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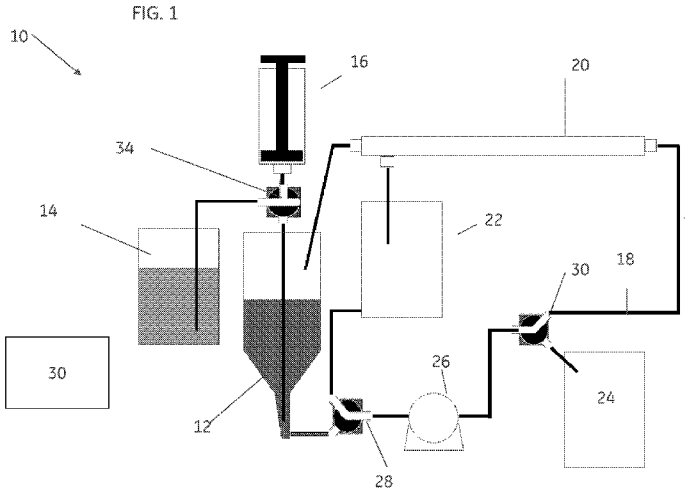
[Continued on next page]

(54) Title: SYSTEM AND METHOD FOR SEPARATING CELLS FROM BODY FLUIDS

FIG. 1



(57) Abstract: Method and systems, for processing biological material, that contain a biological material in a vessel; add an aggregating agent to the material in the vessel and allow the material to separate into two or more distinct submaterials; extract one or more of the submaterials from the vessel; automatically transport one or more of the submaterials remaining in the vessel to a filtration device; and collect a resulting target retentate into a target retentate receptacle.

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System and method for separating cells from body fluids

BACKGROUND

[0001] The invention relates generally to systems, methods and kits for processing complex biological materials into subcomponents.

[0002] Separation of red blood cells (RBC) from whole blood is commonly required prior to analysis or therapeutic use of less abundant cells, such as white blood cells or stem cells. Many conventional blood cell isolation procedures require preliminary red blood cell depletion and sample volume reduction. These are commonly required processing steps for long-term cell banking and regenerative medicine applications where a maximal yield of rare cells is desired in a reduced volume due to storage limitations and/or the small volume requirements needed for direct transplantation. Today, the most common techniques for processing blood-cell containing samples (e.g. cord blood, bone marrow, peripheral blood) involve density-gradient sedimentation using centrifugation with or without the use of a density-gradient media to improve separations. Automated centrifugal systems have recently been developed for closed-system processing of cord blood and bone marrow samples in order to meet the growing needs for high-throughput sample processing. While greatly improving throughput compared to manual techniques, centrifugation-based devices have limited flexibility and portability due to the weight and fixed physical dimensions of the centrifuge bucket.

[0003] Filtration techniques are also used in a number of blood cell separation applications. For example, depth filtration has been used for sometime to achieve removal of leukocytes from whole blood (e.g. for transfusion applications). However these filters are designed for maximal leukocyte depletion (via trapping of cells within the filter) and have not been designed for high cell recovery following the filtration step. In addition, membrane-based plasmapheresis is a common technique for removal and processing of plasma from whole blood. However, these techniques do not involve pre-depletion of the whole blood of red blood cells (RBC) prior to filtration and do not achieve the type of volume reduction that is needed in blood cell banking applications.

[0004] Sedimentation methods, either via gravity or centrifugation, are known in the art for separating different components of blood. One method to facilitate sedimentation of RBCs from

whole blood is to use polymeric large molecules, such as dextran, hetastarch, or gelatin, which are known aggregating agent for RBCs. Depending on the composition and stoichiometric ratio of the aggregating agent in blood, the speed and effectiveness of the RBC sedimentation process can vary widely. Some of the sedimentation-enhancing agents are known, such as potassium oxalate and potassium malonate. The effectiveness of these sedimentation-enhancing agents is largely determined by the concentration of the agent relative to the blood sample. Although potassium oxalate and malonate have previously been demonstrated as effective RBC sedimentation enhancing agents, the clinical utility of these agents is limited by the potential cardiovascular toxicity associated with potassium salt.

