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(54) **Title**: ELECTROCHEMICAL FLOW CELL DETECTOR

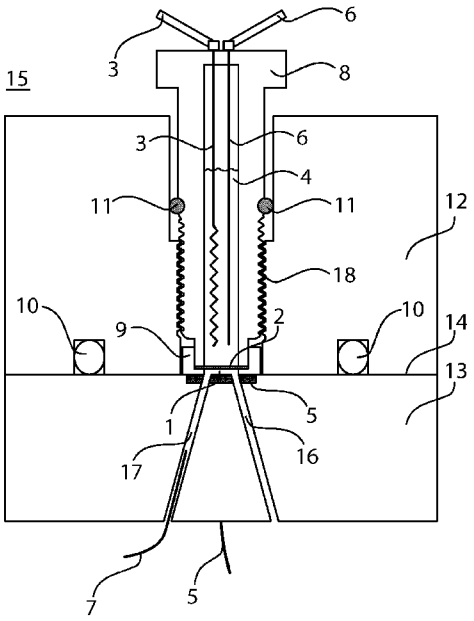

Fig. 2

(57) **Abstract**: Electrochemical Flow Cell Detector comprising a chamber (1) configured to receive a first electrolyte solution; a membrane (2) comprising a second electrolyte in contact with the chamber, and in contact with the first electrolyte solution when present in the chamber; a first electrode (3) in fluid contact with the second electrolyte solution through a third electrolyte solution (4); a second electrode (5) in contact with the chamber; wherein the second electrode (5) forms part of the chamber walls, and is not in direct contact with the membrane (2).

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Electrochemical Flow Cell Detector

Field of the invention

The present invention relates to ion transfer across the Interface between Two Immiscible Electrolyte Solutions (ITIES), and more specifically to measurements of the electric current associated with the ion transfer across ITIES as well as different arrangements of cell volume, electrodes, and the two immiscible electrolyte solutions.

In particular, the present invention relates to devices for, and methods for measuring the electric current associated with the ion transfer across ITIES wherein the electrodes, the two immiscible electrolyte solutions, and cell volume is arranged in an advantageous manner, as well as to a novel electrolyte-containing membrane for use in said flow cell.

Background of the invention

Ion transfer amperometry, such as amperometry at the Interface between Two Immiscible Electrolyte Solutions (typically an organic electrolyte solution and an aqueous electrolyte solution) is quite different from direct amperometry used widely in electrochemical detection of redox species, as it is not dependent of the analyte being a redox species. A further advantage of ion transfer amperometry over classical potentiometric sensors is that the ion selectivity of the former can be tuned by altering the magnitude of the applied potential.

For related state of the art, reference is made to Lee, HJ; Pereira, CM; Silva, AF, Girault, HH (2000): Pulse Amperometric Detection of Salt Concentrations by Flow Injection Analysis Using Ionodes, *Anal. Chem.* **72**, 5562-5566 ("Lee *et al.*") and Sánchez-Pedreño, C; Ortuño, JA; Hernández, J (2002): Chronocoulometric flow-injection analysis with solvent polymeric membrane ion sensors, *Anal. Chim. Acta* **459**, 11-17 ("Sánchez-Pedreño *et al.*").

A device of this description may find use in an electrochemical flow cell detection system. An optimally designed and versatile flow cell of a high sensitivity flow cell detection system requires an optimized, *e.g.* minimized cell volume, reduced distance between reference electrodes, laminar flow, and minimized baseline drift. Furthermore, in order for the flow cell to be versatile and adaptable to varying flows, the cell volume should also be adjustable (wide dynamic range) and at the same time maintain the sensitivity and/or reproducibility to the extent possible.

For making a more simplified device and extending the analytical applications of such an ITIES device, stabilisation of the interface by solidifying or gelifying one side of the interface have been made, as is shown in particular in the initially cited Sánchez-Pedreño *et al*.

Reducing the distance between reference electrodes, *i.e.* reducing the distance between the reference electrode in the aqueous phase and the reference electrode in the organic phase, especially in flow cells of adjustable volume, may generally be made by inserting the aqueous reference electrode, *e.g.* an Ag/AgCl anodized silver wire, at the inlet of the flow cell as near as possible to the interface. However, this way of reducing the distance between reference electrodes is difficult to reproduce, and inappropriate because if the anodized silver wire accidentally touches the interface it would result in a short-circuit, which would damage the equipment connected to the two electrodes such as expensive potentiostats. A silver wire placed in the flow cell at the inlet will further perturb the flow, and result in a less laminar flow compared to no silver wire inserted. An Ag/AgCl anodized silver wire only has a small area exposed, and will be more susceptible to fouling and removal of the AgCl layer resulting in lack of reproducibility and increased baseline drift. Further, a relatively small silver wire will not maintain a constant potential if an analytically relevant current is passed through it.

This way of reducing the distance between reference electrodes is shown in particular in the initially cited Lee *et al*. that also suggest enhancing sensitivity by reducing interfering ionic species present in the eluent.

Summary of the invention

The object underlying the present invention is to provide a remedy for the problem described above. The solution to the problem is to provide a device comprising a chamber (1) configured to receive a first electrolyte solution; a membrane (2) comprising a second electrolyte solution in contact with the chamber, and in contact with the first electrolyte solution when present in the chamber; a first electrode (3) in fluid contact with the second electrolyte solution, optionally through a third electrolyte solution (4); a second electrode (5) in fluid contact with the chamber (1); wherein the first and third electrolyte (4) solution are immiscible with the second electrolyte solution and the second electrode (5) forms part of the chamber walls, and is not in direct contact with the membrane (2).

In an embodiment of the invention the device further comprises at least a third and/or a fourth electrode, wherein the third electrode (6) is in fluid contact with the second electrolyte solution optionally through a third electrolyte solution (4); and the fourth electrode (7) is in fluid contact with the chamber (1).

In an embodiment of the invention the device is a flow cell.

In an embodiment of the invention the chamber receives the first electrolyte solution through at least one inlet (16), and said first electrolyte solution exits the cell through at least one outlet (17).

In an embodiment of the invention the fourth electrode (7) is arranged to not perturb the flow of the first electrolyte in the chamber.

In an embodiment of the invention the fourth electrode (7) forms part of the walls in either the inlet or outlet of the chamber (1); or is placed in the inlet or outlet as near as possible to the chamber (1) thereby maintaining a laminar flow.

In an embodiment of the invention the device is adapted to/for measuring the electric current caused by an ion transfer across the Interface between Two Immiscible Electrolyte solutions (ITIES).

In an embodiment of the invention the device further comprising a detector electrically coupled to at least one of the first electrode (3), second electrode (5), third electrode (6) and fourth electrode (7).

In an embodiment of the invention the detector is an electrochemical detector, *e.g.* a potentiostat, a galvanostat or a current follower.

In an embodiment of the invention the chamber (1) has a volume smaller than 300 μl, *e.g.* smaller than 100 μl and smaller than 20 μl.

In an embodiment of the invention the volume of the chamber (1) may be adjusted, e.g. within the range from 1 μl to 300 μl, from 1 μl to 100 μl and from 1 μl to 20 μl.

In an embodiment of the invention the chamber is constructed to minimize the distance from the membrane (2) to the second electrode (5).

In an embodiment of the invention the membrane (2) and the second electrode (5) are placed on opposing sides of the chamber (1).

In an embodiment of the invention the combined area of the membrane exposed to the fluid in the chamber (1) and the part of the second electrode

(5) that is exposed to the fluid in the chamber (1) is larger than the area of the other sides of the chamber.

In an embodiment of the invention the part of the second electrode (5) that is exposed to the fluid in the chamber is from 10 % of the area of the membrane to the area of the membrane exposed to the fluid in the chamber.

In an embodiment of the invention the area of the membrane exposed to the fluid in the chamber (1) divided by the part of the second electrode (5) that is exposed to the fluid in the chamber (1) is between 0.1 to 10, e.g. between 0.8 to 1.2.

In an embodiment of the invention the volume of the chamber (1) divided by the part of the second electrode (5) that is exposed to the chamber (1) is between 0.001 cm to 6.0 cm.

In an embodiment of the invention at least one of said at least one inlet and/or said at least one outlet traverses the second electrode (5).

In an embodiment of the invention the device is shielded from at least one of the following external influence: Electrical fields and electromagnetic radiation, e.g. by enclosing the device in a Faraday cage; and mechanical shock.

In an embodiment of the invention each of the electrodes 3 and 5 is made out of an insoluble Ag salt (e.g. Ag_2SO_4, AgCl, and AgBr) plated on Ag, and each of the electrodes 6 and 7 are made out of conductive material, e.g. gold, platinum, silver, boron doped diamond, carbon and steel.

Another aspect of the invention is a method for preparing a sample comprising a concentrated ion from a first electrolyte solution comprising the

ion in a lower concentration, the steps comprising: introducing the first electrolyte solution to a device according to any one of the above embodiments; applying a constant potential suitable for the ion to transfer across the interface between the first electrolyte solution and the second electrolyte solution to obtain a sample comprising the concentrated ion from the first electrolyte solution; optionally reversing the constant potential to release the concentrated sample comprising the ion from the second electrolyte solution.

Another aspect of the invention is a method for measuring a ion concentration of one or more charged compounds, which comprises passing said compounds in solution through an electrochemical flow cell according to any one of the above embodiments, and then to a device that provides chemical structure information.

In an embodiment of the invention the one or more charged compounds are selected from the group consisting of: alanine, leucine, acetylcholine, choline and histamine.

In an embodiment of the invention the signal-to-noise ratio is at least 3 when the ion concentration of the particular charged compound being detected is 5 nM.

Brief description of the drawings

Certain illustrative embodiments are described in more detail below with reference to the accompanying figures in which:

Figure 1 is a schematic of a two to four electrode device, in accordance with the invention.

Figure 2 is a more detailed schematic of a four electrode device having a top part (8) and a bottom part (formed by parts 12 and 13), an inlet (16) and outlet (17) and a small chamber (1) that may be varied in volume by turning the upper part (8), in accordance with certain embodiments.

Figure 3 is another schematic of a four electrode device having an inlet (16) and two outlets (17), in accordance with certain embodiments.

Figure 4 is another schematic of a two to four electrode device wherein one of the electrodes (7) is embedded in the walls of the outlet (17) of the cell, and further the volume of the chamber (1) is modified by unscrewing upper part (8) from the bottom part in such a way that the bottom surface of 9 is no longer in contact with surface 14, in accordance with certain embodiments.

Figure 5 shows a top part (8) with membrane (2) and a chamber (1) modifying part (9) that also serves to keep the disc (8) in place, in accordance with certain embodiments. The top part (8) can be used according to certain embodiments of fig 1-4.

Figure 6 shows an assembled top part comprising the top part (8), disc (2) and chamber modifying part (9), in accordance with certain embodiments.

Figure 7 shows different embodiments of the chamber modifying part (9). Fig. 7a results in a cylindrical chamber (1); fig. 7b in a elliptical chamber (1); and fig. 7c results in a rectangular chamber (1) when the bottom surface of 9 is in contact with surface 14, and in accordance with certain embodiments.

Figure 8 shows how different embodiments of the chamber modifying part (9) resulting in different chamber volumes (1) by changing the length of surface (21) from the disc (2) to the hashed line (20) representing the imaginary surface 14, as well by changing the area of the disc (2) exposed to the

chamber (1). Certain illustrative embodiments are shown in fig. 8a-c, and an illustrative embodiment of the chamber modifying part with an O-ring (19) is also shown.

Figure 9 illustrates the principle of pulse amperometry, and depicts a cyclic voltammogram. The upper voltammogram is recorded for the TEA+ ion transfer across the water/plasticized oil phase interface in the electrochemical flow cell described in figure 2 and example 4.

Figure 10 shows a pulse amperogram obtained for the flow injection analysis of tetraethylammonium chloride as described in example 4. Flow rate 0.6 ml/min, injection volume 100 µl.

Figure 11 is based on figure 10 and shows the corresponding current changes versus concentration of TEA^+.

Figure 12 shows a pulse amperogram obtained for the flow injection analysis of tetraethylammonium chloride as described in example 3. Flow rate 0.6 ml/min, injection volume 100 µl. The concentrations 1, 10, 100, 1000, 10000 nM are tested twice.

Figure 13 shows the chamber (1) with inlet (16) and outlet (17), and flow lines showing the 3D simulated flow. Flow rate 0.6 ml/min.

It will be recognized by the person of ordinary skill in the art, given the benefit of this disclosure that certain features shown in figures 1-8 are not necessarily drawn to scale. The dimensions and characteristics of some features in the figures may have been enlarged, distorted or altered relative to other features in the figures to facilitate a better understanding of the illustrative examples disclosed herein.

It will further be recognized by the person of ordinary skill in the art that the individual features of the figures may be interchanged to obtain further embodiments.

Detailed description of the invention

In describing the embodiments of the invention specific terminology will be resorted to for the sake of clarity. However, the invention is not intended to be limited to the specific terms so selected, and it is understood that each specific term includes all technical equivalents which operate in a similar manner to accomplish a similar purpose.

It will be recognized by the person of ordinary skill in the art, given the benefit of this disclosure that the devices and methods herein represent a significant development in devices and methods for detecting analyte with high sensitivity. Devices configured for detection of analytes can be produced, for example, at low cost, with high reproducibility and for use as point of care devices.

