

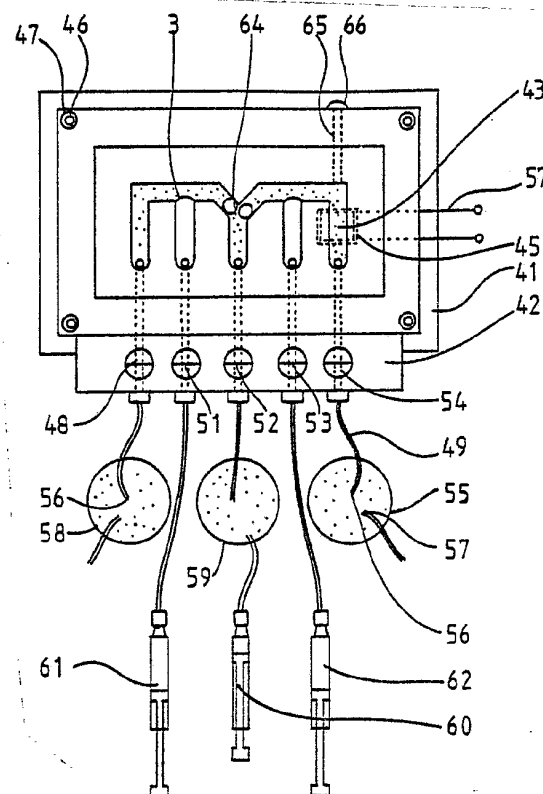


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(54) Title: CHEMICAL DROPLET REACTOR**(57) Abstract**

A method and apparatus for combining chemical reactants wherein the method comprises the steps of: 1) dispensing the reactants into a conduit system in the form of discrete volumes or droplets (5, 15, 21, 22, 36) separated from each other by an inert immiscible liquid (1); and 2) changing the flow of the inert immiscible liquid within the conduit system in a precise sequence in such a manner that predetermined discrete volumes coalesce with each other, thereby combining the reactants; and wherein the apparatus is formed by holding two plates (42, 44) together in face to face contact, where one or both plates possesses a set of indentations (43) on the surface to be mated, thereby forming the conduits for reactant combinations.



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CHEMICAL DROPLET REACTOR

The invention is the subject of British Patent Application No. 8200642.

This invention relates to a method for combining liquid chemical reactants and to apparatus for use therein.

Description of prior art

Automatic analysis apparatus for analysis of liquid samples as a flowing stream was disclosed by Skeggs in U.S. Patent No. 2,797,149 issued June 25 1957 and in U.S. Patent No. 2,879,141 issued March 24 1959. Such a flowing stream is customarily segmented by separating successive segments with air, but it may alternatively be segmented by an inert immiscible liquid, as taught in U.S. 3,479,141 issued to John Smythe November 18 1969. Such methods provide an extremely powerful means of analysing a number of liquid samples by subjecting them to a particular chemical test.

SUMMARY OF THE INVENTION

In contrast with continuous flow systems, the present invention does not operate with a continuous stream, where control is achieved by the selection of appropriate flow rates for each fluid by running a pump or cyclically operated piston at a predetermined rate, but rather the liquid components of the present invention are controlled by a sequence of distinct movements in closed conduits, with each movement or set of movements occurring after the previous movement is completed. For example, a known volume of a reactant is moved through an opening, this movement ceases, then the volume displaced is separated from the parent volume. It should be noted that the volumes of all the reactants to be combined are contained and defined by the immiscible liquid prior to combination, and that individual single reactant samples can easily be handled by virtue of the large amounts of the immiscible liquid present, and of the method of control. Thus each sample is treated by a sequence of changes



of the movements of the liquid components causing it to combine with a reactant or reactants selected at will from a plurality of reservoirs of different reactants. The reactants are thus metered within the conduit system.

It should also be noted that the conduits which are to contain the liquid components are preferably constructed by holding two plates together in face to face contact, where one or both plates possesses a set of indentations or channels on the surface to be mated.

