



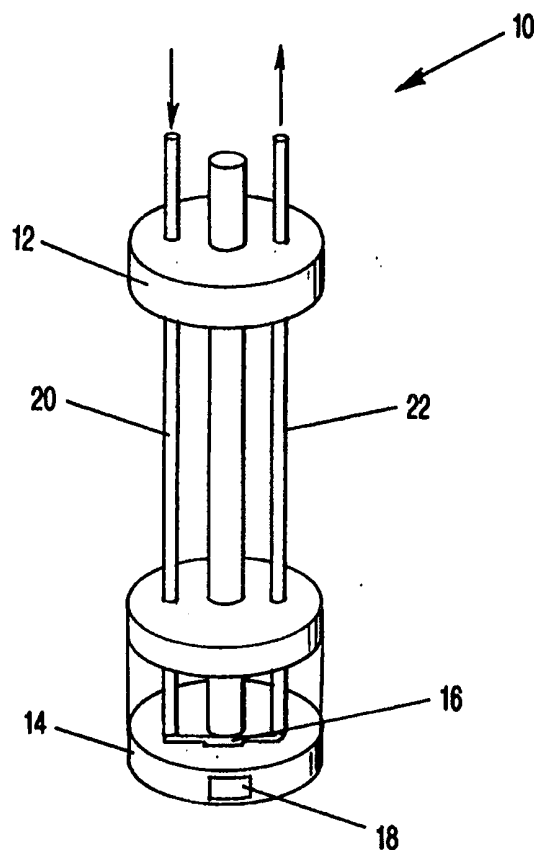
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(54) Title: RENEWABLE-REAGENT ELECTROCHEMICAL SENSOR

(57) Abstract

A new electrochemical probe(s) (10) design allowing for continuous (renewable) reagent delivery. The probe (10) comprises an integrated membrane-sampling/electrochemical sensor that prevents interferences from surface-active materials and greatly extends the linear range. The flow probe of the invention relies on the delivery of a ligand solution through a microdialysis sampling tube (20), followed by transport of the resulting complex to a working electrode (14) positioned within channel (16) at the base of probe (10). The probe(s) (10) is useful for remote or laboratory-based monitoring in connection with microdialysis sampling and electrochemical measurements of metals and organic compounds that are not readily detected in the absence of reacting with the compound. Also disclosed is a method of using the probe(s).



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RENEWABLE-REAGENT ELECTROCHEMICAL SENSOR

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application is a continuation-in-part application of U.S. Patent Application Serial No. 08/383,717, entitled "Remote Electrochemical Sensor", to Joseph Wang, Khri Olsen and David Larsen, filed on February 3, 1995, and the specification thereof is incorporated herein by reference.

GOVERNMENT RIGHTS

10 The U.S. Government may have rights to this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of U.S. Department of Energy (DOE) Grant No. DE-FG07-96ER62306, and by DOE Waste Management Education and Research Consortium (WERC).

BACKGROUND OF THE INVENTION

Field of the Invention (Technical Field):

15 The present invention relates to an electrochemical sensing apparatus and method for continuous monitoring of metals and organic compounds, by means of continuous circulation of reagent and repeated introduction of fresh reagent.

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Background Art:

25 Detection and monitoring of metals and organic compounds is normally done by having an operator collect on-site field samples and then taking the samples back to a laboratory.

30 Attempts have been made to provide both remote field sampling and analysis, on-site. See U.S. Patent No. 5,120,421, entitled "Electrochemical Sensor Detector System and Method," to Glass et al., and U. S. Patent No. 5,296,125, entitled "Electrochemical Sensor Detector System and Method" to Glass et al. However, an operator is still required to be present on-site to collect the sample, and the device does not communicate directly with the laboratory.

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Contamination of hazardous sites and groundwater with toxic heavy metals (e.g., mercury (Hg), lead (Pb), uranium (U), arsenic (As), chromium (Cr), aluminum (Al)) represents a major national problem. Site monitoring and surveillance programs are required for a closer control of metal pollutants. The traditional use of atomic-spectroscopy central-laboratory measurements of heavy metals is too expensive and time consuming. Also, samples often change composition during their collection, transport and delay before analysis, ultimately producing unreliable results. Innovative field deployable methods are highly desired for the task of site characterization and remediation, as they minimize the huge labor analytical costs, and provide timely data for real-time emergencies and decision making. Chemical sensors are particularly attractive for providing real-time, remote monitoring of priority pollutants. While fiber-optic probes have been suggested for monitoring organic contaminants, no chemical sensor technology has demonstrated capability for remote monitoring of trace metals. (See W. Chudyk, et al., J. Anal. Chem. 1985, 57, 1237.) Clearly, a cost effective metal-sensor technology, capable of monitoring the metal both in time and location, is needed to support the characterization and remediation of hazardous waste sites. (See G. Batiaans et al., (Eds.), "Chemical Sensors: Technology Development Planning," U.S. Department of Commerce, Springfield, 1993.)

In the present invention, there is provided a sensor for *in-situ* monitoring of trace metals and organic pollutants. The sensor of the invention is designed both for remote, on-site, use or for use in laboratory applications. The compact instrumentation and low power needs of electrochemical techniques satisfy many of the requirements for on-site metal analysis. Particularly attractive for *in-situ* monitoring of metal contaminants is the remarkably sensitive technique of stripping analysis. (See J. Wang, "Stripping Analysis: Principles, Instrumentation, and Applications," VCH Publishers, Deerfield Beach, Florida 1985). The extremely low (subnanomolar) detection limits of stripping analysis are attributed to its "built-in" pre-concentration step, during which the target metals are deposited onto the working electrode. However, since the performance of stripping analysis as previously achieved, depends on the electrolytic plating of target metals onto the working electrodes of a sensor, conventional stripping analysis alone is not satisfactory for many environmentally-important metals that cannot be readily electroplated. Additionally,

conventional stripping measurements, have suffered from interferences by surfactants (surface-active materials) commonly present in environmental samples that have passivated the electro-surface.

5 SUMMARY OF THE INVENTION (DISCLOSURE OF THE INVENTION)

One of the objectives of the present invention is to extend the concept of metal monitoring, either in remote or laboratory applications, and specifically *in-situ* electrochemical stripping sensors, toward environmentally significant metals that cannot be easily electro-deposited. Such extension is achieved by employing electrochemical monitoring/detection methods, e.g., adsorptive stripping
10 procedures involving the formation and adsorptive accumulation of appropriate complexes of the target sample, and the delivery of a ligand solution through a microdialysis sampling tube, followed by transport of the resulting complex to a downstream detector. The use of alternative (nonelectrolytic) accumulation schemes offers great promise for monitoring priority metal pollutants, including uranium (U), chromium (Cr), nickel (Ni), cobalt (Co), aluminum (Al) or iron (Fe). To
15 achieve this goal, renewable chemistry, involving controlled, continuous reagent (ligand) delivery, is utilized in a manner analogous to that employed in renewable fiber-optic devices.

Accordingly, the preferred sensor of the invention is an electrochemical sensing apparatus comprising an electrode, means for continuously circulating a reagent while reacting said reagent
20 and an analyte proximate said electrode, and means comprising a membrane for ingress of said analyte. The electrode may further comprise a working electrode that is a bare or mercury-coated solid electrode (carbon, gold, platinum, iridium). The working electrode may still further comprise a glassy carbon Hg-coated solid electrode. The reagent may comprise an enzyme or a biologically or chemically modified molecule (utilizing a biological or chemical species).

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The means of continuously circulating a reagent further comprises means comprising reagent delivery inlet and outlet tubes, or further comprises a pump. The means comprising a membrane further comprises reagent delivery inlet and outlet tubes. The analyte further comprises inorganic (metal) pollutant substrates, such as uranium (U), chromium (Cr), nickel (Ni), cobalt (Co),
30 aluminum (Al) or iron (Fe), or numerous organic compounds that react with the reagent to produce

an easily detected electro-active species. Additionally, the membrane inhibits entry of macromolecular surfactants that may passivate the surface.

5 The preferred method of the invention is an electrochemical method of detecting metal and organic pollutants in analytes comprising the steps of providing an electrode, continuously circulating a reagent while reacting the reagent with an analyte proximate the electrode, and providing a membrane for ingress of the analyte. The step of providing an electrode further comprises the step of providing a working electrode; and the step of providing a working electrode further comprises a step of providing a bare or Hg-coated solid electrode (of carbon, gold, 10 platinum or iridium), a glassy carbon mercury-coated electrode, or a modified electrode utilizing a biological or chemical species. The step of continuously circulating reagent further comprises the step of providing reagent delivery inlet and outlet tubes. The step of providing a membrane further comprises the step of providing reagent delivery inlet and outlet tubes.

15 A primary object of the present invention is the detection of environmentally important trace metals and/or organic pollutants that are not readily electroplated.

Another object of the invention is the continuous delivery of reagent and sample to the working electrode.

20

Still another object of the invention is the prevention of surfactant permeation and hence surface passivation.

A primary advantage of the present invention is the combination of ligand delivery through microdialysis sampling tubes.

