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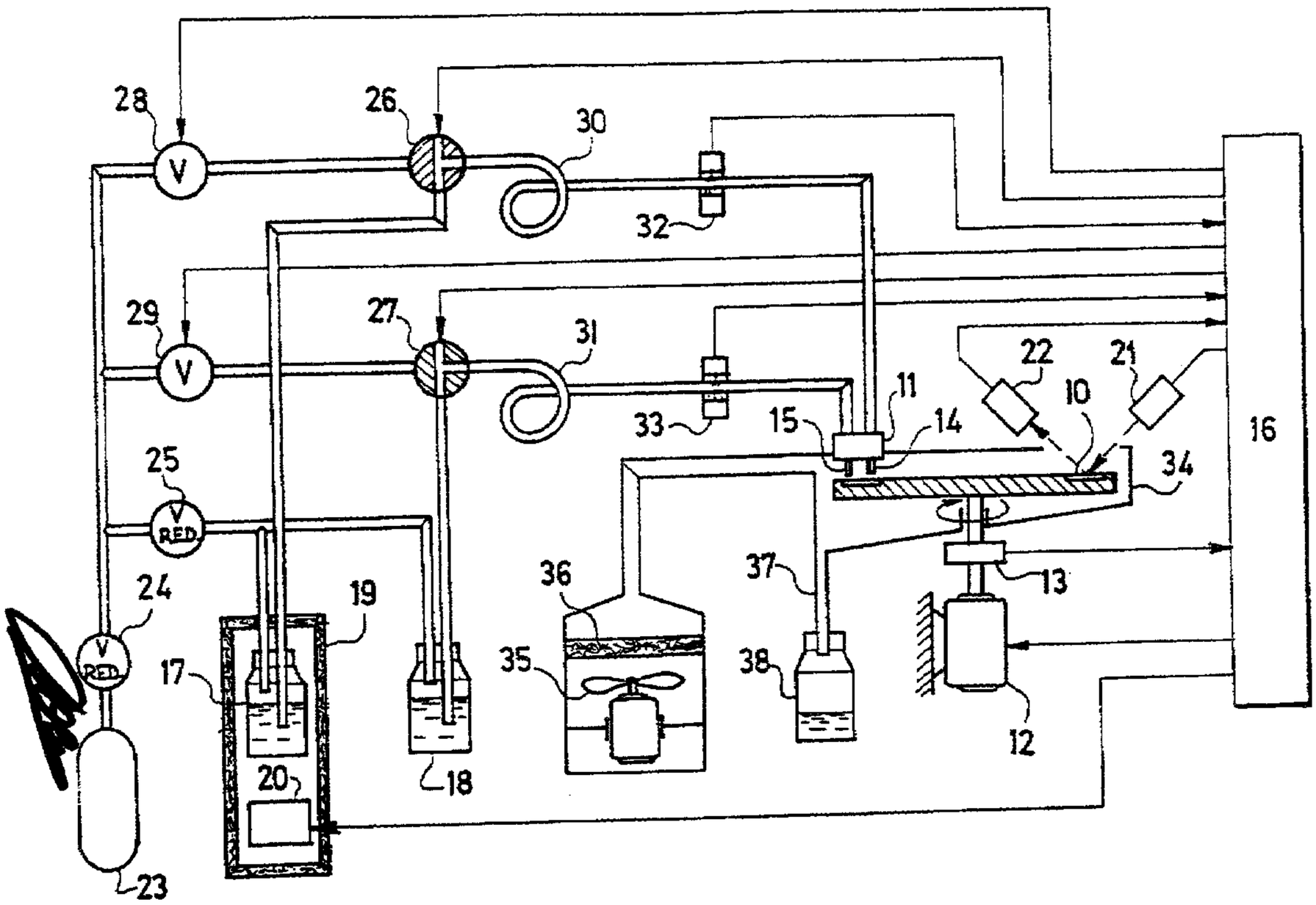
(54) Titre : METHODE POUR EFFECTUER UNE SYNTHESE MULTIPLE DE PEPTIDES SUR UN VECTEUR SOLIDE ET APPAREIL POUR EFFECTUER CETTE METHODE

(54) Title: METHOD OF PERFORMING A MULTIPLE SYNTHESIS OF PEPTIDES ON A SOLID CARRIER AND APPARATUS FOR PERFORMING THIS METHOD



(57) Abrégé/Abstract:
A method and an apparatus for performing a multiple synthesis of peptides on a solid carrier. Active components are successively bonded to functional groups anchored on a carrier, and as the carrier there is applied planar functionalized porous material divided into compartments, into which the needed activated component is put. Common operations of the synthesis are carried out by all compartments of the carrier at the same time.

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ABSTRACT OF THE DISCLOSURE:

A method and an apparatus for performing a multiple synthesis of peptides on a solid carrier. Active components are successively bonded to functional groups anchored on a carrier, and as the carrier there is applied planar functionalized porous material divided into compartments, into which the needed activated component is put. Common operations of the synthesis are carried out by all compartments of the carrier at the same time.

The invention relates to a method and device for carrying out a multiple synthesis on a solid carrier. The synthesis technology of peptides has been developed from classical methods supplied for a synthesis carried out in a solution /the survey is mentioned in Houben--Weyl Methoden der organischen Chemie, Synthese von reptiden, E. Wunsh ed., Thleme, Berlin 1974/, through the synthesis technique developed by Merrifield applying a solid carrier in the form of particles /as to the survey of the hitherto state, see e.g. Stewart J.M. and Young J.D. Solid Phase Peptide Synthesis, Freeman, San Francisco 1985/ which was suitable for automation /see e.g. Merrifield R.B., Stewart J.M. and Jernberg N., Apparatus for the automated synthesis of peptides, US 3,531,258; Brunfeldt K., Reopstorff P. and Halstrom J. Reactions System, US 3,577,077; Kubodera T., Hara T. and Makabe H. Apparatus for synthesis of peptides or the like organic compounds, US 3,647,390; Won Kil Park and Regoli D. system for the solid phase synthesis, US 3,715,190; Bridgham J. et al. Automated polypeptide synthesis apparatus, US 4,668,476; Saneii H.H., Solid phase synthetizer, US 4,746,490/, upto techniques suitable for a parallel synthesis of many peptides /Verlander N.S., Fuller W.D. and Goodman M.Rapid, large scale, automable high pressure peptide synthesis, US 4,192,798; Neimark J. and Briand J.P. Semi-automatic,

