Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2540222 C 2009/11/03

(11)(21) 2 540 222

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(22) Date de dépôt/Filing Date: 2006/03/17

(41) Mise à la disp. pub./Open to Public Insp.: 2006/09/21

(45) Date de délivrance/Issue Date: 2009/11/03

(30) Priorité/Priority: 2005/03/21 (EP05102211.9)

(51) Cl.Int./Int.Cl. *G01N 35/00* (2006.01),
G01N 1/10 (2006.01)

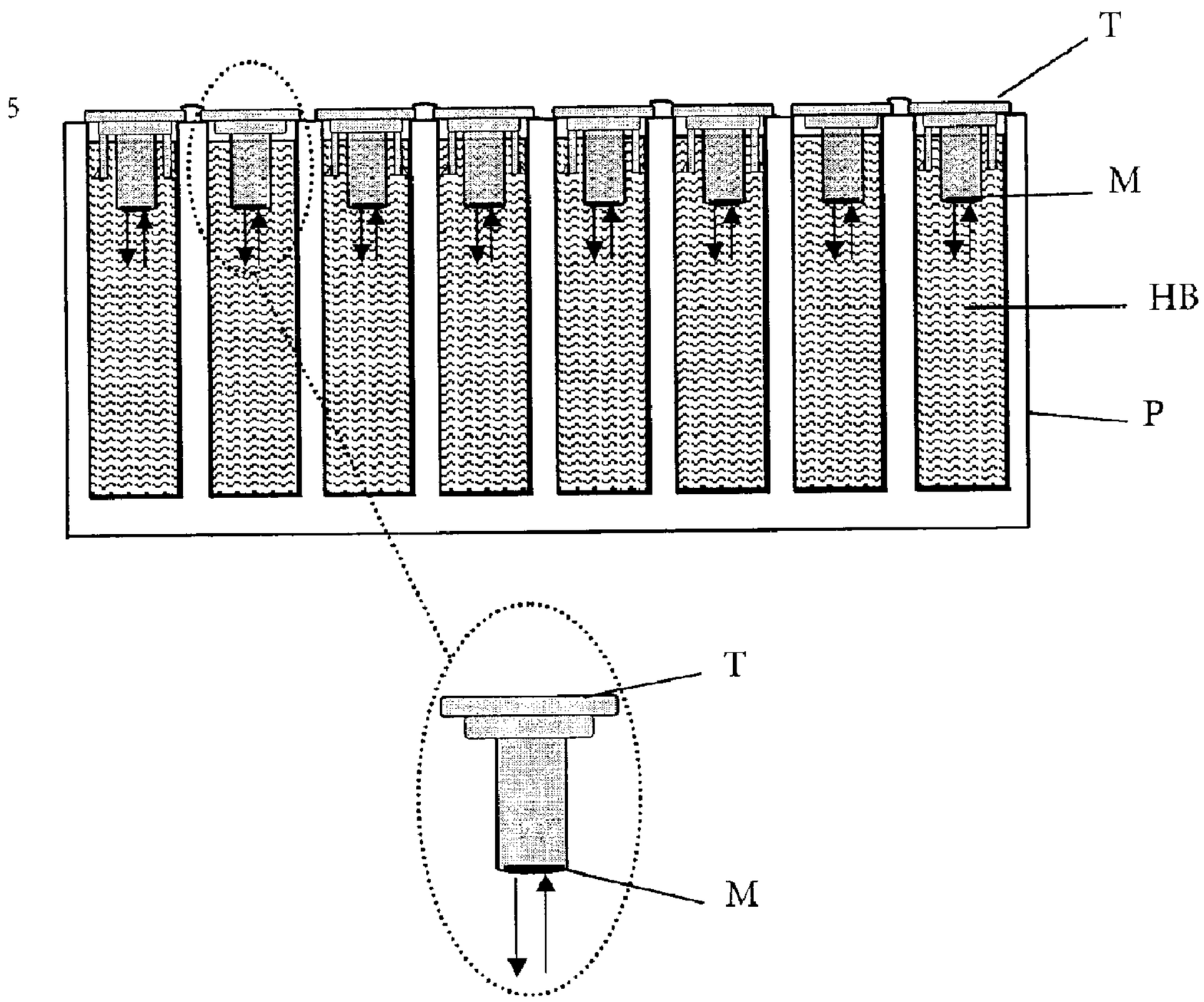
(72) Inventeurs/Inventors:
FISCHER, HOLGER, CH;
KANSY, MANFRED, DE;
WAGNER, BJOERN, DE

(73) Propriétaire/Owner:
F. HOFFMANN-LA ROCHE AG, CH

(74) Agent: GOWLING LAFLEUR HENDERSON LLP

(54) Titre : SYSTEME CAMDIS (CARRIER MEDIATED DISTRIBUTION SYSTEM) POUR LA DETERMINATION DE LA LIPOPHILIE

(54) Title: CARRIER MEDIATED DISTRIBUTION SYSTEM (CAMDIS)



(57) Abrégé/Abstract:
The present invention relates to a novel method for determining the lipophilicity of a compound of interest comprising a) providing a layer, b) impregnating said layer with a solvent A, c) applying a dissolved compound of interest on the impregnated layer, d) adding

CA 2540222 C 2009/11/03

(11)(21) 2 540 222

(13) C

(57) Abrégé(suite)/Abstract(continued):
a solvent B, e) removing the solvent B after the distribution equilibrium has been reached, and f) determining the quantity of the compound of interest in at least one of the solvent phases.

Abstract

The present invention relates to a novel method for determining the lipophilicity of a compound of interest comprising a) providing a layer, b) impregnating said layer with a solvent A, c) applying a dissolved compound of interest on the impregnated layer, d) adding a solvent B, e) removing the solvent B after the distribution equilibrium has been reached, and f) determining the quantity of the compound of interest in at least one of the solvent phases.

***

Carrier Mediated Distribution System (CAMDIS)

The present invention provides a method for determination of high, medium and low lipophilicity values.

Lipophilicity is an important molecular property in drug discovery. The exact knowledge of drug lipophilicity is useful for correlation with pharmaceutical processes such as membrane permeation, solubility, volume of distribution, metabolic stability and protein binding. Lipophilicity is expressed either by log P (octanol-water Partition coefficient for neutral species) or log D (octanol-water Distribution coefficient for charged molecules).

Usually, the lipophilicity is determined by the conventional shake-flask method (M.M. Abraham, H.S. Chadha, J.P.Dixon, and A.J. Leo. Hydrogen bonding. Part 9. The partition of solutes between water and various alcohols. Phys. Org. Chem. 7:712-716 (1994).When performed manually, this method is very time consuming (only 2-5 compounds per day). However, the number of compounds produced in drug discovery increased dramatically due to rapid analogue synthesis and combinatorial chemistry. This situation requests for a fast and efficient method for determining the lipophilicity of compounds.

Further, the methods of the prior art do not work with low soluble compounds. Since 2002 about 35 % of the log D measurements were failed due to the precipitation of compounds in the reference solution or low sample concentrations in the aqueous phase (source: RODIN and SPC database, 2004). On the other hand, there is a need for high throughput measurements of log D > 4, especially for drug targets where high lipophilicity is required.

Therefore, there is a requirement for a method which is fast and which allows the determination of lipophilicity of low soluble compounds.

Summary of the Invention

As an aspect of the invention, there is provided a method for determining the lipophilicity of a compound of interest comprising

a) providing a layer,

b) impregnating said layer with a solvent A,

c) applying the compound of interest on the impregnated layer,

d) adding a solvent B,

e) removing the solvent B after the distribution equilibrium has been reached, and

f) determining the quantity of the compound of interest in at least one of the solvent phases.

Brief Description of the Drawings

Figure 1 shows schematically a method of the prior art. The compound of interest is dissolved in a suitable solvent and added with a hydrophilic buffer (H) in a multi-well plate (W). A lipophilic solvent (L) is added to the buffer. The plate is sealed (S) and the plate is shaken till an equilibrium of distribution is reached. Then the aqueous phase is removed and the quantity of the compound of interest in the hydrophilic buffer is determined. Due to the small volumes the separation of the phases is difficult and often not satisfying.

Figure 2 shows schematically an embodiment of the method of the present invention. The membranes (M) are attached to tubes (T). The membranes are impregnated with octanol and the dissolved compound of interest is applied to the membrane. The tubes (T) are inserted in a plate (P) which is filled with a hydrophilic buffer (HB), whereby the membranes are in contact with the buffer. After reaching the equilibrium the tubes may simply be removed from the plate to get a separation of the phases.

Figure 3 shows a linear regression curve of log D obtained by the method of the present invention (DIFI-log D) and values from literature (Lit.log D). The literature values were found in MEDChem databases (commercial Database from DAYLIGHTChemical Information Systems Inc. 27401 Los Alto, USA), Winiwarter et a1., J. Med. Chem, 41: 4939-49 (1998) and Sirius Technical Application Notes Volume 2 (1995).

Figure 4 shows a linear cuvette array 11. A) top view of the cuvette array, B) cross-section through a plane A-A of the cuvette array in A).

Figure 5 shows a cross-sectional view of one of the cuvettes 12 of the linear cuvette array 11 in Figure 4. A) without layer, B) with attached layer 61.

