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(54) **MICROSCALE TOTAL ANALYSIS SYSTEM**

nIKROMASSSTABGETREUE VORRICHTUNG FÜR CHEMISCHE ANALYSE

SYSTEME D'ANALYSE TOTALE SUR MICROECHELLE

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Description

[0001] This invention relates to apparatus for detecting the presence of a target species in an aqueous sample, and also to apparatus for determining the concentration and reaction kinetics of target species. The invention is applicable to the monitoring of many different molecular interactions, in particular molecular recognition between an immobilised affinity partner and a species in solution, such as immunoglobulin/antigen interaction, DNA hybridisation, haptamer-protein interaction, drug and virus detection and high throughput screening of synthetic molecules.

[0002] As many affinity complexations between two reaction partners are diffusion controlled, the time needed to reach reaction equilibrium is directly dependent on the mass transport of the molecule. The diffusion time of a molecule in a solution is proportional to the square of the path length; typically a small molecule needs less than one second to diffuse through 10 μm while it needs two hours to traverse one millimetre. In order to decrease the equilibrium time of the reaction, the chemical partners must therefore be placed as close as possible to each other; by reducing the reactor size to microdimensions, immobilising one partner on the surface of the reactor and filling the reactor with the second partner, the equilibrium time can be dramatically decreased.

[0003] The use of microreactors not only enhances the speed of affinity assays, but also facilitates the obtaining of information concerning reaction kinetics, which is important in the understanding of the thermodynamic stability of complexes. The affinity constant K_d is the ratio between the forward and reverse reaction rate constants k_+ and k_- , which represent the association and the dissociation constants respectively. A strong complexation is characterised by a very fast association and a very slow dissociation, which in the particular case of sorbent affinity assays are adsorption and desorption from the surface of the microreactor. The understanding of these thermodynamic properties can be used for the study of cross-reactivity between several antigens or of non-specific adsorption of a matrix element during an affinity assay. By modulating the incubation time of the solution, the complexation of higher affinity partners is favoured and the non-specific adsorption is then reduced to a minimum. This fact may be an important factor in the decrease of the detection limit in immunosorbent assays due to the minimisation of the background signal. This method can also be applied to monitor the adsorption of antigens of different molecular weight.

[0004] As the diffusion coefficient of a molecule is proportional to its mass, the diffusion time of the molecule through the reaction chamber is different for small and large molecules. In the case of molecules of different molecular weight with the same epitope, (e.g. fibrine degradation products), the K_d may be the same for all molecules, whereas the diffusion coefficient is different

for each of them. When a kinetics experiment is carried out, the smaller molecules will quantitatively reach the antibodies before the larger ones. The monitoring of the signal resulting from the affinity reaction as a function of time can thus deliver useful kinetics information, which can help in understanding the degradation process. These kinetics events can be followed by modulating the residence time of molecules in contact with their reaction partners, which can most readily be achieved by immobilising an antibody on the walls of a series of microreactors and by incubating different solutions of the analyte of interest for different periods of time.

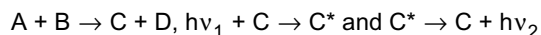
[0005] In the past, analytical procedures of the type described above (such as enzyme-linked immunosorbent assays - ELISAs) have been performed using microtiter plates and have been relatively slow. In recent years, great efforts have been made to reduce the size of analytical devices to micrometer scale with the effect of reducing reaction times. These miniaturised systems have been termed "microscale total analysis systems" (μ -TAS)¹, and they have already been recognised as convenient means of manipulating and analysing small sample quantities²⁻⁸. Most μ -TAS devices to date have been produced by photolithography, wet chemical etching or thin film deposition on substrates such as glass, quartz and silicon^{9, 10}. In order to decrease production costs, plastic substrates have also been micromachined using either silicone rubber casting¹¹⁻¹⁴ injection moulding¹⁵ embossing^{16, 17} or laser photoablation¹⁸. These structures are planar devices with channels of micrometre size that are often sealed by thermal or anodic bonding to a glass cover. Interconnected channels may be fabricated easily, which makes possible the rapid separation and reactions in volumes of few a picoliters. Other advantages of μ -TAS are the reduction of sample and reagent consumption and the increase of precision and reproducibility relative to bench scale apparatus^{21, 22}.

[0006] Competitive immunoassays have also been performed on micro-chips²³⁻²⁵, but the micro-channels have only been used to electrophoretically separate free and bound forms of antigen or antibody. In these assays, antibody and labelled antigen are added in specific quantities to the sample to be analysed. The sample is then incubated with a mixture of the labelled and native antigens that compete for a limited number of antibody binding sites. The micro-channel is then used to separate the free labelled antigen from the complex by capillary electrophoresis, and quantitation is performed by luminescence (fluorescence or chemiluminescence) at the end of the separation channel. The amount of free labelled antigen measured is then related to the analyte concentration in the sample using a previously determined calibration curve. In this type of assay it is essential to avoid adsorption of a reaction partner on the micro-channel walls.

[0007] Another type of immunoassay device has been developed for simultaneous analysis of multiple

samples²⁶. In this case, biotin-labelled antibodies are patterned onto an avidin-coated waveguide so as to form an array of six vertically oriented stripes of captured antibodies immobilised on the waveguide surface by avidin-biotin bridges. Samples are then analysed using a sandwich immunoassay format by patterning another array of six horizontally oriented lines containing the corresponding fluorescent-labelled antigen at various concentrations. Fluorescent complexes on the surface of the waveguide are then excited by a diode laser, and the fluorescence intensities of the 36 square dots is collected by a CCD camera. This immunosensor allows the analysis of multiple samples in parallel and simultaneous detection of more than one analyte per sample.

[0008] Numerous analytical methods utilise luminescence to detect an analyte of interest. Luminescence is the generic term referring to the emission of an electromagnetic radiation (UV, visible or IR) by an excited molecule that relaxes to its ground state, can be induced by photoexcitation (photoluminescence) or by a chemical reaction (chemiluminescence and electrochemiluminescence). Chemifluorescence (CF) is another class of luminescent reactions which combines the reaction mechanisms of both PL and CL. In this case, a fluorogenic substrate A is converted to a fluorescent product C by chemical reaction, and luminescence is generated by excitation of this product:



[0009] For analytical purposes, one of the reactants of the assay system that is capable of generating luminescence can be attached to a molecule in order to "label" it specifically. The presence or absence of an observable label attached to one or more of the binding materials is then used as an indicator of the existence of an analyte of interest. A large body of experiments has been developed to detect and quantitate trace amounts of pharmaceuticals, microorganisms, hormones, viruses, antibodies, nucleic acids and other proteins by such methods. In clinical diagnostics for instance, competitive and sandwich immunoassays using luminescence detection are now used on a routine basis^{27, 28}.

[0010] In enzyme-mediated immunoassays, a molecule is labelled with an enzyme that catalyzes the luminescence reaction. Typical examples are the detection of immunoreagents labelled with Horse Radish Peroxidase (HRP) or Alkaline Phosphatase (ALP) which, in the presence of hydrogen peroxide and hydroxide ions, respectively facilitate the oxidation of luminol and dioxetanes and the hydrolysis of phosphate-containing reagents. Similarly, ALP has been used in CF assays to cleave a phosphate group from a fluorogenic substrate to yield a highly fluorescent product²⁹.

[0011] Luminescence assay methods are widely used in the analysis of peptides, proteins, and nucleic acids.

CL has been shown to be a highly sensitive detection method in both flow injection analysis (FIA) and high-performance liquid chromatography³⁰⁻³², and it has also been employed in capillary electrophoresis (CE)^{33, 34} for the detection of amino acids neurotransmitters³⁵, rare-earth metal ions³⁶ or labelled proteins³⁷. However, it is in immunoassays that luminescence is the most commonly used detection method^{27, 34, 38-43}.

[0012] Among prior art methods for the measurement of enzymatic reaction rate, US 4,621,059 discloses a method in which the light emitted by a luminescent substance flowing through a capillary column and reacting with an immobilised enzyme is collected through a plurality of optical fibers that are arranged along the longitudinal direction of the column in order to determine the enzyme activity or the quantity of analyte of interest from the distribution of the luminescence intensity.

[0013] US 5,624,850 describes a method for performing immunoassays in capillaries in which fluorescence is used to detect an analyte of interest in translucent capillaries having an inner diameter from -0.1 µm to 1.0mm. Similarly, homogeneous chemiluminescence immunoassays can be carried out, for example as described in US 5,017,473, in which a light absorbing material and a luminescent labelled tracer are incubated with the analyte/anti-analyte complex, so that all the emitted light is absorbed by the light-absorbing material except that associated to the bound tracer.

[0014] A method is disclosed in US 5,585,069 in which two or more samples are processed in parallel in a system comprising a plurality of wells that are connected by one or more channels to move a sample from one well to the other using mechanical or electrokinetic pumping. In this apparatus, the channels are simply used as connections between two wells, and are not used as reaction or detection chambers.

[0015] One of the main difficulties associated with the use of µ-TASs is the proper mixing of reagents, due to the low Reynolds numbers of the flows. Another difficulty resides in the accurate timing of fluid entry, which is essential for kinetics studies. In addressing these problems, the applicants have found that fluid entry into a reaction chamber (for example a microchannel) can be accurately be controlled by means of a hydrophobic gate.

[0016] The present application therefore provides, in one aspect, apparatus comprising: at least one reaction chamber; at least one fluid inflow channel communicating with the or each reaction chamber; and gate means adapted to prevent passage of aqueous fluid through the fluid inflow channel(s) into the reaction chamber(s), until such fluid is acted upon by a fluid entry force; wherein the gate means comprises at least a portion of the or each fluid inflow channel having a hydrophobic inner surface.

[0017] Preferably, the apparatus has a plurality of reaction chambers, which take the form of microchannels,

each having an associated fluid inflow channel. Alternatively, a plurality of microchannels may be served by a single inflow channel, feeding into a common conduit communicating with the microchannels. In some preferred embodiments, the fluid entry force is provided by aspiration means connected to a common conduit communicating with each microchannel at its end distal the inflow channel. In other embodiments, the apparatus comprises a rotatable support member and the fluid entry force is provided by centrifugal force upon rotation of the substrate. Conveniently, the support member may form the substrate of the microchannel apparatus, with the microchannels being arranged generally radially. Alternatively the rotatable support member may serve as a support for one or more devices having parallel microchannels.

[0018] The advantage of a common source of fluid entry force for all of the microchannels is that simultaneous filling may be ensured, the fluid samples being prevented from entering the microchannels by the hydrophobic gate means until the fluid entry force is applied. Furthermore, the degree of fluid entry force may also readily be controlled, to ensure rapid filling of the microchannels, and adequate mixing. The microchannels may also be emptied in an efficient and rapid manner, by application of an increased force to the fluid in the channels, for example by increasing the degree of aspiration, or by increasing the rate of rotation of the rotation support member. An exact end point of an assay may thereby be achieved. In many instances it is advantageous for the sample to be expelled before monitoring for bound target species.

[0019] If desired, a liquid reagent or a washing fluid may be supplied in a sealed cavity forming a reservoir, there preferably being one such reservoir per microchannel. The reservoirs may be arranged to communicate with their respective microchannels via normally closed valves, and may be caused to expel their contents through such valves when acted upon by respective pistons. Alternatively, there may be a single reservoir, communicating via a normally closed valve with a common conduit feeding all microchannels.

[0020] Detection of target species with the microchannels may be achieved by conventional means. For example, to permit electrochemical detection, preferred embodiments of apparatus are constructed so that at least a portion of the surface of the microchannel is formed of an electrically conductive material. This may for example be a conductive polymer material or an electrode. In some embodiments, at least a portion of the microchannel walls may be formed of a semi-conductor material such as indium oxide. Preferably, the semi-conductor material is transparent. Alternatively, detection may be achieved by luminesce or fluorescence means, in which case an electromagnetic radiation detector, such as a photodiode or a photomultiplier, is provided.

[0021] One particular advantage of the invention is

that chemical reagents may be immobilised on the inner surfaces of the microchannels, thus providing the possibility of ELISA - type assays in a μ -TAS-type system. A number of different types of reagent may be attached to the microchannel walls, for example oligonucleotides, polypeptides, proteins (such as enzymes), or other natural or synthetic molecules. Conveniently, these may be adsorbed onto the surface of the microchannel walls, or covalently linked thereto, (for example by means of amide bond formation with succinimide), or electrostatically linked thereto (for example by means of a crosslinker such as polylysine). The inner surface of the microchannel and/or of the fluid inflow channel may also be provided with chemically functional groups formed by chemical or physical treatment.

[0022] The invention also extends to a method of manufacturing an apparatus as defined above, comprising the following steps which may be performed in either order or simultaneously: forming at least one reaction chamber; and forming at least one fluid inflow channel communicating with the reaction chamber(s), at least a portion of the or each fluid inflow channel having a hydrophobic inner surface adapted to act as gate means to prevent passage of fluid through the fluid inflow channel into the reaction chamber(s) until such fluid is acted upon by a fluid entry force.

