hydrazines at the part-per-billion level, representing an improvement in detection level of over 5 orders of magnitude in comparison with earlier work which studied the use of lipid membranes for detection of hydrazines. Detection consisted of a time-dependent appearance of a transient ion current peak, where the time dependence could be used to distinguish the presence of different hydrazines, and the peak magnitude was related to the concentration of hydrazine. Repetitive cycles of injection of hydrazines provided transient signals with no signal degradation over 30 sequential injections. A number of inorganic ions and compounds were tested as potential interferents, and validation of the technique for monitoring hydrazine compounds in reference water samples is reported.

EXPERIMENTAL SECTION

Materials and Apparatus. The lipid used throughout this study was lyophilized egg phosphatidylcholine (egg PC, Avanti Polar Lipids, Birmingham, AL). Hydrazine hydrate was purchased from Sigma Chemical Co. (St. Louis, MO). Methylhydrazine, 1,2 dimethylhydrazine dihydrochloride, and phenylhydrazine hydrochloride were products of Aldrich (Steinheim, Germany). **Caution:** The toxicity of hydrazine compounds requires appropriate care. The chemicals and basic techniques used for the oligonucleotide synthesis have been previously reported.[13,14] The filters and (nominal) pore sizes used were GF/F glass microfiber (0.7; Whatman Scientific Ltd., Kent, UK). Water was purified by passage through a Milli-Q cartridge filtering system (Millipore, El Paso, TX) and had a minimum resistivity of 18 MΩ cm. All other chemicals were of analytical reagent grade.

The apparatus for the formation of stabilized BLMs was previously described (Figure 1).[9,15,16] The apparatus consisted of two Plexiglas chambers separated by a Saran-Wrap (PVDC, DowBrands L. P., Indianapolis, IN) partition of a thickness of ~10 μm. This plastic sheet was cut to more than twice the size of the contact area of the faces of the chambers and folded in half; a hole of 0.32-mm diameter was made through the double layer of the plastic film by punching with a perforation tool.[17] A microporous glass fiber disk (diameter of ~0.9 cm) was placed between the two plastic layers and centered on the 0.32-mm orifice. The partition with the filter membrane in place was then clamped between the Plexiglas chambers. One of the chambers was machined to contain an electrochemical cell with a circular shape (diameter 1.0 cm and depth 0.5 cm) connected with plastic tubing for the flow of the carrier solution. A Ag/AgCl reference electrode was immersed in the waste of the carrier electrolyte solution. The second chamber was machined to contain a cylindrical cell having its longitudinal axis perpendicular to the flow of the carrier solution. The upper hole of this cell was circular (surface area of about 0.2 cm^2). The lower was elliptical (with diameters 0.5 and 1.4 cm parallel and vertical, respectively, to the flow of the carrier electrolyte solution) facing the opposing cell. The microporous glass fiber filter was positioned approximately at the center of the cells. A Ag/AgCl reference electrode was placed into the cylindrical cell, and an external 25 mV d.c. voltage was applied across the filter membrane between the two reference electrodes. A digital electrometer (model 614, Keithley Instru-

(10) Krull, U. J.; Seethaler, S. L.; Brennan, J. D.; Nikolelis, D. P. *Thin Solid Films* **1994**, *244*, 917−922.
(11) Siontorou, C. G.; Nikolelis, D. P.; Piunno, P. A. E.; Krull, U. J. *Electroanalysis* **1997**, *9* (14), 1067−1071.
(12) Zeng, J.; Nikolelis, D. P.; Krull, U. J. *Electroanalysis* **1999**, *11*(10−11), 770−773.
(13) Piunno, P. A.; Krull, U. J.; Hudson, R. H. E.; Damha, M. J.; Cohen, H. *Anal. Chim. Acta*, **1994**, *288*, 205−214.
(14) Piunno, P. A.; Krull, U. J.; Hudson, R. H. E.; Damha, M. J.; Cohen, H. *Anal. Chem*. **1995**, *75*, 2635−2643.
(15) Nikolelis, D. P.; Siontorou, C. G. *Anal. Chem*. **1995**, *67*, 936−944.
(16) Nikolelis, D. P.; Siontorou, C. G.; Andreou, V. G.; Viras, K. G.; Krull, U. J. *Electroanalysis* **1995**, *7* (11), 1082−1089.
(17) Nikolelis, D. P.; Krull, U. J. *Talanta* **1992**, *39* (8), 1045−1049.

ments, Cleveland, OH) was used as a current-to-voltage converter. A peristaltic pump (Gilson Minipuls 3) was used for the flow of the carrier electrolyte. Injections of hydrazines were made with a Hamilton repeating dispenser with a disposable tip (Hamilton Co., Reno, Nevada). The electrochemical cell and electronic equipment were isolated in a grounded Faraday cage.