solid-phase peptide multi-synthesizer and process for the production of synthesis peptides by the use of multi-synthesizer, US 4,748,609; Houghten R.A. Means for sequential solid phase organic synthesis and methods using the same, EP 0196174; Geysen H.M., Meloen R.H. and Barteling S.J. Proc.Natl.Acad.Sci. USA 81, 3998, 1984; Frank R. and Doring R. Tetrahedron 44, 6031, 1988; Eichler J., Beyermann M., Bienert M. Collect. Czech. Chem. Commun. 54, 1746, 1989; Krchnák V., Vágner J. and Mach O., Int. J. Pept. Protein Res., 33, 209, 1989/. The application of planar continuous carriers made it possible to carry out the so called continuous synthesis of peptides /Lebl M., Gut V., Eichler J., Krchnák V., Vágner J. and Stepánek J. Method of a continuous peptide synthesis on a solid carrier, Czecho-slovak patent application PV 1280-89/.

The present development of the molecular biology requires the preparation of many peptides and their anchoring onto various carriers which enable their application in many immunological tests. Hitherto described methods for the multiple synthesis of peptides are not suitable for automation /Houghten R.A., Means for sequential solid phase organic synthesis and methods using the same, EP 0196174/, or they give only a limited quantity of yield, the quality of which cannot be verified in an analytical way /Geysen H.M., Meloen R.H. and Barteling S.J. Proc.Natl.Acad.Sci. USA 81, 3998,

1984/. Devices applying a carrier in the form of particles exhibit the drawback residing in the necessity to split off the peptide and its new anchoring for later applications. Another drawback of hitherto methods resides in a high consumption of solvents during the synthesis.

The above mentioned drawbacks are obviated by the method for carrying out a multiple synthesis of peptides on a solid carrier with a successive connectiong of active components onto functional groups anchored on a planar, functionalized, porous carrier and by the apparatus for performing this method according to the invention. The principle of the method resides in that individual activated components are put onto separated carriers, while common synthesis steps of corresponding components of various peptides proceed in all compartments of the carrier at the same time. According to the described method, all liquids and solutions of agents are sucked into the carrier and their removal is carried out by pressing the carrier with a dry porous material or by centrifuging the carrier. The apparatus is formed by a planar carrierdivided into individual compartments and by a frame situated parallelly to the carrier and comprising windows filled with inert porous material, the position of which on the frame corresponds with the position of compartments on the planar carrier, and positions of the carrier and frame are mutually adjustable.

Another variant of the apparatus consists of a planar carrier divided into individual compartments situated along the circumference of a revolvingly seated disk provided with means for connecting a driving device. Over the disk, in the spot into which individual compartments enter, a dosing head is situated. Over the disk head there is situated a source and detector of a light radiation for monitoring the course of condensation reactions of activated components.

An advantage of the invention resides in an automatic parallel performing of condensation reactions causing an increase of a peptidic chain in individual compartments comprising a planar carrier and in a simultaneous washing steps and steps resulting in removing temporary protective groups in all compartments with the planar carrier. An important advantage resides in monitoring the course of the chemical reaction and its computer evaluation, by which the synthesis is considerably shortened and made more effective. Another advantage reside in a considerable decrease of solvent consumption during the synthesis and in the possibility to utilize the peptide bonded on the carrier for further applications.

In enclosed drawings, there is illustrated in Fig. 1, viz, schematically, an apparatus with a linear shift for performing a multiple synthesis of peptides on a planar carrier, in Fig. 2, there is illustrated an embodiment based on a rotation principle, and, in Fig. 3,

there is a block diagram of the apparatus with the utilization of the rotary apparatus according to Figure 2.

The apparatus according to Fig. 1 is formed by a band 1 made of inert material e.g. polyamide or polypropylene on which there is situated a planar carrier divided in compartments 2. A frame 3 comprising windows 4 filled with inert material being able to carry, by means of capillary forces, an agent solution or pure solvent, is situated in such a way that these windows 4 may correspond with defined compartments 2 of the planar carrier. The apparatus is also provided with holding-down rollers 5, situated one opposite the other, on which a porous dry foil 6 is seated.

By pressing down the frame 3 to the carrier, a transfer of liquid from windows 4 to compartments 2 takes place. Material of individual compartments 2 has a higher affinity to transferred liquid and that is why the major part of the solution is transferred. Glass tissue and cotton seam to be a suitable combination of material of windows 4 and compartments 2. In this case, 80% of liquid was transferred /dimethylformamide/ from the window 4 into the compartment 2. The technology of liquid transfer from window 4 into the compartment 2 secures a simultaneous start of condensation reactions in all parts of the carrier. If it is not necessary to comply with this supposition, it is possible to

apply the solution ofthe activated component, as well as solutions used for washing and cleavage of protective groups by means of micropipettes driven by means of a stepping motor. The porousity of individual compartments 2 requires a uniform spreading of applied liquid. After having inserted a solution of the activated component, e.g. symmetrical anhydride amino of protected aminoacid, or respective active esters, eventually of a mixture of the protected aminoacid and activizing agent, advantageously comprising an agent monitoring the condensation source, e.g. bromophenol blue, then a connection of another amino acid into a peptidic chane takes place. The concentration of an active component must be such one that it may be included in the carrier in a sufficient surplus over the present free amino group. Due to the relative high absorption capacity of cotton /1,0 g of DMF for 1 g of cotton/ and relative low substitution applied for the synthesis /0,1 mol/g/ of the concentration 0,5 mol/l of activated component it supplies a sufficient surplus securing a quick course of the reaction. After the reaction has been finished, i.e. after the blue colouring of the carrier has disappeared in case of monitoring with bromophenol blue, liquid is removed from the carrier by passing the carrier together with a porous dry material 6 between rollers 5.

