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(54) Title: ELECTROCHEMICAL LATERAL FLOW AND FLOW-THROUGH DEVICES

(57) Abstract: Lateral flow and flow-through devices containing integrated electrochemical sensors. The devices are useful for carrying out diagnostic assays, particularly methods of electrochemical analysis of analyte binding.

Electrochemical Lateral Flow and Flow-Through Devices

Field of the invention

The invention relates to lateral flow and flow-through devices for use in diagnostic assays containing integrated electrochemical sensors. Such devices are useful in methods of electrochemical analysis of analyte binding.

Background to the invention

Lateral flow and flow-through devices are extant in many areas of point-of-use diagnosis. These devices are sensitive and fast but have limitations in their use as multi analyte testing systems and also in accurate and reproducible quantitation of the analyte measured.

International application PCT/GB98/00548 (WO 98/37409) describes a potentiometric method of electrochemical analysis using an electrochemical sensing electrode comprising a metallic potentiometric electrode coated with a layer of electroconductive polymer containing immobilised bioreceptor molecules which bind specifically to the analyte under test. The presence of analyte is indicated by a change in potential of the sensing electrode upon binding of analyte to the immobilised bioreceptors, using an ion-step detection procedure. This potentiometric analysis of assays, for example ELISA's, has been shown to be sensitive and accurate at low concentrations with simple multiplexing capabilities. Therefore a merger of these two technologies would bring the benefits of both technologies to bear, resulting in a simple fast rapid diagnostic with exquisite quantitative sensitivity, multianalyte specificity and no sample preparation.

EP-A-0 859 230 describes a flow cell or flowing liquid system device for use in performing diagnostic assays in which the means of detection is based on

electrochemical methods. The format of the device allows enzyme by-products to accumulate close to the surface of a working electrode without being swept away in the flow of liquid through the device, thus permitting amperometric detection of the enzyme by-products. This device is particularly suited to low sample volumes.

The present inventors have now developed electrochemical flow-through and lateral flow devices which incorporate a liquid-permeable sensing electrode, such that liquid is able to flow through the electrode when the device is in use. Such devices can permit modes of electrochemical detection other than amperometric detection, for example potentiometric detection, and may be adapted to analysis of large sample volumes.

Description of the invention

In a first aspect the invention provides a lateral flow or flow-through device for use in methods of electrochemical detection of an analyte, which device includes a sensor assembly comprising at least one liquid-permeable sensing electrode and a reference electrode.

An essential feature of the device is the inclusion of a sensor assembly, which permits electrochemical detection of the analyte of interest. The sensor assembly comprises at least one sensing electrode and a reference electrode.

The sensing electrode is liquid-permeable, preferably porous, meaning that liquid sample is able to flow through the sensing electrode when the device is in use. In one embodiment the entire sensor assembly may be liquid-permeable.

In a preferred embodiment the sensor assembly, or at least the sensing electrode parts thereof, may also

function as a filter.

In one embodiment the device may be a flow-through device in the form of a filter funnel, bottle-top filter, or syringe filter. In the case of bottle-top filters, which are generally known in the art, the sensor assembly, which also functions as a filter, may be manufactured as a separate insert replacing the standard filter membrane. Advantageously, the sensor assembly may be manufactured in disposable, single-use format. Flow of liquid through filter funnels and bottle-top filters may be achieved by gravity flow or by application of an external vacuum. These types filter devices allow analysis of samples of large volume.

Disposable syringe filters are well known in the art and are commonly used in filter sterilisation of small volumes of liquid. A sensor assembly, which also functions as a filter, may be incorporated into a syringe filter in place of the standard filter membrane in order to produce a flow-through analytical device. The device may be formed integrally with a syringe or other variable volume chamber but will most preferably be manufactured as a separate unit, typically a disposable single-use unit, which may be fitted onto the end of a standard syringe when in use, for example using a luer lock connection. Liquid is pushed through the device using the syringe, creating flow through the sensor assembly.

Flow-through filter type devices have the advantage that they are very simple in construction and therefore cheap to manufacture. They are also suited to the analysis of large sample volumes (e.g. of the order of several mls up to litres).

In a second aspect the invention provides lateral flow device or flow-through device for use in methods of electrochemical detection of an analyte, which

device includes a sensor assembly comprising at least one sensing electrode and a reference electrode and means for promoting wicking of liquid through the device when in use, such that liquid flows over and/or through the sensor assembly.

In this embodiment liquid sample is "wicked" through the device, which generally has an open, porous structure. Wicking of the sample through the device provides significant advantages as compared to devices that rely on free flow of liquid over an electrochemical sensor in a flow cell. Moreover, devices which rely on wicking of the sample are more simple, and hence less expensive, to manufacture and easier to operate than flow cell devices.

In a preferred embodiment the means for promoting wicking of liquid through the device comprises an absorbent pad positioned to absorb liquid passing through the sensor assembly when the device is in use.

Preferably at least the sensing electrode parts of the sensor assembly are liquid permeable.

In a preferred embodiment flow-though devices and lateral flow devices according to the first and second aspects of invention may be of laminar construction.

In one embodiment the device may include at least one sample pre-separation layer positioned such that any sample to be tested for the presence of analyte passes through the sample pre-separation layer(s) prior to contact with the sensor assembly when the device is in use. The sample pre-separation layer(s) function to remove unwanted components from the sample under test, prior to contact with the sensing electrode.

In a further embodiment the device may include a conjugate pad containing one or more assay reagents

required for detection of analyte. In this embodiment the conjugate pad is preferably positioned such that any sample to be tested for the presence of analyte passes through the conjugate pad prior to contact with the sensor assembly when the device is in use. If the device also comprises pre-separation layer(s), the conjugate pad is preferably positioned between the between the sample pre-separation layer(s) and the sensor assembly.