OBJECTS OF THE INVENTION

Accordingly several objects of my invention are:

1. to carry out chemical reactions using volumes of reactants that may range from more than a litre to less than 10 nanolitres, and
2. to carry out repetitive chemical procedures automatically, and by less complex means than in comparable systems, and
3. to allow greater flexibility during operation, by continuously providing a choice of reactants, and
4. to isolate workers from dangerous substances including radioisotopes and pathogens, and
5. to reduce or eliminate contamination of the reactants, and
6. to prevent accidental mixing spillage or losses of the reactants during transfer, and
7. to provide a method and a means for metering samples and reactants within a conduit system.

DRAWINGS

Figure 1 is a plan view of two conduits being used in accordance with the method for producing droplets of reactants. The stippled areas represent carrier phase.

Figure 2 is a plan view of conduits being used in accordance with an alternative method of producing droplets of reactants.



Figure 3 is a plan view of conduits being used in accordance with a method for coalescing droplets.

Figure 4 is a plan view of an apparatus in accordance with the invention, which has been adapted to allow the separation of substances by electrophoresis.

Figure 5 is an exploded view of one embodiment of an apparatus in accordance with this invention.

Figure 6 is a plan view of the apparatus of figure 5.

DESCRIPTION OF THE INVENTION

The method and apparatus of the invention may be used for initiating and controlling a chemical reaction, or preparing mixtures of reactants, wherein the method comprises steps of:

1. dispensing two or more reactants, optionally in a solvent phase, into a conduit system in the form of discrete volumes, each volume being separated from each other by an inert and immiscible liquid, and
2. moving the immiscible liquid and discrete volumes through the conduit system in such a sequence that predetermined discrete volumes individually coalesce with each other, thereby combining the reactants.

The system is particularly suited to the manipulation of microscopic quantities of reactant with volumes of less than 10 nanolitres, but larger quantities of more than a litre could also be used. The system is capable of treating single volumes of reactants individually.

The discrete volumes may be separated from their parent volumes by passing a small volume of reactant from a reservoir, through a constriction and into a conduit meeting the reservoir at right angles. The discrete volume is now separated from the parent volume in the reservoir by passing carrier phase along the conduit.

The discrete volumes of reactants are coalesced with each other by pressing volumes together by gravity, or by withdrawing the carrier phase from between a plurality of discrete volumes, thereby causing them to move together until they collide, or by causing one volume to remain in a position partially blocking the path of a second discrete volume, then moving this second discrete volume through the opening thus



formed, causing the two discrete volumes to be pressed together and to coalesce.

The reactants can be liquids, or gases capable of being condensed in the apparatus, or solids or gases dissolved in a suitable solvent. This reactant liquid, hereafter referred to as a reactant, will be supported, defined and moved by another, immiscible liquid, referred to hereafter as the carrier phase. Suitable carrier phases include mineral oils, light silicon oils, water, and fluorinated hydrocarbons. The carrier phase may be a mixture of several substances, and preferably has approximately the same density as the reactants. Surface acting agents may be included in the carrier and/or reactant phase to produce suitable surface properties, e.g. to allow efficient merging. Suitable surface acting agents include cholesterol, gelatine, sodium dioxy, succinate Teepol, and Triton-x-100.

The cross-section of the conduits is generally of the same order of magnitude as the cross-section of the droplets which are to be used in them, but smaller conduits, or larger droplets or discrete volumes may be used, causing the droplets to become elongated.

The carrier phase may be moved by any suitable means, including the distortion of flexible tubing or sheeting, blowing or sucking by mouth, pumps or pistons. Pistons can be moved by screw threads turned by electric motors, (which may optionally be controlled electronically using microprocessor technology).

Figure 1 of the accompanying drawings illustrates one method of separating droplets of a predetermined volume from a larger reservoir of reactant. The reactant is introduced into a conduit containing carrier phase 1 from a side arm 2, by being sucked or pushed through a small opening 3. When the correct volume has passed through into the conduit a flow of carrier phase separates it from the reactant in the reservoir, and carries it down the conduit. The volume of the droplet produced can be determined by at least three methods:

1. The piston responsible for pushing the reactant through the opening is moved a known distance, corresponding to the volume of the reagent displaced. When this movement is complete the droplet is broken off by a relatively fast, shortlived current of carrier phase.
2. If large numbers of droplets are required, a continuous flow of carrier phase flows down the conduit. When reactant is simultaneously passed through the opening 3 a series of droplets will be formed, each broken off just before it spans the conduit. The exact size of the droplets thus produced will depend on the magnitudes of the



two currents.

