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(54) Title: NANOSENSOR FOR CAPTURING CHARGED MOLECULES BY USING IN SITU STRIPPING VOLTAMMETRY

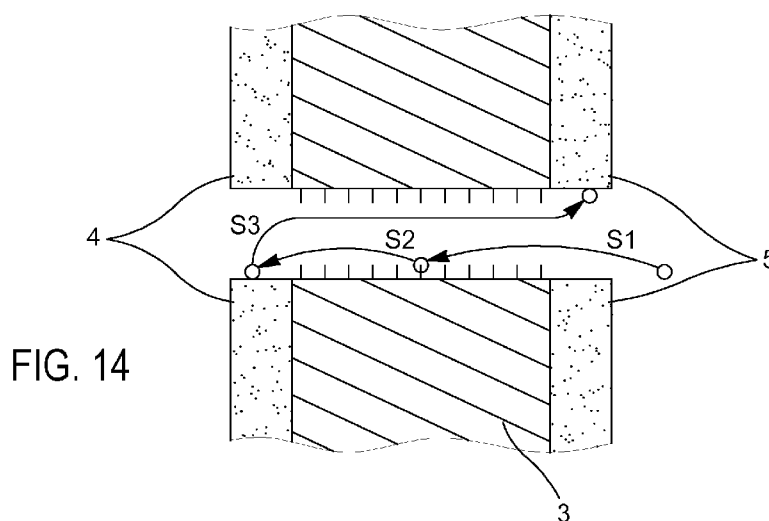


FIG. 14

(57) Abstract: The invention concerns a nanosensor for capturing charged molecules, the nanosensor comprising : - a functionalized radio grafted track-etched membrane (FRTEM) which contains polymer nanopores, - a first electrode arranged on a first side of the functionalized radio grafted track-etched membrane (FRTEM), the first electrode being designed to serve as a working electrode for an in situ stripping voltammetry technique, - a second electrode arranged on a second side of the functionalized radio grafted track-etched membrane (FRTEM), the second electrode being designed to serve as a counter electrode for an in situ stripping voltammetry technique.





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"Nanosensor for capturing charged molecules by using in situ stripping voltammetry."

5 The invention relates to nanosensors, and more particularly, to a device for capturing charged molecules or ions and a method for capturing and quantifying charged molecules and favourably heavy metal ions in a fluid. The present invention advantageously applies to sewage sludge or potable water, but it could apply to biological or medical fields. It could also apply to the
10 detection of other ionic species in fluid, not only heavy metal ions.

 The urban wastewater collection is one of the main facilities used for the disposal of commercial and domestic wastes, both of which may contain heavy metals. Wastewater treatment processes are generally effective in limiting the
15 discharge of potentially toxic elements to the aquatic environment because a high proportion of the contaminant load is extracted and concentrated in the sewage sludge. Application of sewage sludge to agricultural land is the largest outlet for its beneficial use, and this is consistent with EC policy on waste recycling, recovery, and use. The Sewage Sludge Directive 86/278/EEC sets
20 upper limits of trace metals in municipal sewage sludge for use on agricultural land, and revision of the directive is expected to lead to even more stringent limits. This is a critical issue due to the increasing amount of sludge produced (from 5.5M tonnes of dry matter in 1992 to 9M tonnes in 2005), and the fact that other alternatives (incineration and landfill) are not generally considered
25 environmentally acceptable. Consequently, wastewater sludge quality must be protected and improved in order to secure the agricultural outlet as the most cost effective as sustainable solution.

 The most widespread method for the determination of metal concentrations in wastewaters is via grab sampling and subsequent laboratory
30 analysis. This method is both expensive, which limits its application, and time consuming, which means that pollution events can be missed, or detected too late. In the face of increasing levels of sludge production, the expected application of more stringent limits on heavy metal concentrations in sludge, and to identify, survey, and control the sources of input of toxic elements, there
35 is a need for a low cost, time effective, easy to handle and very sensitive

analysis system to determine the concentration of heavy metals in waste waters.

In addition, existing sensors to detect metal residues present following
5 drawbacks:

- metal-based screen-print electrodes or modified glassy carbon are not accurate enough to detect residue (detection limits of 0.5 (Pb^{2+}), 2.0 (Cu^{2+}), 0.9(Hg^+) and 1.4 $\mu\text{g/L}$ (Cd^{2+})),
- most of them suffer from analyte peak distortion, peak overlap, and loss
10 of sensitivity due to interference of analyte ions with each other,
- Hg film coated electrodes are accurate enough at ppb levels ($\mu\text{g/L}$) by ion pre-concentration but not environmentally friendly.

Document WO 2009/147244 concerns a nanosensor for capturing heavy
15 metal ions included in a fluid; said nanosensor comprising a functionalized radio grafted track-etched membrane (FRTEM) which contains nanopores; the membrane surfaces are metallised at both sides to serve as working electrode for an *ex situ* stripping voltammetry. The *ex situ* process comprises steps of immersing the nanosensor in the fluid containing heavy metal ions, removing,
20 rising and immersing the nanosensor into a voltammetric cell to perform the stripping voltammetry, the voltammetric cell containing conductive fluid.

With the nanosensor as disclosed in WO 2009/147244 the nanosensor has to be displaced from the sample medium to a voltammetry cell.

25 An object of the present invention is to overcome drawbacks of prior art by providing an accurate and rapid nanosensor. Another object of the present invention is to improve sensitivity and selectivity of a nanosensor.

In at least preferred embodiments, the present invention provides a
30 nanosensor for capturing charged molecules, the nanosensor comprising:

- a functionalized radio grafted track-etched membrane (FRTEM) which contains polymer nanopores through at least two sides,
- a first electrode arranged on a first side of the functionalized radio grafted track-etched membrane (FRTEM), the first electrode being designed to serve
35 as a working electrode for an *in situ* stripping voltammetry technique,

- a second electrode arranged on a second side of the functionalized radio grafted track-etched membrane (FRTEM), the second electrode being designed to serve as a counter electrode for an *in situ* stripping voltammetry technique.

5 The nanosensor comprises electrodes designed on membrane surfaces for *in situ* analysis due to the ion exchange properties which make the membrane like a solid electrode with a finite resistance. Nanopores constitute low conductivity channels through which current can circulate between the working and counter electrodes.

10

 The nanosensor according to the invention allows for *in situ* analysis, which permits rapid monitoring of the efficiency of wastewater treatment and measure the charged molecules concentration in sewage sludge by functionalized radio grafted track-etched membranes (FRTEM), which are able to
15 capture trace levels of for example heavy metal ions under wastewater flow by absorption.

 According to the invention, the nanosensor further comprises a third electrode arranged on a side of the functionalized radio grafted track-etched
20 membrane (FRTEM), the third electrode being designed to serve as a reference electrode for a stripping voltammetry technique. All three electrodes are independent.

 According to an embodiment of the invention, the membrane is based on
25 a poly(vinylidene fluoride) PVDF film which can be radiografted with vinyl monomers. In other words, in the presence of vinyl monomer, such as acrylic acid (AA), a radical polymerization takes place by radiografting to specifically functionalize the nanopore wall with carboxylate hydrogel.

30 Advantageously, walls of nanopores are functionalized with one or a mixture of elements obtained from following groups:

- poly-acrylic acid,
- styrene,
- taurin,
- 35 - polyphosphine of R_3P type,
- oxidized polyphosphine of $R_3P=O$ type.

- 4 -

- or other functional molecules known to specifically complex certain ions (for example carbamoyl-arms polyazamacrocyle which is known to specifically complex lead ions).

These groups may be used to functionalize the nanopores either by radiografting or coupling reactions.

More generally, depending on the specific functionalization of the nanopores, this nanosensor is useful for the analysis of biological or medical samples or any of the analysis that is performed by anodic stripping voltammetry (ASV) such as those of screen-printed electrodes and other stripping voltammetry techniques such as cathodic stripping voltammetry (CSV) or adsorptive stripping voltammetry (AdSV).

Preferably, the polymer nanopores consist in tubules through the entire thickness of the membrane. The membrane could be in the shape of a cylinder or parallelepiped in which tubules are all parallel and perpendicular to the two opposite sides of the membrane. The thickness of the membrane could substantially be of 9 μm and the nanopores could present a diameter between 20 and 200 nm. Moreover, each side of the membrane could present a diameter of substantially 4mm.