Figure 6 shows a linear cuvette array 31. A) top view of the cuvette array, B) cross-section through a plane A-A of the cuvette array in A).

Figure 7 shows a cross-sectional view of one of the cuvettes 32 of the linear cuvette array 31 in Figure 6. A) without layer, B) with attached layer 71.

Figure 8 shows a top view of a two-dimensional cuvette array 51 with a cuvette holder 52, a matrix array 53 and opening for cuvettes 54, in the two dimensional cuvette array 51 are two linear cuvette arrays 11 inserted.

Figure 9 shows a cross-sectional representation of stacked two-dimensional cuvette arrays 51 and 81.

Figure 10 shows a cross-sectional representation of a two-dimensional cuvette array 51 stacked onto a standard analysis multi-well plate 85.

Detailed Description

The present invention relates to a method of determining the lipophilicity of a compound of interest comprising:

a) providing a layer,

b) impregnating said layer with a solvent A,

c) applying a compound of interest on the impregnated layer,

d) adding a solvent B,

e) removing the solvent B after the distribution equilibrium has been reached, and

f) determining the quantity of the compound of interest in at least one of the solvent phases.

Preferably, the quantity of the compound of interest is determined in the solvent phase sticking to the layer.

The compound of interest may be any chemical or biological compound. The compound of interest may be for example an organic compound, a protein, a peptide or a nucleic acid. An organic compound may include also organic-inorganic molecules.

The term organic-inorganic molecule as used herein refers to an organic molecule in which at least one inorganic atom is bound to a carbon atom. An inorganic atom may i.e. a metal atom such as i.e. silicon (Si) or germanium (Organometallics, i.e. Si or Ge bioisoester of organic molecules).

The compound of interest may be solid or liquid. The compound of interest may be dissolved in a suitable solvent as for example DMSO (dimethyl sulfoxide). The compound of interest may be a lipophilic compound or a hydrophilic compound. A suitable solvent for a hydrophilic compound is preferably a polar solvent; a suitable solvent for a lipophilic compound is preferably a non-polar solvent.

The term "layer" as used herein refers to a carrier for a solvent, whereby the layer is able to completely absorb the solvent. The term "completely absorbed" means that the solvent applied to the layer (i.e. Solvent A) is bound to the layer and may not leak into the other phase (i.e. Solvent B).

The layer has hollows. These hollows may be pores, cavities, holes or slots. The hollows may be formed by the carrier material, such as it is the case e-g. with fibers which form a mesh. The hollows may also be created e.g. by penetrating the layer with ions. Preferably, the layer is permeable.

The layer may be for example a mesh or a membrane (e.g. Westran Clear Signal membrane foil (Whatman), Immobilon-P PVDF (Millipore), PVDF-Plus, Transfer Membrane (Koma Biotech)). Preferably, the layer is a membrane. If the solvent is non-polar the carrier material is preferably hydrophobic such as i.e. polyvinyldenfluorid (PVDF), polytetrafluorethylene (PTFE), cyclic olefin copolymer (COC), polypropylene (PP) or polycarbonate (PC). If the solvent is polar the carrier material is preferably lipophilic such as i.e. cellulose acetate, glass fibres, hydrophilic polyvinylidendifluoride (PVDF), hydrophilized polycarbonate and other hydrophilized filter material.

Solvent A may be a non-polar or polar solvent. Solvent B may also be a non-polar or polar solvent. Solvent A is immiscible or hardly miscible with solvent B. If solvent A is a non-polar solvent, solvent B is a polar solvent, if solvent A is a polar solvent, solvent B is a non-polar solvent.

The term "non-polar solvent" as used herein refers to a hydrophobic solvent. Non- polar solvents are immiscible, or hardly miscible with polar solvents such as for example water. A lipophilic compound has usually the tendency to be more soluble in a non-polar solvent than in a polar solvent. The dielectric constant of a non-polar solvent is usually lower than that of water. Examples of a hydrophobic solvent are organic solvents such as i.e. octanol or aliphatic hydrocarbons (dodecan, hexadecane or halogenized hydrocarbons).

The term "hydrophilic solvent" or "polar solvent" as used herein refers to a solvent that have molecules whose electric charges are not equally distributed and are therefore electronically charged. Polar solvents are immiscible or hardly miscible with non-polar or hydrophobic solvents. Polar or ionizable compounds have the tendency to be more soluble in polar solvents. The polar solvent may be for example a hydrophilic buffer solution which could consist of a buffer salt (i.e. aqueous solutions of phosphate or TAPSO salts buffered at pH 7.4) in water with high buffer capacity within the pH range of interest. The pH of interest may be in the range between pH 0 to 14, preferably the pH is about 7.4.

The term "distribution equilibrium" as used herein refers to the equilibrium of distribution between the polar solvent and the non-polar solvent for compounds of interest after a specific time. Preferably, the distribution equilibrium is achieved between 0.1 - 24 h, more preferably it is achieved within 2h.

The layer may be impregnated by applying the solvent to the layer whereby the layer is able to absorb the solvent completely. The solvent may be applied i.e. by a dispenser. Further methods are known in the art such as for example robotic liquid handling system, which allows to dispense 0.1 µl - 50 µl/cm^2 of the organic modifier on the surface of the layer which may be a filter membrane.

The quantity of the compound of interest in the solvent (solvent A or solvent B) may be determined by methods comprising but not limited to the group consisting of UV- and/or mass spectroscopy, capillary electrophoresis (CE) and high pressure liquid chromatography (HPLC).

Preferably, the layer is attached at the bottom of a tube. A tube comprises but is not limited to, a cuvette, a well and a multi-well-plate. Preferably, said tube to which the

layer is attached to is the tube as described in the European Patent Application EP 1232792. More preferably, the tube to which the layer is attached to is the tube described in EP 1792656.

The preferred cuvette to which the layer is attached to, has an upper chamber 17 and a lower chamber 18 which have a common symmetry axis Y-Y which passes through the centers of both chambers. Upper chamber 17 and lower chamber 18 have each a substantially cylindrical shape. The cross-section of upper chamber 17 at the central part thereof is larger than the cross-section of lower chamber 18 (Figure 5).

This cuvette has a lower chamber 18 with an open lower end 23 and an upper chamber 17 with an open top end 24 and an annular bottom wall 25. This bottom wall has a central circular opening 26 which connects said upper chamber 17 with lower chamber 18 (Figure 5).

The inner surface 27 of bottom wall 25 is part of a conical surface, the cross-section of which forms an angle of about 80 degrees with the symmetry axis Y-Y of the cuvette, so that there is an abrupt change of cross-section between said upper chamber 17 and said lower chamber 18.

Following materials are examples of materials which can be used to manufacture a cuvette: celluloseacetate, polycarbonate, polyvinylidene fluoride (PVDF), polysulfones, polystyrene, polypropylene (PP), cyclic olefin copolymer (COC). Materials with similar shrinkage coefficient (in connection with injection molding) and melting properties may also be used for manufacturing a tube.

Preferably, the cuvette is part of an array 11 (Figure 4). Each of the cuvettes in the array has the same shape and dimensions, and neighboring cuvettes are connected to each other by a single web 15, 16. Each of these single webs 15, 16 has a curved shape.

The symmetry axis Y-Y of every cuvette 12 which forms part of array 11 of cuvettes lies substantially in one and the same plane A-A which is a symmetry plane of cuvette array 11. The upper part of an intermediate cuvette 12 of array 11 is connected by a first single web 15 to a neighboring cuvette 13 which lies on one side of intermediate cuvette 12 and is connected by a second single web 16 to a neighboring

cuvette 14 which lies on the opposite side of intermediate cuvette 12. First single web 15 and second single web 16 lie on opposite sides of said symmetry plane A-A.

Webs 15, 16 are flexible and therefore facilitate the insertion of the cuvettes in a cuvette holder in spite of variations of the length of cuvette array 11 which are due to different shrinkage coefficients of the different materials used for manufacture of cuvette arrays 11 by injection molding.

Some of the cuvettes of cuvette array 11 have latches 21 and 22 (Figure 4B) which are an integral part of the cuvette and which serve for removably connecting the cuvette to a cuvette holder 52. In another preferred embodiment cuvette holder 52 is of substantially rectangular shape and has four centering ribs located each on the outer surface of one of the corners of cuvette holder 52 (Figure 8).

Some of the cuvettes of cuvette array 11 have radially oriented ribs 19, 29 (Figure 4B) which serve for accurately positioning the cuvette into an opening of cuvette holder 32.

The cuvette array 11 is made by injection molding of a selected first plastic material which is particularly suitable for being used in combination with a second selected material of which a layer is made. This layer is adapted to be closely attached to each cuvette of the array of cuvettes for covering at least one opening of each cuvette.

The injection molding apparatus for manufacturing the cuvette array is preferably so configured and dimensioned that injection molding of different materials having different shrinkage coefficients can be carried out with one and the same apparatus.

Following materials are examples of materials which can be used to manufacture cuvette array 11: celluloseacetate, polycarbonate, polyvinylidene fluoride (PVDF), polysulfones, polystyrene, polypropylene (PP). Materials with similar shrinkage coefficient (in connection with injection molding) and melting properties may also be used for manufacturing cuvette array 11.