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Another advantage of the present invention is the relative ease of cleaning electrodes and introducing fresh reagent (ligand) solution.

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Yet another advantage of the present invention is its adaptability to detection of nonelectroactive organic pollutants, via reaction with a flowing redox marker.

Other objects, advantages and novel features, and further scope of applicability of the present invention will be set forth in part in the detailed description to follow, taken in conjunction with the accompanying drawings, and in part will become apparent to those skilled in the art upon examination of the following, or may be learned by practice of the invention. The objects and advantages of the invention may be realized and attained by means of the instrumentalities and combinations particularly pointed out in the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

10 The accompanying drawings, which are incorporated into and form a part of the specification, illustrate several embodiments of the present invention and, together with the description, serve to explain the principles of the invention. The drawings are only for the purpose of illustrating a preferred embodiment of the invention and are not to be construed as limiting the invention. In the drawings:

15

Figure 1 is a schematic diagram of the preferred renewable-ligand electrochemical sensor of the invention.

20 Figures 2(a) and (b), respectively, are chronopotentiograms for 117 $\mu\text{g/L}$ nickel and 1000 $\mu\text{g/L}$ uranium with various times and potentials.

Figures 3(a) and (b), respectively, represent the effect of delivery (flow) rate and ligand concentration on nickel (a) and uranium (b) signals.

25 Figures 4(a), (b), and (c), respectively, represent the effect of dodecyl sodium sulfate and Triton X-100 on the nickel stripping signal, and the effect of Arabic gum on the uranium response.

Figures 5(a) and (b), respectively, represent the effect of surfactant on nickel and uranium measurements in the sensor having a dialysis membrane.

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Figures 6(a) and (b), respectively, represent potentiograms of uranium and nickel with increasing concentrations.

Figures 7(a) and (b), respectively, represent adsorptive stripping responses for 8 $\mu\text{g/L}$ nickel (A,b) and 25 $\mu\text{g/L}$ uranium (B, b).

Figures 8(a) and (b), respectively, represent ten repetitive measurements of 117 $\mu\text{g/L}$ nickel and 1000 $\mu\text{g/L}$ uranium.

Figures 9(a) and (b), respectively, represent the stripping responses for a river water sample and for a groundwater solution spiked with increasing concentrations.

Figure 10 shows a second probe embodiment.

DESCRIPTION OF THE PREFERRED EMBODIMENTS
(BEST MODES FOR CARRYING OUT THE INVENTION)

The invention comprises a renewable-reagent sensor designed to accommodate an electrochemical detecting process, for example, the complex formation and adsorptive accumulation steps of adsorptive stripping protocols, for monitoring environmentally-important metals that are not readily electroplated. The flow probe of the invention relies on the delivery of a ligand solution through a microdialysis sampling tube, followed by transport of the resulting complex to a downstream detector. The microdialysis sampling step minimizes the interference of surface-active macromolecules and extends the linear dynamic range compared to conventional electrochemical, e.g., adsorptive stripping, measurements.

As schematically depicted in Figure 1, the preferred flow probe **10** of the invention comprises Plexiglas housing **12**. Accommodated within probe **10** is a glassy carbon disk or solid working electrode **14** which may be bare or mercury-coated. Working electrode **14** is positioned within channel **16** at the base of probe **10**. A Vycor disk **18** may also be positioned at the base of probe **10** to provide an external conductive connection. In an actual field sampling situation, Ag/AgCl and Pt reference and counter electrodes (not shown) would also be provided within channel **16**.

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Dialysis sampling tube **20** and Teflon drainage capillary **22** are fixed to apertures in probe **10**; a portion of probe **10** is removed to accommodate these tubes. The inlet of dialysis tube **20** is connected to a microsyringe pump (not shown) to provide continuous reagent flow capability. Sampling of analyte occurs by means of permeation through dialysis tube **20**.

5

In operation, ligand solution delivered through dialysis tube **20** reacts with the permeating analyte in dialysis tube **20**. The resulting complex is transported to channel **16** and working electrode **14** and there accumulated. Detection of the trace metal is then effected by a chosen mode of electrochemical analysis, such as chronopotentiometric or voltammetric analysis. The renewable-reagent electrochemical sensor, shown in Figure 1, relies on continuous delivery of the ligand to the working electrode **14**, its complexation reaction with the metal "collected" in the dialysis sampling tube, transport of the complex to the working-electrode **14** in compartment **16**, and electrochemical (e.g., chronopotentiometric or voltammetric) detection of the accumulated complex. The detection step, or the separate cleaning step, remove the chemical complex from the surface to allow drainage through the outlet capillary **22**.

15

The integrated membrane-sampling/electrochemical sensor of the invention was tested in connection with the monitoring of trace uranium and nickel using propyl-gallate (PG) and dimethylglyoxime (DMG) chelating agents. In these tests, established adsorptive stripping protocols for trace uranium and nickel, based on complexation with PG and DMG, respectively, were used for characterizing and testing stripping probe **10** of the invention. Experimental variables, including reagent delivery rate and ligand concentration, were explored in the testing.

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Figure 2 displays chronopotentiograms for non-deaerated solutions of 0.12 mg/L nickel (Figure 2(a)) and 1.0 mg/L uranium (Figure 2(b)), obtained with the flow probe, following different adsorption times (0 - 150 seconds, a-f). The nickel/DMG and uranium/PG chelates yield well-defined reduction peaks at -1.14V and -0.63V, respectively. The longer the accumulation time, the more complex is adsorbed onto the working electrode, and the larger is the peak height. While the nickel peak rises rapidly with the time at first and then more slowly, the uranium signal increases over the entire time scale tested. Convenient measurements at the mg/L level are thus feasible following very short (1-2 minute) accumulation periods. No response is observed without the

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adsorptive accumulation. The favorable background response, obtained for non-deaerated samples, eliminates the need for a time-consuming deaeration step, hence making the chronopotentiometric stripping mode attractive for potential field applications. A short "cleaning" period (at -1.4V Figure 2(a)) or -1.5V Figure 2(b)) is sufficient for desorbing the complex prior to the next measurement.

The influence of various experimental variables affecting the response of the renewable-reagent sensor was tested. Figure 3(a) shows the dependence of the adsorptive stripping response of nickel (Figure 3(a)) and uranium (Figure 3(b)) on the flow rate of the reagent solution. Different profiles are observed for these metals. While the nickel response increases rapidly upon raising the flow rate between 5 and 10 $\mu\text{L}/\text{min}$ and then more slowly, the uranium signal decreases sharply upon increasing the flow rate. Such different profiles are attributed to the fact that the reagent-solution flow rate affects (in a different fashion) various steps of the sensor operation, including the metal sampling, the complex formation and adsorptive accumulation. Obviously, the target metal and the ligand used have a profound effect upon the transport rate through the membrane and upon the rate of the complex formation (leading to different flow rates effects for the different metals).

The influence of the ligand concentration (in the receiving solution) is shown in Figure 3(b). For both nickel (a) and uranium (b), the response rises sharply (to a steady-state value) with the ligand concentration at first, and then decreases slowly. Compared to conventional adsorptive stripping measurements, the ligand and its level affect the response also through their influence on the microdialysis collection of the target metal.

Electrochemical, particularly adsorptive stripping, measurements commonly suffer from interferences by surface-active materials present in environmental samples. Using adsorptive stripping measurements as the example, such substances have a marked effect on the adsorptive stripping response owing to competitive adsorption for surface sites. The microdialysis sampling of the renewable sensor greatly minimizes such matrix effects. Because the membrane does not favor permeation of large macromolecules, the flow probe offers good resistance to surfactant effects. Such resistance is illustrated in Figure 4. Conventional adsorptive stripping measurements

(on the left side of the Figures) result in a substantial depression of the nickel (Figure 4(b)) and the uranium (Figure 4(c)) peaks following the addition of Triton X-100 and Arabic gum (compare a and b). In addition, the presence of dodecyl sodium sulphate causes a severe distortion of the nickel peak (Figure 4(a)). In contrast, no change of the nickel or uranium is observed at the renewable-reagent sensor of the present invention in the presence of similar levels of these surfactants (on the right side of the Figures).

Such protective action is illustrated also in Figure 5. Using the conventional stripping protocol (b), the nickel (Figure 5(a)) and uranium (Figure 5(b)) peaks decrease rapidly upon raising the surfactant concentration (with 90% depressions at 1.3- and 1000 mg/L Triton X-100 and Arabic gum, respectively). In contrast, the flow probe offers a highly stable uranium response up to 2000 mg/L Arabic gum (Figure 5(b)-a). The nickel peak is also not affected by the Triton X-100 concentration up to 0.9 mg/L, but displays a 20% loss at 1.3 mg/L Triton X-100 (Figure 5(a)-a).