In preferred embodiment, the membrane is specifically grafted inside the nanopores. The synthesis of functionalized radio grafted membranes comprises notably steps of irradiation to activate radicals, chemical etching and radiografting. The remanence of the radicals within the nanopore walls after etching allows one to radiograft specifically inside the nanopores and not on the membrane surface. Under wastewater streams, for example, the functionalized radiografted, track-etched membrane (FRTEM) can thus trap cations and concentrate them.

30

The present invention explicitly refers to WO 2009/147244 which provides information relating to an ex situ analysis. The elements disclosed in WO 2009/147244 are incorporated in the present invention insofar as these elements are not incompatible with the teachings of the present invention.

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Advantageously, walls of nanopores further contain bismuth ions, or other ions such as silver or selenium, which constitute an internal standard.

The absorbing ability of the functionalized track-etched nanoporous membranes is used to absorb a determined concentration of specific ions (Bi^{3+} , Ag^+ , Se^{4+} or Co^{2+}) to function as an internal standard for stripping analysis. The bismuth or silver or selenium or cobalt enhances the analysis of the other ions and is stripped last for quantitative comparison as an internal standard.

In order to improve adsorption and screening during stripping voltammetry, the nanosensor further comprises a first and a second piezoelectric contacts respectively on two opposite sides of the functionalized radio grafted track-etched membrane (FRTEM). These piezoelectric contacts can be driven by an alternating voltage.

In other words, voltages may be applied on piezoelectric contacts disposed on the functionalized radio grafted track-etched membrane (FRTEM) in order to deform said functionalized radio grafted track-etched membrane (FRTEM) during capture of charged molecules and/or stripping voltammetry analysis; the functionalized radio grafted track-etched membrane (FRTEM) being designed from a poly(vinylidene fluoride) PVDF film which is radio grafted with vinyl monomer and chelating molecule leading to a polymerization of nanopores walls. Piezoelectric properties of the membranes allow for ultrasonic agitation to enhance the filtering and analysis properties of the sensors.

In another aspect, the invention also enables the provision of a new method for detecting and quantifying charged molecules included in a fluid, said method comprising steps of:

- placing in the fluid a functionalized radio grafted track-etched membrane (FRTEM) which contains polymer nanopores, through two opposite sides; charged molecules being captured inside the polymer nanopores,
- applying an *in situ* stripping voltammetry analysis by using:
 - a working electrode arranged on one side of the functionalized radio grafted track-etched membrane (FRTEM),
 - a counter electrode arranged on a second side of the functionalized radio grafted track-etched membrane (FRTEM), and
 - a reference electrode arranged in the fluid.

The present invention may advantageously be applied in detecting and quantifying charged molecules concentration of which is less than 5 ppb. Inside this range, the nanosensor presents an optimum sensitivity.

- 5 The invention also concerns a system for capturing charged molecules, the system comprising a processing unit equipped with several I/O plugs, each I/O plug comprising:
- a first connector to connect the working electrode,
 - a second connector to connect the counter electrode, and
 - 10 - a third connector to constitute a reference electrode in the fluid.

For the purpose of illustrating the invention, there is shown in the drawings a form that is presently preferred; it being understood, however, that this invention is not limited to the precise arrangements and instrumentalities.

15

Figure 1 is a schematic view illustrating Track-etch process: I) Swift Heavy Ions irradiation forms damaged zones in the polymer bulk along the ion pathway called latent tracks, II) the latent tracks are chemically etched forming nanopores of biconical shape at the early stage and III) if the track etch rate is sufficiently higher than the bulk etch rate, biconical nanopores become cylindrical;

Figure 2 is a graph illustrating the electron paramagnetic resonance (EPR) spectra per mass unit (mg^{-1}) for a heavy ion irradiated film;

Figure 3 is a graph illustrating the radical decay from EPR results versus time;

Figure 4 is a graph illustrating a FTIR spectra of swift heavy ion $^{58}\text{Ni}^{25+}$ irradiated PVDF-g-PAA for different etching times (AA concentration 100% v/v, fluence 10^9cm^{-2} , Mohr's salt 0.25% w/w, radiografting at 60°C for 1 hour); the maximum corresponds to O-C=O stretching (1701cm^{-1}) and gives evidence of COOH groups radiografted PAA chains;

Figure 5 is a schematic view illustrating functionalization on nanosensor walls;

Figure 6 is a schematic view of a membrane cut in order to view nanopore walls;

Figure 7 shows a schematic cut-away side view of a membrane with gold electrodes on top and bottom sides;

Figure 8 shows a perspective view of the membrane of figure 6;

Figures 9-11 are schematic views illustrating the insertion of the membrane onto a membrane-holder constituting a nanosensor;

Figures 12a to 12d illustrate an online analysis in the detour of an effluent stream;

Figure 13 is a schematic view illustrating a handheld multi-way device comprising several input/outputs to connect several nanosensors according to the invention;

Figure 14 is a schematic view illustrating ions displacement through a nanopore wall during an *in situ* stripping voltammetry;

Figure 15 is a schematic view illustrating a nanosensor connected to a potentiostat according to the invention;

Figure 16 is a schematic view illustrating a nanosensor comprising a reference electrode and a working electrode disposed on one side, and a counter electrode disposed on another side;

Figures 17a to 17d illustrate curves concerning ASV calibration; and

Figure 18 is a schematic view illustrating a nanosensor comprising working electrode, counter electrode, and piezoelectric contacts for sonic agitation.

While the invention is susceptible to various modifications and alternative forms, specific embodiments thereof are shown by way of example in the drawings and will herein be described in detail. It should be understood, however, that the drawings and detailed description thereto are not intended to limit the invention to the particular form disclosed, but on the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the present invention as defined by the appended claims.

In accordance with the preferred embodiment, the method and device according to the invention relates to the following materials and processes:

Materials: Poly(vinylidene fluoride) (PVDF) films of 9 μm thickness provided by PiezoTech SA®, Saint Louis (France). Toluene, potassium hydroxide, potassium permanganate, potassium disulfite, acrylic acid (AA), Mohr's salt $((\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O})$, sulphuric acid, EDC ($\text{C}_8\text{H}_{17}\text{N}_3 \cdot \text{HCl}$), phosphate buffer saline (PBS) and Alexa Fluor R 488 hydrazide ($\text{C}_{21}\text{H}_{15}\text{N}_4\text{NaO}_{10}\text{S}_2$)

(C₂₁H₁₅N₄NaO₁₀S₂) purchased from Invitrogen®.

Irradiation: Prior to irradiation, PVDF films are toluene-extracted for 24 h. Swift heavy ion irradiation was performed at the GANIL, Caen (France). Films are irradiated with Kr ions (10.37 MeV/amu, fluence 10⁷ to 10¹⁰ cm⁻²) under He atmosphere. In two cases, samples are stored at -20°C under N₂ atmosphere until chemical etching and radiografting.

Chemical etching: PVDF irradiated films are chemically etched using permanganate solution (0.25 M) in a highly alkaline medium (KOH 10 M) at 65°C with different etching times from 0.5 to 3 h. Membranes obtained are washed in potassium disulfide solution (15%) then dried at 50°C under vacuum.

Radiografting: PVDF films of initial size 20x20 mm², are weighed. The film was immersed at room temperature in a radiografting solution containing acrylic acid and Mohr's salt (0.25%w/w). After 15 minutes of bubbling nitrogen at room temperature, the sample is introduced into a thermostated water bath at 60°C for 1 hour. Membranes are washed with water and then Soxhlet-extracted in boiling water in order to extract free homopolymer. Functionalized membranes are dried at 50°C under vacuum.

Infra-red spectroscopy: FTIR spectra of PVDF films are obtained with a Nicolet Magna-IR 750 spectrometer equipped with a DGTS detector. Spectra are recorded in an attenuated total reflection mode (ATR) using a diamond-crystal with single reflection. Spectra are collected by accumulating 32 scans at a resolution of 2 cm⁻².

Confocal Scanning Laser Microscopy (CSLM): Measurements are performed at LLB (CEA-Saclay, France) with a Leica TCS-SP2 using an Ar laser (488 nm). Samples are observed in water with a 40x dry objective of numerical aperture 0.85.

Electrode fabrication, as shown on figures 5-10: A first gold bottom layer, ~45nm is sputtered (EMITECH K550, UK) through a mask with 3 mm diameter holes. Next, a second gold layer, ~45 nm, is sputtered, through a mask, on the other side of the membrane over the same areas as the first gold area.