Procedures. A stock solution of PC containing 2.5 mg mL^{-1} in *n*-hexane was used for the preparation of lipid solutions of 0.04 mg mL^{-1} lipid in the same solvent that was used for BLM casting in the electrochemical experiments. These dilute lipid solutions were prepared daily, just before the commencement of experiments, and the stock lipid solution was stored in the dark in a nitrogen atmosphere at −4 °C. The BLMs were supported in a 0.1 M KCl electrolyte solution.

The oligonucleotide used was 5′-hexadecyl-deoxythymidylic acid icosanucleotide (dT_{20}-C_{16}) and was synthesized as previously reported.[13,14,18,19] The concentration of the oligonucleotide in normal saline was 124.4 mg mL^{-1}. Further dilutions of the stock solution were also made in normal saline.

Calibration of the response of filter-supported BLMs (with or without incorporated ssDNA) in the presence of hydrazine was done by injections of dilute hydrazine solutions prepared from a standard hydrazine solution (1.03 g mL^{-1} in 0.1 M KCl). The standard solutions of the other hydrazines used were 0.874 mg L^{-1} for methylhydrazine, 0.800 mg mL^{-1} for dimethylhydrazine, and 1.10 mg mL^{-1} for phenylhydrazine. A solution of 0.1 M KCl was also used to prepare the injected dilute hydrazine solutions.

The process of formation of stabilized BLM for flow injection experiments was described recently.[9,15,16] Lipid solution (10 μL) was added dropwise from a microliter syringe to the electrolyte surface in the cylindrical cell near the partition. The level of the electrolyte solution was dropped below the aperture and then raised again within a few seconds. The formation of "solventless" or "solvent-free" BLMs was verified by the magnitude of the ion current obtained and by electrochemical characterization using gramicidin D.[17] An injection of a solution of 19 ng mL^{-1} dT_{20}-C_{16} followed the ion-current stabilization. The flow of the electrolyte solution was stopped and the ion current increased to about 40 pA (within ∼10 s following DNA injection) which indicated the incorporation of ssDNA in BLMs. The flow of the carrier solution was initiated again (at a rate of 0.7 mL min^{-1}) and repetitive injections of 75 μL of hydrazine solutions were made. All experiments were done at 25 ± 1 °C.

RESULTS AND DISCUSSION

Adaptation of Interactions Using Filter-Supported BLMs. The potentiality of BLMs to be the basis for the construction of one-time use biosensors for direct monitoring of hydrazines has recently been suggested.[8] These previous studies have used self-assembled metal-supported BLMs (s-BLMs) with incorporated DNA. The results have shown that increases of ion-current values could appear within 20 s after injection of hydrazines in bulk solution. In an effort to reduce nonselective interferences and apply the technique to real environmental samples, stabilized BLMs supported in microporous filters[9,15,16] were investigated. The oligomer dT_{20}-C_{16} was incorporated in these BLMs by injection of solutions of the oligomer (in a stopped-flow mode) prior to hydrazine monitoring. The results of ssDNA incorporation have shown increases of the ion current values. These current enhancements (on the order of pA) were similar to previous results[11,20] and appeared within ∼10 s after ssDNA injection. These conductance enhancements exhibit the adsorption of the oligomer at filter-supported BLMs and, therefore, that the flow of solution could again be initiated.

