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(54) Title: NOVEL SUPPORTS FOR SOLID PHASE SYNTHESIS

(57) **Abstract:** Novel solid supports and methods for their use in the synthesis of chemical compounds by the sequential addition of chemical building blocks are provided. The solid support comprises a polymer having reactive groups (for example, poly(styrene-co-vinylbenzyl chloride)) grafted to a chemically inert bulk material (such as a Teflon® membrane).

NOVEL SUPPORTS FOR SOLID PHASE SYNTHESIS

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part application of U.S. Patent Application Serial No. 09/216,093 filed December 18, 1998, which is a continuation-in-part application of U.S. Patent Application Serial No. 08/994,802 filed December 19, 1997, the complete disclosure of each is herein incorporated by reference.

BACKGROUND OF THE INVENTION

The present invention is related to the area of chemical synthesis. More specifically, the present invention relates to carrying out chemical synthesis on solid supports, methods for synthesizing said supports, methods for synthesizing compounds using said supports, intermediate compounds linked to said supports and uses therefor.

Solid phase chemistry is well known in the art. The concept of solid phase peptide synthesis was introduced by Merrifield. See, Merrifield, *J. Am. Chem. Soc.* **1963**, 85, 2149-2154; Chem. Rev. **1997,** 97; and *Acc. of Chem. Res.* **1996**, 29 (3), 111-170. The emergence of combinatorial chemistry has revitalized the interest in carrying out organic reactions on polymers. Interestingly, after decades of evaluating alternative options, the resin originally proposed by Merrifield, a crosslinked polystyrene, remains one of the most common solid supports used in solid phase organic chemistry. See Sucholeiki, *Annual Reports in Combinatorial Chemistry and Molecular Diversity* **1997**, 1, 41-49; and Michael Lebl, *Biopolymers (Peptide Science)* **1998**, 47, 397-404. Advantages associated with solid phase synthesis include the ability to drive reactions to completion by use of excess reagents, ease of work up and potential automation of synthetic procedures.

A new class of solid supports for solid phase organic chemistry has been developed by grafting linear polymers, i.e. polystyrene from polyethylene, polypropylene, and fluoropolymers. See, Potapov *et al., Nucl. Acid Res.* **1979**, 6, 2041-2056; Geysen *et al., Proc. Natl. Acad. Sci. USA* **1984**, 81, 3998-4002; Li *et al., Tetrahedron Letters* **1998**, 39, 8581-8584; and Parandoosh *et al., Comb. Chem. & High Throughput Screening* **1998**, 1, 135-142. One of dominant content of the carrier polymer from which the polystyrene is grafted. Moreover, with the exception of fluoropolymers, the other polymeric carriers above do not have sufficient thermal stability and solvent compatibility necessary for solid phase organic chemistry.

There is a need for solid supports that satisfy a wide range of requirements for manual or automated solid phase organic chemistry to allow easy handling, encoding, reaction monitoring, etc. The resins for solid phase organic chemistry are usually used in the form of small 50-200 μm diameter beads. Such beads can not be encoded by imprinting readable characters or by other physical methods. Moreover, they are difficult to handle individually. To circumvent these obstacles, syntheses in tea bags, on tubes, pins, polymer discs, filter paper, cotton, and other membrane materials have been developed. See, Houghten, *Proc. Natl. Acad. Sci. USA* **1985**, 82, 5131-5135; Hird *et al.*, *Tetrahedron* **1999**, 55, 9575-9584; Frank, *Tetrahedron* **1992**, 48, 9217; Eichler *et al.*, *Biochemistry* **1993**, 32, 11035-11041; Daniels *et al.*, *Tetrahedron Letters* **1989**, 30, 4345-4348; and Gao *J. of Biol. Chem.* **1996**, 271, 24634-24638. Unfortunately, these materials suffer from insufficient chemical stability and/or low capacity.

Thus, there is a need for a novel supports for solid phase synthesis. This invention fulfills this and other needs.

SUMMARY OF THE INVENTION

The present invention provides novel solid supports for solid phase synthesis. The supports comprise a polymer having reactive groups grafted to the surface of a porous chemically inert bulk material. More preferably, the bulk material will comprise a Teflon (PTFE) surface. In another preferred embodiment, the bulk material will comprise a membrane, porous sheet, sieve, mesh, porous rod, or thimble (i.e., a round-bottom tube). According to a particularly preferred embodiment, the polymer having reactive groups will comprise poly(styrene-co-vinylbenzyl chloride).

According to a preferred embodiment, the reactive group of the polymer having reactive groups is selected from the group consisting of halogen, amino, and hydroxyl groups.

A further understanding of the nature and advantages of the inventions herein may be realized by reference to the remaining portions of the specification and the attached drawings.

I. Overview of the Invention

The invention provides solid supports and methods for synthesizing chemical compounds by sequential addition of chemical building blocks onto novel solid supports in a parallel manner to produce a combinatorial collection of compounds. The solid supports comprise a polymer having reactive groups which has been grafted onto a porous chemically inert bulk material. The solid supports can be functionalized with a variety of chemical functionalities.

The solid supports further comprise "reaction zones," with a single compound synthesized in each reaction zone. The number of different compounds which can be synthesized is thus equal to the number of reaction zones. The reaction zones are typically arranged in an array, and are preferably maintained at fixed positions relative to one another during synthesis. An important feature of the invention is that the identity of a compound in a particular reaction zone is determined simply from the relative location of that reaction zone in the array. In this way, the need to encode the individual supports is eliminated.

II. The Bulk Material

Suitable bulk materials for these applications include poly(propylene) membranes, poly(ethylene) membranes, PTFE membranes, and fluoropolymer. Alternatively or in addition, a multi-component membrane suitable for use with the invention may be formed by trapping a layer of resin between two sheets of membrane (e.g., PTFE membrane) and fusing the resulting membrane "sandwich," e.g., by pressure and/or heating, in regions outside the reaction zones, forming an array of unfused "pockets" of resin spaced to correspond to reaction zones. Such a multi-component membrane affords an increased surface area of solid support available for reaction at each reaction zone, while preserving the advantages of synthesizing different compounds on a single sheet of membrane as detailed herein.

The bulk materials can be prepared as porous membranes, meshes, sieves, porous sheets, porous rods, and thimbles.

The bulk material should be chemically inert against solvents and reagents, including concentrated acids and alkalis, through a wide range of temperatures. It should be permeable.

A particularly preferred bulk material is a membrane having the following properties: functional pore size, 1-80 microns; thickness, 0.1-0.5 mm; and pore volume, 40-80%.

Particularly preferred examples of such membranes include Zitex A-105, Zitex A-140, Zitex G-108, and Zitex G-115, each of which is available from Norton Performance Plastics, Wayne, NJ and Millipore FSLW/420, which is available from Millipore Corp., Bedford, MA.

Other preferred Teflon surfaces include Teflon rods or thimbles having a pore size of 1-50 microns, which are available from Berghof/America, Concord, CA.

Other preferred Teflon surfaces include porous sheets having a pore size of 30 microns and thickness of 1/16", 1/8", or 1/4", which are available from Porex Corp., Fairburn, GA.

III. Preparation of the Polymer

Generally, radiation grafting is used to graft polymers onto the bulk material. See, Heger, *Technologie der Strahlenchemie von Polymeren*, Carl Hanser Verlag, Muenchen-Wien, **1990**; and Kudoh *et al.*, in *Irradiation of Polymers*, ACS, Washington, DC, **1996,** 2-10. High-energy irradiation such as γ-ray or e^--beam creates radicals in the polymer chains. These radicals can initiate radical chain polymerization with the desired monomer(s). However, Teflon is very sensitive to high-energy radiation, which can cause a loss of mechanical strength and even integrity of the material through main chain scission. By using e^--beam irradiation in an argon atmosphere, these problems can be avoided.

A particularly preferred solid support comprises poly(styrene-co-vinylbenzyl chloride) grafted to a porous Teflon membrane. By way of example, this support was prepared by irradiating a Teflon membrane with e^--beam under argon to form a sufficient number of radicals for grafting. See, Brack *et al.*, Macromol. Symp. **1997**, 126, 25-49. Preferably, a dose of between about 2-6 kGy is used.

Other preferred solid supports include polymers with reactive groups, e.g. poly(acrylic acid), poly(vinyl acetate), poly(acrylamide), poly(isopropyl acrylamide), grafted onto an inert porous polymer, e.g. poly(ethylene), poly(propylene), or a fluoropolymer.

Treatment of the irradiated Teflon membrane with an inhibitor free mixture of styrene and 4-vinylbenzyl chloride (3:1) led to a grafted copolymer. The concentration of the CH_2Cl groups on the membrane was calculated from the weight increase and the monomer ratio. The amount of grafting (%) = $[(W_g - W_0)/W_g] \times 100\%$ where W_g and W_0 are the weight of the grafted and ungrafted membrane, respectively. Loading per weight = $(W_g - W_0)/(W_g \times M_w$ of reactive unit) and loading per area = $(W_g - W_0)/(A \times M_w$ of reactive unit), "A" is the area monomers and their molecular weight.

Preferably, the maximum amount of the grafted polymer is between about 5% and 20%, and more preferably from about 10% to about 15% wherein 15% corresponds to a chloromethyl group loading of 0.32 mmol g^{-1} or 14.4 µmol cm^{-2} (after grafting for 7 days at room temperature in destabilized styrene/4-vinylbenzyl chloride (3:1)). The amount of the grafted polymer can be controlled by varying the temperature, the duration of the polymerization reaction, the irradiation dose, and the concentration of the monomer(s).

The solid supports of the present invention can be obtained with sufficient loading to be used as an alternative to the existing solid supports. These membranes allow rapid mass transfer of reactants and are chemically inert and thermally stable. Additionally, specifically modified membranes can be used as a "scavenger" material, which is a solid support that eliminates excess reagents or side product. The size and shape of the membranes can be tailored to meet the needs of a specific application. For instance, the amount of synthesized material can be easily controlled by the size of the reaction zone.

IV. Derivatized Solid Supports

The chloromethyl groups of the solid support can be quantitatively converted into aminomethyl groups via a reaction with potassium phthalimide followed by hydrazinolysis. The amount of the formed amino groups was determined by allowing the membrane to react with 9-fluorenylmethyloxycarbonyl chloride (Fmoc-Cl). The Fmoc-group was then removed by piperidine and the concentration of the cleaved product, dibenzofulvene, was measured spectrophotometrically. Alternatively, amino groups can be introduced via treatment of the support with $(BOC)_2NH$ followed by cleavage with TFA.

The chloromethyl groups of the solid support can be converted to a hydroxymethyl group via treatment with potassium acetate in 2-methoxyethanol.

One of skill in the art will readily appreciate that the chloromethyl, amino or hydroxyl groups of the support can be converted into other functionalities, including thiol, carboxylic acid, ester, amide, halomethyl, isocyanate and isothiocyanate groups, and the like via reactions well known in the art. The term “functionalities” or “chemical functionalities” is art recognized and refers to a chemically reactive group (i.e., a group capable of reacting with another chemically reactive group).

The amount of compound which can be synthesized per unit area of membrane can be readily determined based on the surface density of derivatizable groups on the membrane (termed "membrane load"), a value can be determined experimentally. Typically, smaller or larger membrane loads may be obtained. Assuming a membrane load of ~0.32 μmole/cm^2, and round shaped reaction zones (diameter = 7mm) could each support the synthesis of about 0.12 μmole of compound.

V. Use of the Solid Support

A. Linkers

The solid support is preferably derivatized with a linking arm, which can be linked to the support directly or via a spacer, as is described in more detail below. Examples of linking arms are known in the art, and include those comprising ester, amide, carbamate, ether, thio ether, urea and amine groups. The linking arm, in turn, preferably terminates in a suitable synthesis initiation site which is optionally protected. Examples of such sites are hydroxyl groups, amine groups and carboxylic acids.

The linking arm is preferably cleavable, i.e., it contains at least one covalent bond which can be cleaved by a specific chemical reaction(s) (including exposure to light in the case of photo-sensitive linkers) to liberate the synthesized compound from the solid support. The chemical reactions employed to break the covalent bond of the linking arm are selected so as to be specific for bond breakage thereby preventing unintended reactions from occurring elsewhere on the compound. The cleavable linking arm is further selected so it does not to interfere with compound synthesis and is not cleaved prematurely.

Cleavable linkage arms include (i) a sulfoester linkage provided by, *e.g.*, a thiolated tagged-molecule and a N-hydroxy-succinimidyl support (cleavage controlled by adjustment of the ammonium hydroxide concentration); (ii) a benzylhydryl or benzylamide linkage provided by, *e.g.*, a Knorr linker (cleavage controlled by adjustment of acid concentration); (iii) a disulfide linkage provided by, *e.g.*, a thiolated tagged-molecule and a 2-pyridyl disulfide support (*e.g.*, thiolsepharose from Sigma; cleavage controlled by adjustment of the DTT (dithiothreitol) concentration); and (iv) linkers which can be cleaved with a transition metal (*e.g.* "HYCRAM").

The invention may be conveniently practiced using photocleavable linkers, such as 6-nitroveratryoxycarbonyl (NVOC), α-methyl-6-nitroveratryl alcohol and other NVOC related linker compounds (*see*, PCT patent publication Nos. WO 90/15070 and WO 92/10092; *see also*, U.S. patent application Serial No. 07/971,181, filed 2 Nov. 1992, now abandoned, incorporated herein by reference); ortho-nitrobenzyl-based linkers (Rich, *et al., J. Am. Chem. Soc.* 97:1575-1579 (1975); Barany, *et al., J. Am. Chem. Soc.* 107: 4936-4942 (1985)); and

39:10 (1985)).

The linking arm may optionally be attached to the support via a spacer, which is typically selected based on the properties it imparts to the molecule being synthesized or the molecule-support construct. For example, a spacer may be selected based upon its hydrophilic or hydrophobic properties to improve presentation of an attached molecule under selected conditions. Suitable spacers include aminoalkyltrialkoxysilanes, hydroxyalkyltrialkoxysilanes, polyethyleneglycols, polyethyleneimine, polyacrylamide, polyvinyl alcohol and combinations thereof. The spacer is attached to the solid substrate via carbon-carbon bonds using, for example, substrates having polytrifluorochloroethylene surfaces, or more preferably, by siloxane bonds (using, for example, glass or silicon oxide as the solid substrate). Siloxane bonds with the surface of the substrate are formed in one embodiment via reactions of derivatization reagents bearing trichlorosilyl or trialkoxysilyl groups. When a spacer is used, it preferably has a reactive site or group for the attachment of a linking arm or first building block, discussed in more detail below. Groups, which are suitable for attaching a linking arm to a spacer include amine, hydroxyl, thiol, carboxylic acid, ester, amide, isocyanate and isothiocyanate.

B. Coupling Building Blocks to the Solid Support

The solid support preferably contains (either directly on its surface or on the exposed ends of linking arms and/or spacers) a reactive moiety such as, for example, a carboxylic acid, alcohol, amine, or halomethyl group. This reactive moiety is used to covalently attach an incoming building block according to standard synthetic methods. In a preferred embodiment, a starting building block is attached to the solid-support prior to beginning the assembly of the library.

The term "building blocks" refers to the chemical components, which are introduced sequentially in a step-by-step manner to generate a desired product or compound. Small molecule compounds suitable for screening for pharmacological activity can typically be synthesized using between 2 and 5 building blocks. To generate a combinatorial library of such compounds, the building blocks are introduced in "sets," where the number of sets is equal to the number of building blocks required to make a final compound. Therefore, to synthesize a combinatorial library of compounds where each compound is synthesized using 3 different building blocks, the methods uses 3 sets of building blocks. The building blocks within each set typically belong to the same "chemical family," so that they will react with building blocks in the other sets in a predictable manner (Gordon et al., 1994). The building blocks may be by the identity of the building block at each position. Alternatively, the building blocks may be selected to react in an interlocking manner, giving rise to non-polymeric three-dimensional structures (Gordon et al., 1994, incorporated herein by reference). The present invention preferably employs 2 or 3 sets of building blocks during a synthesis of a library of compounds.