Anodic stripping voltametry (ASV): Electrochemical quantification of trace metal analytes is done by ASV. This involves the electrochemical reduction of the concentrated metal ions from the membrane on a metal coating on the surface of the membrane, which is the working electrode of an electrochemical cell. The electrode is held at a very negative potential, which reduces all of the

trapped metal ions to their metallic, zero valence, state. The electrode is then scanned slowly toward positive potentials where the ions are oxidized, anodically stripped, and the resulting current is proportional to the concentrations of the ions trapped in the membrane. Different metals oxidize at different potentials so the kind of metal can be identified. The analysis is performed with a potentiostat (HEKA PG310, Germany) with a Ag/AgCl (Metrohm, Switzerland) reference electrode.

The synthesis of functionalized radio grafted membranes is described according to figures 1-4. With respect to figure 1, a track-etch process is described. This process comprises three steps:

- I) latent track formation along the ion pathway through a polymer film 1,
- II) symmetrical attack of latent tracks by hydrolysis at early stage of the process, and
- 15 III) formation of cylindrical pores 2.

In other words, for the preparation of a functionalized radio grafted track-etched membrane 3 (FRTEM), polymer film 1 is first bombarded by swift heavy ions and the formed tracks along the ion passage are revealed under alkaline chemical treatment.

20 The obtained nanoporous polymer membrane 3 does not need to undergo a subsequent e-beam irradiation to increase radical proportion in polymer bulk submicronic pore diameter. Indeed, after etching times inferior to one hour, the radical residues within nanopore walls were found sufficiently numerous to pursue a radiografting from the pore walls.

25 Figure 2 shows an electron paramagnetic resonance (EPR) spectra per mass unit (mg^{-1}) for a heavy ion irradiated film before etching (latent tracks) and after 1 hour of track etching. In the latter case, the non-horizontal baseline is due to paramagnetic impurities (KMnO_4 from the etching bath). Figure 3 shows the radical decay from EPR results versus time. Radicals are always presents even after 1 hour of track etching.

Concerning the functionalization, in the presence of vinyl monomer, such as acrylic acid (AA), a radical polymerization takes place by radiografting process to specifically functionalize the nanopore walls with carboxylate hydrogel as shown from FTIR spectra in figure 4. This polymerization is specific to the

to the nanopore walls and does not occur on the membrane surface. The selectivity of the grafting is checked by labelling of the amine pre-functionalized surface of the PVDF nanoporous membrane by a fluorescent probe specific to the amines and the poly(acrylic acid) inside the pores by a second fluorescent
5 probe specific to the acid groups.

Functionalization is usually accomplished by absorbing functional molecules onto the electrode surface, applying a carbon paste, dipping the electrodes in a solution of the functionalizing agent, or spin coating of a
10 functional polymer such as nafion. The nanoporous PVDF membranes according to the present invention can be functionalized to serve as modified electrodes for stripping voltammetry.

The carboxylate hydrogel located inside the nanopores chelates weakly with transition metal ions. In order to improve the membrane ion affinity, it is
15 possible to further functionalize it by other radical or common coupling reactions with other chelant molecules.

Chelation ("chélation" in French) is the binding or complexation of a mono or bi- or multidentate ligand. These ligands, which are often organic compounds, are called chelants, chelators, chelating agents, or sequestering agent. These
20 molecules should have a very important chelating power to trap even more effectively the heavy metal ions.

These molecules should have different chelating powers or specificities in order to trap even more effectively or selectively the metal ions or other ions (Mono- and polyacides, mono and polyamines, Ethylene Diamine Tetra Acetate and derivatives, cyclodextrines, crownethers or any clathrates complexes).
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In the schematic example of figure 5 a nanosensor 9 is represented. Element 2A represent radicals of chelant molecules used to functionalize nanopore walls. Figure 5 illustrates metallised (gold, carbon or other metal) surfaces 4, 5 and PVDF nanoporous membrane 3. The functionalization,
30 however, takes place before the metallization.

As an example, in order to bring sulfonic acid ion exchange functionalization to the pores the radiografting can for example be performed with 100% styrene (instead of acrylic acid) at 60°C for 30 minutes in a nitrogen atmosphere. The $-C_6H_4SO_3^-$ functionalized pores are strong cation exchange with
35 high capacity that might be more effective for weakly adsorbed ions such as Co, Cr, and Fe. The membranes are then put in a 0.5 M chlorosulfonic acid (SO_2Cl)

in dichloromethane solution at room temperature under a nitrogen atmosphere for 30 minutes. After, it is then possible to hydrolize the membrane in two stages: NaOH 1M during 2 hours then H₂SO₄ 1M during 1 hour.

As another example to impart sulfonate (-SO₃⁻) functionalization to the poly (acrylic acid) functionalized PVDF membranes, a coupling reaction can be performed with Taurin (poly(PPy-co-PPYCONHTaurin)). Taurin coupled to acrylic acid functionalized pores (poly(PPy-co-PPYCONH taurin) due to the -NH groups might have anion exchange effects which could help for metal oxyanions such as AsO₄³⁻, HAsO₄²⁻ or AsO₃³⁻. The membranes may react with 10⁻² molar ethyl-3-(3-dimethylamino-propyl)carbodiimide, C₈H₁₇N₃·HCL (EDC) and 3.2 x 10⁻² molar Taurin C₂H₇O₃NS for 24 h in aqueous medium.

The chemical nature of the polymer that is radiografted in the nanopores will have a direct impact on the metal(s), and the amount of metal, to be trapped and analyzed. It is interesting to have access to a variety of polymers with tunable coordinative abilities to metal centers. The polyphosphine (of R₃P type) polymers are for example well tailored for electron rich metal centres such as Au, Cu, Pt and Zn, whereas the oxidized version (of R₃P=O type) are more efficient for electron poor metal centres such as Co, Cr, Fe, and V. Polymers containing at the same time the two moieties can also be synthesized.

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According to the present invention, the functionalized nanoporous PVDF membranes may be pre-dosed with Bi³⁺ (or other ions such as Ag⁺ or Se⁴⁺ or Co²⁺) for stripping analysis. Bismuth has a large window for voltammetric analysis and is insensitive to dissolved oxygen in sample mediums.

Bismuth is nontoxic and may be integrated inside nanopores as follows: a functionalized, non metallized, nanoporous PVDF membrane is submerged in a specific concentration of bismuth ion solution, Bi³⁺, for a set time, for example 1 ppb Bi solution for 15 mn. The membrane is then removed from the Bi³⁺ solution, rinsed, dried, and metallized. The Bi³⁺ doped membrane is then configured as a stripping voltammetry sensor and placed in a sample medium to absorb metal ions, Mⁿ⁺. (ex. Cd²⁺, Pb²⁺). The stripping voltammetry is normally performed to yield a current/potential plot. The currents peaks can be integrated and their concentration can be determined by comparison with the area of the bismuth peak. This internal standard scheme could be used as a means for controlled dosing of ions into systems. Also, Ag⁺ or Se⁴⁺ or Co²⁺ could be used in the same manner as an internal standard.

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Turning now to figures 6-11, a nanosensor 13 according to the invention is shown. The nanosensor is based on the membrane 3, the shape of which is a cylinder of 9µm thick and of 4.0 mm diameter, with 10^6 to 10^{10} nanopores per cm^2 of 50 nm diameter. Once the membrane has been functionalized, gold is sputtered on each side of the membrane 3 in figure 7 : a bottom electrode 4 of 45nm thick, preferably a value from 30 to 50nm, and a top electrode 5 of 45nm thick, preferably a value from 30 to 50nm,. The sputtering is made in such a way that the nanopores remain open. The gold layers can cover or not the entire side surface. On figure 8 the perspective view of the membrane shows the top electrode 5 and the bottom electrode 4. On figures 9-11, the bottom layer 4 is contacted with silver paste 6A to copper contacts 7 lithographically patterned on 1.5 cm^2 Plexiglas placket 8 with a 4mm diameter hole, 8b. The top layer 5 is contacted with silver paste 6B to other copper contacts 10 on the same placket 8. The membrane 3 is then covered with waterproof tape, Kapton, 3M, except for a 2 mm diameter circular area 11 in the centre of the membrane, which will serve as a window for exposure of the membrane to the wastewater, see figure 11. The diameter of area 11 is less or equal to the diameter of the hole 8b.

Preferably, also electrode 19 is not covered by the waterproof tape so that said electrode 19 is in contact with wastewater.