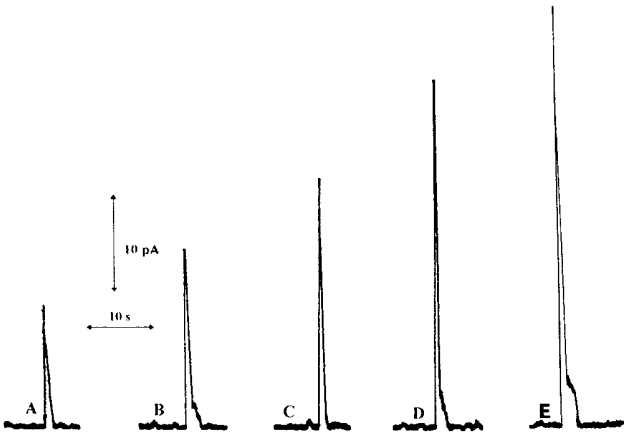

Figure 2. Experimental results obtained for hydrazine at pH 7.3 (0.1 M KCl) with BLMs composed of PC with incorporated 19.0 ng mL^{-1} dT_{20}-C_{16} and supported in glass microfiber filters. Hydrazine concentrations of the solutions injected (75 uL) in the carrier electrolyte solution (ppb): (A) 2.57; (B) 5.15; (C) 7.40; (D) 10.3; (E) 12.5. The injection of each sample was made at the beginning of each recording. The recordings shown were chosen randomly from a number of injections made. The flow rate of the carrier electrolyte solution was 0.7 mL min^{-1}.

Figure 2 shows recordings of the signals obtained with injections of hydrazine in continuous flowing streams of carrier electrolyte solution of pH 7.3 (0.1 M KCl). Experiments were done using a continuous flow of the carrier electrolyte solution and flow rates of 0.7 mL min^{-1}. It can be seen in this figure that a transient current signal as a single event is obtained by the interactions of hydrazine with the filter-supported BLMs with incorporated ssDNA. A constant time delay for the appearance of the transient currents of (6.84 ± 1.31) s is observed in Figure 2. The time period that is related to the appearance of transient peaks is in the range of approximately 1−2.5 s, yet the time related to the passage of the sample across the surface of the filter-supported membrane is about 5−6 s. This difference is due to the fact that the evolution of the transient signal occurs because of dynamic changes at the membrane−solution interface and is not directly influenced by the presence of hydrazines in solution. The hydrazine partition to the surface and, subsequently, changes in capacitance and membrane structure are induced by a secondary associative event. The magnitude of these transient responses is in direct proportion to the hydrazine concentration in the carrier electrolyte solution [ΔI (pA) = 3.05 C (ppb) + 3.37, r^2 = 0.994). The variability of response of the BLMs to repetitive hydrazine injections expressed by the relative standard deviation was between 3.5 and 7.5% (N = 5). The detection limit of hydrazine monitoring expressed as three times the signal noise was 2.5 ppb.

(18) Hirschfeld, T. B. U.S. Patent 5,242,797, 1993.
(19) Vo-Dinh, T.; Houck K.; Stokes, D. L. *Anal. Chem.* **1994**, *66*, 3379−3383.
(20) Siontorou, C. G.; Oliveira Brett, A.-M.; Nikolelis, D. P. *Talanta* **1996**, *43*, 1137−1144.

Table 1. Comparison of Results of Signal Magnitude and Time of Appearance of Signal Between BLMs with Incorporated sDNA with Respect to Unmodified Lipid Membranes

Concn of Hydrazine	Ion Current Magnitude (Peak Current)	
	planar BLMs	filter BLMs
BLMs without Incorporated ssDNA		
1030 ppm	15 pA (18.5 s)	14 pA (17.4 s)
1543 ppm	22 pA (17.5 s)	22.4 (18 s)
2056 ppm	35 pA (16 s)	34.5 (17.5 s)
BLMs with Incorporated ssDNA		
5.15 ppb	22.4 pA (7.6 s)	19.9 (7 s)
10.3 ppb	41.2 pA (6 s)	38.5 (6.5 s)

The goal of this research was to prepare a sensitive biosensor for hydrazines that could operate at the ppb concentration level. While filter-supported lipid membranes have been previously reported, the novelty in this work is in the use of immobilized ssDNA to significantly increase the sensitivity of the detector. The ssDNA is modified to contain a C16 hydrocarbon chain that helps ensure incorporation into a lipid membrane. The selection of dT_{20}-C_{16} as a modifying agent was made on the basis of experience and knowledge of the kinetics and adsorption characteristics of this material onto lipid membranes from earlier work.[11,12] Our experimental work has also included investigation of adsorption of dT_{20}-C_{16} and mixed base pair sequences up to 25mer in length. These sequences adsorb to membranes, although apparently with different partition equilibria than those for dT_{20}-C_{16}. The dT_{20}-C_{16} provides a high degree of electrostatic modification of lipid membranes as seen by ion current changes and is relatively strongly adsorbed as determined by washing experiments that show negligible loss of material over time courses of relevance to the experiments with hydrazines. Long strands of natural DNA and unmodified (no C16 tail) DNA have not been used since preliminary results show that they are more weakly adsorbed than short strands of DNA with a C16 tail. The ssDNA does not act as a selective receptor for hydrazines. Rather, the presence of the ssDNA alters the electrochemical sensitivity of the BLM so that perturbations of the charge and structure of the membrane are amplified to provide greater changes in signal (ion current). Table 1 provides a summary that indicates the analytical advantages in sensitivity that can be established by incorporation of ssDNA into lipid membranes with respect to unmodified lipid membranes. The results of Table 1 serve to indicate that the presence of ssDNA can increase the detection limit of a filter-supported lipid membrane sensor by at least 5 orders of magnitude for detection of hydrazines.