[0005]

BRIEF DESCRIPTION

[0006] The invention is adapted to address the need for a functionally closed-system high throughput system and method for processing biological materials, such as whole blood, while achieving high target cell (such as stem cell) recoveries and viabilities for downstream cell therapy applications. Filtration is a commonly used technique for blood processing application including hemodialysis and plasmaphersis but has not previously been used in blood cell banking applications where there is a need to process biological materials such as whole blood in order to remove red blood cells and excess plasma to achieve a concentrated white blood cell (WBC) sample. This is due to the challenges associated with separating abundant red blood cells from less abundant white blood cells and even less abundant stem cells of similar size. One of the embodiments of the systems and methods comprises a two-step process involving an initial RBC aggregation and gravity sedimentation step for bulk erythrocyte removal, followed by a filtration step for cell concentration and removal of excess plasma.

[0007] One embodiment of the closed system for processing biological material comprises: a vessel for containing and enabling the biological material to separate into two or more distinct submaterials; an extraction device for removing at least one of the submaterials from the vessel; a filtration device; a conduit that transports one or more submaterials between the vessel and the filtration device; and a control device for at least transporting one or more of the submaterials between the vessel and the filtration device via the conduit. The system may comprise one or more receptacles for at least temporarily storing one or more filtrates, wherein at least one of the receptacles is a waste filtrate receptacle and at least one of the receptacles is a target retentate receptacle. The system may further comprise a valve along the conduit for

selectively directing target retentate into the target retentate receptacle; and a valve along the conduit for selectively recirculating the waste filtrate at least partially through the conduit. A pump, in fluid communication with the conduit, may also be incorporated into the system for facilitating the transport of one or more submaterials between the vessel and the filtration device.

[0008] The vessel of the system may be adapted to separate the material into submaterials at least in part based on the relative weight of two or more submaterials. The submaterials may separate into sedimentary layers, wherein the extraction device is adapted to draw off or otherwise extract one or more of the sedimentary layers. In one embodiment, the extraction device is adapted to draw off a lowermost layer within the vessel, and in another embodiment, the extraction device may alternatively, or additionally, draw off an uppermost layer within the vessel, or one or more layers in between the lowermost and uppermost.

[0009] The system may further comprise a valve, in fluid communication with an agent receptacle, to selectively remove a determined amount of agent from the agent receptacle and introduce the determined amount of agent into the vessel. The extracting device in this example may be further adapted to draw a determined amount of material from the vessel, into which the agent has previously been introduced, into the extracting device and then return the drawn material back into the vessel, to facilitate mixing of the material with the agent. The system may further comprise a sensing device for determining a location or level of at least one of the submaterials in the vessel.

[0010] The entire system, or a portion of the system such as the transportation of one or more of the submaterials between the vessel and the filtration device, may be automated.

[0011] An example of the methods for processing biological material generally comprises: providing a biological material in a vessel; adding an aggregating agent to the material in the vessel and allowing the material to separate into two or more distinct submaterials; extracting one or more of the submaterials from the vessel; automatically transporting one or more of the submaterials remaining in the vessel to a filtration device via a conduit; and directing a resulting target retentateinto a target retentatereceptacle.

[0012] One example of the methods comprises processing blood samples for subsequent cryopreservation and/or direct therapeutic applications, e.g. to reduce sample volume, achieve

high recovery and viability of nucleated cells, and remove the majority of red blood cells present in the starting sample.

[0013] One example of the methods enables one to isolate a white blood cell (WBC) fraction, which comprises pluripotent stem cells, from whole cord blood, bone marrow, or peripheral blood (including GCSF stimulated peripheral blood). At least one of the example methods of the invention is capable of achieving high leukocyte recoveries (>80%), >95% CD34 recovery, and high leukocytecell viabilities (>95%), while providing flexibility in handling a broad range of starting volumes and sample types based on adjustment of filtration times and filter cartridges used.