The attachment of the layer to each cuvette can be effected e.g. by gluing the layer and the cuvette or by a welding process. The layer attached to each individual

cuvette is attached only to this individual cuvette and has no connection with any other cuvette or with a foil attached to a different cuvette.

The attachment of the layer to the cuvette must ensure a medium tight connection (liquid tight connection) of these components.

A more preferred tube to which the layer is attached is a cuvette which has an upper chamber 37 and a lower chamber 38 and a common symmetry axis Y-Y which passes through the centers of both chambers. Upper chamber 37 and lower chamber 38 have each a substantially cylindrical shape. The cross-section of upper chamber 37 at the central part thereof is larger than the cross-section of lower chamber 38 (Figure 7A).

Lower chamber 38 has an open lower end 33. Upper chamber 37 has an open top end 34 and an annular bottom wall 35. This bottom wall has a central circular opening 36 which connects said upper chamber 37 with lower chamber 38.

The inner surface of bottom wall 45 is part of a conical surface, the cross-section of which forms an angle of about 80 degrees with the symmetry axis Y-Y of the cuvette, so that there is an abrupt change of cross-section between said upper chamber 37 and said lower chamber 38.

The cuvette array 31 is made by injection molding of a selected first plastic material which is particularly suitable for being used in combination with a second selected material of which a layer is made. This layer is adapted to be closely attached to at least one cuvette of the array of cuvettes for covering at least one opening of the cuvette. The same plastic material may be used for said first plastic material and said second plastic material.

The attachment of the layer to one or more cuvettes can be effected e.g. by gluing the layer and the one or more cuvettes or by a welding process. The layer attached to one individual cuvette is attached only to this individual cuvette and has no connection with any other cuvette or with a layer attached to a different cuvette.

The attachment of the layer to the cuvette must ensure a medium tight connection (liquid and/or gas tight connection) of these components.

Every cuvette of array 31 has the same shape and dimensions and neighboring cuvettes are connected to each other by a single web 35, 36. Each of these single webs 35, 36 is flexible and has a curved shape (Figure 6).

The symmetry axis Y-Y of every cuvette 32 which forms part of array 31 of cuvettes lies substantially in one and the same plane A-A which is a symmetry plane of cuvette array 31. The upper part of an intermediate cuvette 32 of array 31 is connected by a first single web 35 to a neighboring cuvette 33 which lies on one side of intermediate cuvette 32 and is connected by a second single web 36 to a neighboring cuvette 34 which lies on the opposite side of intermediate cuvette 32.

The single webs 35, 36 are flexible and therefore facilitate the insertion of the cuvettes in a cuvette holder, e.g. cuvette holder 52, in spite of variations of the length of cuvette array 31 which are due to different shrinkage coefficients of the different materials used for manufacture of cuvette arrays 31 by injection molding. These single webs 35, 36 may lie on either of two opposite sides of the plane A-A (Figure 5).

At least two of the cuvettes of the array 31 have means which are an integral part of the cuvette for removably connecting the cuvettes to cuvette holder. Preferably, these connecting means are latches 41 and 42. In a preferred embodiment the cuvette holder is of substantially rectangular shape and has four centering ribs located each on the outer surface of one of the corners of a cuvette holder like the cuvette holder 52 in Figure 8.

Preferably, the distribution of cuvettes with connecting means over the array is equitable. If two cuvettes have connecting means preferably the first and the last cuvettes has each connecting means, or second and the last but one cuvette has each connecting means, or the third and the last but two cuvette has each connecting means, and so on.

In a preferred embodiment, in array of eight cuvettes, the first cuvette, the third cuvette, the fourth, the fifth, the sixth and the eighth cuvette has each connecting means.

As can be appreciated from Figure 8, a two-dimensional array 51 of cuvettes according to the cuvette holder 52 having a matrix array 53 of openings 54 for receiving cuvettes 12, 32 of at least one linear cuvette array 11, 31 having the above described

features. Each of the cuvettes 12, 32 of cuvette array 11, 31 has a shape and dimensions that snugly fits into one of openings 54 of cuvette holder 52.

As shown by Figure 9, two or more two-dimensional cuvette array 51 and 81 each of which has the structure described above with reference to Figure 8 can be stacked on each other to form a three-dimensional cuvette array. The components of such an array are so configured and dimensioned that cuvettes having the same relative position in their respective holders are accurately positioned one above the other with coincidence of their symmetry axis, one of said cuvettes taking the position of an upper cuvette 91 and the other cuvette taking the position of a lower cuvette 92. In a preferred embodiment a portion of the lower part of each upper cuvette 91 lies within the upper chamber of the corresponding lower cuvette 92 and the lower end of the upper cuvette 91 is at a predetermined distance from the bottom wall of the upper chamber of the lower cuvette 92.

As shown by Figure 10, a two-dimensional cuvette array 51 which has the structure described above with reference to Figure 8 can be stacked also on a standard holder plate 85 for a standard multi-well plate.

One embodiment of the present invention relates to a method of determining the lipophilicity of a lipophilic compound of interest comprising:

a) providing a hydrophobic layer,

b) impregnating said layer with a non-polar solvent,

c) applying the compound of interest on the impregnated layer,

d) adding a hydrophilic solvent,

e) removing the hydrophilic solvent after the distribution equilibrium has been reached, and

f) determining the quantity of the compound of interest in the lipophilic solvent phase sticking to the hydrophobic layer.

The term "hydrophobic layer" as used herein refers to a carrier for non-polar solvent. Preferably, the hydrophobic layer is a hydrophobic membrane. Such membranes may be formed as a mesh out of the hydrophobic material or a layer with pores. Preferably, the pore size or mesh size is in the range between 0.01 - 100 μm. The

hydrophobic membrane carrier material comprises but is not limited to PVDF, PTFE, cyclic olefin copolymer (COC), PP or PC.

A lipophilic compound of interest may be for example polycyclic aromatic or aliphatic hydrocarbons, fat soluble vitamins, hydrophobic drugs like fungicides, halogen containing aromatic or aliphatic hydrocarbons, nitrogen and oxygen containing aromatic or aliphatic hydrocarbons.

The non-polar solvent is immiscible or hardly miscible with the polar solvent. The preferred non-polar solvent is octanol (octan-1-ol). The preferred polar solvent is water or a buffer.

Another embodiment of the present invention relates to a method of determining the lipophilicity of a hydrophilic compound of interest comprising:

a) providing a hydrophilic layer,

b) impregnating said layer with a polar solvent,

c) applying the compound of interest on the impregnated layer,

d) adding a non-polar solvent,

e) removing the non-polar solvent, and

f) determining the quantity of the compound of interest in the hydrophilic phase sticking to the hydrophilic layer.

The term "hydrophilic layer" as used herein refers to a carrier for polar solvent. Preferably, the hydrophilic layer is a hydrophilic membrane. Such a membrane may be formed as a mesh of hydrophilic material or as a layer with pores. Preferably, the pore size or mesh size is in the range between 0.01 - 100 µm. The hydrophilic carrier material comprises but is not limited to cellulose acetate, glass fibres, hydrophilic polyvinylidendifluoride (PVDF), hydrophilized polycarbonate and other hydrophilized filter material.

The non-polar solvent is immiscible or hardly miscible with the polar solvent. The preferred non-polar solvent is octanol (octan-1-ol). The preferred polar solvent is water or a buffer.

The present invention further pertains to the use of a layer impregnated with a solvent for determination of lipophilicity of a compound of interest.

The layer may be a hydrophobic or hydrophilic layer. The solvent may be a non-polar (hydrophobic) or polar (hydrophilic) solvent. If the solvent is a non-polar solvent the layer is preferably hydrophobic. If the solvent is a polar solvent, the layer is preferably hydrophilic.

The compound of interest may be any chemical or biological compound. The compound of interest may be for example an organic compound, a protein, a peptide or a nucleic acid. An organic compound may include also organic-inorganic molecules. The term organic-inorganic molecule as used herein refers to an organic molecule in which at least one inorganic atom is bound to a carbon atom. An inorganic atom may i.e. a metal atom such as i.e. silicon (Si) or germanium (Organometallics, i.e. Si or Ge bioisoester of organic molecules).

The compound of interest may be solid or liquid. The compound of interest may be dissolved in a suitable solvent as for example DMSO (dimethyl sulfoxide). A suitable solvent for a hydrophilic compound is preferably a polar solvent; a suitable solvent for a lipophilic compound is preferably a non-polar solvent.

The compound of interest may be a lipophilic compound or a hydrophilic compound. If the compound of interest is a lipophilic compound, the layer may be a hydrophobic layer impregnated with a non-polar solvent. Preferably, the non-polar solvent is octanol. If the compound is a hydrophilic compound, the layer may be a hydrophilic layer impregnated with a polar solvent. Preferably, the non-polar solvent is water or a buffer.

In a preferred embodiment, the layer is attached at the bottom of a tube. The tube comprises but is not limited to, a cuvette, a well and a multi-well-plate. More preferably said hydrophobic layer is attached to a tube as described in the European Patent Application EP 1232792 or EP 1792656. A preferred tube was described at another place in this document.

