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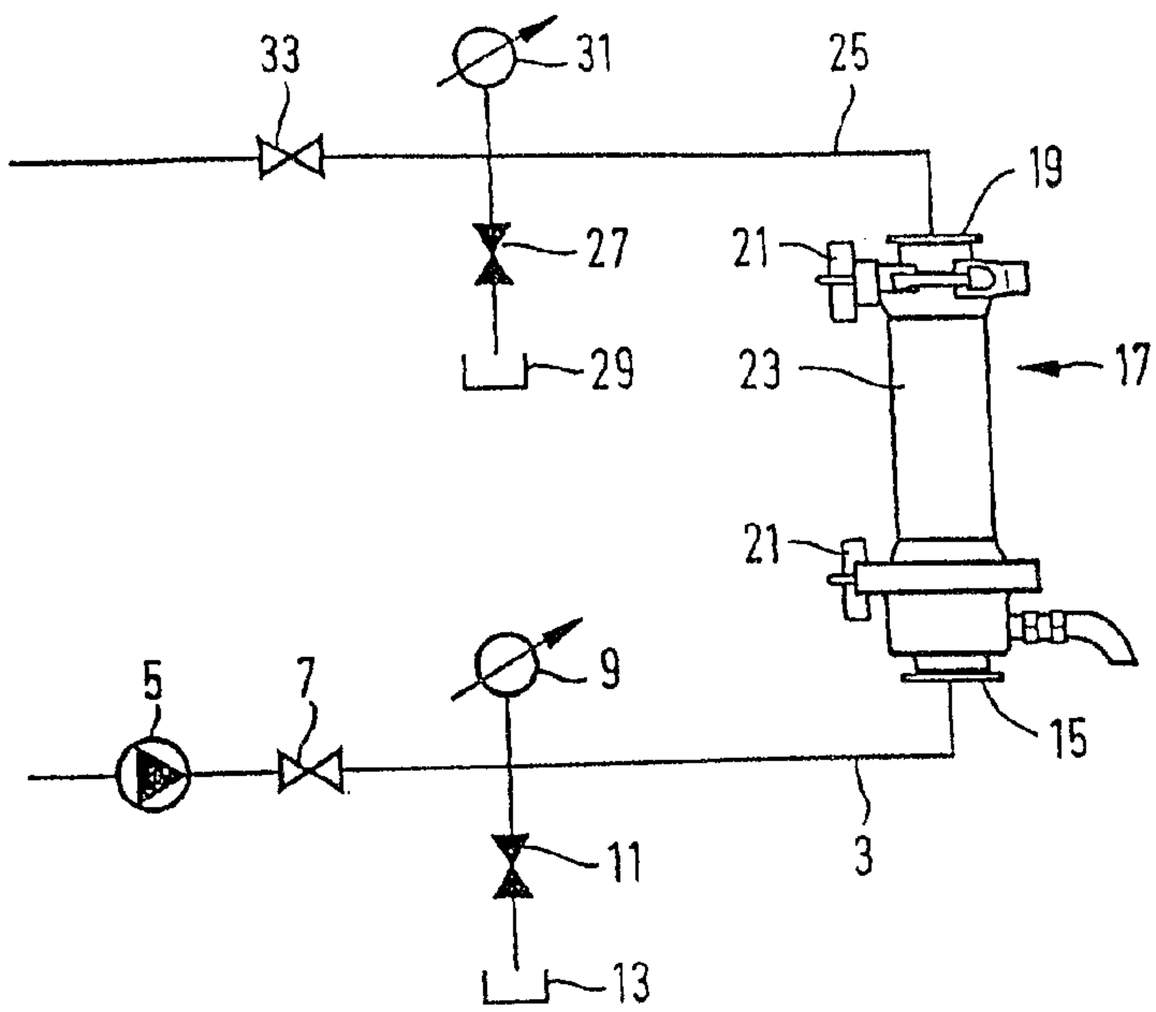
(54) **PROCESSUS PERMETTANT DE PROVOQUER LA DEPLETION DE VIRUS DANS DES SOLUTIONS ET DE DETERMINER LE TAUX DE DEPLETION DES VIRUS**

(54) **PROCESS FOR DEPLETING VIRUSES IN SOLUTIONS AND FOR DETERMINING THE DEPLETION RATE OF THE VIRUSES**



(57) In order to remove viruses from organic material, the material to be purified is passed through a filter or a filtration unit, the removal rate of which has previously been determined, in such a way that the filtration unit is inoculated with viruses of the leviviridae family. The virus titer is determined before and after filtration which establishes the removal rate. By following up on the removal of a marker substance the virus removal can be controlled in the ongoing process.

Abstract

In order to remove viruses from organic material, the material to be purified is passed through a filter or a filtration unit, the removal rate of which has previously been determined, in such a way that the filtration unit is inoculated with viruses of the *leviviridae* family. The virus titer is determined before and after filtration which establishes the removal rate. By following up on the removal of a marker substance the virus removal can be controlled in the ongoing process.

Disclosure

The invention relates to a method to remove viruses and to determine the removal rate of viruses in organic material.