The copper leads are not completely covered by the tape and remain outside of the solution so they can be connected to a potentiostat via a bridge 12.

For the stripping voltammetry, electrode 4 is the working electrode; electrode 5 is the counter electrode. A reference electrode 19 can be realised, but not necessary, on the membrane 3. On figures 9-11, reference electrode 19 is not sputtered on the membrane 3 but is on the Plexiglas packet 8 so that it will be inside the fluid during a stripping voltammetry.

The nanosensor 13, 13A according to the present invention is very compact and easy to handle.

The nanosensor 13, 13A can then be immersed into wastewater for a preset time to absorb ions based on calibration. According to an embodiment of the invention, the nanosensor could be used by placing it in a bypass circuit of the fluid or for example in a small detour 14 of a waste-water effluent pipe 15 as shown on figure 12a. The detour 14 comprises an inlet pipe equipped with an

inlet valve A, and an outlet pipe equipped with an outlet valve B. Between said valves, is disposed the nanosensor 13, 13A. The detour 14 also comprises two evacuation pipes equipped respectively with valves C and D.

Figures 12 a-d illustrate an on-line analysis. In figure 12a), valves A and B open and valves C and D closed, the nanosensor is in the detour 14 of the effluent stream. In this mode the sensor can be absorbing ions for a calibrated time. In figure 12b), valves A and B open and valves C and D closed, the sensor could be in a conductivity mode for constantly measuring the resistance of the effluent. The conductivity measurements are discussed hereafter. The conductivity mode could serve as an alarm for pollution events such as a dramatic increase in effluent conductivity. In figure 12c, valves A and B closed and valves C and D open, a supporting electrolyte could be injected into the sensor chamber and the sensor could be connected to a potentiostat for ASV analysis. In figure 12d), valves A and B closed and valves C and D open, a cleaning solution, 0.1 M HNO_3 or EDTA, could be injected into the sensor chamber after analysis in-order to clean the sensor for reuse.

Figure 13 illustrates a mobile device D1 for capturing charged molecules. This device D1 comprises several input/outputs to connect several nanosensors D2, D3 according to the invention. One input/output can receive a nanosensor functionalized with any suitable molecule. The device may be connected to different nanosensors functionalized with different molecules respectively. Thus, each nanosensor may be adapted to detect a specific ion. Device D1 contains a processing unit controlling one or a plurality of potentiostats which feed nanosensors D2, D3.

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For stripping voltammetry it is necessary to use three electrodes: a working electrode, a reference electrode, and a counter electrode. The potential is controlled between the working electrode and the reference electrode and the current is measured between the working electrode and the counter electrode.

Since the current is measured between the working and counter electrodes the ionic transport is between the working and counter electrodes. The problem with *in situ* measurements is that the ionic concentration of the sample medium is uncontrolled and often very low. This hampers current response because of the high resistance. According to the present invention, the functionalized nanoporous PVDF membrane is designed as a conducting or ion exchange medium between the working and counter electrodes in order to allow

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for *in situ* voltammetry. More precisely, when the membrane is placed into a sample medium, the ions of interest are absorbed from the sample medium to nanopore walls as stated by S1 figure 14. To perform an *in situ* stripping voltammetry, a potentiostat is used to control the working, counter and reference electrodes while the membrane stays in the sample medium. At step S2 on figure 14, ions migrate to the working electrode 4 by a negative potential -1200 mV applied between the reference electrode 19 and the working electrode 5. Ions are then reduced onto the electrode 4 at the potential of -1200 mV. At step S3, the potential is then scanned in a positive sense from -1200 mV to +400 mV, which oxidizes (dissolves) the ions into the sample medium and creates a current which is carried through the membrane between the working electrode 4 and the counter electrode 5. Advantageously the current passes through the nanopores. The resulting current intensity and position, measured between the working and the counter electrodes 4, 5 of the membrane 3 indicate the type and concentration of the heavy metal ions. The resulting currents are proportional to the contaminate ions concentration based on previous calibration.

Figure 15 shows the membrane attached by wires to pin connectors for connection to a potentiostat. Since the counter and working electrodes are both gold they can be interchanged to suit different situations. The reference electrode 19 is connected to the potentiostat 20 and its free end is either freely disposed in the sample medium (figure 15) or metallised on a part of a side of the membrane as shown on figure 16 (figure 15 dashed line). With the membrane configuration shown in figure 15, the functionalized membrane functions to absorb ions from a sample medium and the current for analysis is carried by the membrane functionalization groups between the working and counter electrodes and the potential is controlled between the working and reference electrodes. In this way, even high resistance mediums can be measured, even non liquid mediums could be analysed such as air or exhaust.

An application of the nanosensor according to the present invention concerns *in situ* conductivity measurements. Preferably, two sensors may be used, the first sensor including a functionalized radio grafted track-etched membrane (FRTEM) which is positively functionalized, preferably with a poly(PPy-co-PPYCONHtaurin), the second sensor including a functionalized radio

grafted track-etched membrane (FRTEM) which is negatively functionalized, preferably with a poly(acrylic acid).

In general, a resistance, $R(\Omega)$ of a medium depends on the size and shape of the measuring cell and the resistivity of the medium. For a uniform cell
5 with an area, $A \text{ (m}^2\text{)}$, and length, $L \text{ (m)}$ the resistivity, $\rho(\Omega\text{m})$, is:

$$\rho = RA/L$$

The conductivity of a cell, $\kappa \text{ (Sm}^{-1}\text{)}$; where $S = 1/\Omega$, is the reciprocal of the resistivity:

$$\kappa = L/RA$$

10 To have fast and stable measurements of mediums with low conductivity, i.e. high resistance, it is convenient to have a cell with a small separation between electrodes, L , and a large area of overlap, A . This then can be used to define a cell constant (γ):

$$\gamma = L/A$$

15 The nanosensor according to the present invention is ideal for *in situ* conductivity measurements of low conductivity mediums such as pure water and air because the electrodes are very close together providing a very low cell constant, γ . The nanosensor increases the concentration of ions near the electrodes because of the functionalization of the nanopores and the
20 improvement of the hydrophilicity of the nanopores due to the functionalization and thus increases the sensitivity of the measurements. This is very important for conductivity analysis since it is usually done *in situ* in very low conductivity media such as pure water. As it is possible to functionalize the membrane nanopore walls with different chemicals in order to make the adsorption more
25 specific, it would be useful to have two sensors: one with positively charged functions more sensitivity to anions (such as poly(PPy-co-PPYCONH taurin) and another one with negatively charged functions more specific to cations (such as polyacrylic acid).

As a matter of fact, conductivity measurements may be made
30 continuously in real time or after a delay of immersion, depending for example on the resistivity of the solution to measure. If the solution is not conductive enough to perform an efficient analysis, a conductive electrolyte may be added into the solution.

Figure 17a) illustrates an ASV analysis of a nanosensor according to the
35 invention after immersion in 10 ml of a stirred 8.4 ppb ($\mu\text{g/L}$) Ni, 24.8 ppb Co, 23.8 ppb Pb, and 9.9 ppb Cu multi-ion ion solution for 1 hour. Figure 17b)

illustrates Square-wave ASV curves of a nanosensor according to the invention after immersion in 50 ml of a stirred Pb^{2+} ion solution for 30 minutes in different concentrations. The curve from a blank analysis is also plotted. Figure 17c) illustrates a Square wave ASV calibration curve for Pb^{2+} ions. Concentrations as low as 0.1 ppb (0.1 $\mu\text{g/L}$) have been repeatedly measured. Figure 17d) shows a log - charge plot of the calibration curve demonstrating Temkin isotherm adsorption behaviour of the PAA functionalised β -PVDF nanopores.

According to another embodiment of the present invention, the nanosensor comprises piezoelectric contacts. Piezoelectric effect arises from the application of an electric field to a poled (stretched) materials such as the PVDF polymer family: polyvinylidene fluoride PVDF, P(VDF-TrFE) or terpolymers of PVDF such as P(VDF-TrFE-CFE). The electric field causes length, tensile, thickness, and shear deformation. Ultrasonic transducers have been made with PVDF, designed according to the present invention, that operate at kHz frequency.

Ultrasonic agitation enhances the diffusion of solutions through membranes and enhances solution transport. This is used to improve the efficiency of filtration if the membranes are made of piezoelectric materials such as the PVDF family of polymers.