The inclusion of a sensor assembly for electrochemical detection provides significant advantages over prior art lateral flow and flow-through devices, which generally rely on a visual/optical determination of the assay result, for example by labelling one of the assay reagents with a coloured label. Electrochemical detection provides for accurate quantitative measurement and also allows for electronic tracking and recording of assay results, whilst retaining the advantages provided by the lateral flow format of simplicity of manufacture and ease of use.

Embodiments of the present invention will now be described, by way of example only, with reference to the accompanying Figures in which:

Figure 1 is a schematic cross-section through one type of flow-through device according to the invention;

Figure 2 is a schematic cross-section through one a further flow-through device according to the invention;

Figure 3 (a) is a schematic cross-section and Figure 3 (b) a plan view of a sensor assembly for incorporation into a multi-unit flow-through device;

Figure 4 is a schematic view of a lateral flow device of laminar construction;

Figure 5 is a schematic view of a further lateral flow device of laminar construction;

Figures 6(a) and 6(b) are plan views of electrode assemblies for incorporation into devices according to the invention. 6(a) single analyte porous electrode assemble; 6(b) multianalyte porous sensor assembly;

Figure 7 is a schematic illustration of a syringe filter type device according to the invention;

Figure 8 is a schematic illustration of a bottle-top filter type device according to the invention.

There is shown in Figure 1 a cross-section of a first flow-through analytical device according to the invention. The main body of the device comprises a housing 1, typically formed of an inert plastics material. The sample to be tested for the presence of analyte 2 is introduced dropwise via an opening 7 in the housing. The device itself is of a multi-layered construction, with each successive layer performing a different function. Sample 2 introduced into the device via opening 7 flows downward through the device and encounters the successive layers.

In the particular embodiment shown in Figure 1 the sample 2 is introduced onto a pre-separation layer 3, which functions to separate undesirable components from the sample prior to detection of the analyte(s) of interest. The precise nature of the pre-separation layer may vary depending on the nature of the sample and hence the nature of the components which it is desired to remove from the sample. Multiple pre-separation layers of differing properties may be included if required to achieve the desired separation. By way of example, in devices for detection of analytes in samples of whole blood the

device may incorporate a single pre-separation layer formed of a material that serves to remove blood cells, allowing filtered plasma to pass through. Suitable materials for separation/filtering of whole blood include, for example, polyester membranes (e.g. Hemacep™) or polyethersulfone membranes.

Material passing through the pre-separation layer 3 next encounters a conjugate pad 4, which may contain assay reagents required for the detection of the analyte of interest. The conjugate pad is typically formed of a permeable material (e.g. borosilicate glass wool). The assay reagents are stored dry in this layer prior to use of the device and are collected by the sample as it flows through.

Material passing through the conjugate pad 4 next encounters the sensor assembly 5, which is a planar structure. The sensor assembly itself comprises at least one sensing electrode which is liquid permeable. Each sensing electrode in the sensor assembly has associated therewith a reference electrode. The sensor assembly may comprise an array of liquid permeable sensing electrodes, each having an associated reference electrode. In certain embodiments, depending on the nature of the electrochemical detection to be performed using the device, each pair of sensing and reference electrodes may have associated therewith a third counter-electrode. Such an arrangement is required for amperometric detection, as described below.

Electrochemical determination of the presence of analyte takes place at the sensor assembly 5, as described below. The sensing electrodes within the sensor assembly function as porous, sieve-like structures, which capture analyte from the liquid flowing through the assembly. An absorbent pad 6 or absorbent material, e.g. cellulose paper, is positioned immediately below the sensor assembly. The

absorbent pad 6 absorbs liquid flowing through the device and thus promotes "wicking" of the sample downward through the multi-layered structure of the device.

In the embodiment shown in Figure 1 the absorbent pad 6, sensor assembly 5, conjugate pad 4 and pre-separation layer 3 may be assembled directly overlaying one another, with no intervening supports.

In Figure 2 there is shown a further embodiment of a flow-through device according to the invention. This device also has a multi-layered construction, similar to the device shown in Figure 1. However, in the device of Figure 1 the pre-separation layer is carried on a separation cartridge 8, which is separate from the main body of the device but co-operates therewith. The bottom surface 9 of cartridge 8 provides a support for the pre-separation layer 3. Bottom surface 9 is liquid-permeable. A top surface 10 of the main body of the device, which overlays the conjugate pad 4, is also liquid permeable. When the device is in use cartridge 8 is placed on the main body of the device. In this position bottom surface 9 of the cartridge 8 and top surface 10 of the main body define a space in which liquid 11 passing through the pre-separation layer may collect.

In the embodiment shown in Figure 2 the sensor assembly 5 is supported on a layer of liquid-permeable analytical membrane 12 (e.g. nitrocellulose). In a particularly preferred construction the sensing and reference electrodes making up the sensor assembly may be printed directly onto the layer of analytical membrane.

Figure 3 shows a multi-unit device made up of multiple flow-through sensors of the type illustrated in Figure 1. Any of the flow-through devices according to the invention may be arranged in groups

to form multi-unit devices. Such multi-unit devices provide for simple manufacture, enabling easy quality control. They may also be adapted for multi-analyte testing, i.e. simultaneous testing of a single sample for multiple different analytes.

Figure 3(a) shows a plan view of a sensor assembly suitable for incorporation into a multi-unit flow-through device. The assembly is substantially planar and is comprised of six separate pairs of sensing and reference electrodes 13, each pair is made up of a substantially circular, liquid permeable sensing electrode 14 surrounded by a circumferential reference electrode 15. In this embodiment each of the sensing and reference electrode pairs has independent electrical connections 16. Each of the sensing electrodes within the assembly may be specific for a different analyte. Sensing electrodes may be rendered specific for different analytes by immobilising different analyte receptor molecules on the surface of the sensing electrode (as described herein). In another embodiment the individual pairs of sensing and reference electrodes may be compartmentalised within the multi-unit device, i.e. separated by barriers or walls to prevent cross-flow of liquids. This will allow different assay reagents to be applied to the compartmentalised electrodes. In one embodiment each of the compartmentalised electrodes may be overlaid with a separate conjugate pad containing different assay reagents. These may in turn be overlaid with a common pre-separation layer.

