



- (51) International Patent Classification:
G01N 15/12 (2006.01)
- (21) International Application Number:
PCT/IB2017/000468
- (22) International Filing Date:
25 April 2017 (25.04.2017)
- (25) Filing Language:
Italian
- (26) Publication Language:
English
- (30) Priority Data:
102016000043032 27 April 2016 (27.04.2016) IT
- (71) Applicant: NATIONAL SYSTEMS, S.R.L. [IT/IT]; Via Goito, 60, 21019 Somma Lombardo (VA) (IT).
- (72) Inventors: GIACOMETTI, Marco; Piazza Insubria, 10, 20137 Milano (MI) (IT). CARMINATI, Marco; Piazza

Imperatore Tito, 8, 20137 Milano (MI) (IT). CHIODINI, Sergio; 20087 Robecco sul Naviglio (MI) (IT). SAMPIETRO, Marco; Via Giuseppe Ferrari, 14, 22100 Como (CO) (IT). FERRARI, Giorgio; Via Medaglie d'Oro, 47, 21019 Somma Lombardo (VA) (IT).

(74) Agent: PREMUR, Rok; via Aliprandi, 13, 20900 Monza (MB) (IT).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC,

(54) Title: DEVICE AND METHOD FOR DETECTING NONELECTROLYTE PARTICLES IN TWO FLOWS OF LIQUID SOLUTIONS CONTAINING AN ELECTROLYTE

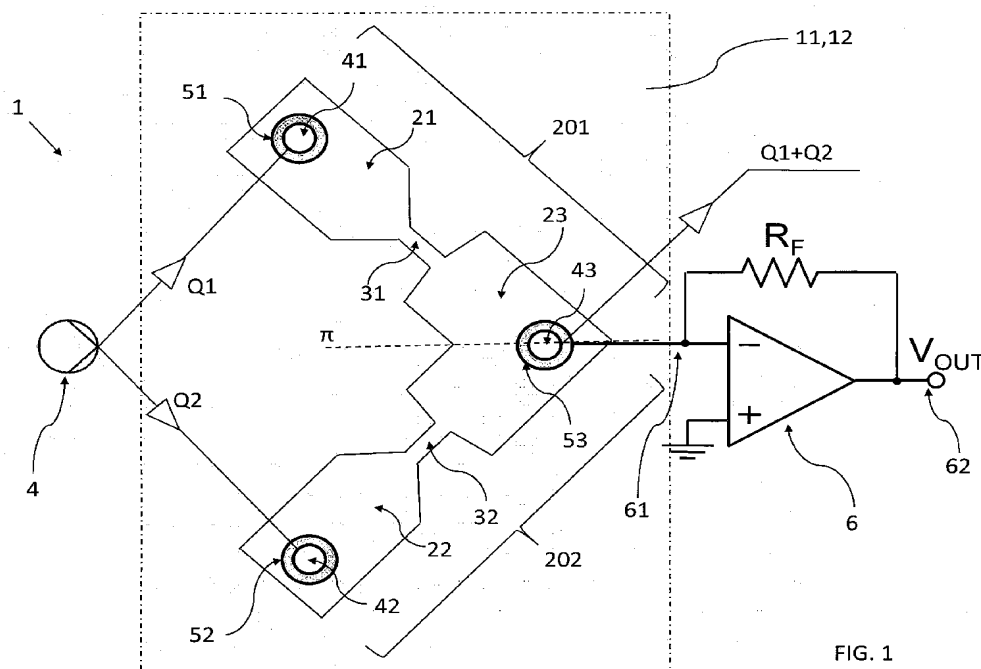


FIG. 1

(57) Abstract: A device is herein disclosed for detecting corpuscles of nonelectrolyte particles contained in two flows of solutions containing an electrolyte, comprising a first channel (201); a second channel (202); a first orifice (31) located in an intermediate point of said first channel (201); a second orifice (32) located in an intermediate point of said second channel (202); a first electrode (51), located on a first side with respect to said first orifice (31) with a first voltage (VI) applied thereto; a second electrode (52), located on a first side with respect to said second orifice (32) with a second voltage (V2) applied thereto; a third electrode (53; 53', 53'') located on a second side with respect to said first and second orifices (31, 32), said third electrode having an intermediate electric voltage (V3) between said first and second voltages (VI, V2); and Means for measuring the current in said



SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR,
TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

DEVICE AND METHOD FOR DETECTING NONELECTROLYTE PARTICLES IN TWO FLOWS OF LIQUID SOLUTIONS CONTAINING AN ELECTROLYTE.**Description**

A device is herein disclosed for detecting nonelectrolyte particles in two flows of electrically conductive liquid solutions, e.g. aqueous solutions containing an electrolyte.

Particularly, a device is herein disclosed for detecting nonelectrolyte particles or corpuscles such as agglomerates and/or aggregates of proteins, fragments of nucleic acids (DNA, RNA), residues of physico-chemical reactions contained in electrolytic solutions, bacteria and cells, in two flows of electrically conductive liquid solutions.

The device as described below is not only able to count nonelectrolyte particles in two flows of liquid electrolytic solutions, but can estimate the size of the counted particles.

The device as described below is suitable, for instance, to detect aggregates of monoclonal antibodies in a saline solution containing complex physiologic electrolytes.

Therefore, the device as described below will be used, for instance, in processes for the production of drugs containing monoclonal antibodies.

Processes for continuous production of monoclonal antibody-based drugs are known.

The molecules of these antibodies can be only effective if they are present in the solution without being bound to other molecules.

Errors in the parameters of the production processes such as, by way of example and without limitation, compound concentrations, reaction temperature, pH, reaction time, may cause the formation of aggregates of antibody molecules.

These aggregates may even have a diameter equivalent to about 1-100 μm (note that a molecule of a monoclonal antibody may have a diameter equivalent to about 5-10 nm).

The aggregation of molecules of antibodies decreases effectiveness in the action of such antibodies.

Therefore, there is the problem of monitoring in real time the amount of aggregates in drugs being produced to correct, if needed the drug production process parameters and/or reject the production part with an excessive amount of antibody aggregates.

Optical devices are currently known, which detect cells, agglomerates and aggregates in a solution.

Nevertheless, when these sensors are used for detection of antibody aggregates, they exhibit various drawbacks, namely in reliable counting of particles, particle size measurement and cost problems.

Also, the size of these instruments makes it difficult to parallelize measurements, for analysis of large volumes of a solution in a short time.

Particle counters using electric measurements are known, namely as Coulter counters, and can count the number and size of suspended particles (e.g. Cells, bacteria, viruses, or protein agglomerates or aggregates) in a flow of an aqueous solution that has become conductive due to an electrolyte.

A conventional Coulter counter includes a sensor comprising two chambers, communicating via an orifice.

The orifice has a passage section that is slightly larger than the maximum size of the particles to be monitored, as well as a pair of electrodes located upstream and downstream from the orifice between the two chambers respectively.

When the particles pass through the orifice that connects the two chambers, the electric resistance between the two electrodes increases in proportion to the particle volume, which will result in a corresponding decrease of ion current (when operating at constant voltage and current is measured between the electrodes) or in an increase of voltage (when operating at constant current and voltage is measured between

the electrodes).

Any resistance variation detected by the counter will indicate the passage of a particle.

The amplitude of electric resistance variation may be related to the equivalent diameter of the particle.

Coulter counters may use a direct current (voltage) or an alternate current (voltage) depending on the type of particles to be monitored and the type of electrodes being used.

Nevertheless, these known Coulter counters are affected by certain drawbacks.

For example, noise-related errors occur, which affect measurement reliability.

Also, currently available Coulter counters are quite bulky and expensive; therefore problems may arise from the use of many counters in parallel, when large volumes of solutions are required to be analyzed in a short time.