Sonic agitation can break up sediments and micelles and disassociate complexes and precipitates to release contaminants for stripping voltammetry. With piezoelectric effects incorporated into the nanosensor, its sensitivity is improved and it is more useful for *in situ* applications such as rivers and lakes where the pollutant ions are tied up in sediments, micelles, complexes and precipitates and in general improve solubility.

Sonic agitation cycle may be added to the stripping voltammetry protocol during the extraction of adsorbed ions from the membrane to the cathode surface and stopped for the anodic stripping cycle. This could shorten the extraction time and improving its efficiency. This could aid the cleaning of the membrane for reuse. Another sonic agitation cycle could be added to the beginning of the adsorption cycle in order to release trapped ions and then stopped in order to allow the ions to be adsorbed by the membrane.

The nanosensor as described on figure 18 contains nanopores area 3 localized to the centre by the use of masks during the irradiation of the piezoelectric polymers. The piezoelectric electrodes 22, 23 may be by sputtering

or evaporation through a mask but are not required to be very thin as the electrodes 4 and 5.

5 With the nanosensor according to the invention, specific charged molecules may functionalize nanopores. The nanosensor may be useful for the analysis of biological or medical samples or any of the analysis that is performed by stripping voltammetry such as those of screen-printed electrodes. The ability to configure the functionalized nanoporous PVDF membrane for in situ analysis makes it potentially useful in non liquid mediums such as air or exhaust. The
10 internal standard scheme could be used as a means for controlled dosing of ions.

Numerous variations and modifications will become apparent to those skilled in the art once the above disclosure is fully appreciated. It is intended
15 that the following claims be interpreted to embrace all such variations and modifications.

CLAIMS

1. Nanosensor for capturing charged molecules, the nanosensor comprising:
 - a functionalized radio grafted track-etched membrane (FRTEM) which
 - 5 contains polymer nanopores,
 - a first electrode arranged on a first side of the functionalized radio grafted track-etched membrane (FRTEM), the first electrode being designed to serve as a working electrode for an *in situ* stripping voltammetry technique,
 - a second electrode arranged on a second side of the functionalized radio
 - 10 grafted track-etched membrane (FRTEM), the second electrode being designed to serve as a counter electrode for an *in situ* stripping voltammetry technique.

2. Nanosensor according to claim 1, characterized in that it further comprises
- 15 a third electrode arranged on a side of the functionalized radio grafted track-etched membrane (FRTEM), the third electrode being designed to serve as a reference electrode for a stripping voltammetry technique.

3. Nanosensor according to claim 1 or 2, characterized in that walls of
- 20 nanopores are functionalized with one or a mixture of elements obtained from following groups:
 - poly-acrylic acid,
 - styrene,
 - taurin,
 - 25 - polyphosphine of R_3P type,
 - oxidized polyphosphine of $R_3P=O$ type.
 - other functional molecules known to specifically complex certain ions.

4. Nanosensor according to any of preceding claims, characterized in that
- 30 walls of nanopores further contain bismuth ions or silver ions or selenium ions or cobalt ions which constitute an internal standard.

5. Nanosensor according to any of preceding claims, characterized in that it further comprises a first and a second piezoelectric contacts respectively on
- 35 two opposite sides of the functionalized radio grafted track-etched membrane (FRTEM).

6. Method for detecting and quantifying charged molecules included in a fluid, said method comprising steps of:

- placing in the fluid a functionalized radio grafted track-etched membrane (FRTEM) which contains polymer nanopores; charged molecules being captured inside the polymer nanopores,
- applying an *in situ* stripping voltammetry analysis by using:
 - a working electrode arranged on one side of the functionalized radio grafted track-etched membrane (FRTEM),
 - a counter electrode arranged on a second side of the functionalized radio grafted track-etched membrane (FRTEM), and
 - a reference electrode arranged in the fluid.

7. Method according to claim 6, characterized in that the stripping voltammetry analysis is an anodic stripping voltammetry (ASV), a cathodic stripping voltammetry (CSV), or an Adsorptive Stripping Voltammetry (AdSV).

8. Method according to claim 6 or 7, characterized in that walls of nanopores further contain a predetermined concentration of bismuth or silver ions or selenium ions or cobalt ions which constitute an internal standard.

9. Method according to any of preceding claims 6-8, characterized in that voltages are applied on piezoelectric contacts disposed on the functionalized radio grafted track-etched membrane (FRTEM) in order to deform said functionalized radio grafted track-etched membrane (FRTEM) during capture of charged molecules and/or stripping voltammetry analysis; the functionalized radio grafted track-etched membrane (FRTEM) being designed from a poly(vinylidene fluoride) PVDF film which is radio grafted with vinyl monomer and chelating molecule leading to a polymerization of nanopores walls.

10. Method according to any of preceding claims 6-9, characterized in that the functionalized radio grafted track-etched membrane (FRTEM) is placed in a fluid in a bypass circuit.

11. Application of the nanosensor according to claims 1-5, as a sensor for in situ conductivity measurements.

12. Application according to claim 11, characterize in that two sensors are used, the first sensor including a functionalized radio grafted track-etched membrane (FRTEM) which is positively functionalized, preferably with a poly(PPy-co-PPYCONHtaurin), the second sensor including a functionalized radio grafted track-etched membrane (FRTEM) which is negatively functionalized, preferably with a poly(acrylic acid).

10

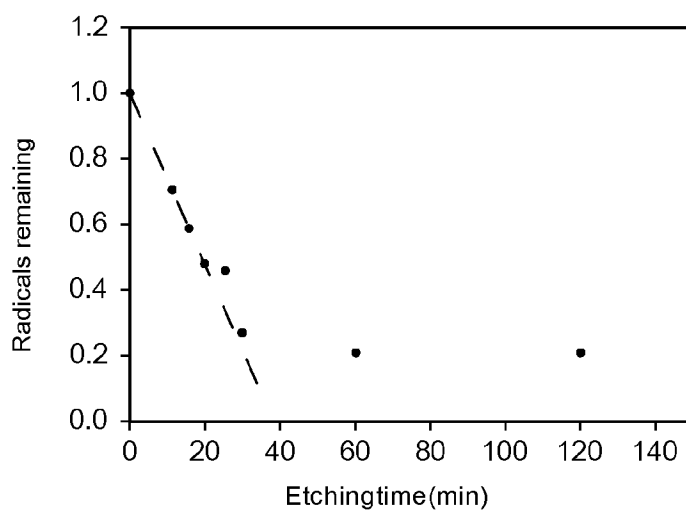
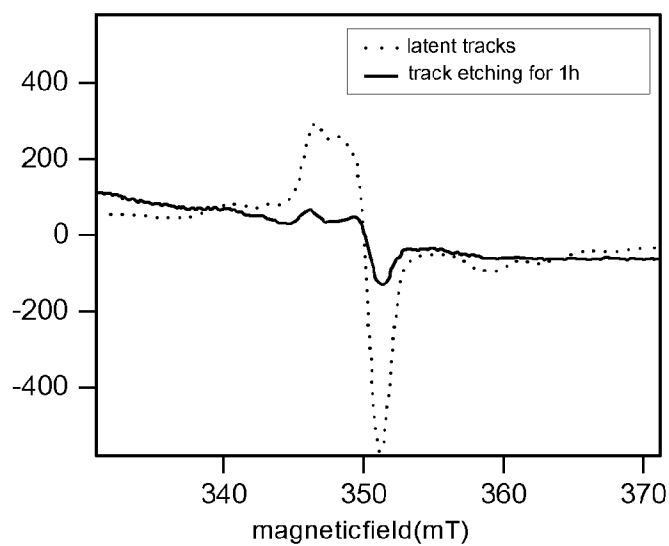
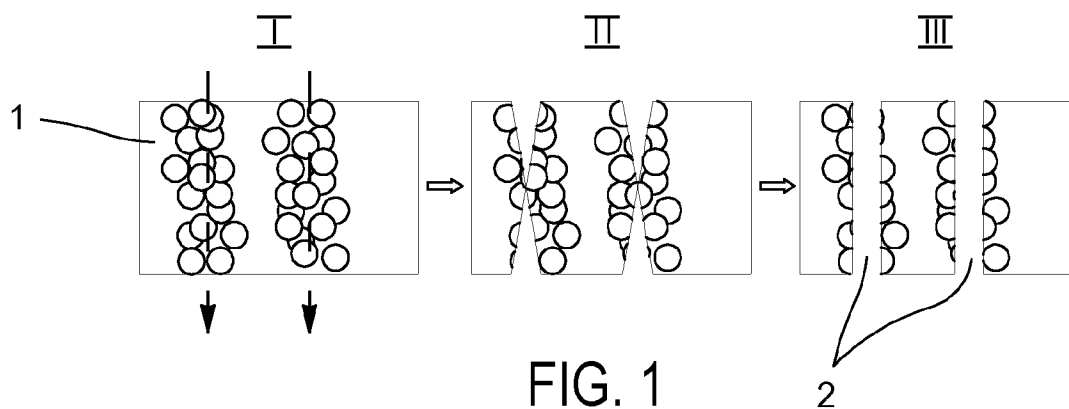
13. Application of the method according to claims 6-10, in detecting and quantifying charged molecules concentration of which is less than 5 ppb.