3. The volume of the droplet before separation 5, is estimated visually, optionally using a telescope or microscope with a graduated eye piece. When the correct volume is achieved it is separated by a current of carrier phase.

Figure 2. illustrates another method of forming droplets. In this case the reactant 16 is passed into a conduit of considerably narrower width 11 than the droplets to be produced. The conduit is graduated relative to the opening 12 of a side arm 13. The reactant is passed into the narrow conduit to a certain graduation 14, whereupon carrier phase is introduced from the side arm, thus separating a droplet of the required size 15.

Figure 3. illustrates a method of coalescing droplets, which can be of different sizes. The coalescence is effected at a site where two conduits, 23 and 25 in figure 3, merge to form a single conduit 24, preferably in a Y-configuration as shown. A current from conduit 23 to conduit 24 is produced which carries a droplet 21 to the site for coalescence. When the surface of this droplet protrudes into the space where conduits 23 and 25 converge, the valves feeding conduit 23 are closed. A current from conduit 25 to conduit 24 now carries a second droplet past the first, and in doing so the two droplets are pressed together, and coalesce to form a single droplet.

If either droplet is smaller than the other, then the conduit through which it emerges should be correspondingly narrower. All conduits should have diameters approximately the same as the droplets to be used in them.

The various movements of the fluids are produced by connecting the conduits to pistons, pumps or to reservoirs containing compressed gas or partial vacuum. Control is achieved by conventional valves.

Conduits can be heated by electric circuits or coils or by electromagnetic radiation, allowing coalesced droplets to be incubated at the desired temperature. Solid state heat pumps can be used to cool conduits both for incubation at low temperatures and to allow the storage of unstable reactants. Heating can be achieved by reversing the heat pumps. Heating and cooling can also be achieved by circulating a fluid through separate ducts.

After coalescence the reactants can be thoroughly mixed by moving the droplet up and down a conduit, or if required by passing it through a constriction several times.



Ports can be provided to allow droplets to be removed or introduced with a syringe needle.

The droplet can be passed into a tube and transferred to any standard instrument of chemical or biochemical analysis. Alternatively parts of the device itself can be adapted to form the sample chambers of such instruments. For example conduits can be formed with two plain transparent walls to form the sample chambers of photometers. A single transparent wall opposite a reflecting surface could also be used.

Conduits can also be packed with the materials used for electrophoresis and chromatography.

Figure 4. shows a conduit which is adapted for electrophoresis. The electrophoretic conduit 31, is packed with electrophoretic material. The sample to be separated by electrophoresis is introduced as a droplet from conduit 32, and this droplet is merged on one side with the surface of the electrophoretic material (e.g. agarose, polyacrylamide or cellulose) and on the other side with a semipermeable membrane. This semipermeable membrane 33 encloses a chamber filled with buffer, containing an electrode 34 and provided with a gas vent 35 if required. Another droplet 36 is merged on to the other end of the electrophoretic material and to another semipermeable membrane and electrode assembly 37. A current is passed between the electrodes which causes the constituents to migrate at various speeds. The fastest fractions migrate into the receiving droplet first. This droplet is now exchanged for another 38 after a period, allowing the collection of a series of droplets containing different fractions.

The ability to handle very small samples makes the system suited to use with High Performance Liquid Chromatography.

The system can be connected to a conventional H.P.L.C. system by fine bore tubing or to a mass spectroscopy system.

The progress of droplets can be automatically monitored by detecting fluctuations in the light propagated through the conduit as the droplets pass. Similarly, when two droplets merge the light scattered from a beam passing through both droplets suddenly decreases. Windows in an opaque coating of the conduits can be used, and in cases where the monitoring of many sites is required, optic fibres could carry light to or from a central emitter or detector. The position of droplets could also be detected when they pass between an infra-red emitter and detector, or when they pass between the



plates of a conductance cell, or by the resultant change in the refractive index of the contents of the conduits. In any automatic embodiment of the device, all pressure generating and monitoring means can be controlled by microprocessors or computers.