Particular methods of attaching components to a solid support are well known in the field of solid phase chemistry. In general, it is understood that for attachment of an amine derivative to a solid support, an additional functional group should be present on the amine derivative, which can be covalently bonded to a functional group on the linker. If the presence of the amine would render attachment more difficult, the amine can be suitably protected. Alternatively, attachment of a stabilized sulfene precursor to a solid support will require additional functionality on the precursor, which can be used to covalently bond to a functional group on the linker. The additional functionality should not interfere with either the formation of a stabilized sulfene, or reaction of the sulfene moiety with the complementary compound.

C. Preparation of Libraries

The solid supports of the invention can be used as a support for combinatorial synthesis or parallel synthesis. The synthesis of combinatorial libraries is well known in the art (see, e.g., Gordon *et al., J. Med. Chem.,* 37:1385-1401 (1994); and Gallop, *J. Med. Chem.,* 37:1233-1251 (1994)). For example, libraries of small organic molecules can be synthesized on the solid supports of the present invention.

The present invention may be used in the synthesis of oligomeric as well as non-oligomeric compounds. Solid phase syntheses of oligomeric compounds, such as polynucleotides, polypeptides peptide-nucleic acids (PNAs), and the like, are well-known (see, e.g., SOLID PHASE SYNTHESIS - PEPTIDES, PROTEINS AND NUCLEIC ACIDS; BIOLOGICAL AND BIOMEDICAL APPLICATIONS. Roger Epton, Ed., Mayflower Worldwide Limited, Birmingham, England, UK, 1994; incorporated herein by reference). Solid phase techniques suitable for combinatorial synthesis of non-oligomeric small molecules are also known in the art (see, e.g., Gordon, E.M., *et al, J. Med. Chem.* 37:1385 (1994); Thompson, L.A. and Ellman, J.A., *Chem. Rev.* 96:555-600 (1996); Früchtel, J.S. and Jung, G., *Angew. Chem.* 35:17-41 (1996); and Patel, D.V., and Gordon, E.M., *Drug Disc. Today* 4:134-144 (1996); all incorporated herein by reference). Such techniques have been applied, for example, to the preparation of libraries of compounds based on a wide variety of heterocyclic structures, including benzodiazepines (Bunin, B.A., and Ellman, J.A., *J. Am. Chem. Soc.* 114:10997 (1992); Bunin, B.A., *et al., Proc. Natl. Acad. Sci. U.S.A.* 91:4708 (1994); Plunkett, M.J. and Ellman, J.A., *J. Am. Chem. Soc.*

(1993)), pyrrolidines (Murphy, M.M., *et al.*, *J. Am. Chem. Soc.* 117:7029 (1995); Gallop, *et al.*, U.S. Patent Serial Number 5,525,734, Issued June 11, 1996; Maclean, D., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 94:2805 (1997), thiazolidinones (Holmes, C.P., *et al.*, *J. Org. Chem.* 60:7328 (1995); Holmes, C.P., U.S. Patent Serial Number 5,549,974, Issued Aug. 27, 1996), thiazolidines (Patek, M., *et al.*, *Tetrahedron Lett.* 36:2227 (1995)), diketopiperazines (Gordon, D.W. and Steele, J., *Bioorg. Med. Chem. Lett.* 5:47 (1995); Szardenings, A.K., *et al.*, *Tetrahedron* 53:6573 (1997)), diketomorpholines (Szardenings, et al., 1997), tetrahydrofurans (Beebe, X., *et al.*, *J. Am. Chem. Soc.* 114:10061 (1992)), lactones (Moon, H.-S., *et al.*, *J. Org. Chem.* 57:6088 (1992)), isoxazoles and isoxanolines (Pei, Y. and Moos, W.H., *Tetrahedron Lett.* 35:5825 (1994)), and other compounds. Accordingly, these techniques and others can be used in conjunction with the methods and devices of the present invention.

By way of example, to evaluate the new solid support, triazine (**6**) was prepared. Some triazines have anti-tumor activity and were previously synthesized on solid supports. See, Golfin *et al.*, *Bull. Cancer* **1979**, 66, 61-66; Foster *et al.*, *Cancer Treatment Rev.* **1986**, 13, 197-217; Masquelin *et al.*, *Heterocycles* **1998**, 48, 2498-2505; Matsuno *et al.*, *Chem. Pharm. Bull.* **1997**, 45 (2), 291-296; and Stanková *et al.*, *Molecular Diversity* **1996**, 2, 75-80.

The acid labile Fmoc-protected Knorr-linker (p-{(R,S)-α-{1-(9H-Fluoren-9-yl)-methoxyformamido]-2,4-dimethoxybenzyl]-phenoxyacetic acid is available from Calbiochem-Novabiochem, Corp.) was coupled to the amino groups of the membrane. The Fmoc-group was removed and the membrane was reacted with cyanuric chloride. The two remaining chlorine atoms of compound (**3**) were sequentially substituted with *iso*-amylamine and morpholine. After the cleavage from the membrane with 95% TFA in DCM, the final product (**6**) was obtained in 46% yield. The purity of the crude material was over 95% (by HPLC and ^{1}H-NMR).

The creation and analysis of diverse chemical products typically proceeds by synthesizing diverse collections of molecules onto a plurality of solid supports. For many applications, it is desirable to simultaneously synthesize a wide variety of compounds onto the solid supports in a high throughput mode. In this manner, numerous compounds may be simultaneously synthesized in a single process, commonly referred to as parallel synthesis.

An apparatus capable of the parallel synthesis of compounds is described in U.S. Patent Application Serial No. 09/216,093 filed December 18, 1998 which is a continuation-in-part application of U.S. Patent Application Serial No. 08/994,802 filed December 19, 1997, each of which is incorporated herein by reference. Such an instrument is capable of performing up to 100 or more parallel reactions simultaneously by distributing the reaction mixture or control, to the various channels for pooling, mixing, and redistribution.

D. Applications

Following synthesis, the compounds of the library are typically tested for a desired activity, e.g., agonist or antagonist activity, in an *in vitro* biological assay. Although the compounds are typically eluted from the solid supports prior to such assays, they may, if desired, be left attached to the supports. Performing such a subsequent assay serves at least two purposes: (i) discovery of new compounds with biological activity in a given screening assay, and (ii) development of a relationship between the structural variations contained within the series and biological potency (i.e., a structure activity relationship (SAR)).

Examples of suitable assays include receptor binding assays (screening for receptor agonists or antagonists), functional enzyme assays (measuring competitive or noncompetitive inhibition of the catalyzed reaction), and the like. For instance, compounds synthesized using methods of the present invention may used in an assay to isolate compounds having antibacterial activity. Compounds are synthesized and eluted as described above, and individual compounds are spotted onto a lawn of bacteria. Compounds with antimicrobial activity will generate a bacteria-free zone around the point where the compound was contacted with the plate.

Alternatively or in addition, affinity for an enzyme such as a bacterial D-Ala-D-Ala carboxypeptidase (involved in bacterial cell wall construction) can be studied by exposing the solid support to the enzyme and assaying binding of the enzyme to the solid support. Binding of the enzyme on the solid support may be assayed by labeling the enzyme with a radioactive or fluorescent label, and scanning the surface of the solid support for the presence of the label.

Assays such as are described above may be used to screen for pharmaceutical agents, veterinary agents, agricultural agents, diagnostic reagents, and the like. Typical compounds and pharmaceutical applications include:

(1) nitrogen containing heterocyclic compounds; imidazopyridines having antiulcer or anxiolytic activities, dihydropyridines having calcium antagonist activity, nucleoside and nucleoside analogs having antiviral activity, indazoles having 5HT3 antagonist activity, piperidines having antidopamine, antiserotonin, antidepressant, or antihistamine activities, benzazepines having antiparkinsonism and antidopamine activities, indoles and condensed indoles with 5HT antagonist activities, quinolines and isoquinolines having anti-infective and

aminopyrimidines having antihypertensive activities, pyrrolizidines having antiarrhythmic activities, guanidines having anticancer activities, tetrazoles having antiallergenic;

(2) oxygen containing heterocycles; benzopyrans having potassium agonist and antagonist activities, coumarins having antiplatelet aggregating and antithrombotic activities, prostaglandins and prostacyclins having antiplatelet, antiulcer, labor inducing activities, psoralens having antipsoriasis activities, tetrahydrofurans and pyrans having antidiabetic activity;

(3) nitrogen and sulfur containing compounds; beta-lactams and cephalosporins having anti-infective activities;

(4) carbocyclic compounds; tocopherol analogs having antipsoriasis activities, vitamin D analogs having antipsoriasis activities, steroids having anti-inflammatory, bronchiodilating, antihyperplasia and antifertility activities, naphthalenes having antifungal activities, anthracene analogs having anticancer activities;

(5) alicyclic compounds; polyunsaturated alkenes having antithrombotic activities, hydroxypropanolamines having adrenergic blocking activities, benzofused bicyclic amines having analgesic activities, aryl amides having anesthetic, gastroprokinetic, antidepressant, and anti-inflammatory activities; and

(6) cyclic peptides and cyclic nucleotide having anti-infective and antiautoimmune activities; and the like.

The methods described herein have a number of applications beyond those described above. For example, the methods may be applied to the optimization of chemical reactions, e.g., reaction yields or reaction times. In this application, the same reaction is performed in all reaction vessels, but reaction variables are systematically varied across the array. For example, the reagent concentration and/or reagent equivalency (mole percentage) can be varied from near zero to the maximum achievable with an undiluted reagent. Typically, one varies this variable from 0.001 to 25 M. Reaction times can also be varied within the array by withdrawal of the contents of the reaction well, followed by for example work-up, and/or isolation, and/or purification, and/or quantitation of the final solution. Statistical experimental design strategies or quantitative structure activity relationship (QSAR) strategies may also be implemented to select a subset of locations within the original array which will provide the necessary information for final analysis and conclusions, thus reducing the number of reactions necessary in the final array.

The following examples are included for the purpose of illustrating the invention and are not intended to limit the scope of the invention in any manner.

Examples 1-5

Grafting of irradiated Teflon membranes in styrene (S), 4-vinylbenzene chloride (VBC), and in a mixture of both

Zitex A-105 membranes were irradiated under argon by e^--beam to a total dose of 60 kGy. The membranes were kept at -78°C until grafting. The membranes and monomer were placed in a Schlenk tube and exposed to 3 freeze-thawing cycles under vacuum. The Schlenk tubes were heated at 50°C for 18 hours. After thorough rinsing with methanol, toluene, and DCM the membranes were dried in a vacuum oven at 90°C. An additional extraction in toluene didn't show any weight decrease of the membrane.

Example	Monomer	Solvent	Weight increase (%)	Grafted amount (%)
1	S	methanol	10.3	9.3
2	VBC	methanol	7.6	7.1
3	S:VBC = 1:1	methanol	13.1	11.6
4	S	methanol in 0.2M H_2SO_4	14.4	12.6
5	S	none	23.3	18.9

Example 6

Grafting of a Zitex A-140 membrane, which was irradiated with 20 kGy

Experimental conditions as in examples 1-5. The grafting monomer was styrene, which was used without a solvent. Duration of the grafting was 24 hours. The obtained membrane showed a weight increase of 16.7% (the corresponding grafted amount is 14.3%).

Examples 7 and 8

Grafting of a Zitex A-105 membranes, which were irradiated with 60kGy and 100kGy

Experimental conditions are as in example 1-5. The grafting solution was a 1:1 mixture of VBC and toluene. The weight increase of the membrane, which was irradiated with 60 kGy was 5.3% (grafted amount 5.0%). The weight increase of the membrane, which was irradiated with 100 kGy was 5.5% (grafted amount 5.2%).

Grafting of two different kinds of Teflon membranes in S, VBC, and a mixture of both

Zitex G-108 and Millipore FSLW/420 membranes were irradiated under argon by e^--beam to a total dose of 6 kGy. In comparison to the pure Teflon membrane G-108, the Millipore membrane contains a polyethylene backing. The membranes were kept at -78°C until grafting. Schlenk tubes were filled with monomer and membranes were exposed to 3 freeze-thawing cycles. The Schlenk tubes were then put in a heating bath for 40 hours at 40°C. After intensive rinsing with methanol, toluene, and DCM the membranes were dried in a vacuum oven at 90°C.

Example	Membrane	Monomer	Weight increase (%)	Grafted amount (%)
9	G-108	S	7.3	6.8
10	FSLW/420	S	48.3	32.6
11	G-108	S:VBC = 3:1	6.5	6.1
12	FSLW/420	S:VBC = 3:1	10.5	9.5
13	G-108	S:VBC = 1:1	3.4	3.3
14	FSLW/420	S:VBC = 1:1	7.8	7.2

Examples 16 and 17

Zitex G-108 and G-115, irradiated with 6kGy

Two PTFE membrane sheets (10"x10"), differing in thickness and pore size (Zitex G-108 and G-115, irradiated with 6kGy), were placed in a flat custom-made reaction box filled with a 3:1 mixture of S and VBC. Argon was bubbled for 10 minutes into the monomer mixture before the box was closed and heated for 3 days t 60°C. After thorough rinsing with toluene and DCM the membranes were dried and weighed. The G-115 membrane increased 8.8% by weight (grafted amount 8.1%), the G-108 membranes by 8.5% (grafted amount 7.8%).

Zitex G-115, irradiated with 4kGy

15 PTFE membrane sheets (10"x10", Zitex G-115, irradiated with 4kGy) were rolled together and placed in a cylindrical reaction vessel filled with a 3:1 mixture of S and VBC. Argon was bubbled for 10 minutes into the monomer mixture before it was closed at kept for 7 days at room temperature. After thorough rinsing with toluene and DCM the membranes were dried and weighed. The membranes showed weight increases between 11.8% and 17% (grafted amount 10.6 and 14.6%).

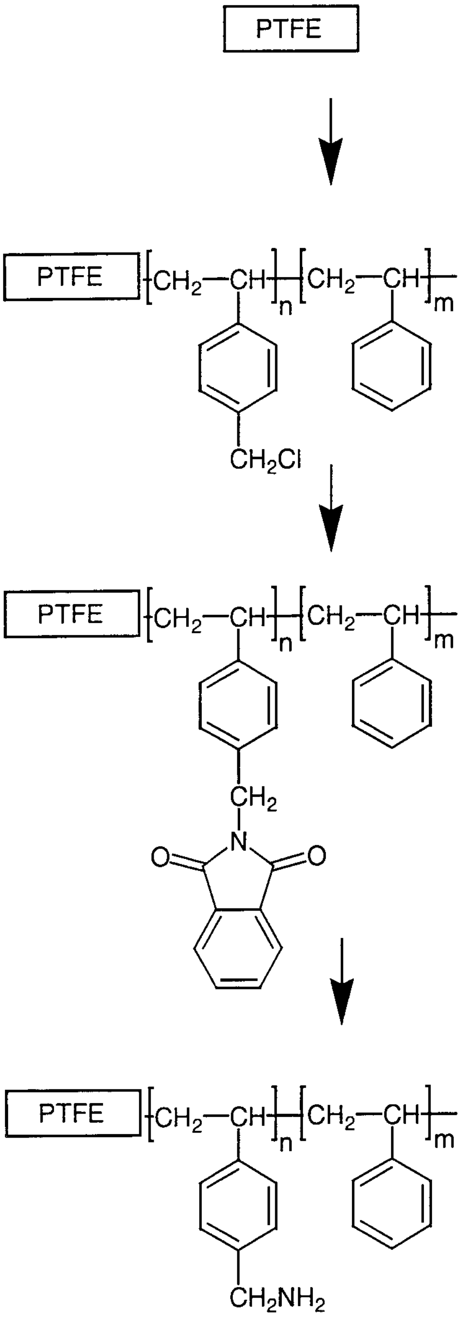
Example 19

Grafting of a poly(styrene-co-vinylbenzyl phthalimide) grafted Teflon Membrane

A Teflon Membrane (Zitex G-115, irradiated with 6kGy) was placed in a Schlenk tube filled with a 8:1 mixture of S and 4-vinylbenzyl phthalimide. Argon was bubbled for 10 minutes into the monomer mixture before it was closed and heated for 40 hours to 50°C. After thorough rinsing with toluene and DCM the membrane was dried and weighed. The membrane showed a weight increase of 2.6% (grafted amount 2.6%).