14. System for capturing charged molecules, the system comprising a processing unit equipped with several I/O plugs, each I/O plug comprising:

15

- a first connector to connect the working electrode,
- a second connector to connect the counter electrode, and
- a third connector to constitute a reference electrode in the fluid.

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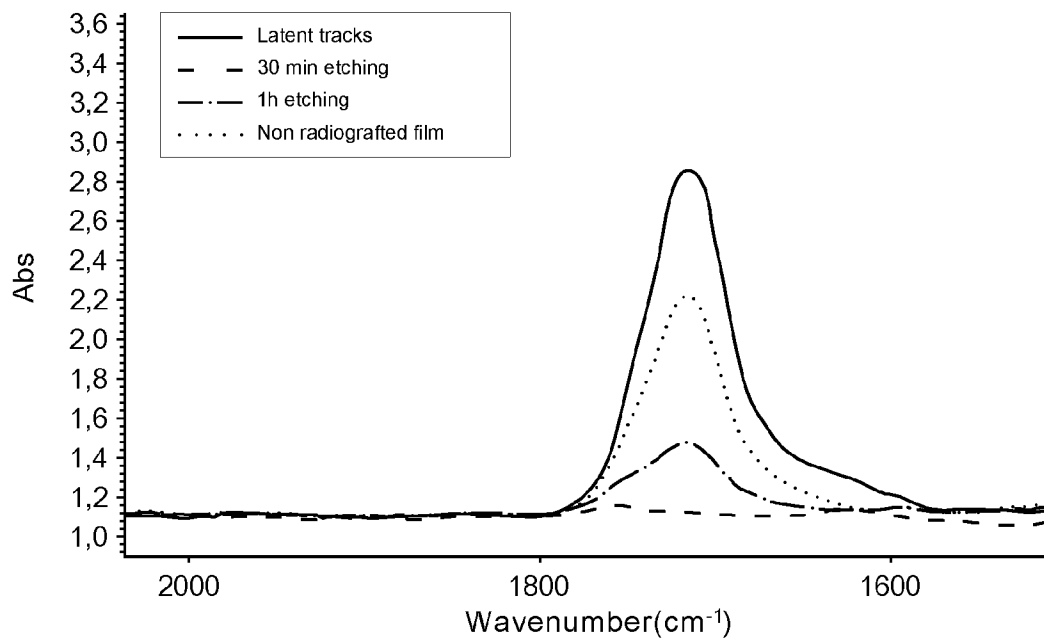


FIG. 4

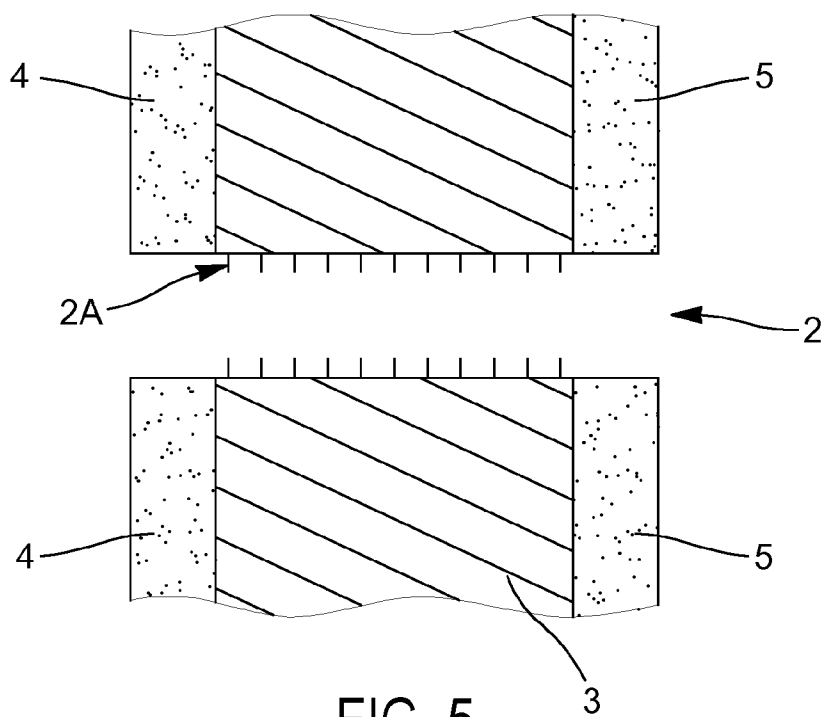


FIG. 5

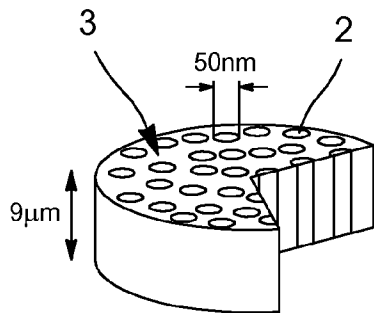


FIG.6

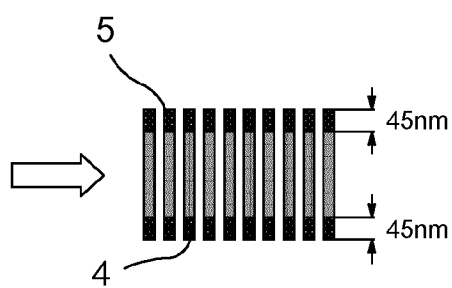


FIG. 7

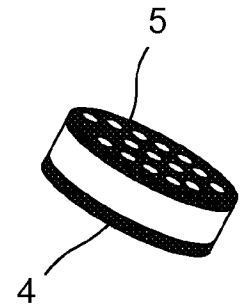


FIG. 8

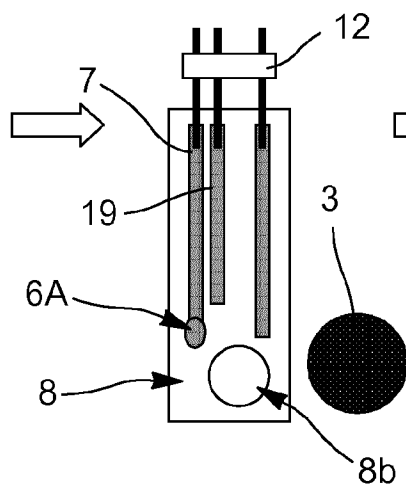


FIG. 9

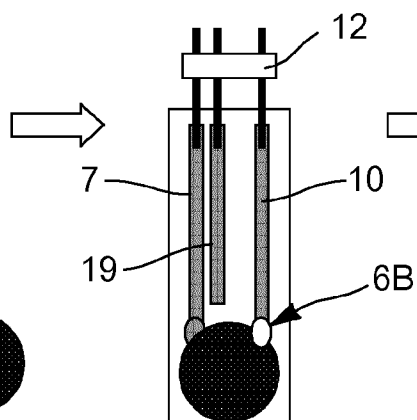


FIG. 10

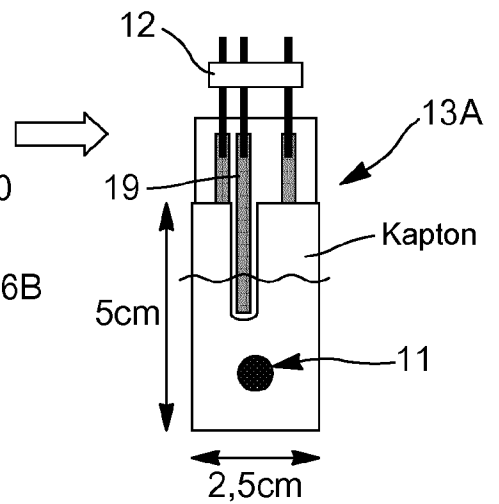


FIG.11

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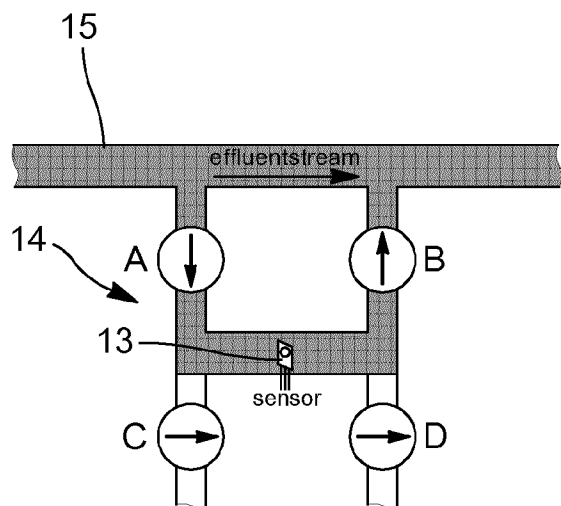