The renewable-reagent electrochemical sensor also displays a well-defined concentration dependence. Figure 6 shows stripping potentiograms obtained for nickel (Figure 6(a)) and uranium (Figure 6(b)) solutions of increasing concentrations (60-300 $\mu\text{g/L}$ and 200-1200 $\mu\text{g/L}$, respectively). The defined peaks observed over these ranges offer convenient quantitation following 2 (Figure 6(a)) and 4 (Figure 6(b)) minute adsorption. The six measurements shown in Figure 6 are a part of 8 (Figure 6(a)) or 10 (Figure 6(b)) concentration increments up to 600 and 2000 $\mu\text{g/L}$, respectively. The resulting calibration plots are also shown in the Figures. For both metals, a linear relationship exists between the peak area and the bulk concentration over the entire ranges examined. Least-squares treatment of these data yields slopes of 1.025 (Figure 6(a)) and 0.041 (Figure 6(b)) $\text{ms.L}/\mu\text{g}$ (correlation coefficients, 0.999 (Figure 6(a)) and 0.997 (Figure 6(b))). Apparently, conditions of low surface coverage (linear adsorption isotherms) exist. The very wide linear range (compared to conventional adsorption stripping measurements) is attributed to the "built-in" dilution action associated with the microdialysis sampling.

An analogous conventional (batch) adsorptive stripping calibration experiment for uranium yielded a nonlinear calibration plot, with a leveling off at 600 $\mu\text{g/l}$; the sensitivity (slope of the linear portion) was 20-fold higher than that of the flow probe. The different sensitivities observed with the

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renewable-reagent sensor for uranium and nickel are attributed in part to their different transport rates through the microdialysis membrane (with a more facile collection of the smaller nickel ion). The use of sampling membranes with higher molecular weight cutoffs would increase the recovery of the target metals, and would further enhance the sensitivity. In addition, the sensitivity and the dynamic range may be changed by adjusting the ligand delivery rate or the ligand concentration.

Despite internal dilution, the renewable-flow probe 10 of the invention results in extremely low detection limits. These were estimated from the adsorptive stripping response for 8 $\mu\text{g/L}$ nickel and 25 $\mu\text{g/L}$ uranium (Figure 7(a) and 7(b), respectively). Well defined peaks are observed for these low levels following 5 and 20 minutes accumulation (Figure 7(a) and 7(b), respectively). Detection limits of 0.9 $\mu\text{g/L}$ (1.5×10^{-8} M) nickel and 10 $\mu\text{g/L}$ (4.2×10^{-8} M) uranium were estimated from the signal-to-noise characteristics of these data (S/N=3). Even lower detection limits are expected with longer accumulation times, use of more permeable membranes or in connection to a stopped-flow operation.

The renewable-ligand electrochemical sensor yields a reproducible and stable response. Figure 8 displays stripping potentiograms for 10 repetitive measurements of uranium (Figure 8(a)) and nickel (Figure 8(b)). The peak area and shape are maintained throughout these operations, yielding relative standard deviations of 4.0% (Figure 8(a)) and 3.7% (Figure 8(b)). High stability (RSD = 1.7%) was observed also in a longer (60 minutes) unbroken series involving 20 successive measurements of 400 $\mu\text{g/L}$ nickel after a 2 minute accumulation (not shown). Such data reflect the efficiency of the electrochemical "cleaning" step, i.e., regeneration of a "metal-free" surface prior to each run.

Figure 9 demonstrates the applicability of the renewable-reagent sensor to measurements of trace metals in environmental samples. While no response is observed for the unpolluted river (Figure 9(a)) or groundwater (Figure 9(b)) samples, subsequent additions of 0.11 mg/L nickel (Figure 9(a), b-d) or 0.5 mg/L uranium (Figure 9(b), b-d) yielded defined adsorptive stripping peaks in connection with short accumulation times (2 and 3 minutes, respectively). No major interferences are indicated from the response of the unspiked sample.

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It has been demonstrated that renewable-reagent flow probes are very suitable for adsorptive-stripping electrochemical sensing of trace metals. In summary, detection limits of 1.5×10^{-8} M nickel and 4.2×10^{-8} M uranium were obtained following 5-minute and 20-minute adsorption times, and a relative standard deviation of 1.7% was obtained for prolonged operations of, e.g., 20 runs.

Integration of microdialysis sampling with electrochemical, e.g., adsorptive stripping, detection extends the scope of electrochemical sensors to additional metals that are not readily electroplated. Near real-time monitoring of numerous metals can thus be accomplished via a judicious choice of the complexing ligand. The sensor of the invention is designed for laboratory use or for use in the field. The remote monitoring capability is coupled to minimization of interferences from surface-active materials and extension of the linear dynamic range (compared to conventional electrochemical, e.g., adsorptive stripping, measurements).

Integrating the reference and counter electrodes within the body of the flow probe, coupling the probe to a long shielded cable, and designing multichannel sensor arrangements for the simultaneous monitoring of several metals (with each channel carrying the desired complexation reaction) readies probe 10 for field detection. Considering the versatility of the renewable-flow electrochemical concept, probe 10 is easily adaptable to various environmental or industrial monitoring scenarios. The renewable-reagent electrochemical sensor is particularly applicable to assays of river and groundwater samples, and holds great promise for monitoring a variety of trace metals, either in the laboratory or remotely.

Conventional stripping measurements, that also suffer from interference of surfactants, may also benefit from the isolation of the target metals from large macromolecules. Other electrochemical detection schemes (e.g., biosensing of pollutants) may also benefit from the versatility of the renewable-reagent strategy.

30 Industrial Applicability

The invention is further illustrated by the following non-limiting examples.

Probe Design

The probe **10** is based on a Plexiglas cylindrical body **12** (0.8-inch diameter, 2-inch length) that accommodates the glassy carbon disk working electrode **14** and the reagent delivery and drainage capillaries, **20** and **22**, respectively. The glassy carbon electrode **14** (Model MF-2012, BAS Inc.) was inserted through a 0.223-inch diameter hole drilled in the center of the Plexiglas cylinder **12**. The electrode terminates in an 80 μ L thin-layer channel **16** at the base of the sensor body, which formed by fixing (with epoxy) a plastic end cap. A 3-mm diameter Vycor disk **18** (BAS Inc.) was fixed at the center of the end cap, below the thin-layer channel **16**, to provide conductivity to the external reference (Ag/AgCl) electrodes and Pt-wire counter electrodes (located in the sample solution). The dialysis sampling tube **20** and the Teflon drainage capillary **22** were fixed to holes in the Plexiglas body. A portion of the Plexiglas body was removed for accommodating these sampling and drainage tubes. A band of four "Regenerated Cellulose (RC) Hollow Fibers" (MWCO 13,000; 200- μ m i.d.; Spectrum Medical Industries Inc.) serves for the dialysis sampling. Teflon tubing connects a microsyringe pump (2.5mL volume, Model MD-100/MF-5127, BAS Inc.) with the inlet of the dialysis tubing. The outlet of the dialysis tubing is connected to the thin-layer working electrode channel **16** through a hole in the Plexiglas body.

Apparatus

Potentiometric stripping analysis (PSA) was performed with a TraceLab system (PSU20, Radiometer Inc.), in connection with an IBM PS/55XS (not shown).

Reagents

Stock solutions (1000 mg/L) of nickel and uranium (atomic adsorption standard, Aldrich) were diluted as required. DMG and PG were also received from Aldrich. A 0.02M ammonia buffer solution (pH 9.2) containing 5×10^{-5} M DMG served as the receiving solution for the nickel system. A 0.05 M of acetate (Aldrich) buffer solution (pH 4.5) containing 1×10^{-4} M PG was used for uranium measurements. Dodecyl sodium sulfate and Triton X-100 were obtained from J. T. Baker Chemical Co., and Arabic gum was obtained from Sigma. Rio Grande river samples were collected in Las Cruces, New Mexico, while the groundwater sample was collected at Hanford site (Richland, WA). Tap water samples were obtained at the NMSU laboratory. All solutions were prepared from double-distilled water. All chemicals used were analytical grade.

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Procedure: Monitoring of Nickel

A Mercury film was preplated by immersing the polished glassy carbon disk electrode **14** in a 10mL cell containing a stirred 100 mg/L Hg and 0.1 M HCl solution, for 5 minutes while holding the potential at -0.6V. Following the mercury deposition, the glassy carbon electrode **14** was
5 incorporated into the flow probe body **10**. The probe was then immersed into a 100 mL cell, containing 40 mL of a tap water solution. Adsorptive accumulation proceeded (usually for 2 minutes) while holding the potential at -0.7V, and pumping the DMG/ammonia-buffer reagent solution at 10 μ L/minutes. After the accumulation, the potentiogram was recorded by applying a suitable constant current. A "cleaning" step was followed by holding the sensor at -1.4V for 10
10 seconds.

Procedure: Monitoring of Uranium

The glassy carbon based mercury film electrode was prepared using the procedure reported above for the measurement of nickel, prior to the uranium experiments, by preplating
15 mercury at -0.6 V for 5 minutes from a 100 ppm Hg and 0.1M HCl solution. The coated electrode **14** was inserted into the flow probe body **10** and ready for use. The probe, along with the reference and counter electrodes, was then dipped in a 100 mL cell containing 40 mL of a 0.03 M NaCl solution. The reagent solution (1x10⁻⁴ M PG in 0.05 M acetate buffer solution, pH 4.5) was allowed to flow at 10 μ L/minute, while applying an accumulation potential of -0.05V for a selected
20 time. After the accumulation step, a potentiogram was recorded by applying a constant negative current (-20 μ A). The surface "cleaning" was carried out by holding the potential at -1.5V for 60 seconds. Following each addition, a 3-minute "waiting period" was used to allow for the microdialysis sampling and transport to the working-electrode compartment. All experiments were carried out at room temperature.