There is shown in Figure 4 a schematic view of a lateral-flow device of laminar construction. The device is built up in layers upon a backing support 17 of an inert, liquid impermeable material. Sample 2 is introduced onto a pre-separation layer, which is overlays a conjugate pad 4, which in turn overlays a first (upper) layer of analytical membrane 12. The sensor assembly 5 is sandwiched between this first

(upper) layer of analytical membrane and a second (lower) layer of analytical membrane 12' in a region of overlap between the two analytical membranes. The sensor assembly may be printed directly onto the top surface of the lower analytical membrane 12' in the region of overlap. In the vicinity of the sensor assembly the upper analytical membrane 12 may be very thin to allow maximum exposure of sample to the sensing electrodes. An absorbent pad 6 is placed on the lower layer of analytical membrane 12' and functions to promote "wicking" of liquid through the layers of the device. Liquid sample 2 added to the device passes sequentially through the pre-separation layer 3, which functions to remove unwanted components of the sample, into the conjugate pad 4, where it may pick up assay reagent components. The sample then passes over and through the sensor assembly 5 as it is wicked from the top analytical membrane 12 into the bottom analytical membrane 12'. Electrochemical determination of the presence of analyte in the sample takes place at the sensor assembly.

In an alternative embodiment of the lateral flow device, illustrated in Figure 5, the sensor assembly 5 may be printed directly onto a plastic support 17, which is placed beneath the analytical membrane 12 between the conjugate pad 4 and wicking pad 6. The analytical membrane 12 may be very thin over the sensor assembly 5 to allow for maximum exposure of sample analytes to sensing surface. In this embodiment it is not absolutely essential for the sensor assembly to be liquid-permeable. The device functions by lateral wicking of liquid sample through the device and over the sensor assembly. This provides significant advantages over prior art lateral flow devices, which generally rely on a visual/optical determination of the assay result, for example by labelling one of the assay reagents with a coloured label. Electrochemical detection provides for accurate quantitative measurement and also allows for

electronic tracking and recording of assay results, whilst retaining the advantages provided by the lateral flow format of simplicity of manufacture and ease of use.

Lateral flow devices provide inherent sample preparation, i.e. removal of unwanted components from the sample, such as blood cells or flocculates or particles, as it passes through the pre-separation layer. Such devices also provide the ability to select particular chemical characteristics by inclusion of selective membranes between sample addition and the sensor assembly, and simplicity of providing dried assay reagents on specific conjugate pads in the path of the sample being "wicked" through the device. In devices that rely on wicking the rate of flow of sample through the device may be controlled just by varying the nature and porosity of the materials making up the layers of the device (generally specialist papers). The path of liquid through the device may be controlled very simply, for example by printing impermeable ink onto the wicking pad.

Figure 6(a) shows a single analyte porous electrode assembly for incorporation into devices according to the invention. The assembly is composed of a substantially circular sensing electrode, a reference electrode 15 and external electrical connections. Figure 6(b) shows a multi-analyte sensor assembly, comprising a number of sensing electrodes and a single reference electrode. In this embodiment the sensing electrodes are formed in a substantially grid like arrangement positioned substantially centrally in the membrane. The reference electrode is formed as a spot or line near the sensing electrodes. Other arrangements of the sensing and reference electrodes are envisaged.

There is shown in Figure 7 a further embodiment

of the invention which is a syringe filter type device consisting of a filter housing 18, which will generally be formed of an inert plastic material, having a liquid inlet 19 and filter outlet 20. In a preferred embodiment the liquid inlet 19 may be a luer lock outlet, enabling secure connection to a luer lock syringe. The liquid outlet will preferably be a simple luer slip outlet. The filter housing 18 is substantially disc-shaped, and is substantially identical to the disposable syringe filters known in the art for use in filter sterilisation (e.g. those commercially available from Millipore Corporation.). A planar, liquid-permeable sensor assembly 5 is located centrally in the housing, perpendicular to the flow of liquid through the device. The sensor assembly 5 is entirely enclosed within the housing 18, but external electrical connection 16 is provided from the sensor assembly to enable connection to an external electrical measuring device (not shown) and, if required, an external power source (not shown). When the device is in use liquid sample is introduced into the housing via liquid inlet 19 and flows through the sensor assembly 5. At least the sensing electrode portion(s) of the sensor assembly are liquid permeable. Any analyte present in the liquid sample is captured as it flows through the sensing electrode(s) of the sensor assembly.

The device may further comprise one or more pre-separation layers located above the sensor assembly, such that liquid flowing through the device passes through the pre-separation layer prior to contact with the sensor assembly. The pre-separation layer functions to remove unwanted components or debris from the sample which might otherwise clog or impair the function of the sensor assembly. A conjugate pad containing assay reagents required for detection of a particular analyte may also be included, positioned above the sensor assembly such that liquid flowing through the device passes through the conjugate pad

and picks up the reagents prior to contact with the sensor assembly. The syringe filter device is conveniently manufactured in a disposable, single-use format. In this embodiment the sensor assembly 5 will be fitted into a filter housing 18 during manufacture and any processing of the sensor assembly required to render it specific for a particular analyte (e.g. polymer coating, coating with capture receptors specific for a particular analyte) may be carried out before it is assembled into the filter housing. The devices may this be supplied in a ready-to-use format, having specificity for a particular analyte. The syringe filter device is intended for use in conjunction with a syringe, or other variable volume chamber, by means of which liquid sample may be introduced into the filter device.