Example 20

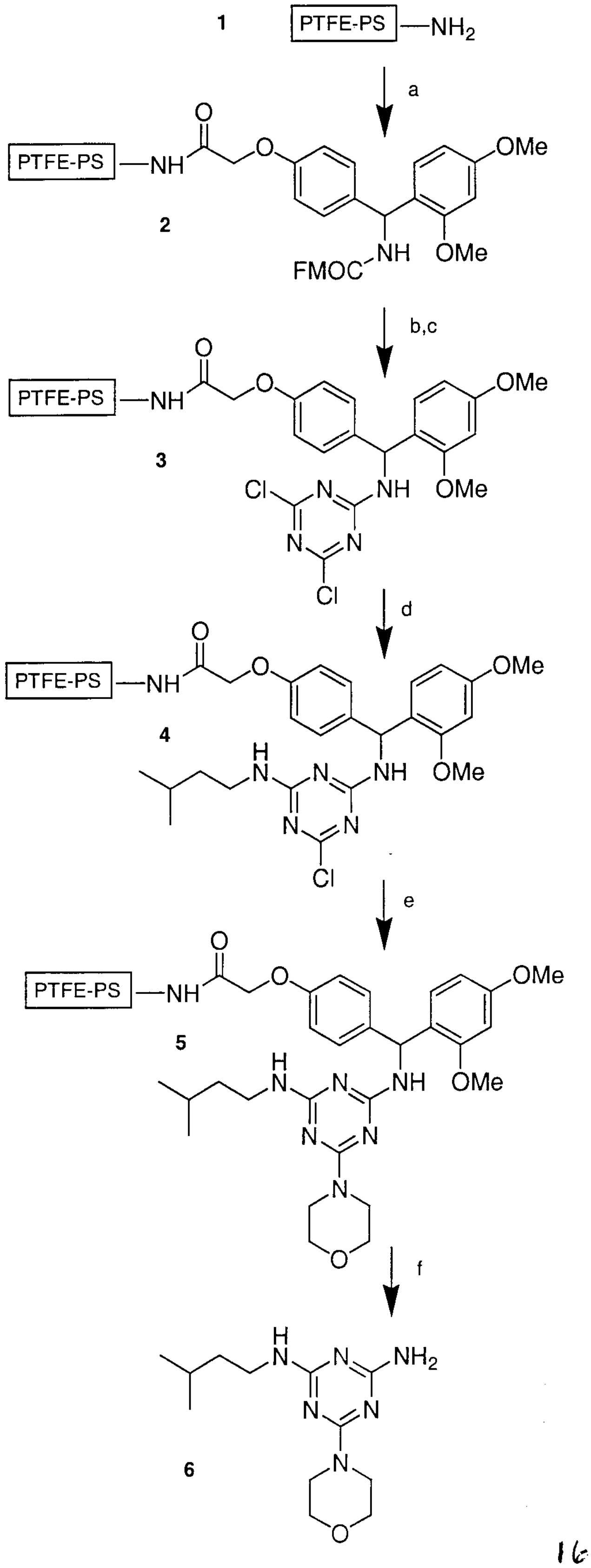
Modification of the CH_2Cl groups of poly(styrene-co-vinylbenzyl chloride) grafted Teflon Membranes (A-105, A-140, G-108, G-115) to CH_2-NH_2 groups



In the first step the membrane was reacted with a 0.5M solution of potassium phthalimide and the phase transfer catalyst TBAI (0.25M) in NMP at 85 °C overnight. After rinsing with water, methanol and DCM the membrane was treated with 20% methylhydrazine in DCM. The membranes were carefully rinsed and dried at 90°C in a vacuum oven.

Example 21

Synthesis of a triazine compound on a membrane containing amino groups

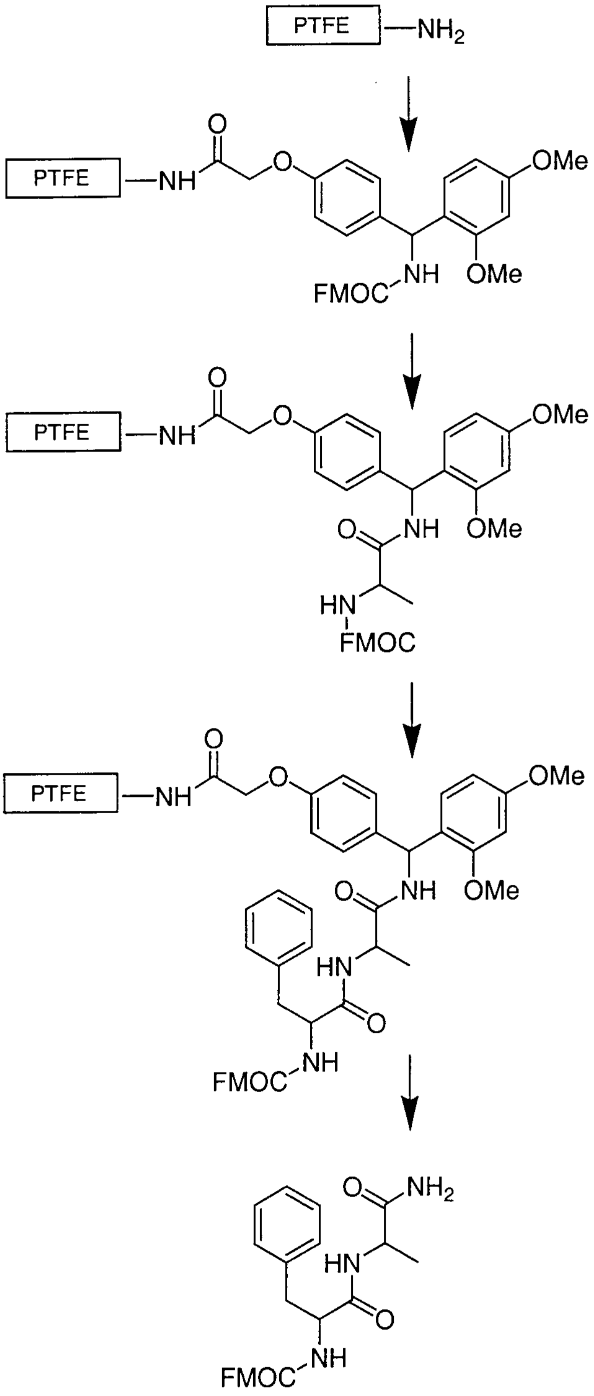


The membrane was carefully rinsed after each reaction step to remove unreacted reagents.

A piece of an aminomethylated-polystyrene grafted Teflon membrane (0.733g, loading 0.23 mmol/g) was coupled with the Fmoc protected Knorr linker by shaking it in a CH_2Cl_2/DMF (4:1) solution of the linker (0.1M), HATU=[O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate (0.1M), and DIEA=N,N-diisopropylethylamine (0.2M) for 16h at room temperature. The Fmoc protecting group was removed by treatment with 20% piperidine in NMP for 0.5h. Cyanuric acid was bound to the free amino groups by reacting in a 0.5 M cyanuric chloride solution, DIEA, CH_2Cl_2 for 16h at room temperature. The first of the two remaining chlorine atoms was substituted by reacting with a 0.2M solution of *iso*-amylamine in chloroform (16 hours at room temperature). The other chlorine was substituted via a reaction with a 1.0M morpholine in acetonitrile (3h at 75°C). The final product (**6**) was obtained in 46% yield by cleaving with 95% TFA in DCM. The purity of the crude material was over 95% (by HPLC and ^{1}H-NMR).

Example 22

Synthesis of a dipeptide on an amino-group containing polystyrene grafted Teflon membrane



Pieces of the membrane (0.54g, 0.076mmol) were placed in a glass column (15cmx10mm), which was connected to a pump to ensure a flow of solvents/reagents. After each reaction step appropriate solvent was pumped through the system to eliminate unreacted chemicals.

of a Fmoc protected Knorr linker (3.5g, 6.5mmol), HATU (2.47g, 6.5mmol), DIEA (2.26ml, 13mmol) in 10 ml of DCM/DMF 4:1 at room temperature overnight.

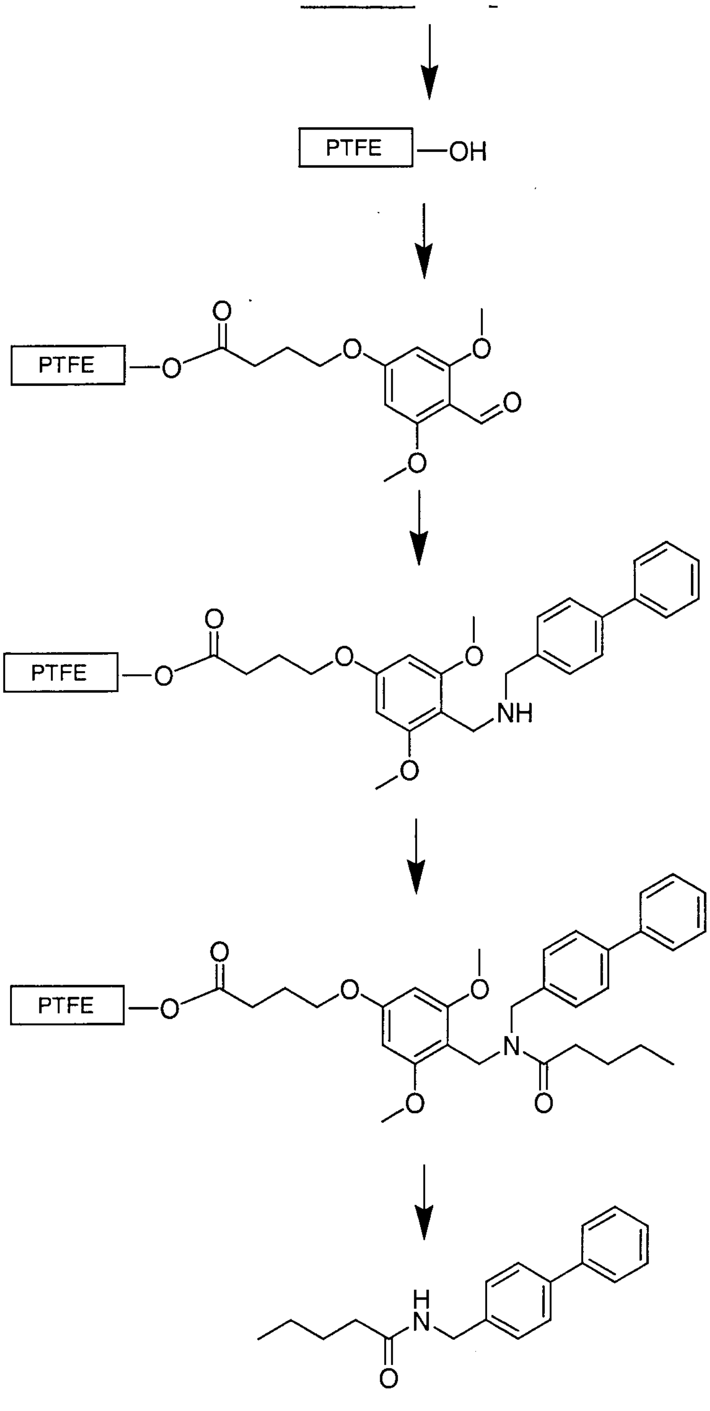
Coupling of Fmoc-Ala. A solution of 20% of piperidine in DMF was recirculated for 1 hour to remove the protection group. After the solution was removed, the strips were washed sequentially with DMF, dioxane and DCM. A solution of Fmoc-Ala (3 mmol, 1g), HATU (2.8 mmol,1g), DIEA (10.12 mmol, 1.76 ml) in 10 ml of DMF was recirculated through the column overnight. The second coupling was was carried out.

Coupling of Fmoc-Phe. The addition of the second amino acid was accomplished by recirculating the solution of Fmoc-Phe (9.2mmol, 3.56g), HATU (8.36mmol, 3.17g), DIEA (5.18mmol, 5.28ml) in 30 ml of DMF. The coupling was repeated.

Cleavage from Support. The strips were removed from the glass column, placed in a solid phase reaction vessel, and treated with 50% TFA in DCM for 3 hours on a shaker. The solution was collected in a flask, and the strips were washed several times with the same mixture. The HPLC of the lyophilized product (24mg = 70% yield) indicated only one peak, and the ^{1}H-NMR spectra for the compound was consistent with the structure.

Example 23

Modification of a poly(styrene-co-vinylbenzyl chloride) grafted Teflon membrane to a hydroxy-group containing membrane, which was used as solid support for the synthesis of an amide by reductive amination



0.94g of a poly(styrene-co-vinylbenzyl chloride) grafted Teflon membrane (loading 0.21mmol/g) was heated in a 1M solution of potassium acetate in 2-methoxy ethanol overnight under reflux. The obtained poly(styrene-co-vinylbenzyl alcohol) grafted Teflon membrane was thoroughly washed and then coupled with an acid labile Bal-linker. This was done by shaking the membrane in a solution of Bal linker (0.4M), 2,6-dichlorobenzoyl chloride (0.44M), and pyridine (0.44M) at room temperature. After carefully rinsing the aldehyde of the sodium triacetoxyborohydride (0.5M), and acetic acid (catalytic amount) in dichloroethane. After rinsing, the obtained secondary amine was acylated with 1M valeroyl chloride in anhydrous DCM, under basic conditions (1.2 M pyridine) in the presence of catalytic amounts of DMAP. The product was cleaved from the solid support by a 95% solution of TFA in DCM. After evaporating of solvents, the product (yield 63%) was found to be pure by LC-MS and HPLC.

The above description is illustrative and not restrictive. Many variations of the invention will become apparent to those of skill in the art upon review of this disclosure. Merely by way of example a wide variety of process times, reaction temperatures, and other reaction conditions may be utilized, as well as a different ordering of certain processing steps. The scope of the invention should, therefore, be determined not with reference to the above description, but instead should be determined with reference to the appended claims along with their full scope of equivalents. Although certain embodiments and examples have been used to describe the invention, changes may be made to those embodiments and examples without departing from the scope of the following claims or spirit of the invention.

The disclosures in this application of all articles and references, including patent documents, are incorporated herein by reference.

1. A solid support comprising a polymer having reactive groups grafted to a chemically inert bulk material.

2. The solid support of Claim 1, wherein said polymer having reactive groups comprises poly(styrene-co-vinylbenzyl chloride).

3. The solid support of Claim 1, wherein said chemically inert bulk material comprises a Teflon (PTFE) surface.

4. The solid support of Claim 3, wherein said chemically inert bulk material comprises a membrane, porous sheet, sieve, mesh, porous rod, or thimble.