Pharmaceuticals produced from cell cultures, organs or blood of animal or human origin are potentially contaminated with animal or human pathogenic viruses. Considering the broad spectrum of viruses that are likely to occur in any given specimen, it is obviously impossible to test the source material for all existing viruses. Also, there is no method ac-curate and sensitive enough to identify all virus groups with absolute certainty. For this reason it is essential that a purification or inactivation process be employed that will reduce the number of pathogenic viruses present, thus making sure that, even when source materials or intermediate products are massively infected, no problems will arise. This purification or removal process has to be so effective that virus concentrations are reduced by a factor of up to 10^{12}.

In order to verify elimination or removal rates, samples of material have to be inoculated with viruses in extremely high concentrations (spiking) and the virus titers have to be checked. Since viruses differ considerably in their physicochemical behaviour, the material to be used in the production process has to be inoculated with a spectrum of at least 4 different virus groups, a costly and time-consuming procedure, as viruses of a potentially pathogenic nature for humans have to be employed as well. Descriptions of the complicated procedures involved in the preparation of coagulation factors from human serum have been published by Heimburger, Schwinn, Gratz, Lüben, Kumpe and Herchenhahn, Faktor VIII-Konzentrat, hochgereinigt und in Lösung erhitzt, Arzneimittelforschung 31 (1981), 612-622; Mauler and Hilfenhaus: Inaktivierung von Viren in Faktor VIII-Konzentrat

durch Erhitzen in Lösung, Arzneimittelforschung 34 (1984), 1524-1527; Hilfenhaus, Mauler, Friis and Bauer: Safety of human blood products; inactivation of retroviruses by heat treatment at 60°C, Proc. Soc. Exp. Biol. Med., 178 (1985), 580-584.

In the production of pharmaceuticals it has been a long- time standard procedure to remove bacteria by practising sterile filtration, and this is considered to be a safe decontamination procedure for these types of potential pathogens. Thereby the sterile filters are randomly inoculated by the manufacturer with *Pseudomonas diminuta*, the minutest bacterium known outside the groups of mycoplasms and L-form bacteria. If the "bacteria challenge test" shows evidence of a pre-set amount of removal for this bacterium, the manufactured lot is considered to be safe. This type of procedure is described by Wallhäußer, Practice of Sterilization, Thieme Verlag, Stuttgart, 1988, Seiten 324 ff.

For the removal of viruses, filtration procedures are also useful methods; for that purpose the filter pores have to be so small that they successfully prevent the passage of molecules and particles of more than 1 million daltons. These ultra-filters are available in various forms. In contrast to sterile filters, however, they do not form an absolute barrier, i.e. molecules and particles bigger than 1 million daltons are not completely removed but only retained to a very high degree. The retention rate does not only depend on the type of filter but can vary from batch to batch. This is the reason why ultra-filters have not been used so far in the removal of viruses but at most contributed to the overall elimination of viruses in a multi-step production process (Werner and Langlius-Gane, Meeting the Regulatory Requirements for Pharmaceutical Production of Recombinant DNA

Derived Products, Arzneimittel-Forschung, vol. 39 (1989), 108-111). The unreliability of ultra-filters is caused by the production process. In ultra-filters designed for a specific molecular cut-off, always wider pores may develop permitting, for instance, viruses to pass through. Another problem is that ultra-filters cannot be tested for their density by the so-called bubble-point method like microfilters can.

It was therefore object of the invention to provide a method for removing viruses that would attain a removal rate of at least 10^{12}. A further object of the invention was to provide a method according to which the removal rate of viruses can be determined simply and precisely and which method gives information on the question by which filtration process and with how many filtration steps a removal rate can be obtained that would be considered safe.

This object is achieved by a method for removing viruses in solutions which is characterized in that the solution to be purified is passed through a filter or filtration unit, the removal rate of which has previously been determined, in such a way that a solution containing viruses of the *leviviridae* family is passed through the filter and that before and after the filtration the virus titer is determined and therefrom the removal rate is derived. According to the invention also other bacteriophages of equivalent size are equally useful. These bacteriophages can best be detected through simple plaques that have developed on a lawn of bacteria.

According to the invention it is also possible to use only the ultra-filtration method to guarantee a safe virus removal, without having to take any additional purification or inactivation measures, by consistently checking the removal rate of the viruses in the ongoing process. If this

removal rate of the production batch is determined before and after filtration and the difference is found to be higher than 10^{12}, a virus contamination of that product can be ruled out with great certainty. Up to the present, validating tests were only performed with animal or human pathogenic viruses, which meant that for safety reasons the validated filters had to be discarded and replaced by new filters with possibly different removal rates. Such a validation could consequently apply to only one single filter and, because of the complicated nature of the technique, only be done once before or after the removal for demonstration purposes. In contrast thereto, according to the invention, it is possible to observe and keep track of the removal of viruses during the ongoing process, i.e. to control in process.

Based on these findings the solution is passed through a filter or a filtration unit, the removal rate of which has previously been determined. By using viruses of the *leviviridae* group as test viruses, it is possible to determine the removal rate safely and conveniently.