There is shown in Figure 8 a further embodiment of a filter-type device, which is in the form of a conventional filter funnel or bottle-top filter. The device consists of a filter funnel housing 21, which may be formed of any inert material, e.g. plastic, inert polymer, glass, etc. A planar, liquid-permeable sensor assembly 5 is situated in the neck of the filter funnel. External electrical connection to the sensor assembly is provided 16 to enable connection to an external measuring instrument and, if required, an external power source. The sensor assembly 5 is designed to fit snugly within the neck of the filter funnel housing 21, such that all liquid passing through the funnel is forced to pass through the filter. One or more pre-separation layers may be included to remove unwanted components or debris from the liquid sample which would otherwise clog or impair the function of the sensor assembly. A conjugate pad containing assay reagents required for detection of a particular analyte may also be included, positioned above the sensor assembly such that liquid flowing through the device passes through the conjugate pad and picks up the reagents prior to contact with the

sensor assembly. In a preferred embodiment, the sensor assembly 5 may be fitted into the filter funnel 21 during manufacture and supplied as a ready-to-use unit. Filter funnels with an integral sensor assembly may be supplied as disposable, single use units. Alternatively, the sensor assembly 5 and filter funnel housing 21 may be supplied as separate components, to be fitted together prior to use. This enables the filter funnel housing to be re-used, fitting a fresh sensor assembly prior to each use. Conveniently, the filter funnel housing 21 may be manufactured as a two-part filter assembly, consisting of separate, co-operating funnel top and funnel stem components that are sealed together (e.g. with a clamp) prior to use. Such two-part filter assemblies are well known in the art. The sensor assembly may be supplied as a separate component and sealed between with funnel top and funnel stem prior to use. This arrangement is convenient as it allows separate processing of the sensor assembly (e.g. polymer coating, coating with capture receptors specific for a particular analyte) before it is assembled into the filter device. Filter funnel devices according to the invention may be adapted for use in conjunction with vacuum filtration apparatus. Filter funnel devices are suited to analysis of liquid samples of very large volumes.

In the syringe filter and filter funnel embodiments the sensor assembly also functions as a filter, meaning that it captures and retains at least one component (at least the analyte of interest) from liquid flowing through the device when in use, whilst allowing passage of the bulk flow of liquid.

Sensor assemblies for incorporation into flow-through and lateral flow devices according to the invention may be manufactured in a variety of ways, for example using screen-printed liquid permeable membranes or sandwiches of sieve-like metallic sensors between liquid-permeable membranes. Arrays of

reference and working electrodes may be assembled by screenprinting, etching and gold plating, or using thin film technologies, which are generally known in the art. Conveniently, electrode arrays may be manufactured on a flat porous sheet or substrate (e.g. nitrocellulose membrane), which may then form the central portion of the device. Independent electrical connections 16 are provided from each of the sensing and reference electrodes through to the side of the device. The tracks can be connected to a single edge connection point/plug, which enables the whole unit to be plugged into a detection instrument.

For convenience the sensor assembly in the device can be linked to a measuring instrument by means of a special holder equipped with electrical contacts for connection of the sensing electrode(s) and reference electrode(s) and connected to the measuring instrument by a cable or other means. A holder integral with the measuring instrument may also be used, making it possible to miniaturise the measuring system in terms of its overall dimensions.

In a further embodiment each arrangement of sensing (multi or single) and reference electrodes incorporated into the sensor assembly may have associated therewith a third counter-electrode. The combination of sensing and reference electrodes is sufficient to allow potentiometric measurement, but the inclusion of a third electrode enables other forms of electrochemical analysis to be carried out, for example amperometric measurement. In one embodiment the third counter-electrode and sensing electrode may be formed as interdigitating electrodes, or as closely separated parallel lines in a variety of shapes. Such arrangements enable alternative forms of electrochemical analysis to be carried out, for example amperometry, impedance, voltammetry, polarography, chronoamperometry, chronocoulometry and chronopotentiometry.

The sensing electrodes may be essentially any suitable electrodes comprising a conductive or semi-conductive layer. Suitable electrodes include standard potentiometric electrodes possessing metallic or quasi-metallic conductivity that are stable in aqueous media, e.g. gold and other noble metal electrodes. In a preferred embodiment the sensing electrode may comprise a membrane support or substrate with a conductive layer (preferably gold or other noble metals) electrochemically plated or directly screen-printed onto the membrane.

Sensing electrodes can be used without any further processing, or they can be electrochemically coated, preferably on at least a portion of at least one major surface, with a layer of electroconductive polymer, e.g. polypyrrole, polythiophene, polyfuran, polyaniline, etc.

Preferred types of reference electrodes include the regular Ag/AgCl or calomel electrode.

In embodiments wherein one or more of the sensing electrodes are coated with an electroconductive polymer film as part of the sensing element, a thin film is deposited onto the surface of an electrically conductive electrode by electrochemical synthesis from a monomer solution. The electrically conductive electrode is preferably a standard potentiometric electrode possessing metallic or quasi-metallic conductivity that is stable in aqueous media. Electrodeposition of the electroconductive polymer film may be carried out using a solution containing monomers, a polar solvent and a background electrolyte, according to procedures, which are known in the art (see WO 00/11473 and WO 98/37409). Pyrrole is the preferred monomer, but other monomers such as thiophene, furan or aniline may also be used. Combinations of two or more of these monomers may also

be used, leading to the production of conductive co-polymers.

The preferred supporting electrolyte is sodium dodecylsulphate but other electrolytes may be used. The electrolyte also serves as a doping agent. Deionised water is preferably used as the polar solvent.

The electrochemical polymerisation solution generally consists of an aqueous solution of monomers and supporting electrolyte. However, other components may be added to the polymerisation solution such as, for example, components that provide specific functional groups that can be used as linkers for bioreceptors or for chemical modification of the sensor surface (see WO 00/11473 and WO 98/37409).