FIG. 12a

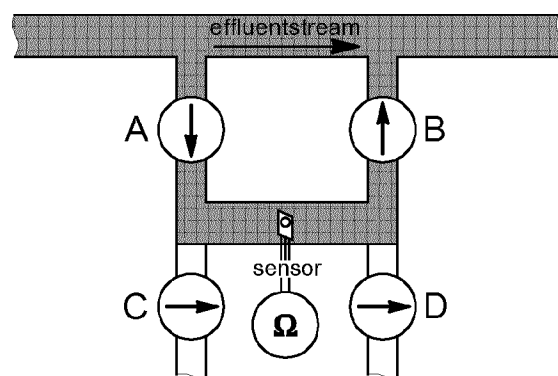


FIG. 12b

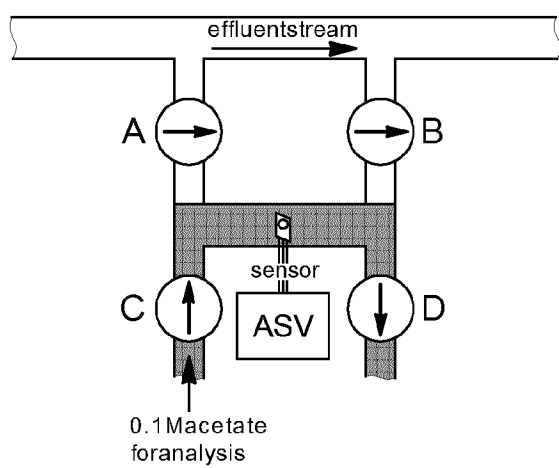


FIG. 12c

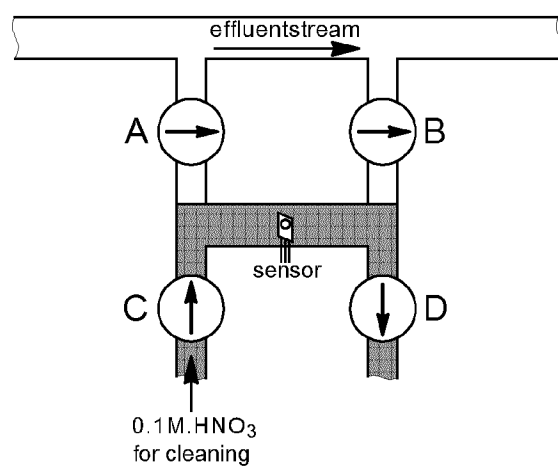


FIG. 12d

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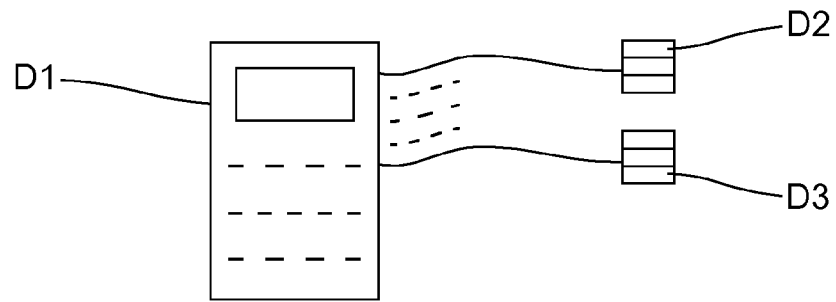


FIG. 13

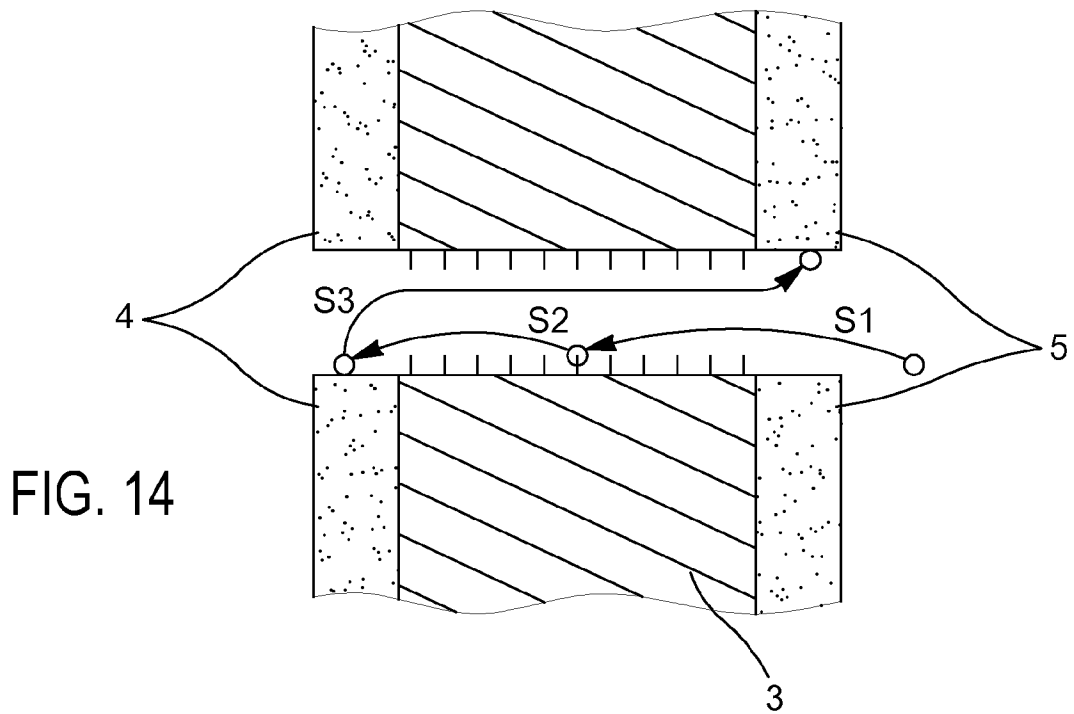


FIG. 14

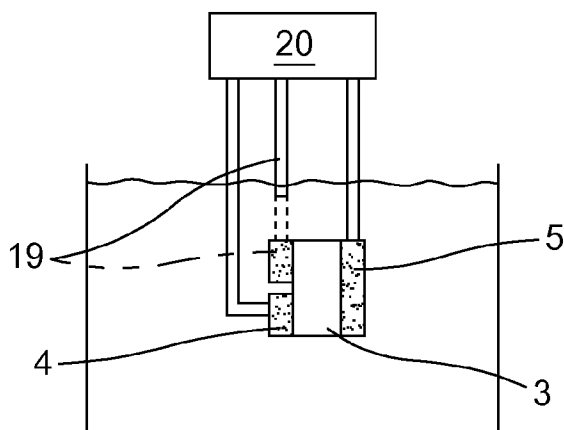


FIG. 15

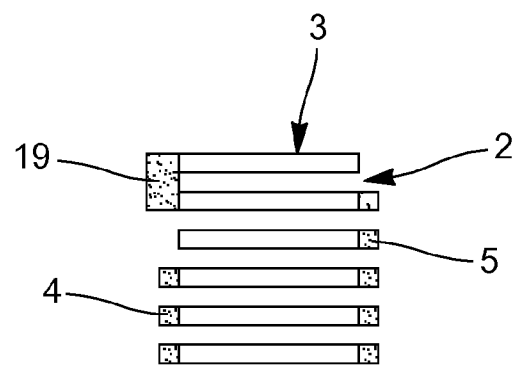


FIG. 16

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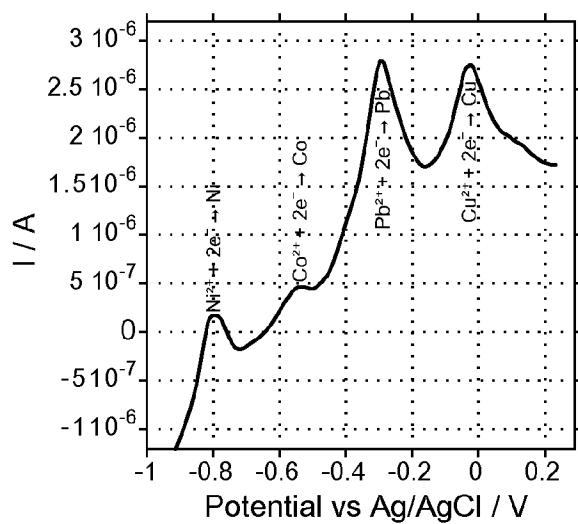