Leviviridae viruses, measuring 23 nm in diameter and having a molecular weight of 1.4 million daltons, are smaller than animal or human pathogenic viruses (H. Fraenkel-Conrat, The Viruses, Catalogue, Characterization and Classification, Plenum Press, 1982). They only infect specific F^+-strains of the harmless intestinal bacterium *Escherichia coli* and can, being RNA viruses, already be hydrolyzed in 10 mM NaOH within a short period of time leading to a breakdown into their molecular constituents. The smallest animal and/or human pathogenic viruses belong to the *picorna* virus group, having a diameter of 27 nm and weighing 2.5 million daltons. The *leviviridae* viruses are therefore well suited for the

validation of size-selective ultra-filters. Subsequent rinsing of the filters with 0.1 M NaOH also ensures the removal of pyro-gens generated by *Escherichia coli*. After that, the filters are ready for re-use in the production process. All conditions required for an in-process control have thus been fulfilled, namely by:

- a simple and accurate validation procedure feasible within a short period of time;
- a re-usability of the tested filters without any additional risk of contamination of the product.

Another advantage is that *leviviridae* can be grown to extremely high titers of up to 10^{14} pfu/ml and are reliably isolated in a simple plate method even in concentrations of 1 pfu/ml.

For the determination the filter is inoculated with a virus solution or suspension having a titer of more than 10^{10} pfu/ml. The concentration of the phages in the filtrate and in the retained suspension is determined, which can be done according to a generally known technique (such as the top-agar method, for example, developed by N.H. Adams (1959), Bacteriophages, Interscience Publishers, New York). The phages are mixed with suitable host bacteria (such as E. *coli* 3300, ATCC No. 19853) and applied in a layer of 0.6% agar-agar over the surface of a nutrient medium (such as 1% bactotrypton, 0.5% yeast extract, 0.5% NaCl, 0.1 mM $CaCl_2$, 1.5% agar-agar). 10^7 to 10^8 bacteria and less than 100 phages should be applied onto every plate. The plates are incubated at 37°C developing a lawn of bacteria after 10 hours. Plaques on the lawn will indicate the presence of viruses and the number of plaques will indicate the virus titer in pfu (plaque-forming units). Since one single virus can bring forth one plaque, it is

possible to detect 1 virus/ml on a standard agar plate. In this way a virus concentration range of more than 10^{14} up to 1 pfu/ml can be covered.

By determining the virus concentration in the filtrate and in the suspension prior to filtration, the virus removal rate will become apparent. By determining the virus titer in the concentrate (i.e. in the suspension retained by the filter) it can be figured out whether viruses were lost to the filter through absorption or through inactivation, which adds an even higher degree of safety to the validating process.

Since the elimination behaviour of filtration membranes may differ not only from manufacturer to manufacturer but also considerably from one production batch to another, it is essential to determine the virus elimination rate for each individual filter.

Preferably the filter or the filtration unit chosen for the purification of the organic material should be examined under precisely defined pressure conditions that have to be strictly adhered to later on in the purification process.

After having determined the elimination rate, the filters can simply be rinsed with caustic soda to remove bacteriophages and other residues such as pyrogens, and can subsequently be used for the purification of the organic materials. This is another advantage of the method of the invention, since this would not be possible when animal or human pathogenic viruses are used to determine the elimination rate due to the great danger of contamination with said viruses.

From the group of the *leviviridae*, the viruses MS2, f2, f4, Qß, Vk, ST, R17 or equivalent strains (described by H. Fraenkel, Conrat, The Viruses-Catalogue, Characterization and

Classification, Plenum Press, New York, 1982) have proven to be the best choice. Particularly recommended is the bacteriophage fr as a test virus, filed at ATCC under No. 15767-B1 and described in Knolle and Hoffmann-Berling, Virology, vol. 123, 271-273 (1964). This phage consists of a round protein-RNA -complex in form of a polyhedron measuring 23 nm in diameter and weighing 1.4 million daltons.

In a preferred embodiment the organic material is purified by ultra-filtration in spiral cartridges, whereby the cartridges are charged via a pump. Before the cartridges are put into operation, the virus elimination factor is established with the help of the test virus. The test solution containing a known amount of test viruses is passed across the cartridge in a tangential flow always making sure that the exactly defined pressure conditions are maintained. Then the virus titer is determined from the filtrate with the help of any current method. After that, the organic material is purified in the same cartridge following the same conditions.

The test viruses can, before they are used for the method of the invention, be grown with any current technique to a titer of up to 10^{14}. As a host bacteria for the bacteriophages, *Escherichia coli* 3300, ATCC No. 19853, qualifies, for instance, very well. There are commonly known culture media suitable for the growth of phages. A suitable medium is described, for example, by Luria and Bertani which contains 10g bactotrypton, 5 g yeast extract and 5 g NaCl in 1 l distilled water, having a pH of 7.5 which can be easily adjusted with NaOH, if necessary. With the help of a precipitating agent (for *instance* polyethylenglycol PEG6000) the desired viruses are precipitated from the culture medium. The viruses are then resuspended in a buffer solution, and this solution is adjusted to the desired titer. Suitable for this purpose is, for example, a tris-HCl-buffer with a pH of

7.5 containing 100 mM NaCl and 3 mM $CaCl_2$. The titer can be determined with the top-agar method. After that, the diluted phage suspension is subjected to the filtration procedure required for the organic material. After having conducted this filtration, the virus titer is determined, from which the elimination factor can be derived. The virus titer is indicated in "pfu" (plaque-forming units) and stands for the number of plaques on the lawn of bacteria which are the result of the virus infection. The filtration is, after rinsing the cartridge with caustic soda and neutralizing it with distilled water, repeated as often as necessary until the desired elimination rate is obtained. It is also possible to set up a row of filters one behind the other, thus permitting a continuous filtration progression. After determination of the elimination factor, the pyrogens introduced by the phages are removed by rinsing the cartridge with caustic soda and neutralizing it with distilled water. The cartridge is ready for re-use in the production process.