Effect of Temperature and pH. The applications reported here were done at ambient temperature (25 °C) since it was clear that under these conditions the sensitivity for detection of hydrazines was sufficient for the purpose of our work. The adjustment of temperature provides another method to optimize signal-to-noise, and this is an area of further research in our laboratories. An increase of temperature to 30 °C has shown that the signal remained practically constant.

The pH range that was examined was between 3.0 and 9.0.[21] The signal was found to be constant within experimental uncertainty for the pH range between 3.0 and 8.0 and decreased marginally by about 10% at pH 9.0. Consideration of the ionization properties of primary and secondary phosphates, amines, and the enol-OH in DNA suggests that the ssDNA used in our work will have a pI in the range of pH 2−3. In this pH range the ionization of primary phosphates and that of amine is at about the same percentage (enol effects are not significant in this pH range).[22] Hydrazine is a diprotic base with a pK_1 value of 6.05.[23]

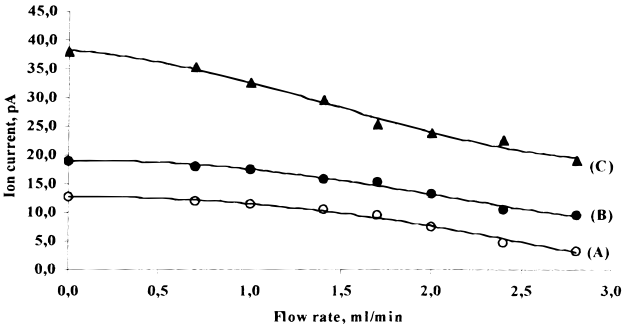

Figure 3. Effect of flow rate on signal magnitude. Hydrazine concentrations of the solutions injected in the carrier electrolyte solution (ppb): (A) 2.57; (B) 5.15; (C) 10.3.

Effect of Flow Rate. Figure 3 shows the effect of the flow rate of the carrier electrolyte solution on the magnitude of the transient signal. The equations which relate the magnitude of ion current as a function of flow rate are Boltzman sigmoidals and are given by the equations

$$\mathrm{I\ (pA)} = 0.555 + 12.2/1 + \mathrm{e}^{(2.14 - x)/-0.504}\ (r^2 = 0.995) \quad (1)$$

$$\mathrm{I\ (pA)} = 7.084 + 12.1/1 + \mathrm{e}^{(1.99 - x)/-0.540}\ (r^2 = 0.991) \quad (2)$$

$$\mathrm{I\ (pA)} = 17.58 + 22.7/1 + \mathrm{e}^{(1.41 - x)/-0.607}\ (r^2 = 0.990) \quad (3)$$

where x represents the flow rate in mL/min and equations (1), (2) and (3) correspond to hydrazine concentrations of 2.57, 5.15, and 10.3, respectively. The results of Figure 3 do not reflect a constant proportionality between signal (transient height) and concentration, nor do they indicate a constant proportionality between signal and flow rate at any one concentration. This provides some clues to the relationships between partitioning of hydrazine to the membrane and the electrochemical effects that are caused by the presence of hydrazines.

Linearity of the signal-magnitude increase with concentration of hydrazines is not observed at static conditions (no flow) or low flow rates in Figure 3. The ion current signal observed for a hydrazine concentration of 2.57 ppb is proportionally larger in comparison with the background current than that observed for hydrazine at higher concentrations. The signal magnitude does not show linear proportionality when comparing the increases in current between the 5.15 and 10.3 ppb concentrations at static conditions. These results suggest that the electrochemical system is disproportionally and advantageously more sensitive to low

(21) Nikolelis, D. P.; Brennan, J. D.; Brown, R. S.; Krull, U. J. *Anal. Chim. Acta* **1992**, *257*, 49−57.

(22) Smith, J. D. *The Nucleic Acids*; Chargaff, E.; Davidson, J. N., Eds.; Academic Press: New York, 1955; Vol. 1.