Patent DE 21 45 531 A1 (Licentia GmbH) discloses a fluidic device for counting the number of particles in a liquid flow and for estimating the volume and cross section of each particle being detected.

The flow of particles is hydrodynamically focused, for the particles to move along a single row.

Namely DE 21 45 531 A1 discloses a device that can count leukocytes and erythrocytes in a blood sample.

The device as disclosed in DE 21 45 531 A1 includes an open tank with two parallel partitions that divide the tank into three compartments (two end compartments and a middle compartment), which are hydraulically connected in series via a first orifice formed in the first partition and a second orifice formed in the second partition.

The tank contains an electrolytic solution and the sample of liquid containing the particles to be counted and measured is released from a nozzle, which has to be aligned with the two orifices (otherwise no hydrodynamic focusing would be

obtained).

Three electrodes are also provided in the three compartments of the tank respectively, with three different electric potentials applied thereto.

Therefore, a first ion current is created through the first orifice and a second ion current is created through the second orifice.

The three electrodes belong to two measuring circuits, which measure ion current at the ends of the first orifice and at the ends of the second orifice respectively.

The passage of a particle through an orifice is indicated by an abrupt drop in ion current.

The length of the first orifice is smaller than the average length of the particles to be measured; therefore current decrease may be related to the cross section of the passing particles .

The length of the second orifice is greater than that of the particles to be measured; therefore the volume of the passing particles may be related to current decrease.

The device as disclosed in DE 21 45 531 suffers from various drawbacks.

The quality of measurement achieved with the device of DE 21 45 531 is based on the assumption that perfect hydrodynamic focusing may be obtained.

This is not necessarily true.

Alignment errors between the nozzle and the orifices, vibrations or other disturbances will affect the correlation between the measurement of particles that pass through the first orifice and the measurement of particles that pass through the second orifice.

In other words, there is no certainty that the particle that passes through the second orifice corresponds to the last particle that has passed through the first orifice, therefore volume and cross section measurements may relate to two

distinct particles.

The invention has the object of at least partially solving the problems of the prior art, and particularly those discussed above.

Namely, one object of the invention is to provide a device that reduces measurement errors caused by electrical, mechanical and thermal disturbances.

A further object of the invention is to provide a device that affords increased flexibility of use as compared with prior art devices.

This object is fulfilled by a device and a method as defined in the independent claims.

Further advantages are achieved by means of the additional characteristics of the dependent claims.

A possible embodiment of a device for detecting nonelectrolyte particles in two flows of electrolytic solutions as defined in the claims will be described hereinafter with reference to the annexed drawings, in which:

Figure 1 is a schematic view of a possible embodiment of a device for counting nonelectrolyte particles in two flows of conductive liquid solutions, e.g. two aqueous solutions, containing an electrolyte.

Figure 2e is a schematic view of an alternative embodiment of the device.

Figure 3 shows an electrical equivalent of a detail of the device of Figure 1;

Figure 4 is a schematic sectional view of a detail of the device of Figure 1;

Figure 5 shows a first possible curve of the output voltage provided by the device of Figure 1 when the passage of two successive particles, one for each flow, is detected;

Figure 6 shows another possible curve of the output voltage provided by the device of Figure 1 when the passage of two successive particles, one for each flow, is detected;

Figure 7 is a schematic view of a closed-loop control system applied to the device of Figure 1;

Figure 8 is a more detail view of a feedback control system applied to the device of Figure 1.

Referring to the accompanying drawings, a device 1 is described for detecting nonelectrolyte particles in two flows of aqueous solutions containing an electrolyte and nonelectrolyte particles of corpuscles.

The aqueous solutions containing the particles to be counted are caused to flow in the device 1, for counting to occur.

The device 1 can also (indirectly) measure the size of the particles that are being detected.

The device 1 as described below may be used, for example, to detect aggregates of monoclonal antibodies in aqueous saline solutions.

The device 1 comprises a first channel 201 and a second channel 202, for the passage of two flows Q1, Q2 of liquid solutions containing ions to be conductive.

In the exemplary embodiment hereof, the two liquid solution flows Q1, Q2 which flow in the two channels 201, 202 come from a single source of an aqueous solution, i.e. one solution is divided into two flows.

The device 1 may be operated by a pump 4 for creating an electrolyte flow which is divided into a first flow Q1 and a second flow Q2, flowing the first and second channels 201, 202 at the same time.

The operating pressure of the pump 4 may range, for instance, from 200 Pa to 2,500 Pa.

If the aqueous solution to be introduced into the two channels 201, 202 is already under pressure, the pump 4 may be omitted.

The first channel 201 has a first orifice 31 in an intermediate location thereof.

The second channel 202 has a second orifice 32 in an intermediate location thereof.

In the illustrated embodiment, the two channels 201, 202 have two distinct ends and one common end, thereby assuming a generally V-shaped configuration.

With this configuration, one flow may be divided into two distinct flows Q1, Q2, or two distinct flows Q1, Q2 may be joined together.

In a possible embodiment, the second orifice 32 has substantially and/or generally the same shape and size as the first orifice 31.

There are a first electrode 51 with a voltage V1 applied thereto, a second electrode 52, with a voltage V2, other than V1, applied thereto, and a third electrode 53, with an intermediate voltage between V1 and V2 applied thereto.

The first and second electrodes 51, 52 are introduced into the two channels 201, 202, to be both on the same sides relative to the orifices 31, 32.

In other words, considering the flow direction of the liquid solution in the two channels 201, 202, the first and second electrodes 51, 52 shall be both upstream from the orifices 31, 32 or both downstream from the orifices 31, 32.

The third electrode 53 is placed on the opposite side of the orifices 31, 32 from the second electrode 51, 52.

This means that, if the first and second electrodes 51, 52 are located upstream from the orifices 31, 32, then the third electrode 5 will be located downstream from the orifices 31, 32 or vice versa.

The third electrode 53 may be replaced by two distinct electrodes 53', 53'' (not shown), provided that they are electrically connected to each other to have the same potential.

The option of using two distinct third electrodes is required, for instance, if the two channels 201, 202 do not have a common end.

From an electrical point of view, the portion of the hydraulic

portion between the first and third electrodes 51, 53 (in which the first flow of electrolyte flows) forms the first electric dipole 81, having a resistance R_1 , and the portion of the hydraulic circuit between the second and third electrodes 52, 53 (in which the second flow of electrolyte flows) forms a second electric dipole 82, having a resistance R_2 .

The dipoles 81 and 82 have a terminal in common (the third electrode 53) whereby they form a resistive tripole.

A first ion current I_1 flows in the first bipole 81, and a second ion current I_2 flows in the second bipole 82.

Means are further provided, as described in greater detail below, for measuring the difference between the currents I_1 , I_2 flowing in the two bipoles 81, 82.

When nonelectrolyte particles are not present, or when such particles are much smaller than the flow section of the orifices 31, 32, then the overall resistances R_1 , R_2 of the two bipoles 81, 82 are substantially constant with time.

In this state, the bipoles 81, 82 have constant currents I_1 , I_2 flowing therethrough, which are a function of the voltages V_1 , V_2 , V_3 applied to the first, second and third electrodes 51, 52, 53.

When no particles pass through the orifices 31, 32, the differences between the currents I_1 , I_2 is constant with time; therefore, the current I_3 at the third electrode 53 is constant with time.

When a nonelectrolyte particle having a significant size as compared with the flow section of the orifice, passes through one of the two orifices 31 or 32, then a temporary condition will be established, for an interval of time Δt , during which the flow section available to the ion charges in the orifice 31 or 32 is significantly reduced and hence the overall resistance of the first resistive dipole 81 or the second resistive dipole 82 is significantly increased.

The duration of the time interval Δt depends on the flow rates

in the channels 201 and 202.