The devices and methods disclosed herein may be configured to detect one or more analytes, such as biomarkers in a body fluid. The device may be configured in cartridge form with an “on-board” detector such that it may be used without any additional equipment or devices, or it may be configured to interface with other devices or equipment such as, for example electrochemical detectors or light absorption or emission detectors, mass and NMR spectrometers. In some examples, the devices may be configured such that indicia are provided if the level of biomarker exceeds a threshold value. Such indicia include, but are not limited to, switching on of a light, beeping, flashing lights or the like. In other examples the device may output the level of the analyte/biomarker. In yet other examples, the device may be configured such that no result is provided unless the level of analyte such as

a biomarker in a body fluid exceeds a threshold value. Additional advantages and configurations of the device are discussed below.

Fig. 1 shows a schematic of a two to four electrode device, in accordance with certain embodiments of the invention. The device comprises a chamber (1) configured to receive a first electrolyte solution. In some embodiments the first electrolyte solution is an aqueous solution comprising an analyte. The device further comprises a membrane (2) in contact with the chamber, and in contact with the first electrolyte solution, when the electrolyte solution is present in the chamber (1). In some embodiments the membrane comprises a gelified or solidified organic phase as the second electrolyte solution, such as for example the one described in example 1 and 2 and serves as a miniaturisation of the second electrolyte phase. Please note that a gelified or solidified organic phase is not a solution *per se*. The use of the word solution has nevertheless been used to describe a phase comprising an organic gelified/solidified electrolyte. The first and second electrolyte solutions may be any electrolytes, as long as they are substantially immiscible in order to measure ion transfer across the Interface between Two Immiscible Electrolyte solutions (ITIES). Typically the first and second electrolytes are an aqueous and a non-aqueous solution. In order to measure ion transfer across the ITIES, at least one electrode (3) should be in fluid contact with the second electrolyte solution, either through direct contact with the second electrolyte solution, or optionally through a third electrolyte solution (4), whose purpose is to improve contact between the electrode (3) and the second electrolyte solution, and a second electrode (5) should be in fluid contact with the first electrolyte in the chamber (1). The second electrode (5) forms part of the chamber walls, and is not in direct contact with the membrane (2). When measuring ITIES the second electrode (5) is a reference electrode with a well defined potential, such as *e.g.* anodized Ag/AgCl. The first electrode (3) is also a reference electrode with a well defined potential, such as *e.g.* anodized Ag/AgCl. A potential can be applied

over the first and second reference electrode, and the current may be measured using the same set of electrodes.

The concept of how to measure ion transfer across the Interface between Two Immiscible Electrolyte solutions (ITIES) are well known to the skilled person. Reference is made to both the aforementioned two publications, Lee *et al*. and Sánchez-Pedreño *et al*., and to Vanysek, P; and Ramírez, LB (2008): Interface Between Two Immiscible Liquid Electrolytes: A Review, *J. Chil. Chem. Soc.* **53(2)**, 1455-1463 that are hereby incorporated by reference with respects to the concept of how to measure ion transfer across the ITIES.

The device may also advantageously further comprise at least a third and/or a fourth electrode, wherein the third electrode (6) is in fluid contact with the second electrolyte solution optionally through a third electrolyte solution (4); and the fourth electrode (7) is in fluid contact with the chamber (1). Such 3 and 4 electrode devices are well-known to the person skilled in the art.

The device may be made out of any non-conducting/insulating material, such as for example poly(methyl methacrylate) (PMMA), and polyaryletheretherketone (PEEK).

The chamber may take any shape or size. However, shapes and/or sizes that promote laminar flow are preferred. Laminar flow as opposed to turbulent flow means that the flow is regular, and the fluid simply moves in layers (possibly with different velocities). Laminar flows are characterised by low Reynolds numbers (*e.g*. less than 2000). The Reynolds number *Re* is a dimensionless parameter characterising the fluid flow in a pipe, given by the relation

$$Re = \frac{\rho \bar{v} d}{\mu}$$

where d is the diameter of the flow channel, ρ is density, μ is the dynamic viscosity and $\bar{v}$ is the average velocity.

In general the Reynolds number is the ratio of inertial forces to initial forces (different geometries have different equations to determine the absolute value). A more general definition, which requires the knowledge of local fluid velocities, is:

$$Re = (\rho u^2) / (\mu u / L) = \rho u L / \mu = u L / v$$

L is a “characteristic length”, u the fluid velocity and v the kinematic viscosity.

In the detection chamber (1) the Re is estimated to be lower as the flow path is widened. When determining the Reynolds number, the following equation is used

$$Re = \frac{\rho \bar{v} d}{\mu}$$

Unless it is obvious to the skilled person that the Reynolds number, *i.a.* the ratio of inertial forces to initial forces could be determined with higher accuracy with a different equation.

Shapes that promote laminar flow are characterised by low Reynolds numbers. In some embodiments the chamber has a shape resulting in a Reynolds number lower than 100, e.g. lower than 50. The actual design described in Fig. 2 has a Reynolds number lower than 30 for a flow rate of 0.6 ml/min. In some embodiments the chamber has the following general shape: Cylinderical, truncated cone, half a cylinder, square box, rectangular box, elliptical cylinder, and shapes derived thereof. These shapes are simple to generate, and typically results in a low Reynolds number, *e.g.* a Reynolds number lower than 100.

The chamber (1) is configured to receive a first electrolyte solution. This may be through one or more inlets and/or outlets to allow fluid to enter and leave the chamber. When the device is used as a flow cell it typically comprises at least one inlet and one outlet to allow fluid to enter and leave the chamber simultaneously.

The fluid received by the chamber is called the first electrolyte solution.

The first electrolyte solution comprises the analyte, which carries a net charge at the pH of the first electrolyte solution, when the device is operated as an ITIES device. Examples of charged analytes that may be detected using the device are for example the class of local anaesthetics (*e.g.* Procaine, Tetracaine, Oxybuprocaine, Prilocaine, Mepivacaine, Lidocaine, Bupivacaine, Dibucaine, Benzocaine, Cocaine), β-blockers (*e.g.* Acebutolol, Alprenolol, Atenolol, Bisoprolol, Carazolol, Carteolol, Carvedilol, Clonidine, Metipranolol, Metoprolol, Oxprenolol, Papaverine, Penbutolol, Pindolol, Propranolol, Sotalol, Timolol), Cationic drugs/compounds such as quaternary ammonium drugs/compounds (*e.g.* Acetylcholine, S-Butyrylthiocholine, Carbamoylcholine, 1-Ethylquinoline, Homidium, N-Methylderamciclane, Methylhomatropine, Methylquinidine, 14-Methylrutecarpine, Neostigmine, Propantheline, Pyridostigmine, Tetra-N-butylammonium, Tetra-N-ethylammonium, Tetra-N-methylammonium, Tetra-N-penthylammonium, Tetra-N-propylammonium, Trantheline), anionic drugs/compounds (*e.g.* Phenol, 2-Nitrophenol, 3-Nitrophenol, 4-Nitrophenol, 2,4-Dinitrophenol, 2,5-Dinitrophenol, 7-Isoxicam, 4-Bromobenzoic acid, 4-Chlorobenzoic acid, 3-Chlorobenzoic acid, 4-Iodobenzoic acid, 1-Naphtoic acid, Ketoprofen, Suprofen, Naproxen, Piroprofen, Flurbiprofen, Ibuprofen, Carprofen, Indomethacin, Sulindac sulfide, Sulindac, Sulindac sulfone, Phenylbutazone, Sulfinpyrazone Sulfide, Sulfinepyrazone, Sulfinepyrazone Sulfone), Zwitterionic, monobasic and dibasic drugs/compounds (*e.g.* Azapropazone, Cetirizine, Labetalol, Tenoxicam, Isoxicam, Raclopride,

Eticlopride, Piroxicam, Hydroxyzine, Amferpramone, N-methylephedrine, 3,5,N,N-tetra-methyl aniline, N,N-diethylaniline, Trimetazidine, Quinine, N-(p-methylbenzyl)hexylamine, Lauric acid, Pyridine, Diclofenac, Nicotine, Hydrazine, Phenylalanine, Paraquat, Ethambutol, Fexofenadine, Cephaloridine, and all amino acids, and peptides).

The membrane (2) forms the interface between the second electrolyte and the first electrolyte. In some embodiments the membrane may be prepared on a solid support, such as PVDF, PVC or polypropylene (PP). The membrane comprises the second electrolyte that may be gelified or solidified. One example of gelifying the second electrolyte has been described in example 1. However, the skilled person will appreciate that there are numerous ways of gelifying and/or solidifying a second electrolyte. In some embodiments the solid support as described above is not present. In these embodiments the membrane consists of the gelified or solidified second electrolyte solution. The membrane may, *e.g.* be prepared on a glass template.

An electrolyte solution is any substance comprising free ions that behaves as an electrically conductive medium.

The second electrolyte solution should be substantially immiscible with the first electrolyte solution when the device is operated as an ITIES device. Substantial immiscible may be understood as being unable to mix to form a homogeneous mixture, such as the well-known immiscible water and oil. Substantial immiscible liquids are unable to mix and will eventually (at equilibrium) distribute in layers or distinct phases. Examples of substantially immiscible first and second electrolyte solutions may be combinations of the following solvent pairs comprising free ions: nitrobenzene:water; o-nitrophenyl octyl ether (NPOE):water; 1,2-dichloroethane:water; 1,6-dichlorohexane:water; 2-octanone:water.

Examples of free ions comprised in the electrolyte solutions are: Phosphate buffered saline in water comprising the analyte as the first electrolyte solution and hexadecyl-trimethylammonium tetrakis(pentafluorophenyl) borate (HTMATPFB) or Tetrabutyl-ammonium tetraphenylborate (TBATPB) in NPOE as the second electrolyte solution. Examples of the third electrolyte solution that facilitates contact between the electrode (3) and/or (6) and the second electrolyte solution may be *e.g.* water comprising hexadecyl-trimethylammonium bromide (HTMABr) or tetrabutylammonium chloride (TBACl). The salts in the second and third electrolyte solutions are selected so that the interface between the second and third electrolyte solution appears non-polarisable. This may be accomplished by having a common ion in the second and third electrolyte solution.

In the context of this description there are two types of electrodes.
Counter electrodes, which are typically used to measure the current, may be made out of any conductive material e.g. gold, platinum, silver, boron doped diamond (BDD), stainless steel, *etc.*
Reference electrodes are well-known to the skilled person, and are made out of a conductive material with a well defined reference potential. The well defined reference potential is necessary to apply a correct potential across two reference electrodes. The fact that reference electrodes maintains a constant well-defined potential further ensures that the change in applied potential effectively takes place between the first and second electrolyte solution. Reference electrodes may for example be an anodized metal, such as for example insoluble Ag salt (*e.g.* Ag_2SO_4, AgCl, and AgBr) plated on Ag, Fe_2O_3 plated on Fe, or a Pd/H electrode.

When the device is operated in 2-electrode mode, the first electrode (3) in contact with the second electrolyte solution, and the second electrode (5) in fluid contact with the chamber (1) acts both as reference electrodes and as

counter electrodes meaning that a potential is applied over the two electrodes, and the resulting current is measured between the same two electrodes (3) and (5). The electrodes (3) and (5) are preferably made out of a conductive material with a well defined reference potential. The third (6) and fourth electrode (7) as shown in the figures may be absent or present. However, the third and fourth electrode are inactive meaning that they do not form a circuit. If present the third (6) and fourth electrode (7) may also be short-circuited with the corresponding first (3) and second electrodes (5).

It is important that both electrode 3 and 5 maintains stable potentials, *i.e.* do not change potential to any practical extent as a result of an analytically relevant current passing through the electrode, otherwise the baseline will drift, and analytical data be difficult to obtain reproducibly.

In typical electrode electrochemistry a 3-electrode potentiostat is used, having a working, a counter and a reference electrode. In principle, two electrodes are needed; the 3-electrode setup allows the reference electrode not to pass any current and therefore maintain a stable reference potential, and the current is supplied by the counter (or auxiliary) electrode. Such setup allows compensating for the resistance of the solution. In electrochemistry at ITIES two electrodes, preferably reference electrodes are needed as the potential differences between two liquid phases have to be controlled. Therefore a special potentiostat is used, which has input for 2 reference electrodes, rather than for one. Additionally, two counter electrodes are used. A number of commercial potentiostats allow this connection either directly or after some modifications.

There are two ways of operating the device in 3-electrode mode.

Method 1: The first electrode (3) in contact with the second electrolyte solution, and the second electrode (5) in fluid contact with the chamber acts as reference electrodes, and a potential is applied across these two

electrodes. The third electrode (6) is present (and the fourth electrode (7) is either absent or inactivated), and the resulting current is measured between the third electrode (6) and the second electrode (5). Example 3 describes how to operate the device in 3-electrode mode using method 1.

Method 2: The first electrode (3) in contact with the second electrolyte solution, and the second electrode (5) in fluid contact with the chamber acts as reference electrodes, and a potential is applied across these two electrodes. The fourth electrode (7) is present (and the third electrode (6) is either absent or inactivated), and the resulting current is measured between the fourth electrode (6) and the first electrode (3).

When using 3-electrode mode, such as *e.g.* method 1 or 2, it is important that the references electrodes through which the current is passed (electrode 5 in method 1 and electrode 3 in method 2) maintains a stable potential, *i.e.* do not change potential to any practical extent as a result of current passing through the electrode, as this will give rise to baseline drift as explained above. This may be accomplished in practice by electrode 3 or 5 having a sufficiently large area compared to the membrane (2).