(23) (a) Sillen, L. G., Martell, A. E., Eds. *Stability Constants of Metal-Ion Complexes*; The Chemical Society: London, 1964; p 158. (b) Budavari, S., Ed. *The Merck Index*; Merck Research Laboratories: NJ, 1996; p 4807.

concentrations, although linearity of response is compromised. At the highest flow rates examined in this work, the ion current was linearly dependent on the concentration of hydrazine. This could reflect the concentration-dependent Fickian kinetics of partitioning (presuming a constant partition coefficient) of hydrazine to the surface of the membrane. The magnitude of the signal at high flow rates provides evidence for rapid kinetics of adsorption of hydrazine to a membrane.

Investigation of Hydrazine Interactions with BLMs and Mechanism of Signal Generation. The data in Table 1 indicates that hydrazine concentration as well as the presence or absence of ssDNA on a lipid membrane surface each have a significant effect on the time dependence of the appearance of an analytical signal. Increase of the concentration of hydrazine has a measurable effect that can be seen as a small decrease in the time needed for signal appearance. This observation is consistent with the proposal that there may be a two-step process involving partitioning of hydrazine to the surface of a membrane followed by a secondary event for signal generation. Higher concentrations of hydrazine would accelerate the first step involving adsorption on a surface. A longer time is required for signal development by planar BLMs and filter-supported membranes relative to the length of time that it takes for the sample to pass by the membrane surface. This time is also longer relative to the time dependence of signal generation by membranes that contain ssDNA. This suggests that there may be an adsorptive step followed by a slow second process that ultimately leads to signal generation. We have tested whether there may have been some form of chromatographic event occurring that is responsible for the time dependence of transient signal generation. Table 1 includes data from a series of experiments that compare the time dependence of signal appearance from planar BLMs and filter-supported membranes, where the only significant difference in the two experimental systems was the presence of the filter-support. The results demonstrate that planar BLMs and filter-supported membranes behave similarly and that the filter support is not responsible for a chromatographic artifact in the results.

The presence of ssDNA substantially decreases the time required for signal generation and suggests that both planar and filter-supported membranes that contain ssDNA either adsorb hydrazines more quickly or provide different kinetics for the proposed second step of association. Membranes could also be simply more sensitive to the second step and that is the reason the threshold concentration required for the production of transient ion current is reached more quickly.

Repetitive Flow Monitoring of Hydrazines Using Filter-Supported BLMs. There is significant variability of peak area and shape for the ion current transients. There are a number of concurrent processes involved with the evolution of a signal, and the electrochemical event is indirectly an indication of kinetic adsorption, association, and loss processes. The tailing that is often seen is an indication of extraction of hydrazine from the surface of a membrane. This will be convoluted with the requirement for a threshold concentration of hydrazine to be present to see an electrochemical effect. The variability of peak area suggests that peak height is a better indicator to use in the determination of concentration of hydrazines. The mechanism of signal generation has allowed the signal to exhibit good reversibility. This was

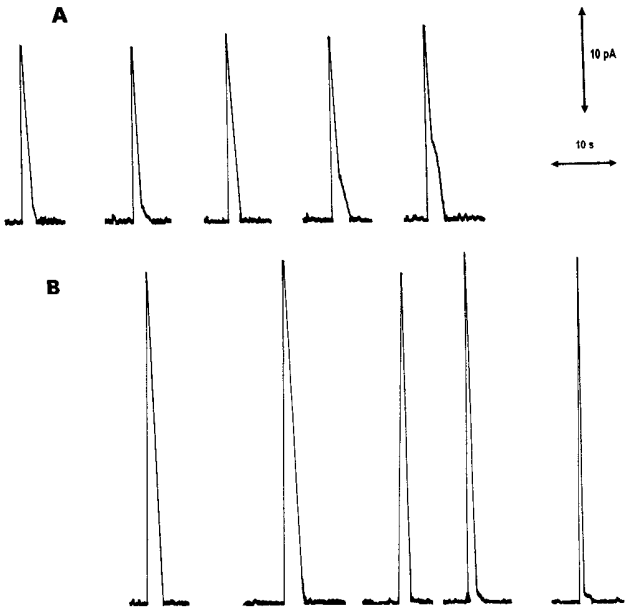

Figure 4. Recordings showing the variability of response of the BLMs to repetitive hydrazine injections. Hydrazine concentration used was (A) 5.15 ppb; (B) 10.3 ppb.

observed with repetitive determinations of hydrazine in our system which has shown no sample carryover or membrane memory effects (Figure 4, the relative standard deviation was 4.1 and 2.5% for 5.15 and 10.3 ppb of hydrazine concentration, respectively). The return to the baseline after each measurement was almost instantaneous, and this permits repetitive hydrazine determinations with a rate of ~8 samples/min. A number of cycles of repetitive injections were done, and no reduction of the signal magnitude was observed during each cycle (Figure 4). The number of sequential injections performed in each cycle was ~30, and a larger number of injections was practically limited by the syringe capacity used in the Hamilton repeating dispenser (i.e., 2.5 mL).