The preferred method of construction of the apparatus is by producing indentations or channels of the appropriate configuration in the surface of a plate, and to clamp or hold this plate against another, producing closed ducts or conduits. The second plate may have a planar surface, or it may have indentations, either in the mirror image of the first plate's or in a different configuration. Also it is possible to produce three dimensional configurations, where conduits cross over each other, by using three or more plates. Preferably at least one of the plates is transparent, e.g. glass or perspex, allowing visual inspection of the procedures. The other plate may be Kel-F or Teflon (materials suited for use with aqueous reactants) or glass, perspex, metal, polyvinyl-chloride, polypropylene etc.

Indentations in glass can be etched. Plastics and metals can be etched, moulded or machined. Electrical contacts can be produced by depositing a metallic layer of the desired configuration on either plate.

Micro-organisms can be cultured in droplets containing the appropriate nutrients and incubated at the correct temperature.

In place of droplets, larger segments occupying a considerable length of conduit can be separated from reservoirs of reactants and coalesced with other segments in the same way as droplets.

Contamination of the walls can be reduced by:

1. constructing conduits with highly polished walls,
2. constructing conduits with walls coated with or constructed from a material which repels the reactant phase but attracts the carrier phase,
3. washing the conduits with segments of a cleaning liquid prior to the passage of each volume of reactant.
4. preventing the droplets or segments from remaining stationary by oscillating the flow of carrier phase thus maintaining a film of carrier phase around the reactant.



An apparatus can be constructed in accordance with the method of the invention by joining tubes and pipes in the desired configuration.

This invention has applications in many branches of medicine, chemistry, biochemistry, geology, etc., notably in procedures which utilize very small volumes, such as forensic and molecular biology work. The invention could be used to perform biological or chemical assays of sub-samples during purification procedures, and to produce chemical derivatives of samples prior to analysis by chromatography or mass spectroscopy. It could also be used for chemical analysis or synthesis in outer space, in conditions of very low gravity and pressure.

OPERATION OF INVENTION

Figure 5 is an exploded view of one embodiment of an apparatus in accordance with the method of the invention. This embodiment is capable of mixing predetermined volumes of two aqueous reactants under sterile conditions.

The device comprises a steel base 41 supporting a Teflon block 42 in which U-sectioned indentations 43, of the configuration shown, have been machined. A sheet of siliconized glass 44 is pressed against this block by a clamp 45 which is tightened by nuts 46 and bolts 47. The five branches of the U-sectioned conduits thus formed lead to five stop-cock valves 48. The middle and outer pair of these conduits are connected by Teflon tubing 49 to reservoirs of the carrier phase, which is mineral spirit. The remaining two branches are connected to glass syringes, each containing one of the two reactants to be mixed.

Figure 6 is a plan view of the device of figure 5 and uses the same reference numerals. Reservoirs 55 and 58 are half-filled with mineral spirit with the ends of the tubing 56 opening below the surface of the mineral spirit, while the end of an air vent 57 opens above the surface in each. A syringe containing air 60 is connected to reservoir 59 above the surface of the mineral spirit.

The ports serving the valves 51 and 53 lead to two syringes 61 and 62 containing the two reactants. The conduits are initially filled with white spirit.

An operating sequence for one cycle is as follows:

1. Valves 48, 51, and 52 are opened. Valves 53 and 54 are closed.
2. Syringe 61 is moved in until reactant reaches constriction 3.



3. Syringe 61 is moved further by the required amount, pushing a known volume of reactant through the constriction 3.
 4. Valve 51 is closed.
 5. Syringe 60 is moved out until the volume of reactant protruding through constriction 3 is broken off, and the resultant droplet moves towards the Y-junction 64. Valve 48 is closed.
 6. Valves 53 and 54 are opened and a similar sequence of events produces a droplet of the reactant from syringe 62.
 7. Syringe 60 moves out until the droplet originating from syringe 62 protrudes into the Y-junction. Valves 53 and 54 are closed. The positions of the droplets are illustrated at this stage in Figure 6.
 8. Valve 48 is opened and syringe 60 moves out until the droplet of the reactant from syringe 61 moves into the Y-junction 64, where it is pressed against the other droplet causing the droplets to coalesce.
 9. If a colour change reaction is involved, such a colour change can be recorded immediately, or after incubation using a thermostatically controlled coil 57. The droplet can be discarded by passing it into a reservoir 59 of mineral spirit.
- If the droplet is to be analysed using external instrumentation, it can be moved to a site opposite port 65, all valves closed, plug 66 removed and the droplet impaled and removed by a needle and syringe and transferred to the instrument. The plug is then replaced.
10. Syringe 60 is disconnected, emptied of air, and reconnected, leaving the device primed for another cycle of operation.