Surprisingly it was found that the method of the invention is particularly suited to be used for the removal or elimination of viruses during the production of sterile extracts obtained from biological material as a so-called in-process control. It was, in fact, found that the elimination rate of marker substances in the specimen to be purified correlates with that of viruses. This enables a convenient follow-up of the virus elimination by simply determining the elimination of the marker substance.

According to the invention the elimination rate of virus vs. marker is determined and the ratio of both elimination factors is ascertained, which means that a calibration curve is drawn up and within the ongoing process the decrease of the virus concentration is followed by determining the elimination of the marker with the aid of said calibration curve.

As markers, those easily identifiable substances are commonly preferred which are already present in the system to be purified. It is, however, also possible to add marker substances to that system. Preferred marker substances are proteins, peptides and/or nucleic acids. Synthetic substances, especially oligos and polymers, are also suitable. Experienced lab workers generally know how to select the polymer required for the system in question by simple tests. According to the invention, BSA is especially preferred.

Preferred preparations to be purified are biological materials, especially those derived from plant or animal organisms. They are preferably collected from organs, tissues and/or cells. In one embodiment the animal tissue is human tissue, including human or other animal organs. Preferably organs like spleen, thymus and/or bone marrow are used. The method described herein is, however, also suitable for the purification of biological material that was collected from body fluids or from bacterial or viral material, particularly from pathogenic material.

In a particularly preferred embodiment of the invention the elimination factor is determined with four different viruses.

The invention is further explained by figures 1 and 2 and the following examples.

Fig. 1 is a diagram of a filtration system which is on the market under the name of Amicon S1.

Fig. 2 shows the filtration cartridge of the filtration system in fig. 1.

Fig. 1 illustrates a filtration system for the ultra-filtration of organic suspensions. From a storage tank (not shown) the suspension is conveyed through a conduit 3 which is equipped with a rotary pump 5, a throttle valve 7, a manometer 9, a shut-off valve 11, and a discharge 13, via the inlet 15 into the filtration device 17. This device 17 which is equipped with said inlet 15 and an outlet 19 and possessing clamps 21, contains a spiral cartridge 23. From the filtration device 17 the filtrate runs through a conduit 25 equipped with a shut-off valve 27, an outlet 29, a manometer 31 and a check valve 33, into another storage tank (not shown).

Fig. 2 shows the filtration device 117. This device 117 has an inlet 115 which is equipped with a manometer 116 and a clamping device 121. A permeate port 126 leads into the inlet. The filtration device 117 has a spiral cartridge 123. On the top part of the filtration device 117 an outlet 119 is arranged that is provided with a check valve 120 and a clamping device 121.

Example 1

Testing a Sartorius polysulfone membrane with a molecular cut-off of 100.000 daltons, for virus elimination:

A filtration cartridge made of polysulfone by Sartorius (Göttingen, Germany) was inoculated with a phage suspension in a tangential flow, strictly complying with the conditions described in the prototype procedure. Table 1 summarizes the results of these test runs with 3 different partial pressures. With all three, the phage was only removed by a power of 10. In order to achieve an elimination factor of 10^{10}, the filtration would have to be repeated at least ten times and verified each time by adding the phage fr.

Example 2

In this example the filtration system Amicon S1, equipped with an ultrafiltration membrane having a molecular cut-off of 30.000 daltons, was assessed for virus elimination. The technical principle of the filtration system is demonstrated in fig. 1, the filtration cartridge is illustrated in fig. 2. The suspension to be filtrated runs in a tangential flow across the membrane. Part of the suspension is filtered out by the transmembrane pressure (P_t) above the membrane. The pressure at inlet 15 (P_a) and outlet 19 (P_b) of the system is measured by two manometers. The transmission pressure forming across the membrane is calculated by

$$P_t = \frac{P_a + P_b}{2} - P_p$$

whereby P_p stands for filtrate pressure which is generally at zero and ≠ P_a. The specimens were pumped by a peristaltic pump 31 into the cartridge 23 at 130 rpm and an inner tube diameter of 8 mm. The transmission pressure was adjusted by the drain valve to 0.2, 0.4 and 0.7 bar.

In order to test the cartridge, phage suspensions with a titer of 7.8×10^9 pfu (plaque-forming units) per ml (in 10 mM tris-Cl buffer, pH 7.5, diluted in 100 mM NaCl with 1 mg bovine serum albumin per ml) were pumped through the membrane. For every test 1.5 l phage suspension was filtered. In between filtrations the cartridge was cleansed with 0.1 M NaOH and then rinsed with a phosphate-buffered NaCl solution until the eluate was neutralized. These cleansing measures ensured a complete inactivation of any phages possibly remaining in the system. The cartridge was stored in a solution of 10 mM NaOH.