In the production of drugs containing monoclonal antibodies, the time interval Δt is indicatively of the order of 10^{-5} - 10^{-2} secs.

Due to constant-voltage operation during this time interval Δt , a variation results in the ion current I_1 or I_2 that flows through the corresponding dipole 81 or 82, leading to a variation in the ion current I_3 that flows through the third electrode 53 (which is equal to the difference between the first ion current I_1 and the second ion current I_2).

The use of the currents that flow through the two orifices 31, 32 as information, will make the device 1 less sensitive to errors caused by disturbances that simultaneously act on both channels 201 and 202 such as: changes in electrolyte concentration, changes in the temperature of the aqueous solution containing the antibodies, thermal expansions of the material used to form the channels 201, 202, electromagnetic noise caused by electrical equipment placed near the device 1.

In order to use the current signal available on the third electrode 53 to count the particles that pass through the orifices 31, 32, a current meter 6 is required.

As used herein, the term "current meter" is intended to designate any device that can read the current on the third electrode 53 and provide an output signal that is proportional to the current measured on the third electrode 53.

The current meter 6 may comprises a current to current converter or a current to voltage converter, also known as a transimpedance amplifier 6.

The transimpedance amplifier 6 has an output 62 with an output voltage V_{out} that is proportional to the current in the third electrode 53.

In a possible embodiment of the device, the voltages V_1 , V_2 and V_3 are selected to obtain two ion currents I_1 , I_2 , whose difference, when no particles of monoclonal aggregates are

found in the solution, is zero.

The voltage differences required to obtain the desired values of the two ion currents I_1 , I_2 may change as a function of any size differences of the channels 201, 202 and particularly the orifices 31, 32.

The modulus $|\Delta V_{1-3}|$ of the voltage difference between the first electrode and the second electrode 51, 52 preferably ranges from about 0.3 to 1 volt.

Likewise, the modulus $|\Delta V_{2-3}|$ of the voltage difference between the first electrode and the third electrode 51, 53 preferably also ranges from about 0.3 to 1 volt.

Greater voltage differences are generally not desired, as they cause water hydrolysis that may lead to measurement errors.

However, with moderate water hydrolysis, measurement errors were found to be negligible, even when voltages of 1-10 Volts are applied.

In the illustrated embodiment 52, the first and second channels 201, 202 are formed in a body 11, 12 (made of an electrically insulating material) and have one communicating end in the body itself, whereas the other two ends are allowed to communicate via an external joint or connector 51, 52.

In the illustrated embodiment the device 1 has a first chamber 21, accommodating the first electrode 51, a second chamber 22, accommodating the second electrode 52, and a third chamber 23, accommodating the third electrode 53.

The first chamber 21 communicates with the third chamber 23 via a first orifice 31 and the second chamber 22 communicates with the third chamber 23 via a second orifice 32.

The first chamber 21, the first orifice 31 and the third chamber 23 define the first channel 201, whereas the second chamber 22, the second orifice 32 and the third chamber 23 define the second channel 202.

Preferably, the second orifice 32 has substantially and/or generally the same shape and size as the first orifice 31.

Then, a first hole 41 connecting the first chamber 21 with the outside, a second hole 42 connecting the second chamber 22 with the outside and a third hole 43 connecting the third chamber 23 with the outside are provided.

In the illustrated embodiment, the hydraulic circuit comprising the three chambers 21, 22, 23, the two orifices 31, 32 and the three holes 41, 42, 43 is substantially and/or generally symmetric with respect to a plane of symmetry π .

In the illustrated embodiment, the chambers 21, 22, 23 and the orifices 31, 32 are formed as recesses or grooves on one side of a first plate-like body 12; these recesses are closed by a second plate-like body 11, which acts as a cover.

Therefore, the channels 201, 202 are adapted to contain a pressurized liquid.

The plate-like body 12 may be made of a silicone elastomer material, such as polydimethyl siloxane (PDMS).

The recesses that form the hydraulic circuit may be formed, for example, by conventional photolithography and soft lithography. With this technology, channels with a square or rectangular section, and sides having a minimum size of 0.1 μm , may be formed.

The support plate 11 may be made of electrically insulating materials, such as glass, quartz or silicon dioxide (SiO_2).

The first plate-like body 11 and the second plate-like body 12 may be coupled together by mutual contact after exposure to oxygen plasma.

Other known technologies may be used to form the hydraulic circuit for the two flows of the solution to be monitored.

In the illustrated embodiment, a pump 4, e.g. a positive-displacement pump is provided, for generating two electrolyte flows Q1, Q2, which respectively enter the first and second holes 41, 42.

The flow Q1 that enters the first hole 41 flows through the first chamber 21 and the first orifice 31 and reaches the third

chamber 23.

The flow Q2 that enters the second hole 42 flows through the second chamber 22 and the second orifice 32 and reaches the third chamber 23.

The electrolyte flows Q1 and Q2 that reach the third chamber 23 come out together from the third hole 43.

In an alternative embodiment, not shown, the flow of the electrolyte in the hydraulic circuit follows a reverse path.

In this case, the pump 4 introduces an electrolyte flow Q1+Q2 into the third hole 43.

The electrolyte introduced into the third chamber 23 is divided into a first flow Q1, which reaches the first chamber 21 via the first orifice 31, and a second flow Q2, which reaches the second chamber 22 via the second orifice 32.

The flows Q1, Q2 that reach the first and second chambers 21, 22, come out through the first and second holes 41, 42.

As discussed above, with a consistent specific resistivity of the the electrolytic solution, and assuming that the electrolytic solution does not contain nonelectrolyte corpuscles, the overall resistance of each resistive dipole 81, 82 is found to be a function of the flow section and the length of the path of the two channels 201, 202 (as well as of the characteristics of the electrolytic solution).

In this case, the flow section between the first and third electrodes 51, 53 and the flow section between the second and third electrodes 52, 53 have a profile that varies according to the location in the path.

Therefore, the electric resistance of each resistive dipole 81, 82 may be deemed to be the sum of a first resistance R11, R21, corresponding to a section located on one side of the orifice 31, 32 (e.g. upstream from the orifice, in the solution flow direction), a second resistance R12, R22 corresponding to the orifice 31, 32 and a third resistance R13, R23 corresponding to the section located on the other side of the orifice 31, 32

(e.g. downstream from the orifice, in the solution flow direction).

In a possible embodiment, the sizes of the chambers 21, 22, 23 and the sizes of the orifices 31, 32 are in such mutual relation that the electric resistance R_1 , R_2 of each dipole 81, 82 mainly depends on the flow section and length of the orifices 31, 32 and only to a minor extent on the flow section and length of the rest of the channels (here the chambers 21, 22, 23).

The flow section of the first and second orifices 31, 32 is slightly larger than the maximum size of the sizes of the corpuscles to be monitored.

If the device for monitoring the presence of monoclonal antibody aggregates is used, the orifices 31, 32 may have a square or rectangular flow section, with sides ranging from 16 to 25 μm and may have a length of about 20 μm .

Orifices with these dimensions are suitable for detecting protein macroaggregates with an equivalent diameter of 2.0 to 16.0 μm , with total flow rates of up to 25 $\mu\text{l}/\text{min}$.

The flow rate for each channel 201, 202 may be controlled by adjusting the liquid injection pressure in the hydraulic circuit.

Also, multiple devices may be used in parallel, to increase the overall flow rate of the solution being monitored.

When nonelectrolyte particles are not present, or when such particles are much smaller than the flow section of the orifices 31, 32, then the overall resistances R_1 , R_2 of the two bipoles 81, 82 are constant.

In this state, the bipoles 81, 82 have currents I_1 , I_2 flowing therethrough, which are a function of the voltages V_1 , V_2 , V_3 applied to the first, second and third electrodes 51, 52, 53.