It is emphasised that Figure 5 shows a very simple embodiment of the invention. Embodiments are envisaged having reservoirs for the introduction of more than twenty different reactants. It is also proposed to operate the valves automatically, and heat and cool regions of the conduits to allow incubation of reacting mixtures, and the storage of reactants for long periods.

While the above description contains many specificities, these should not be construed as limitations on the scope of the invention, but rather as an exemplification of a few



preferred embodiments thereof. Many other variations are possible, for example it is possible to press together and coalesce droplets by gravity, and to use water as the carrier phase for oil soluble reactants. Accordingly, the scope of the invention should not be determined by the embodiments illustrated but by the following claims and their legal equivalents.



CLAIMS

1. A method of combining chemical reactants comprising the steps of:

dispensing two or more liquid reactants into a conduit system in the form of discrete volumes of liquid, each volume being separated from each other and from all undispensed reactants by an inert immiscible liquid, thereby metering the reactants within the conduit system; and

changing the flow of said immiscible liquid and said discrete volumes through said conduit system in a precise sequence, in such a manner that predetermined discrete volumes are individually coalesced with each other, thereby combining the reactants.

2. A method as claimed in claim 1 in which said discrete volumes of chemical reactants are sufficiently small in relation to said conduits to form substantially spherical droplets with diameters less than the conduits.
3. A method as claimed in claim 1 in which said discrete volumes of chemical reactants are sufficiently large in relation to said conduits to be elongated by the conduits.
4. A method as claimed in claim 1 in which each of said discrete volumes of reactants is formed by passing each chemical reactant from a reservoir, through an opening, into a conduit initially containing said carrier phase, then moving said carrier phase causing a volume of said reactant to become separated from the reactant in the reservoir, said opening being optionally constricted, thereby metering the reactant within the conduit system.
5. A method as claimed in claim 4 in which the volume of reactant passed out of the reservoir is estimated by assuming it to be spherical, and by measuring the diameter



of such a sphere optically, after which said volume is adjusted and separated.

6. A method as claimed in claim 4 in which the volume of reactant passed out of the reservoir is estimated before separation by measuring the length of conduit which it occupies, after which said volume is adjusted and separated.
7. A method as claimed in claim 4 in which the volume of reactant passed out of the reservoir is determined by displacing said volume by moving a piston a known distance, or by means of a well calibrated pump, prior to the separation of said volume.
8. A method as claimed in claim 1 in which the immiscible liquid is moved until a discrete volume of reactant partially blocks the opening of a conduit, whereupon another discrete volume of reactant is moved out of or into said opening causing these two discrete volumes to be pressed together, causing them to coalesce.
9. A method as claimed in claim 1 in which said discrete volumes of reactants contain suspended micro-organisms.
10. A method as claimed in claim 1 in which surface acting chemical agents are dissolved in said chemical reagents or in said immiscible liquid, or in both.
11. An apparatus for combining chemical reactants comprising two or more plates held in face to face contact, in which one or more plates possesses a set of indentations or channels, and bores, on the surface to be mated, such that closed conduits are formed, these conduits being in communication with reservoirs to contain two or more reactants and an inert immiscible liquid and with means for producing and controlling movements of the liquid components, said plates having surfaces with low affinity for the reactant to be used, which apparatus includes at least one zone where at least three conduits meet, which zones allow the formation and coalescence of chemical reactants.
12. An apparatus as claimed in claim 8 but in which said conduits are constructed by joining a set of tubular elements in the appropriate conformation.
13. An apparatus as claimed in claim 11 or 12, comprising three conduits which join in a T or Y configuration, these three conduits leading to reservoirs for an immiscible liquid, each reservoir possessing means for moving said immiscible liquid, in which one or more of said conduits communicates with reservoirs for a plurality of reactants.