The rotary apparatus according to Fig. 2 and Fig. 3 is formed by a disk 8 made of inert material provided

on its circumference with compartments 10. Over the disk 8, in the spot in which individual compartments 10 enter, a dosing head 11 is situated. Over the level of the disk 8 there is also situated an optical device consisted of a source 21 of the light radiation and detector 27 of the reflected radiation. The disk 8 is seated on the same axle as the driving motor 12 and rotary incremented position pick-up 13. The disk 8 is situated in a tank 34 provided with an exhaust device 35 with a separator 36 and with waste piping 37 which is led out into a waste vessel 38. The dosing head 11 comprises outlets 14 of activated components and outlets 15 of washing solutions and solutions used for removing the protecting groups. Outlets 14 are connected by means of piping to reservoirs 17 of activated components situated in cooled boxes 19, the temperature of which is controlled with the controller 20. The outlets 15 to reservoirs 18 of washing solutions and solutions used for removing the protecting groups.

The dosing system is formed by a container 23 of compressed inert gas, first and second pressure reducing valve 24, 25, first and second two-way valve 28, 29, first and second three-way valve 26, 27, measuring loop 30 of activated components consisting of a transparent hose and sensor 32 of the activated component presence and by a measuring loop 31 of washing solutions and solutions applied for removing the protecting groups together with the sensor 13 of the solution presence. All controlled elements, such as the motor 12, valves

and the like, or pick-up elements are connected to a control computer 16.

Number of outlets 14, 15 of the dosing head 11 results from the number of activated components applied for the synthesis of peptides and from the number of washing solutions determined for removing the protecting groups. The dosing and transport of activated components and solutions is carried out by means of pressure of inert gas. For the process there are utilized two pressure levels controlled with pressure reducing valves 24, 25. The first pressure reducing valve controls pressure needed for transporting the measured quantity of liquids into the dosing head 11 and from it to the respective compartment 10, by means of the second pressure valve 25 one determines optimum velocity of transfer of the measured liquid in measuring loops 30, 31. The application of activated components and solutions may be carried out also with a higher number of dosing heads 11, situated over individual compartments 10 along the circumference of the disk 8. After having supplied the memory of the computer with parameters of the process, from which the most important is the number and sequence of bonded activated components, the synthesis may be started.

The motor 12 turns the disk 8 in such a way that successively into each compartment 10 with a functionalized carrier there may be sprayed, from the reservoir 17, by means of the dosing device, the respective activated

components. The measuring of the dose of the activated component is carried out in such a way that after having stabilized the position of the respective compartment 10 under the dosing head 11, then the activated component, after the liquid way has been opened between the reservoir 17 and the first measuring loop 30 by means of the three-way valve 26, is pressed out, due to pressure of the inert gas, through the transparent pipe for such a long time till the sensor 32 of the activated component presence is put in function. In this moment, the first three-way valve 26 is changed over in such a way that it interconnects the dosing loop 30 and the pressure gas inlet, and, after a needed delay, the first two-way valve 28 is opened, which, by means of inert gas pressure set up with the pressure reducing valve 24, pulls out the measured quantity of the activated component via the respective outlet 14 of the dosing head 11 from the measuring loop 30 onto the carrier. By a successive turning of the disk 8 under the dosing head 11, all needed hydraulic ways are activated in this way from reservoirs 17 of activated components, till all compartments 10 of the disk are attended. The motor 12 goes on turning slowly the disk 8, and one watches, by means of an optic device consisting of the source 21 and detector 22 of the light radiation, the course of the electrical reaction, in this case condensation, in individual compartments by comparing the colour of active compartments 10 with the reference compartment. For watching the

the course of the reaction with optical device, the solution of the activated component must be completed with a respective agent, e.g. bromophenol blue. In the moment when it is found out by means of the optical device that in all active compartments in the reaction proceeded well, the disk 8 is rotated to such revolutions that residuals of unbonded active components may be centrifuged. The centrifuging having been finished, the disk 8 is turned slowly again in order that it would be possible, by means of the hydraulic way through valves 25 and 27 and measuring loop 31 and sensor 33 of solution presence, to measure and then by means of valves 24, 29 and 27 to spray the defined quantity of the washing solution through the outlet 15 of the dosing head 11 onto all compartments 10 in an analogous way as it was described above at dosing active components. After centrifuging, this step may be repeated several times. Then, in the same way, the application of the solution used for removing the protecting groups, as well as the repeated centrifuging, take place. After several steps, when the washing solution is applied and then centrifuged, the synthesis may go to the next step in which the further component is bonded in the described way. The sequence of bonded activated components in individual compartments 10 of the disk 8 is determined in this way on the basis of the peptide sequence determined by the computer, and the synthesis velocity depends on the slowed condensation from all simultaneously proceeding condensations.

The interval for bonding individual activated components is limited and if e.g. in some compartment the bonding was not successful, the application of the same component is repeated in the next cycle, eventually the synthesis of this peptide does not continue in the following cycles.

Examples of synthesis which do not limit the mentioned technology but illustrate it only, are mentioned beneath.