[0014] Unlike current methods, the methods and systems of the invention enable automated processing of complex biological fluids without requiring users to purchase and use a separate centrifuge. The methods and systems of the invention are also readily adaptable to handle a range of starting volumes, to concentrate a sample to a user-specified final volume, and for use in multiplexing processes (e.g. increasing/decreasing number of samples processed/run).

[0015] In general, the methods and kits of the invention provide sedimentation-enhancing agents that are biocompatible and significantly increase the efficiency of blood separation methods and systems and thereby increase the recovery of total nucleated cells (TNC). At the concentration range specified, these sedimentation-enhancing agents are considered non-toxic and safe to use in vivo.

[0016] One or more examples of the method to sediment cells in a sample comprising blood cells comprises adding an aggregating agent; and a non-toxic enhancer having a final concentration range from about 10 mM to about 100 mM.

[0017] In some of the examples of the method to sediment cells is provided in a sample comprising blood cells comprises addition of an aggregating agent and the non-toxic enhancer comprises sodium citrate or sodium succinate or a combination thereof.

[0018] In some embodiments of the kit to sediment cells, the kit comprises an aggregating agent; and a non-toxic enhancer wherein the non-toxic enhancer comprises sodium citrate or sodium succinate or a combination thereof.

[0019] In some embodiments of the kit to sediment cells, the kit comprises an aggregating agent wherein the aggregating agent is selected from the group consisting of dextran, hetastarch or gelatin and a non-toxic enhancer wherein the non-toxic enhancer comprises sodium citrate or sodium succinate or a combination thereof.

[0020] Some embodiments of the method to sediment cells improve the resulting recovery of an increased percentage of total nucleated cells from a sample comprising red blood cells, wherein the method comprises the steps of adding an aggregating agent, a non-toxic enhancer, incubating the sample to aggregate plurality of RBCs, and recovering the total nucleated cells.

DRAWINGS

[0021] These and other features, aspects, and advantages of the present invention will become better understood when the following detailed description is read with reference to the accompanying drawings in which like characters represent like parts throughout the drawings, wherein:

[0022] FIG. 1 is a schematic drawing of an embodiment of the system of the invention showing a biological sample and an RBC aggregating agent (w/ or w/o enhancer) in a mixing vessel and an agent receptacle, respectively.

[0023] FIG. 2 is a schematic drawing of the embodiment shown in FIG. 1 showing the aggregating agent (w or w/o enhancer) drawn into an extraction device.

[0024] FIG. 3 is a schematic drawing of the embodiment shown in FIG. 2 showing the aggregating agent (w or w/o enhancer) agent mixed into the biological sample in the vessel.

[0025] FIG. 4 is a schematic drawing of the embodiment shown in FIG. 3 showing a portion of the agent/sample mixture drawn into the extraction device.

[0026] FIG. 5 is a schematic drawing of the embodiment shown in FIG. 4 showing the drawn portion returned to the vessel.

[0027] FIG. 6 is a schematic drawing of the embodiment shown in FIG. 5 showing the mixture in a state of settling.

[0028] FIG. 7 is a schematic drawing of the embodiment shown in FIG. 6 showing the lowermost layer of the settled mixture drawn into the extraction device.

[0029] FIG. 8 is a schematic drawing of the embodiment shown in FIG. 7 showing a syringe valve between the extraction device and the vessel in a closed position.

[0030] FIG. 9 is a schematic drawing of the embodiment shown in FIG. 8 showing a pump valve between the vessel and a pump in an open position and the mixture flowing through the system from the vessel through a conduit to a filtration device.

[0031] FIG. 10 is a schematic drawing of the embodiment shown in FIG. 9 showing the filter waste being collected in a waste filtration receptacle and the sample recirculating through the system.