When a nonelectrolyte particle having a significant size as compared with the flow section of the orifice, passes through one of the two orifices 31 or 32, then a temporary condition

will be established (for an interval of time Δt depending on the flow rate and indicatively of the order of 10^{-5} - 10^{-2} secs) during which the flow section available to the ion charges in the orifice is significantly reduced and hence the overall resistance of the dipole 81, 82 is significantly increased.

During this time interval Δt , a variation results in the ion current I_1 or I_2 that flows through the corresponding dipole 81 or 82, leading to a variation in the ion current I_3 at the third electrode 53 (which is equal to the difference between the first and second ion currents I_1 and I_2).

The third electrode 53 is connected to the input of the current meter 6.

As mentioned above, as used herein, the term "current meter" is intended to designate any device that can provide a signal that is proportional to the current measured on the third electrode 53.

In the illustrated example, the current meter 6 is a current-to-voltage converter, namely a transimpedance amplifier 6.

In the illustrated embodiment, the non-inverting input of the transimpedance amplifier 6 is grounded, whereby the third electrode 53 has a zero voltage V_3 .

Then, the transimpedance amplifier 6 receives a current signal I_3 at its input, which is a function of V_1 and V_2 and of the presence of particles, if any, in one of the two orifices 31, 32, and converts it into a voltage signal at the output 62.

Knowledge of the voltage values V_1 , V_2 applied to the electrodes 52, 53 and detection of the output voltage 62 of the transimpedance amplifier 6 allows detection of any resistance variation in any of the two dipoles 81, 82, and hence ascertainment of the number and sizes of nonelectrolyte corpuscles that pass through any of the two orifices 31, 32.

In an alternative embodiment (not shown), the third electrode 53 is connected to the input of a current amplifier (not

shown).

When no particles pass through the orifices 31, 32, the voltage V_{out} at the output 62 is substantially and/or generally constant, e.g. has a zero value.

The chart of Figure 4 shows the voltage curve at the output 62 of the transimpedance amplifier 6 when the particles that pass through one of the two orifices 31, 32 have a length that is smaller than the length of the orifices 31, 32.

Here, pulses 65, 66 are obtained, whose duration is substantially independent of the size of the particle and whose amplitude is proportional to the volume of the particle.

The pulse sign (positive or negative) depends on the channel 201, 202 along which the particle passes.

The chart of Figure 5 shows the curve of the voltage V_{out} at the output 62 of the amplifier in case of particles having a substantially constant cross section, and a length that is greater than the length of the orifices 31, 32.

Here, pulses 67, 68 are obtained whose duration is a function of the length of the particle and whose amplitude is proportional to the section of the particle that passes through the orifice 31, 32.

In a possible embodiment, the first, second and third electrodes 51, 52, 53 are tubular elements that are partially inserted in the first, second and third holes 41, 42, 43.

This arrangement allows a single component to be used both for electric connection and for hydraulic connection.

In the illustrated embodiment, the first, second and third electrodes 51, 52, 53 are tubular elements made of silver.

Preferably, the silver electrodes 51, 52, 53 are plated with silver chloride (AgCl).

The choice of this type of electrodes provides particular benefits when operating with aqueous solutions containing chlorine ions (e.g. sodium chloride solutions), as it minimizes the electric potential required to create the ion current.

Here, the first and second electrodes 51, 52 receive supply voltages with alternating components, e.g. square wave forms, having the same frequency and a duty cycle of 50%.

The period T of the two square waves may be, for example, about 10 seconds.

With this arrangement, the thickness of the silver chloride plating on the electrodes may be kept substantially constant with time, which will protect the electrodes from erosion.

As an alternative to silver, platinum may be used as a material to form the electrodes 51, 52, 53.

In a possible embodiment, the voltages V1, V2 applied to the first and second electrodes 51, 52 have opposite signs and the third electrode 53 is kept at zero electric potential by the transimpedance amplifier 6.

In a possible embodiment, the device 1 comprises a negative-feedback closed-loop control 100, as generally shown in Figure 6, which controls the voltage of the first and second electrodes 41, 42 to maintain the current I3 in the third electrode 43 (with no particles passing through the orifices 31, 32) at a desired value.

The control loop 100 comprises a controller 101 for controlling the voltages V1, V2 on the first and second electrodes 51, 52 and a device 102 for reading the electric signal at the output of the third electrode 53.

The set point 103 of the control loop 100 may be, for instance, zero, so that a zero current may be obtained on the third electrode 53 (with no particles passing through the orifices 31, 32).

Figure 7 shows the closed-loop control 100 in greater detail.

The controller 101 comprises two PID (Proportional-Integral-Derivative) controllers 1001, 1002, a reference voltage generator 1003, a clock generator ϕ and an inverted clock generator $\bar{\phi}$.

A digital-to-analog converter 1008, 1009 of the desired voltage

at the output of each PID controller 1001, 1002.

The clock ϕ determines the voltage inversion interval at the electrode 41, 42 (the period T is typically of the order of seconds or tens of seconds).

With the "high" clock the reference voltage V_{ref} is applied to the second electrode 42, whereas a voltage determined by the PID controller 1001 is applied to the first electrode 41, to set the first electrode 41 to the voltage required for the output voltage 62 of the transimpedance amplifier 6 to be equal to the set point ($-V_{ref}$ in case of identical channels and zero set points).

With the "low" clock, the voltage V_{ref} is connected to the electrode 41 and the PID controller 1002 is activated to set the second electrode 42 to the voltage required to obtain the voltage at the output 62 of the transimpedance amplifier 6.

In terms of implementation, a single PID controller is required, whose state variables are changed every time the clock is switched.

This arrangement allows management of voltage switching at the electrodes 41, 42, 43 with reduced settling transients.

The output signal of the transimpedance amplifier 6 is converted to digital form and sent to a data acquisition system 1005.

A device as described above may be used to carry out a method of detecting corpuscles of nonelectrolyte particles in a solution, particularly an aqueous saline solution containing monoclonal antibodies.

This method first includes the step of simultaneously creating two flows Q_1 , Q_2 of the same electrolytic solution.

Each of the two flows Q_1 , Q_2 flows through an orifice 31, 32.

This is followed by the generation of a first ion current I_1 , which flows through a section 81 of the first flow Q_1 (including the orifice 31) and a second ion current I_2 which flows through a section 82 of the second flow Q_2 (including the

orifice 32).

The ion currents I_1 , I_2 are obtained by operating with constant voltage supplies.

Then a current signal I_3 is obtained, which is equal to the difference between said first and second ion currents I_1 , I_2 .

Each of the two ion currents I_1 , I_2 may be temporarily reduced when nonelectrolyte particles pass through one of the two throats 31, 32.

This will lead to a temporary variation of the current signal I_3 , which will depend on the size of the detected particle.

Conveniently, the current signal I_3 that is equal to the difference between the first and the second ion currents I_1 , I_2 may be amplified and converted to a voltage signal.

The device 1 is able to distinguish the particles that pass through the first orifice 31 from the particles that pass through the second orifice 32 by relating the sign of the current pulse with the voltages applied to the electrodes as the particle passes.

In a possible variant, the two ion currents I_1 , I_2 have alternating components, e.g. square wave forms, with a duty cycle of 50%.

The device 1 as described above may be also used to simultaneously compare two flows of electrolytic solutions having the same electric conductivity and different particle concentrations and/or different particle sizes (i.e. Coming from different sources).

In this case, two distinct pumps may be used, to inject the liquid solutions into the two channels 201, 202 (namely the chambers 21 and 22).

This may take place, for instance, when there is a first flow, or reference flow, and a second flow that might be the flow of a production process.

In this alternative mode, the device 1 may be also used to identify and recognize viral and/or bacterial forms by causing

the same fluid containing the virus or the bacterium to flow in both conduits 31 and 32 and adding appropriate antibodies to one of the flows 32 or 33 before causing it to flow in its conduit.