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For comparison, conventional adsorptive stripping potentiometric measurements were carried out in a 10mL cell (Model VC-2, BAS), using a stirred (ca. 500rpm) sample solution, and accumulation periods, stripping currents and "cleaning" steps, similar to those using the flow probe.

Additional Embodiments

Figure 10 depicts another embodiment that is trace metal and organic pollutant probe **30** in which the membrane is held differently from the probe **10** of Figure 1. Probe **30** comprises housing **35**, further comprising grooves **34** for accommodating reagent inlet and outlet tubes. Gold working electrode **36** is positioned proximate the reagent flow. Screw-on cap **38** secures O-ring **39** against permeable membrane **32**; membrane **32** permits sample (analyte) flow toward electrode **36**, but excludes surfactants.

Again, as in the preferred embodiment, continuous reagent flow over the working electrode enhances monitoring, prevents surfactant contamination, greatly extends the linear range of measurements, and promotes ease of electrode cleaning. Similarly, detection of organic pollutant levels upon reaction with a suitable flowing reagent, is rendered possible. For example, the reagent may be an enzyme if the enzyme chosen is used as part of the reagent and the derivitization reaction to convert the analyte (an organic substrate) to a detectable species. Effective monitoring of several environmentally significant substrates can be accomplished by the enzymes listed in Table 1, which can be used as reagents for monitoring/detecting the appropriate target pollutant substrates as shown. Further, the reagent may be another biological molecule, such as an ssDNA-modified or a dsDNA-modified molecule, as long as it is included in the reagent solution.

TABLE 1: Enzymes and Target Substrates

Enzyme	Substrate (Target Pollutants)
Nitrate reductase	Nitrate
Formaldehyde dehydrogenase	Formaldehyde
Horseradish peroxidase	Hydrogen peroxide, organic peroxide
Sulfite oxidase	Sulfite
Tyrosinase	Chlorophenols
Nitrilase	Organonitriles
Cholinesterase	Organophosphate Pesticides, Cyanide

In addition to detection of organic or mixed waste, *in-situ* monitoring of various toxins inhibiting biocatalytic activity is possible with enzyme reagents. For example, inhibition of acetylcholinesterase can monitor organophosphate pesticides.

For other than natural water pH ranges, the probes of Figures 1 and 10 permit "optimal" pH delivery.

Another variation involves the use of electrodes for real-time monitoring of mutagens and carcinogens, either remotely or in laboratory applications. The association of various damaging agents with flowing ds-DNA reagent can be monitored via changes in the anodic response of the nucleic acid. Such changes may result from chemical, structural or conformational variations of the nucleic acid probe.

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

Although the invention has been described in detail with particular reference to these preferred embodiments, other embodiments can achieve the same results. Variations and modifications of the present invention will be obvious to those skilled in the art and it is intended to cover in the appended claims all such modifications and equivalents. The entire disclosures of all references, applications, patents, and publications cited above, and of the corresponding application(s), are hereby incorporated by reference.

CLAIMS

What is claimed is:

1. An electrochemical sensing apparatus comprising:
an electrode;
means for repeatedly introducing a reagent;
means for continuously circulating the reagent; and
means for electrochemically detecting a target sample.
2. The apparatus of claim 1 wherein said means for electrochemically detecting is selected from the group consisting of voltammetric detecting means, chronopotentiometric detecting means, and adsorptive stripping means.
3. The apparatus of claim 1 wherein said means for repeatedly introducing a reagent comprises means for delivering the reagent through a microdialysis sampling tube.
4. The apparatus of claim 1 wherein said means for continuously circulating reagent comprises means for forming a target complex and adsorptively accumulating the complex in the absence of electrodepositing the target complex on said electrode and means for delivering the accumulated target complex to said means for electrochemically detecting.
5. The apparatus of claim 1 further comprising means for passivating surfactant contamination.
6. The apparatus of claim 5 wherein said means for passivating surfactant contamination comprises means for preventing surfactant permeation.
7. The apparatus of claim 1 further comprising means for cleaning the electrode.

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8. An adsorptive stripping sensor apparatus comprising:
an electrode;
means for continuously circulating a reagent while reacting the reagent and an analyte proximate said electrode;
means comprising a membrane for ingress of the analyte; and
means for performing electrochemical measurement of the analyte.
9. The apparatus of claim 8 wherein said electrode is a working electrode selected from the group consisting of solid working electrodes, bare solid working electrodes, Hg-coated solid working electrodes, and glassy carbon Hg-coated electrodes.
10. The apparatus of claim 9 wherein said electrode is a solid working electrode selected from the group consisting of gold, carbon, platinum and iridium electrodes.
11. The apparatus of claim 8 wherein the reagent comprises a reagent selected from the group consisting of propyl gallate, dimethylglyoxime, enzymes, dsDNA, and ssDNA.
12. The apparatus of claim 8 wherein the analyte is selected from the group consisting of metal analytes and organic analytes.
13. The apparatus of claim 12 wherein the analyte comprises a metal selected from the group consisting of chromium, uranium, iron, aluminum, nickel and cobalt.
14. An electrochemical method of detecting metal and organic pollutants in analytes, comprising the steps of:
- a) providing an electrode;
 - b) continuously circulating a reagent while reacting the reagent with an analyte proximate the electrode; and
 - c) providing a membrane for ingress of the analyte.

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15. The method of claim 14, wherein the step of providing an electrode comprises the step of providing electrodes selected from the group consisting of working electrodes, solid working electrodes, bare solid working electrode, Hg-coated solid working electrodes and glassy carbon Hg-coated electrodes.

16. The method of claim 15 wherein the step of providing an electrode comprises the step of providing a solid working electrode selected from the group consisting of carbon, gold, platinum and iridium electrodes.

17. The method of claim 14 wherein the step of continuously circulating a reagent comprises the step of circulating a reagent selected from the group consisting of propyl gallate, dimethylglyoxime, enzymes, dsDNA and ssDNA.

18. The method of claim 14 wherein the step of continuously a circulating reagent comprises the step of providing reagent delivery inlet and outlet tubes.

19. The method of claim 14 wherein the step of providing a membrane comprises the step of providing reagent delivery inlet and outlet tubes.

20. The method of claim 14 further comprising the step of performing electrochemical measurement selected from the group consisting of voltammetric measurement, chronopotentiometric and adsorptive stripping measurement.

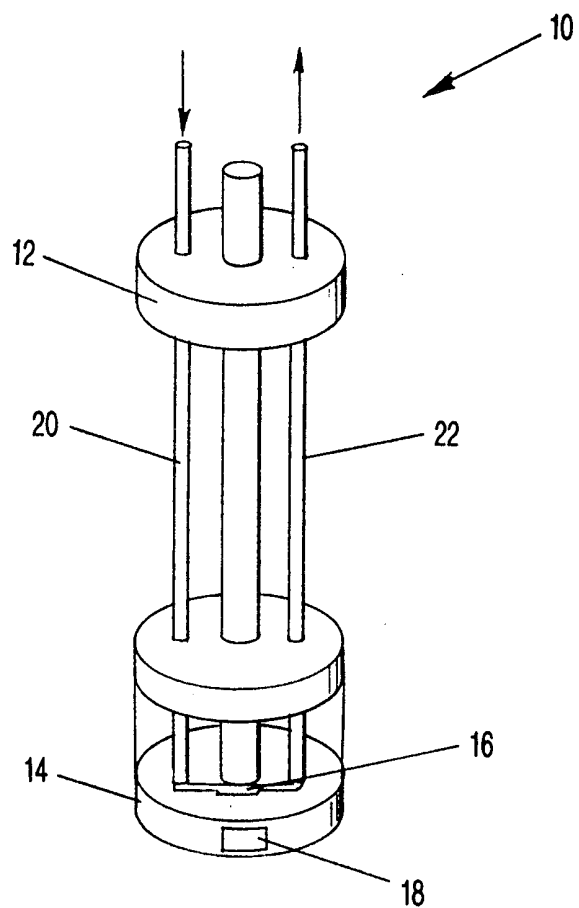


FIG-1

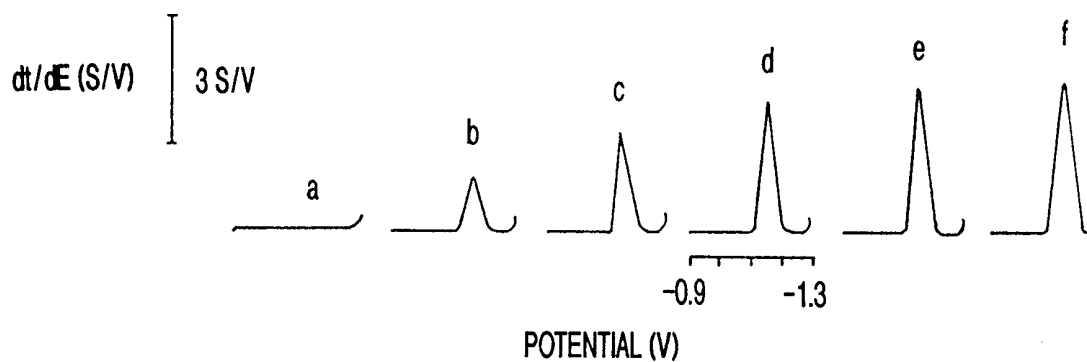


FIG-2(a)