Table 2 summarizes the results of this test conducted with filtration cartridge S1Y30, serial No. 8864. Elimination factors of 4.53 $\log_{10}$ were achieved right after operation start-up and 4.4 $\log_{10}$ after storage in 10 mM NaOH for several months after first filtration.

Example 3

Determination of the elimination rate in a filtration unit with the bacteriophage fr:

In this example the spiral cartridge S1Y30, serial No. 8864 made by Amicon was assessed. 600 ml each of a phage suspension with a starting titer of 3×10^{10} were filtrated in 5 parallel runs three times in row with the aid of the above cartridge. In between every filtration step the cartridge was rinsed with 2 l RO water (water purified by reverse osmosis). Table 3 shows that in all five parallel runs after three filtrations across the cartridge no infectious phage fr could be detected in 1 ml of the filtrate.

Example 4

Elimination of the test virus by a factor 12:

In this example the filtration cartridge S1Y30, serial No. 10330, was tested. The phage suspension consisted of 600 ml phage buffer with 600 mg bovine serum albumin and 50 ml phage concentrate (titer: 1.3×10^{12} pfu (plaque-forming units)/ml). To begin with, three filtrations were done in a row. The volume of the filtrate dropped trom 600 to 400 and further down to 380 ml. Every filtration step lasted approx. 20 minutes. In between filtrations the cartridge was cleansed with 1 1 10 mM caustic soda to inactivate phage residues, and then rinsed with distilled water until neutralized (measured with a pH-electrode).

The virus content of every single filtrate was measured and is given in Table 4.

Since 1 ml filtrate was already free of the bacteriophage fr after the 2nd filtration, the last filtrate (380 ml) was inoculated once more with 40 ml virus concentrate (titer: 5.2×10^{12} pfu/ml) and filtrated three times in a row. As can be noted from table 1, no phages were identifiable in 1 ml filtrate after the 2nd filtration.

From the virus elimination data in table 4 it is evident that the virus titer dropped by 6.92 $\log_{10}$ and 7.22 $\log_{10}$ after the first filtration done with the ultra-filtration cartridge under consideration. In both cases, after the 2nd filtration no phages were any longer detectable. This shows that the amount of viruses was reduced after the first two filtrations by a total decimal power of 11 and after two more filtrations by a decimal power of 11.7 which is a total virus reduction by a decimal power of 22.7 after four filtrations. Consequently, an elimination by a decimal power of 12 to 16 which is commonly recommended in the literature is exceeded by a decimal power of 18.22 after only three filtrations in the here presented experiments. With 7 $\log_{10}$ the virus elimination in this production run is more effective than in the production runs of examples 2 and 3 that showed an elimination

of approx. 4.5 $\log_{10}$ (tables 3 and 4). Considerable differences can be noted in the virus elimination among various production batches, which only underlines the need for careful validation of any test virus.

Example 5

Observation of the virus elimination in a thymus extract by determining BSA:

The thymus glands of calves were homogenized and made into an extract by a generally known technique. To this extract the bacteriophage fr (ATCC No. 15767-B1; Knolle and Hoffmann Verlag, Virology, vol. 123, 271-273 (1964)) was added as a test virus. The BSA content as well as that of purine and pyrimidine bases were determined by means of the HPCL analysis by a generally known technique.

The specimen inoculated with the test phage was then filtrated, as described in examples 3 and 4, across the filtration cartridge S1Y30, serial No. 10330 (Amicon Div.; W.R. Grace & Co.; Danvers, Ma. USA). The virus elimination as well as the BSA reduction were determined. There was a correlation between the virus reduction and the BSA reduction.

The bacteriophage fr (ATCC 15767-B1) was filed on November 19th, 1964 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852-1776, USA and is freely available on the market since that date.

E. coli 3300 (ATCC 19853) was filed on January 12th, 1967, with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852-1776, USA and is freely available on the market since that date.

Table 1

Ultra-filtration conducted with the Sartorius Tangential Flow System
Ultra-filter: polysulfone membrane with a molecular cut-off of 100.000 daltons
Module type: 303 145 69 01 W

Test conditions:

1st run: filtrate flow - 4 ml/sec, partial pressure - 0.8 bar
Volume of the eluate: 660ml, duration of filtration: 2 min 31 sec
Volume of the concentrate: 66 ml

2nd run: filtrate flow - 2.3 ml/sec, partial pressure - 0.4 bar
Volume of the eluate and of the concentrate as above, duration of filtration: 4 min 32 sec

3rd run: filtrate flow - 5ml/sec, partial pressure - 1.6 bar
Volume of the eluate and of the concentrate as above, duration of filtration: 1 min 56 sec

Distribution of the bacteriophages:

Run No.	Starting Susp. pfu/ml	Concentrate pfu/ml	Filtrate pfu/ml	Phage Elimination Rate (log10 pfu 1 / pfu 2)
1	4.30E+06	5.00E+06	1.80E+05	1.378196
2	4.30E+06	6.35E+06	1.50E+05	1.4573772
3	1.70E+09	7.40E+09	1.60E+08	1.0263289

Table 2

Test of the ultra-filtration system Amicon S1Y30
as for permeability for test phage fr

1.5ml each of a phage test suspension (bacteriophage fr suspended in 10mM tris-Cl, pH 7.5, 100 mM NaCl and 1mg bovine serum albumin per ml) was filtered twice with the spiral cartridge, following the required transmission pressures.