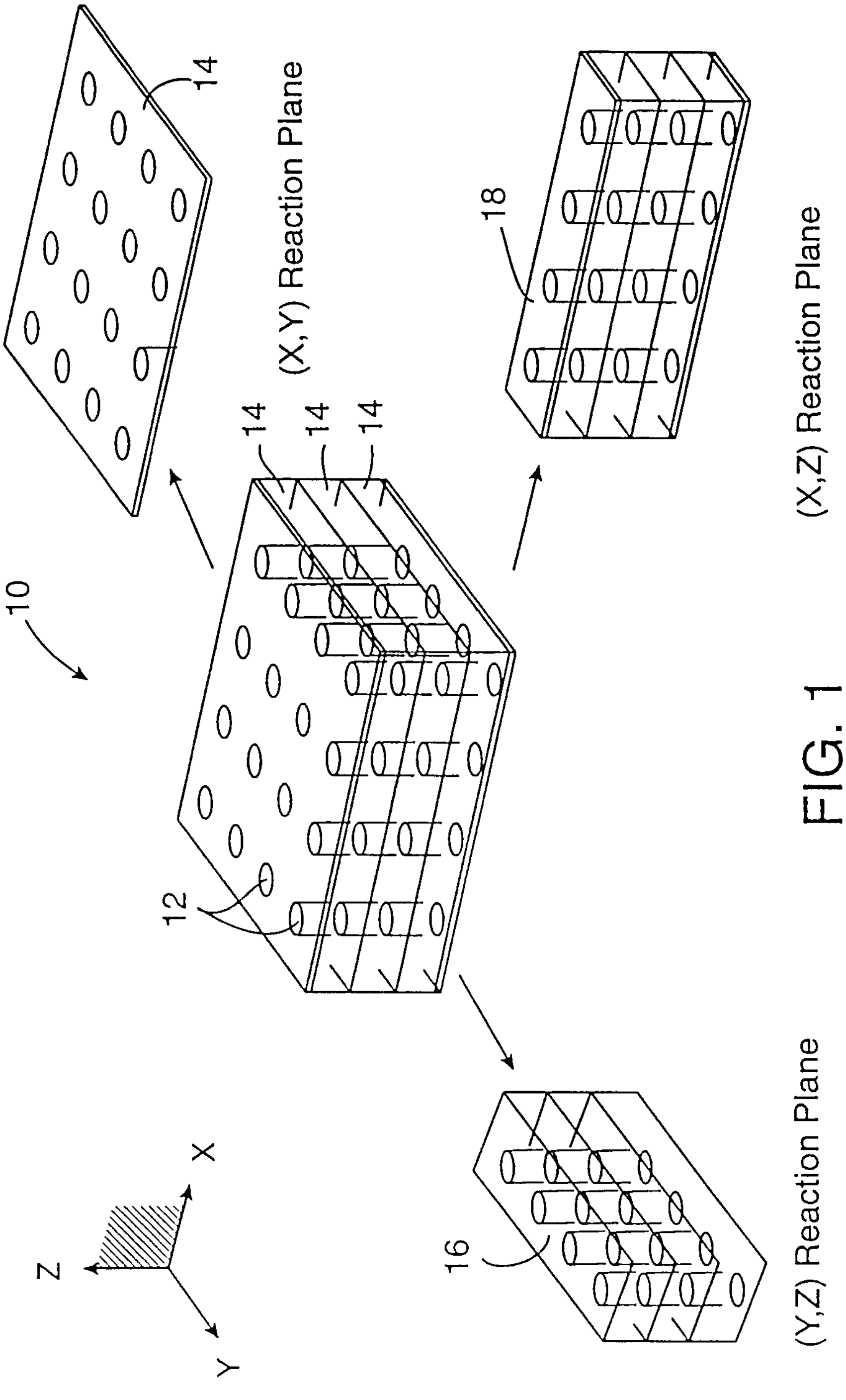

10
12
14
14
14
14
16
18
(X,Y) Reaction Plane
(X,Z) Reaction Plane
(Y,Z) Reaction Plane
X
Y
Z