Example 1

A cotton strip /3 x 27 cm/ was esterified with Fmoc-Gly at it was described in Czechoslovak Patent Application PV-1280-89 and then there was added to it the arm HO-CH2C6H4O/CH2/3COOH. The carrier modified in this way was separated in nine parts and three of them were situated on a glass pad. Into these parts of the carrier, in each of them, there were added 200 µl of a solution comprising Fmoc-Met /F-moc-Leu, F-moc-Nle/, diisopropylcarbodiimide, hydroxybenzotriazole /all 0,5 M/ and dimethyl amino pyridine /0,15 M/. The putting in was carried out in such a way that solutions were laid on at first into the square of the glass tissue /3 x 3 cm/ which was then pressed onto the cotton carrier and in this way the transfer of the liquid into the carrier was realized. After twelve hours, the parts of the carrier were washed with dimethyl formamide and dichloromethane. The following

solutions were added in a stepwise way using the above mentioned techniques into above mentioned parts in the quantity of 200 µl in the sequence:

1. dimethylformamide /3 x 1 min/
2. 20% of piperidine in dimethylformamide /1 x 2 min and 1 x 10 min/
3. dimethylformamide /5 x 1 min/
4. solution of Fmoc-amino acid, N-hydroxybenzotriazole and diisopropylcarbodiimide /all 0,5 M in dimethylformamide/ and bromophenol blue /0,5 mM in dimethylformamide/
5. dimethylformamide /3 x 1 min/

After the mentioned time of action, solutions were removed by pressing the carrier together with filtering paper and another portion of the solution was laid on. After the laying on of the solution 4, the carriers were getting blue, and the other step was carried out after the carrier had been decolored. In individual parts of the carrier, there were connected in a stepwise way the following derivatives: Fmoc-Phe, Fmoc-Gly-Gly and Fmoc-Tyr/But/. In this way three various peptidic sequences were obtained at the same time /Tyr-Gly-Gly--Phe-Met, Tyr-Gly-Gly-Phe-Leu, Tyr-Gly-Gly-Phe-Nle/. These peptides, after having been cleaved from the carrier /90% of trifluoroacetic acid, 5% dimethylsulfide, 5% thioanisole, 3 hours at room temperature/, were purified by means of HPLC and characterized in a standard way.

Example 2

A strip of polypropylene modified with a hydroxypropyl group /Milligen Bioresearch, USA/ was esterified in the same way as a cotton tissue, and a carrier was obtained of a substitution 0,1 mmol/g /determination by means of a cleavage of Fmoc group/. Then the synthesis was carried out in the same way as in example 1, only with the distinction that one put on less solutions /60 µl/ with respect to the lower specific weight of this carrier. The same peptides as in example 1 were prepared on this carrier.

Example 3

The synthesis of the above mentioned analogs of enkephalin was carried out on a cotton carrier as it was mentioned in example 1, only with the distinction that all solutions were laid onto the carrier by means of a micropipette. The quality of obtained products was identical with the peptide quality yielded in example 1.

Example 4

The synthesis of the above mentioned analogs of enkephalin was carried out on a cotton carrier as it was mentioned in example 1, only with the distinction that the compartmentized carrier was connected onto the disk circumference and all solutions were removed from the carrier by centrifuging. The quality of obtained products was identical with the quality of peptides yielded in example 1.

Example 5

Six square pieces of cotton (3 x 3 cm, modified by Fmoc-Gly, substitution 0.09 mmol/g) were placed on the perimeter of a planar rotor (diameter 25 cm) with six shallow compartments (3.2 x 4.5 x 0.2 cm). To the center of the cotton piece the solutions in the order given at the particular example were added. After given time the rotor was spinned for 30 seconds at 2500 r.p.m. and next solution was added.

Typical synthetic protocol for the attachment of one amino acid residue consists of the following steps:

Cleavage:

S.1) Addition of 20% piperidine in dimethylformamide (0.2 ml)

S.2) Waiting 10 min

S.3) Spinning

Washing:

W.1) Addition of dimethylformamide (0.4 ml)

W.2) Waiting 1 min

W.3) Spinning

Coupling:

C.1) Addition of 0.1% solution of bromophenol blue in dimethylformamide spiked with N-hydroxybenzotriazole (80µl)

C.2) Spinning

C.3) Addition of the solution of activated protected amino acid (0.4 ml)

C.4) Waiting until the blue color of the dot formed in step C.1 disappears (5-120 min)

C.5) Spinning

Example 6

Synthesis of Acyl Carrier Protein 65-74

In the first step of the synthesis performed according to the example 5 Fmoc-Gly-OCH2C6H4OCH2CH2CH2COOH was coupled to the cotton pieces in all six compartments. In the next steps the following amino acid derivatives were coupled to the modified carrier: Fmoc-Asn-OH, Fmoc-Ile-OH, Fmoc-Tyr(But)-OH, Fmoc-Asp(OBut)-OH, Fmoc-Ile-OH, Fmoc-Ala-OH, Fmoc-Ala-OH, Fmoc-Glu(OBut)-OH, Fmoc-Val-OH.

The protected amino acid (0.08 mmol) was dissolved in dimethylformamide (0.4 ml) together with N-hydroxybenzotriazole (0.08 mmol) and diisopropylcarbodiimide (0.08 mmol)

was added. After 2 minutes the solution was added to the carrier. In the synthesis, the following protocol was used:

Cleavage
Washing (3x)
Coupling
Washing (3x)

In the step S.1 (see Example 5) of cleavage, various concentrations of piperidine and cleavage times in particular cotton pieces were used:

Compartment 1 - 20% piperidine, 5 min
2 - 20% piperidine, 10 min
3 - 20% piperidine, 20 min
4 - 50% piperidine, 2 min
5 - 50% piperidine, 5 min
6 - 50% piperidine, 10 min

(Cleavage was started in different times so that it could be terminated in all compartments simultaneously by spinning.) At the end of the synthesis the compartments were washed by ethanol and dried. The peptides were cleaved by 50% trifluoroacetic acid, 2% anisole (1h at room temperature), solution was evaporated in vacuo, dissolved in 3M acetic acid and lyophilized. The crude material was analyzed by HPLC (Vydac C18, 25 x 0.4 cm, gradient 20-100% methanol in 0.05% trifluoroacetic acid in 40 min). The quality of peptides synthesized in compartments 4-6 were slightly worse than that from compartments 1-3. The optimal result was obtained from compartment 1. The product was characterized by amino acid analysis (Asp 2.05, Glu 1.04, Gly 1.14, Ala 2.03, Val 0.91, Ile 1.97, Tyr 0.85) and FAB Mass spectroscopy (M+H+ = 1064; theory 1064).