When the device is operated in 4-electrode mode, the first electrode (3) in contact with the second electrolyte solution, and the second electrode (5) in fluid contact with the chamber acts as reference electrodes, and a potential is applied across these two electrodes, while the resulting current is measured between the third (6) and fourth electrode (7) acting as counter electrodes. Example 3 describes how to operate the device in 4-electrode mode.

The advantage of operating the device in 4-electrode mode is that the current is passed through the counter electrodes (6) and (7), and not through one or both of the reference electrodes (3) and (5). The reason being that passing the current through one or both of the reference electrodes (3) and (5) results in redox reactions taking place at the reference electrode, *e.g.* AgCl/Ag.

These redox reactions ultimately destroy the reference electrode, e.g. by depleting the AgCl layer on the Ag/AgCl reference electrode. This leads to an unstable and non-controllable applied potential.

The second electrode (5) forms part of the chamber walls. By "forming part" is meant that it should be part of the surface of the chamber and follow the shape of the chamber. One way of making the second electrode part of the chamber walls are to *e.g.* embed the electrode in the chamber walls, or *e.g.* to coat part of the chamber walls with the electrode.

It is essential that the second electrode (5) forms part of the chamber walls, as it allows the placement of the two reference electrodes (3) and (5) close to each other while maintaining laminar flow, which results in a higher sensitivity of the device. The first electrode is placed as close to the membrane (2) comprising the second electrolyte solution. This advantage and other advantages resulting from the second electrode (5) forming part of the chamber walls will be described in more detail in relation to some embodiments of the invention.

The second electrode (5) must not be in direct contact with the membrane (2) comprising the second electrolyte solution, as this would create a short-circuit and/or give rise to inaccurate measurements. By the second electrode (5) not being in direct contact with the membrane (2) comprising the second electrolyte solution is meant that the second electrode (5) should be insulated from the second electrolyte solution, and/or insulated from the membrane (2) comprising the second electrolyte solution.

In some embodiments of the invention the device is a flow cell. As opposed to a stagnant system, where no flow is present in the chamber (1), a flow cell further comprises an inlet and an outlet to accommodate flow. This inlet and outlet may be made to be compatible with commercially available connectors

and fittings, known to the skilled person, such as e.g. luer lock, or HPLC fittings, PEEK fittings. The device being a flow cell allows for it to be part of hyphenated techniques, and for example serve as a detector for a LC system.

In accordance with certain examples, the devices and systems disclosed herein may be hyphenated in serial or parallel to one or more additional devices. These additional devices include, but are not limited to, liquid chromatographs such as those commercially available from Waters Corp. (Milford, MA), Thermo Fisher Scientific, Inc. (Waltham, MA), Shimadzu (Japan), Agilent Technologies, Inc. (Santa Clara, CA), mass spectrometry devices, such as those commercially available from PerkinElmer, Inc. (Waltham, MA), Thermo Fisher Scientific, Inc., Agilent Technologies, Inc. (Santa Clara, CA), Waters Corp. (Milford, MA), Bruker (Billerica, MA), Shimadzu (Japan), electrophoretic equipment, and the like, or may be hyphenated to other analytical devices, such as ultraviolet/visible light detectors, fluorescence detectors, an evaporative light scattering detectors (ELSDs), chemiluminescence detectors (CLNDs), infrared detectors, electrophoretic equipment, and nuclear magnetic resonance devices commercially available from Bruker, Varian, Inc. (Palo Alto, CA) and Agilent Technologies, Inc. and other manufacturers. The devices disclosed herein are particularly useful to detect species in a fluid flow stream that elutes from a separation column.

In some embodiments of the invention the chamber receives the first electrolyte solution through at least one inlet (16), and said first electrolyte solution exits the cell through at least one outlet (17).

In some embodiments of the invention the fourth electrode (7) is arranged to not perturb the flow of the first electrolyte in the chamber. In some embodiments of the invention the fourth electrode (7) forms part of the walls

in either the inlet or outlet of the chamber (1); or is placed in the inlet or outlet as near as possible to the chamber (1) thereby maintaining a laminar flow. By letting the fourth electrode form part of the walls in the inlet or outlet of the chamber, insulated from the second electrode (5), maintains the laminar flow, which is an advantage when using hyphenated techniques, where a turbulent flow may result in peak broadening.

In some embodiments of the invention the device is adapted to/for measuring the electric current caused by an ion transfer across the Interface between Two Immiscible Electrolyte solutions (ITIES). Examples of how to adapt the device for measuring is to connect the electrodes to equipment capable of measuring and applying potentials and currents. In some embodiments of the invention the device further comprising a detector electrically coupled to at least one of the first electrode (3), second electrode (5), third electrode (6) and fourth electrode (7). In some embodiments of the invention the detector is an electrochemical detector, *e.g.* a potentiostat, a galvanostat or a current follower. A potentiostat may be used to control the device by applying potentials and measuring currents. A galvanostat (amperostat) is a control and measuring device used to keep constant the current flowing though an electrochemical device and measuring a potential. In a current follower the output current follows or tracks the input current.

It is preferable to use a potentiostat as the electrochemical detector, as it provides means for manipulating and measuring the potential and current in e.g. a 2-, 3-, or 4-electrode device. A potentiostat such as the Autolab PGSTAT 30 (Eco-chemie, NL) may be used, or e.g. The BioStat System (ESA Bioscience, Chelmsford, MA) may also be used. The BioStat System allows precise amperometric or potentiometric measurements to be made independently on up to four channels for multiple testing, with the system handling signals of either polarity, employing either 2- or 3- electrode configurations.

In some embodiments of the invention the chamber (1) has a volume smaller than 300 µl, *e.g.* smaller than 100 µl and smaller than 20 µl, and in some embodiments of the invention the volume of the chamber (1) may be adjusted, *e.g.* within the range from 1 µl to 300 µl, from 1 µl to 100 µl and from 1 µl to 20 µl. The adjustment may be done by making the device out of at least two parts that can move in relation to each other and in such a fashion increase or decrease the volume of the chamber. Figure 2-4 describes specific embodiments of how adjustment may be done by screwing and unscrewing the second part (8). Consequently the chamber size may be adjusted in a stepless/ungraduated way. The adjustment may in some embodiments be controlled by a computer. Another way of adjusting the chamber size is by having the chamber dimensions be determined by a template, and consequently change the template to another template in order to adjust the volume of the chamber. Figure 2-4 describes specific embodiments of how a template (9) may be used to define the chamber size, and figure 7-8 shows different template sizes and shapes.

The method of adjusting the chamber size is advantageous, as only one cell is necessary to cover a large range of volumes.

In some embodiments of the invention the chamber is constructed to minimize the distance from the membrane (2) to the second electrode (5). If the distance from the membrane (2) to the second electrode (5) then the first electrode (3) in fluid contact with the second electrolyte solution may be placed as close to the second electrode (5) as possible, which will reduce resistivity between the two reference electrodes (3) and (5) and thereby increase sensitivity.

In some embodiments of the invention the membrane (2) and the second electrode (5) are placed on opposing sides of the chamber (1). In a situation,

where the distance from the membrane (2) to the second electrode defines the height of the chamber, it will be advantageous to minimize chamber height (21) as much as possible in order for the two reference electrodes (3) and (5) to be placed as close to one another as possible.

In some embodiments of the invention the combined area of the membrane exposed to the fluid in the chamber (1) and the part of the second electrode (5) that is exposed to the fluid in the chamber (1) is larger than the area of the other sides of the chamber. The area of the membrane exposed to the fluid in the chamber (1) actually makes up the ITIES, and in order to increase sensitivity, it is advantageous that one or both of the area of the ITIES and the area of the part of the second electrode (5) that is exposed to the fluid in the chamber (1) is large compared to the other sides of the chamber, such that the combined area of the ITIES and the area of the part of the second electrode (5) that is exposed to the fluid in the chamber (1) is larger than the other sides of the chamber. By area is meant the surface area. The surface area may be significantly larger than the immediate surface area, if e.g. a porous membrane is employed.

In some embodiments of the invention, the second electrode (5) is a porous electrode with a porous surface area that is greater than the immediate “2-D” surface area, i.e. the surface area that can be measured using e.g. a ruler. The porous surface area may be larger than a factor 2 greater than the immediate surface area, e.g. factors larger than 5, 10 and 100.

In some embodiments of the invention the part of the second electrode (5) that is exposed to the fluid in the chamber is at least 10 % of the area of the membrane to the area of the membrane exposed to the fluid in the chamber. For example from 10 % of the area of the membrane to the area of the membrane exposed to the fluid in the chamber. The area of the membrane exposed to the fluid in the chamber is the ITIES. When the area of the

second electrode (5) is at least 10 % of the area of the ITIES the further advantages of increased sensitivity, and less baseline drift is realised, since the second electrode (5) will be less susceptible to fouling and removal of the anodized layer, e.g. AgCl in the case of the second electrode (5) being a Ag/AgCl reference electrode. In the following the Ag/AgCl electrode is used as an example of the second electrode (5). However, the skilled person would understand that the advantages are not associated exclusively with an Ag/AgCl electrode, but rather that the advantages can be realised with any electrode that is susceptible to fouling and/or removal of the anodized layer. Other specific examples could be Ag/AgBr or Ag/Ag_2SO_4 electrodes.

It is especially advantageous that the area of the second electrode (5) is at least 10 % of the area of the ITIES when the device of this description is operated in 3-electrode mode, since passing the current through the reference electrode would result in redox reactions taking place at the reference electrode, *e.g.* AgCl/Ag. These redox reactions ultimately destroy the reference electrode, e.g. by depleting the AgCl layer on the Ag/AgCl reference electrode leading to an unstable and non-controllable applied potential.

In some embodiments of the invention the area of the second electrode (5) is at least 20, 30, 40, 50, 60, 70, 80, 90, 100 % of the area of the ITIES. In some embodiments the area of the second electrode (5) is larger than the ITIES. Large areas of the second electrode (5) allows for higher currents to be studied, as the larger the area of the second electrode (5) the better the stability of the electrode.

In some embodiments of the invention the area of the membrane exposed to the fluid in the chamber (1) divided by the part of the second electrode (5) that is exposed to the fluid in the chamber (1) is between 0.1 to 10, e.g. between 0.8 to 1.2.

Typical volumes of the chamber (1) may be from 1-300 µl, for example 1, 5, 20, 50, 100, 150, 200, 250, 300 µl. It is also possible to have sub µl volumes of the chamber, such as for example nl. Typical areas of the second electrode (5) that is exposed to the chamber (1) may be from 0.7 mm^2 to 10 mm^2, such as for example 0.7, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 mm^2. The ratio of the volume of the chamber (1) divided by the part of the second electrode (5) that is exposed to the chamber (1) is: 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 0.6, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 10.0, 20.0, 30.0, 40.0.

In some embodiments of the invention the volume of the chamber (1) divided by the part of the second electrode (5) that is exposed to the chamber (1) is between 0.0001 cm to 40 cm, more narrow between 0.0001 cm to 10.0 cm, more narrow between 0.01 cm to 0.6 cm, *e.g.* between 0.01 cm to 6.0 cm. In general the ratio should be as small as possible (low dead volume – no dilution effect) for increased sensitivity. However, if it is too small that the diffusion layer is comparable to the height of the detection chamber (1), lower sensitivity in terms of molar concentration of the analyte is likely to be observed.

In an embodiment of the invention at least one of said at least one inlet and/or said at least one outlet traverse the second electrode (5). This is advantageous, as the chamber may be made smaller compared to not having the inlet(s) and/or outlet(s) traverse the second electrode (5).

In an embodiment of the invention the device is shielded from at least one of the following external influence: Electrical fields and electromagnetic radiation, e.g. by enclosing the device in a Faraday cage; and mechanical shock. By shielding the device from external influences the signal-to-noise ratio may be improved. In some embodiments the device may be shielded

from mechanical shock by placing it on a material that dampens such mechanical shock, e.g. rubber or springs.

In some embodiments of the invention each of the electrodes 3 and 5 is made out of an insoluble Ag salt (e.g. Ag_2SO_4, AgCl, and AgBr) plated/anodized on Ag, and each of the electrodes 6 and 7 are made out of conductive material, *e.g.* gold, platinum, silver, boron doped diamond, carbon and steel.

Another aspect of the invention is a method for preparing a sample comprising a concentrated ion from a first electrolyte solution comprising the ion in a lower concentration, the steps comprising: introducing the first electrolyte solution to a device according to any one of the above embodiments; applying a constant potential suitable for the ion to transfer across the interface between the first electrolyte solution and the second electrolyte solution to obtain a sample comprising the concentrated ion from the first electrolyte solution; optionally reversing the constant potential to release the concentrated sample comprising the ion from the second electrolyte solution.

Some biomolecules, such as proteins do not transfer across the interface between the first and second electrolyte solution, but is adsorped onto the membrane/interface. The application of a constant potential suitable for the ion to transfer across the interface between the first electrolyte solution and the second electrolyte solution to obtain a sample comprising the concentrated ion from the first electrolyte solution also comprise the situation, wherein the ion is adsorped onto the membrane/interface.