[0023] For ease of fabrication, the apparatus is preferably formed in two main parts: a substrate in which the microchannels (and possibly also the inflow channels) are formed as depressions (for example by injection moulding, hot embossing, photoablation, casting or polymerisation on a mould); and an overlying layer applied over the substrate and over the depressions, to form the microchannels (and optionally also the inflow channels). In embodiments in which the inflow channels are not produced in the substrate they may, for example, be produced by drilling through a laminated overlying layer using a laser, or by depositing above the inlet of the reaction chamber a joint made of a hydrophobic material such as polydimethylsiloxane (PDMS).

[0024] The apparatus may be formed from any suitable material, for example, ceramics, glass, semiconductors, polymers, or combinations thereof. In a particularly preferred embodiment, both the substrate and lamination layer are formed of polymer material, which not only permits ready formation of the microchannels (for example by photoablation), but also allows the two components to be fused together by a thermal lamination technique. For this purpose, it is preferred that at least one of the polymers is of a material which has a relatively low melting point, for example polyethylene with a melting point of under 200°C. The lamination layer may with advantage be of an elastomeric material, such as polydimethyl siloxane (PDMS). In apparatus for use in conjunction with optical detection means, it is preferred that the lamination layer be formed of a substantially transparent material, and the substrate of a substantially opaque material (such as a ceramics material or a car-

bon-filled polymer).

[0025] In another aspect, the invention extends to a method of operating an apparatus as defined, comprising the steps of: placing at least one sample of an aqueous solution under test at the end of at least one fluid inflow channel distal at least one reaction chamber; causing the sample to enter the reaction chamber(s) via the fluid inflow channel(s) by applying a fluid entry force; and monitoring the sample in the reaction chamber(s) for the presence or concentration of a target substance.

[0026] In embodiments of apparatus having aspiration means, the fluid entry force is preferably applied by activating the aspiration means to apply reduced pressure to the microchannels for a period of time in the range 0.1 to 100s. In order to evacuate the microchannels, the aspiration means may then be activated to provide an even lower pressure to the microchannels, optionally in conjunction with the supply of washing fluid from a reservoir.

[0027] In embodiments of apparatus constructed using rotatable substrates or supports, the fluid entry force is preferably applied by rotating the substrate or support at an angular velocity in the range 1 to 1,000 revolutions per minute for a period of time in the range 1 to 100s. The microchannels may then be evacuated by rotating the substrate or support at an increased angular velocity, in the range 10 to 100,000 revolutions per minute, for a period of time in the range 1 to 100s.

[0028] The invention is hereinafter described in more detail by way of example only, with reference to the accompanying drawings, in which:

Fig 1 is a schematic cross section of an embodiment of apparatus according to the invention illustrated a) after deposition of an aqueous sample drop on the hydrophobic gate, and b) after sample loading;

Fig 2 is a schematic plan view of an embodiment of apparatus according to the invention, illustrating the steps of parallel sampling, loading and washing, achieved by aspiration;

Fig 3 is a schematic plan view of an alternative embodiment of apparatus according to the invention, in which parallel filling and washing step are achieved by centrifugal force;

Fig 4 is a schematic plan view of a further embodiment of apparatus according to the invention, in which the solution is loaded by slight aspiration and washed by strong aspiration;

Fig 5a is a partial plan view of an embodiment of apparatus according to the invention, incorporating a fluid reservoir adjacent the fluid inflow channel;

Fig 5b consists of two vertical cross sections of the Fig. 5a apparatus together with an associated piston,

illustrating the action of the piston in penetrating the sealed reservoir and expelling its contents through a valve into the reaction chamber;

Fig 6 is a top plan representation of an embodiment of apparatus according to the invention manufactured by UV-Laser photoablation of a polycarbonate compact disk, the embodiment being constructed substantially as the apparatus of Fig 3;

Fig 7 is a calibration curve obtained from a fluorescence imager using ALP-DDi solution in the microchannel of an embodiment of apparatus according to the invention;

Fig 8 is a graph illustrating fluorescence results indicating the level of binding between DDi-ALP in a test involving use of two microchannels of apparatus according to the invention, one incubated solely with BSA, and the other with BSA and anti-DDi (noted as BSA + Ab in the figure);

Fig 9 is a fluorescence image obtained using apparatus of the type illustrated in Fig 6, in which different microchannels were incubated with different concentrations of DDi-ALP;

Fig 10 is a graph illustrating the intensity of fluorescence obtained from different concentrations of ALP-DDi in an apparatus of the type illustrated in Fig 6, having antibody-coated microchannels;

Fig 11 is a graph illustrating variation of fluorescence intensity with time from an incubation of ALP-DDi in an embodiment of apparatus according to the invention, having at least one antibody coated microchannel; and

Fig 12 is a graph illustrating fluorescence intensity (indicating bound ALP-DDi) in a competitive immunoassay with D-Dimer utilising an embodiment of apparatus according to the invention, having at least one antibody-coated microchannel.

[0029] The microchannel devices of Figs 1 to 6 are produced by UV-Laser photoablation of commercially available polymers such as PET or polycarbonate. The photoablation procedure is performed in known fashion, for example as described previously by the present applicants⁴⁴. Briefly, a polymer sheet is rinsed with distilled water and ethanol and then mounted on an X,Y machining stage (Microcontrol, France). UV-Laser pulses (193 nm) (Lambda Physik LPX 205 i, Germany) are then fired at the polymer substrate target through a photomask and a 10:1 lense with a frequency of 50 Hz at 200 mJ/pulse, corresponding to a fluence per pulse of 1 J/cm² on the surface. During the photoablation process, the polymer substrate is moved horizontally with a

X,Y stepping motor (Microcontrol, France) at a speed of 0.2 mm/s resulting in linear channels 22 mm long. The microchannels are typically between 1 and 1,000µm in width, and in this example are approximately 100µm wide. The depth of the channels was fixed at 40 µm, by controlling the number of laser pulses used (each pulse photoablates approximately 150 nm). The channels are then sealed by thermal lamination of a layer of polyethylene over the base polymer sheet, the channels then exhibiting a trapezoidal shape in which three walls are composed of the substrate polymer (PET or Polycarbonate) and the top is composed of the lamination (Polyethylene). Fluid inflow channels (or "gates") (1) are opened either by firing enough laser pulses or are mechanically drilled through the hydrophobic lamination layer. The gates, which may have a diameter between 10µm and 10mm, have hydrophobic inner surfaces due to the nature of the polymer, and therefore inhibit passage of aqueous fluids.

[0030] The precise arrangement of microchannels is not crucial to the operation of the invention, though two general geometries have been developed and tested by the applicants and proved to be of benefit. In the first of these, a plurality of microchannels are arranged parallel to each other, conveniently on a generally rectangular substrate. The inflow channel "gates" of the various microchannels are aligned with each other, to permit rapid and efficient loading with test solutions from a linear multiple pipette device (see Fig 2). In the second configuration, the microchannels are arranged radially on a generally circular substrate, either with the inflow channel gates towards the centre of the circle and the opposite (outflow) ends of the microchannels towards the circumference (Figs 3 and 6), or vice versa (Fig 4).

[0031] A number of different means may be employed to provide the fluid entry force, of which the preferred means are aspiration and centrifugal force. In the apparatus of Fig 2, a common conduit (3) is supplied at the outflow ends of the microchannels (2), to which a reduced pressure is applied during operation of the device, to draw fluid into the microchannels through the fluid inflow gates. As illustrated in the last representation in Fig 2, the aspiration means may also be utilised to supply a stronger aspirating force in order to expel the microchannel contents to a drain, optionally in conjunction with the supply of a washing fluid. The apparatus illustrated in Fig 4 operates in a similar fashion, with aspiration being applied to the common outflow drain (6). In the apparatus illustrated in Figs 3 and 6, fluid is compelled to pass through the fluid inflow gates and into the microchannels by spinning the substrate to produce centrifugal force. In the illustrated arrangement, each microchannel has its own drain (7).

[0032] Typically, in an aspiration driven device (as in Figs 2 and 4) a 2 µl sample is placed with a pipette on each gate (1). The solution is then loaded into the microchannel by a brief aspiration from the common conduit (3;6). This technique ensures homogeneity of the

solution over the whole microchannel. After incubation, the microchannel is aspirated and rinsed three times with 2 µl. It is worth noting that the washing solution volume is much larger than that of the microchannel (about 100 nl) thus ensuring efficient washing. Using devices driven by centrifugal pressure, the filling and washing procedures may be achieved by placing 2 µl of solution over each gate (1). Slow rotation results in loading of the sample into the microchannel(s), and faster rotation is subsequently used to expel the sample from the microchannel(s).

[0033] Fig 5 illustrates an optional modification of apparatus according to the invention, in which each microchannel has an associated fluid reservoir (10) formed by a sealed cavity situated adjacent the fluid inflow gate (1). The reservoir communicates with microchannels (2) by means of a normally closed valve (12), which comprises valve member (13) which may be deformed under pressure into depression (14). Reservoir (10) is capped by seal (15), which may be broken by downward pressure applied by piston (11), which is profiled to be a close fit within reservoir (10). Downward movement of piston (11) within reservoir (10) increases the fluid pressure within the reservoir, thus opening valve (12) and allowing fluid from the reservoir to enter the microchannel. Depending upon the requirements of any particular assay, the reservoir may either be filled with a reagent or with wash fluid.

[0034] By way of example, various tests were carried out to establish the utility of apparatus according to the invention in performing an immunoassay for D-Dimer. D-Dimer is used as a diagnostic indicator in thromboembolic events: deep vein thrombosis and pulmonary embolism can be diagnosed by monitoring D-Dimer concentration in blood. In the past, the most reliable assay of D-Dimer have been performed by ELISA techniques, for example the "Asserachrom D-Di" of Diagnostica Stago. However, standard ELISA techniques are not suited for emergency situations, and alternative membrane-based techniques have been developed which use colour based detection systems⁴⁸. However, these suffer from the disadvantage that the detection mechanism is too subjective.

[0035] In the present tests, the detection of the enzyme was effected by a chemifluorescent substrate solution (VCR, Amersham). This system is based on the fluorescent detection of the AttoPhos substrate hydrolysed by ALP. The microchannels were then exposed to a Fluorescence Imager screen (MP840, Molecular Dynamics) and every channel was read for 1 minute. The image was then quantified using Image Quant software (Molecular Dynamics). The calibration of the enzyme in the microchannel was achieved by mixing the substrate solution with different concentrations of enzyme and incubating for 5 minutes. The microchannels were then filled with the mixtures and analysed with the fluorescence imager. In the actual tests, the enzyme was immobilised on the surface of the microchannels, and the

VCR solution was added to the channels with fluorescence being measured 5 minutes later.

[0036] Immobilisation of the proteins was achieved by physisorption for 1 hour at room temperature. The mouse IgG antibody (Serbio, France) was immobilised by placing either 10 or 100 $\mu\text{g/ml}$ in the microchannel, followed by incubation for 1 hour in a wet chamber. The surface was then washed with PBS and 20 % Tween (Tween/Water :0.2 ml/L, Fischer Germany), and blocked for 1 hour with a solution of 50 $\mu\text{g/ml}$ of heat shocked BSA (Sigma, USA) in the washing buffer solution. After another washing step, the channels were individually filled with the antigen solution. After five minutes (except for the kinetic experiment, where other periods are specified below), the microchannels were rinsed and a solution of 10 $\mu\text{g/ml}$ of alkaline phosphatase labelled antigen (ALP-DDi) was introduced and rinsed again after five minutes.

[0037] Fluorescence dependence on enzyme concentration after 5 minutes of incubation is presented graphically in Figure 7. The detection limit is reached in the range of 1 ng.ml^{-1} . The non-linear detection range is due to the fact that the product of the hydrolysis is not highly soluble and may precipitate on the surface at higher concentrations. Nevertheless, this system can be used for quantifying the enzyme concentration in the microchannel.

[0038] In order to study the activity of the adsorbed antibodies in the microchannel, two different incubation procedures were undertaken. Firstly, a few channels were incubated only with BSA. Secondly, some further channels were incubated with Ab and then with BSA. Every channel was then filled with the DDi-ALP, incubated for 1 hour and washed by aspiration following the procedure described above. The fluorescence intensity of each channel was then measured and the results are presented in Figure 8. The channels incubated only with BSA exhibited a low fluorescence which is not significantly different from that of the polymer substrate itself. In contrast the channels incubated with the antibodies were much more fluorescent, thereby demonstrating that DDi-ALP was adsorbed on the antibodies. This experiment shows that some of the adsorbed antibodies are still active on the surface and that BSA is an effective blocker against the non-specific adsorption of the DDi-ALP complex.