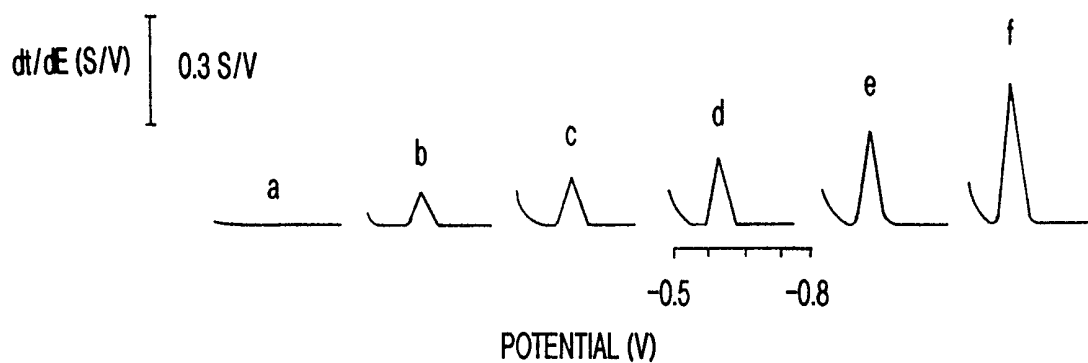


FIG-2(b)

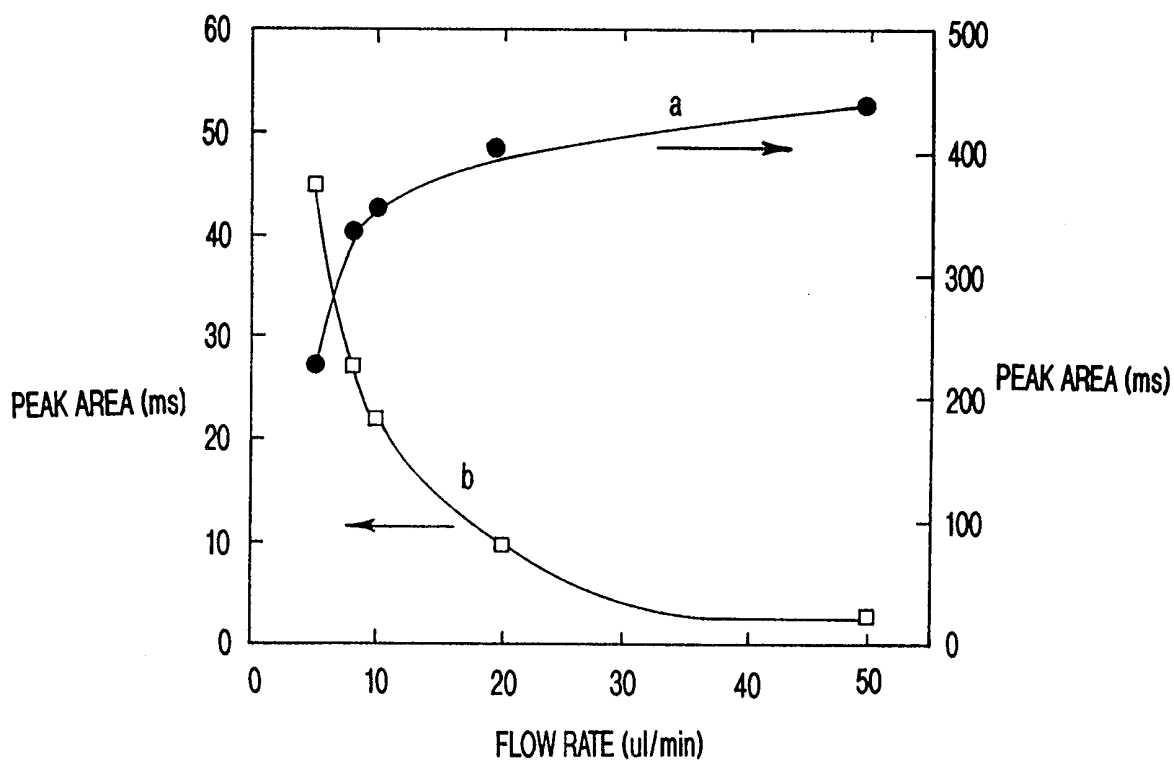


FIG-3(a)

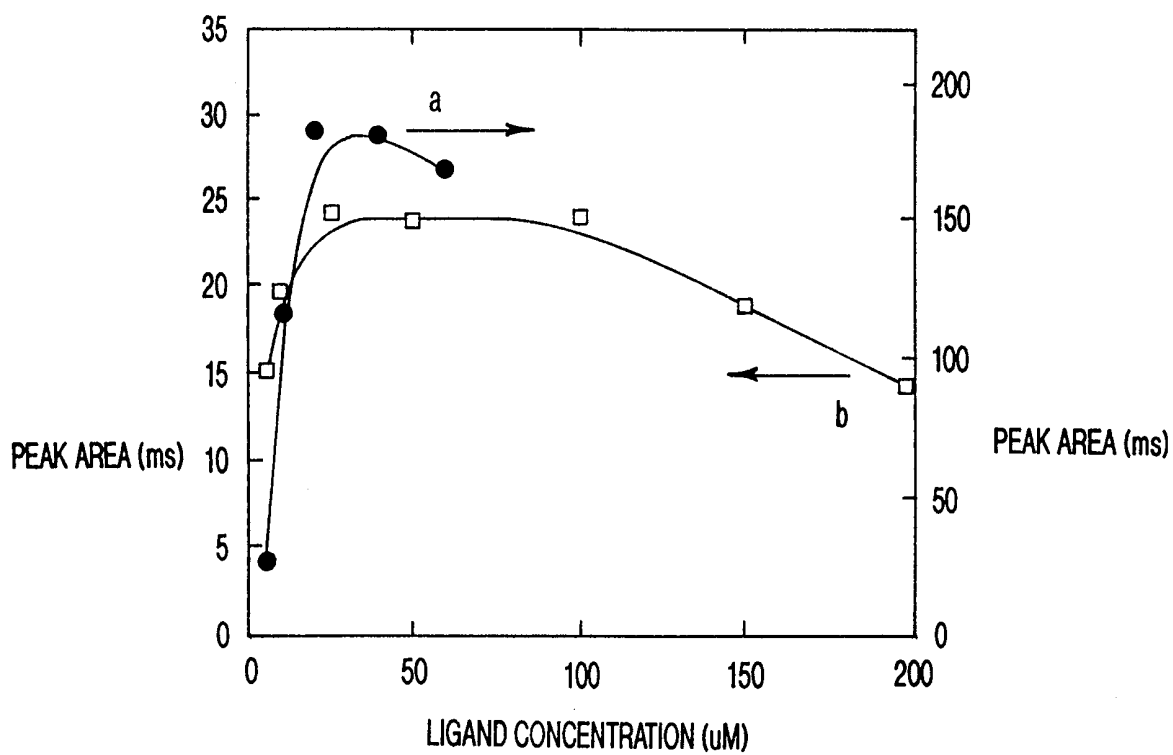
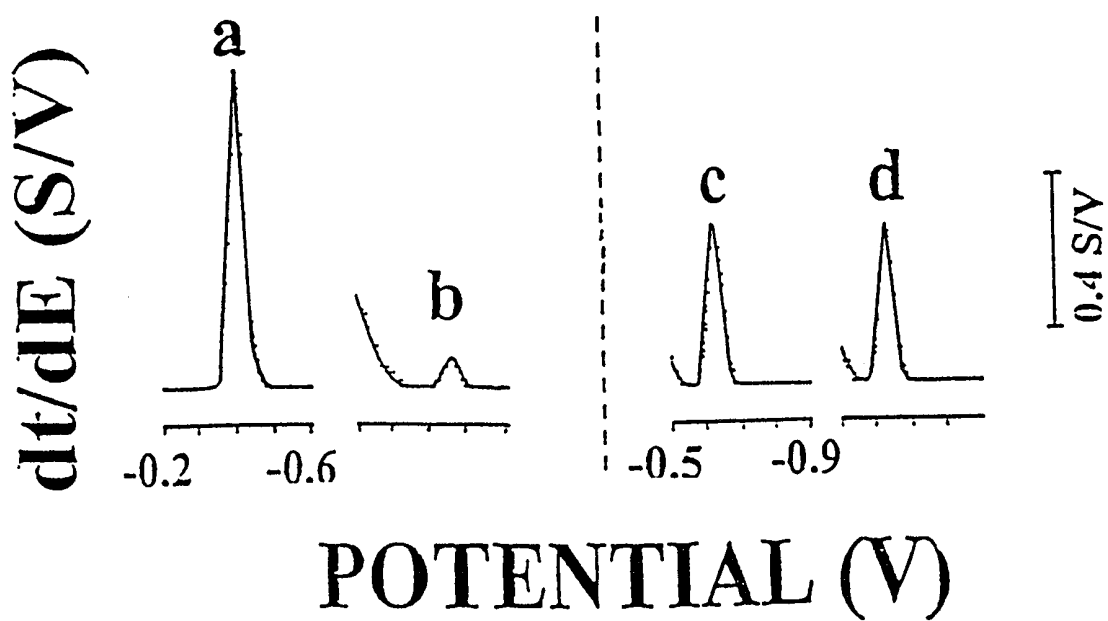