Electrochemical polymerisation is typically carried out in a three-electrode cell comprising of sensor electrode(s) to be coated, the auxiliary electrode and the reference electrode. Suitable assemblies have been described in the prior art (see WO 00/11473 and references contained therein). Multiple sensor electrodes can be combined in a block with one electrical contact. An entire array of sensing electrodes may be coated in a single polymerisation reaction. This may use either a single auxiliary electrode or one auxiliary electrode per pair of sensing and reference electrodes. For example, arrays that include a third counter-electrode associated with each pair of sensing and reference electrodes (e.g. for amperometric analysis) may be coated using the third counter-electrode as the auxiliary electrode. In a further arrangement, the reference electrodes may be used as the auxiliary (counter) electrode for the polymerisation step. The reference electrode may be manipulated to function as an auxiliary electrode for polymerisation, for example with Ag/AgCl electrodes the ratio of Ag/AgCl may be

temporarily altered such that it functions as an auxiliary electrode for polymerisation, and then restored to function as a reference electrode after polymerisation.

As is well known to persons skilled in the art, electroconductive polymers are often doped at the electrochemical synthesis stage in order to modify the structure and/or conduction properties of the polymer. The ease with which ion exchange takes place and the rapidity with which ion equilibrium is attained for electroconductive polymers immersed in a solution are essentially dependent on the size of the dopant anion introduced at the electrodeposition stage: the larger the ionic radius of the dopant anion, the more readily ion-exchange reactions take place and the more rapidly a state of equilibrium is reached. This is directly linked to the value and rate of change of the potential of the "metal electrode - electroconductive polymer" system in response to variation in the ion composition of the solution [6]. The type of the response (anionic, cationic, redox) and its rate can be determined during the polymerisation [5, 6].

A typical dopant anion is sulphate (SO_4^{2-}), which is incorporated during the polymerisation process, neutralising the positive charge on the polymer backbone. Sulphate is not readily released by ion exchange and thus helps to maintain the structure of the polymer.

It is possible to provide potentiometric sensitivity of the electroconductive polymer to one particular cation or anion. The ions of background electrolyte are immobile and able to react specifically with the ion of interest, e.g. calcion (cation), which specifically reacts with calcium and gives precipitated product (salt).

For redox and pH sensitive sensors it is

preferred to use a salt whose anions have a large ionic radius as the background electrolyte when preparing the electrochemical polymerisation solution. In this case ion response is minimised and redox or pH response predominate, potentiometric response is provided by electron exchange between the polymer film and surrounding solution.

Suitable salts whose anions have large ionic radius include sodium dodecyl sulphate and dextran sulphate. The concentration of these salts in the electrochemical polymerisation solution is varied according to the type of test within the range 0.0001 - 0.05 M.

Redox response can be increased by incorporating into the polymer dopant ions, which can change their redox state due to the changes in the surrounding solution giving the sensor the additional change in redox state. The dopant should be in reduced form if one of the solution components is oxidized and vice versa. $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ can be given as an example for both cases. The concentration of these electrolytes in the electrochemical polymerisation solution can be varied within the range 0.001 - 0.1 M to meet specific requirements of the test.

The surfaces of electroconductive polymer-coated electrodes can be further modified by coating with biological molecules or other functional groups which can be used as linkers for biological molecules or for chemical modification of the sensor surface (see WO 00/11473, WO 98/37409 and WO 96/02001).

Biological molecules, for example molecules capable of specifically binding to the analyte under tested, can be immobilised onto a sensor using well known techniques for solid phase coating. Biological molecules may be incorporated into the electroconductive polymer during the polymerisation

reaction, or they may be adsorbed onto the surface of the coated sensing electrode in a separate modification step after the polymer coating step, or they may be covalently linked to the polymer coating (see WO 00/11473, WO 98/37409 and WO 96/02001).

In a particular embodiment the biological molecules may be "adaptor molecules" which enable the attachment of further molecules, or even whole cells, to the surface of the sensor via a specific binding interaction. With the selection of appropriate adaptor molecules it is also possible to manufacture "universal" sensing electrodes containing adaptor molecules capable of binding to a whole range of different receptor molecules. Specificity for the analyte under test is conferred on the "universal" sensing electrode simply by binding to the adaptor molecules receptors of the appropriate specificity.

The proteins avidin and streptavidin are preferred for use as adaptor molecules. Investigations carried out by the authors of the present invention have shown that avidin and streptavidin immobilised in an electroconductive polymer film, retain their native properties for an extended period of time (at least one year and possibly longer) and can be used throughout this period to link with biotin conjugated receptors. Techniques that allow the conjugation of biotin to a wide range of different molecules are well known in the art. Thus sensing electrodes with immobilised avidin or streptavidin can easily made specific for a given analyte merely by binding of the appropriate biotinylated receptors via biotin/avidin or biotin/streptavidin binding interactions.

Although avidin and streptavidin are the preferred adaptor molecules it is within the scope of the invention to use alternative adaptor molecules, for example protein A, protein G, lectins and FITC.

The incorporation of adaptor molecules enables other biomolecules or whole cells to be attached to the surface of the sensing electrode, for example via protein A/antibody, protein G/antibody, FITC/anti-FITC or lectin/sugar binding interactions. Biological molecules or whole cells may alternatively be absorbed directly or covalently bound to the polymer-coated sensor surface.

Sensing electrodes coated with a layer of electroconductive polymer may also be modified by further coating with biological cells immobilised in, adsorbed to or attached to the layer of electroconductive polymer. The cells can be any type of prokaryotic or eukaryotic cell that it is desired to study. In this embodiment the electroconductive polymer layer performs a dual function, serving both to bind the cells to the surface of the sensing electrode, and to render the sensing electrode sensitive to variations in the composition of a bathing electrolyte solution (i.e. when liquid is passed over or through the sensor assembly). In particular, changes in the composition of the electrolyte solution that affect the redox composition of the electroconductive polymer result in a corresponding change in the steady state potential of the sensing electrode.

Biological cells may be adsorbed directly onto the surface of a polymer-coated sensing electrode, or the surfaces of polymer-coated sensing electrodes can be modified by coating with biomolecules or other functional groups which can in turn be used to link biological cells to the electrode surface. In a particular embodiment the biomolecules may be "adaptor molecules" which enable the attachment of cells, or further molecules (e.g. receptors) capable of binding cells, to the surface of the sensor via a binding interaction.