[0039] Figure 9 shows the fluorescence of the substrate in the channels after adsorption of different concentrations of ALP-DDi on the 10 $\mu\text{g.ml}^{-1}$ adsorbed antibodies. The fluorescence intensity of the microchannel lines clearly shows the gradient of concentration in the different microchannels. The relative intensity of every microchannel is shown graphically in Figure 10. Saturation of channels is reached at about 30 $\mu\text{g/ml}$.

[0040] Figure 11 shows the fluorescence intensity of microchannels that have been incubated for different periods of time. For short incubation times (<5 min), the intensity grows linearly, showing that the antigens are

very quickly captured by the antibodies. It is thought that all the antigens have still not reached the surface by diffusion. This first slope approximately follows the diffusion of the molecules to the walls. The molecules then react rapidly and the reaction becomes quasi diffusion-controlled. After 5 minutes of incubation, the reaction is controlled by slower kinetics driven by two different phenomena. Firstly, large molecules diffuse much more slowly and therefore reach the surface after a long time. In this case, the molecules can be partially degraded fibrin products, of which the molecular weight can be larger than 1000 kD. Secondly, there is a tendency for non-specific adsorption; such reactions are much slower than immunological recognition and are driven by electrostatic or hydrophobic interactions that require reorganisation at the molecular level. This type of non specific adsorption may therefore be excluded by short incubation times.

[0041] Figure 12 shows the fluorescence dependence of the D-Dimer concentration after a competitive immunoassay. In the low concentration range, most of the immobilised antibody sites are not occupied by the DDi, allowing the DDi-ALP to be present in a large amount and therefore to hydrolyse more fluorescent substrates. At concentrations higher than 1000 ng.ml^{-1} , D-Dimer molecules are present on most of the antibody sites and therefore only a few sites are available for DDi-ALP. The central part of the concentration range (100-1000 ng.ml^{-1}) shows the strong concentration dependence of the system, the two orders of magnitude detection range being in the range of interest for diagnostics applications⁴⁹.

[0042] These experiments demonstrate the feasibility of ELISA techniques in microchannels. Benefitting from fast equilibration times and rapid filling and rinsing procedures, the time taken to complete an assay (including calibration) may be reduced to less than 10 minutes, compared to a typical time of 3 hours for an ELISA in a microtiter plate. Hundreds of microchannels may be provided on a substrate, if desired, and the ability to ensure simultaneous filling provides the possibility of highly efficient parallel assays.

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Claims

1. Apparatus for performing chemical assays involving aqueous fluids, the apparatus comprising: at least one reaction chamber; at least one fluid inflow channel communicating with the or each reaction chamber; and gate means adapted to prevent passage of aqueous fluid through the fluid inflow channel(s) into the reaction chamber(s), until such fluid is acted upon by a fluid entry force; wherein said reaction chamber has a hydrophilic inner surface and said gate means comprises at least a portion of the or each fluid inflow channel having a hydrophobic inner surface.
2. Apparatus according to claim 1, wherein the reac-

tion chamber comprises a microchannel having at least one dimension in the range 1 to 1,000µm.

3. Apparatus according to claim 1 or claim 2, wherein the fluid inflow channel is formed in a substrate at least a portion of which is composed of a hydrophobic material.
4. Apparatus according to any of claim 1 or claim 2, wherein the fluid inflow channel is formed in a substrate at least a portion of which is physically or chemically treated to render it hydrophobic.
5. Apparatus according to any preceding claim, wherein the fluid inflow channel has a cross-sectional area in the range 10µm² to 1,000mm².
6. Apparatus according to any preceding claim, wherein the fluid inflow channel is moulded to be complementary in shape to a standard pipette (for example of the Eppendorf^(RTM) type).
7. Apparatus according to any preceding claim, wherein the fluid entry force is provided by piston pressure.
8. Apparatus according to any preceding claim, comprising a plurality of separate reaction chambers, each communicating with an inflow channel and associated gate means.
9. Apparatus according to claim 8, wherein each reaction chamber is provided with a separate inflow channel.
10. Apparatus according to claim 8, wherein there is one inflow channel forming a common conduit to all reaction chambers.
11. Apparatus according to claims 8, 9 or 10, as appendant to claim 2, wherein each microchannel communicates, at its end distal the inflow channel, with a common conduit, the common conduit being connected to aspiration means adapted selectively to apply reduced pressure to the conduit and thus to draw fluid through the microchannel in operation.
12. Apparatus according to claim 10 or claim 11, wherein the common conduit has a cross-sectional area in the range 0.01mm² to 25cm².
13. Apparatus according to any preceding claim, wherein the microchannels are arranged generally parallel to each other.
14. Apparatus according to claim 13, as appendant to any of claims 10 to 12, wherein the microchannels are arranged generally perpendicularly to the com-

mon conduit.

15. Apparatus comprising a plurality of apparatuses according to claim 14 mounted together on a tape.

16. Apparatus according to claim 11, comprising a substantially circular substrate the microchannels being arranged substantially radially, each having its inflow channel towards the circumference of the circle and its opposite end communicating with a central chamber connected to said aspiration means.

17. Apparatus according to claim 8 or claim 9, comprising a substantially circular substrate the microchannels being arranged substantially radially, the inflow channel(s) being disposed towards the centre of the circle, and each microchannel having a waste chamber at the opposite end towards the circumference of the circle.

18. Apparatus according to claim 16 or claim 17, wherein the thickness of the substantially circular substrate is in the range 50 to 5,000µm.

19. Apparatus according to claim 16, wherein the circular substrate is rotatable, and the fluid entry force is provided by centrifugal pressure as the substrate is caused to rotate.

20. Apparatus comprising a plurality of apparatuses according to claim 13 or claim 14 disposed on a rotatable support member, and wherein the fluid entry force is provided by centrifugal pressure as the support member is caused to rotate.

21. Apparatus according to any preceding claim, wherein the or each reaction chamber is provided, proximate thereto, with a sealed cavity forming a reservoir filled with an aqueous fluid.

22. Apparatus according to claim 21, wherein the reservoir communicates with the reaction chamber via a normally closed valve, which may be caused to open by the application of increased pressure to the aqueous fluid within the cavity.

23. Apparatus according to claim 22, further comprising a piston member having an outer profile shaped to fit within the cavity, the cavity being capped by a breakable seal, which, in operation, is broken by the piston, wherein movement of the piston into the cavity provides the increased pressure necessary to urge the aqueous fluid from the cavity via the valve into the reaction chamber.

24. Apparatus according to any preceding claim, wherein at least a portion of the surface of the reaction chamber is formed of an electrically conduc-

tive material, and the apparatus further comprises electrical detection circuitry connected to said conductive portion, to enable detection of a target species within the reaction chamber by electrochemical means.

25. Apparatus according to claim 24, wherein said conductive portion is formed of a conductive polymer material.

26. Apparatus according to claim 24, wherein said conductive portion is formed by an electrode.

27. Apparatus according to claim 26, wherein said electrode is of a semiconductor material.

28. Apparatus according to claim 27, wherein said semiconductor material is substantially transparent.

29. Apparatus according to claim 28, wherein the semiconductor material is indium oxide.

30. Apparatus according to any of claims 1 to 23, further comprising electromagnetic radiation detection means adapted to detect radiation emitted by a target species in the reaction chamber.

31. Apparatus according to claim 30, wherein the detection means comprises at least one photodiode or at least one photomultiplier array arranged along at least a portion of the reaction chamber.

32. Apparatus according to any preceding claim, wherein a chemical reagent is immobilised on at least a portion of the inner surface of the reaction chamber, the reagent being adapted to interact with a target species whose presence or concentration is to be determined.

33. Apparatus according to claim 32, wherein the reagent comprises an oligonucleotide, a polypeptide, a protein or another natural or synthetic molecule.

34. Apparatus according to claim 32 or claim 33, wherein the reagent is adsorbed onto said inner surface of the reaction chamber.

35. Apparatus according to claim 32 or claim 33, wherein the reagent is covalently attached to said inner surface of the reaction chamber.

36. Apparatus according to claim 35, wherein the covalent bonding is achieved via a succinimide bonding agent.

37. Apparatus according to claim 32 or claim 33, wherein the reagent is electrostatically attached to said inner surface of the reaction chamber via a

crosslinker.

38. Apparatus according to claim 37, wherein the crosslinker is polylysine.

39. Apparatus according to any preceding claim, wherein at least a portion of the inner surface of the reaction chamber and/or of the fluid inflow channel is provided with chemically function groups formed by chemical or physical treatment of the surface.

40. Apparatus according to any preceding claim, comprising a substrate, in which the reaction chamber and/or the fluid inflow channel are formed as a depression(s), the reaction chamber and/or the fluid inflow channel being sealed by an overlying layer applied over the substrate.

41. Apparatus according to claim 40, wherein the substrate and overlying layer are formed of polymer materials, the melting point of at least one of the materials being sufficiently low to permit the substrate and overlying layer to be sealed together by thermal lamination.

42. Apparatus according to claim 41, wherein said at least one material is polyethylene.

43. Apparatus according to claim 40 or claim 41, wherein the overlying layer is formed of an elastomeric material.

44. Apparatus according to claim 43, wherein the elastomeric material is polydimethylsiloxane (PDMS).

45. Apparatus according to claim 40, as appendant to claim 30 or 31, wherein at least a portion of the substrate is formed of a substantially opaque material and the overlying layer is formed of a substantially transparent material.

46. Apparatus according to claim 45, wherein the substantially opaque material comprises a carbon-filled polymer or a ceramics material.

47. A method of manufacturing an apparatus according to any of claims 1 to 46, comprising the following steps which may be performed in either order or simultaneously:

forming at least one reaction chamber having a hydrophilic inner surface; and forming at least one fluid inflow channel communicating with the reaction chamber(s), at least a portion of the or each fluid inflow channel having a hydrophobic inner surface adapted to act as gate means to prevent passage of fluid through the fluid inflow channel into the reaction chamber

until such fluid is acted upon by a fluid entry force.

48. A method according to claim 47, wherein the apparatus is formed from polymeric material.

49. A method according to claim 48, wherein the apparatus is formed by injection moulding, hot embossing, photoablation, casting, or polymerisation on a mould.

50. A method according to claim 48 or claim 49, comprising the steps of forming a substrate having at least one depression therein, and applying an overlying layer over the substrate to seal the or each depression so as to form at least one fluid inflow channel and/or at least one reaction chamber.

51. A method according to claim 50, wherein the overlying layer is sealed with the substrate by thermal lamination.

52. A method according to claim 47, wherein at least a part of the apparatus is formed of a ceramics material, glass, a conductor or a semi-conductor material.

53. A method of operating an apparatus according to any of claims 1 to 46, comprising the steps of: placing at least one sample of an aqueous solution under test at the end of at least one fluid inflow channel distal at least one reaction chamber, at least a portion of the or each fluid inflow channel having a hydrophobic inner surface; causing the sample to enter the reaction chamber(s) via the fluid inflow channel(s) by applying a fluid entry force; and monitoring the sample in the reaction chamber(s) for the presence or concentration, of a target substance.

54. A method according to claim 53, wherein the sample(s) is caused to exit the reaction chamber(s) before the reaction chamber (s) or the expelled sample is monitored for the presence or concentration of a target substance.

55. A method according to claims 53 or 54, wherein the or each sample is applied by means of a pipette, a syringe, or an electrically operated injector.

56. A method according to any of claims 53 to 55, for operating an apparatus according to claim 11 or any claim appendant thereto, wherein the fluid entry force is provided by aspiration means, the aspiration means being activated to apply reduced pressure to the or each reaction chamber for a period of time in the range 0.1 to 100s.

57. A method according to any of claims 53 to 55, for

operating an apparatus according to either claim 19 or claim 20, or any claim appendant thereto, wherein the fluid entry force is provided by spinning the substrate or the support member at an angular velocity in the range 1 to 1,000 revolutions per minute for a period of time in the range 1 to 100s.

58. A method according to claim 57, wherein the sample is expelled from the reaction chamber by spinning the substrate at a greater angular velocity, in the range 10 to 100,000 revolutions per minute, for a period of time in the range 1 to 100s.

59. A method according to any of claims 53 to 55, for operating an apparatus according to claim 7 or any claim appendant thereto, wherein the fluid entry force is provided by piston pressure.

Patentansprüche

1. Vorrichtung zur Durchführung chemischer Assays, die wässrige Fluida beinhalten, wobei die Vorrichtung umfasst: mindestens eine Reaktionskammer, mindestens einen Fluidzuflusskanal in Verbindung mit der oder jeder Reaktionskammer, und Schrankenmittel, die zur Verhinderung des Durchflusses von wässrigem Fluid durch den Fluidzuflusskanal/die Fluidzuflusskanäle in die Reaktionskammer(n) angepasst sind, bis eine Fluideintrittskraft auf das Fluid einwirkt, wobei die Reaktionskammer eine hydrophile Innenfläche hat und das Schrankenmittel mindestens einen Teil des oder jedes Fluidzuflusskanals mit einer hydrophoben Innenfläche umfasst.

2. Vorrichtung nach Anspruch 1, wobei die Reaktionskammer einen Mikrokanal mit mindestens einer Dimension im Bereich von 1 bis 1000 µm umfasst.