FIG-3(b)

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POTENTIAL (V)

Fig. 4(c)

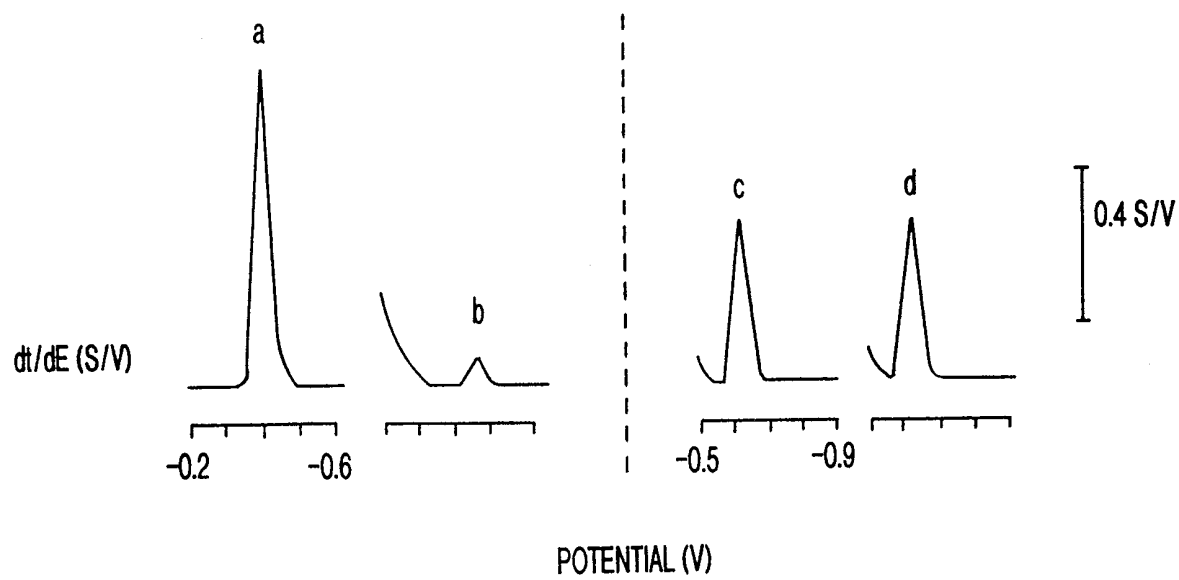


FIG-4(c)

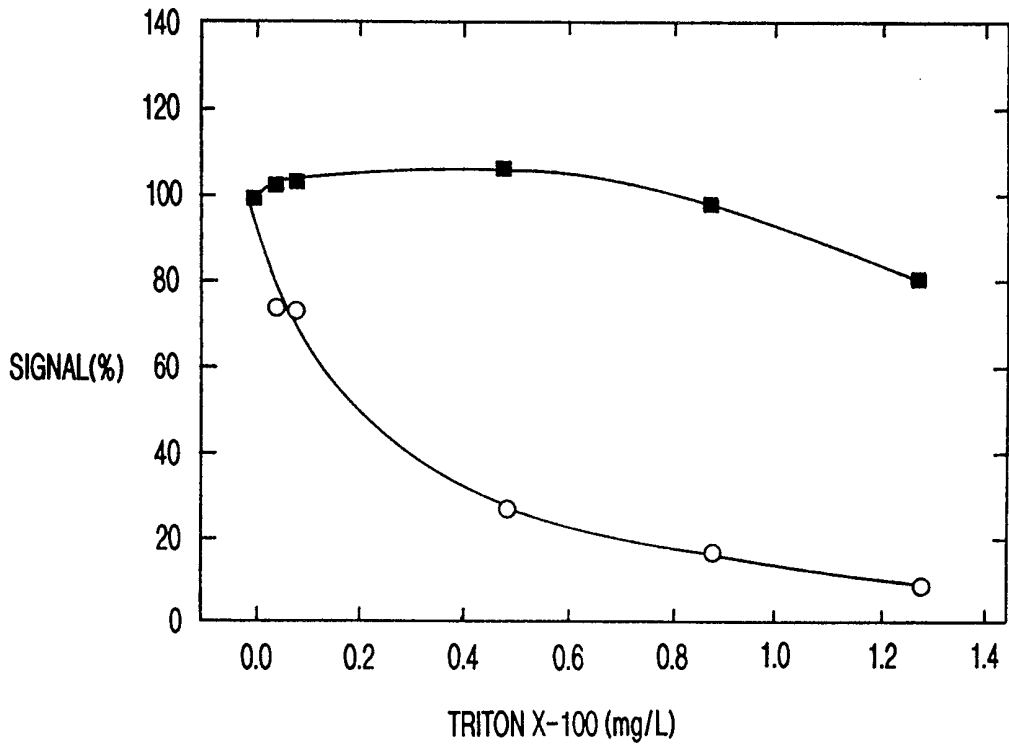


FIG-5(a)

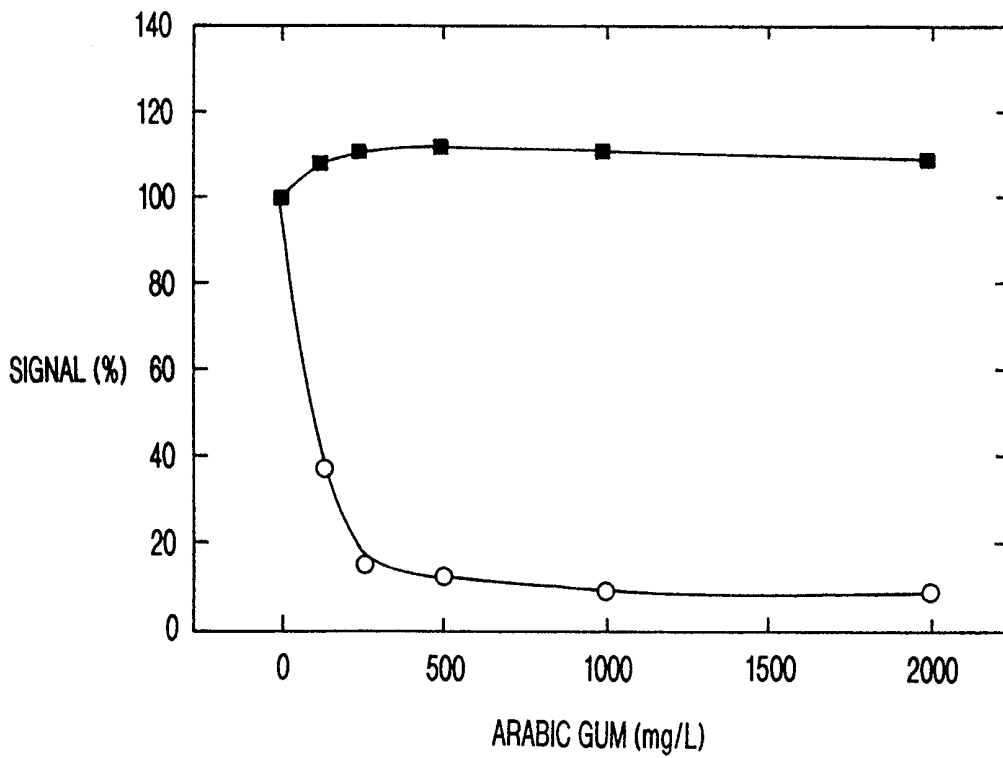


FIG-5(b)

SUBSTITUTE SHEET (RULE 26)