FIG. 1

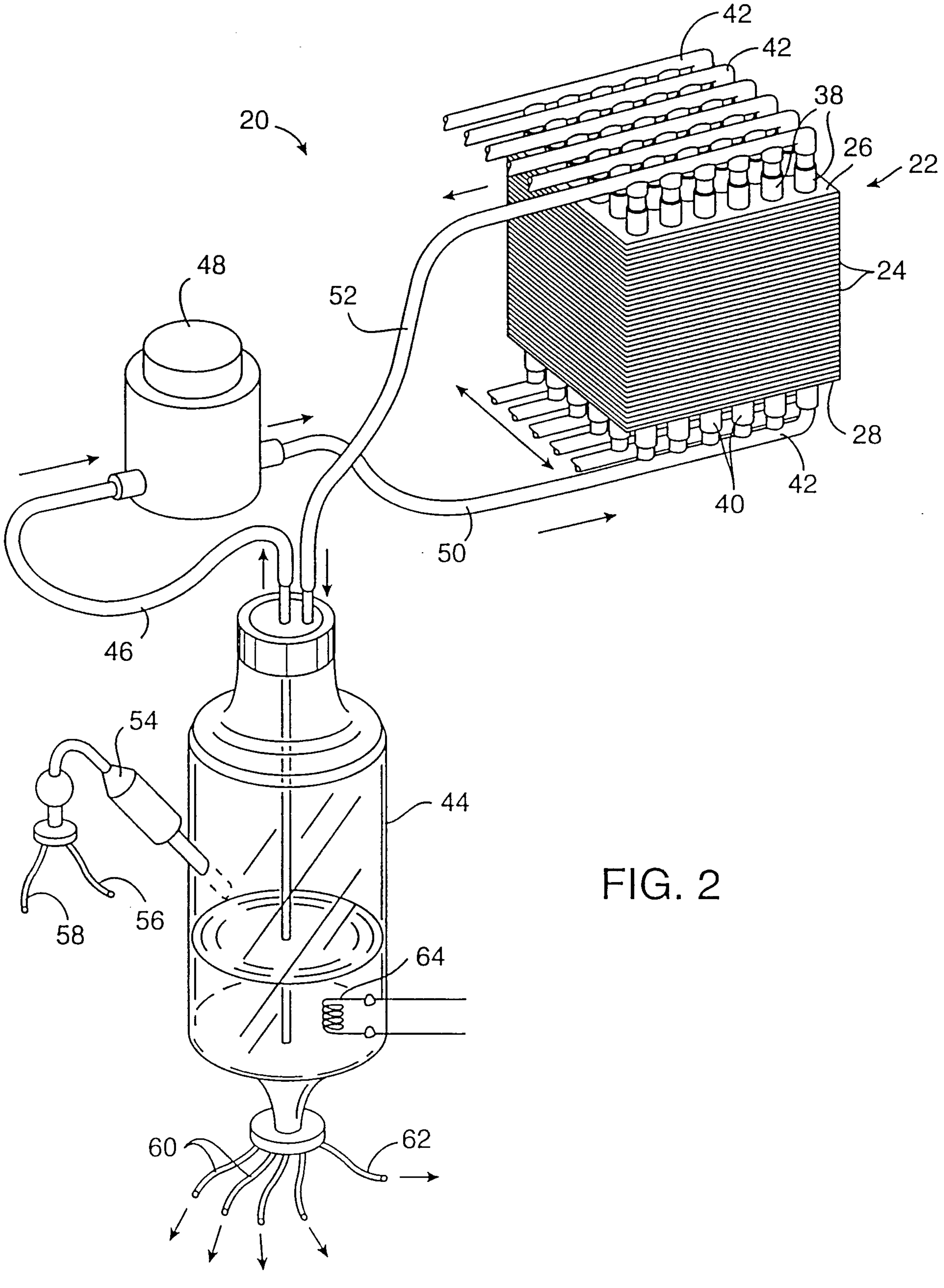


FIG. 2

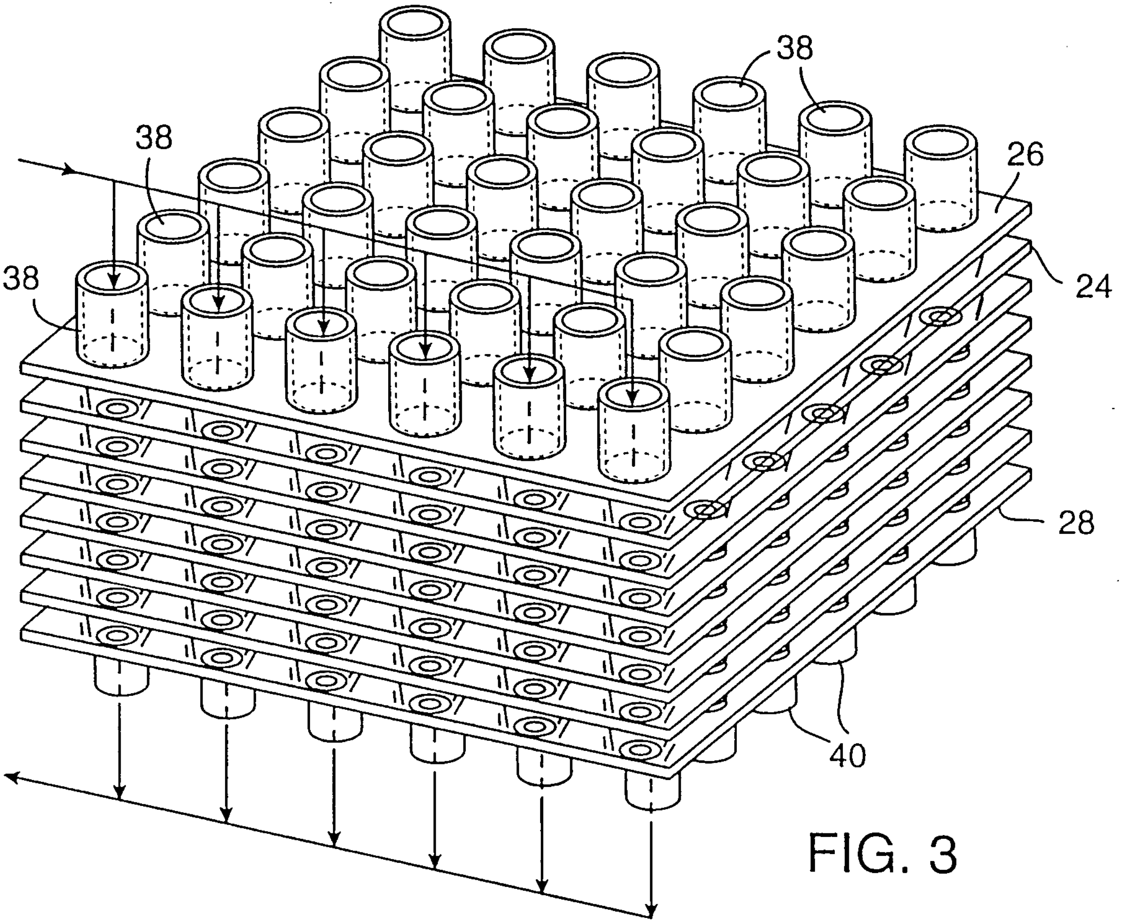


FIG. 3

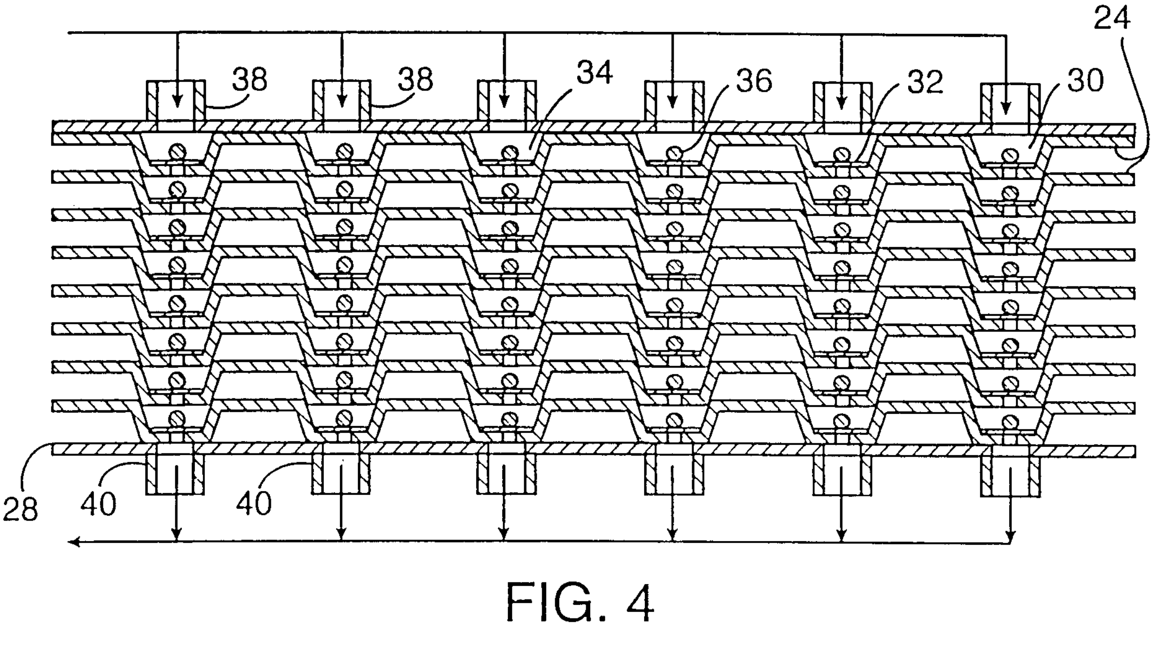


FIG. 4

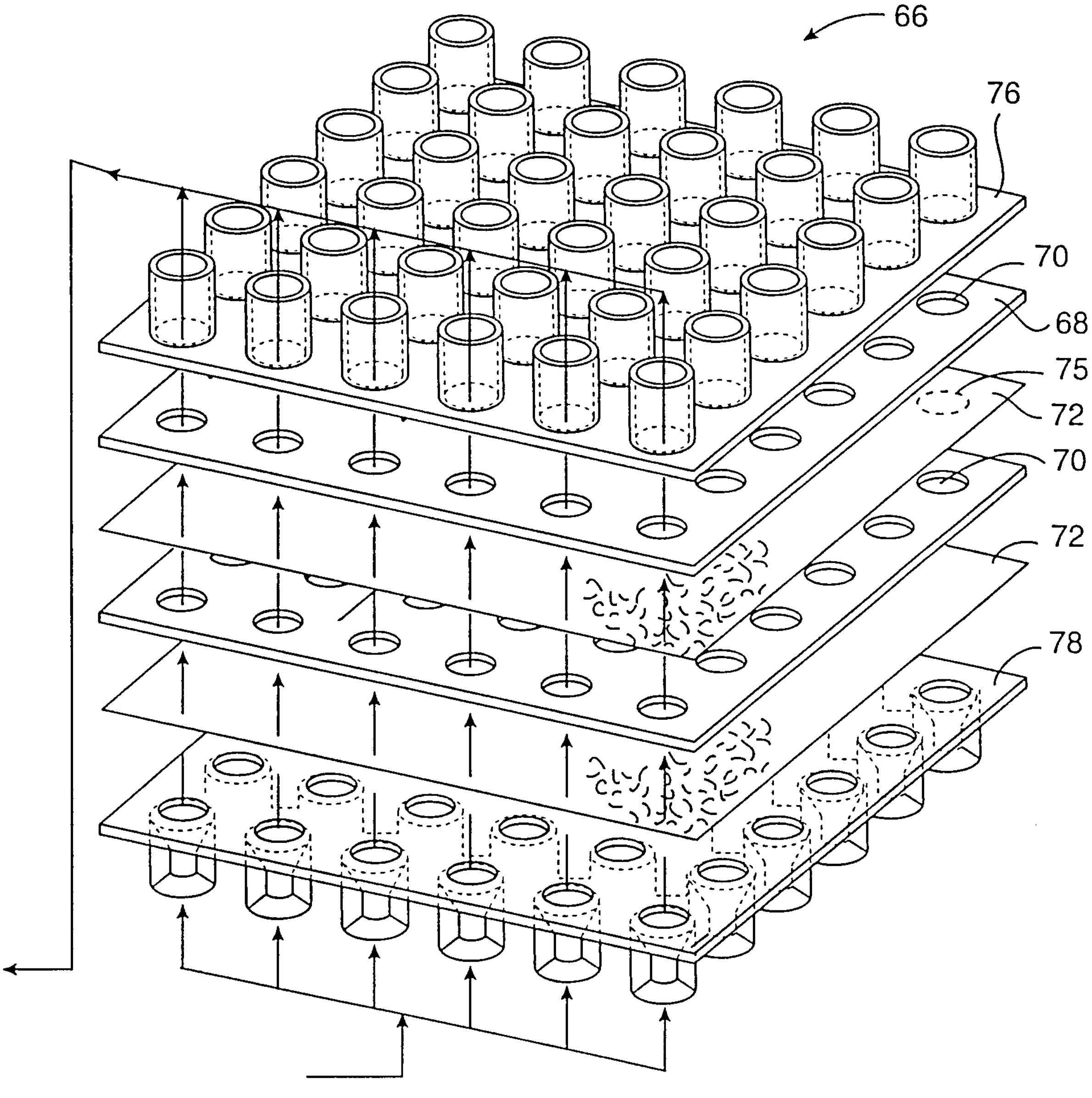


FIG. 5

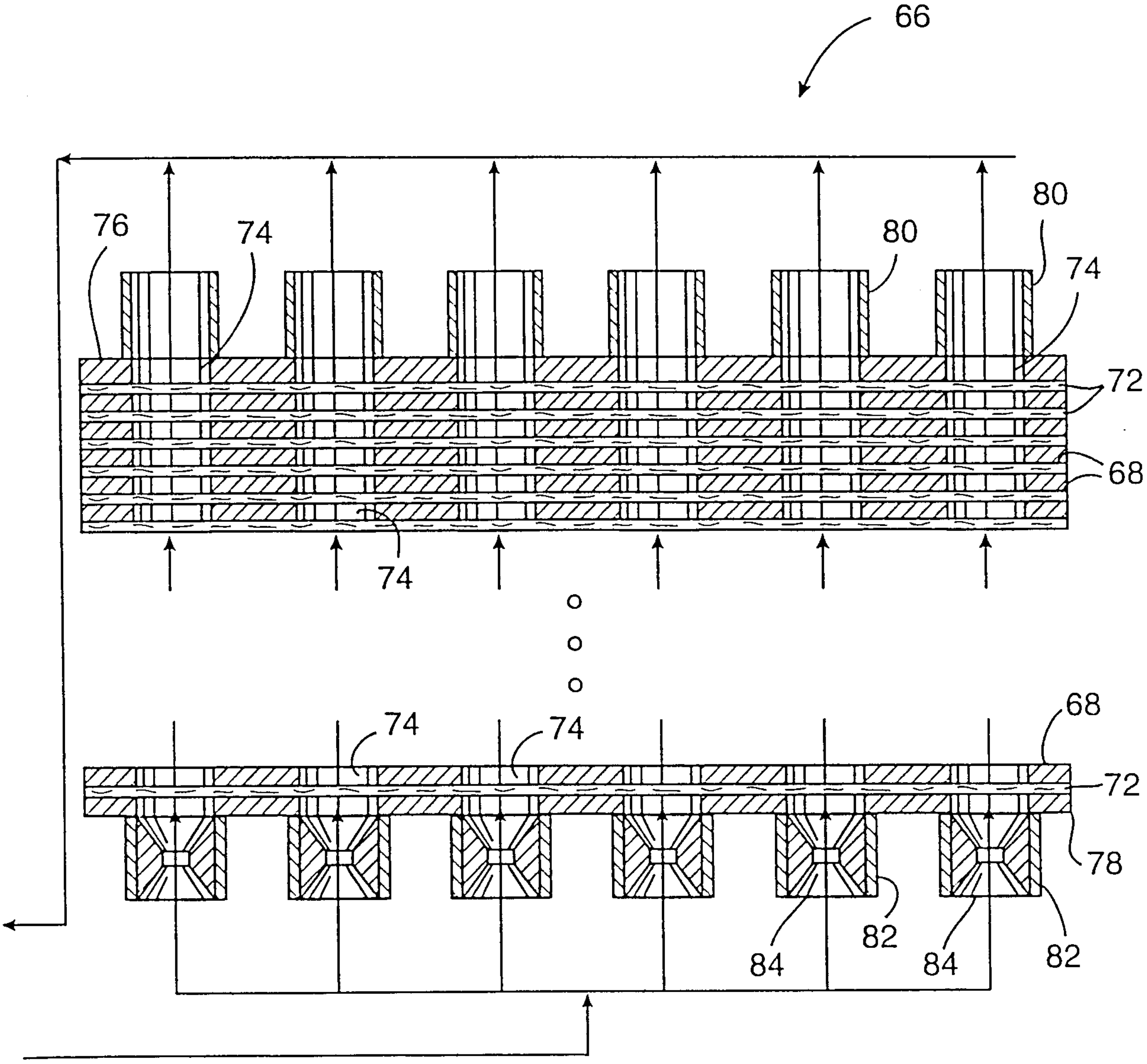


FIG. 6

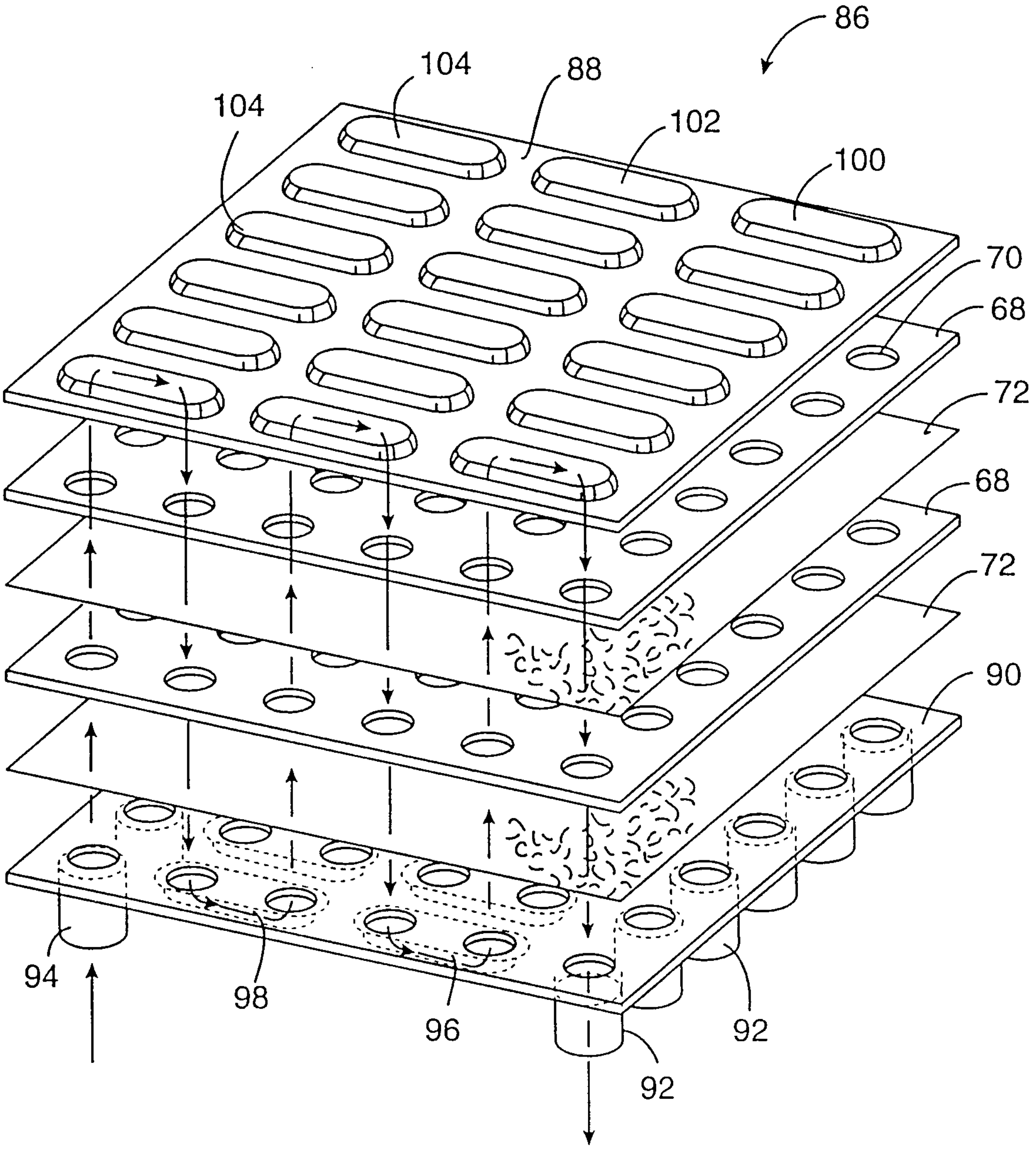


FIG. 7

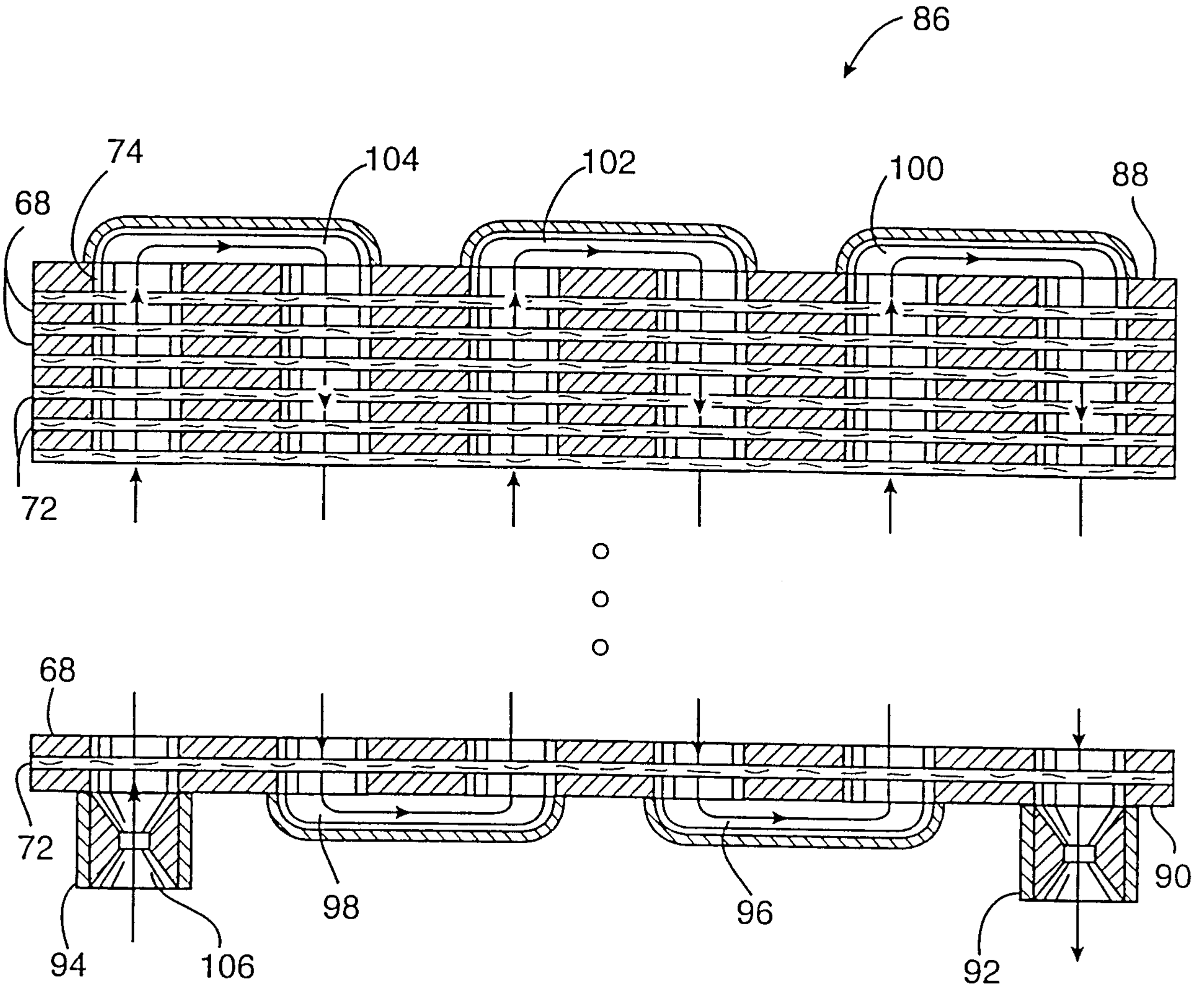


FIG. 8