3. Vorrichtung nach Anspruch 1 oder Anspruch 2, wobei der Fluidzuflusskanal in einem Substrat gebildet ist, von dem mindestens ein Teil aus einem hydrophoben Material besteht ist.

4. Vorrichtung nach Anspruch 1 oder Anspruch 2, wobei der Fluidzuflusskanal in einem Substrat gebildet ist, von dem mindestens ein Teil physikalisch oder chemisch behandelt worden ist, um es hydrophob zu machen.

5. Vorrichtung nach einem der vorhergehenden Ansprüche, wobei der Fluidzuflusskanal eine Querschnittfläche im Bereich von 10 µm² bis 1000 mm² hat.

6. Vorrichtung nach einem der vorhergehenden Ansprüche, wobei der Fluidzuflusskanal als Komple-

mentärform zu einer Standardpipette (beispielsweise vom Eppendorf^(RTM)-Typ) geformt ist.

7. Vorrichtung nach einem der vorhergehenden Ansprüche, wobei die Fluideintrittskraft durch Kolbendruck bereitgestellt wird.

8. Vorrichtung nach einem der vorhergehenden Ansprüche, umfassend mehrere separate Reaktionskammern, die jeweils mit einem Zuflusskanal und zugehörigem Schrankenmittel in Verbindung stehen.

9. Vorrichtung nach Anspruch 8, wobei jede Reaktionskammer mit einem separaten Zuflusskanal ausgestattet ist

10. Vorrichtung nach Anspruch 8, wobei ein Zuflusskanal vorliegt, der eine gemeinsame Leitung für alle Reaktionskammern liefert.

11. Vorrichtung nach den Ansprüchen 8, 9 oder 10, soweit abhängig von Anspruch 2, wobei jeder Mikrokanal an seinem von dem Zuflusskanal entfernten Ende mit einer gemeinsamen Leitung in Verbindung steht, wobei die gemeinsame Leitung an ein Aspirationsmittel angeschlossen ist, das angepasst ist, um selektiv verminderten Druck an die Leitung anzulegen und so im Betrieb Fluid durch den Mikrokanal zu ziehen.

12. Vorrichtung nach Anspruch 10 oder Anspruch 11, wobei die gemeinsame Leitung eine Querschnittfläche im Bereich von 0,01 mm² bis 25 cm² hat.

13. Vorrichtung nach einem der vorhergehenden Ansprüche, wobei die Mikrokanäle allgemein parallel zueinander angeordnet sind.

14. Vorrichtung nach Anspruch 13, soweit abhängig von einem der Ansprüche 10 bis 12, wobei die Mikrokanäle allgemein senkrecht zu der gemeinsamen Leitung angeordnet sind.

15. Vorrichtung, die mehrere Vorrichtungen nach Anspruch 14 gemeinsam auf einem Band angebracht umfasst.

16. Vorrichtung nach Anspruch 11, die ein im Wesentlichen kreisförmiges Substrat umfasst, wobei die Mikrokanäle im Wesentlichen radial angeordnet sind, wobei ihr jeweiliger Zuflusskanal in Richtung der Peripherie des Kreises weist und ihr entgegengesetztes Ende mit einer mittigen Kammer in Verbindung steht, die an das Aspirationsmittel angeschlossen ist

17. Vorrichtung nach Anspruch 8 oder Anspruch 9, die

ein im Wesentlichen kreisförmiges Substrat umfasst, wobei die Mikrokanäle im Wesentlichen radial angeordnet sind, der Zuflusskanal bzw. die Zuflusskanäle in Richtung der Mitte des Kreises angeordnet sind und jeder Mikrokanal eine Abfallkammer am entgegengesetzten Ende in Richtung der Peripherie des Kreises aufweist.

18. Vorrichtung nach Anspruch 16 oder Anspruch 17, wobei die Dicke des im Wesentlichen kreisförmigen Substrats im Bereich von 50 bis 5000 µm liegt. 10
19. Vorrichtung nach Anspruch 16, wobei das kreisförmige Substrat drehbar ist und die Fluideintrittskraft durch Zentrifugaldruck bereitgestellt wird, wenn das Substrat zum Rotieren gebracht wird. 15
20. Vorrichtung, die mehrere Vorrichtungen nach Anspruch 13 oder Anspruch 14 auf einem drehbaren Trägerelement angeordnet umfasst, und wobei die Fluideintrittskraft durch Zentrifugaldruck bereitgestellt wird, wenn das Trägerelement zum Rotieren gebracht wird. 20
21. Vorrichtung nach einem der vorhergehenden Ansprüche, wobei die oder jede Reaktionskammer daran anschließend mit einem versiegelten Hohlraum ausgestattet ist, der ein mit einem wässrigen Fluid gefülltes Reservoir bildet. 25
22. Vorrichtung nach Anspruch 21, wobei das Reservoir über ein normalerweise geschlossenes Ventil mit der Reaktionskammer in Verbindung steht, das durch Ausübung von erhöhtem Druck auf das wässrige Fluid in dem Hohlraum zum Öffnen gebracht werden kann. 30
23. Vorrichtung nach Anspruch 22, die ferner ein Kolbenelement mit einem Außenprofil umfasst, das so geformt ist, dass es in den Hohlraum passt, wobei der Hohlraum durch eine zerreibare Siegelung verschlossen ist, die im Betrieb durch den Kolben zerrissen wird, wobei die Bewegung des Kolbens in den Hohlraum den erforderlichen erhöhten Druck liefert, um das wässrige Fluid aus dem Hohlraum über das Ventil in die Reaktionskammer zu drücken. 35
24. Vorrichtung nach einem der vorhergehenden Ansprüche, wobei mindestens ein Teil der Oberfläche der Reaktionskammer aus einem elektrisch leitenden Material gebildet ist, und die Vorrichtung ferner elektrische Detektierungsschaltkreise aufweist, die mit dem leitenden Teil verbunden sind, um die Detektion einer Zielspezies in der Reaktionskammer durch elektrochemische Mittel zu ermöglichen. 40
25. Vorrichtung nach Anspruch 24, wobei der leitende 45

Teil aus einem leitenden Polymermaterial gebildet ist

26. Vorrichtung nach Anspruch 24, wobei der leitende Teil durch eine Elektrode gebildet ist. 5
27. Vorrichtung nach Anspruch 26, wobei die Elektrode aus einem Halbleitermaterial ist.
28. Vorrichtung nach Anspruch 27, wobei das Halbleitermaterial im Wesentlichen transparent ist.
29. Vorrichtung nach Anspruch 28, wobei das Halbleitermaterial Indiumoxid ist.
30. Vorrichtung nach einem der Ansprüche 1 bis 23, die ferner elektromagnetische Strahlungsdetektionsmittel umfasst, die angepasst sind, um von einer Zielspezies in der Reaktionskammer emittierte Strahlung zu detektieren.
31. Vorrichtung nach Anspruch 30, wobei das Detektierungsmittel mindestens eine Photodiode oder mindestens eine Sekundärelektronenvervielfältigergruppierung "(photomultiplier array)" umfasst, die über mindestens einen Teil der Reaktionskammer angeordnet ist.
32. Vorrichtung nach einem der vorhergehenden Ansprüche, wobei ein chemisches Reagenz auf mindestens einem Teil der Innenfläche der Reaktionskammer immobilisiert ist, wobei das Reagenz zur Wechselwirkung mit einer Zielspezies angepasst ist, deren Anwesenheit oder Konzentration bestimmt werden soll.
33. Vorrichtung nach Anspruch 32, wobei das Reagenz ein Oligonukleotid, ein Polypeptid, ein Protein oder anderes natürliches oder synthetisches Molekül umfasst.
34. Vorrichtung nach Anspruch 32 oder Anspruch 33, wobei das Reagenz auf der Innenfläche der Reaktionskammer adsorbiert ist.
35. Vorrichtung nach Anspruch 32 oder Anspruch 33, wobei das Reagenz kovalent an die Innenfläche der Reaktionskammer gebunden ist
36. Vorrichtung nach Anspruch 35, wobei die kovalente Bindung über ein Succinimid-Bindungsmittel erreicht ist.
37. Vorrichtung nach Anspruch 32 oder Anspruch 33, wobei das Reagenz elektrostatisch über einen Vernetzer an die Innenfläche der Reaktionskammer gebunden ist. 50

38. Vorrichtung nach Anspruch 37, wobei der Vernetzer Polylysin ist.
39. Vorrichtung nach einem der vorhergehenden Ansprüche, wobei mindestens ein Teil der Innenfläche der Reaktionskammer und/ oder des Fluidzuflussskanals mit chemisch funktionellen Gruppen ausgestattet ist, die durch chemische oder physikalische Behandlung der Oberfläche gebildet sind. 5
40. Vorrichtung nach einem der vorhergehenden Ansprüche, die ein Substrat umfasst, in dem die Reaktionskammer und/oder die Fluidzuflussskanäle als Vertiefung(en) gebildet sind, wobei die Reaktionskammer und/oder der Fluidzuflussskanal durch eine Deckschicht versiegelt sind, die über dem Substrat aufgebracht ist. 10
41. Vorrichtung nach Anspruch 40, wobei das Substrat und die Deckschicht aus Polymermaterialien gebildet sind, wobei der Schmelzpunkt von mindestens einem der Materialien ausreichend niedrig ist, um zu ermöglichen, dass das Substrat und die Deckschicht durch thermische Laminierung miteinander versiegelt werden. 15 20 25
42. Vorrichtung nach Anspruch 41, wobei mindestens eines der Materialien Polyethylen ist.
43. Vorrichtung nach Anspruch 40 oder Anspruch 41, wobei die Deckschicht aus einem elastomeren Material gebildet ist. 30
44. Vorrichtung nach Anspruch 43, wobei das elastomere Material Polydimethylsiloxan (PDMS) ist. 35
45. Vorrichtung nach Anspruch 40, soweit abhängig von Anspruch 30 oder 31, wobei mindestens ein Teil des Substrats aus einem im Wesentlichen opaken Material gebildet ist und die Deckschicht aus einem im Wesentlichen transparenten Material gebildet ist. 40
46. Vorrichtung nach Anspruch 45, wobei das im Wesentlichen opake Material ein kohlenstoffgefülltes Polymer oder ein Keramikmaterial umfasst. 45
47. Verfahren zur Herstellung einer Vorrichtung nach einem der Ansprüche 1 bis 46, umfassend die folgenden Stufen, die in beliebiger Reihenfolge oder simultan durchgeführt werden können: 50
- Bilden mindestens einer Reaktionskammer mit einer hydrophilen Innenfläche und Bilden mindestens eines Fluidzuflussskanals in Verbindung mit der Reaktionskammer bzw. den Reaktionskammern, 55
- wobei mindestens ein Teil des oder jedes Fluidzuflussskanals eine hydrophobe Innenfläche aufweist, die so angepasst ist, dass sie als Schrankenmittel wirkt, um den Durchgang von Fluid durch den Fluidzuflussskanal in die Reaktionskammer solange zu verhindern, bis eine Fluideintrittskraft auf dieses Fluid wirkt.
48. Verfahren nach Anspruch 47, wobei die Vorrichtung aus polymerem Material gebildet ist.
49. Verfahren nach Anspruch 48, wobei die Vorrichtung durch Spritzgießen, Heißprägen, Photoablation, Gießen oder Polymerisation auf einer Form gebildet ist.
50. Verfahren nach Anspruch 48 oder Anspruch 49, umfassend die Stufen:
- Bilden eines Substrats mit mindestens einer Vertiefung darin und Aufbringen einer Deckschicht über dem Substrat, um die oder jede Vertiefung zu versiegeln, um so mindestens einen Fluidzuflussskanal und/ oder mindestens eine Reaktionskammer zu bilden.
51. Verfahren nach Anspruch 50, wobei die Deckschicht durch thermische Laminierung mit dem Substrat versiegelt wird.
52. Verfahren nach Anspruch 47, wobei mindestens ein Teil der Vorrichtung aus einem Keramikmaterial, Glas, einem Leiter- oder einem Halbleitermaterial gebildet ist.
53. Verfahren zum Betreiben einer Vorrichtung nach einem der Ansprüche 1 bis 46, umfassend die Stufen: Aufbringen von mindestens einer Probe einer zu testenden wässrigen Lösung am Ende mindestens eines Fluidzuflussskanals entfernt von mindestens einer Reaktionskammer, wobei mindestens ein Teil des oder jedes Fluidzuflussskanals eine hydrophobe Innenfläche aufweist; Herbeiführen des Eintretens der Probe in die Reaktionskammer(n) über den Fluidzuflussskanal bzw. die Fluidzuflussskanäle durch Ausüben einer Fluideintrittskraft und Überwachen der Probe in der Reaktionskammer bzw. den Reaktionskammern auf Anwesenheit oder Konzentration einer Zielsubstanz.
54. Verfahren nach Anspruch 53, wobei die Probe(n) zum Verlassen der Reaktionskammer(n) gebracht wird bzw. werden, bevor die Reaktionskammer(n) oder die ausgeworfene Probe auf Anwesenheit oder Konzentration einer Zielsubstanz überwacht wird.
55. Verfahren nach den Ansprüchen 53 oder 54, wobei

die oder jede Probe mittels einer Pipette, einer Spritze oder einer elektrisch betriebenen Einspritzvorrichtung aufgebracht wird.