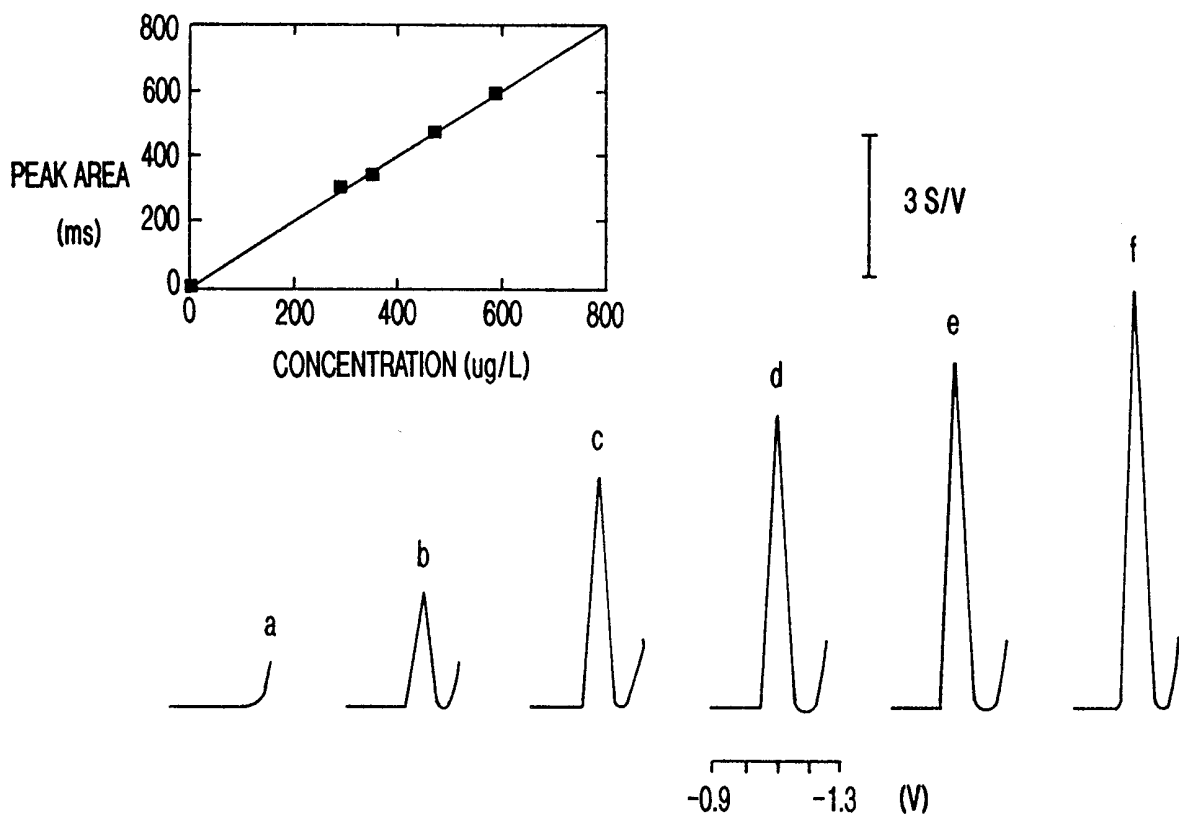


FIG-6(a)

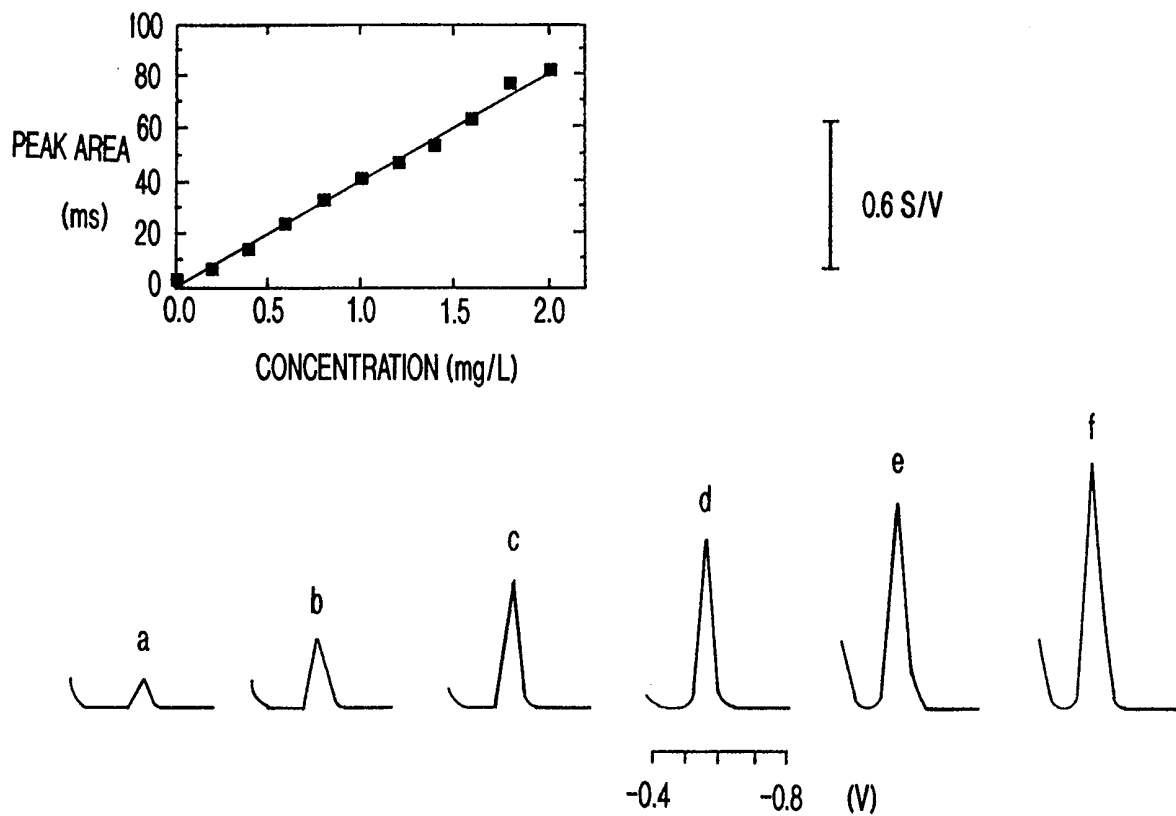


FIG-6(b)

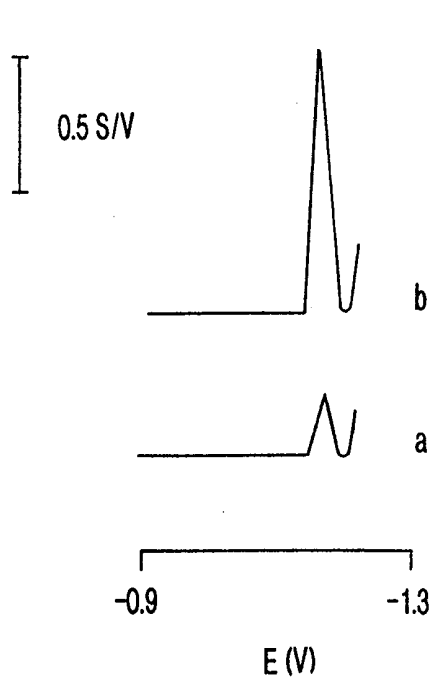


FIG-7(a)

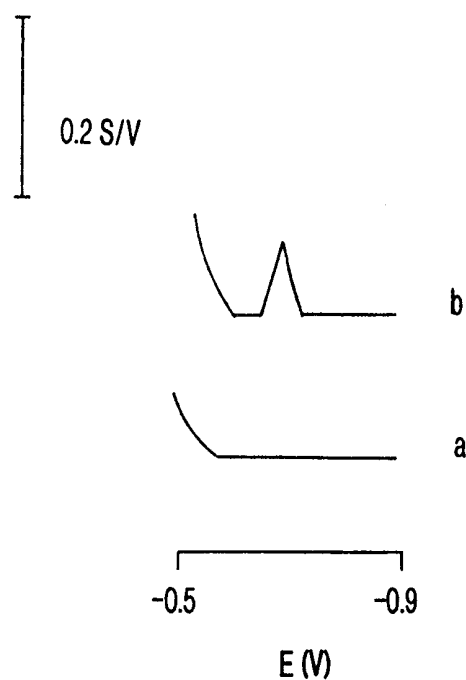


FIG-7(b)

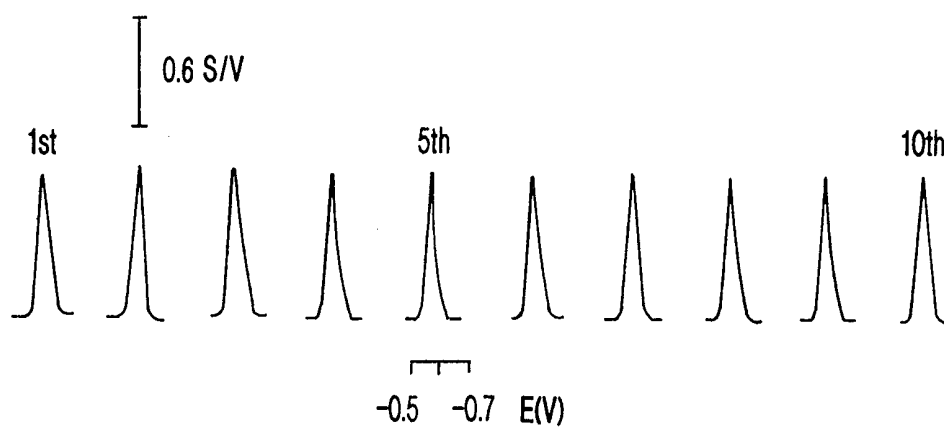


FIG-8(a)