FIG. 17a

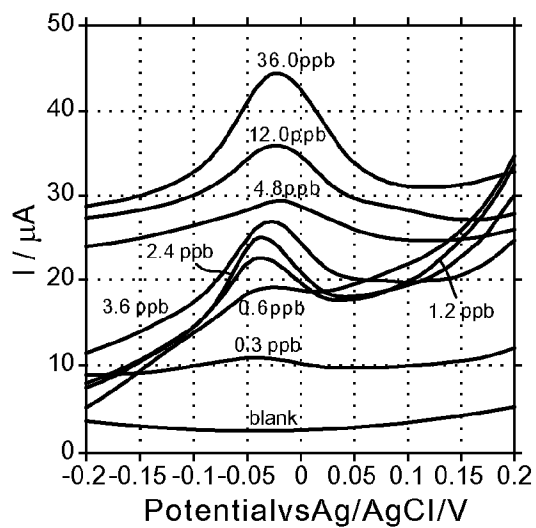


FIG. 17b

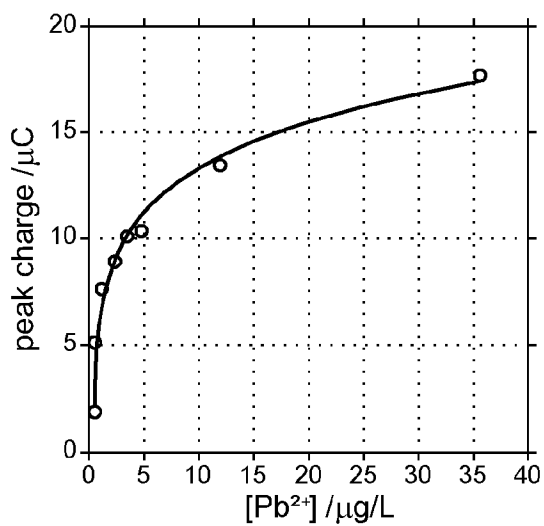


FIG. 17c

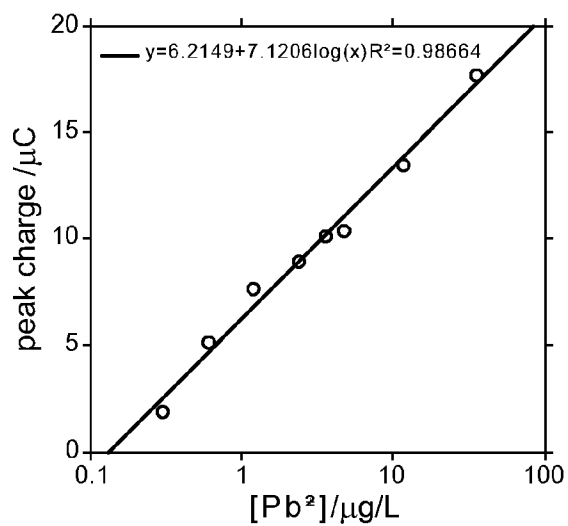


FIG. 17d

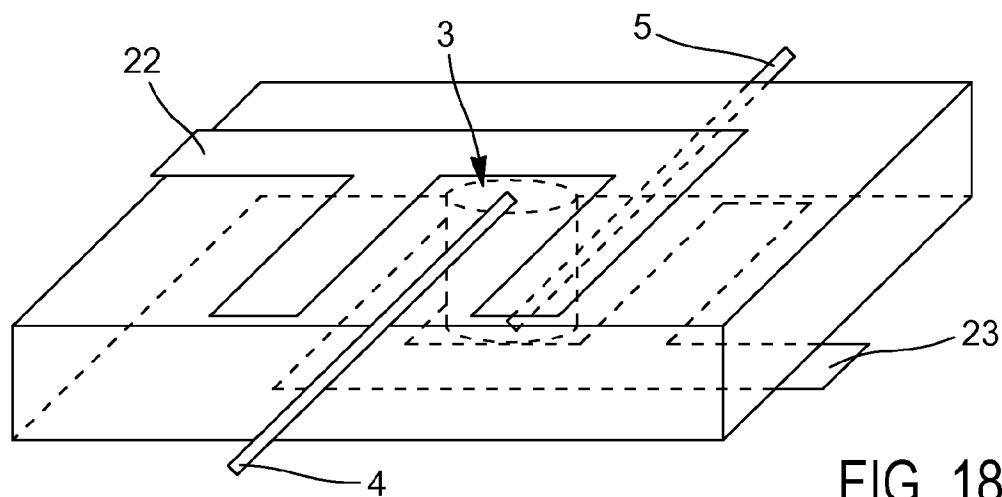


FIG. 18

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/051897

A. CLASSIFICATION OF SUBJECT MATTER

INV. B82Y30/00 G01N27/12 G01N27/48 G01N33/18
ADD.

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)

B82Y G01N

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)

EPO-Internal, BIOSIS, EMBASE, FSTA, INSPEC, MEDLINE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2 230 511 A1 (ECOLE POLYTECH [FR]; CENTRE NAT RECH SCIENT [FR]; COMMISSARIAT ENERGIE) 22 September 2010 (2010-09-22)	1-3
Y	abstract	6,7,
A	paragraph [0011]	10-14
	paragraphs [0014] - [0023]	4,5,8,9
	paragraphs [0030] - [0035]	
	claims 1-3	

X	EP 2 131 189 A1 (ECOLE POLYTECH [FR]; COMMISSARIAT ENERGIE ATOMIQUE [FR]; CENTRE NAT RE) 9 December 2009 (2009-12-09)	1-3
Y	abstract	6,7,
	paragraphs [0006] - [0010]	10-14
	paragraphs [0012], [0013]	
	paragraphs [0026], [0027]; figures 1-5	

	-/--	



Further documents are listed in the continuation of Box C.



See patent family annex.

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"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

"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

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"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

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Date of the actual completion of the international search

15 May 2012

Date of mailing of the international search report

23/05/2012

Name and mailing address of the ISA/

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Authorized officer

Michalitsch, Richard

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/051897

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	page 3, lines 10-20 page 3, last paragraph; figure 2 page 5, lines 23-34 page 9 - page 10, line 2 page 11, line 15 - page 12, line 17; example 1B page 14 - page 15, line 5; example 2 -----	6,7
X	CUSCITO ET AL: "Nanoporous beta-PVDF membranes with selectively functionalized pores", NUCLEAR INSTRUMENTS & METHODS IN PHYSICS RESEARCH, SECTION - B:BEAM INTERACTIONS WITH MATERIALS AND ATOMS, ELSEVIER, AMSTERDAM, NL, vol. 265, no. 1, 17 November 2007 (2007-11-17), pages 309-313, XP022349433, ISSN: 0168-583X	1-3
Y	Section: Results; page 309, right-hand column	6,7, 10-14
A	Section: Nanopores diameter; page 310 Section: Conclusion; page 312 -----	4,5,8,9
X	WO 2005/033685 A2 (EPIGEM LTD [GB]; HARVEY THOMAS GRIERSON [GB]) 14 April 2005 (2005-04-14) page 4, line 4 - page 5, line 2 page 6, lines 17-26 page 9, lines 4-14 page 10, lines 1-12 page 12, line 5 - page 14, line 2 -----	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2012/051897

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