The electrochemical prepared flow devices can be used in the same manner as normal rapid diagnostic flow devices. The only difference is in that these are electronic and possibly multi-analyte detection systems, being electrochemical (e.g. potentiometric, or amperometric if a three electrode array format is employed) with associated benefits of quantitation and multiple analyte detections with one device.

The flow devices of the invention are inexpensive to manufacture and so for convenience can be produced in a disposable format, intended to be used for a single electrochemical detection experiment or a multianalyte detection measurements and then thrown away.

Electrochemical flow devices according to the invention may be used in methods of electrochemical analysis of analytes, such as, for example the methods described in the applicant's published International patent application WO 00/11473.

The devices may be adapted to perform quantitative assays for a wide range of analytes. In particular, the device may be used to perform electrochemical binding assays in sandwich or competitive formats.

A typical sandwich assay requires a "capture" binding agent having specificity for the analyte of interest (e.g. an antibody or receptor). This capture binding agent is typically immobilised on the surface of the sensing electrode(s), for example it may be adsorbed to the surface of a polymer-coated electrode or incorporated into the polymer coating. When the device is in use, any analyte present in the sample flowing through the sensing electrode will bind to the immobilised capture binding agent. Electrochemical detection of bound analyte requires a secondary binding agent, capable of binding to the analyte at a

site spatially distinct from the site of binding to the capture agent. The secondary binding agent is typically conjugated with a charge or enzyme label that permits electrochemical detection, as described in WO 00/11473.

The affinity reaction steps of the above-described assay are equivalent to a standard sandwich binding assay well known to those skilled in the art. The sandwich format of analysis is particularly useful for the detection of polyvalent antigens, in which case the capture agent and labelled secondary binding agent used in the test are preferably antibodies that bind to different, spatially distinct epitopes on the antigen. The sandwich format may also be used where the antigen carries two or more identical epitopes, which are spatially separated. In this latter case, the capture agent and labelled secondary binding agent used in the test may be antibodies of identical specificity.

A typical competitive binding assay also requires a "capture" binding agent having specificity for the analyte of interest, which is typically immobilised on the surface of the sensing electrode(s), as described for the sandwich assay. When the device is in use, any analyte present in the sample flowing through the sensing electrode will bind to the immobilised capture binding agent. Competing molecules (e.g. an analogue of the analyte) also capable of binding to the capture agent compete with the analyte for binding to the available capture agent. The competing molecules are labelled with a label that enables electrochemical detection, e.g. a charge or enzyme label.

In this competitive electrochemical assay the competing molecules may be labelled analyte or labelled structural analogs of the analyte that are capable of binding to the same analyte binding site on the immobilized/adsorbed capture agent. The use of

labelled analyte as the competing molecule is particularly preferred for the detection of small analyte molecules. Alternatively, the competing molecule may bind to a different site on the immobilized/adsorbed capture agent. For example, if the immobilized capture agent is an antibody, the competing molecule could be an anti-immunoglobulin antibody (preferably Fab-specific) or even an anti-idiotype antibody of the appropriate specificity. Competitive detection methods are usually dependent on there being an excess of capture sites on the surface of the sensing electrode. Those capture agents which do not bind analyte will be available for binding to the competing molecule. Assuming that the total number of capture binding sites remains constant, the amount of bound competing molecule will be inversely proportional to the amount of analyte present.

In order to transduce the chemical signal associated with the concentration of the analyte into a measurable electrical signal it is preferred that one of the assay reagents is labelled with a charge or enzyme label (see WO 00/11473). Suitable charge labels include gold, ferrocene and latex microspheres. The magnitude of the charge on the charge label affects the redox composition of the electroconductive polymer coating on the sensing electrode, resulting in a detectable change in potential difference between the sensing electrode and the reference electrode.

Suitable enzyme labels include enzymes capable of converting a substrate which directly affects the redox composition of the electroconductive polymer coating of the sensing electrode into a product which has no detectable effect on the redox composition of the electroconductive polymer; or enzymes capable of converting a substrate which has no detectable effect on the redox composition of the electrochemical polymer coating of the sensing electrode to a product capable of directly or indirectly affecting the redox

composition of the electroconductive polymer, e.g. peroxidase.

The use of charge or enzyme labels will be further understood with reference to the applicant's published International patent application WO 00/11473, the contents of which are incorporated herein by reference.

An example protocol for potentiometric ELISA detection comprises;
addition of sample to pre-separation layer, this is separated and cleaned up and passes into the conjugate pad where the labelled assay reagents are present. These are resolubilised in the sample and taken along into the analytical membrane past the prepared potentiometric sensor assembly, which captures the analyte complexes of interest. Subsequently the first measuring solution is applied which washes out the sample and any unbound complexes. A first measurement of electrochemical potential difference is taken. The second measuring solution is applied, which replaces the environment over the immobilised immunocomplex and initiates the enzymic reaction, which is subsequently detected by the sensor. The change in potential is proportionally to the concentration of analyte in the sample.

Claims:

1. A lateral flow or flow-through device for use in methods of electrochemical detection of an analyte, which device includes a sensor assembly comprising at least one liquid-permeable sensing electrode and a reference electrode.

2. Device according to claim 1 wherein the sensor assembly functions as a filter.

3. Device according to claim 2, which is a syringe filter device.

4. Device according to claim 2, which is a filter funnel device.

5. Device according to claim 4, which comprises a filter funnel housing and sensor assembly supplied as separate components.

6. A lateral flow device or flow-through device for use in methods of electrochemical detection of an analyte, which device includes a sensor assembly comprising at least one sensing electrode and a reference electrode and means for promoting wicking of liquid through the device when in use, such that liquid flows over and/or through the sensor assembly.

7. Device according to claim 6 wherein the means for promoting wicking of liquid through the device comprises an absorbent pad positioned to absorb liquid passing over and/or through the sensor assembly when the device is in use.