This method may advantageously be used together with hyphenated techniques (e.g. MS, UV, IR, NMR, and electrophoresis) as described elsewhere in this description, especially when the analyte is to diluted to be

detected or be detected satisfactory with the hyphenated techniques. By employing the method described a diluted analyte may be concentrated in the second electrolyte solution. When enough analyte has passed the chamber, and concentrated analyte in the second electrolyte is satisfactory, it may be released by reversing the constant potential, or alternatively remain isolated in the membrane (2). The membrane (2) comprising the second electrolyte and the concentrated analyte may be removed and subjected to further isolation and/or purification, such as solvent extraction, blotting on another medium, or used directly in e.g. MALDI-MS, DESI-MS, EASI-MS, DAPPI-MS, MIMS or other surface sensitive analytical techniques.

Another aspect of the invention is a method for measuring a ion concentration of one or more charged compounds, which comprises passing said compounds in solution through an electrochemical flow cell according to any one of the above embodiments, and then to a device that provides chemical structure information. The device that provides chemical structure may for example be the hyphenated techniques previously described.

In some embodiments of the invention the one or more charged compounds are selected from the group consisting of: alanine, leucine, acetylcholine and histamine, and/or the charged analytes mentioned in table 1 and 2 one page 9 and 10 in WO 2007/092331 A2 to ESA Biosciences, inc.

These one or more charged compounds may advantageously be detected at the nanomolar level, *e.g.* at lower than 500 nM, lower than 100 nM, lower than 40 nM or lower than 10 nM, *i.e.* well below the 10 ng level of all compounds listed in table 1 and 2 provided that the compounds distribute into the membrane under the influence of an applied potential at an optimal pH. The device of this description is able to detect the low nM concentrations (e.g. lower than 40 nM or lower than 10 nM) of these analytes contrary to the prior art, Sánchez-Pedreño *et al.* and Lee *et al.*. The device of this

description may advantageously be used e.g. in parallel or series with the equipment described in WO 2007/092331, in order to provide further detection means. It would especially be advantageous to use the device of this description in relation to the following compounds: Alanine (Ala), Leucine (Leu), Acetylcholine (ACh), Histamine (HSN), and Riboflavin (RF), as the specific EC array described in WO 2007/092331 did not detect these.

In some embodiments of the invention the signal-to-noise ratio is at least 3, *e.g* at least 6 when the ion concentration of the particular charged compound being detected is 5 nM. In some embodiments of the invention the LOD is better than 10 nM, e.g. better than 5 nM, better than 4 nM, better than 3 nM, better than 2.5 nM, better than 2.2 nM.

Unless stated otherwise the lower limits of detection (LOD) were estimated based on the signal-to-noise ratio (S/N) obtained for a 5 nM concentration of TEA, with the LOD being defined as S/N = 3.

The LOD for the device as described in figure 2, with a chamber volume of 50 µl is (5 nM / 6.86)*3 = 2.2 nM. The data is based on the pulse amperogram in figure 10 obtained for the flow injection analysis of tetraethylammonium chloride as described in example 4.

LOD with an EC array may be significantly improved when analysis is targeted to specific analytes. Furthermore, preprocessing , *e.g.* smoothing, signal averaging and background subtraction may also be used to improve the LOD. Shielding the device from external influences may also be employed to improve the LOD. Using an extraction step as previously described may also improve the LOD. No preprocessing as described above has been applied in the examples 2-4.

Furthermore, in embodiments, where the membrane (2) and the second electrode (5) is placed on opposing sides offers the advantage that the distance between the reference electrodes (3) and (5) is uniform and consistent at the various volumes of the chamber.

In the following, reference is made to the accompanying figures, which show by way of illustration how the invention may be practiced.

Fig. 2 shows a schematic of a four electrode device having a top part (8) and a bottom part (formed by parts 12 and 13), an inlet (16) and outlet (17) and a chamber (1) that may be varied in volume by turning the upper part (8), in accordance with certain embodiments. The bottom part (12) and (13) may be made in one piece, but are depicted in fig. 2 as two pieces. The bottom part being made out of two pieces allows for easy cleaning of the cell, including the electrode (5). The bottom part (12) and (13) may be held together by several means, such as screws, clamps, rubber bands and the like in order to form a tight seal (14). The O-ring (10) is a safety measure in case the seal (14) is not tight, then the leak will be contained, and the risk of short-circuit is minimized due to a leak in the seal (14). The bottom part includes threads (18) fitting the threads on the top part (8) and allowing the top (8) and bottom (12) and (13) part to move in an ungraded/stepless fashion to modify the volume of the chamber (1). In some embodiments the volume of the chamber (1) may be adjusted between 1 μl and 300 μl. O-ring (11) is a safety measure in case the seal formed by template (9) connected with the top part with the sides of the bottom part (12) and (13), then the leak will be contained, and the risk of short-circuit is minimized due to a leak in the seal between template (9) and the sides of the bottom part (12) and (13). There are many ways of fitting a top part (8) with a bottom part (12) and (13), and using a thread (18) is an example. The thread (18) may for example not be present, which will make the top part (8) more like a piston in a cylinder (the bottom part (12) and (13)). The O-ring (11) will in addition to being a safety measure

also function as a means for holding the piston/top part (8) in the desired position. The device (15) contains two electrodes (3) and (6) in contact with the membrane (2) comprising the second electrolyte solution, optionally through a third electrolyte solution (4), although figure 2 has been drawn such that the two electrodes (3) and (6) is in contact with the membrane (2) comprising the second electrolyte solution through the third electrolyte solution (4). The first electrode (3) may be a reference electrode, e.g. a Ag/AgCl reference electrode, and is placed as close to the membrane (2) comprising the second electrolyte solution. The third electrode (6) may be a counter electrode, *e.g.* made out of Pt. The device (15) contains two electrodes (5) and (7) in contact with the chamber (1). The second electrode (5) may be a reference electrode, *e.g.* a Ag/AgCl electrode that forms part of the chamber walls, and is placed opposite of the membrane (2). The fourth electrode (7) is placed in the outlet (17) of the device (15) thereby not disturbind the flow in the chamber. The device (15) may be operated in 4-electrode mode, or in 3- or 2-electrode mode. When operated in 3-electrode mode the fourth electrode may be absent and only electrodes (3), (5) and (6) be in use (method 1) or electrodes (3), (5) and (7) may be used (method 2). The template (9) serves the purpose of holding the membrane (2) in place, and providing a minimum distance from the membrane (2) to the electrode (5). The shape of the template (9) may be altered to modify the volume and shape of the chamber (1).

In some embodiments where the device is operated as an ITIES device, a potential difference is applied between the reference electrodes (3) and (5) which cause an ion transfer across the interface/membrane (2) to take place. In order to maintain electroneutrality, a current is passed through the two platinum counter electrodes (6) and (7). In a typical experiment the potential is varied linearly in time while the current passing through the platinum counter electrodes (6) and (7) is measured. Potential control and current measurement is performed using a 4 electrode potentiostat. A typical

experiment is shown in figure 9: At low potential differences transfer of anions from the first electrolyte solution is observed, in the middle of the potential window the transfer of the analyte ion of the first electrolyte (in this case tetraethylammonium, TEA) is observed and finally at high potentials the transfer of cations of the first electrolyte is observed. The formal transfer potential of the analyte ion (*e.g.* TEA in this example) is calculated as the average of the two peak potentials. The formal transfer potential is proportional to the log P (partition coefficient) of the analyte ion. The current is proportional to the concentration of the compound.

Fig. 7 discloses three exemplary embodiments of template (9). Fig. 7a results in a cylindrical chamber (1); fig. 7b in a elliptical chamber (1); and fig. 7c results in a rectangular chamber (1) when the bottom surface of 9 is in contact with surface 14, and in accordance with certain embodiments. Several other shapes of the template (9) may be envisaged.

Fig. 8 shows different embodiments of the chamber modifying part (9) resulting in different chamber volumes (1) by changing the length of surface (21) from the membrane (2) to the hashed line (20) representing the imaginary surface 14, as well by changing the area of the disc (2) exposed to the chamber (1). Certain illustrative embodiments are shown in fig. 8a-c, and an illustrative embodiment of the chamber modifying part with an O-ring (19) is also shown. Reducing the length of the surface (21) from the membrane (2) to the hashed line (20) representing the imaginary surface (14) results in the two reference electrodes (5) and (3) being close to each other. Several different widths and heights (21) of the template (9) can be envisaged, and some embodiments have been described in figure 8.

Different embodiments of figure 7 may be combined with different embodiments of figure 8 to obtain new embodiments. These templates may

in turn be combined with all possible embodiments of figure 1, 2, 3 and 4 to obtain devices of this description.

The device (15) has been shown with a top part designed for ITIES measurements. However, if the top part is exchanged with a modified top part (not shown), wherein the membrane (2) is exchanged with a solid electrode such as *e.g.* platinum, gold, silver, carbon, glassy carbon, boron doped diamond, iron, carbon steel, stainless steel connected to (3). Many other embodiments exist that modifies the top part into a solid electrode. It is advantageous to be able to exchange the top part for a solid electrode, as it allows the one and same device to be used with different top parts (8) to operate as both regular electrochemical cells (direct amperometric cells) and ITIES cells in conjunction with different chamber volumes. Consequently a single device may be used for different volumes and for different types of electrochemistry. In some embodiments the device (15) may be a kit comprising the bottom part (12) and (13) and several top parts (8) suited for different purposes, and several templates (9) to allow different chamber volumes.

Fig. 3 shows a second embodiment of a device according to the invention. The device has one inlet (16) and two outlets (17). Other configurations such as 3 or more outlets (17), or 2, 3 or more inlets (16) may also be envisaged.

Fig. 4 is another schematic of a two to four electrode device wherein one of the electrodes (7) is embedded in the walls of the outlet (17) of the cell, and further the volume of the chamber (1) is modified by unscrewing upper part (8) from the bottom part in such a way that the bottom surface of 9 is no longer in contact with surface 14, in accordance with certain embodiments.

In device claims enumerating several means, several of these means can be embodied by one and the same item of hardware. The mere fact that certain

measures are recited in mutually different dependent claims or described in different embodiments does not indicate that a combination of these measures cannot be used to advantage.

Certain specific examples are described in more detail below to illustrate further the novel technology disclosed herein.

Examples

Apparatus & Reagents

A four-electrode potentiostat AUTOLAB PGSTAT 30 (Eco-Chemie, Netherlands) equipped with IR drop compensation connected to a computer was used for electrochemical measurements. A Merck-Hitachi L-7100 pump, a Merck-Hitachi L-7200 autosampler, connecting tubing of 0.5 mm bore, PTFE tubing and various end fittings and connections were used to construct the flow injection system.

Reagents. All aqueous solutions were prepared using deionized water from a Milli-Q system (Millipore) and 2-nitrophenyloctyl ether (NPOE) (Fluka, Switzerland) was used as the organic phase. High molecular weight poly(vinyl chloride) (PVC) and tetrahydrofuran (THF) were supplied from Fluka. The organic phase supporting electrolyte salt hexadecyl-trimethylammonium tetrakis(pentaflurophenyl) borate (HTMATPFB) was prepared by metathesis of equimolar quantities of the corresponding salts, lithium tetrakis(pentafluorophenyl) borate (Boulder Scientific company, USA) and hexadecyl-trimethylammonium bromide (HTMABr) (Fluka, Switzerland) in a minimum amount of a 2:1 methanol:water mixture 14. Tetraethylammonium chloride (TEACl) was supplied by Sigma. Polyvinyldifluoride (PVDF) membrane with a pore size of 0.45 µm was purchased from Millipore. All inorganic salts were provided by Merck, Germany.

Example 1 – Preparation of membrane comprising organic phase

Membranes may be prepared by dissolving 4.8 mg hexadecyl-trimethylammonium tetrakis(pentafluorophenyl) borate (HTMATPFB), 500 mg ortho-nitrophenyl octyl ether (NPOE) and 300 mg polyvinylchloride (PVC) in 4 ml tetrahydrofuran (THF). 10 µl of this solution was then placed on a 5 mm diameter polyvinyldifluoride (PVDF) membrane (pore size 0.45 µm) and left overnight to allow the solvent (THF) to evaporate slowly. The membrane was then placed in the flow cell. PVDF membrane was used for the additional stabilization of the oil phase and for the convenience of flow cell sampling.

Example 2 – optimising membrane composition

The second electrolyte solution comprised the organic solvent NPOE and the organic salt HTMATPFB resulting in a solution of 10 mM HTMATPFB in NPOE. A solution of 5 mM HTMATPFB in NPOE was also prepared.

The gelifying/solidifying solution comprised PVC (300 mg) in THF (4 ml).

Different mixtures of the second electrolyte solution (SE) with the gelifying/solidifying solution (GEL) was prepared (SE-GEL ratio of 1:4, 1:2 and 1:1) , and different volumes (3, 5, 10 µl) of the mixtures were placed on 5 mm diameter polyvinyldifluoride (PVDF) membrane (pore size 0.45 µm) and left overnight to allow the THF solvent to evaporate slowly. Mixtures of the second electrolyte solution (SE) with the gelifying/solidifying solution (GEL) without the PVDF membran was also prepared.

The membranes were tested in a device according to figure 2, using the method described in Example 4 – 4-electrode mode to obtain a cyclic voltammogram using differential pulse amperometry, and tetraethylammonium (TEA) as the analyte.

The best results were obtained with the following parameters:

-- *Volume*: 10 µl was chosen for the 5 mm diameter PVDF membrane, as a smaller volume resulted in short membrane stability in the flow, and a larger drop increased the resistance in the system (which lead to fall in the sensitivity).