[0032] FIG. 11 is a schematic drawing of the embodiment shown in FIG. 10 showing the pump inlet valve in a closed position relative to the vessel and in an open position relative to the waste filtration receptacle and the waste filtrate recirculating through the conduit and filtration device.

[0033] FIG. 12 is a schematic drawing of the embodiment shown in FIG. 11 showing the waste filtrate pumped through the system until it has replaced a target retentate trapped in the fluid path.

[0034] FIG. 13 is a schematic drawing of the embodiment shown in FIG. 12 showing the pump inlet valve in a closed position relative to the waste filtrate receptacle and in an open position relative to the vessel, and a pump outlet valve, between the pump and a target retentate receptacle, in an open position.

[0035] FIG. 14 is a schematic drawing of the embodiment shown in FIG. 13 showing the target retentate being collected in the target retentate receptacle.

[0036] FIG. 15 is a schematic drawing of the embodiment shown in FIG. 14 showing the remaining amount of the target retentate at the bottom of vessel being transported to the target retentate receptacle.

[0037] FIG. 16 is a schematic drawing of the embodiment shown in FIG. 15 showing a residual amount of target retentate in the conduit between the vessel land the target retentate receptacle.

[0038] FIG. 17 is a schematic drawing of the embodiment shown in FIG. 16 showing the pump inlet value in an open position relative to the waste filtrate receptacle.

[0039] FIG. 18 is a schematic drawing of the embodiment shown in FIG. 17 showing the waste filtrate being transported through the conduit until the waste filtrate has pushed the residual target retentate in the conduit into the target retentate receptacle.

[0040] FIG. 19 is a schematic drawing of the embodiment shown in FIG. 18 showing the pump outlet value in a closed position relative to the target retentate receptacle and the waste filtrate being transported to the vessel.

[0041] FIG. 20 is a schematic drawing of the embodiment shown in FIG. 19 showing the waste filtrate and any remaining target retentate in the system being collected in an auxiliary filtrate receptacle.

[0042] FIG. 21 is a graph and table showing examples of the volume of recovered TNC for dextran alone, dextran combined with sodium citrate and dextran combined with sodium succinate.

[0043] FIG. 22 is a graph showing an example of the sedimentation efficiency of sodium citrate.

DETAILED DESCRIPTION

[0044] To more clearly and concisely describe and point out the subject matter of the claimed invention, the following definitions are provided for specific terms, which are used in the following description and the appended claims. Throughout the specification, exemplification of specific terms should be considered as non-limiting examples.

[0045] As used herein, the term "vessel" refers to any object capable of containing a liquid within its confines for at least a temporary period of time having at least one port.

[0046] As used herein, the term "biological material" refers to any material of a biological nature that can be aggregated into two or more submaterials. Non-limiting examples of biological materials are whole blood, cord blood and bone marrow that can be separated via aggregation and sedimentation/removal of RBCs while nucleated cells remain in a plasma solution. Nucleated cells include WBCs and rare stem cells.

[0047] One embodiment of the methods for processing nucleated cells generally comprises the separation and enrichment of nucleated cells, such as, but not limited to, rare stem cells, from cell samples including, but not limited to, blood and bone marrow. The filtration-based embodiment comprises two general steps. The first step comprises contacting the cell sample with a settling solution, such as a red blood cell aggregating agent (e.g. Dextran) with or without the addition of an enhancing agent (e.g. sodium citrate, sodium succinate). The enhancing agent in this example embodiment is added to enhance the RBC sedimentation rate and/or reduce the final RBC packed volume following sedimentation. Subsequently the aggregated RBCs are removed from the upper fraction containing plasma and nucleated cells by drainage, drawing off or other suitable means of transfer. The second step comprises volume reduction and nucleated cell concentration by filtering the RBC-depleted sample. One example of filtration uses a hollow-fiber filtration cartridge (General Electric Healthcare, Piscataway, NJ). This embodiment provides high cell recoveries (e.g. minimal cell trapping), minimal cell damage, and fast processing times. This example of the method is adaptable for use in the automated closed-system system. The methods and systems are adaptable for sterile processing of complex biological materials such as but not limited to cord blood and other cell sample materials.