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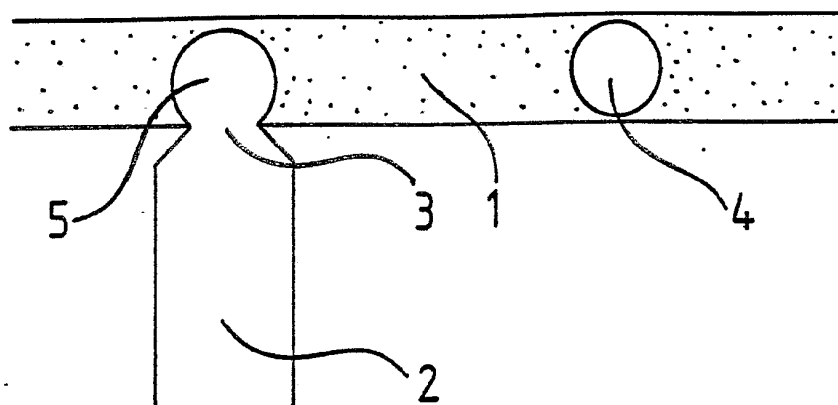


Figure 1.

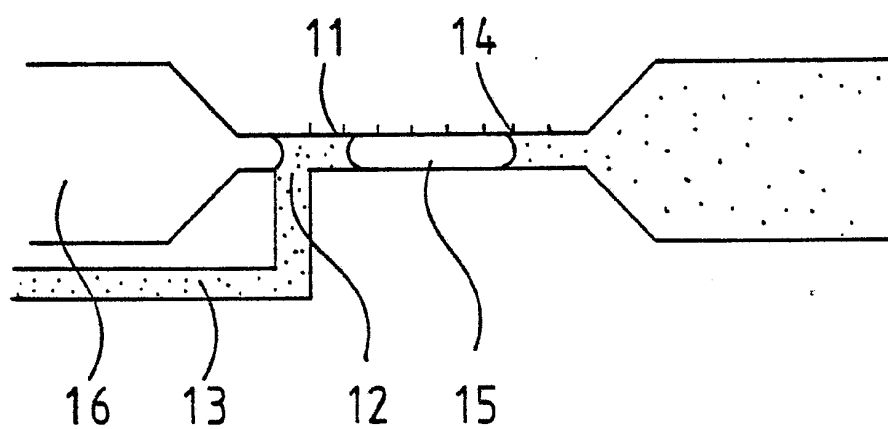


Figure 2.

SUBSTITUTE SHEET



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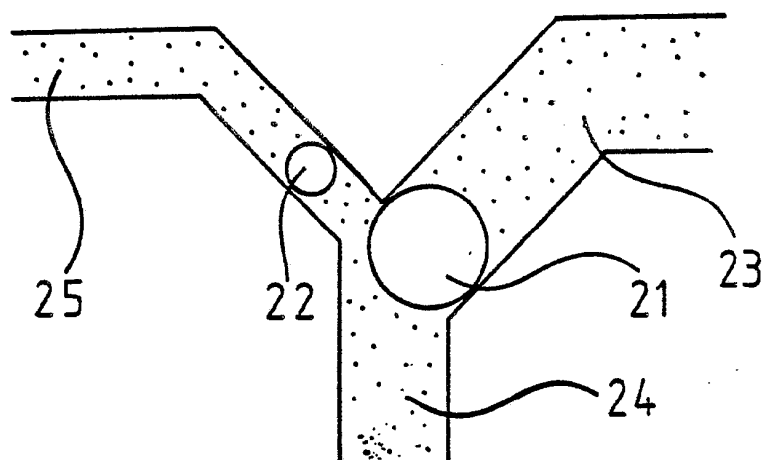


Figure 3.