Transient signals similar to those of hydrazine were obtained with injections of methylhydrazine, dimethylhydrazine, and phenylhydrazine in continuous-flowing solution streams of a carrier electrolyte solution of pH 7.3 (0.1 M KCl). However, the time delay for the appearance of these transients was (35.8 ± 1.67) s for methylhydrazine, (36.9 ± 2.01) s for dimethylhydrazine, and (58.8 ± 2.28) s for phenylhydrazine. The range of time delay for the appearance of the transient signals was between 5 and 8 s for hydrazine ($N = 11$), 34−38 s for methylhydrazine ($N = 11$), 34−39 s for dimethylhydrazine ($N = 11$), and 56−62 s for phenylhydrazine ($N = 11$). The difference in time for appearance of signals from the different hydrazines is likely due to their relative hydrophobicities. Fluorescence microscopy[12] of lipid membranes that were exposed to fluorescent C_{16}-dT_{20} indicates strong partitioning of the oligomers with a dense coverage at the solution−lipid interface. This suggests that the planar and filter-supported membranes that were exposed to ssDNA in the present work would have a polar negatively charged surface, in contrast with membranes prepared from PC lipid alone that would have an electrically neutral surface. The adsorption of molecules in solution to a membrane surface would, therefore, be driven by the relative hydrophilicity, and this trend is followed in terms of the sequence

of hydrazines that produce electrochemical signals. The present results suggest that each transient signal causes a temporary change in the membrane and that each of these disturbances can happen without interference from very similar compounds. These current transients appeared as singular events similar to those of hydrazine (no further transients were observed over periods of 10 min), and their magnitude was also found to be increased with the concentration of methylhydrazine or dimethylhydrazine and phenylhydrazine. The magnitude of the transient signals could be used to quantify the concentrations of methylhydrazine, dimethylhydrazine, and phenylhydrazine in the carrier electrolyte solution. Statistical treatment of results gave regression equations: ΔI (pA) = 35.8 C (ppb) + 1.67, r^2 = 0.990, for methylhydrazine; ΔI (pA) = 36.9 C (ppb) + 2.01, r^2 = 0.980, for dimethylhydrazine; and ΔI (pA) = 20.4 C (ppb) + 0.65, r^2 = 0.999, for phenylhydrazine. In general, a good linear correlation was achieved, as observed from the obtained values of r^2, and replicate analyses of hydrazine samples indicated that the reproducibility was better than 6%. The calibration graph for the determination of hydrazines was linear in the concentration range between 2.5 and 12.5 ppb for hydrazine, 0.2−1.2 ppb for methylhydrazine, 0.2−1.2 ppb for dimethylhydrazine, and 0.3−1.5 ppb for phenylhydrazine. The detection limits in the present studies are on the order of 2.5, 0.1, 0.2, and 0.25 ppb for hydrazine, methylhydrazine, dimethylhydrazine, and phenylhydrazine, respectively (for S/N = 3).

Flow Injection Analysis of Hydrazine Compounds in Mixtures. The differences observed in the delay time for the appearance of signal for the hydrazines tested has allowed the investigation of a simultaneous determination of a mixture of hydrazine, methylhydrazine or dimethylhydrazine, and phenylhydrazine. Figure 5 shows recordings obtained for such mixtures containing variable amounts of the four hydrazines. This figure shows that a discrete signal is obtained for each hydrazine in the mixture. An integral response (i.e., a single transient corresponding to the effect of the hydrazines in the mixture) of the BLM occurs only for methylhydrazine and dimethylhydrazine. The resolution of the peaks of each hydrazine obtained in such mixtures was sufficient to permit reliable selective monitoring of these in the mixture. The results shown in Figure 5 were used for quantification of each hydrazine in mixtures, and the results are summarized in Table 2. This table shows that the recovery of the hydrazines in mixture was complete. However, analysis of mixtures of methylhydrazine and dimethylhydrazine provided an integral response, and the results shown are expressed as total amounts.