	1st filtration*)			2nd filtration*)		
Transmission pressure	filtration suspension	filtrate	Concentrate (10% of the starting suspension)	Suspension before filtration	filtrate	Concentrate (10% of the starting suspension)
3 psi	7.8×10^9	2.3×10^5	1.3×10^{10}	2.3×10^5	0	9.6×10^5
6 psi	7.8×10^9	6.9×10^5	6.4×10^9	6.9×10^5	1.5×10^1	2.8×10^6
10 psi	7.8×10^9	7.3×10^5	1.07×10^{10}	7.3×10^5	1.5×10^1	3.3×10^6

*) The phage titer is stated in plaque-forming units (pfu)/ml.

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Table 3

Validation of the untra-filtration cartridge S1Y30 with test phage fr [a)]

	Titer of the test phage fr in plaque-forming units [b)] per milliliter (pfu/ml)			
	in the starting suspension	in the filtrate of the 1st filtration	in the filtrate of the 2nd filtration	in the filtrate of the 3rd filtration
1st test run	3×10^{10}	3.4×10^{6}	2.9×10^{2}	0
2nd test run	3×10^{10}	4.5×10^{6}	7.3×10^{1}	0
3rd test run	3×10^{10}	3.9×10^{6}	1.1×10^{2}	0
4th test run	3×10^{10}	7.0×10^{6}	7.0×10^{1}	0
5th test run	3×10^{10}	5.4×10^{6}	7.6×10^{1}	0
Means		4.84×10^{6}	1.24×10^{2}	
Standard deviations		1.2×10^{6}	8.4×10^{1}	

a) 600 ml each of the phage test suspension (bacteriophage fr suspended in phage buffer: 10mM tris-Cl, 10mM $CaCl_2$, 0.15 M NaCl and 1mg bovine serum albumin per ml) were filtrated three times in a row with the Amicon ultra-filtration cartridge S1Y30 using a transmission pressure of 3 psi.

b) The titer of the phages was determined with Escherichia coli strain 3300 on agar plates according to the technique described by Davis and Sinsheimer ("Top-Agar-Methode"), J. Mol. Bio., 1963, vol. 2, 203-207); the filtrates in the phage buffer were diluted (see a)). Deviating from the original directions, the titration was done on Luria-Bertani culture medium (10 g trypton, 5 g yeast extract, 5 g NaCl in 1 l RO-water, pH adjusted to 7.5 with NaOH). Also, 3mM $CaCl_2$ were added to the "Top-Agar".

Table 4

Virus titer measured in the filtrate of every single filtration step with the aid of the ultra-filtration cartridge Amicon S1Y30

Filtration step	Virus titer (pfu/ml)
Starting suspension	1×10^{11}
1st filtration	1.2×10^{4}
2nd filtration	0
3rd filtration	0
Subsequent addition of test phages	5×10^{11}
1st filtration	3×10^{4}
2nd filtration	0
3rd filtration	0

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A method to remove biological material selected from the group consisting of a virus and a pathogenetic substance, from organic material, characterized in that the material to be purified is passed through an ultra-filter for which the removal capacity has previously been calibrated by admitting into the filter test viruses selected from the group consisting of the leviviridae family and other bacteriophages equivalent in size, and that before and after the filtration, the titer of the viruses is determined from which the removal rate is established.

2. Method according claim 1, characterized in that MS2, f2, f4, Qß, Vk, ST or R17 are used as test virus.

3. Method according to any one of claims 1 or 2, characterized in that the bacteriophage fr, ATCC No. 15767-B1 is used as test virus.

4. Method according to claim 1 wherein the ultra-filter is part of an ultra-filtration unit.

5. Method to determine the removal rate of viruses in organic material, characterized in that a virus of the leviviridae group is added to a specimen of the material as test virus, that this specimen is subjected to the purification procedure chosen for said material and that a virus count is made before and after purification from which the removal rate is determined.

6. Method according to any one of claims 1, 2 or 5 characterized in that the removal rate of a marker substance is determined which was either inherent in or subsequently added to the material to be purified, that the ratio of the removal rates between the marker substance and the virus is determine and the removal of

the virus is controlled by closely keeping track of the removal of the marker substance.

7. Method according to any one of claims 1, 2 or 5 characterized in that the material to be purified is collected from one of the group consisting of plants, animal tissue, bacteria and viruses.

8. Method according to claim 7 wherein the animal tissue is human tissue.

9. Method according to claims 7 or 8 wherein the animal tissue or human tissue includes human or animal organs.

10. Method according to any one of claims 1, 2 or 5 characterized in that the material to be purified is extracted from one of the group consisting of spleen, thymus and bone marrow.