-- *The second electrolyte solution:* the 10 mM HTMATPFB in NPOE solution yielded the best results.

-- *Ratio of the second electrolyte solution (SE) to the gelifying/solidifying solution (GEL):* The use of a SE-GEL ratio of 1:1 did not lead to the detection of TEA in any of the tested concentrations. SE-GEL ratio of 1:2 lead to the detection of TEA with a limit of detection of 5 nM. SE-GEL ratio of 1:4 lead to the detection of TEA with a limit of detection of 50 nM. The SE-GEL ratio of 1:2 was subsequently chosen.

-- *With or without PVDF membrane:* The PVDF membrane was used for the additional mechanical stabilization the mixture of the second electrolyte solution (SE) with the gelifying/solidifying solution (GEL), and it is preferred that the PVDF membrane is present, as the gelified/solidified mixture of SE and GEL are difficult to handle, and place in the membrane holder due to its fragility.

Example 3 – 3-electrode mode

The 3-electrode mode according to method 1 was tested. The conditions were as described for the 4-electrode mode described in example 4, except for the electrode connections.

The signal (S) to noise (N) ratio for 10 nM TEA^+ concentration as measured in figure 12:

S = 48 nA; N = 7.5 nA => S/N = 6.4

Example 4 – 4-electrode mode

The device according to figure 2 was used.

The Ag/AgCl reference and Pt counter electrodes are immersed into the 5 mM HTMABr water solution. 2 mM phosphate buffer (pH 7) containing 10 mM NaCl was used as a flowing buffer solution.

The electrochemical cell has the following cell diagram:

$$\mathrm{Ag}\,|\,\mathrm{AgCl}\,|\,5\ \mathrm{mM\ HTMABr}\,\Big|\begin{array}{c}20\%\ \mathrm{w/w\ PVC-NPOE}\\ 10\ \mathrm{mM\ HTMATPFB}\\ \mathrm{on\ PVDF\ membrane}\end{array}\,\Big\|\,\begin{array}{c}2\ \mathrm{mM\ buffer\ in}\\ 10\ \mathrm{mM\ NaCl}\\ +\,\mathrm{analyte}\end{array}\,\Big|\,\mathrm{AgCl}\,|\,\mathrm{Ag}$$

The buffer solution was pumped through the flow system at a flow rate of 0.6 ml/min. The injections were performed using an autosampler with a 100 µl injection loop. Tetraethylammonium chloride (TEACl) at different concentrations was used as the analyte.

Electrochemical measurements were carried out using a four-electrode potentiostat with IR drop compensation. The differential pulse amperometric method was performed with voltage pulses of the duration τ = 0.25 s and amplitude ΔE ($\Delta E = E_f - E_b$) at a constant interval Δt = 0.5 s. All the above experiments were carried out at room temperature (20±2 ºC).

The principle of the differential pulse amperometric method and cyclic voltammogram for the transfer reaction of the tetraethylammonium-ion (TEA-ion) are presented in figure 9. The electrode potential is held between a base potential E_1 (0.2 V) at which no ion transfer occurs and the final potential E_2 (0.6 V) where analyte ions are transferred to the oil phase. From this base potential, voltage pulses of short duration τ (0.25 s) at an interval Δt (0.5 s) were applied. The reason to choose these potentials are to maximize the current difference measured, resulting in the optimal sensitivity of the

method. The current is sampled at the end of each pulse and recorded as the difference between the value at the end of the pulse and at the end of the period as a function of real time. It can also be expressed by the following equation, where the current is measured as the difference between the current value at the final potential and at the base potential:

$$I_{diff} = I_{fpulse} - I_{baseline} \qquad (6)$$

Returning the electrode potential to the base potential leads to a negative faradaic current for the reverse transfer of the ion from plasticized oil phase to the water phase. Thus, before the next pulse is applied, the electrode conditions are returned to the initial state and the oil phase is regenerated.

The formal transfer potential of the analyte ion (*e.g.* TEA^+ in this example) is calculated as the average of the two peak potentials. The formal transfer potential is proportional to the log P (partition coefficient) of the analyte ion. The current is proportional to the concentration of the compound.

The signal (S) to noise (N) ratio for 5 nM TEA^+ concentration as measured in relation to figure 10:

S = 2.6 nA; N = 0.35 nA => S/N = 6.86

Claims

1. Device comprising:
   - a chamber (1) configured to receive a first electrolyte solution;
   - a membrane (2) comprising a second electrolyte solution in contact with the chamber, and in contact with the first electrolyte solution when present in the chamber;
   - a first electrode (3) in contact with the second electrolyte solution, optionally through a third electrolyte solution (4);
   - a second electrode (5) in contact with the chamber;

   wherein the first and third electrolyte (4) solution are immiscible with the second electrolyte solution and the second electrode (5) forms part of the chamber walls, and is not in direct contact with the membrane (2).

2. The device according to claim 1, further comprising at least a third and/or a fourth electrode, wherein:

   the third electrode (6) is in contact with the second electrolyte solution optionally through a third electrolyte solution (4); and

   the fourth electrode (7) is in contact with the chamber (1).

3. The device according to claim 1 or 2, wherein the device is a flow cell.

4. The device according to any one of claims 1 to 3, wherein the chamber receives the first electrolyte solution through at least one inlet (16), and said first electrolyte solution exits the cell through at least one outlet (17).

5. The device according to claim 3 or 4 wherein the fourth electrode (7) is arranged to not perturb the flow of the first electrolyte in the chamber.

6. The device according to claim 5, wherein the fourth electrode (7):

forms part of the walls in either the inlet or outlet of the chamber (1); or

is placed in the inlet or outlet as near as possible to the chamber (1) thereby maintaining a laminar flow.

7. The device according to any one of claims 1 to 6, wherein the device is adapted to/for measuring the electric current caused by an ion transfer across the Interface between Two Immiscible Electrolyte solutions (ITIES).

8. The device according to any one of claims 1 to 7, further comprising a detector electrically coupled to at least one of the first electrode (3), second electrode (5), third electrode (6) and fourth electrode (7).

9. The device according to claim 8, wherein the detector is an electrochemical detector, *e.g.* a potentiostat, a galvanostat and a current follower.

10. The device according to any one of claims 1 to 9, wherein the chamber (1) has a volume smaller than 300 µl, *e.g.* smaller than 100 µl and smaller than 20 µl.

11. The device according to any one of claims 1 to 10, wherein the volume of the chamber (1) may be adjusted, e.g. within the range from 1 µl to 300 µl, from 1 µl to 100 µl and from 1 µl to 20 µl.

12. The device according to any one of claims 1 to 11, wherein the chamber is constructed to minimize the distance from the membrane (2) to the second electrode (5).

13. The device according to claim 12, wherein the membrane (2) and the second electrode (5) are placed on opposing sides of the chamber (1).

14. The device according to any one of claims 1 to 13, wherein the combined area of the membrane exposed to the fluid in the chamber (1) and the part of the second electrode (5) that is exposed to the fluid in the chamber (1) is larger than the area of the other sides of the chamber.

15. The device according to any one of claims 1 to 14, wherein the part of the second electrode (5) that is exposed to the fluid in the chamber is from 10 % of the area of the membrane to the area of the membrane exposed to the fluid in the chamber.

16. The device according to any one of claims 1 to 15, wherein the area of the membrane exposed to the fluid in the chamber (1) divided by the part of the second electrode (5) that is exposed to the fluid in the chamber (1) is between 0.1 to 10, e.g. between 0.8 to 1.2.

17. The device according to any one of claims 1 to 16, wherein the volume of the chamber (1) divided by the part of the second electrode (5) that is exposed to the chamber (1) is between 0.001 cm to 6.0 cm.

18. The device according to any one of claims 4 to 17, wherein at least one of said at least one inlet and/or said at least one outlet traverse the second electrode (5).

19. The device according to any one of claims 1 to 18, wherein the device is shielded from at least one of the following external influence:

    a) Electrical fields and electromagnetic radiation, e.g. by enclosing the device in a Faraday cage; and
    b) mechanical shock.

20. The device according to any one of claim 1 to 19, wherein each of the electrodes 3 and 5 is made out of an insoluble Ag salt (e.g. Ag_2SO_4, AgCl, and AgBr) plated on Ag, and each of the electrodes 6 and 7 are made out of conductive material, e.g. gold, platinum, silver, boron doped diamond, carbon and steel.

21. A method for preparing a sample comprising a concentrated ion from a first electrolyte solution comprising the ion in a lower concentration, the steps comprising:
    a) introducing the first electrolyte solution to a device according to any one of claims 1 to 20;
    b) applying a constant potential suitable for the ion to transfer across the interface between the first electrolyte solution and the second electrolyte solution to obtain a sample comprising the concentrated ion from the first electrolyte solution;
    c) optionally reversing the constant potential to release the concentrated sample comprising the ion from the second electrolyte solution.

22. A method for measuring a ion concentration of one or more charged compounds, which comprises passing said compounds in solution through an electrochemical flow cell according to any one of claims 1-20, and then to a device that provides chemical structure information.

23. The method according to claim 22, wherein the one or more charged compounds are selected from the group consisting of: alanine, leucine, acetylcholine and histamine.

24. The method according to claim 22 or 23, wherein the signal-to-noise ratio is at least 3 when the ion concentration of the particular charged compound being detected is 5 nM.