56. Verfahren nach einem der Ansprüche 53 bis 55 zum Betreiben einer Vorrichtung nach Anspruch 11 oder irgendeinem davon abhängigen Anspruch, wobei die Fluideintrittskraft durch Aspirationsmittel bereitgestellt wird, wobei die Aspirationsmittel zum Anlegen von vermindertem Druck an die oder jede Reaktionskammer für einen Zeitraum im Bereich von 0,1 bis 100 s aktiviert werden.

57. Verfahren nach einem der Ansprüche 53 bis 55 zum Betreiben einer Vorrichtung nach Anspruch 19 oder Anspruch 20 oder irgendeinem davon abhängigen Anspruch, wobei die Fluideintrittskraft durch Drehen des Substrats oder des Trägerelements mit einer Winkelgeschwindigkeit im Bereich von 1 bis 1000 Umdrehungen pro Minute für einen Zeitraum im Bereich von 1 bis 100 s bereitgestellt wird.

58. Verfahren nach Anspruch 57, wobei die Probe aus der Reaktionskammer ausgeworfen wird, indem das Substrat mit einer höheren Winkelgeschwindigkeit im Bereich von 10 bis 100.000 Umdrehungen pro Minute für einen Zeitraum im Bereich von 1 bis 100 s gedreht wird.

59. Verfahren nach einem der Ansprüche 53 bis 55 zum Betreiben einer Vorrichtung nach Anspruch 7 oder irgendeinem davon abhängigen Anspruch, wobei die Fluideintrittskraft durch Kolbendruck geliefert wird.

Revendications

1. Dispositif pour effectuer des essais chimiques impliquant des fluides aqueux, le dispositif comprenant: au moins une chambre de réaction; au moins un canal d'entrée de fluide communiquant avec la ou chaque chambre de réaction; et une barrière adaptée pour empêcher le passage de fluide aqueux à travers le canal ou les canaux d'entrée de fluide jusqu'à ce qu'une force d'entrée de fluide agit sur celui-ci; dans lequel ladite chambre de réaction présente une surface intérieure hydrophile, et ladite barrière comprend au moins une partie du ou de chaque canal d'entrée de fluide présentant une surface intérieure hydrophobe.

2. Dispositif selon la revendication 1, dans lequel la chambre de réaction comprend un micro-canal présentant au moins une dimension comprise dans la gamme allant de 1 à 1000 μm .

3. Dispositif selon la revendication 1 ou la revendica-

tion 2, dans lequel le canal d'entrée de fluide est formé dans un substrat dont au moins une partie est composée d'une matière hydrophobe.

4. Dispositif selon la revendication 1 ou la revendication 2, dans lequel le canal d'entrée de fluide est formé dans un substrat dont au moins une partie est traitée physiquement ou chimiquement pour devenir hydrophobe.

5. Dispositif selon l'une quelconque des revendications précédentes, dans lequel la surface de la section transversale du canal d'entrée de fluide est comprise dans la gamme allant de 10 μm^2 à 1000 mm^2 .

6. Dispositif selon l'une quelconque des revendications précédentes, dans lequel le canal d'entrée de fluide est moulé de telle sorte que sa forme soit complémentaire à celle d'une pipette standard (par exemple du type Eppendorf^(RTM)).

7. Dispositif selon l'une quelconque des revendications précédentes, dans lequel la force d'entrée de fluide est fournie par la pression d'un piston.

8. Dispositif selon l'une quelconque des revendications précédentes, comprenant une pluralité de chambres de réaction séparées, chaque chambre communiquant avec un canal d'entrée et une barrière associée.

9. Dispositif selon la revendication 8, dans lequel chaque chambre de réaction comporte un canal d'entrée séparé.

10. Dispositif selon la revendication 8, dans lequel un canal d'entrée forme un conduit commun à toutes les chambres de réaction.

11. Dispositif selon la revendication 8, 9 ou 10, lorsqu'elles dépendent de la revendication 2, dans lequel chaque micro-canal communique, à son extrémité distante du canal d'entrée, avec un conduit commun, le conduit commun étant connecté à un moyen d'aspiration adapté sélectivement pour appliquer une pression réduite au conduit et donc pour aspirer le fluide à travers le micro-canal lors du fonctionnement.

12. Dispositif selon la revendication 10 ou la revendication 11, dans lequel la surface de la section transversale du conduit commun est comprise dans la gamme allant 0,01 mm^2 à 25 cm^2 .

13. Dispositif selon l'une quelconque des revendications précédentes, dans lequel les micro-canaux sont arrangés d'une façon essentiellement parallèle

les uns par rapport aux autres.

14. Dispositif selon la revendication 13, lorsqu'elle dépend de l'une quelconque des revendications 10 à 12, dans lequel les micro-canaux sont arrangés d'une façon essentiellement perpendiculaire par rapport au conduit commun. 5
15. Dispositif comprenant une pluralité de dispositifs selon la revendication 14 montés ensemble sur une bande. 10
16. Dispositif selon la revendication 11, comprenant un substrat essentiellement circulaire, les micro-canaux étant arrangés essentiellement radialement et comprenant chacun leur canal d'entrée orienté vers la circonférence du cercle et dont l'extrémité opposée communique avec une chambre centrale reliée audit moyen d'aspiration. 15
17. Dispositif selon la revendication 8 ou la revendication 9, comprenant un substrat essentiellement circulaire, les micro-canaux étant arrangés essentiellement radialement, le canal ou les canaux d'entrée étant disposé(s) en direction du centre du cercle, et chaque micro-canal comprenant une chambre de rebut à l'extrémité opposée tournée vers la circonférence du cercle. 20
18. Dispositif selon la revendication 16 ou la revendication 17, dans lequel l'épaisseur du substrat essentiellement circulaire est comprise dans la gamme allant de 50 à 5000 μm . 25
19. Dispositif selon la revendication 16, dans lequel le substrat circulaire est rotatif, et la force d'entrée de fluide est fournie par une pression centrifuge lorsque le substrat est entraîné à tourner. 30
20. Dispositif comprenant une pluralité de dispositifs selon la revendication 13 ou la revendication 14 disposés sur un élément de support rotatif, et dans lequel la force d'entrée de fluide est fournie par une pression centrifuge lorsque l'élément de support est entraîné à tourner. 35
21. Dispositif selon l'une quelconque des revendications précédentes, dans lequel la ou chaque chambre de réaction comprend, à proximité de celle-ci, une cavité étanche formant un réservoir rempli d'un fluide aqueux. 40
22. Dispositif selon la revendication 21, dans lequel le réservoir communique avec la chambre de réaction par l'intermédiaire d'une valve normalement fermée, qui peut être entraînée à s'ouvrir sous l'application d'une pression accrue sur le fluide aqueux à l'intérieur de la cavité. 45
23. Dispositif selon la revendication 22, comprenant en outre un élément de piston dont le profil extérieur est configuré pour s'adapter à l'intérieur de la cavité, la cavité étant fermée par un couvercle cassable qui, lors du fonctionnement, est cassé par le piston, dans lequel un déplacement du piston dans la cavité procure la pression accrue nécessaire pour pousser le fluide aqueux hors de la cavité à travers la valve dans la chambre de réaction. 50
24. Dispositif selon l'une quelconque des revendications précédentes, dans lequel au moins une partie de la surface de la chambre de réaction est constituée d'une matière électriquement conductrice, et le dispositif comprend en outre un circuit de détection électrique connecté à ladite partie électriquement conductrice et destiné à permettre la détection d'espèces cibles à l'intérieur de la chambre de réaction par un procédé électrochimique. 55
25. Dispositif selon la revendication 24, dans lequel ladite partie conductrice est constituée d'une matière polymère conductrice.
26. Dispositif selon la revendication 24, dans lequel ladite partie conductrice est formée par une électrode.
27. Dispositif selon la revendication 26, dans lequel ladite électrode est constituée d'une matière semi-conductrice.
28. Dispositif selon la revendication 27, dans lequel ladite matière semi-conductrice est essentiellement transparente.
29. Dispositif selon la revendication 28, dans lequel ladite matière conductrice est l'oxyde d'indium.
30. Dispositif selon l'une quelconque des revendications 1 à 23, comprenant en outre un moyen de détection de rayonnement électromagnétique adapté pour détecter un rayonnement émis par une espèce cible dans la chambre de réaction.
31. Dispositif selon la revendication 30, dans lequel le moyen de détection comprend au moins une photodiode ou au moins un réseau photomultiplicateur arrangé(e) le long d'au moins une partie de la chambre de réaction.
32. Dispositif selon l'une quelconque des revendications précédentes, dans lequel un réactif chimique est immobilisé sur au moins une partie de la surface intérieure de la chambre de réaction, le réactif étant adapté pour interagir avec une espèce cible dont la présence ou la concentration doit être déterminée.

33. Dispositif selon la revendication 32, dans lequel le réactif comprend un oligonucléotide, un polypeptide, une protéine ou une autre molécule naturelle ou synthétique.
34. Dispositif selon la revendication 32 ou la revendication 33, dans lequel le réactif est adsorbé sur ladite surface intérieure de la chambre de réaction.
35. Dispositif selon la revendication 32 ou la revendication 33, dans lequel le réactif est attaché d'une façon covalente sur ladite surface intérieure de la chambre de réaction.
36. Dispositif selon la revendication 35, dans lequel la liaison covalente est réalisée à l'aide d'un agent de liaison succinimide.
37. Dispositif selon la revendication 32 ou la revendication 33, dans lequel le réactif est attaché d'une façon électrostatique sur ladite surface intérieure de la chambre de réaction à l'aide d'un agent réticulant.
38. Dispositif selon la revendication 37, dans lequel l'agent réticulant est la polylysine.
39. Dispositif selon l'une quelconque des revendications précédentes, dans lequel au moins une partie de la surface intérieure de la chambre de réaction et/ou du canal d'entrée de fluide est pourvue de groupes de fonction chimique formés via un traitement physique ou chimique de la surface.
40. Dispositif selon l'une quelconque des revendications précédentes, comprenant un substrat, dans lequel la chambre de réaction et/ou le canal d'entrée de fluide est/sont formé(s) comme une ou des dépression(s), la chambre de réaction et/ou le canal d'entrée de fluide étant scellé(e) par une couche de recouvrement appliquée sur le substrat.
41. Dispositif selon la revendication 40, dans lequel le substrat et la couche de recouvrement sont constitués de matières polymères, le point de fusion d'au moins une de ces matières étant suffisamment bas pour permettre au substrat et à la couche de recouvrement d'être scellés l'un avec l'autre via un laminage thermique.
42. Dispositif selon la revendication 41, dans lequel ladite au moins une matière est le polyéthylène.
43. Dispositif selon la revendication 40 ou la revendication 41, dans lequel la couche de recouvrement est constituée d'une matière élastomère.
44. Dispositif selon la revendication 43, dans lequel la matière élastomère est le polydiméthylsiloxane (PDMS).
45. Dispositif selon la revendication 40, lorsqu'elle dépend de la revendication 30 ou 31, dans lequel au moins une partie du substrat est constituée d'une matière essentiellement opaque, et la couche de recouvrement est constituée d'une matière essentiellement transparente.
46. Dispositif selon la revendication 45, dans lequel la matière essentiellement opaque comprend un polymère chargé en carbone ou une matière céramique.
47. Procédé pour fabriquer un dispositif selon l'une quelconque des revendications 1 à 46, comprenant les étapes suivantes, qui peuvent être exécutées dans n'importe quel ordre ou simultanément, et consistant à former au moins une chambre de réaction présentant une surface intérieure hydrophile; et former au moins un canal d'entrée de fluide communiquant avec la ou les chambre(s) de réaction, au moins une partie de la ou de chaque canal d'entrée de fluide présentant une surface intérieure hydrophobe adaptée pour agir comme barrière destinée à empêcher le passage de fluide à travers le canal d'entrée de fluide dans la chambre de réaction jusqu'à ce qu'une force d'entrée de fluide ait agit sur ledit fluide.
48. Procédé selon la revendication 47, dans lequel le dispositif est constitué d'une matière polymère.
49. Procédé selon la revendication 48, dans lequel le dispositif est formé par moulage par injection, emboutissage à chaud, photo-ablation, coulée ou polymérisation dans un moule.
50. Procédé selon la revendication 48 ou la revendication 49, comprenant les étapes consistant à former un substrat comportant au moins une dépression, et appliquer une couche de recouvrement sur le substrat de manière à sceller la ou chaque dépression en vue de former au moins un canal d'entrée de fluide et/ ou au moins une chambre de réaction.
51. Procédé selon la revendication 50, dans lequel la couche de recouvrement est scellée avec le substrat via un laminage thermique.
52. Procédé selon la revendication 47, dans lequel au moins une partie du dispositif est constituée d'une matière céramique, de verre ou d'une matière conductrice ou semi-conductrice.
53. Procédé de fonctionnement d'un dispositif selon l'une quelconque des revendications 1 à 46, comprenant les étapes consistant à: placer au moins un

échantillon d'une solution aqueuse à tester à l'extrémité d'au moins un canal d'entrée de fluide à distance d'au moins une chambre de réaction, au moins une partie de chaque canal d'entrée de fluide présentant une surface intérieure hydrophobe; entraîner l'échantillon à pénétrer dans la ou les chambre(s) de réaction par l'intermédiaire du canal ou des canaux d'entrée de fluide en appliquant une force d'entrée de fluide; et contrôler l'échantillon dans la ou les chambre(s) de réaction dans le but de déterminer la présence ou la concentration d'une substance cible.