Example 7

Synthesis of [Ser5,15]MCH

In the first step of the synthesis performed according to example 5 N-Fmoc-4-methoxy-4'-(3-carboxypropyloxy)-benzyhydrylamine was coupled to the cotton pieces in all six compartments. In the next steps, the following amino acid derivatives were coupled to the modified carrier: Fmoc-Asp(OBut)-OH, Fmoc-Thr(But)-OH, Fmoc-Met-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Ser(But)-OH, Fmoc-Met-OH, Fmoc-Val-OH, Fmoc-Gly-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Val-OH, Fmoc-Tyr(But)-OH, Fmoc-Arg(Mtr)-OH,

Fmoc-Pro-OH, Fmoc-Ser(But)-OH, Fmoc-Trp-OH, Fmoc-Glu(OBut)-OH, Fmoc-Val-OH.

The synthesis was performed in the same way as in example 6 with the exception of the step S.1 where different bases were used for the cleavage of the Fmoc protecting group.

Compartment 1 - 20% piperidine, 10 min
2 - 2M 4-benzylpiperidine, 10 min
3 - 0.05M 4-piperidinopiperidine, 10 min
4 - 0.5M 4-(aminomethyl)piperidine, 10 min
5 - 0.5M tris(2-aminoethyl)amine, 10 min
6 - 1M 1-(2-aminoethyl)piperazine, 10 min

The finished peptides were cleaved and analyzed in the same manner as in the example 6. The peptides from compartments 1 and 2 were indistinguishable, other bases afforded the product of inferior quality. Amino acid analysis: Asp 1.09, Thr 1.00, Ser 1.94, Glu 1.10, Pro 1.06, Val 3.25, Met 1.78, Tyr 0.91, Arg 2.85, FAB mass spectrum: 2069.

Example 8

Synthesis of Acyl Carrier Protein 65-74

The synthesis was performed in the same way as in example 6. The base used for the cleavage was 20% piperidine in dimethylformamide. In particular cotton pieces the different protocol (number of washing) was applied:

Compartment 1 - Cleavage, Washing (1x), Coupling, Washing (1x)
2 - Cleavage, Washing (2x), Coupling, Washing (2x)
3 - Cleavage, Washing (4x), Coupling, Washing (4x)
4 - The same protocol as in compartment 3, but the modification of the cotton was performed by periodate oxidation and hexamethylenediamine treatment
5 - Cleavage, Washing (1x), Coupling, Washing (1x)
6 - Cleavage, Washing (4x), Coupling, Washing (4x)

In the compartments 5 and 6 the solution of protected amino acid (0.08 mmol) and HOBt (0.08 mmol) in 0.2 ml dimethylformamide was added to the carrier separately from the 0.4 M solution of diisopropylcarbodiimide in dimethylformamide (0.2 ml).

After the cleavage and analysis performed in the same way as in example 6 all peptides were found indistinguishable.

Example 9

Synthesis of model peptides

In the first step of the synthesis acid-labile amide linker (N-Fmoc-4-methoxy-4'-(3-carboxypropyloxy)-benzhydrylamine) was coupled to the cotton squares in compartments 1 to 5. The synthesis was performed in the same manner as in the example 6, but the different sequence was assembled in all compartments:
Compartment 1: Ala-Val-Leu-Gly-His-Asp-Glu-Ala-Ala-Tyr-Ser-Lys-Asn-Arg-Arg-Ala-Val

2: Asp-Thr-Met-Arg-Ser-Met-Val-Gly-Arg-Val-Tyr-Arg-Pro-Ser-Trp-Glu-Val

3: Tyr-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Val

4: Tyr-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-D-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Val

5:Tyr-Ala-Ala-Ala-Ala-Ala-Ala-D-Ala-Ala-Ala-Ala-Ala-Ala-Ala-D-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Val

6: Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Ala-Val-Gly

Peptides from the cotton carrier in compartments 1 to 5 were cleaved by trifluoroacetic acid - phenol - water - thioanisole -ethanedithiol (82.5:5:5:5:2.5) mixture (1h, r.t.) and worked up and characterized in the way described in example 6. Cotton from compartment 6 was treated with 1M NaOH for 1 h, washed and extracted by trifluoroacetic acid. This extract was worked up in the usual way. All products were found more than 80% pure by HPLC. They had correct amino acid analysis and FAB mass spectrum.

Example 10

Polystyrene resin (153 mg, 1% divinylbenzene, 300-400 mesh) was placed in the "tea bag" according to EP 0196174 (Houghten R. A.) and dimethylformamide was soaken into it. The cotton piece 3 x 3 cm (160 mg) was soaken with dimethylformamide too. The content of solvent in the carrier was determined by weighing. Both carriers were centrifuged (2000 r.p.m., 2 min) and the content of solvent was determined again. Results of the experiment, together with the attempt to eliminate the liquid from the cotton by its compression together with the dry filtration paper are given in the table 1.

Table 1

Solvent content in carriers after different treatment

		DMF content after					
Material	Dry weight (mg)	Soaking		Compression		Centrifugation	
		mg	%	mg	%	mg	%
Cotton	160	182	114	38	24	10	6.2
Polystyrene	153	268	175	*		101	66

*Not determined

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A method for performing a multiple synthesis of peptides, comprising the steps of:

providing a solid planar carrier divided into a plurality of individual compartments, each compartment containing an inert porous material, a path being defined on said carrier, said compartments being spaced along said path, a functional group of an amino acid residue being anchored onto the inert porous material of each compartment to form a plurality of individual functionalized compartments;

arranging a dosing head at a fixed location adjacent said path, the dosing head including means for directly dispensing measured quantities of at least one liquid component from a common reservoir of said component;

positioning the carrier so that one of the individual functionalized compartments is positioned to receive a liquid component directly dispensed by the dosing head;

directly dispensing a measured quantity of the liquid component to said individual functionalized compartment from the common reservoir thereof, via the dosing head, the liquid component dispensed thereto providing an amino acid to form a covalent bond with the functional group of the amino acid residue in said individual functionalized compartment positioned to receive the liquid component for peptide synthesis in said individual functionalized compartment receiving the measured quantity;

moving the carrier to position another individual functionalized compartment along the path to receive the liquid component applied by the dosing head; and

subsequently dispensing at least one additional measured quantity of one of said liquid component providing an amino acid to said other individual functionalized compartment in at least one other step for peptide synthesis.