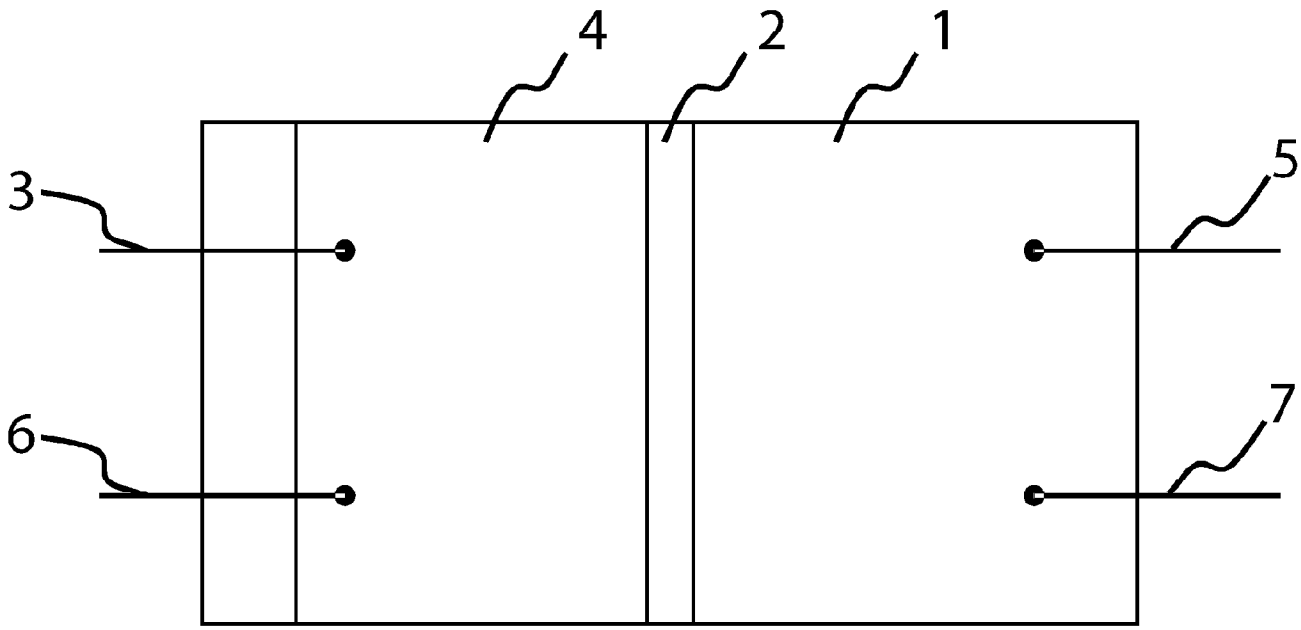


Fig. 1

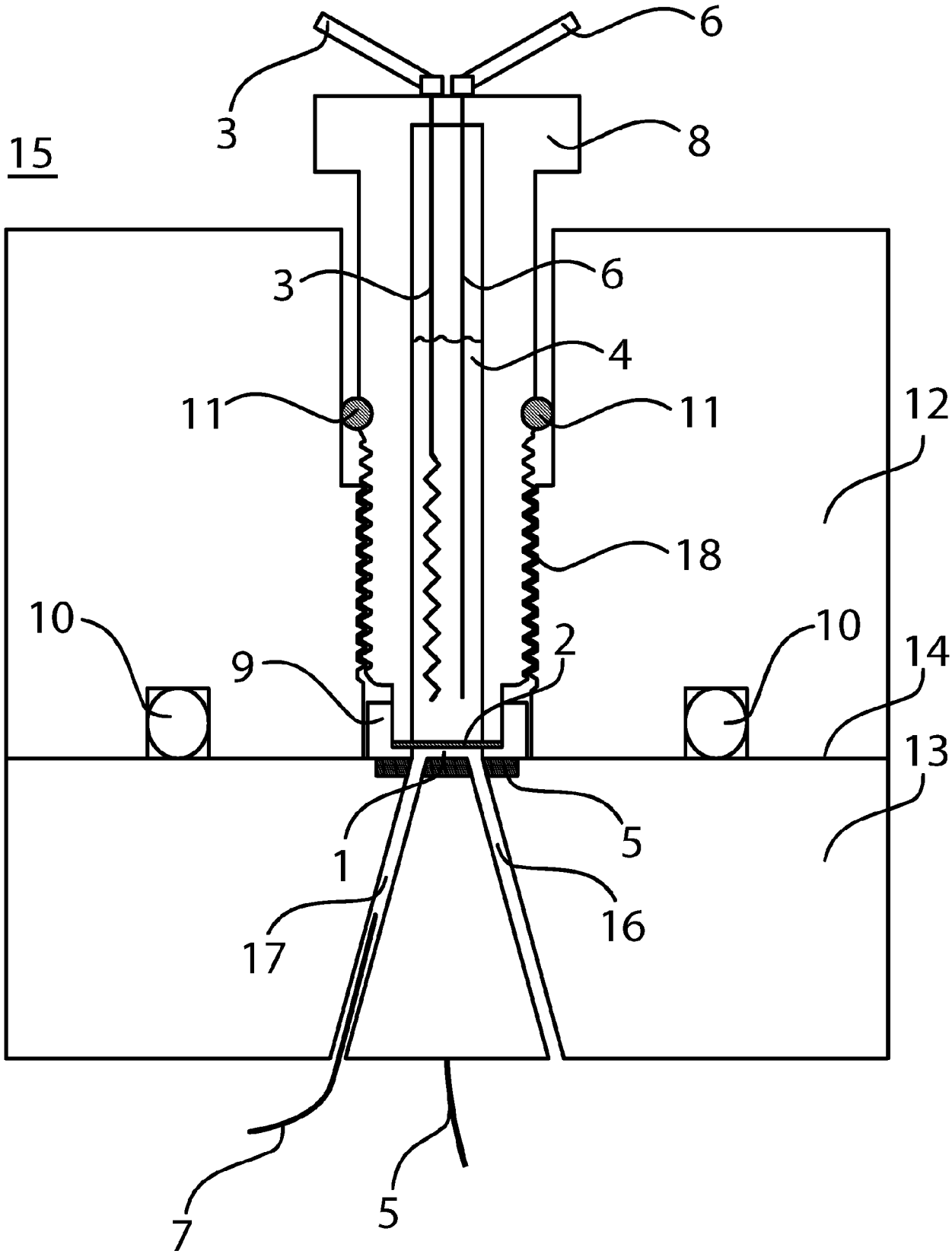


Fig. 2

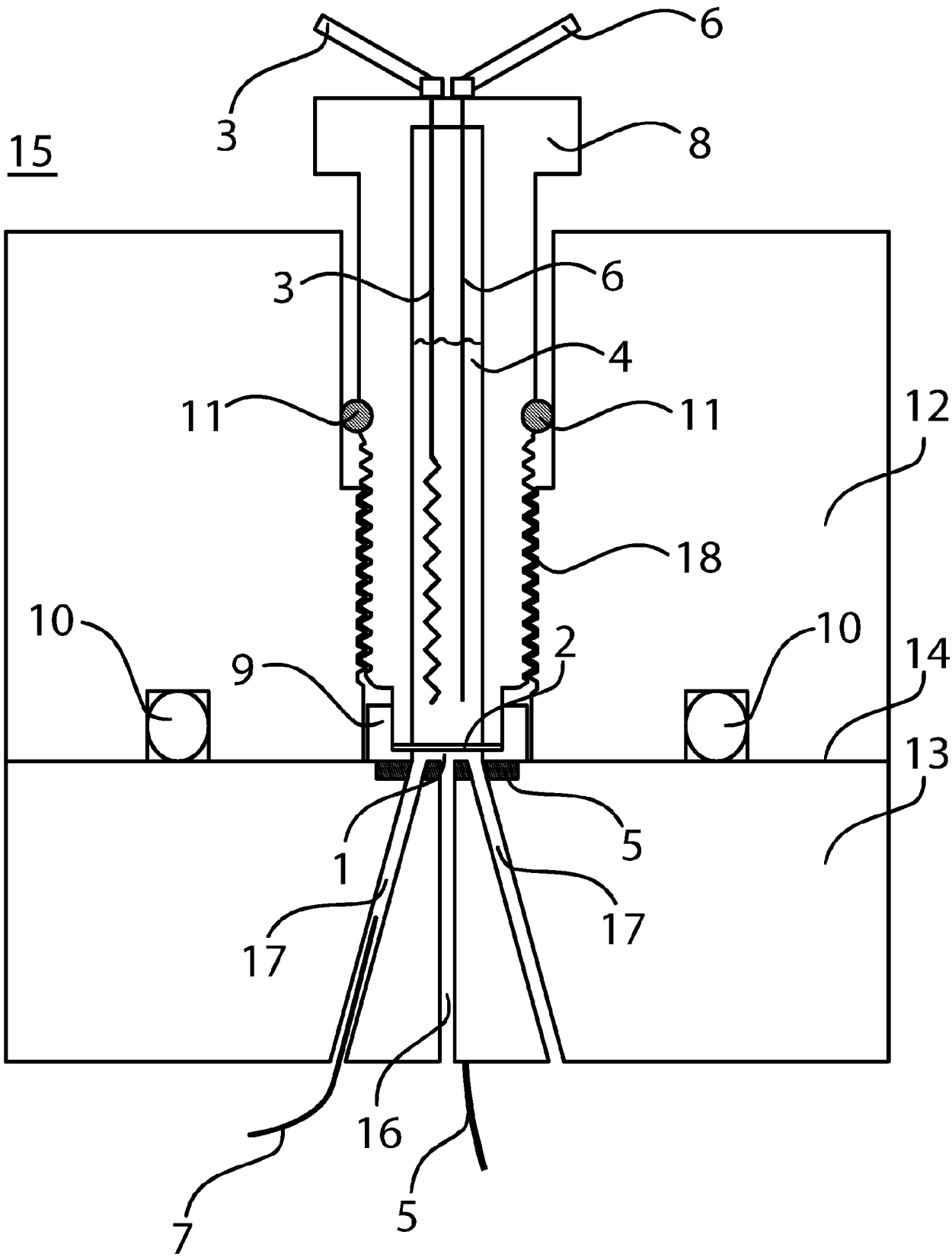


Fig. 3

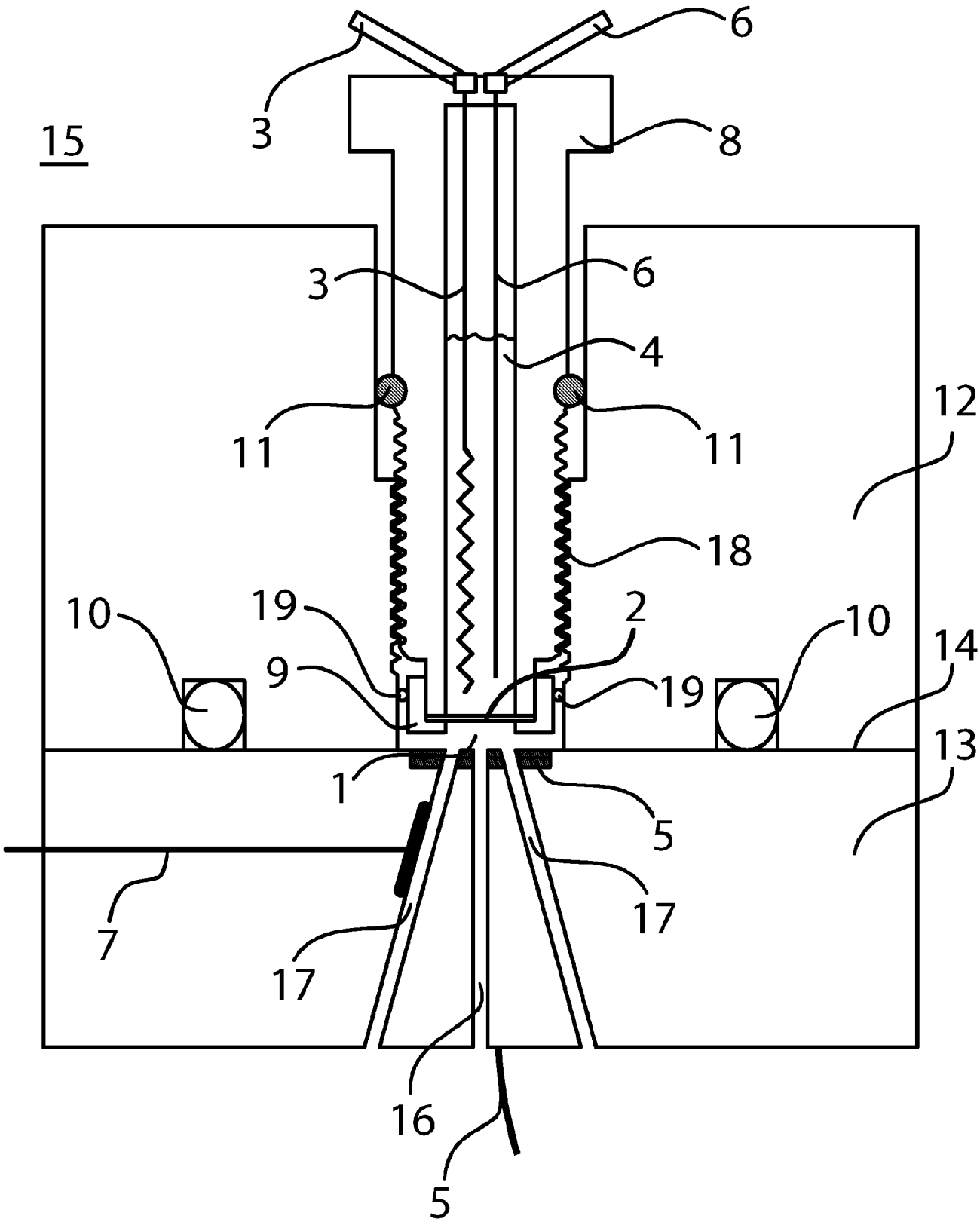


Fig. 4

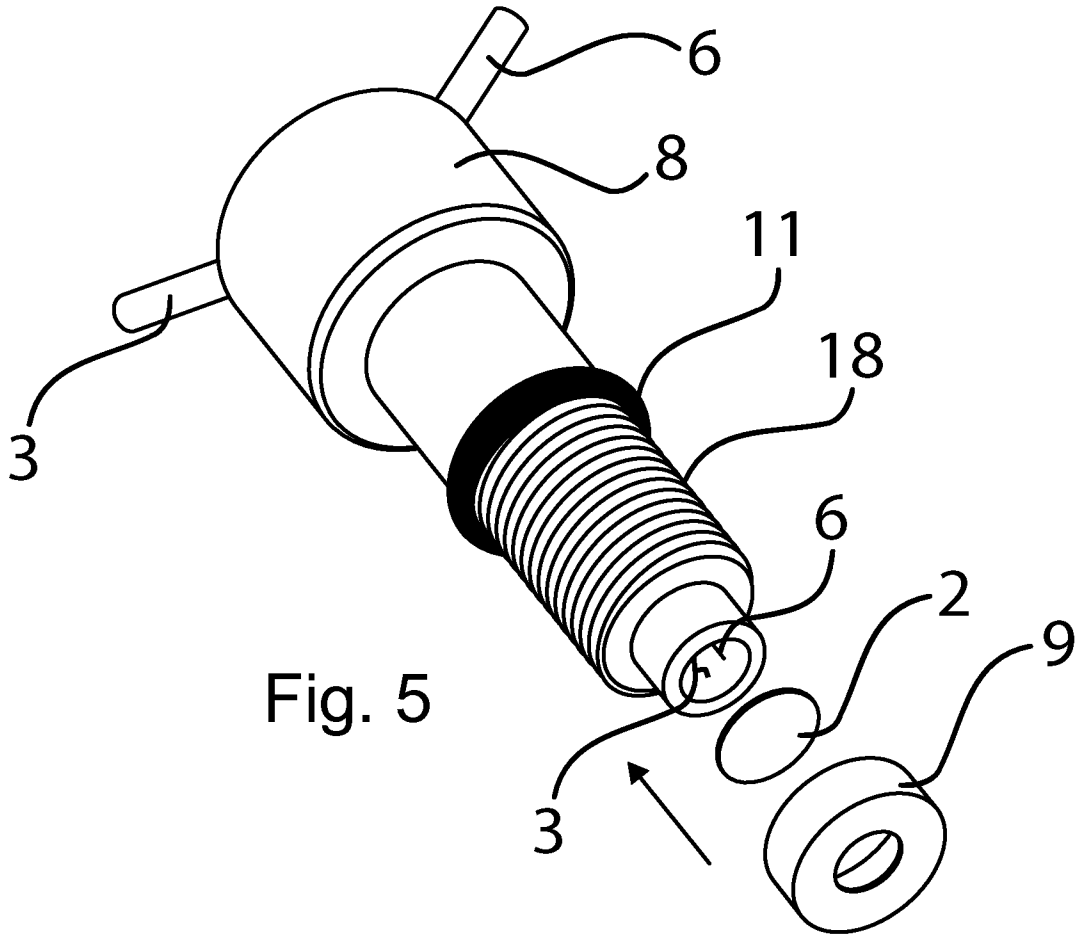