11. Method according to claim 6, characterised in that a protein or a nucleic acid is used as marker substance.

12. Method according to claim 7, characterized in that a protein or a nucleic acid is used as marker substance.

13. Method according to claim 10, characterized in that a protein or a nucleic acid is used as marker substance.

14. Method according to claim 6, characterized in that BSA is used as marker substance.

15. Method according to claim 7, characterized in that BSA is used as marker substance.

16. Method according to claim 10, characterized in that BSA is used as marker substance.

17. Method according to claim 11, characterized in that BSA is used as marker substance.

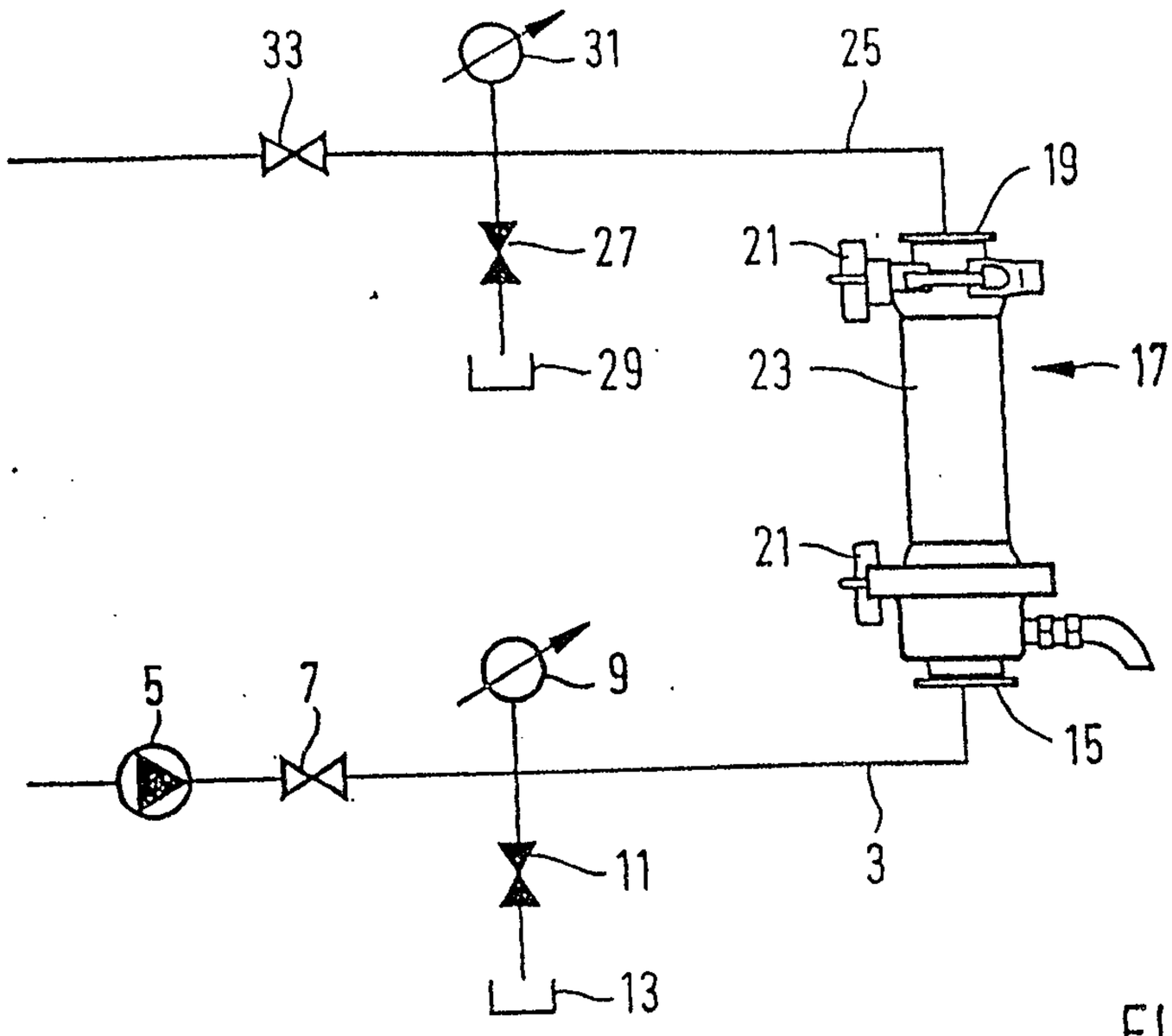


FIG.1

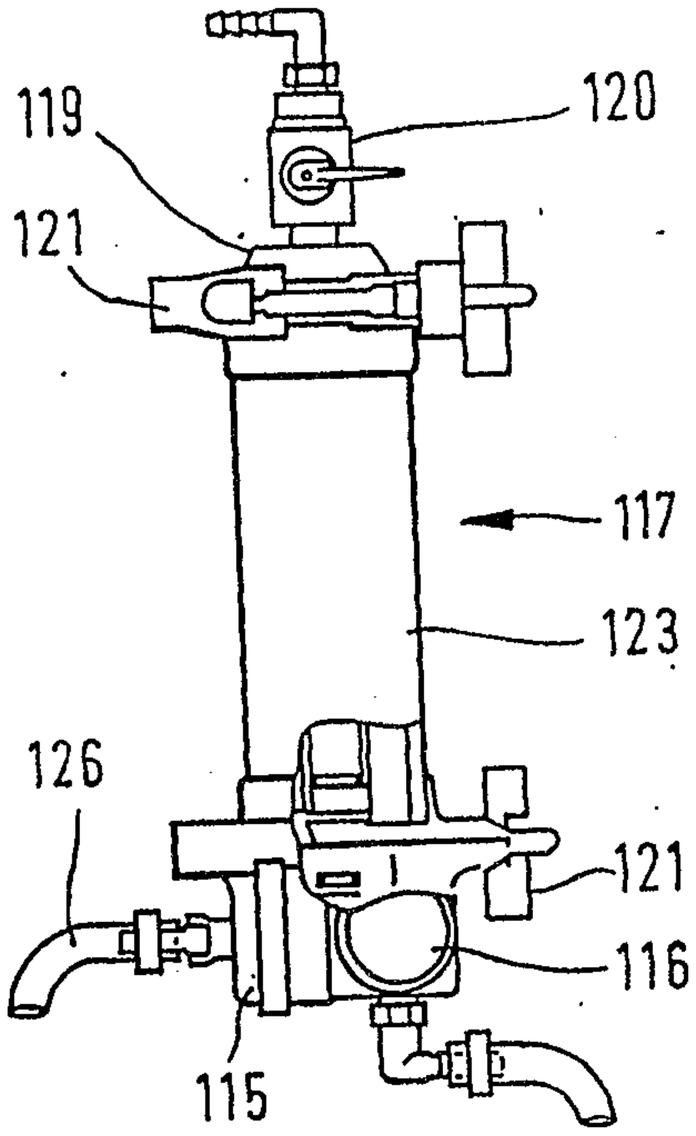
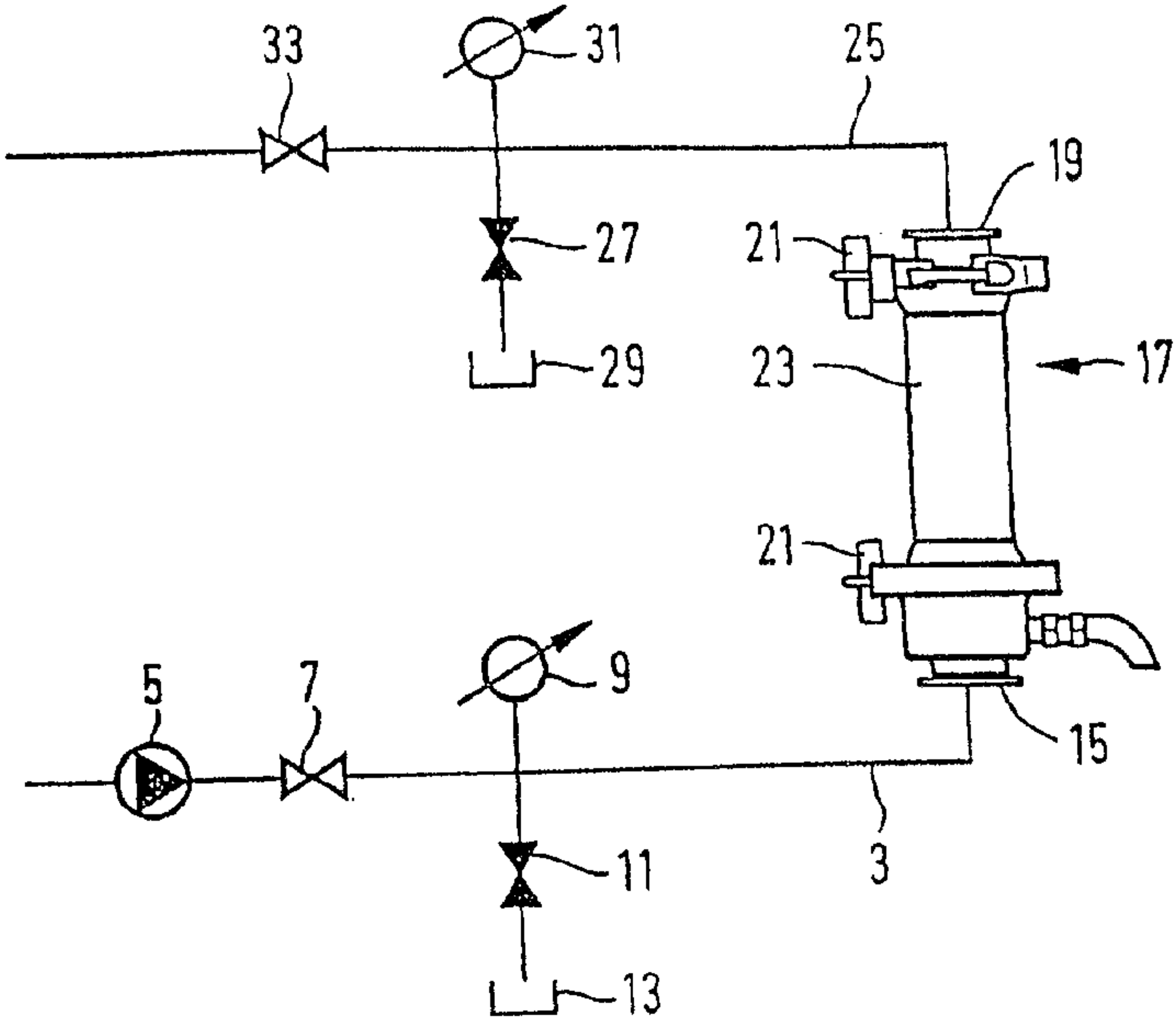


FIG.2

Gowling, Strathy & Henderson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