This addition will generate an increase of the physical size of the viruses or bacteria, due to antibody aggregation on the antigen surface of viruses or bacteria.

This size increase, which does not occur in viruses or bacteria in the other flow, allows identification through comparison of the sizes of aggregates in the flows of the two conduits 31 and 32.

Claims

1. A device for detecting nonelectrolyte particles in two flows of liquid solutions containing an electrolyte, comprising:
 - a first channel (201);
 - a second channel (202);
 - a first orifice (31) located in an intermediate point of said first channel (201);
 - a second orifice (32) located in an intermediate point of said second channel (202);
 - a first electrode (51), located on a first side with respect to said first orifice (31) with a first voltage (V1) applied thereto;
 - a second electrode (52), located on a first side with respect to said second orifice (32) with a second voltage (V1), other than the first voltage (V1), applied thereto;
 - a third electrode (53; 53', 53'') located on a second side with respect to said first and second orifices (31, 32), said third electrode having an intermediate electric voltage (V3) between said first and second voltages (V1, V2);
 - a current meter (6) for measuring the current in said third electrode (53; 53', 53'').
2. A device as claimed in claim 1, wherein said current meter (6) comprises a transimpedance amplifier (6) having an input (61) and an output (62), said input (61) being connected to said third electrode (53; 53', 53''), said transimpedance amplifier (6) providing an output voltage signal representative of the current signal applied at its input.
3. A device as claimed in claim 1 or 2 wherein said first channel (201) comprises a first chamber (21) and a third chamber (23), said first chamber (21) communicating with said third chamber (23) via said first orifice (31), and wherein
 - said second channel (202) comprises a second chamber (22) and a third chamber (23), said second chamber (22) communicating

with said third chamber (23) via said second orifice (32); and wherein

- said first chamber (21) communicates with the outside via a first hole (41);

- said second chamber (22) communicates with the outside via a second hole (42);

- said third chamber (23) communicates with the outside via a third hole (43).

4. A device as claimed in claim 1, 2 or 3, wherein said first, second and third electrodes (51, 52, 53) are tubular elements adapted to allow the passage of the electrolytic solution.
5. A device as claimed in claim 1, 2 or 3, wherein said first, second and third electrodes (51, 52, 53) are made of silver and are plated with silver chloride (AgCl) and wherein said first and second electrodes (51, 52) receive supply voltages with alternating components, particularly a square wave form, with a duty cycle of 50%.
6. A device as claimed in any of the preceding claims, wherein said second orifice (32) has substantially and/or generally the same size as said first orifice (31).
7. A method of detecting corpuscles of nonelectrolyte particles in two flows of electrically conductive solutions, comprising the steps of:
 - creating a first electrolytic solution flow (Q1), said first flow (Q1) passing through a first orifice (31);
 - creating a second electrolytic solution flow (Q2), said second flow (Q2) passing through a second orifice (32), at least one of said first and second flows (Q1, Q2) containing nonelectrolyte particles;
 - creating a first ion current (I1) in a first section (81) through which said first flow (Q1) passes, said section (81) comprising said first orifice (31);
 - creating a second ion current (I2) that passes through a second section (82) of said second flow (Q2) passes, said second

- section (82) comprising said second orifice (32);
- obtaining a current signal (I3) which is equal to the difference between said first and second ion currents (I1, I2).
8. A method as claimed in claim 7, wherein said first and second flows (Q1, Q2) come from the same source.
9. A method as claimed in claim 7 or 8, wherein said current signal (I3) equal to the difference between said first and second ion currents (I1, I2) is converted into a voltage signal.
10. A method as claimed in claim 7, 8 or 9, wherein said two first and second ion currents (I1, I2), when no particles are contained in the solutions being monitored, have a substantially identical value.
11. A method as claimed in claim 7, 8, 9 or 10, wherein said two ion currents (I1, I2) have alternating components with a duty cycle of 50%.