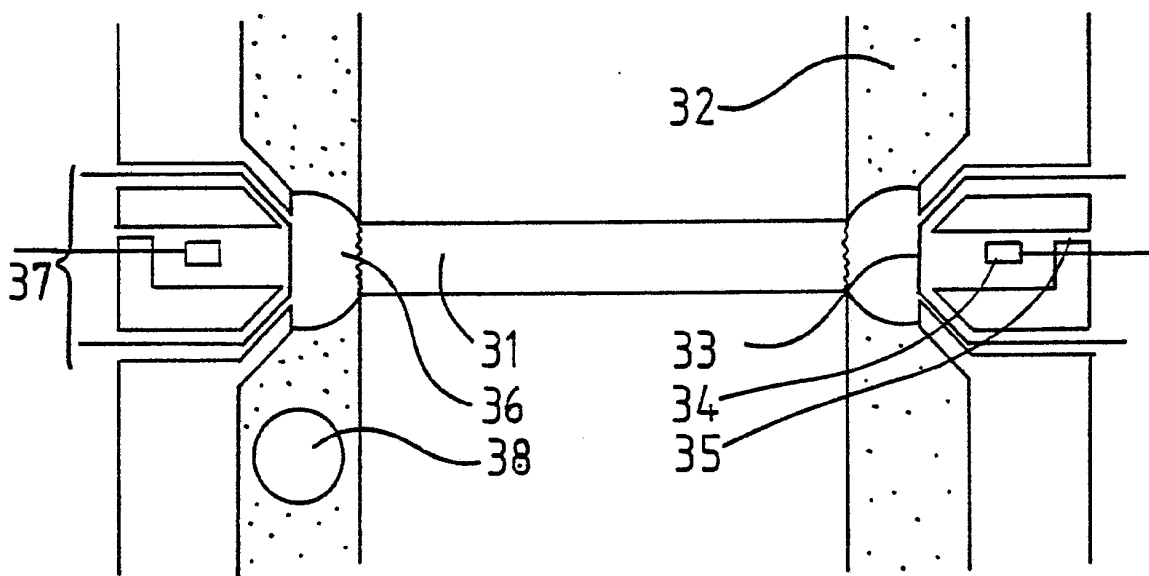
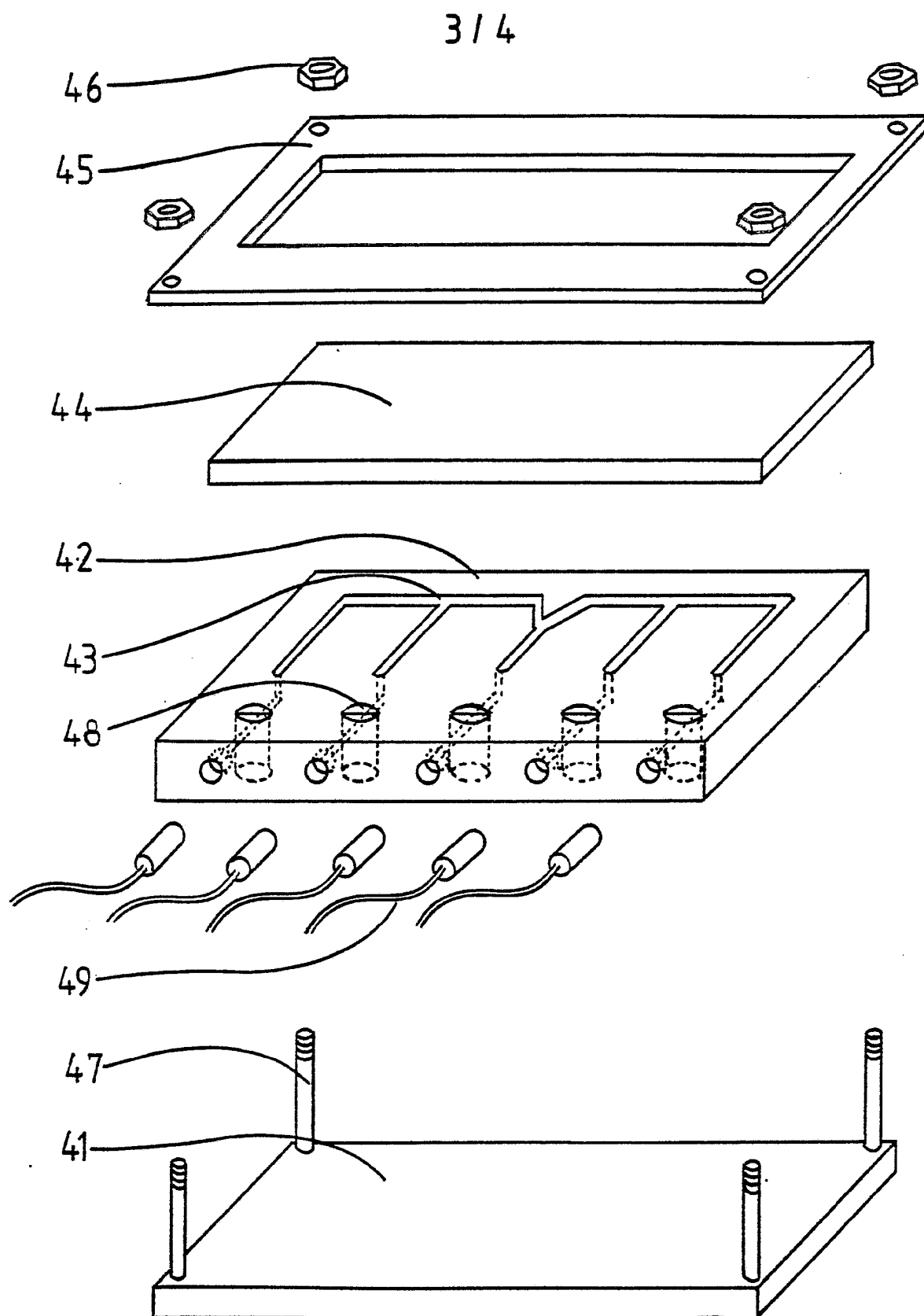