Fig. 5

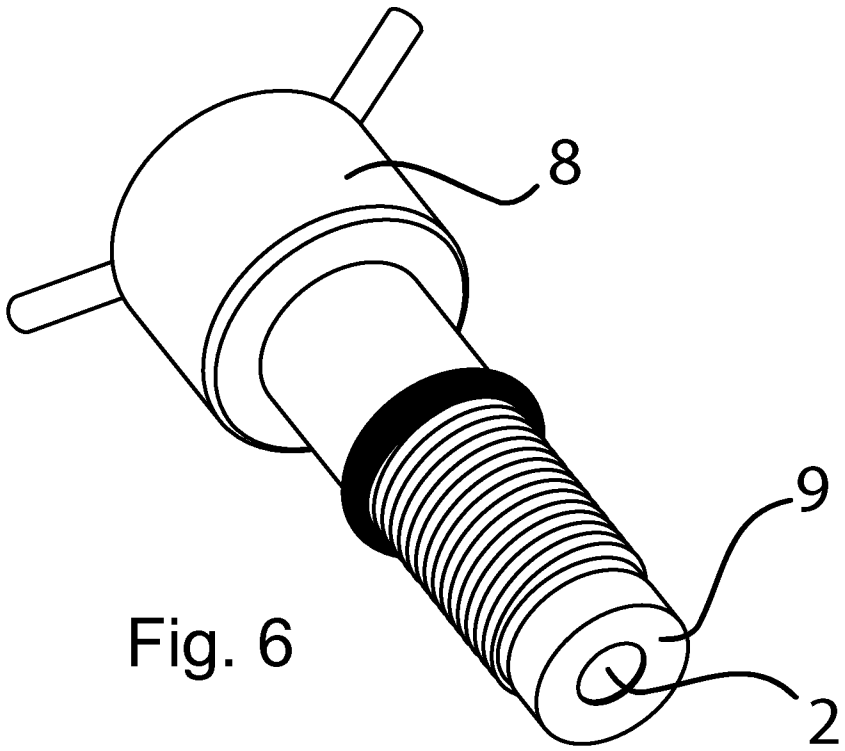


Fig. 6

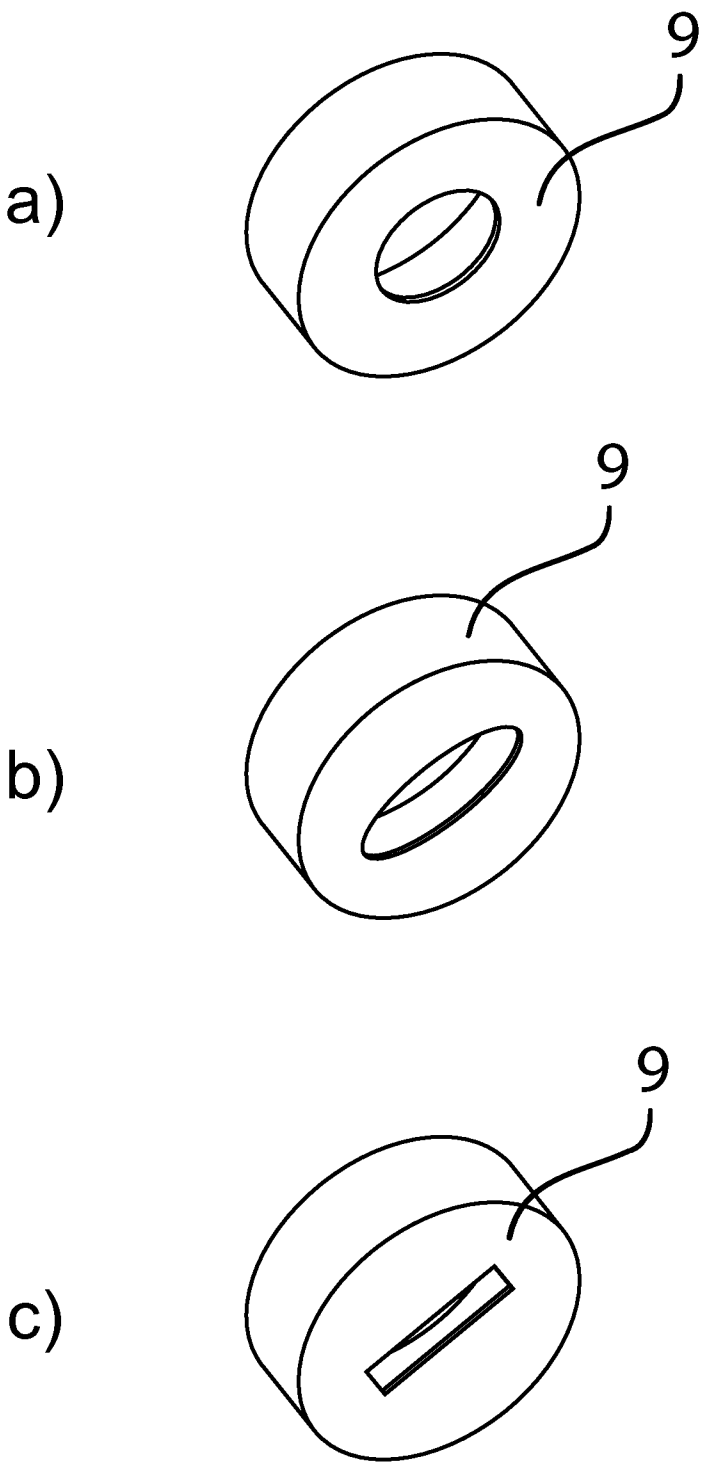


Fig. 7

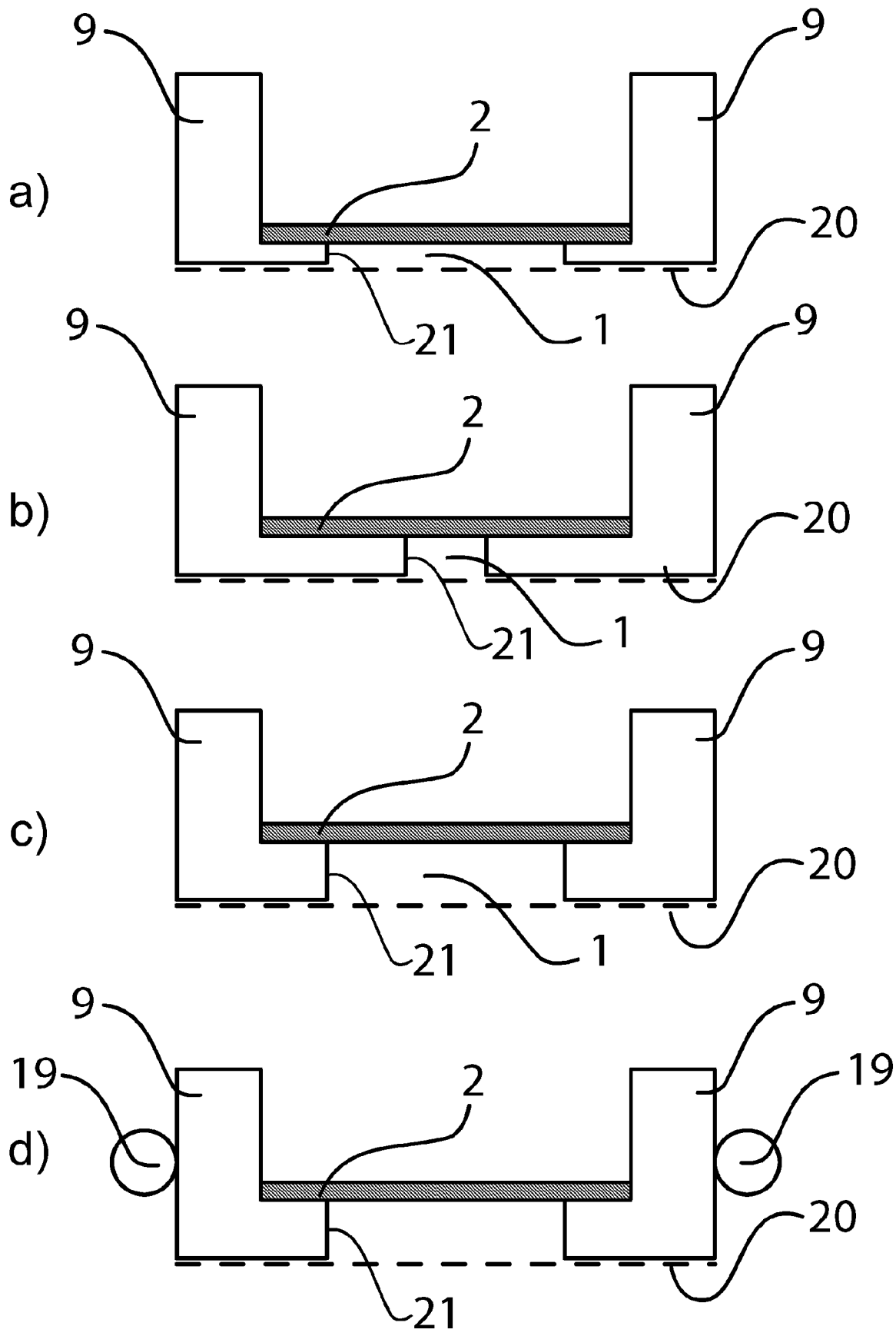


Fig. 8

Fig. 9

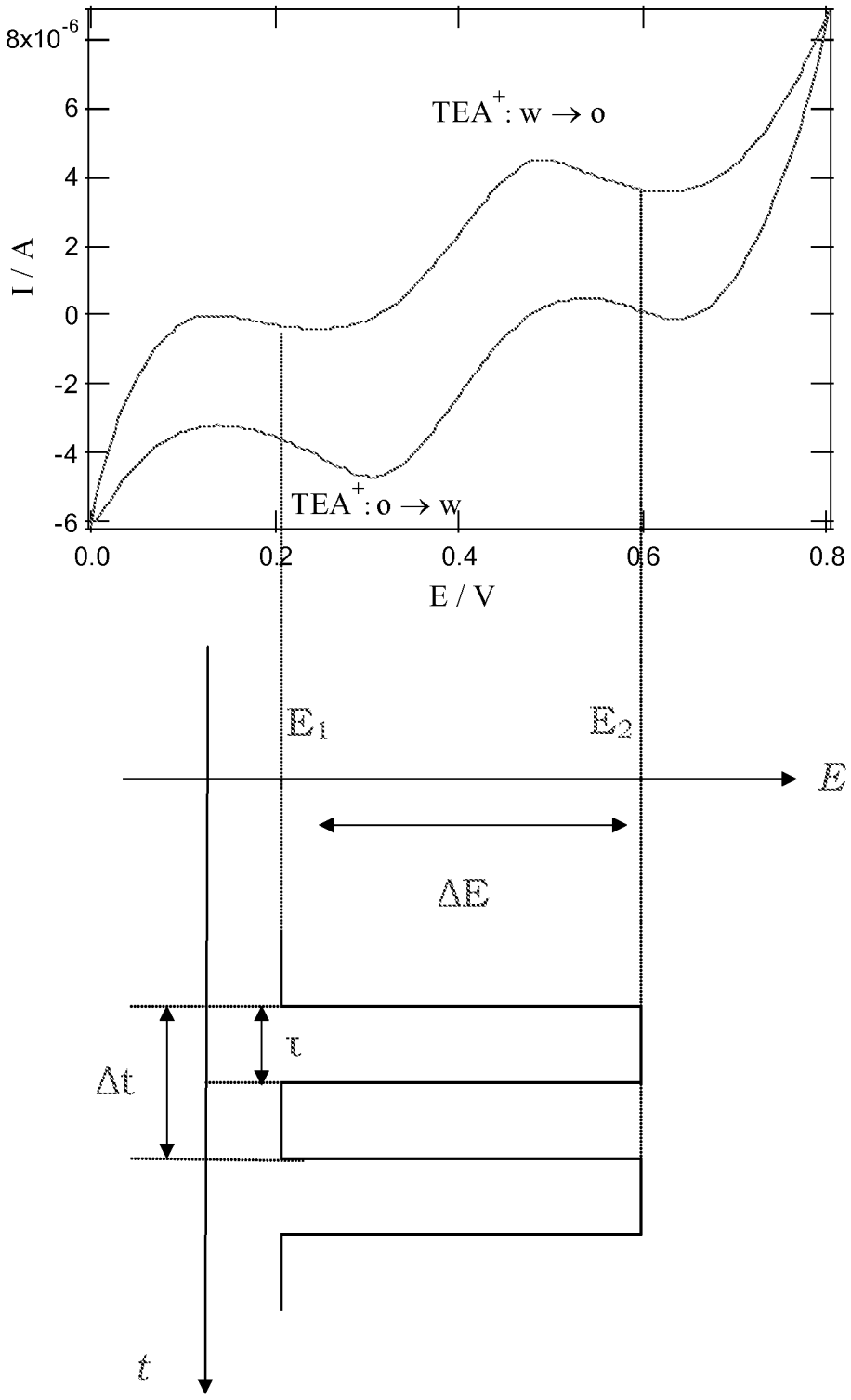
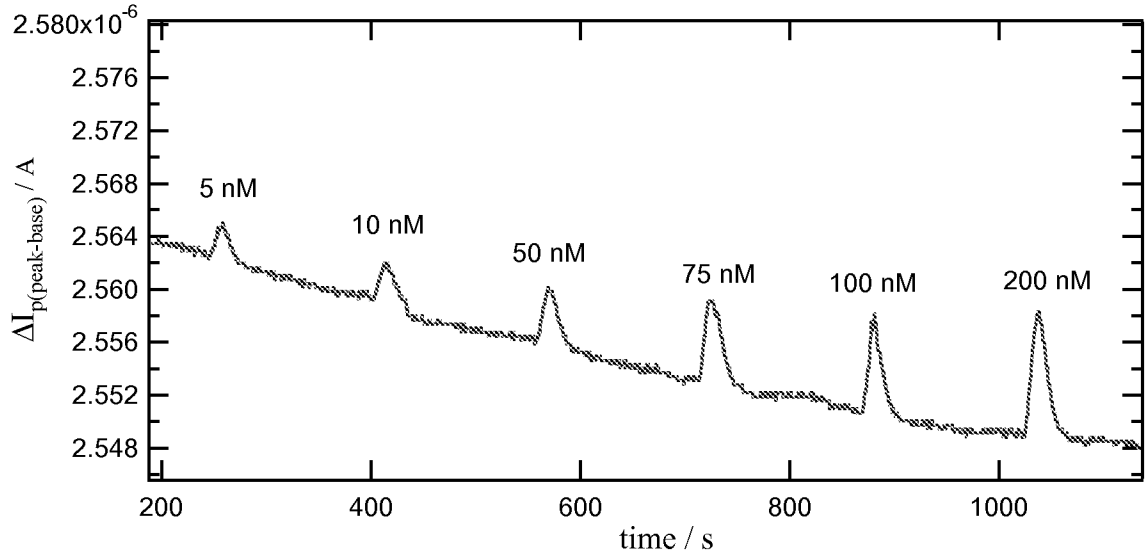