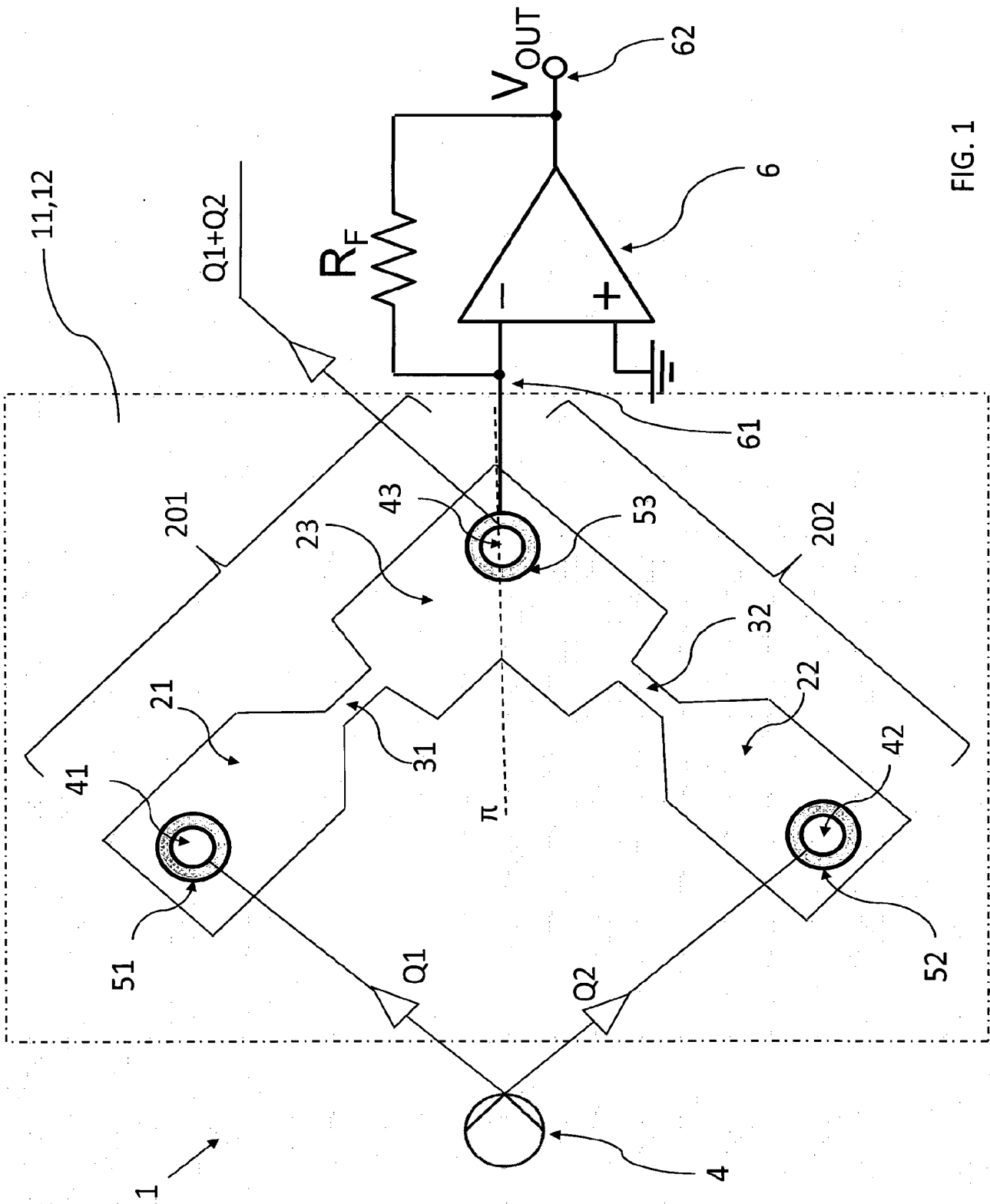


FIG. 1

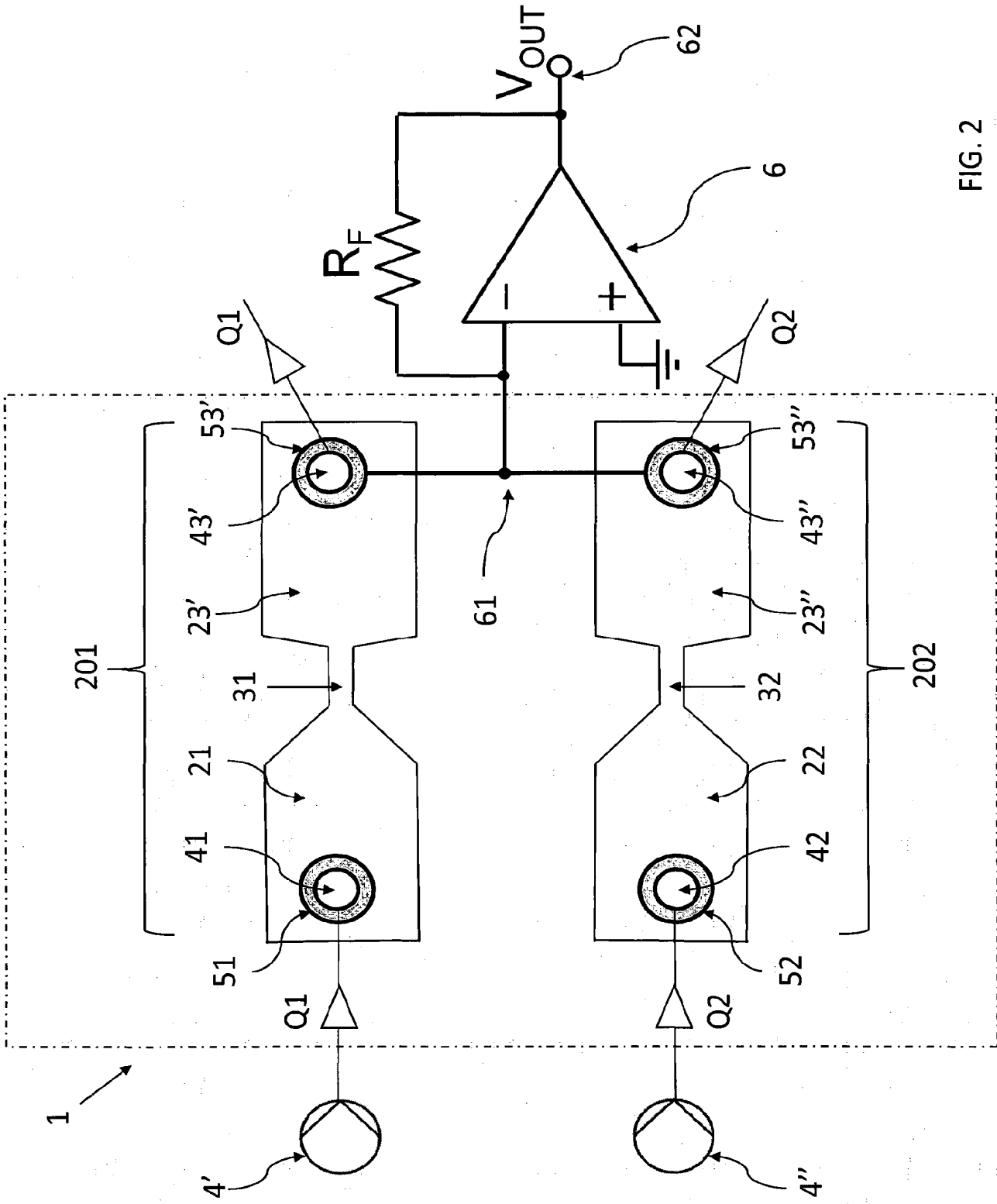


FIG. 2

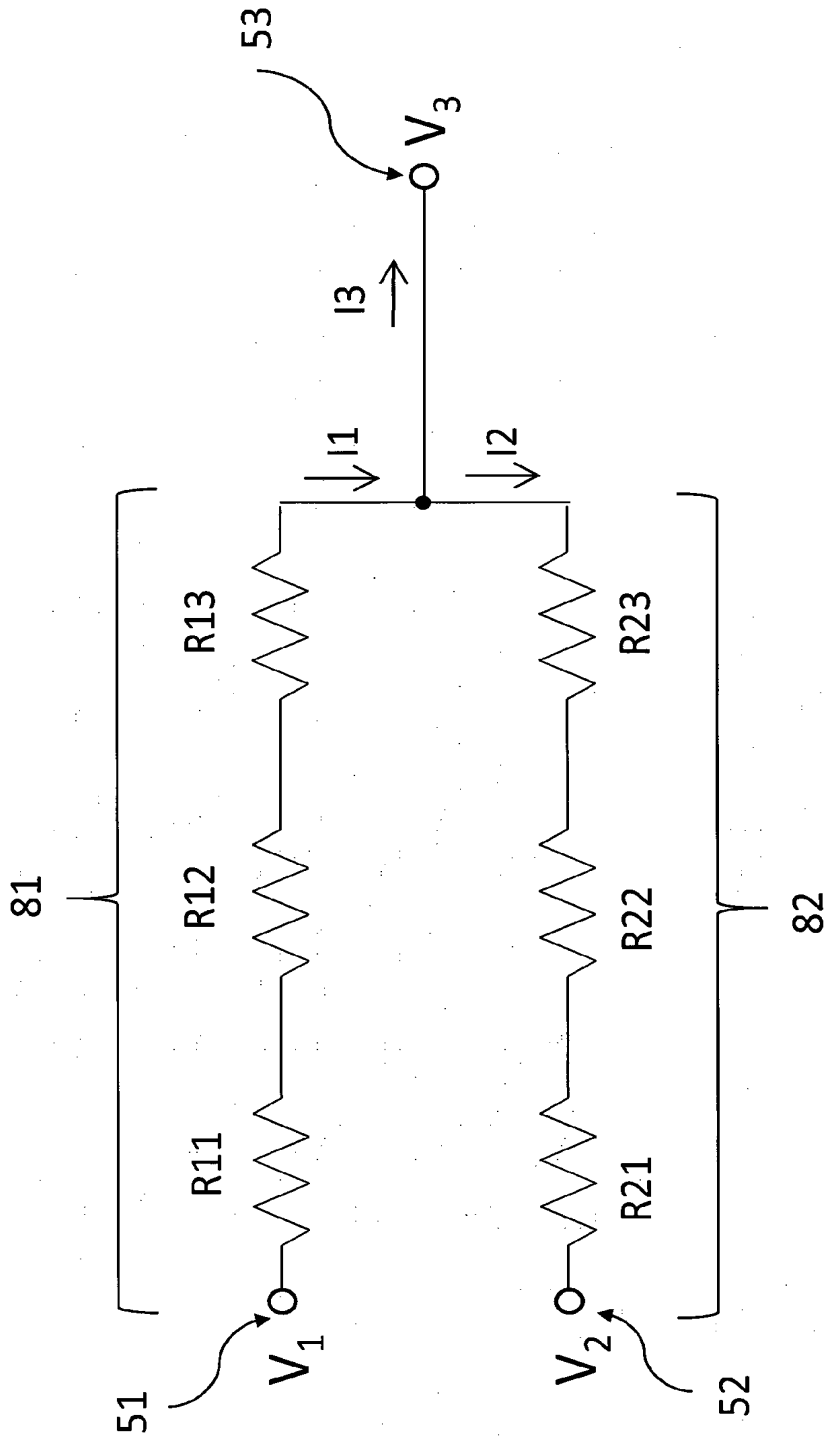


FIG. 3

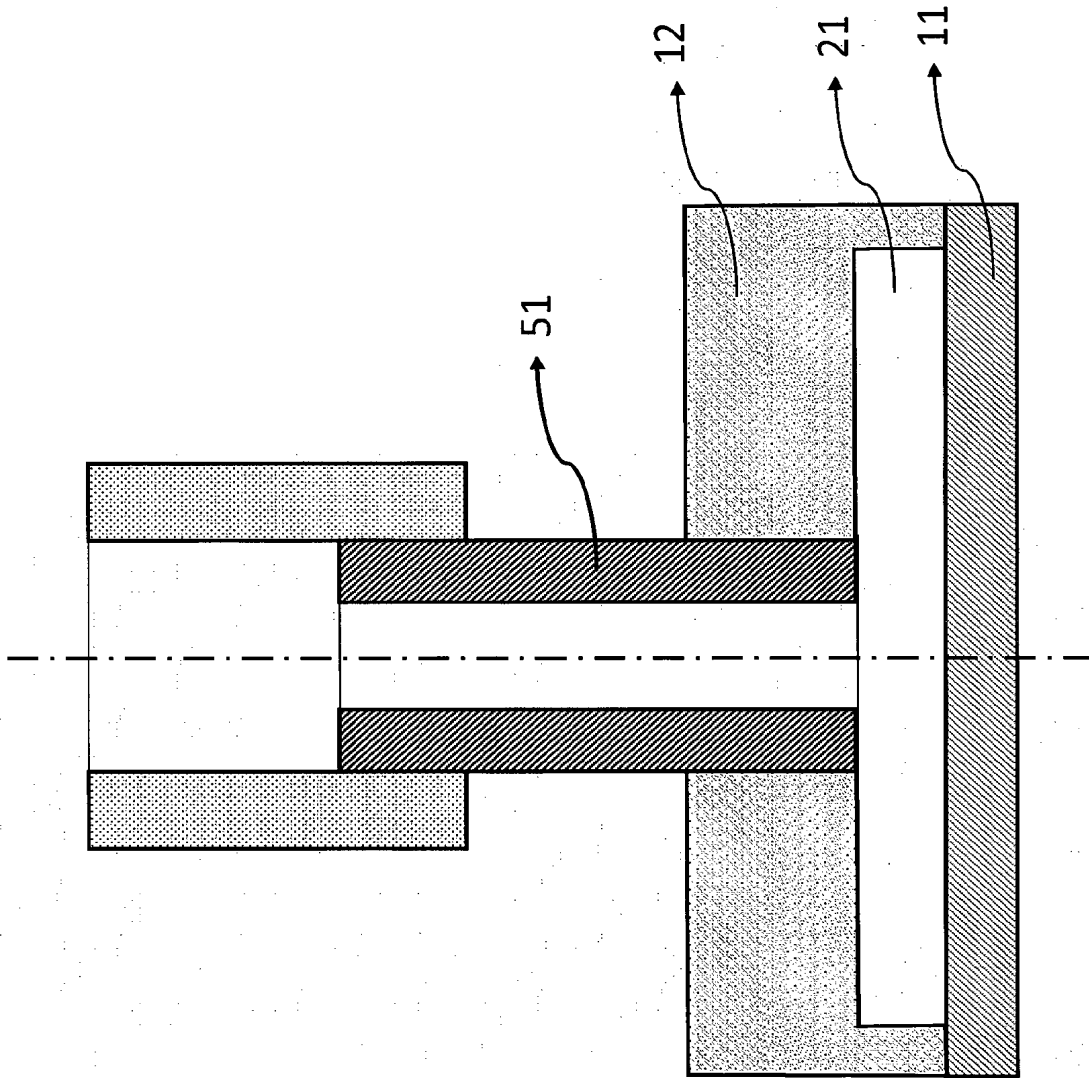


FIG. 4

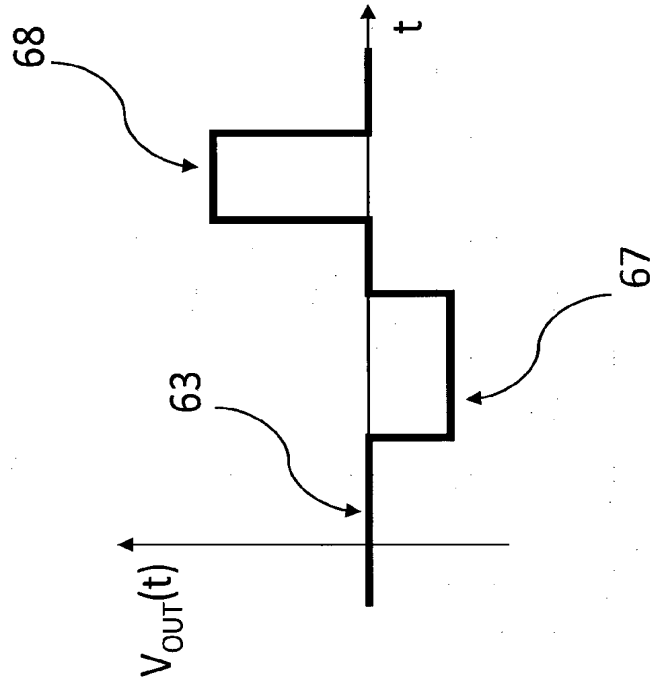


FIG. 5

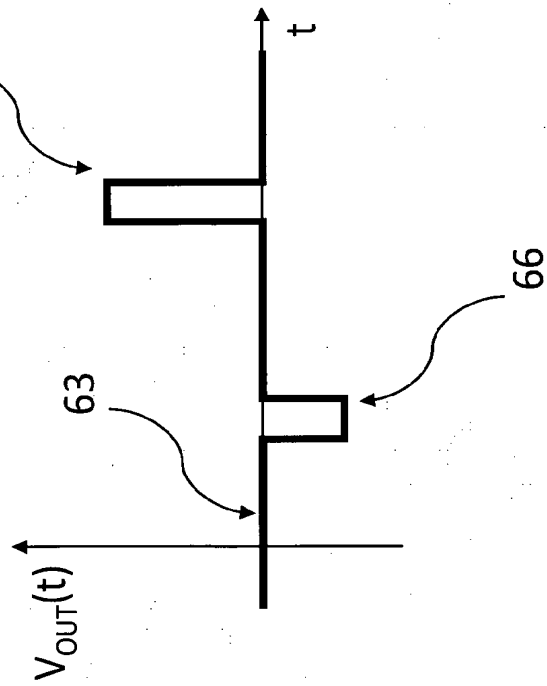


FIG. 6

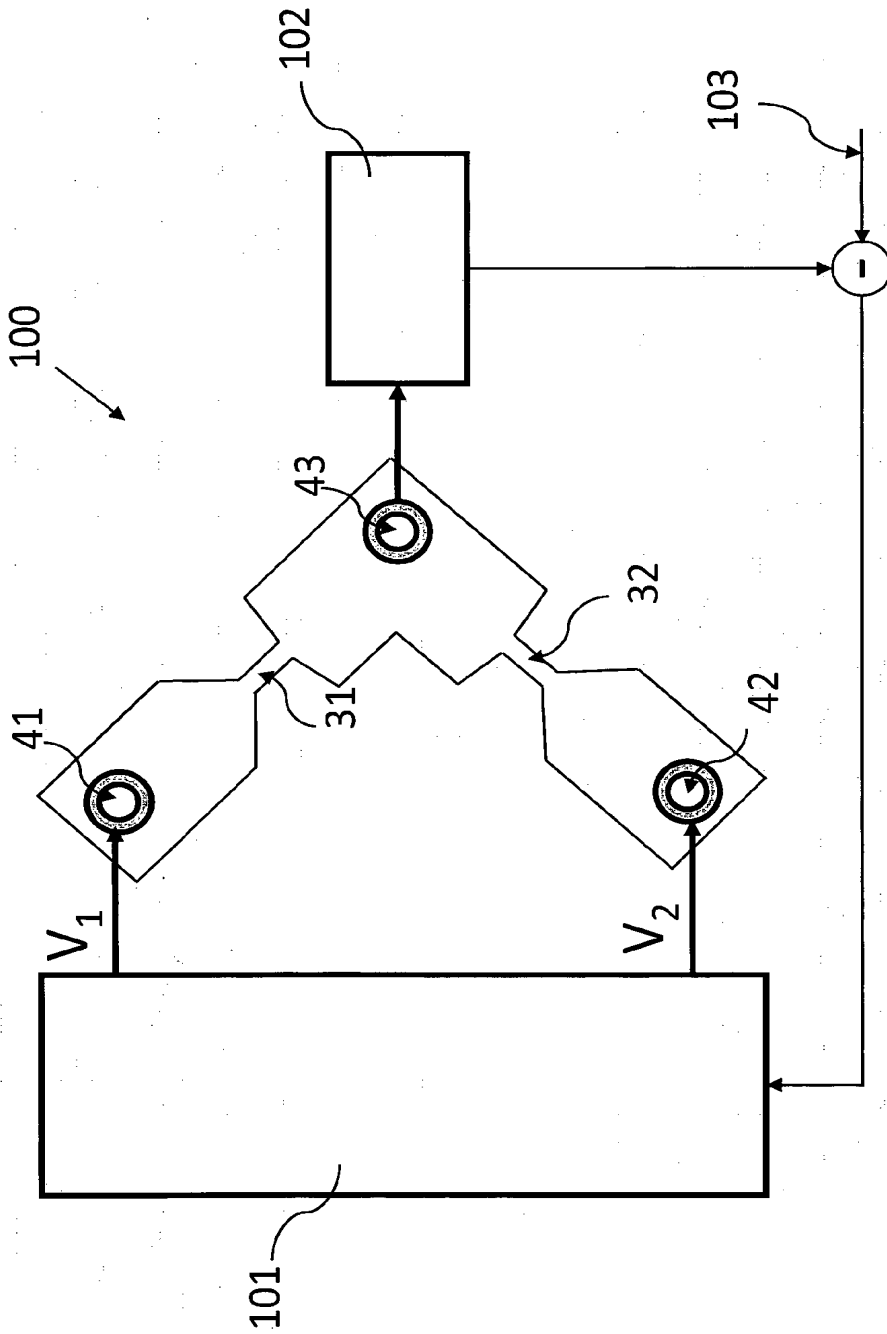


FIG. 7

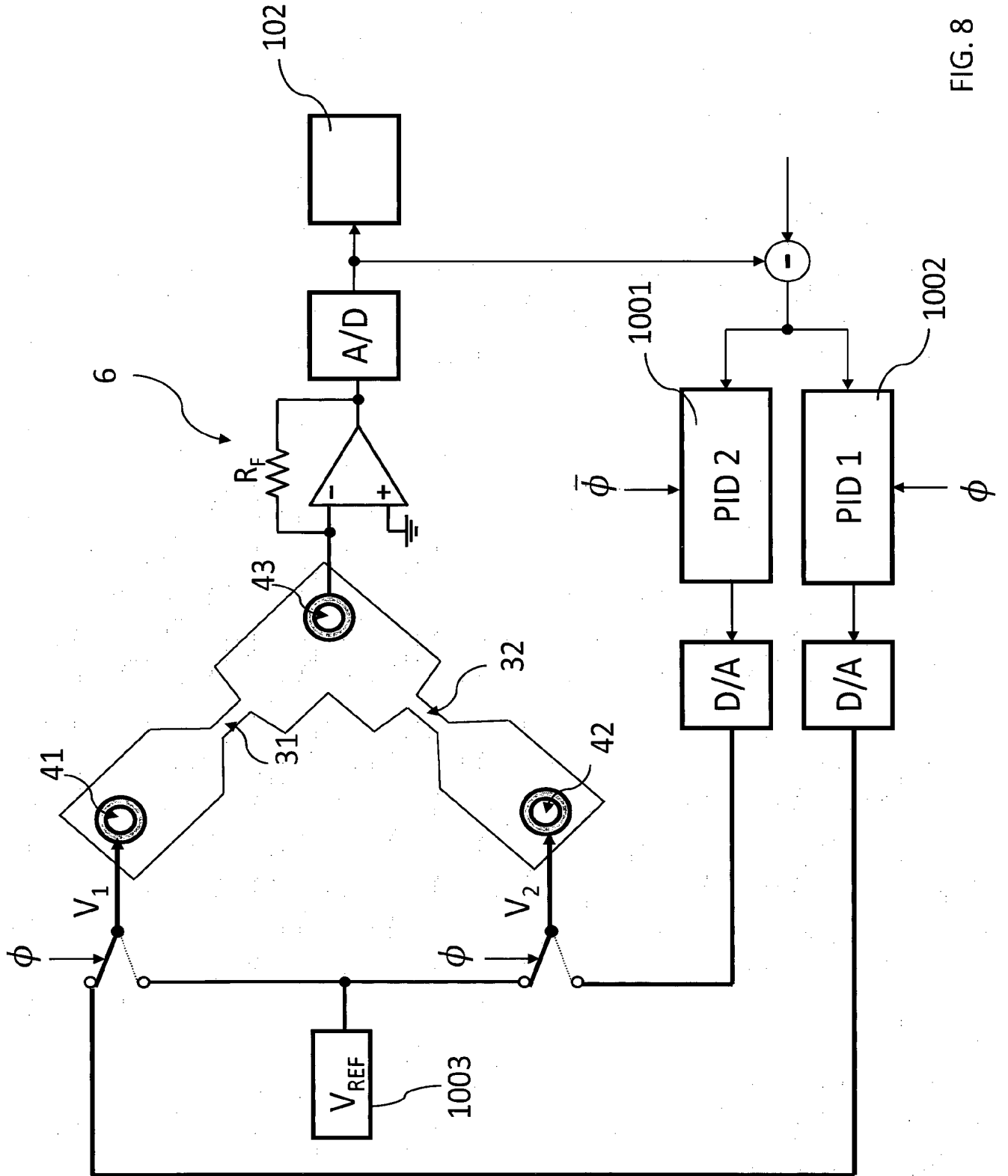


FIG. 8

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2017/000468

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N15/12
ADD.

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)
G01N

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