2. The method as in claim 1, wherein all liquid components are absorbed into the carrier and removal of said liquid components is carried out by pressing the carrier together with dry porous material or by centrifuging the carrier.

3. An apparatus for performing the method as in claim 1, comprising:
a solid planar carrier divided into a plurality of individual compartments, each compartment containing an inert porous material, a path being defined on said carrier, said compartments being spaced along said path, a functional group for peptide synthesis being anchored onto the inert porous material of each compartment to form a plurality of individual functionalized compartments;
at least one reservoir containing an activated agent for peptide synthesis and a wash reservoir containing a washing solution;
a dosing head arranged at a fixed location adjacent said path, the dosing head including means for selectively drawing and dispensing said activated agent from said at least one reservoir or said washing solution from said wash reservoir; and
means for positioning the carrier relative to the dosing head so that selected ones of the individual compartments are positioned successively to selectively receive a said activated agent or washing solution directly dispensed by the dosing head.

4. An apparatus for performing the method as in claim 1, comprising:
a planar carrier on a disk, said carrier divided into a plurality of individual compartments, each compartment containing an inert porous material, a circular path being defined around said disk, said compartments being circumferentially spaced along said circular path, a functional group for peptide synthesis being anchored onto the inert porous material of each compartment to form a plurality of individual functionalized compartments;

at least one reservoir containing an activated agent for peptide synthesis and a wash reservoir containing washing solution;

a dosing head arranged at a fixed location adjacent said circular path, the dosing head including means for selectively drawing and dispensing said activated agent from said at least one reservoir or said washing solution from said wash reservoir; and

means for rotating the disk so that selected ones of the individual compartments are positioned to selectively receive said activated agent or washing solution directly by the dosing head.