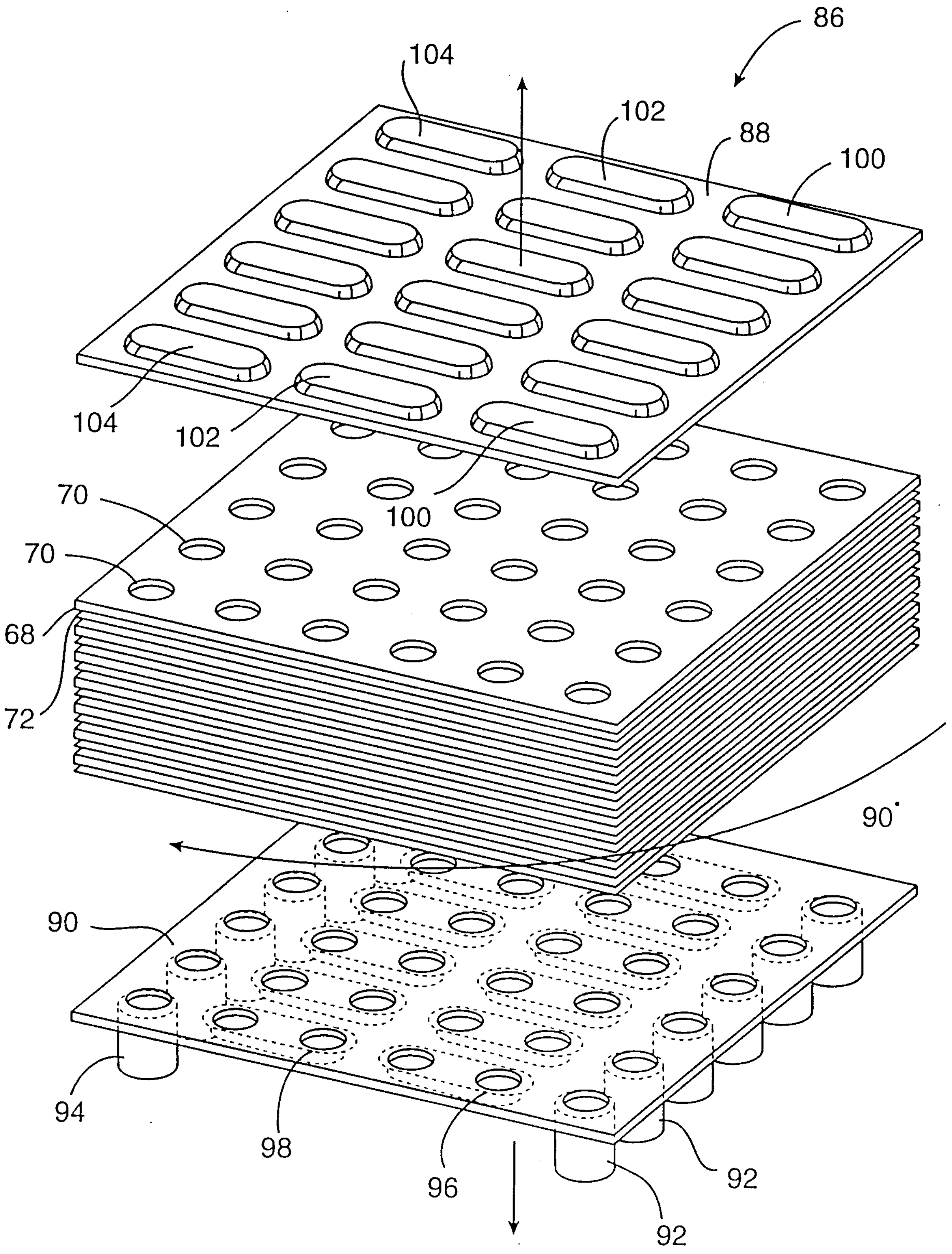


FIG. 9

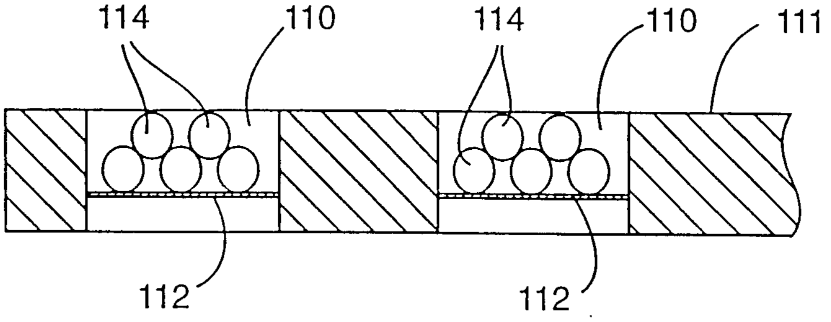


FIG. 10

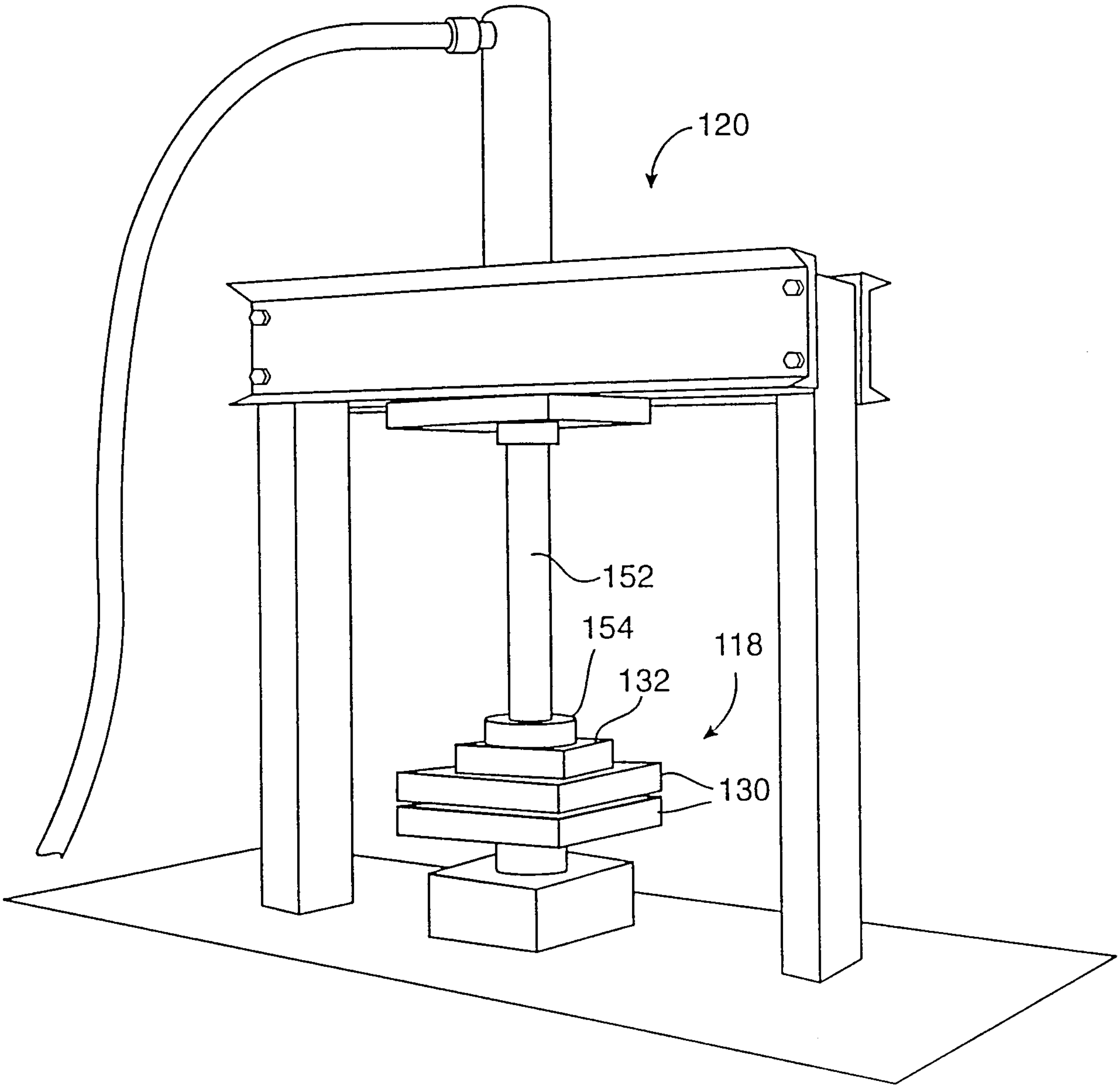
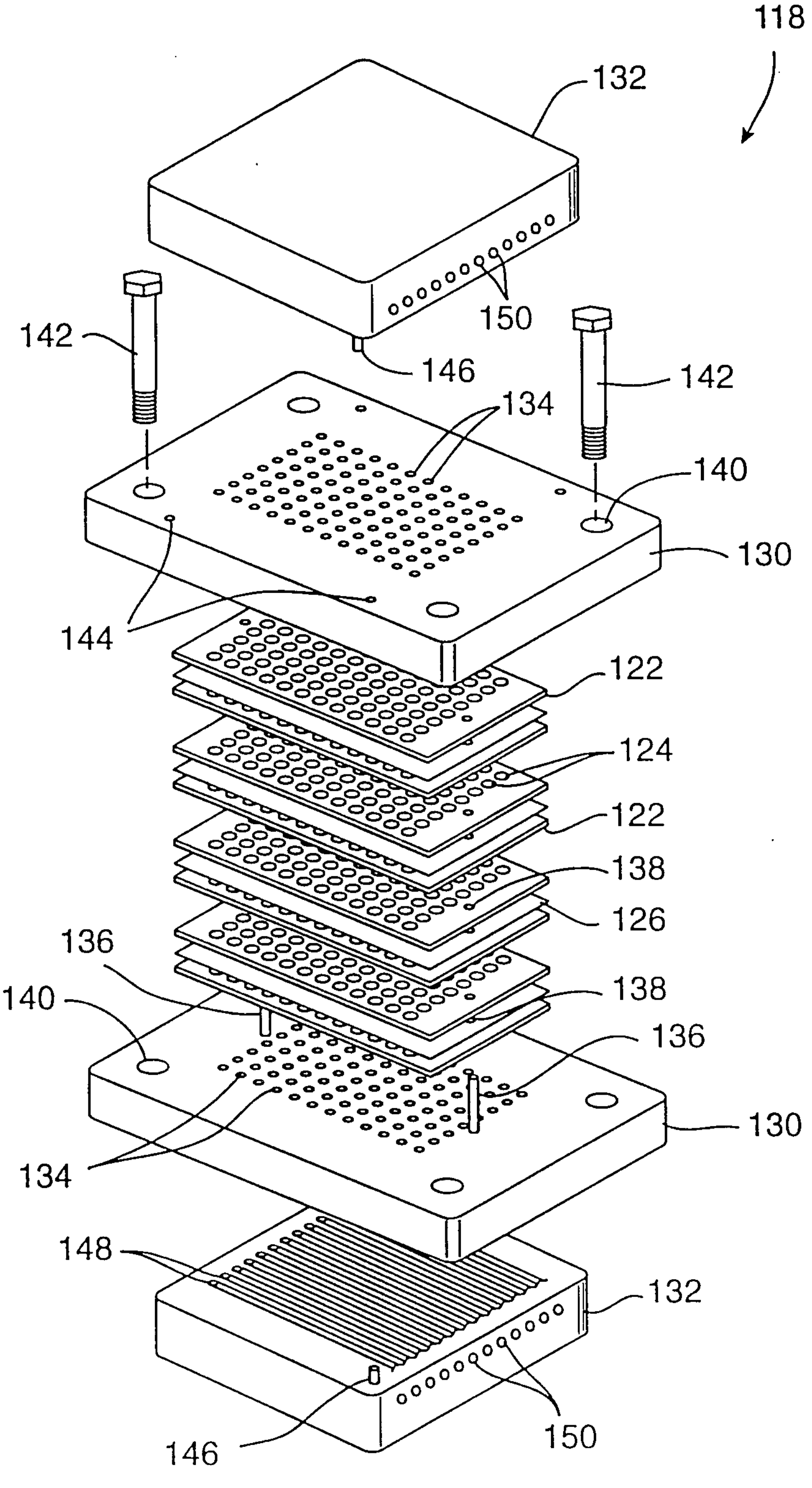


FIG. 11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150

FIG. 12

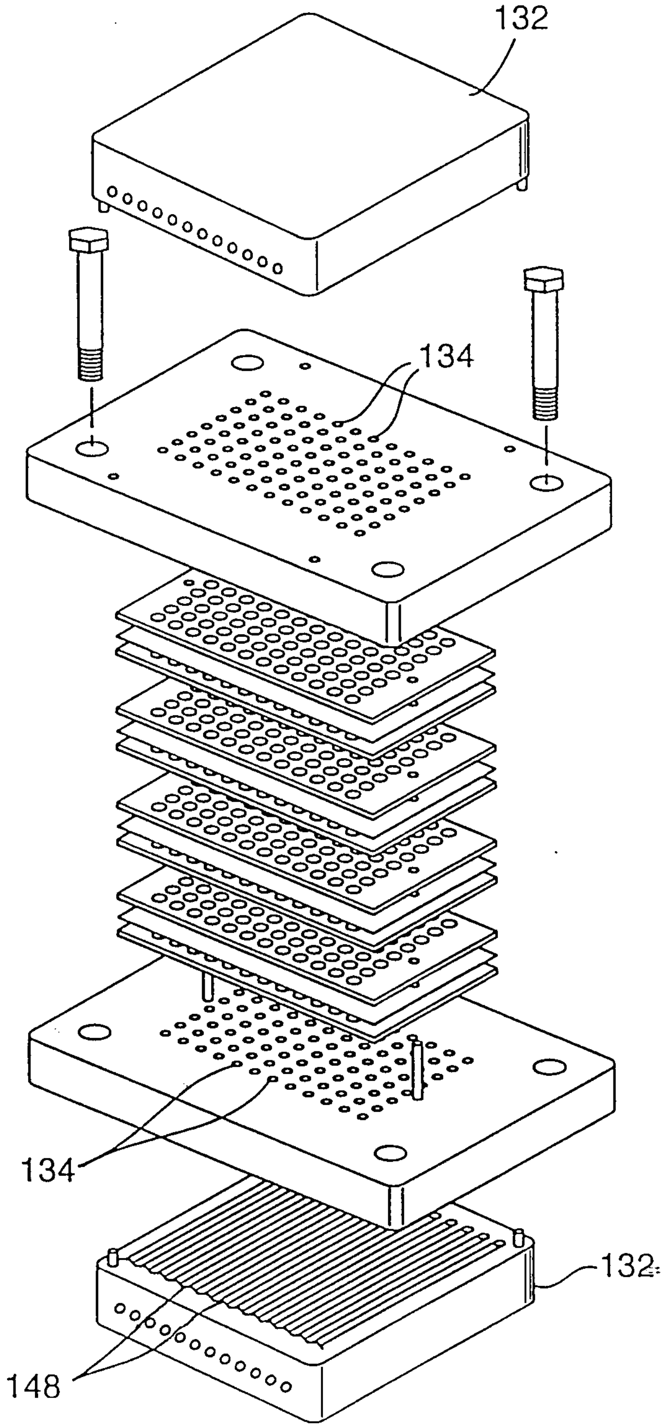


FIG. 13

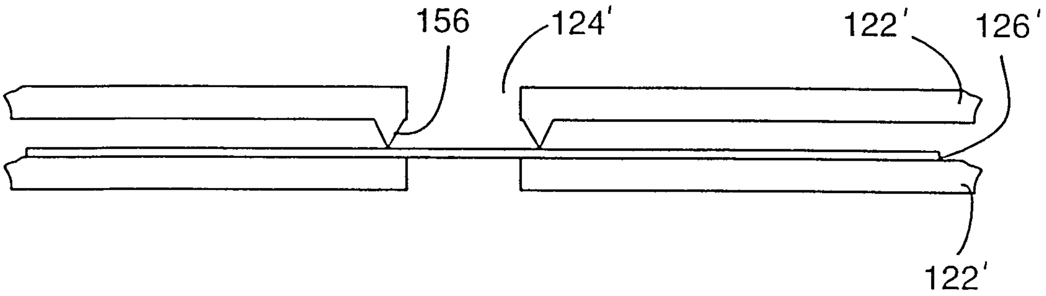
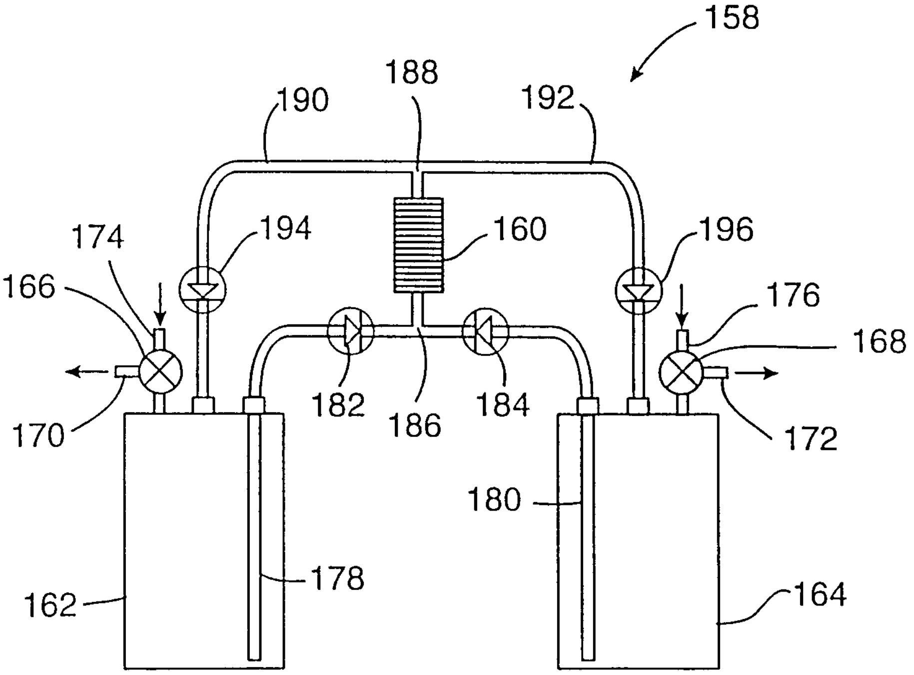