8. Device according to claim 6 or claim 7 wherein the sensing electrode(s) is/are liquid permeable.

9. Device according to any one of claims 1 to 8

which further comprises at least one sample pre-separation layer positioned such that liquid sample to be tested for the presence of analyte passes through the sample pre-separation layer(s) prior to contact with the sensor assembly when the device is in use.

10. Device according to any one of claims 1 to 9 which further comprises a conjugate pad containing one or more assay reagents for detection of an analyte, wherein the conjugate pad is positioned between the sensor assembly and any sample pre-separation layer(s) included in the device such that liquid sample to be tested for the presence of analyte passes through the conjugate pad prior to contact with the sensor assembly when the device is in use.

11. Device according to any one of claims 1 to 10 wherein the sensor assembly is supported on an analytical membrane layer.

12. Device according to any one of claims 1 to 11 wherein the sensing electrode(s) comprise an electroconductive electrode coated with a layer of electroconductive polymer.

13. Device according to claim 12 wherein the sensing electrode(s) are further coated with biomolecules immobilised in, adsorbed to or attached to the layer of electroconductive polymer.

14. Device according to claim 13 wherein the electrode is still further coated with cells attached to the biomolecules immobilised in, adsorbed to or attached to the layer of electroconductive polymer.

15. Device according to any one of claims 12 to 14 wherein the layer of electroconductive polymer has been doped with mobile anions of large ionic radius.

16. Device according to any one of claims 12 to

14 wherein the layer of electroconductive polymer has been doped with anions that are immobile in the electroconductive polymer.

17. Device according to any one of claims 12 to 14 wherein the layer of electroconductive polymer has been doped with anions carrying a large amount of negative charge.

18. Device according to any one of claims 12 to 14 wherein the layer of electroconductive polymer has been doped with anions capable of specific interaction with cations.

19. Device according to any one of claims 12 to 14 wherein the layer of electroconductive polymer has been doped with anions capable of changing their redox state.

20. Device according to any one of claims 1 to 19, which further comprises a counter-electrode associated with each of the sensing electrodes.

21. Method for detecting the presence of an analyte in a liquid sample comprising passing said liquid sample through a device according to any one of claims 1 to 20, whereby the analyte is directly or indirectly detected by the sensor assembly.

FIG. 1.

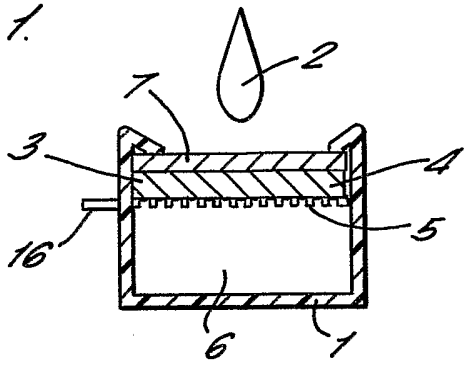


FIG. 2.

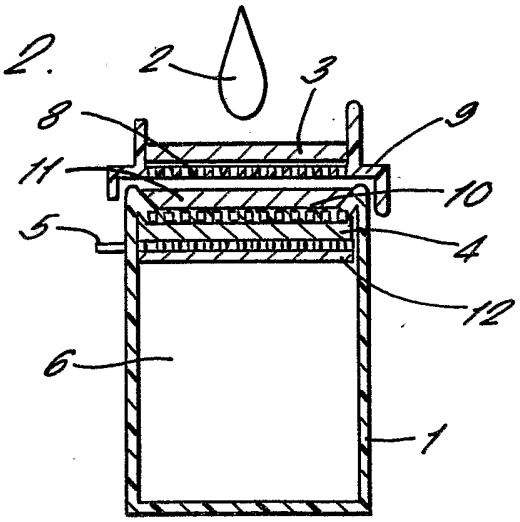


FIG. 3a.

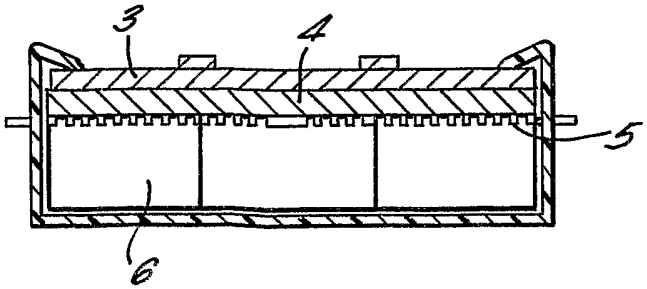


FIG. 3b.

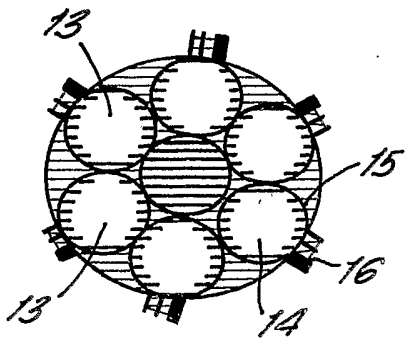


FIG. 4.

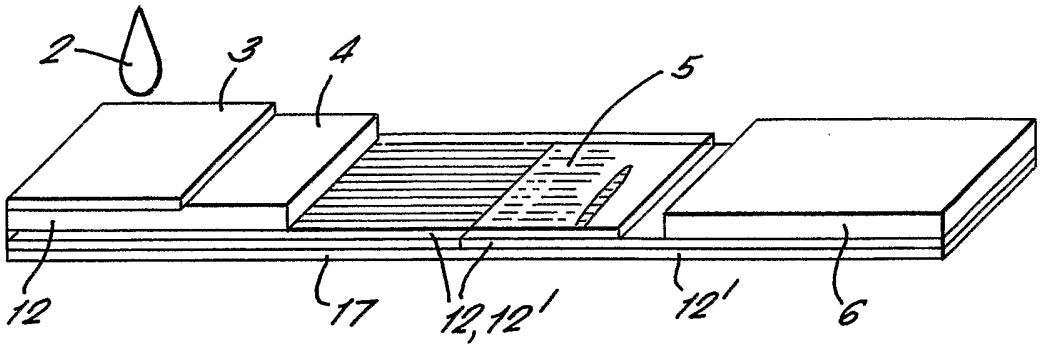


FIG. 5.