The results of Figure 5 were consistent in various flow rates, i.e., the magnitude for each of the hydrazine compounds was flow-rate-dependent, but the sequence of appearance of each hydrazine compound was constant and independent of the flow rate. The evolution of the multiple signals at various flow rates has shown that the sequence of appearance was always hydrazine, methyl- or dimethylhydrazine, and phenylhydrazine. These results show that the tubing did not act in a way analogous to a capillary column (i.e., as a chromatographic support).

Application of the Method to the Direct Determination of Hydrazines in Water Standards. As shown in Table 3, a number of reference Water Standards (AlliedSignal, Riedel-deHaen, Seelze, Germany) have been artificially contaminated with hydrazines at various concentrations, and the hydrazine content has been assayed using the above method and a calibration equation. Recoveries within the range of ~94−103% have been obtained.

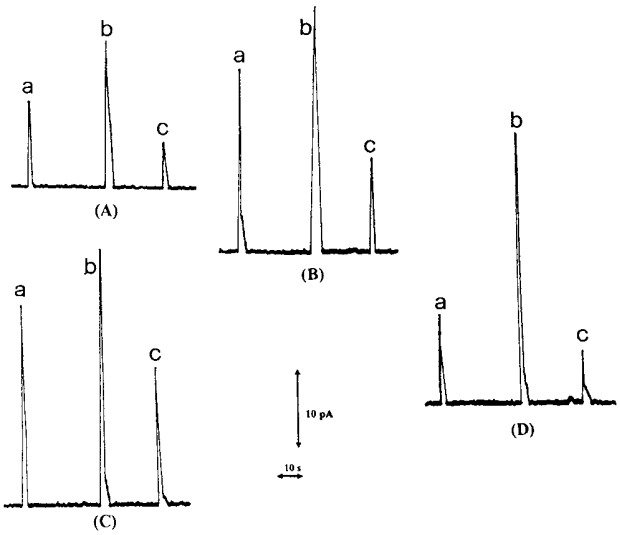

Figure 5. Experimental results obtained for mixtures of hydrazine (a), methylhydrazine and dimethylhydrazine (b), and phenylhydrazine (c) at pH 7.3 (0.1 M KCl) with BLMs composed of PC with incorporated 19.0 ng/mL dT_{20}-C_{16} and supported in glass microfiber filters. The solutions injected (75-μL) in the carrier electrolyte (at a flow rate of 0.7 mL min^{-1}) contained: (A) hydrazine, 2.57 ppb; methylhydrazine, 0.22 ppb; dimethylhydrazine, 0.20 ppb; phenylhydrazine, 0.28 ppb. (B) hydrazine, 7.73 ppb; methylhydrazine, 0.22 ppb; dimethylhydrazine, 0.60 ppb; phenylhydrazine, 0.55 ppb. (C) hydrazine, 7.73 ppb; methylhydrazine, 0.22 ppb; dimethylhydrazine, 0.60 ppb; phenylhydrazine, 0.85 ppb. (D) hydrazine, 2.57 ppb; methylhydrazine, 0.44 ppb; dimethylhydrazine, 0.40 ppb; phenylhydrazine, 0.28 ppb. The injection of each sample was made at the beginning of each recording.

Table 2. Results of Quantification of Mixtures of Hydrazines (Numbers in Parentheses are the Taken Amounts of Hydrazines)

sample no.	hydrazine (ppb)	total methyl- and dimethylhydrazine (ppb)	phenylhydrazine (ppb)
1	2.40 (2.57)	0.404 (0.420)	0.251 (0.280)
2	7.49 (7.72)	0.810 (0.820)	0.524 (0.550)
3	7.49 (7.72)	0.854 (0.820)	0.883 (0.850)
4	2.65 (2.57)	0.847 (0.820)	0.261 (0.280)
5	2.70 (2.57)	0.730 (0.820)	0.500 (0.550)
6	9.70 (10.0)	0.800 (0.820)	0.300 (0.280)
7	9.70 (10.0)	0.840 (0.820)	0.600 (0.550)
8	7.49 (7.72)	0.810 (0.820)	0.350 (0.300)
9	2.70 (2.57)	0.730 (0.820)	0.800 (0.850)
10	10.2 (10.0)	0.240 (0.220)	0.500 (0.550)
11	7.49 (7.72)	0.810 (0.820)	0.810 (0.850)
12	2.39 (2.57)	0.415 (0.420)	0.550 (0.550)
13	10.6 (10.3)	0.835 (0.840)	1.05 (1.10)
14	2.48 (2.57)	0.857 (0.840)	0.272 (0.280)
15	7.48 (7.72)	0.586 (0.600)	0.572 (0.550)

Comparison with Other Hydrazine Biosensors. A biosensor for the rapid determination of hydrazines has been described in the literature.[7] However, this biosensor lacks selectivity and cannot discriminate between hydrazine compounds. The present BLM-based system is able to monitor each hydrazine in mixtures.