Following materials are examples of materials which can be used to manufacture a tube: celluloseacetate, polycarbonate, polyvinylidene fluoride (PVDF), polysulfones, polystyrene, polypropylene (PP) or cyclic olefin copolymer (COC). Materials with similar shrinkage coefficient (in connection with injection molding) and melting properties may also be used for manufacturing a tube.

The method of the present invention allows a rapid determination of high lipophilicity values. In addition, less material has to be employed to test one compound and the method can be easily automated with standard liquid-handling workstation. Furthermore, no aqueous reference solutions with risk of precipitation are required.

Having now generally described this invention, the same will become better understood by reference to the specific examples, which are included herein for purpose of illustration only and are not intended to be limiting unless otherwise specified.

List of reference numbers

11 linear cuvette array

12 cuvette

13 cuvette

14 cuvette

15 web

16 web

17 upper chamber

18 lower chamber

19 rib

21 latch

22 latch

23 open low end

24 open top end

25 bottom wall

26 opening

27 inner surface of bottom wall 25

29 rib

31 array

32 cuvette

33 neighboring cuvette

34 neighboring cuvette

35 single web

36 single web

37 upper chamber

38 lower chamber

41 rib

42 rib

43 lower top end

44 open top end

45 bottom wall

46 opening

51 two-dimensional cuvette array

52 cuvette holder

53 matrix array of openings

54 opening (for receiving cuvettes)

61 layer

71 layer

81 two-dimensional cuvette array

82 cuvette holder

83 holder plate

85 standard holder plate for a standard multiwell plate

91 upper cuvette

92 lower cuvette

Examples:

Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated.

Example 1:

Lipophilicity experiments were carried out in 96-deepwell microtiterplates in combination with the novel DIFI-tubes as described in the European patent application EP 1232792.

All compounds of the validation set were dissolved in dimethylsulfoxide at a concentration of 10 mM (DMSO-stock).

The experiment started with the accurate coating of 0,45 µm hydrophobic PVDF membranes (Immobilon-P PVDF (Millipore) and PVDF-Plus (Whatman, Headquater: 27 Great West Road, Brentford, Middlesex, TW8 9BW, UK), which were fixed on the bottom of the DIFI-tubes.

Each membrane is impregnated with exactly 1 µl octanol and 1 µl DMSO-stock.

After a short incubation of 10 min the tubes with the coated membranes were connected to a 96-deepwell plate which had been prefilled with exactly 1600 µl of the selected buffer solution (50 mM TAPSO (*FLUKA*, BioChemika, *ArtNo.93357)*, pH 7.4). The resulting sandwich construct guarantees, that the membrane is completely dipped in the buffer solution (Figure 2).

The plate was then sealed and shaken for two hours. During this time the substance was distributed between the membrane, the octanol and the buffer solution accordingly.

After the distribution equilibrium was reached (after 2 h) the tubes were carefully disassembled from the top of the deepwell plate in order to analyze the octanol phase which still stucks to the membrane.

Therefore, the remaining compound in the octanol phase was washed out with additional octanol as eluent. The concentration of the substance in the eluent was then determined by UV spectroscopy at absorption wavelengths between 250 and 450 nm.

In order to know the exact sample concentration before incubation with buffer a reference plate was generated under the same conditions as the described sample plate above. The reference plate was directly treated with the eluent and the resulted reference concentration in the octanol phase was measured by UV spectroscopy.

EVALUATION

The lipophilicity value log D may be calculated from the analyzed sample concentration after incubation with buffer (C_o) and the reference concentration before distribution (C_{ref}).

$$\log D = \log\left(\frac{C_o}{C_{ref} - C_o} \cdot \frac{V_w}{V_o}\right)$$

Due to the fact, that all UV measurements were carried out under the same conditions, the concentration term were replaced by UV-absorption values (Abs) at the same wavelength.

$$\log D = \log\left(\frac{Abs_o}{Abs_{ref} - Abs_o} \cdot \frac{V_w}{V_o}\right)$$

In this equation, V_W is the volume of the aqueous phase was divided by the volume of the octanol phase V_o.

VALIDATION

A set of 14 well-characterized, chemically diverse drugs with known log D values from literature was used to validate this method. For each compounds three determinations were performed according to the described method above. The preparation of the reference and sample plate was performed manually.

The obtained log D values (DIFI-log D) were then compared with the values from literature (Lit.log D). Table 1 summarizes the results of the validation. Corresponding data were used in Figure 3.

Table 1. Validation set of 14 drugs with known log D values from literature (Lit.log D). A comparison of literature values from different sources with the data obtained by the new method validates the method of the present invention.

No.	Compound	Lit.log D	Source	DIFI-log D
1	Astemizole	4.10	a)	4.12
2	Carvedilol	3.37	a)	3.38
3	Chlorprothixene	4.19	a)b)	4.24
4	Clotrimazole	4.99	a)	4.91
5	Dexamethasone	1.83	a)	1.83
6	Diltiazem	2.32	a)b)	2.33
7	Felodipine	4.80	a)	4.84
8	Imipramine	2.65	a)	2.66
9	Mitotane	4.87	a)	4.78
10	Nimodipine	3.73	a)	3.61
11	Progesterone	3.87	a)	3.77
12	Quinine	2.10	a)	2.15
13	Testosterone	3.32	a)	3.33
14	Verapamil	2.70	c)	2.73

a) MEDChem01, MEDChem03

b) calculated from logP and pKa

c) Winiwarter, S., Bonham, N. M., Ax, F., Hallberg, A., Lennernas, H., Karlen, A., J. Med. Chem., 41, 4939-4949 (1998)

d) Sirius Technical Application Notes Volume 2 (1995)

In comparison with the HTlog D the method of the present invention requires an extremely low octanol-water ratio for the determination of high log D values. Further advantages lies in the reduced sample consumption and in the easy separation of the octanol phase and the transfer of the carrier fixed non-polar solvent to multiple wells with polar solvent, thus increasing the distribution volume and therefore the measurement range for highly lipophilic compounds (Table 2).

Table 2. HTlog D in comparison to the new approach.

	Required DMSO-stock with 10 mM1 (Without dead volume)	**Analyzed phase**	Octanol/Water ratio	**Separation of the Octanol phase possible?**
HTlog D	32 µl	Water phase	3 / 180	NO
Method of the present invention	6 µl	Octanol phase	1 / 1600 (or multiple of the polar **solvent volume** e.g. 1/3200, 1/4800)	YES

Claims

1. Method for determining the lipophilicity of a compound of interest comprising

a) providing a layer,

b) impregnating said layer with a solvent A,

c) applying the compound of interest on the impregnated layer,

d) adding a solvent B,

e) removing the solvent B after the distribution equilibrium has been reached,

and

f) determining the quantity of the compound of interest in at least one of the solvent phases.

2. A method according to claim 1 wherein the quantity of the compound of interest is determined in phase sticking to the layer.

3. A method according to claim 1 or 2 wherein the layer is a hydrophobic layer.

4. A method according to claim 3 wherein solvent A is a non-polar solvent and the solvent B is a polar solvent.

5. A method according to claim 1 or 2 wherein the layer is a hydrophilic layer.

6. A method according to claim 5 wherein the solvent A is a polar solvent and the solvent B is a non-polar solvent.

7. A method according to any one of the claims 4 or 6 wherein the non-polar solvent is octanol.

8. A method according to any one of the claims 1 to 7 wherein the layer is a membrane.

9. A method according to any one of the claims 1 to 8 wherein the layer is attached to the bottom of a tube.

Figure 1:

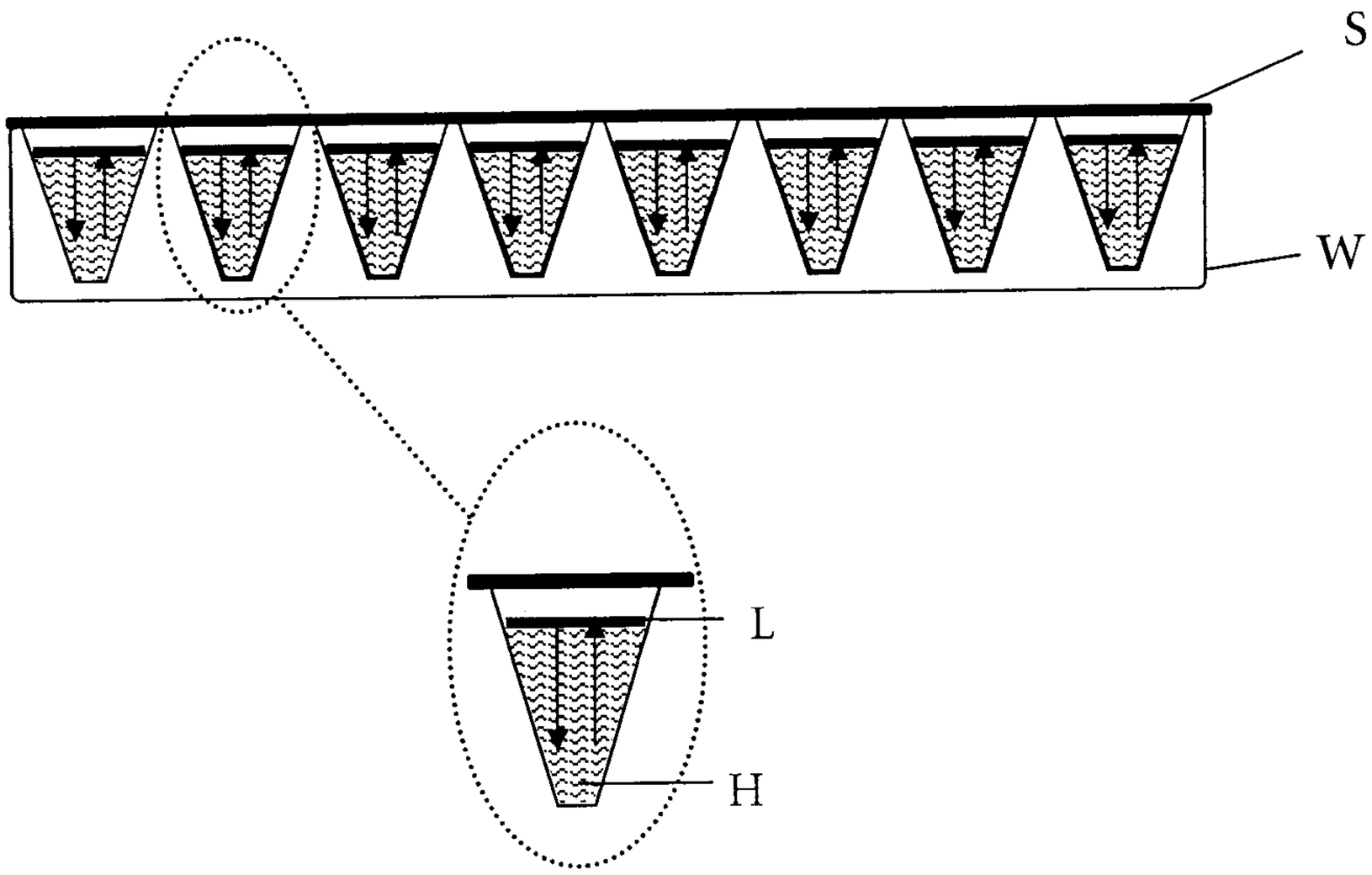


Figure 2:

Figure 3:

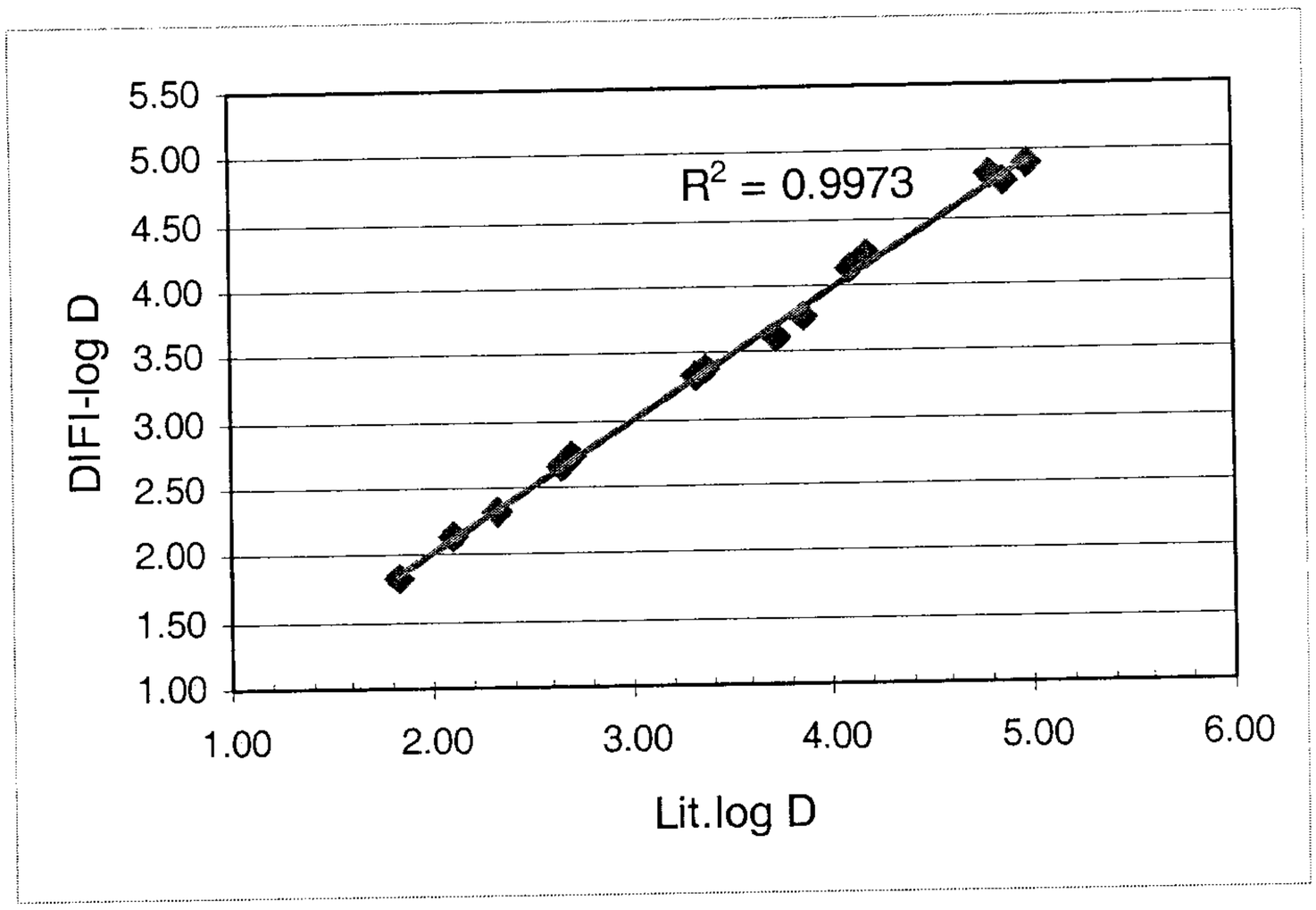

5.50
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
DIFI-log D
R^2 = 0.9973
1.00
2.00
3.00
4.00
5.00
6.00
Lit.log D