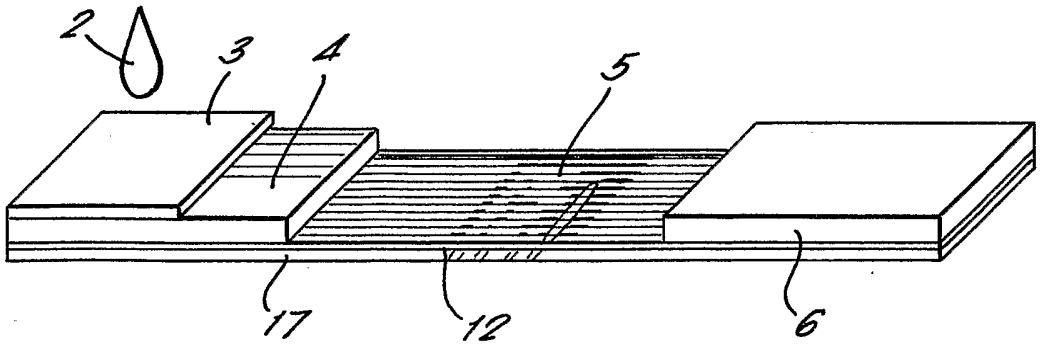


FIG. 6.

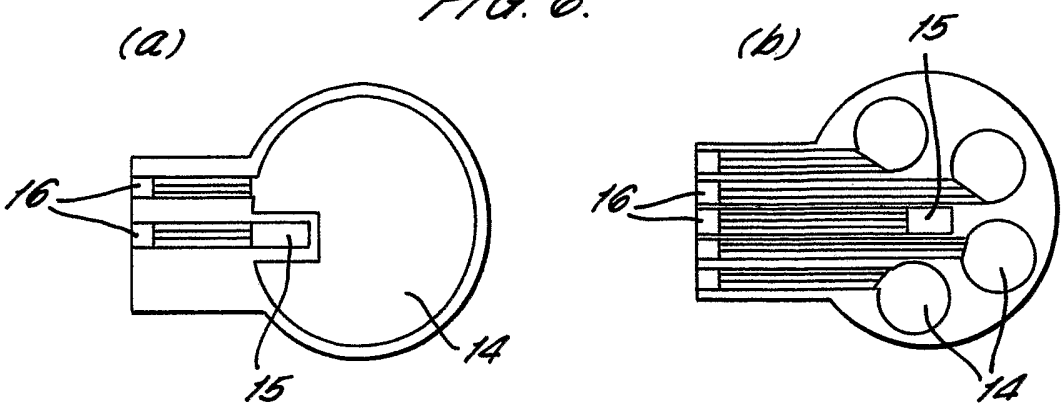
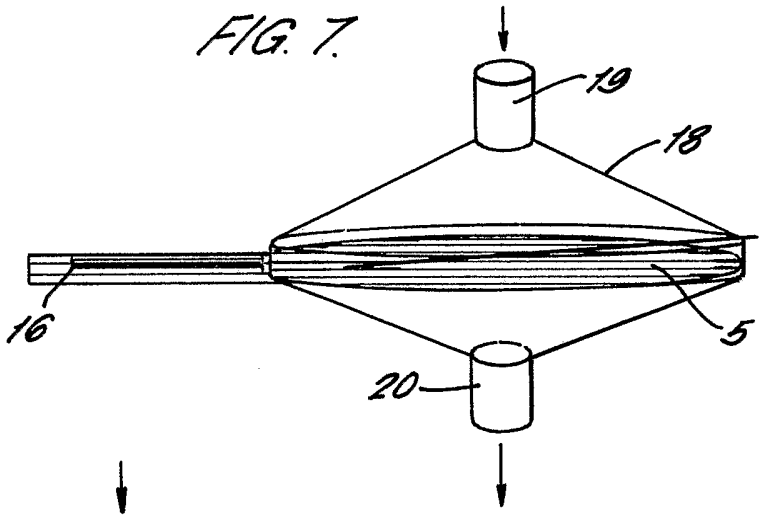


FIG. 7.



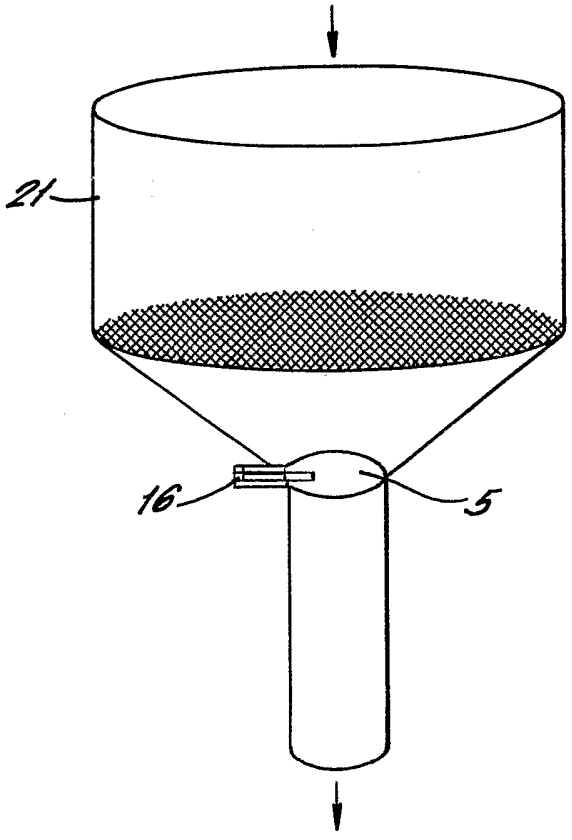


FIG. 8.