156
124'
122'
126'
122'

FIG. 14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164

FIG. 15

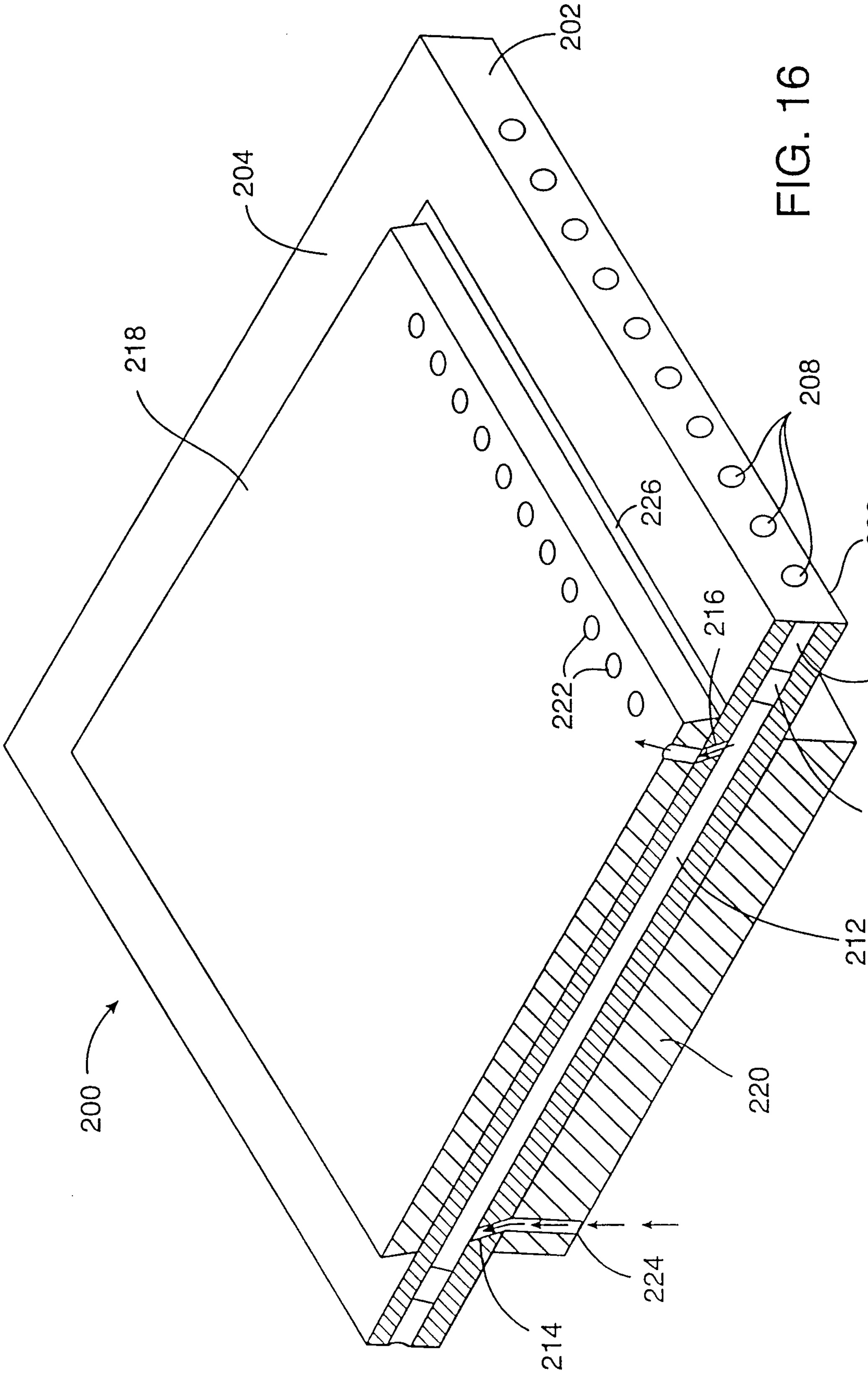


FIG. 16

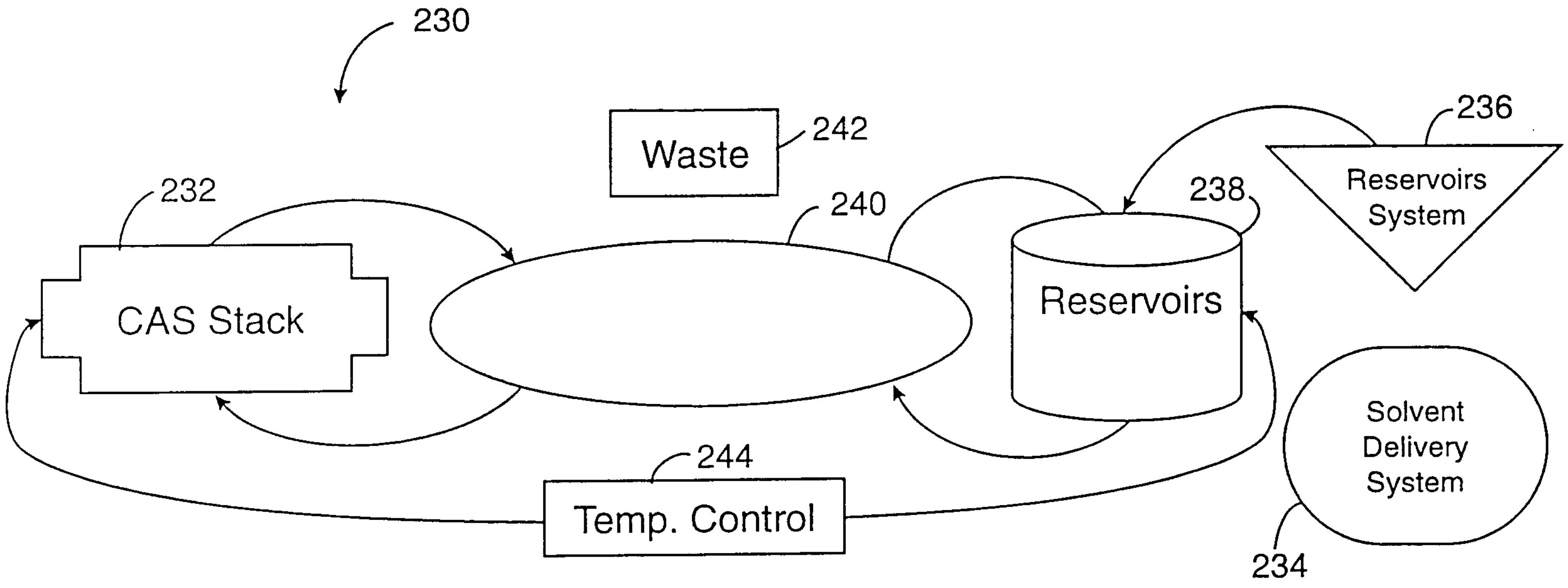
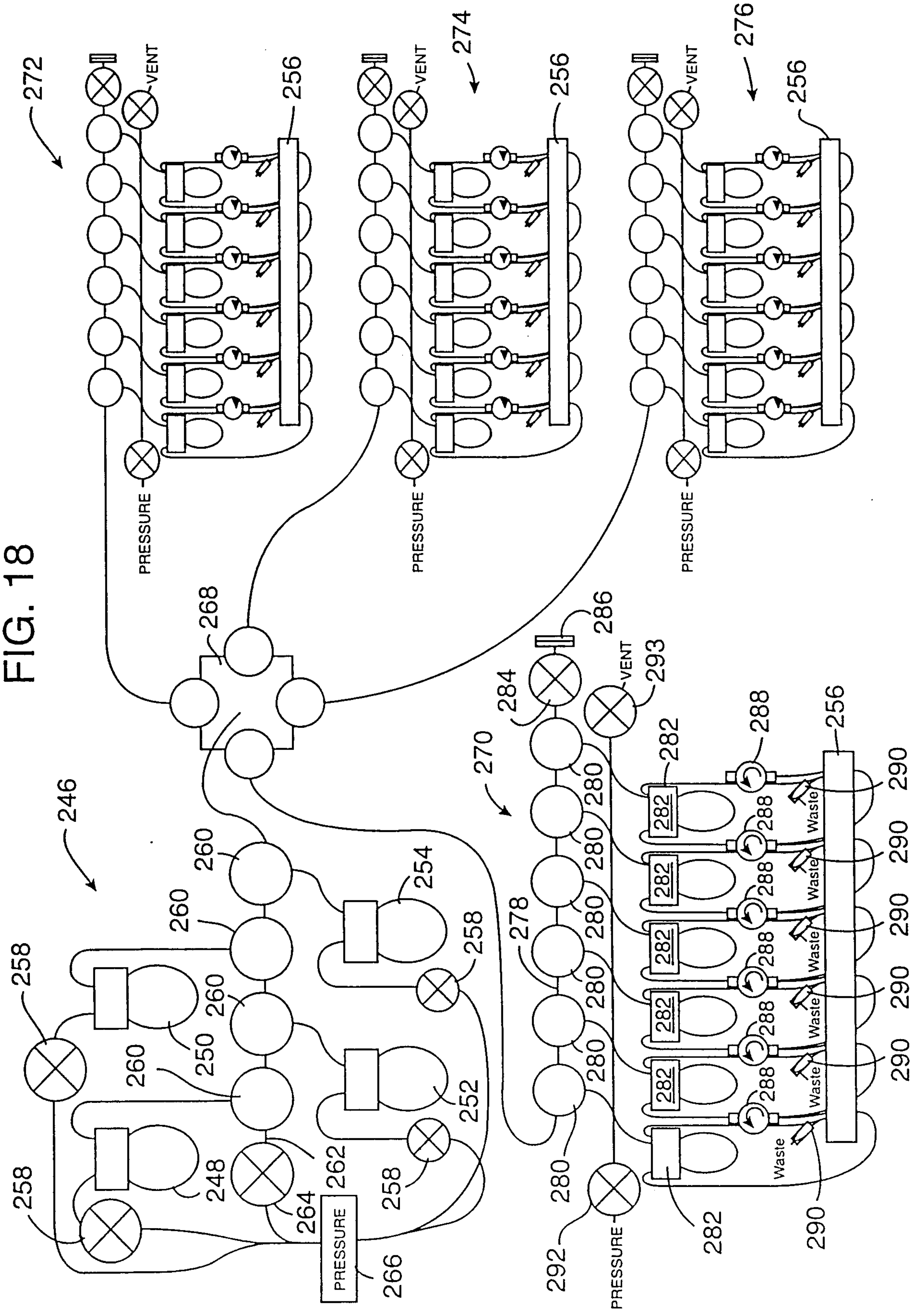

230
Waste
242
232
240
CAS Stack
236
Reservoirs System
238
Reservoirs
Solvent Delivery System
244
Temp. Control
234

FIG. 17

FIG. 18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276
256
VENT
PRESSURE
268
286
284
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Waste
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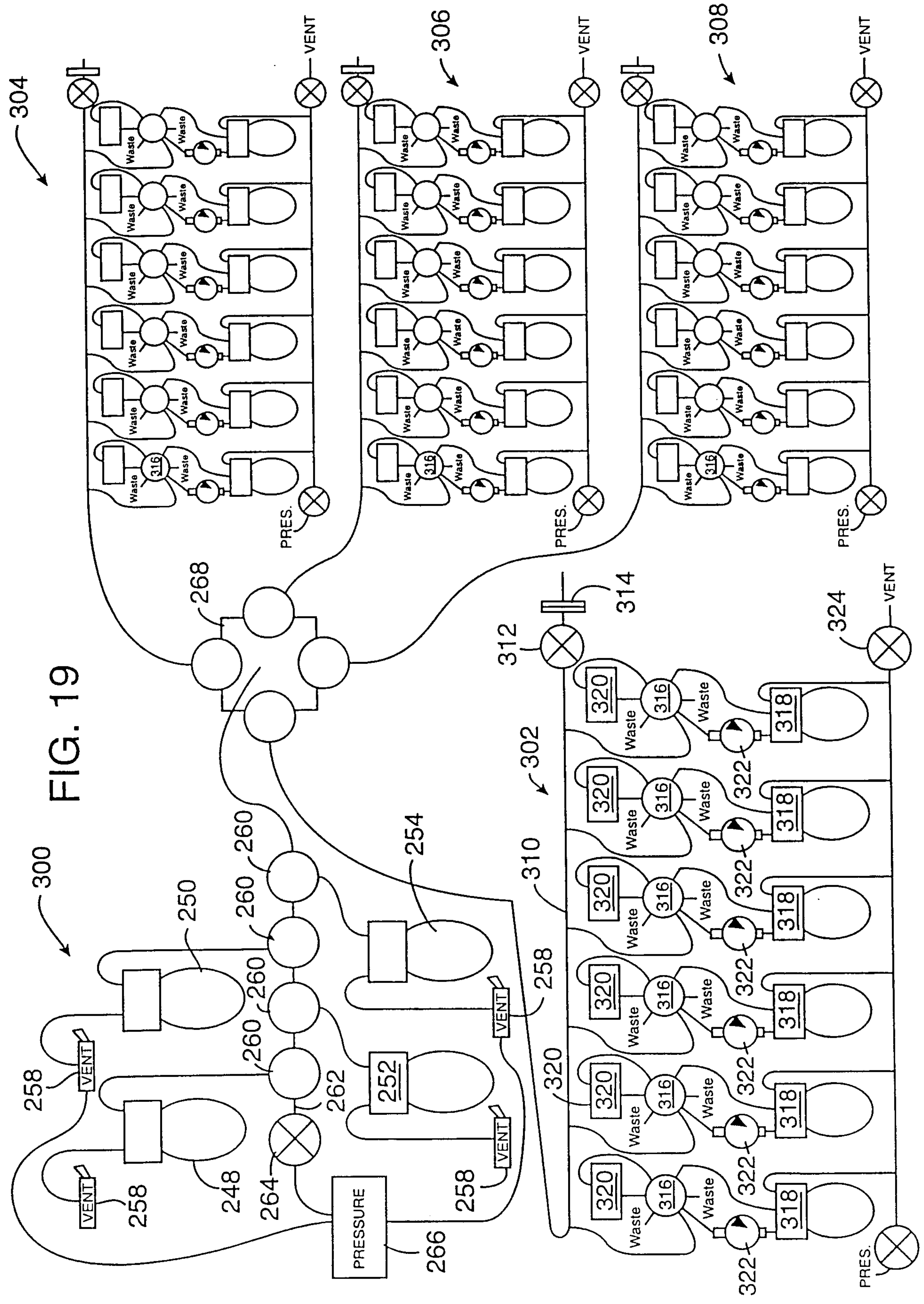
266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VENT
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PRESSURE
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322
324
Waste
VENT
PRES.