Figure 4

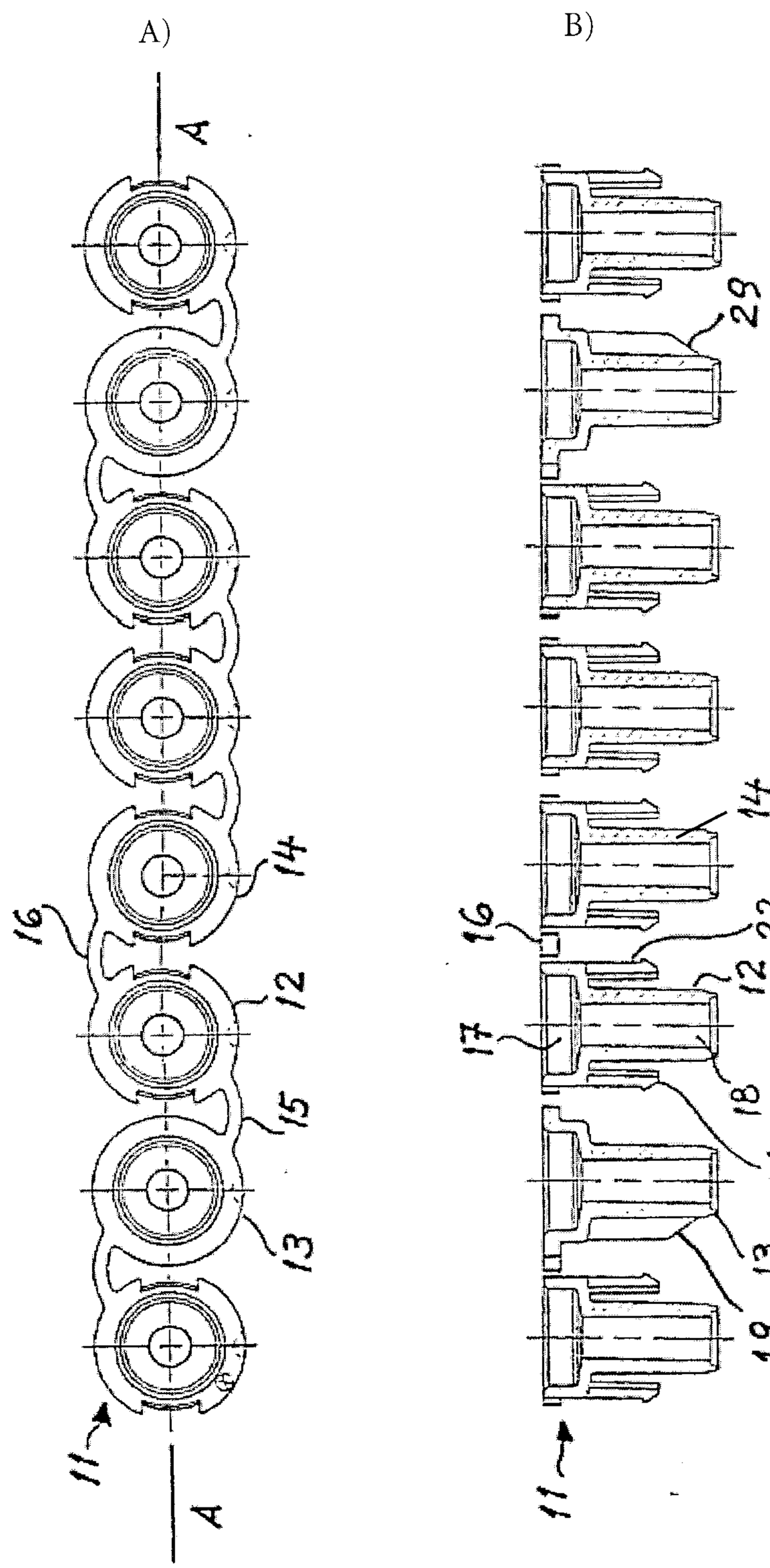
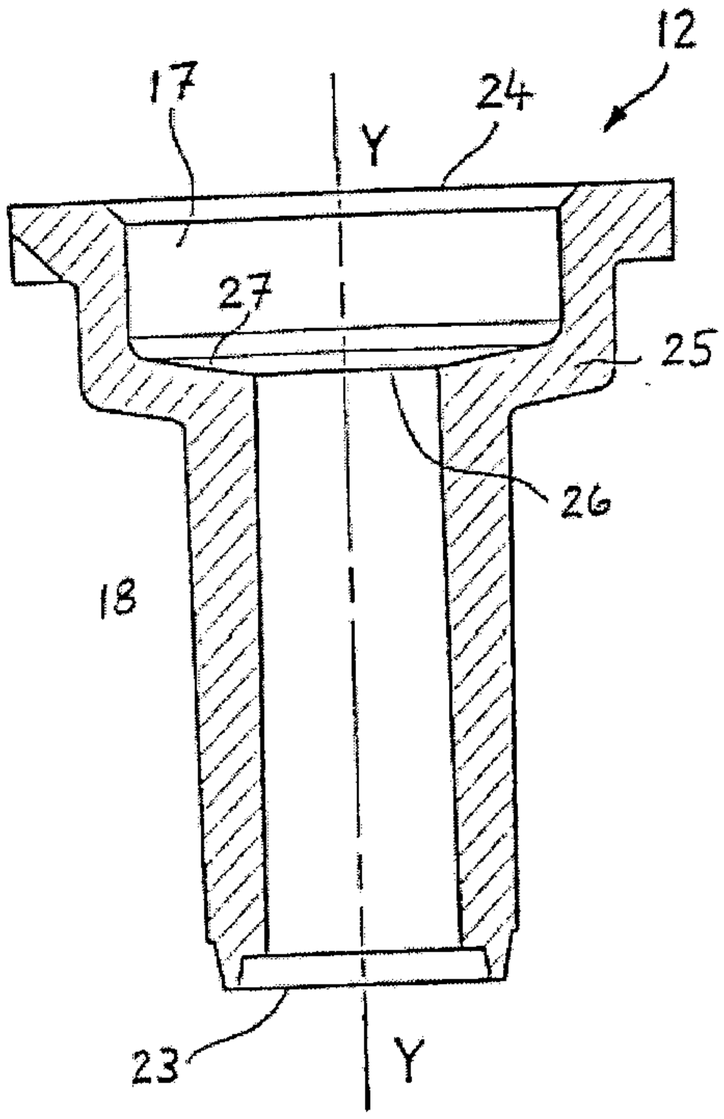


Figure 5

A)



B)