Example

[0048] The two-step automated example methods, that combine separation followed by filtration, rather than mere filtration, centrifugation, or magnetic separation alone, provide (1) increased total nucleated cell (TNC) recovery, (2) increased RBC removal, and (3) greater flexibility in handing a range of sample volumes (e.g. 50 to 300mL blood) than centrifugation due to the fixed physical dimensions of the centrifuge's sample holder. Unlike a centrifuge with a fixed sized, the filters used in the systems and methods may be scaled according to the sample volume.

[0049] The volume of starting material is determined (e.g. by weight, visual inspection). The required amount of RBC aggregating reagents is calculated based on the desired stoichiometric ratio (typically 1:1 or 1:2, blood to Dextran).

[0050] The cell sample starting material is transferred to a processing vessel. The RBC aggregating reagent(s) are also transferred to the processing vessel. The sample and reagents are then mixed and allowed to incubate ~ 20min for RBC aggregation and gravity sedimentation.

[0051] The aggregate RBC fraction is then extracted from the vessel from the upper white blood cell (WBC)/plasma fraction (e.g. by pumping, pipetting, or drainage). The remaining WBC/plasma fraction is then transferred (e.g. by pumping, positive or negative pressure) to a filtration device (e.g. hollow fiber cartridge) having a suitable pore size (e.g. approximately 0.65 um pores). Excess plasma passes through the filtration device and is collected in a waste filtrate receptacle, while WBCs are retained.

[0052] The fraction sample is recirculated through the filtration device until the sample volume is concentrated to the desired final volume (e.g. 5-20 ml). The sample is transferred to a target retentate receptacle, typically for longterm cryo-storage. The filter and tubing is then purged to recover cells present in this "dead volume", typically using plasma and/or air. This material is then added to the concentrated sample. High total nucleated cell recovery (>85%) and viability (>95%) are achieved.

[0053] FIG. 1 is a schematic drawing of an embodiment of the system showing a biological sample and an enhancing agent in a mixing vessel and an agent receptacle, respectively. The embodiment of the system shown and generally referred to in FIG. 1 as system 10 comprises vessel 12 for containing and enabling the biological material to separate into two or more distinct submaterials; extraction device 16 for removing at least one of the submaterials from the vessel; filtration device 20; conduit 18 that transports one or more submaterials between the vessel and the filtration device; and control device 30 for controlling at least the transporting of one or more of the submaterials between the vessel and the filtration device via the conduit. This embodiment is only an example configuration of the system. The number and type of components can be varied as needed for a given set up and the order and flow of materials through the system may be varied as well as needed. For example, the materials may be extracted, stored, flushed and mixed using various configurations and components.

[0054] In the embodiment shown in FIG. 1, vessel 12 has an opening at the top through which the extracting device, which in this example is a syringe that is in fluid communication with valve 34, introduces the agents and withdraws materials and submaterials from vessel 12 at various times during the process.

[0055] System 10 also comprises receptacles for at least temporarily storing one or more filtrates. One of the receptacles in this embodiment is waste filtrate receptacle 22 and target retentate receptacle 24. System 10 further comprises valve 30 along the conduit for selectively directing target retentate into the target retentate receptacle; valve 28 along the conduit for selectively recirculating the waste filtrate at least partially through the conduit, and valve 34 for selectively introducing one or more agents into vessel 12, extracting materials from vessel 12 to mix the agents with the sample, and extracting one or more of the submaterials from vessel 12 after aggregation of the submaterials.