FIG. 19

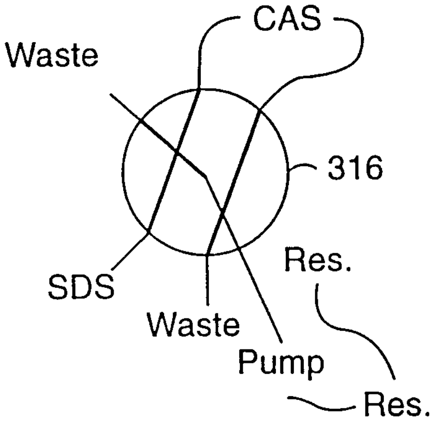


FIG. 20D

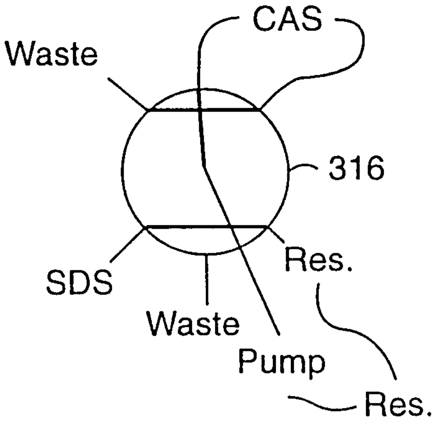


FIG. 20C

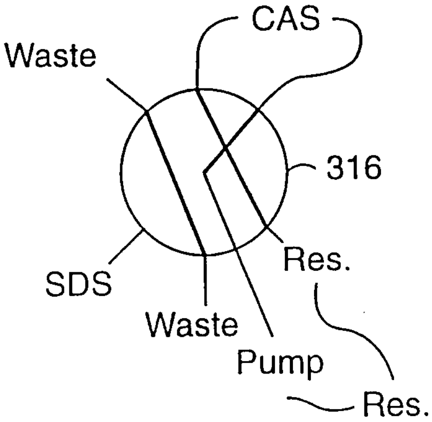


FIG. 20B

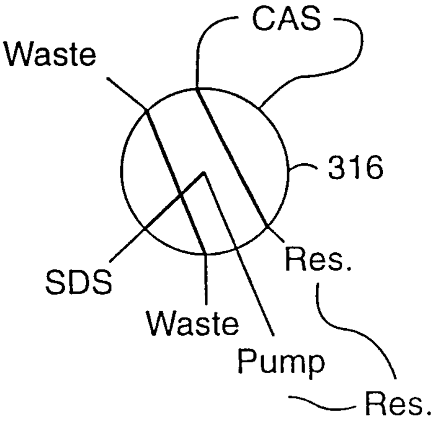


FIG. 20A

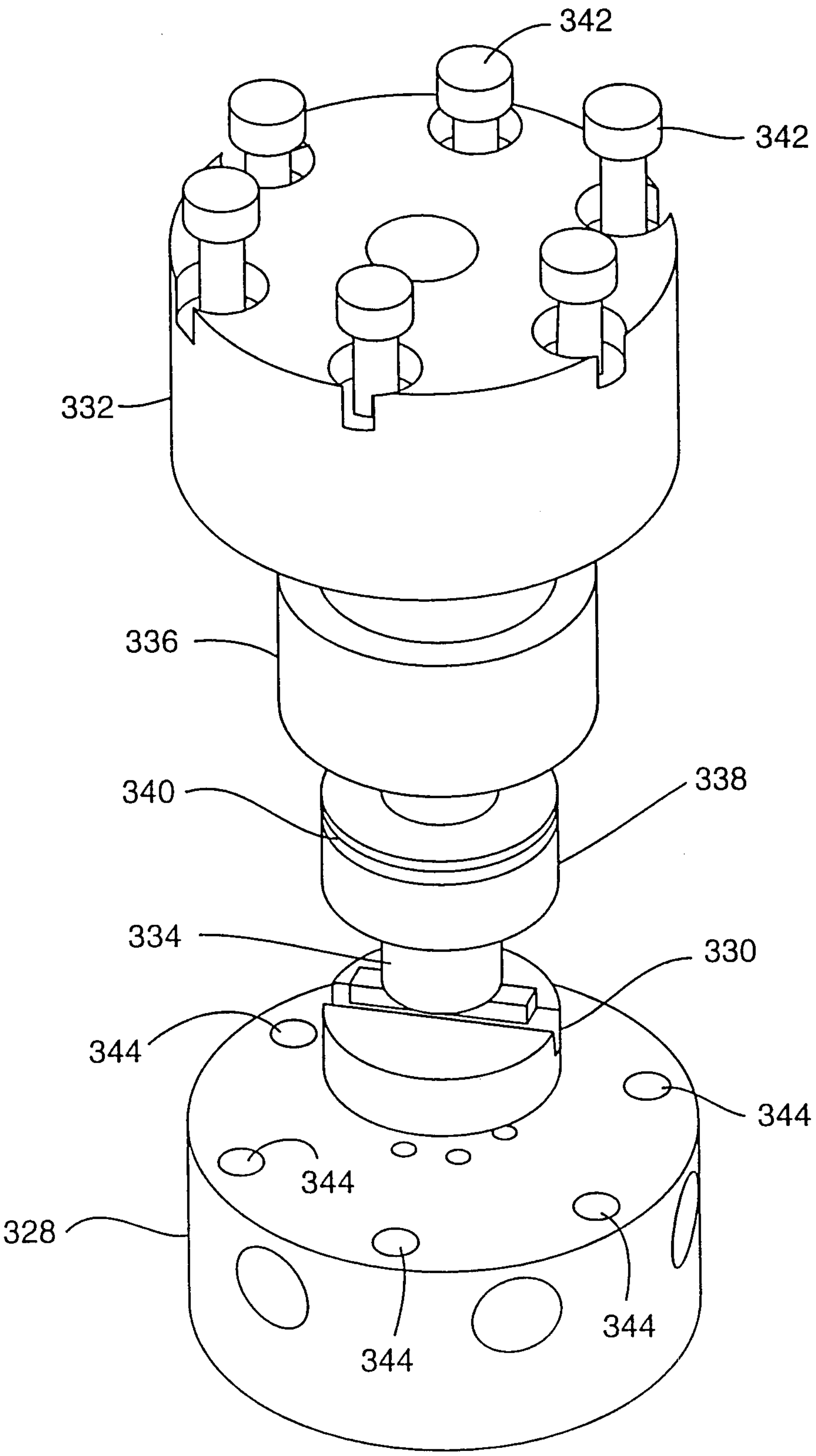


FIG. 21

FIG. 23A

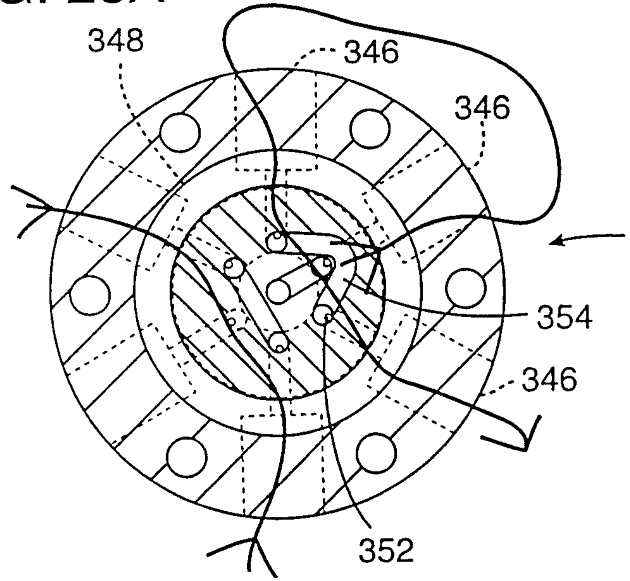


FIG. 23C

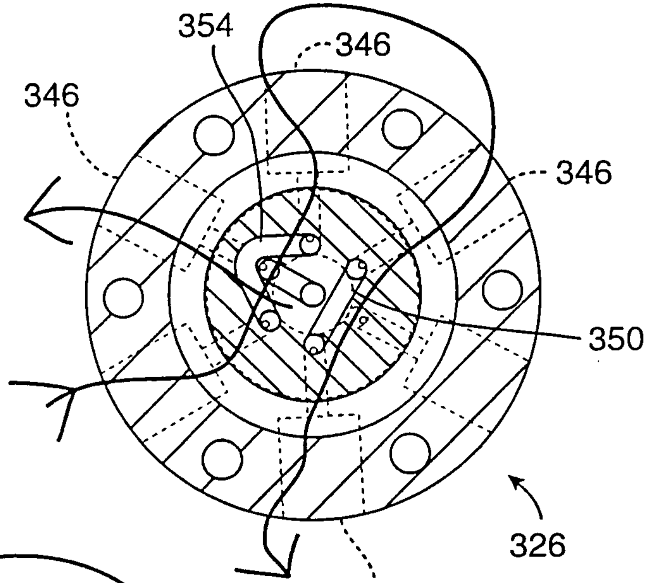
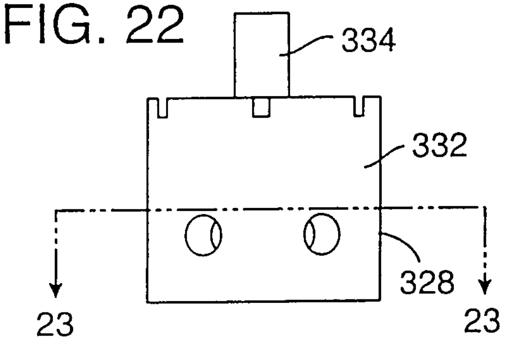


FIG. 22



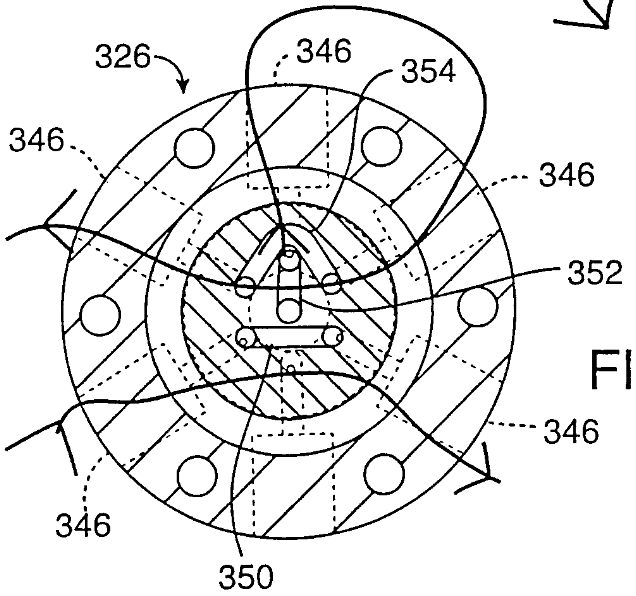


FIG. 23B

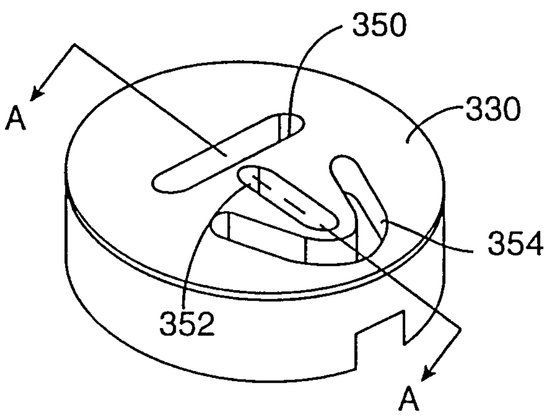


FIG. 24

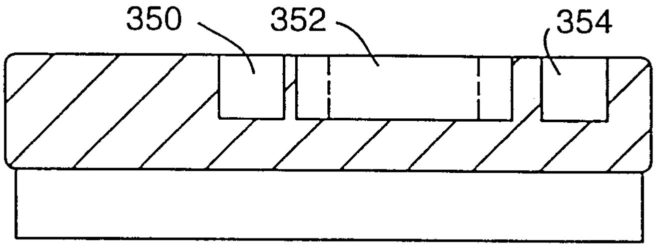
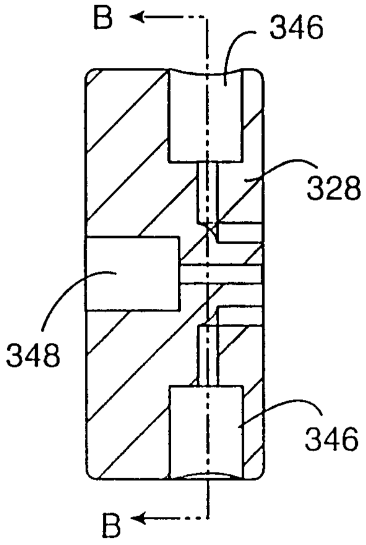


FIG. 24A

FIG. 25A



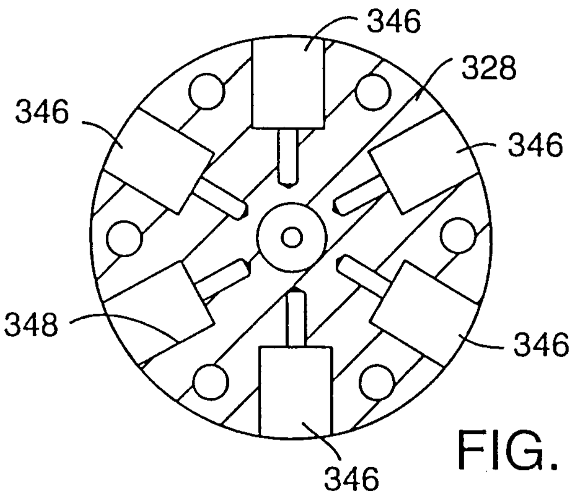


FIG. 25B

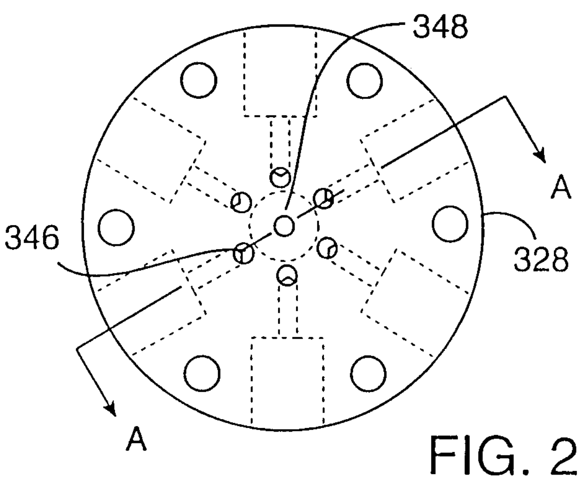


FIG. 25

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/33202

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL : 436/518, 528, 529, 531; 536/1.11, 23.1; 530/300+, 85, 86; 526/72
According to International Patent Classification (IPC) or to both national classification and IPC .

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/518, 528, 529, 531; 536/1.11, 23.1; 530/300+, 85, 86; 526/72

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EAST, CAPLUS, MEDLINE, BIOSIS, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US 5,624,711 A (SUNDBERG et al) 29 April 1997, see the entire document.	1, 4 ----- 2, 3
X ---- Y	ITSUNO et al. New Solid-Phase Catalysts for Asymmetric Synthesis: Cross: Linked Polymers Containing a Chiral Shiff Base-Zinc Complex. J. Org. Chem. 1990. Vol. 55. No. 1, pages 304-315, see entire document.	1, 2 ------ 3, 4
X	US 5,472,672 A (BRENNAN) 05 December 1995, see the entire document	1-4

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:
"A"	document defining the general state of the art which is not considered to be of particular relevance
"E"	earlier document published on or after the international filing date
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O"	document referring to an oral disclosure, use, exhibition or other means
"P"	document published prior to the international filing date but later than the priority date claimed
"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&"	document member of the same patent family

Date of the actual completion of the international search 05 FEBRUARY 2001	Date of mailing of the international search report 22 MAR 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer BARBA M. KOROMA TERRY J. DEY PARALEGAL SPECIALIST TECHNOLOGY CENTER 1600 Telephone No. (703) 308 0196

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

G01N 33/543, 33/544, 33/545; C07H 1/00, 21/02, 21/04; A61K 38/00; C08F 2/00