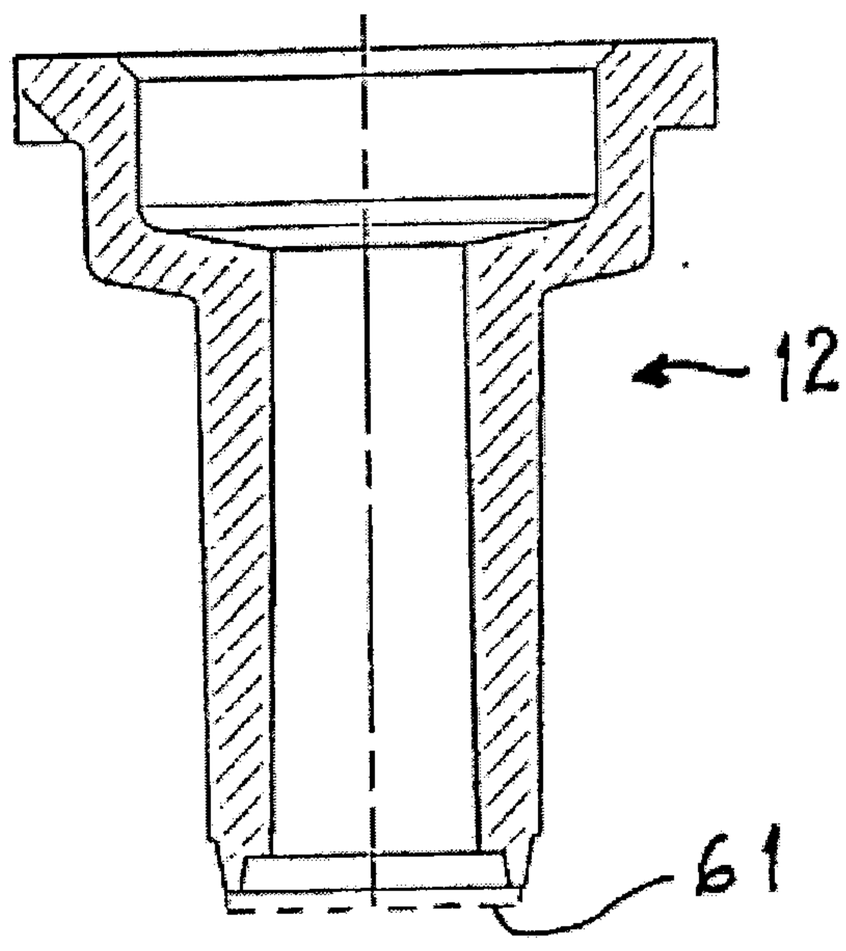


Figure 6

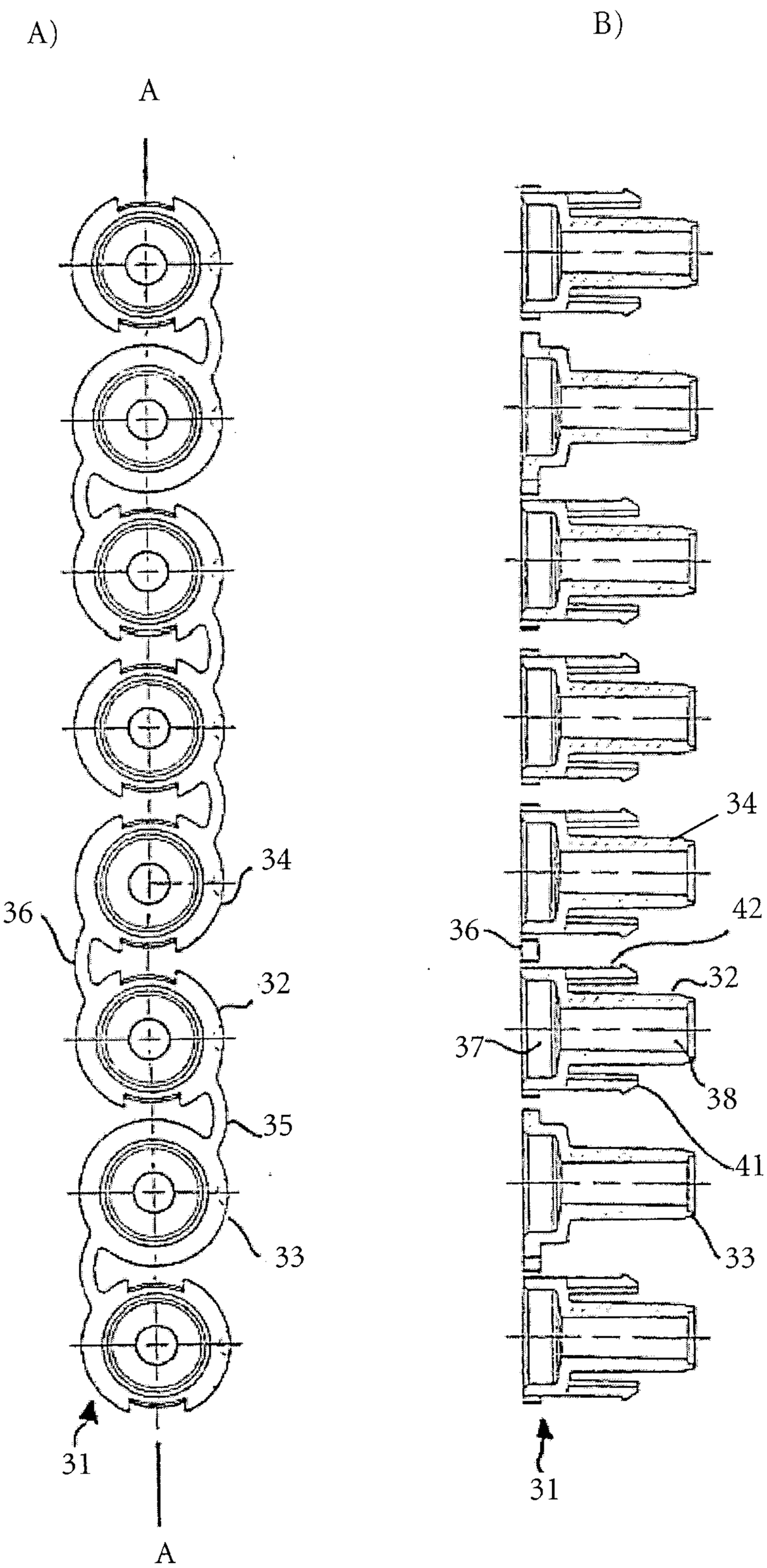
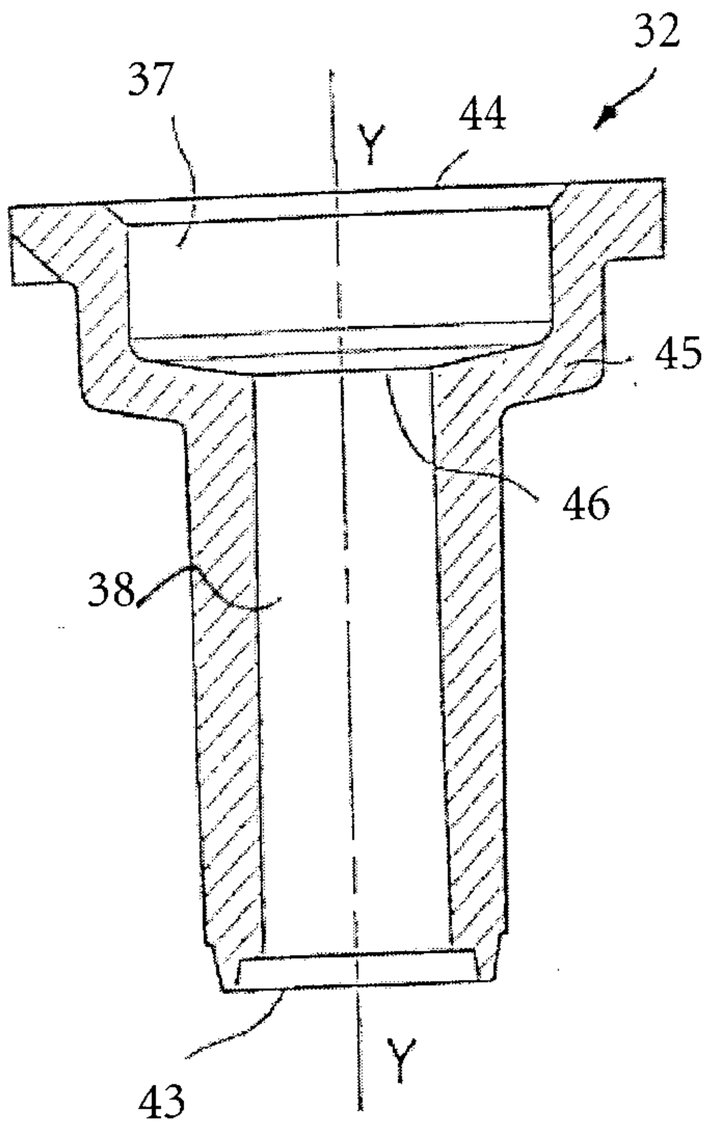


Figure 7

A)



B)