5. The apparatus according to claims 3 and 4, wherein the carrier is made of cotton or functionalized polypropylene.

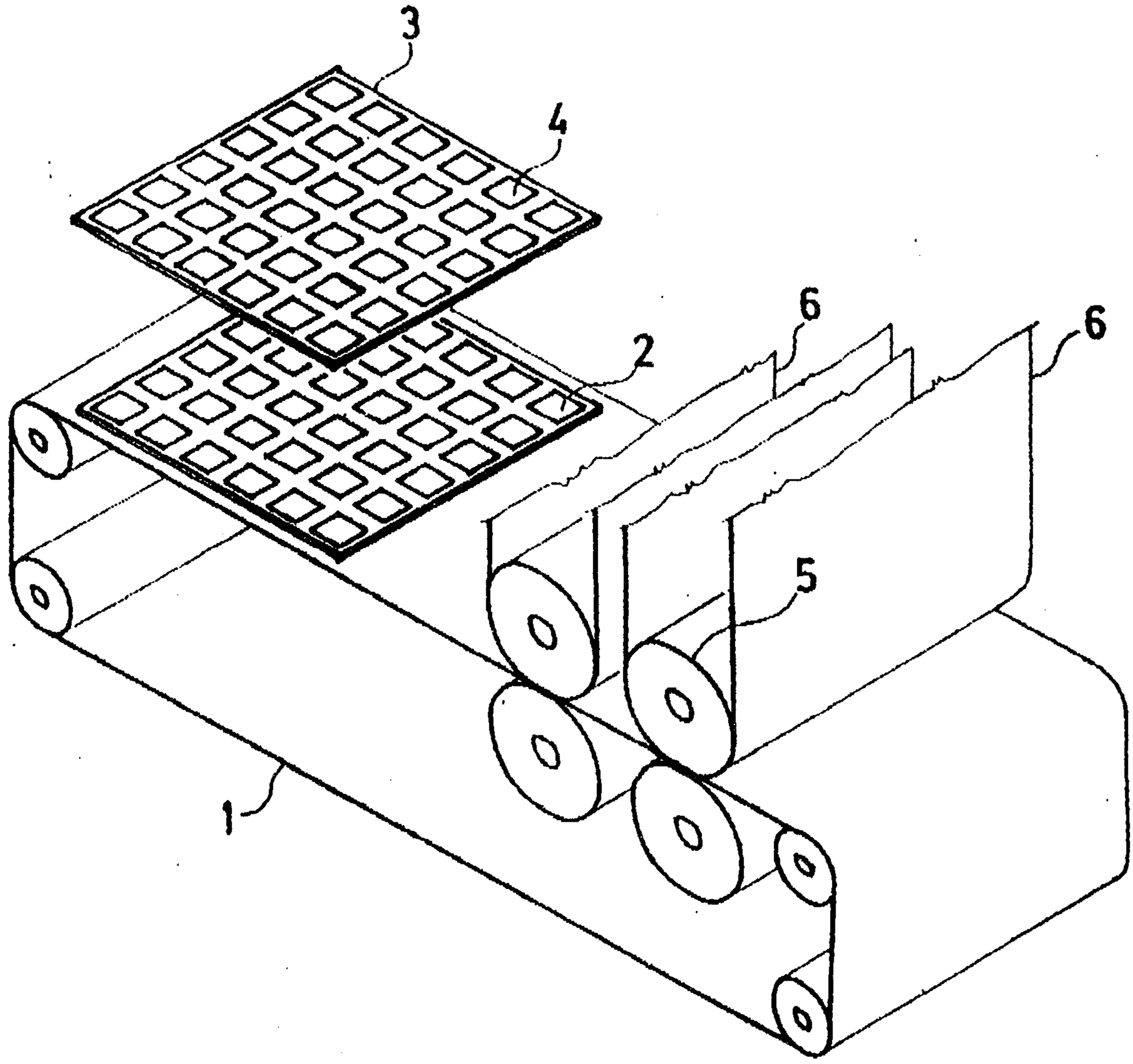

Fig. 1

Patent Agents

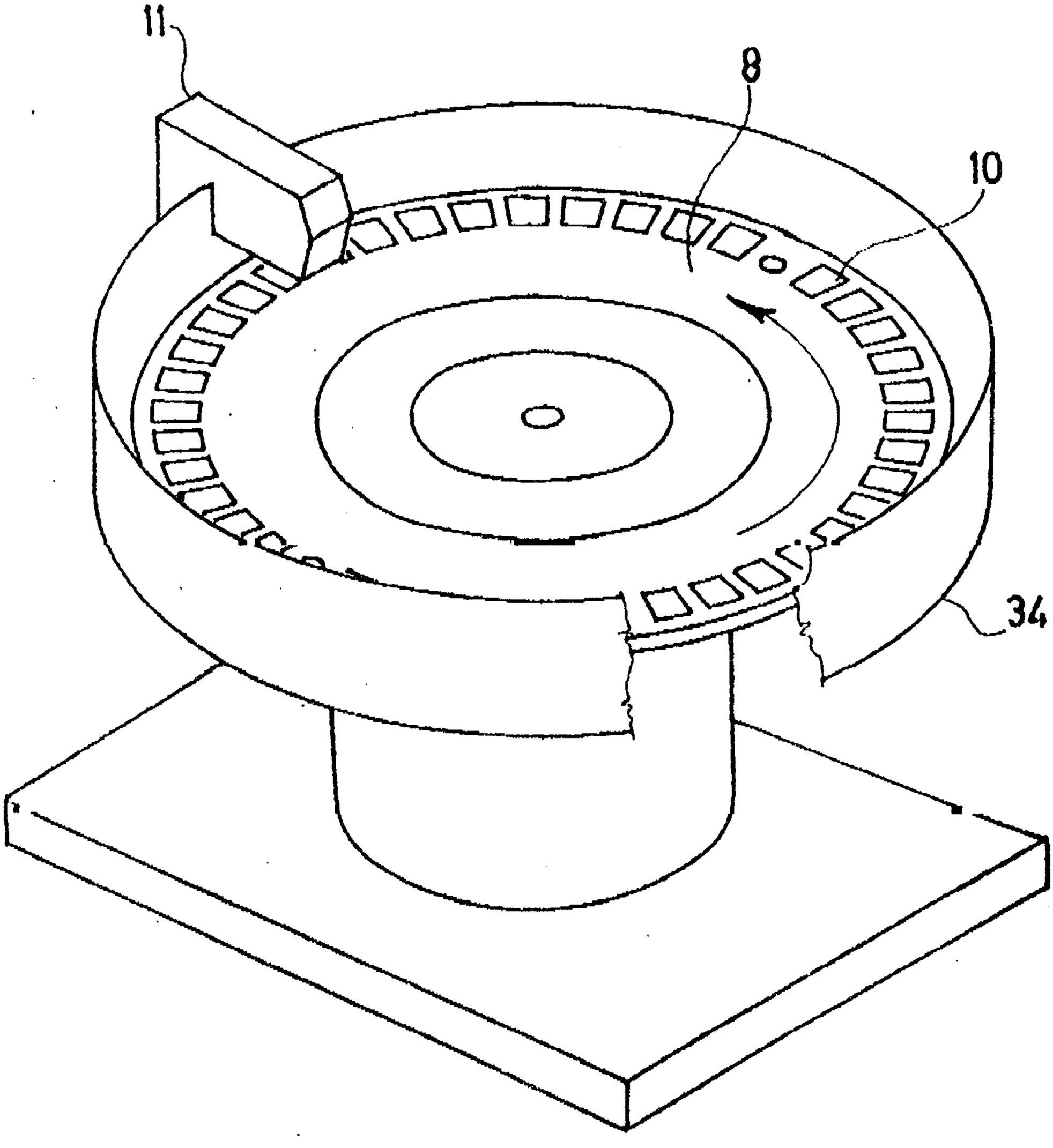

Fig. 2