Figure 4.

SUBSTITUTE SHEET





SUBSTITUTE SHEET



INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 82/00319

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ³		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC ³ : G 01 N 1/00; G 01 N 35/08		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁴		
Classification System	Classification Symbols	
IPC ³	G 01 N 1/00; G 01 N 35/00	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched ⁵		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴		
Category [*]	Citation of Document, ¹⁵ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
X	EP, A, 0047130 (VICKERS LTD.) 10 March 1982 see page 2, line 25 - page 3, line 35; page 6, line 31 - page 7, line 19; page 8, line 1 - page 9, line 1; figures	1,4,7,12,13
X	US, A, 4315754 (J. RUZICKA et al.) 16 February 1982 see column 6, lines 4-34; figures 2,4,6	1,4
A	US, A, 4253846 (W.J. SMYTHE et al.) 3 March 1981 see column 5, line 13 - column 6, line 18; column 7, lines 6-49; figures 2,4-6	1,7
A	US, A, 4022575 (E.H. HANSEN et al.) 10 May 1977 see column 5, lines 6-30; figure 2	7
A	US, A, 4130394 (K.M. NEGERSMITH) 19 December 1978	./.
<div style="display: flex; justify-content: space-between;"> <div style="width: 48%;"> <p>[*] Special categories of cited documents: ¹⁶</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the International filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 48%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search ¹	Date of Mailing of this International Search Report ¹	
17th June 1983	18 JUL 1983	
International Searching Authority ¹	Signature of Authorized Officer ²⁰	
EUROPEAN PATENT OFFICE	G.L.M. Kruidenberg	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No ¹⁸
A	see column 2, lines 18-33; column 4, line 62 - column 5, line 20; figure 1A --	6
	US, A, 4210809 (M.H. PELAVIN) 1 July 1980 see column 2, lines 47-67 -----	6

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/GB 82/00319 (SA 4439)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 11/07/83

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0047130	10/03/82	None	
US-A- 4315754	16/02/82	GB-A- 2062225	20/05/81
		JP-A- 56036053	09/04/81
		FR-A- 2469714	22/05/81
		DE-A- 3031417	11/06/81
US-A- 4253846	03/03/81	BE-A- 886132	13/05/81
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		GB-A- 2047880	03/12/80
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For more details about this annex :
see Official Journal of the European Patent Office, No. 12/82