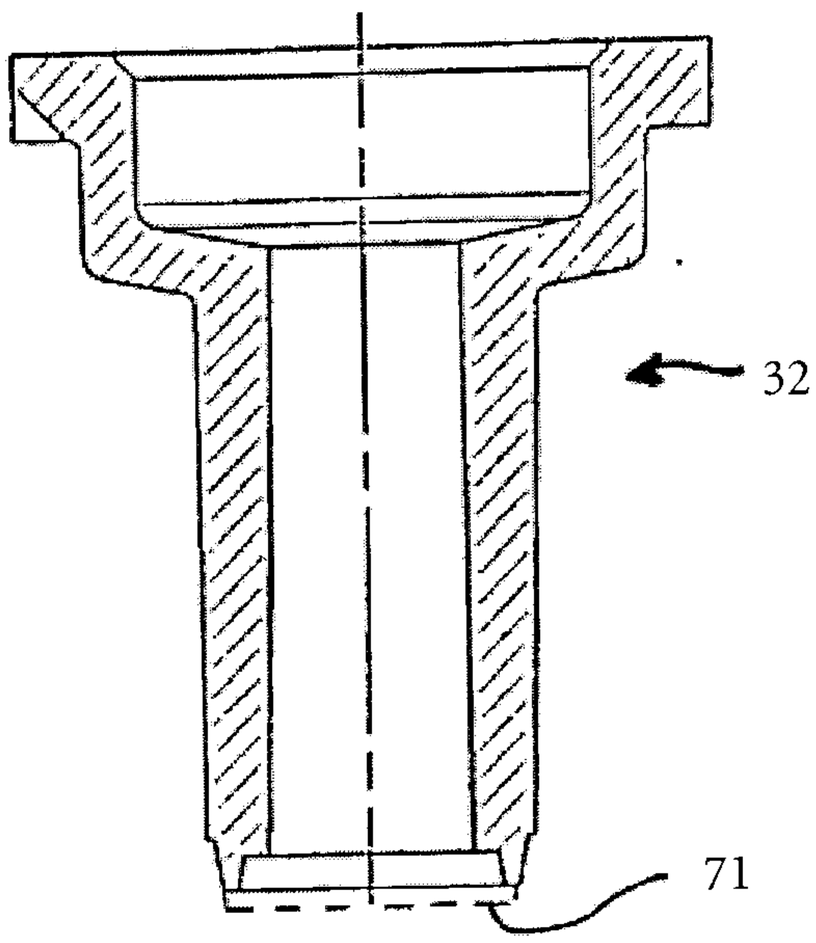


Figure 8

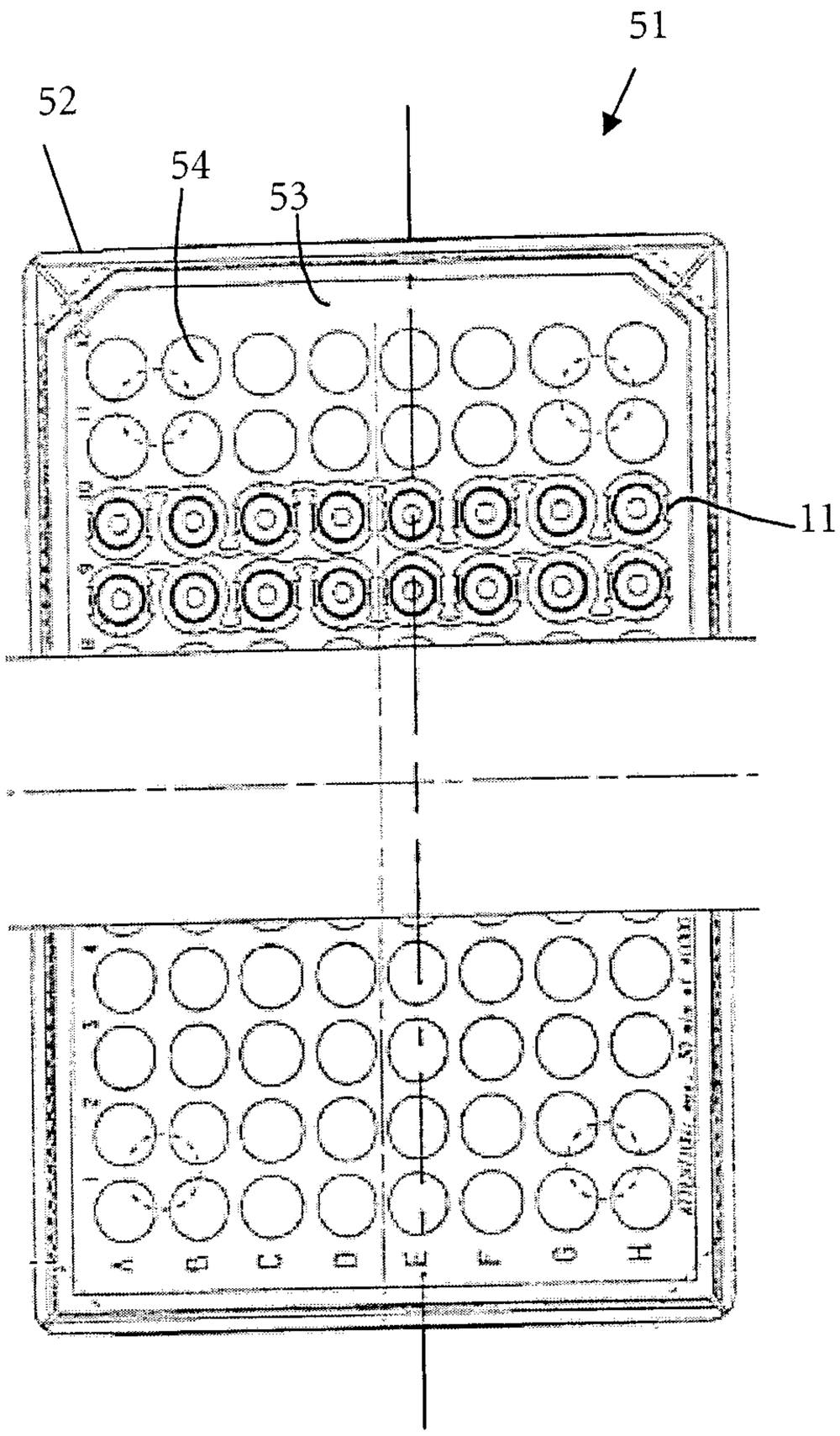


Figure 9

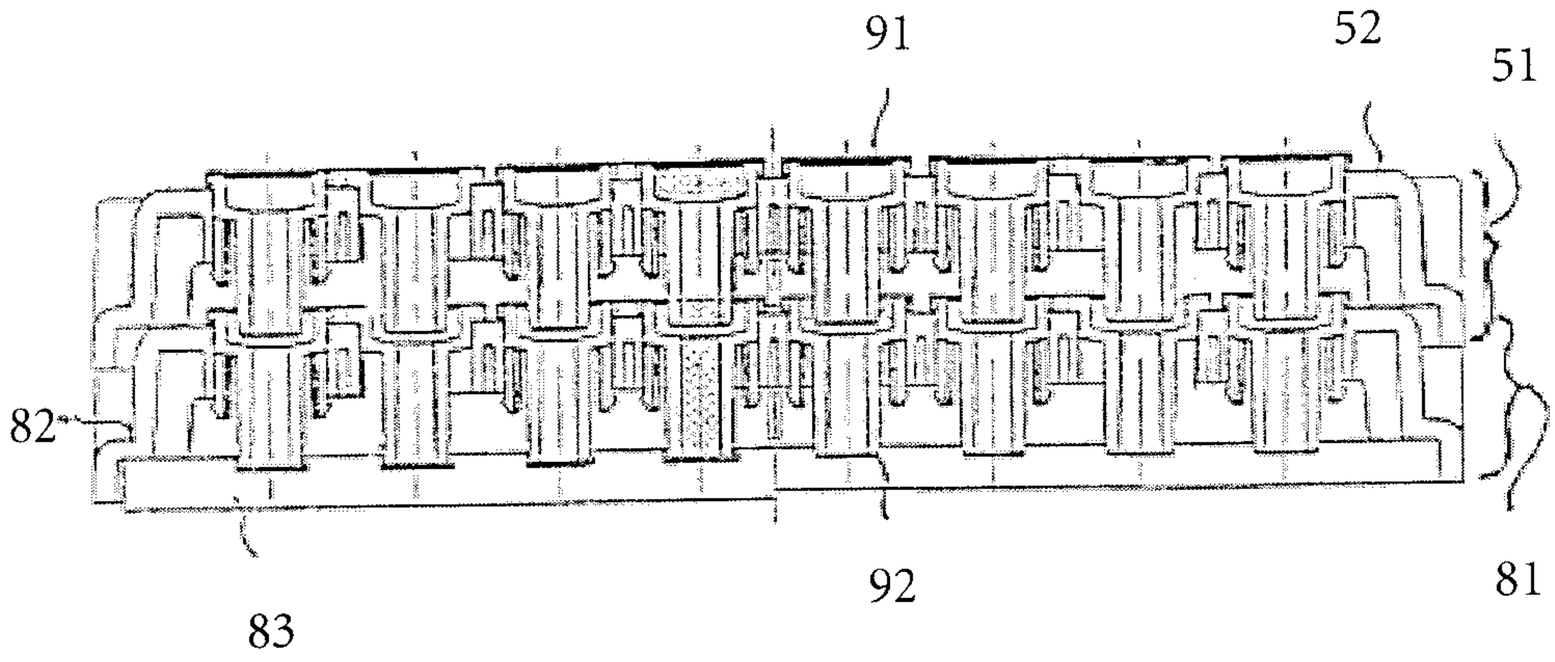
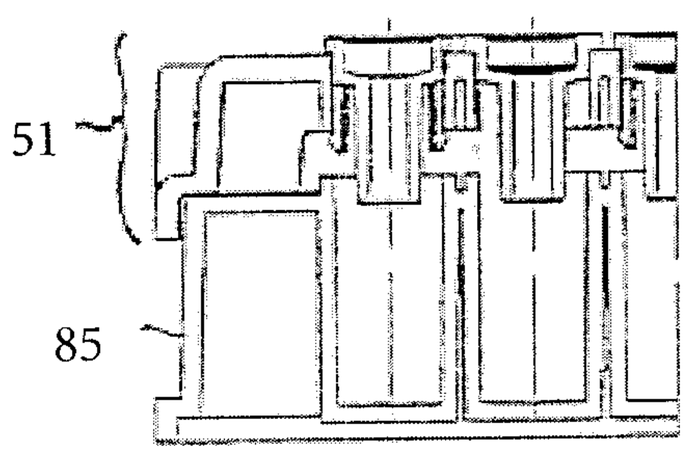
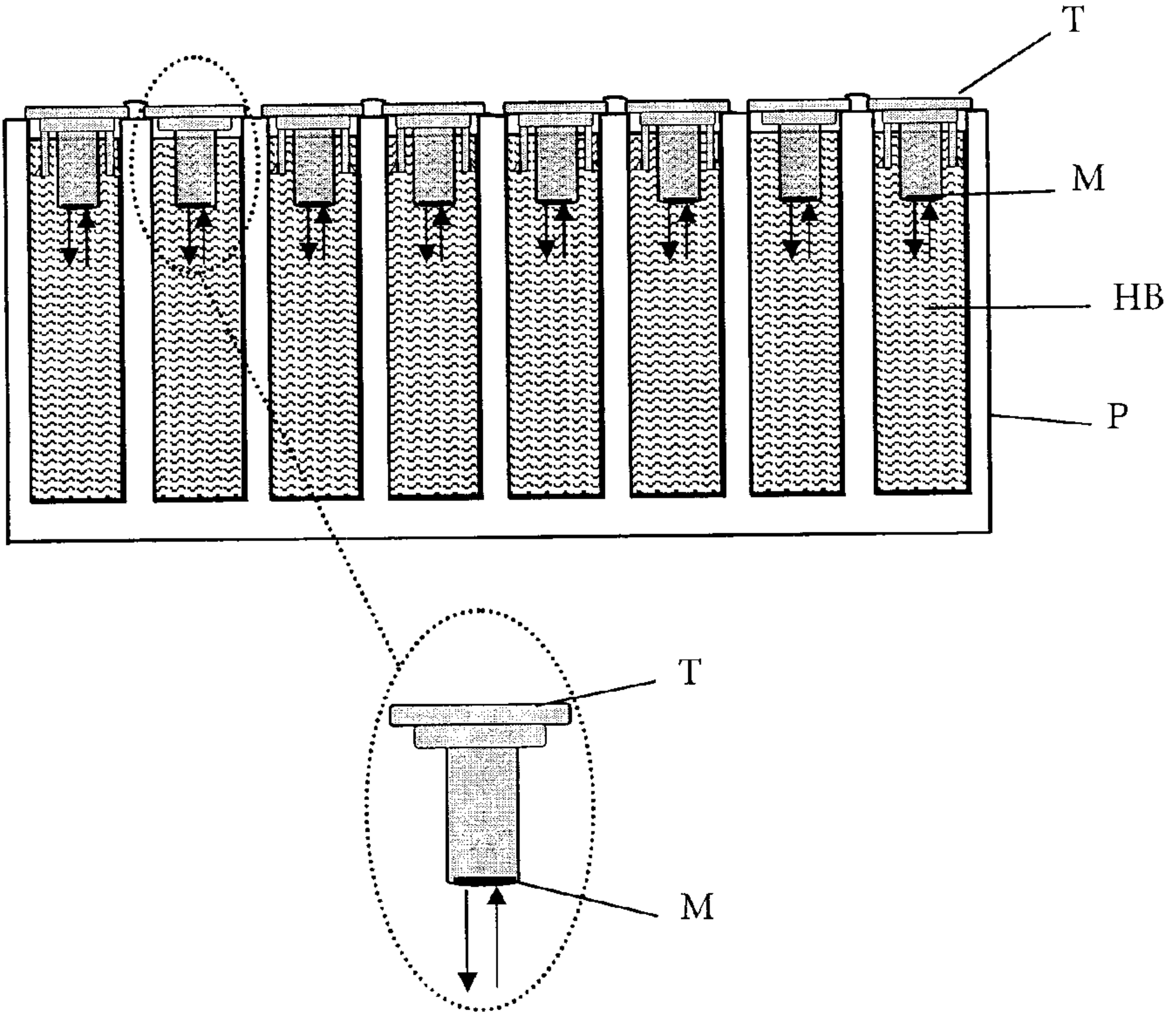


Figure 10



5


T
M
HB
P
T
M