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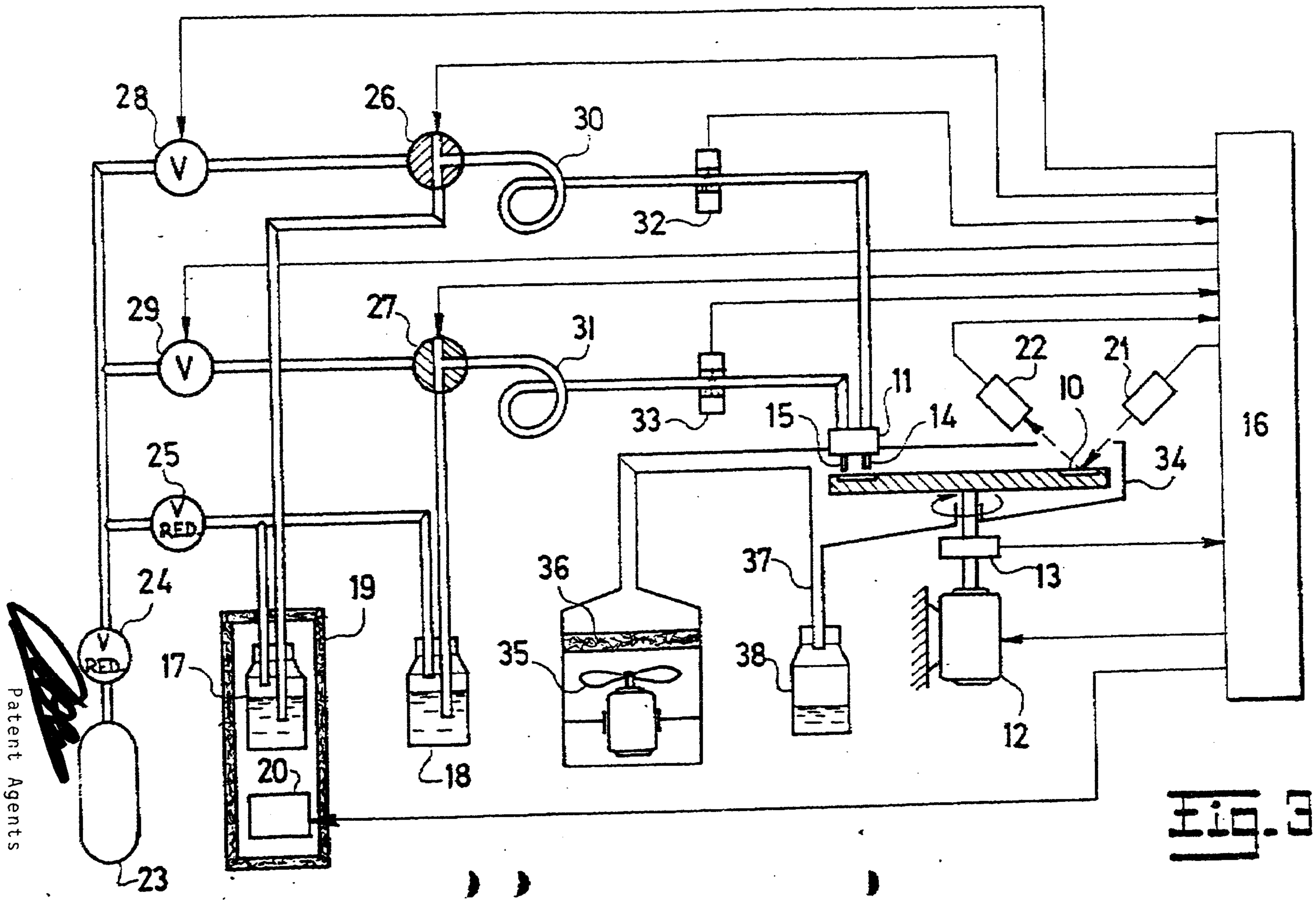

Fig. 3

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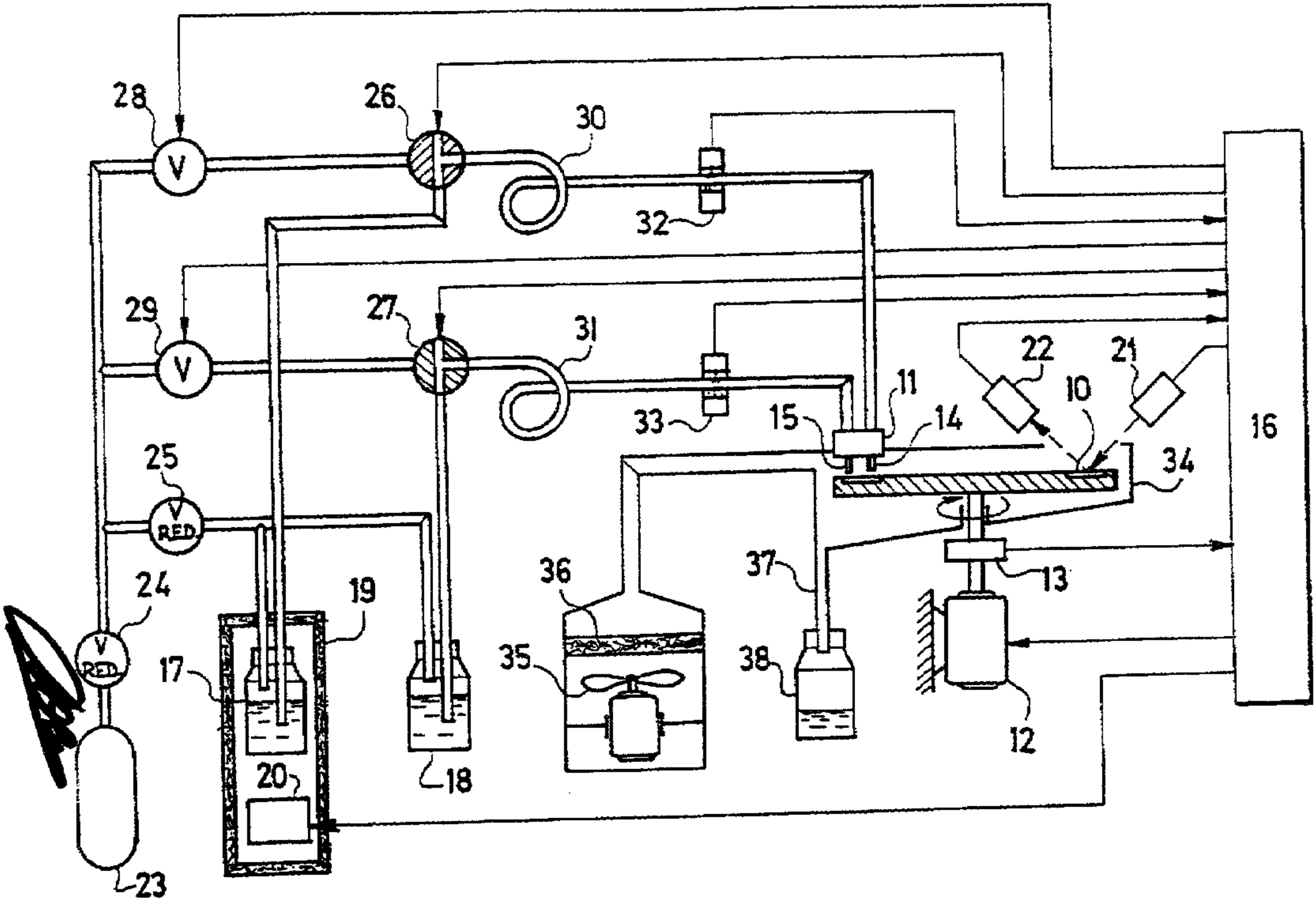
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