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

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 21 45 531 A1 (LICENTIA GMBH) 15 March 1973 (1973-03-15)	1-6
A	page 13, paragraph 1 - page 14, paragraph 1; figure 4 page 15, paragraph 2 - page 16, paragraph 1	7-11

A	US 4 296 373 A (ANGEL HENRY R ET AL) 20 October 1981 (1981-10-20) column 6; figure 4 column 1, line 63 - column 2, line 26	1-11

A	EP 0 368 241 A2 (HITACHI LTD [JP]; KIKUCHI YUJI [JP]) 16 May 1990 (1990-05-16) column 3, line 44 - column 4, line 29; figure 1	1-11

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 application or patent but 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

17 July 2017

Date of mailing of the international search report

25/07/2017

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Mauritz, Jakob

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2017/000468

Patent document cited in search report	Publication date	Publication date	Patent family member(s)	Publication date
DE 2145531	A1	15-03-1973	NONE	
US 4296373	A	20-10-1981	DE 3066583 D1	22-03-1984
			EP 0022568 A1	21-01-1981
			US 4296373 A	20-10-1981
EP 0368241	A2	16-05-1990	DE 68918223 D1	20-10-1994
			DE 68918223 T2	02-02-1995
			EP 0368241 A2	16-05-1990
			JP 2685544 B2	03-12-1997
			JP H02130471 A	18-05-1990
			US 5023054 A	11-06-1991