Table 3. Results of Quantification of Hydrazines Added in Water Standards (Numbers in Parentheses are the Taken Amounts of hydrazines)

sample ID/ Hydranal Catalog no.	hydrazine (ppb)	total methyl- and dimethylhydrazine (ppb)	phenylhydrazine (ppb)
34847	2.46 ± 0.15[a] (2.57)	0.413 ± 0.030[a] (0.420)	0.268 ± 0.022[a] (0.280)
34828	2.55 ± 0.11[a] (2.57)	0.220 ± 0.012[a] (0.220)	0.564 ± 0.031[a] (0.550)
34813	2.47 ± 0.10[a] (2.57)	0.826 ± 0.055[a] (0.840)	1.05 ±0.030[a] (1.10)
34803 (with 0.05 M potassium sodium tartate)	2.53 ± 0.11[a] (2.57)	0.222 ± 0.009[a] (0.220)	1.06 ± 0.050[a] (1.10)

[a] Average of 5 determinations ± 1 SD.

The method also offers response times in less than one minute, which are the fastest times reported for any similar detectors. The detection limits in the present studies are similar to those obtained by using fluorescence detection.[6] However, the present method offers a simultaneous and repetitive mode of detection of hydrazines. The present method is faster and has a lower cost than that based on fluorescence or another mode of detection.[6] The results of the flow injection analysis of mixtures of hydrazines by using the BLM-based detection scheme show an analogy to the resolution of the peaks obtained by using chromatographic procedures.

In conclusion, the results demonstrate that hydrazines can be monitored by using stabilized BLM-based sensors in conjunction with flow injection analysis to achieve fast response times of less than one minute. The approach also allows quantitative analysis of mixtures of the hydrazines. Analysis of these environmental pollutants can be done in protein-free water samples, however, proteins or small peptides should be eliminated from the samples prior to analysis. These macromolecules may cause a nonselective interference due to interaction with ssDNA20. The negative charge from the phosphate groups of the DNA that are localized on the surface of the BLMs will provide electrostatic attraction of positively charged proteins and peptides to the surface. This is dependent on the PI of protein and the local pH at the interface.[24] Note that selective binding interactions of proteins with DNA have been reported, with one example being the general zinc-finger proteins.[25] Our results indicate that concentrations of albumin greater than 10 μg mL^{-1} provide ion current transients from BLM-based sensors in experiments that use 12 ng mL^{-1} of ssDNA. Such interference from albumin was achieved at a threshold concentration of 8 and 6 μg mL^{-1}, when the concentration of ssDNA was increased to 19 and 21 ng mL^{-1}, respectively. A number of other compounds were also tested as potential interferents, including: calcium ion (0.01 M) and magnesium ion (0.01 M), liquid humus (1.0 g L^{-1}, liquid organic acids, Actagro, LLC, Kerman, CA), Nobel of calcium (1:10 dilute solution, Agrilip Co., Iraklion, Greece), and potassium sodium tartrate (0.005 M). Nobel of calcium contains 10% complex of calcium−EDTA with proteins, 4% free calcium ions, 6% nitrogen (as urea, amino acids, etc.), and 25% organic substance. These compounds were not found to cause any transient signals even at concentration levels provided in the parentheses. The present technique has detection limits similar to those obtained by chromatographic methods. However, it offers significant advantages based on analysis times, sample volumes, and size and cost of chromatographic instrumentation which limit this technology for monitoring applications in the field. The applications presented herein show that a BLM-based biosensor can provide an attractive alternative to chromatographic devices.

(24) Selinsky, B. S. In *The Structure of Biological Membranes*; Yeagle, P., Ed.; CRC Press: Boca Raton, 1992; Chapter 14.
(25) Sluyser, M., Ab, G., Brinkmann, A. O., Blankenstein, R. A., Eds. *Zinc-Finger Proteins in Oncogenesis*; New York Acad. Sci.: New York, 1993; Vol. 684.

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