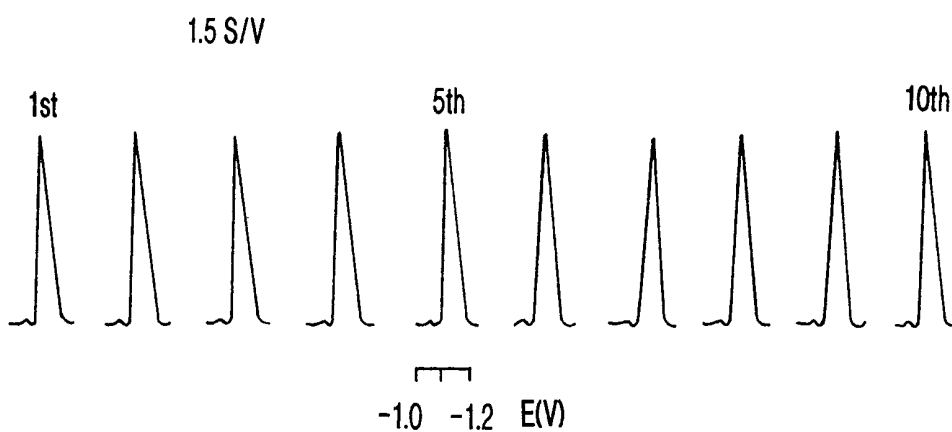


FIG-8(b)

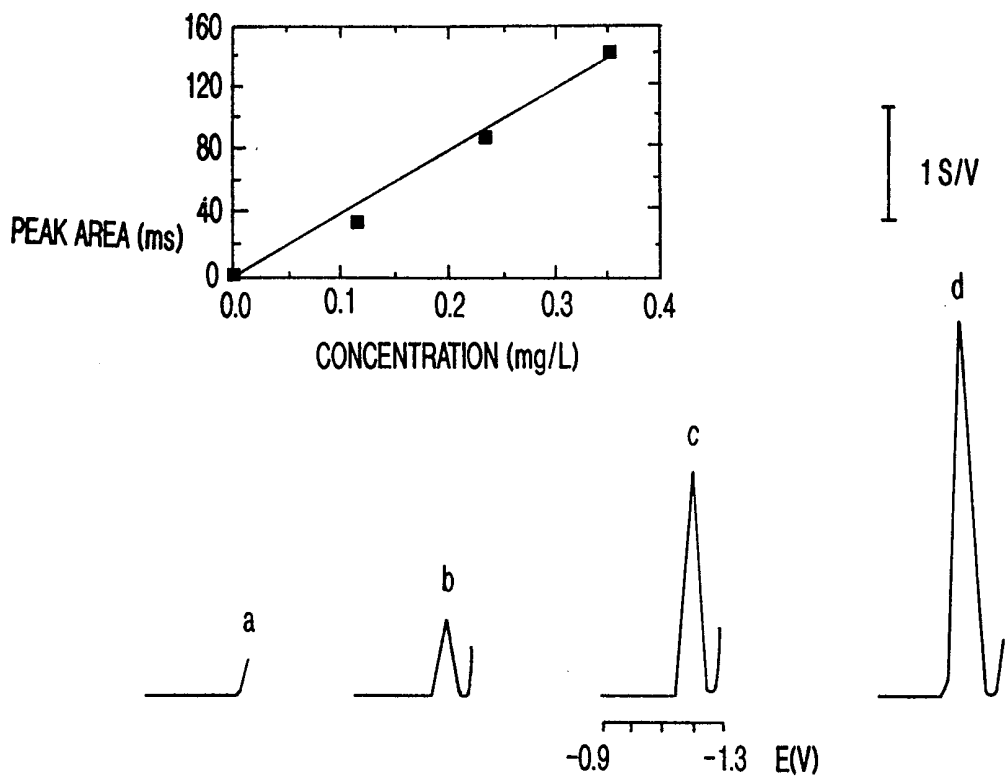


FIG-9(a)

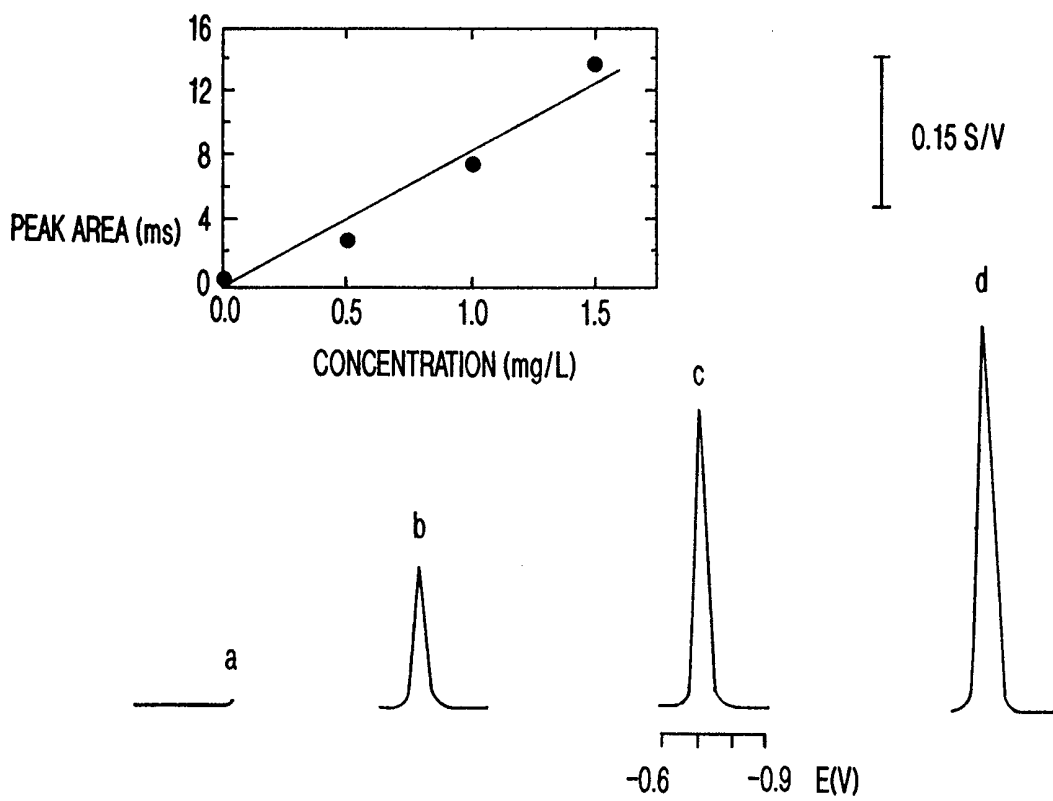


FIG-9(b)
SUBSTITUTE SHEET (RULE 26)

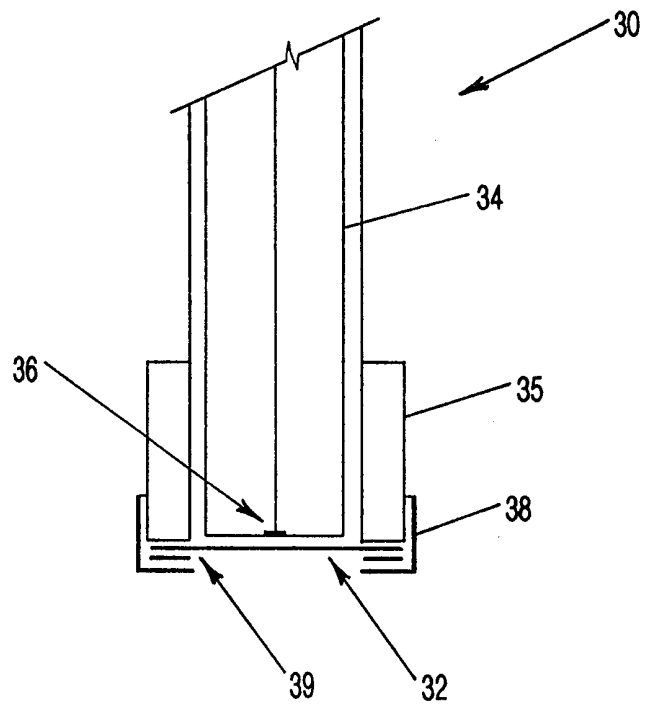


FIG-10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/17464

A. CLASSIFICATION OF SUBJECT MATTER
 IPC(6) : G01F 1/64
 US CL : 205/777.5
 According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
 Minimum documentation searched (classification system followed by classification symbols)
 U.S. : 205/777.5,778,787,798.5,790,792,793
 204/409,412,415,434

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 4,172,770 A (SEMERSKY et al) 30 October 1979 (30-10-79), column 3, lines 53-57, column 4, lines 3-6, line 33, line 63, column 7, lines 50-56.	1,2,5,6 7
X --- Y --- A	US 5,462,645 A (ALBERY et al) 31 October 1995 (31.10.95), column 4, lines 44-45, lines 64-65; column 5, lines 20-21, lines 44-49.	1,2,5,6, 8-18,20 ----- 4 ----- 3,19
Y	US 5,298,129 A (ELIASH) 29 March 1994 (29.03.94), column 2, lines 40-55; column 4, lines 22-31	4,7

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	* & * document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 04 NOVEMBER 1998	Date of mailing of the international search report 02 DEC 1998
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