Fig. 10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2.576
2.572
2.568
2.564
2.560
2.556
2.552
2.548
ΔIp(peak-base) / A
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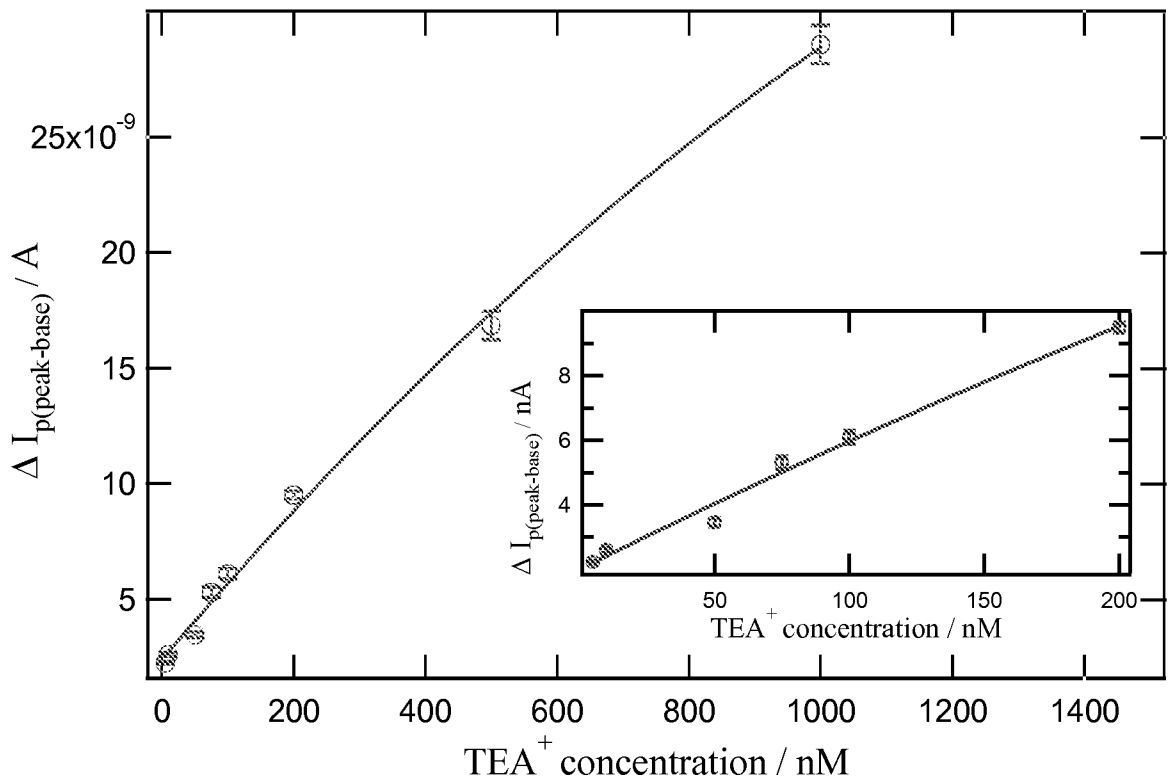
10 nM
50 nM
75 nM
100 nM
200 nM
200
400
600
800
1000
time / s

Fig. 11


25x10-9
20
15
10
5
Δ Ip(peak-base) / A
0
200
400
600
800
1000
1200
1400
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8
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4
Δ Ip(peak-base) / nA
50
100
150
200
TEA+ concentration / nM

Fig. 12

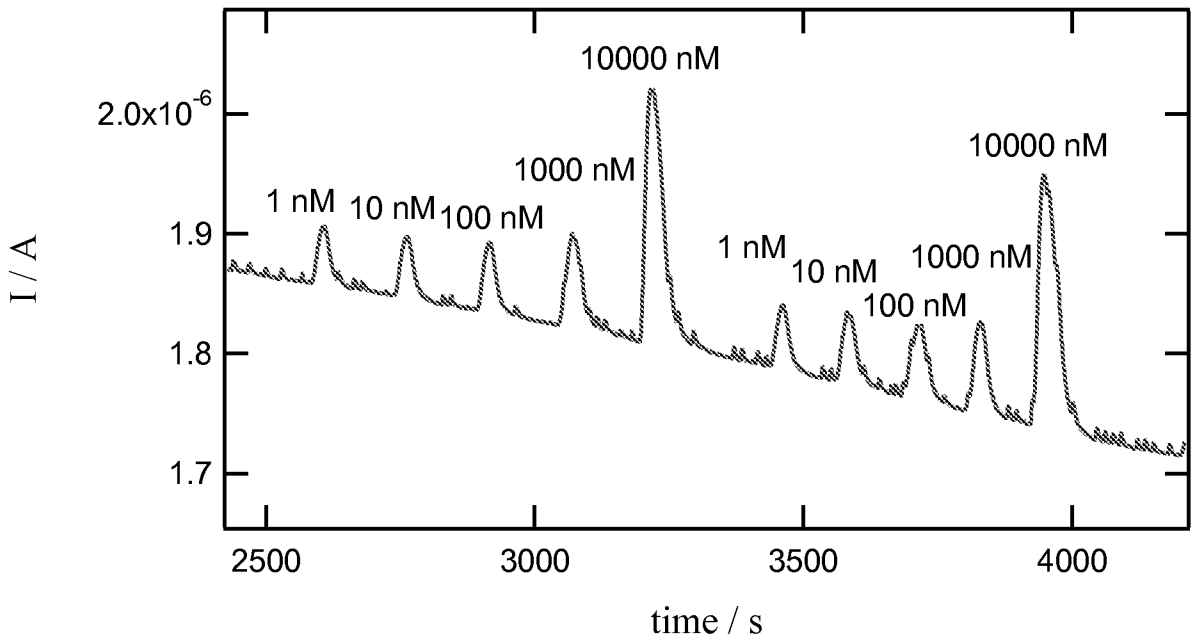
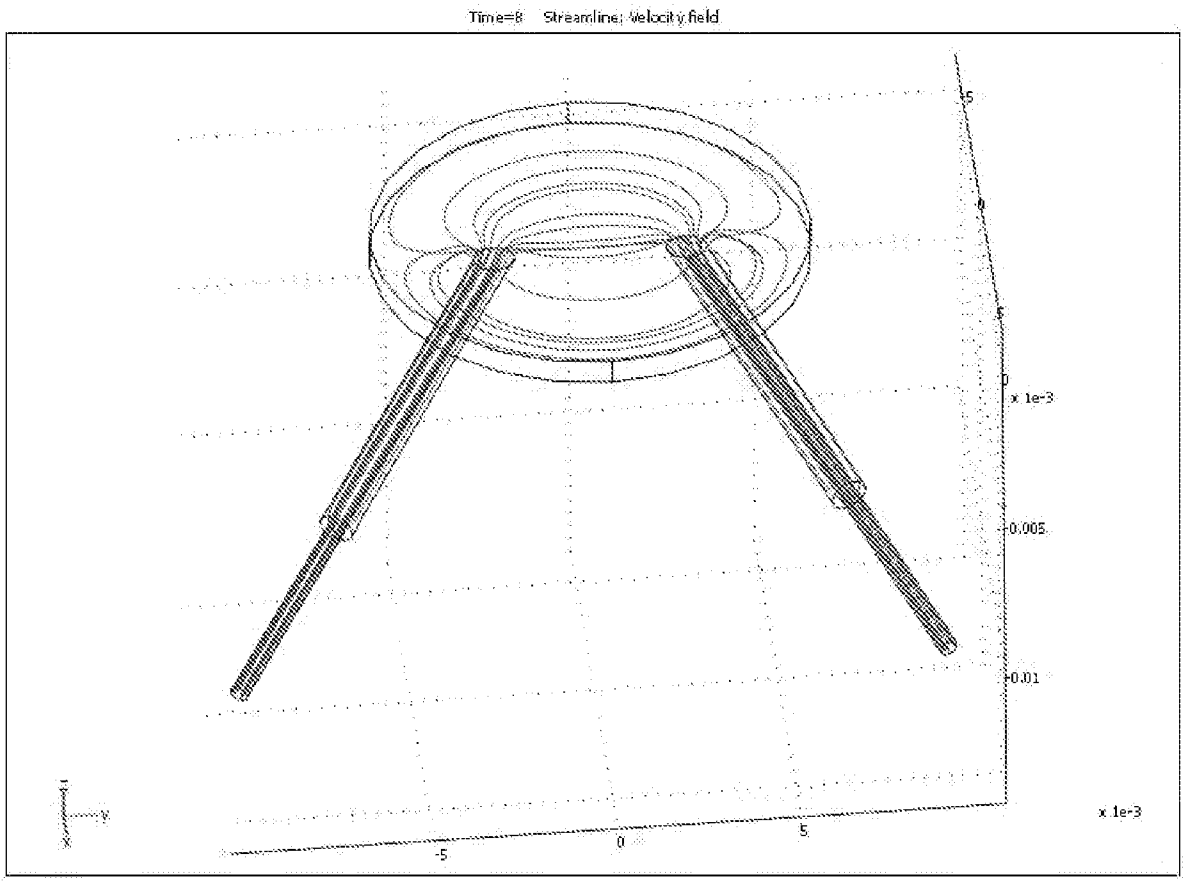

10000 nM
2.0x10-6
1000 nM
10000 nM
1 nM
10 nM
100 nM
1 nM
10 nM
1000 nM
100 nM
1.9
1.8
1.7
I / A
2500
3000
3500
4000
time / s

Fig. 13


Time=8 Streamline: Velocity field
5
0
-5
x 1e-3
0.005
0.01
x 1e-3
-5
0
5
y
x

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/053454

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N27/333 G01N27/49
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)
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 practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MARIAULLE P ET AL: "Application of electrodes modified with ion-exchange polymers for the amperometric detection of non-redox cations and anions in combination to ion chromatography" ELECTROCHIMICA ACTA, ELSEVIER SCIENCE PUBLISHERS, BARKING, GB LNKD-DOI:10.1016/S0013-4686(01)00636-3, vol. 46, no. 23, 10 August 2001 (2001-08-10), pages 3543-3553, XP004299612 ISSN: 0013-4686	1-6,8-24
Y	paragraph [2.4.]; figure 1	7

-/--

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

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"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)

"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

"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

"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.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 June 2010	29/06/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Wilhelm, Jörg

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/053454

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PETR VAN SEK AND LUIS BASÁEZ RAMÍREZ: "INTERFACE BETWEEN TWO IMMISCIBLE LIQUID ELECTROLYTES: A REVIEW" CHILEAN CHEMICAL SOCIETY. JOURNAL, SOCIEDAD CHILENA DE QUIMICA, PAICAVI, CL, vol. 53, no. 2, 1 January 2008 (2008-01-01), pages 1455-1463, XP007913496 ISSN: 0717-9324 cited in the application figures 4,5	7
X	MARTINEZ-BARRACHINA SILVIA ET AL: "Potentiometric flow injection system for the determination of polyethoxylate nonionic surfactants using tubular ion-selective electrodes" ANALYTICA CHIMICA ACTA, ELSEVIER, AMSTERDAM, NL, vol. 438, no. 1/2, 3 July 2001 (2001-07-03), pages 305-313, XP007913548 ISSN: 0003-2670	1,3, 8-12, 14-24
Y	paragraph [2.1.]; figure 1	2,4-6,13
Y	SANCHEZ-PEDRENO C ET AL: "Chronocoulometric flow-injection analysis with solvent polymeric membrane ion sensors" ANALYTICA CHIMICA ACTA, ELSEVIER, AMSTERDAM, NL LNKD- DOI:10.1016/S0003-2670(02)00100-9, vol. 459, no. 1, 1 January 2002 (2002-01-01), pages 11-17, XP007913495 ISSN: 0003-2670 cited in the application paragraph [2.3.]; figure 1	2,4-6,13