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54. Procédé selon la revendication 53, dans lequel le ou les échantillons) sont entraînés à sortir de la ou des chambre(s) de réaction avant que la ou les chambre(s) de réaction ou l'échantillon expulsé soit (en)t contrôlé(es) pour déterminer la présence ou la concentration d'une substance cible.

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55. Procédé selon la revendication 53 ou 54, dans lequel le ou chaque échantillon est appliqué au moyen d'une pipette, d'une seringue ou d'un injecteur à commande électrique.

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56. Procédé selon l'une quelconque des revendications 53 à 55, pour commander un dispositif selon la revendication 11 ou toute revendication dépendant de celle-ci, dans lequel la force d'entrée de fluide est fournie par un moyen d'aspiration, le moyen d'aspiration étant activé pour appliquer une pression réduite à la ou à chaque chambre de réaction pendant une période de temps comprise entre 0,1 et 100 secondes.

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57. Procédé selon l'une quelconque des revendications 53 à 55, pour commander un dispositif selon la revendication 19 ou la revendication 20, ou toute revendication dépendant de celles-ci, dans lequel la force d'entrée de fluide est fournie en faisant tourner le substrat ou l'élément de support à une vitesse angulaire comprise dans la gamme allant de 1 à 1000 révolutions par minute pendant une période de temps comprise entre 1 et 100 secondes.

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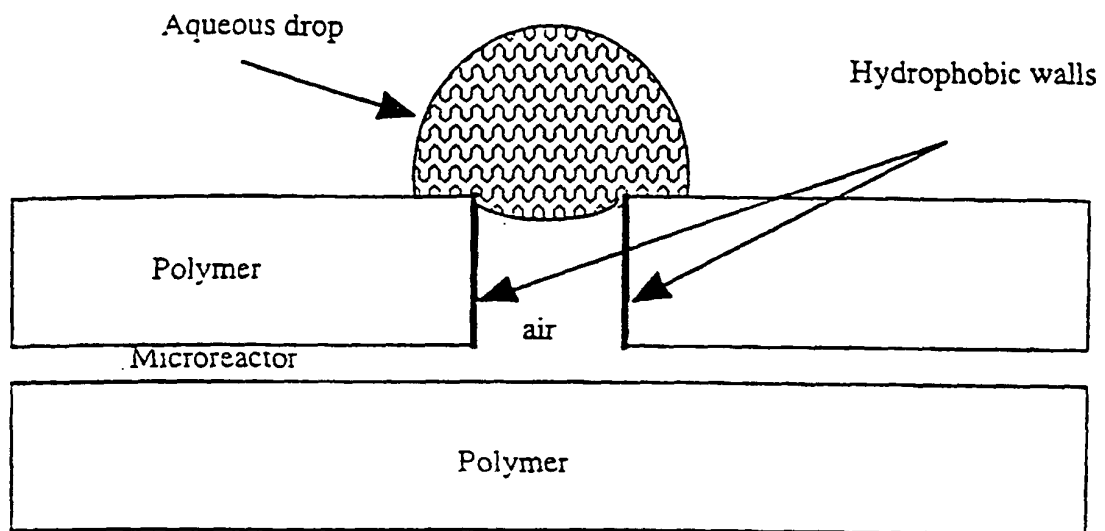
58. Procédé selon la revendication 57, dans lequel l'échantillon est expulsé de la chambre de réaction en faisant tourner le substrat à une vitesse angulaire plus élevée, comprise dans la gamme allant de 10 à 100000 révolutions par minute, pendant une période de temps comprise entre 1 et 100 secondes.

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59. Procédé selon l'une quelconque des revendications 53 à 55, pour commander un dispositif selon la revendication 7 ou toute revendication dépendant de celle-ci, dans lequel la force d'entrée de fluide est fournie par la pression d'un piston.

55

a)



b)

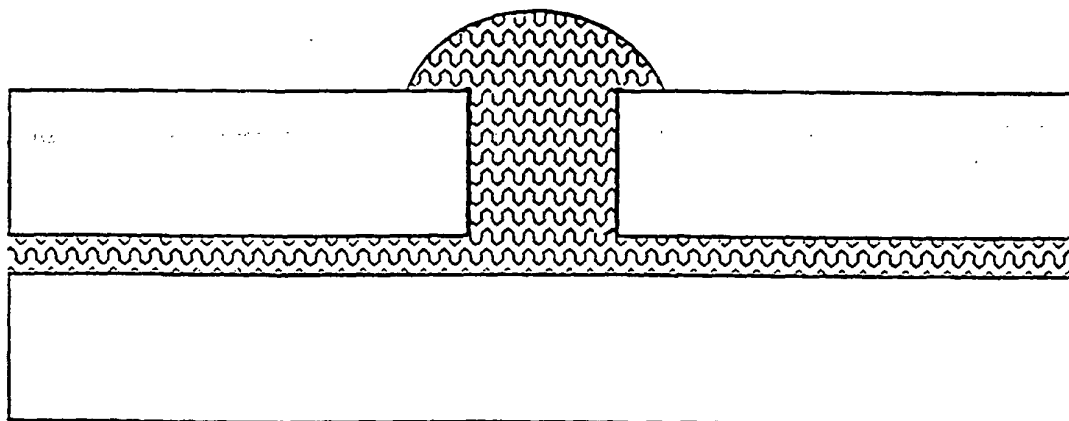


FIG 1

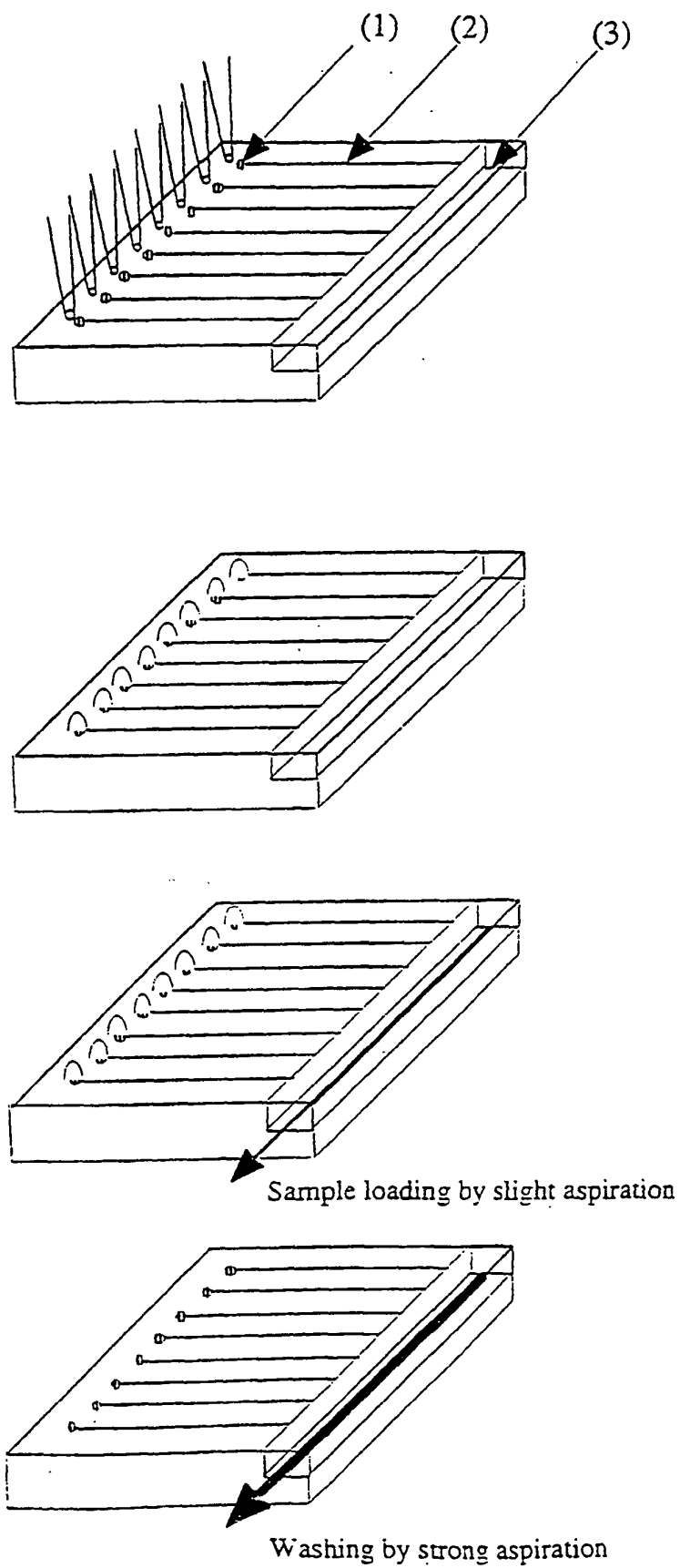


FIG 2

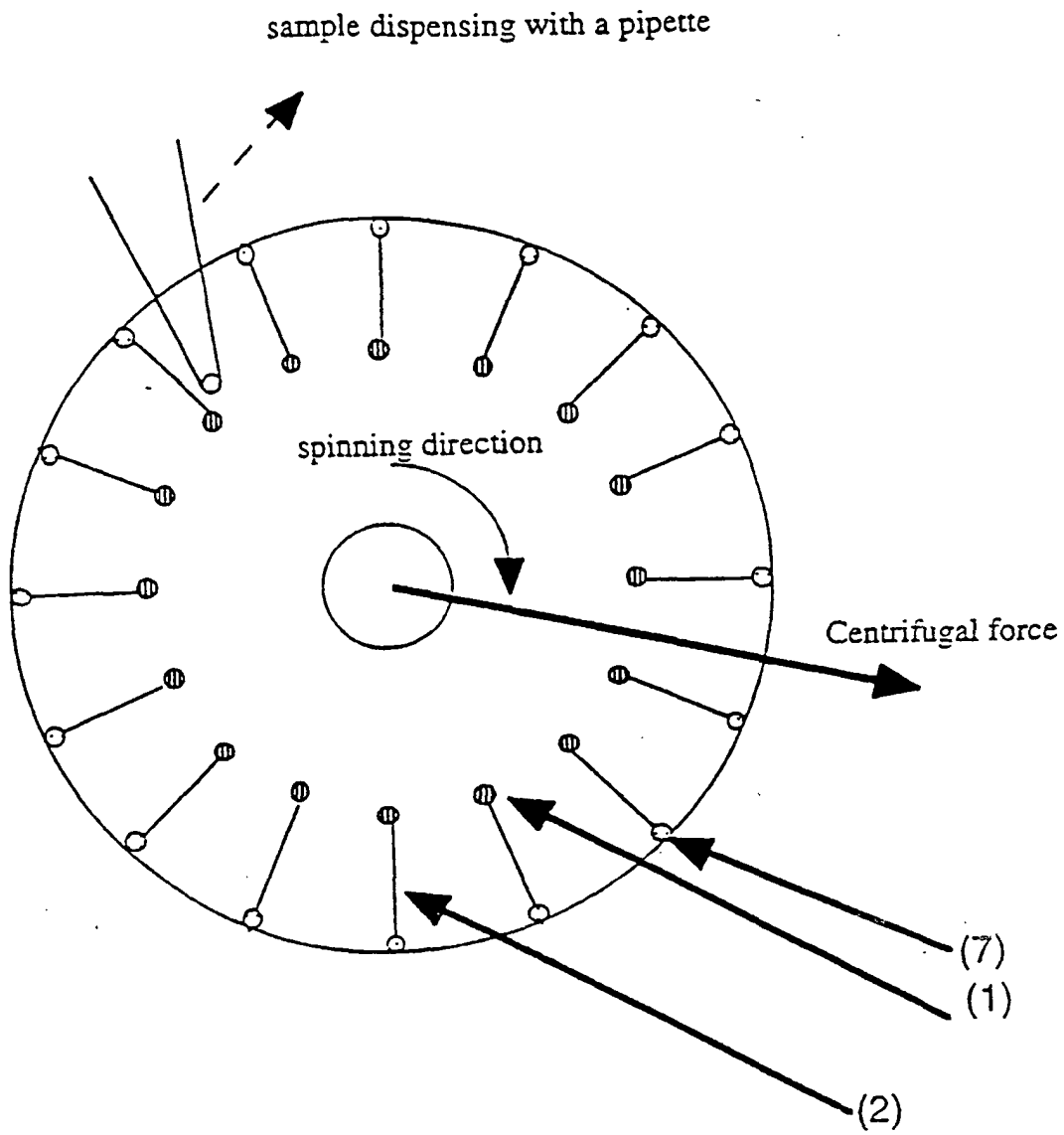


FIG 3

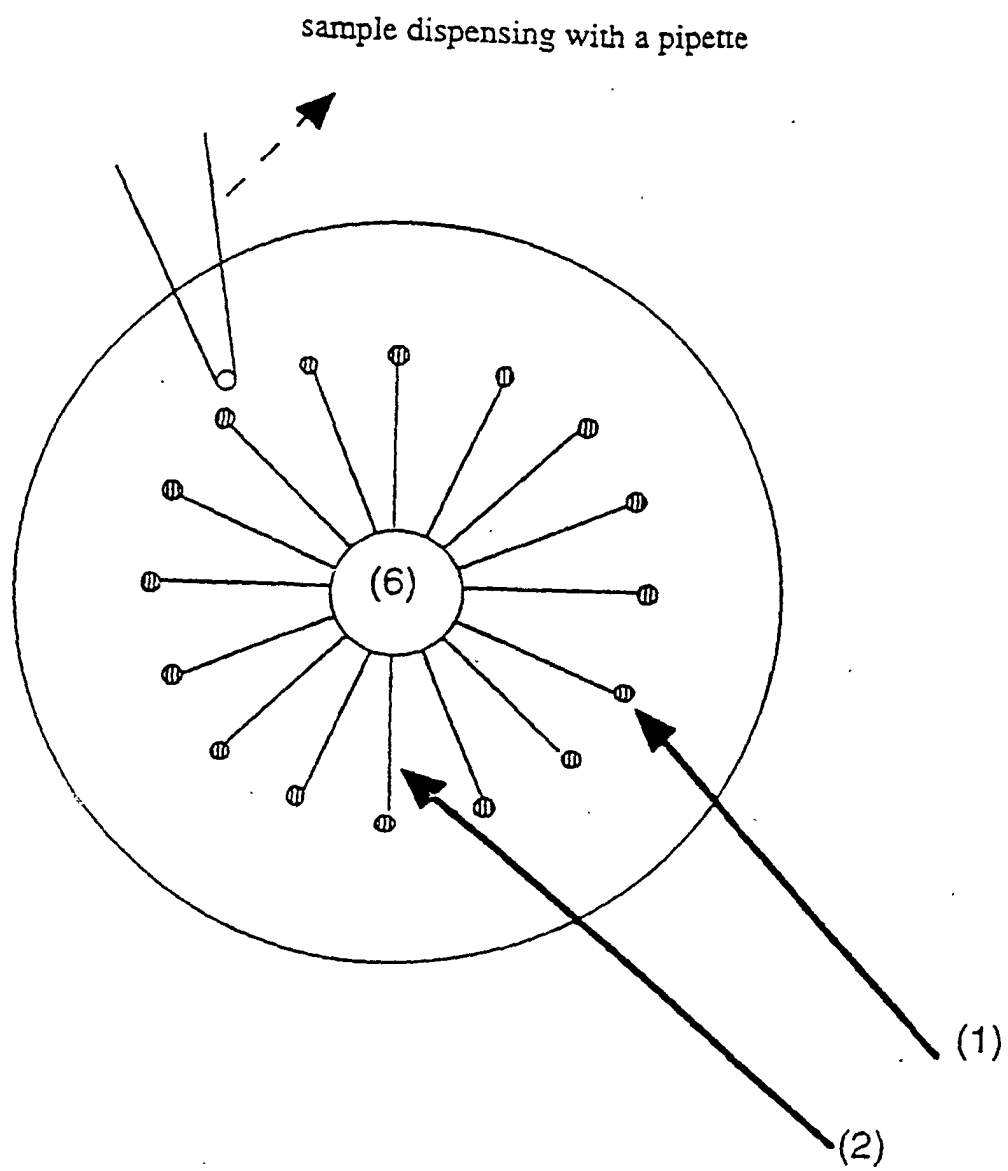


FIG 4

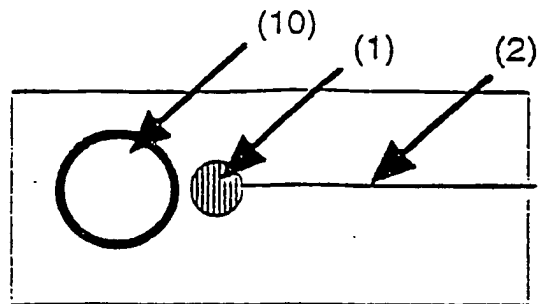


FIG 5a

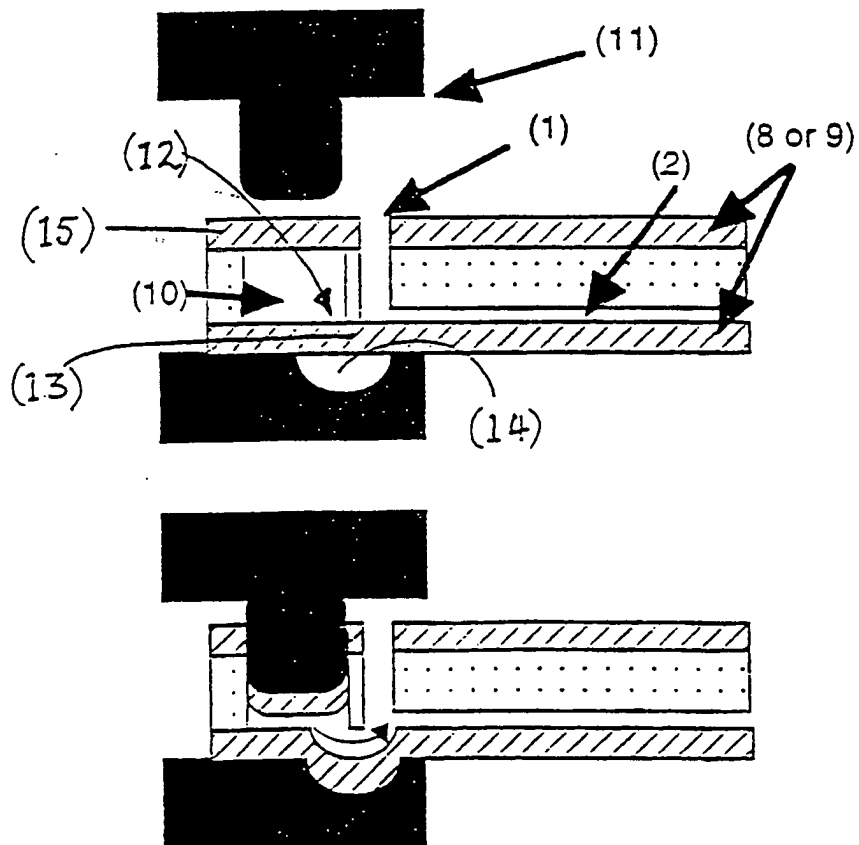
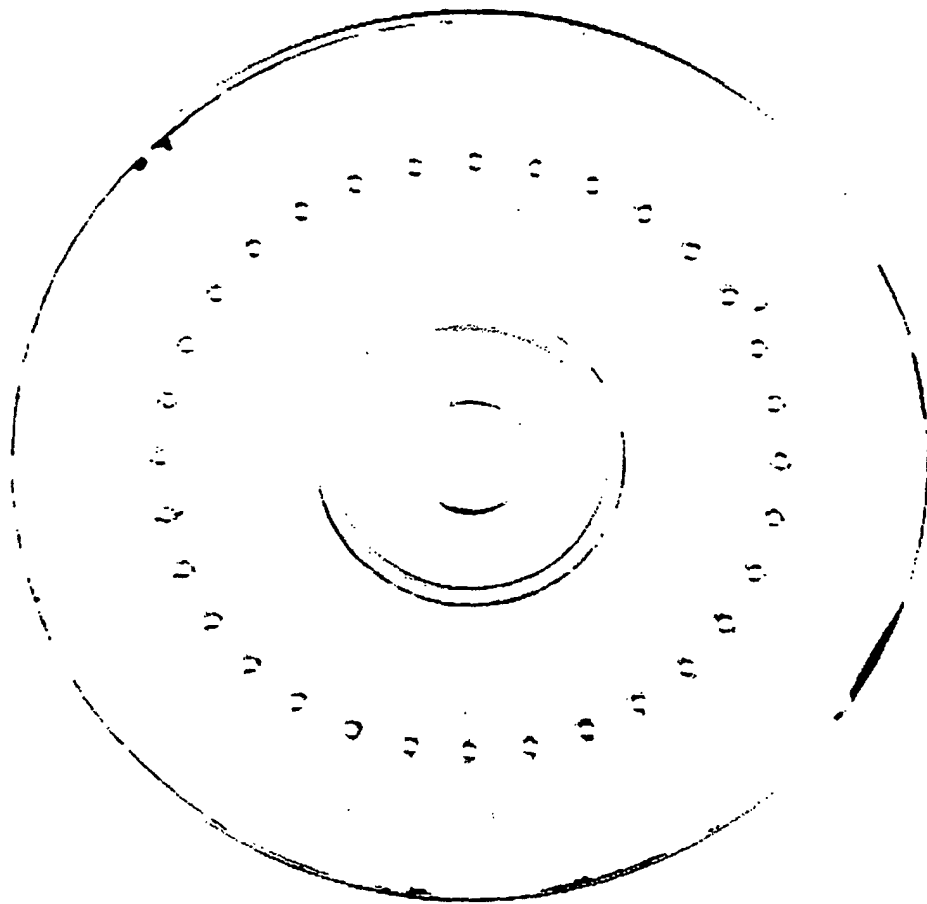


FIG 5b

Figure 6:



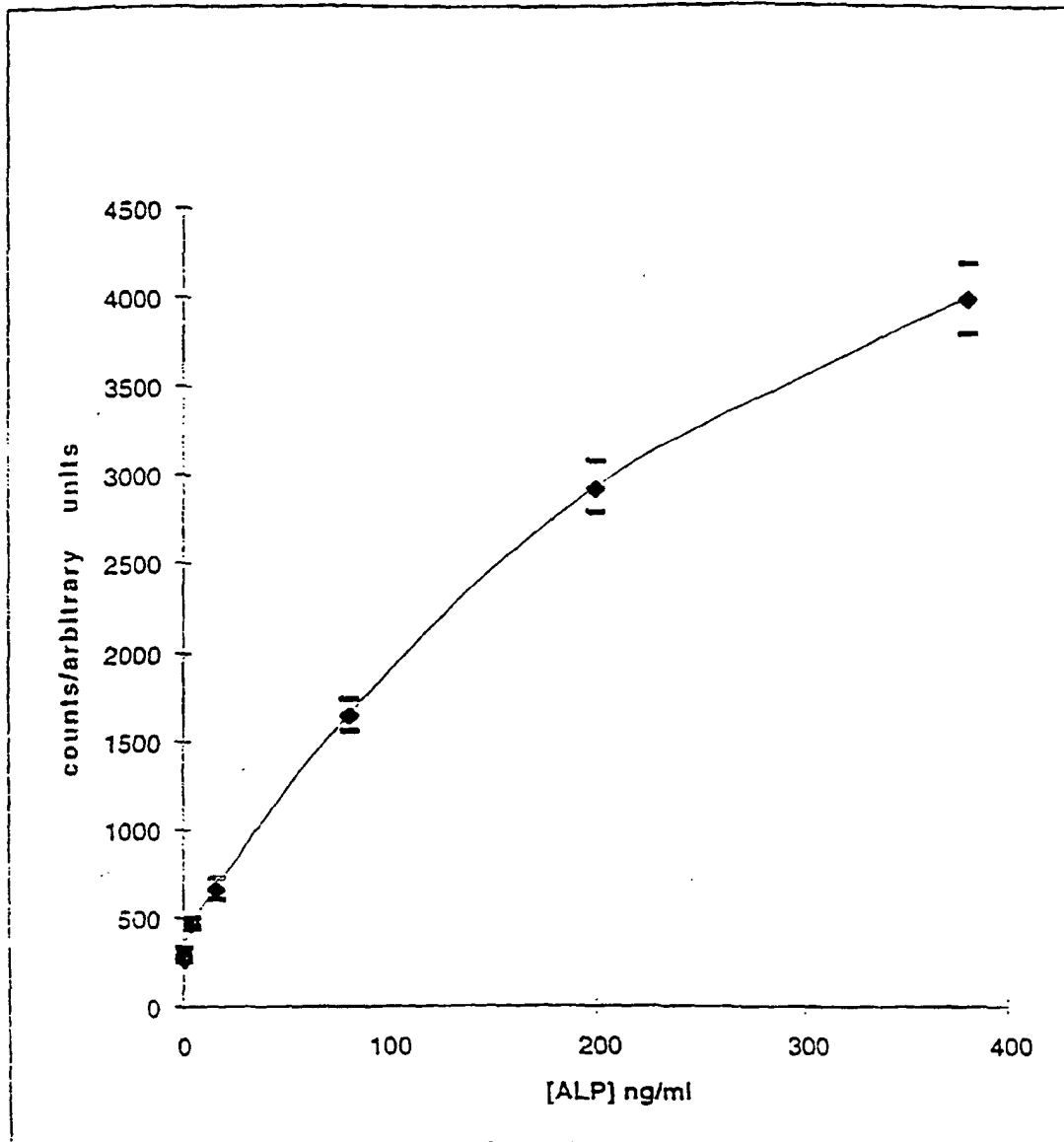


FIG 7

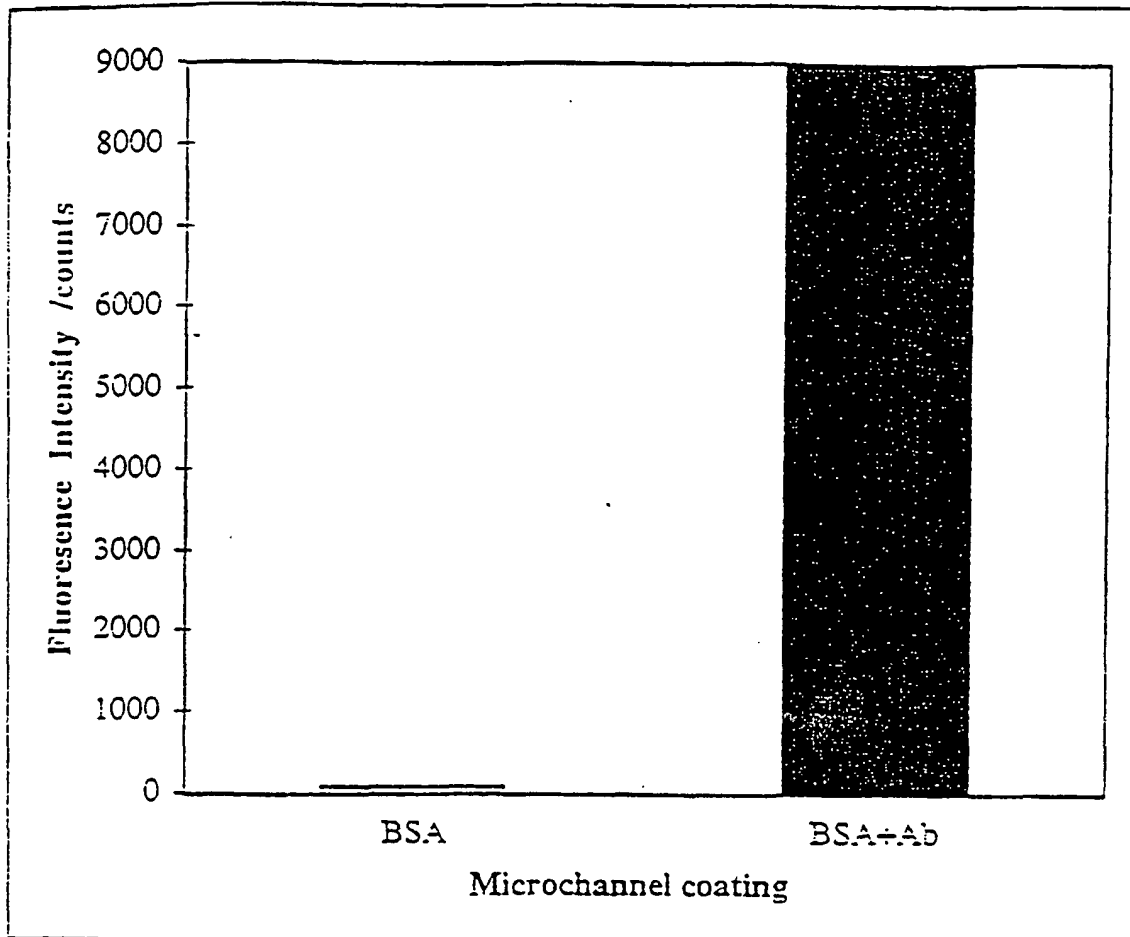
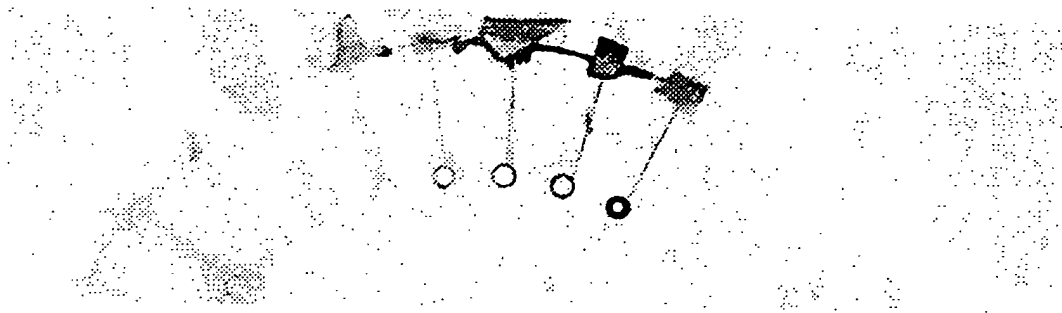


FIG 8

Figure 9:



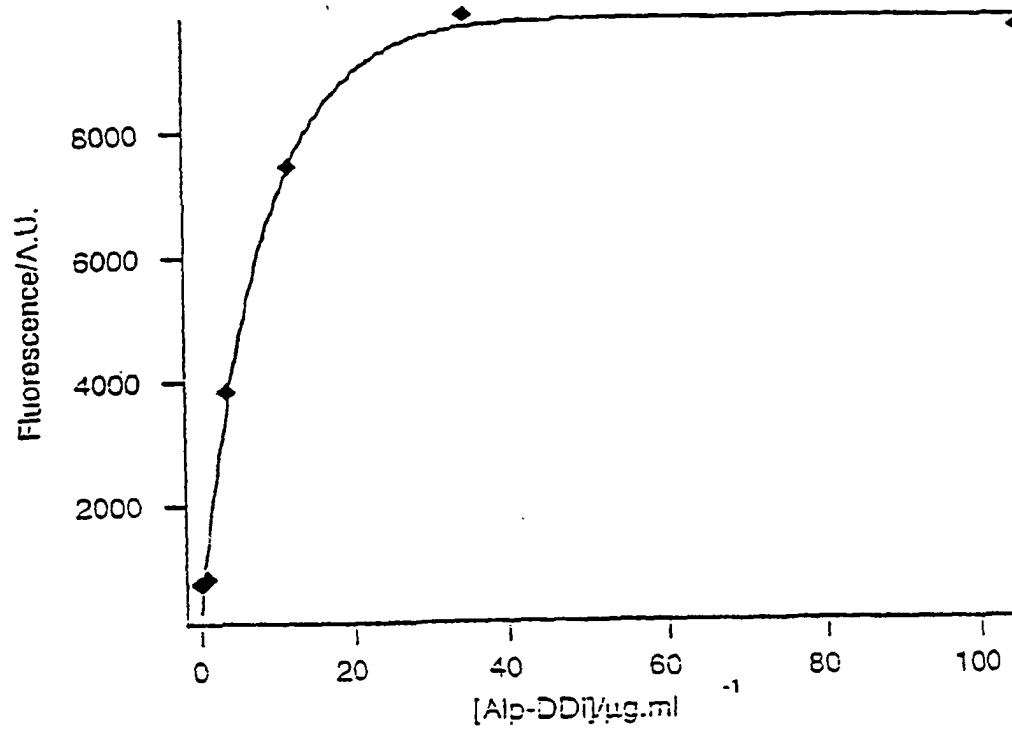


FIG 10

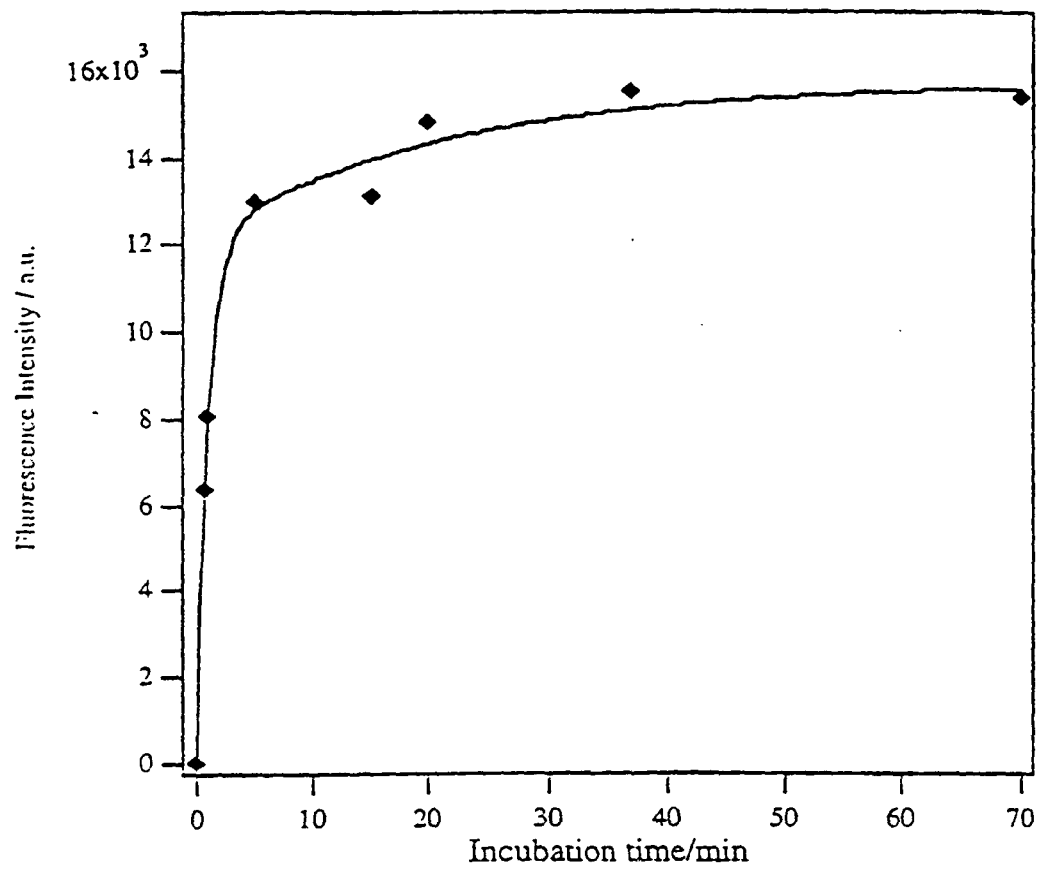


FIG 11

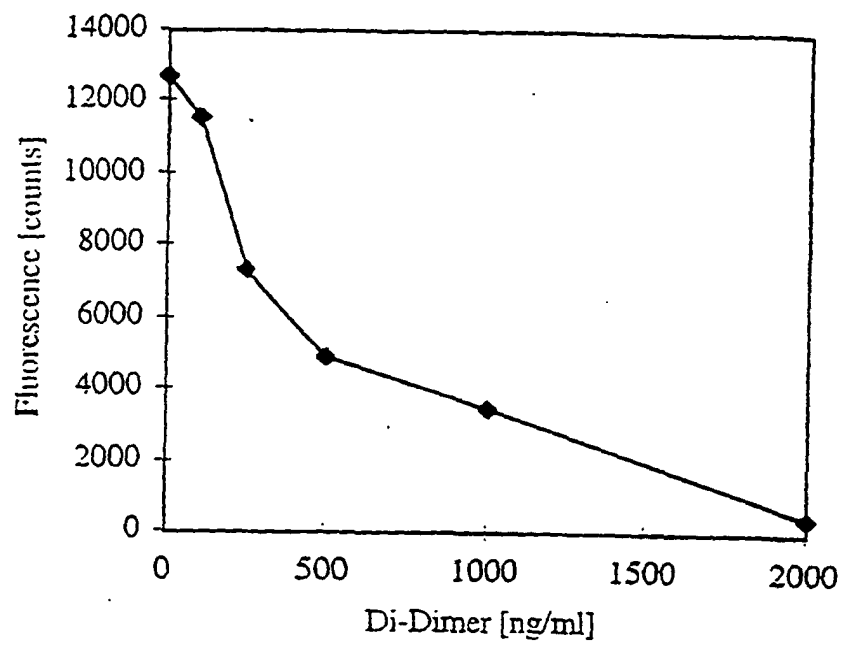


FIG 12