[0056] System 10 may also comprise one or more sensors such as sensor 32. Sensor 32 may be used to sensing one or more parameters of the materials in vessel 12 including but not limited to the presence of a submaterial at a given location within the vessel; the environmental conditions within the vessel such as but not limited to, temperature, pH, humidity, and pressure; and qualities or characteristics of the biological materials or submaterials. The sensors may be, but are not limited to, optical sensors, ultrasonic sensors, piezoelectric sensors, motion sensors, RFID sensors, electromagnetic sensors and load sensors.

[0057] System 10 also comprises a control subsystem 30 (also referred to herein as a controller) for automating and coordinating pump 26, extraction device 16 and valves 28, 34 and 30. Control subsystem 30 may also be configured to receive input from the user of the system and automatically determine the amount and/or type of agents to be added to vessel 12 based on the amount and type of materials introduced into vessel 12 to be process using the system. The system may be fully or partially automated by the control subsystem depending on the configuration of the a given system. The agents may be contained within a removable cassette that is inserted into a port in the system as needed depending on the type or amount of materials and submaterials to be processed.

[0058] System 10 further comprises pump 26, in fluid communication with the conduit, to facilitating the transport of one or more submaterials between the various components of the system. Pump 26 in this embodiment is a peristaltic pump but may comprise any type of pump suited to the configuration of the system.

[0059] Vessel 12 of the system may be adapted to separate the material into aggregated submaterials at least in part based on the relative weight of two or more submaterials. The submaterials separate into sedimentary layers and the extraction device in this embodiment is adapted to draw off or otherwise extract one or more of the sedimentary layers. In the

embodiment shown in FIG. 1, extraction device 16 comprises a pick up line with a distal end located towards the bottom of vessel 12 to draw off a lowermost layer within the vessel once the submaterials have separated into their respective sedimentary layers. The extraction device may alternatively, or additionally, draw off an uppermost layer within the vessel, or one or more layers in between the lowermost and uppermost, depending on the configuration of the extraction device relative to the vessel.

[0060] Syringe 16 together with valve 34, in fluid communication with agent receptacle 14, selectively remove a determined amount of agent from the agent receptacle and introduce the determined amount of agent into vessel 12 as shown in FIGs. 2 and 3. The extracting device in this example may be further adapted to draw a determined amount of material from the vessel, into which the agent has previously been introduced, into the extracting device and then return the drawn material back into the vessel, to facilitate mixing of the material with the agent as shown in FIGs. 4 and 5. To carry out the mixing step, valve 34 closes relative to receptacle 14 and opens relative to vessel 12 open the fluid communication between syringe 16 and vessel 12. Once the aggregating agents are mixed with the materials (e.g. whole blood) in vessel 12, the mixture typically needs time to settle into its various sedimentary layers. For whole blood or cord blood mixed, for example, with Dextran and sodium citrate, settling should occur within 20 minutes as shown in FIG. 6.

[0061] A sensing device such as sensor 32 may be used for determine when the submaterials have aggregated and separated into their respective layers by determining the location or level of at least one of the submaterials in the vessel.

[0062] Non-limiting examples of possible agents, for use in this example in which whole blood is being processed, are dextran (an aggregant), and sodium citrate and sodium succinate, which both enhance aggregation. These three examples of agents enhance the methods and systems by acting as aggregating agents and/or aggregation enhancing agents to initiate and accelerate the aggregation and sedimentation of the different types of submaterials, such as WBCs and RBCs, in the biological sample.

[0063] FIG. 7 is a schematic drawing of the embodiment shown in FIG. 6 showing the lowermost layer of the settled mixture drawn into syringe 16. The system may be configured to extract one or more of the layers, such as RBCs shown in FIG. 7, until one or more of the layers reaches a predetermined set point. Sensor 32 may be used to determine when a set point is reached. Once the RBCs are withdrawn into syringe 16, valve 34 closes between syringe 16 and

vessel 12 to prevent the RBCs from leaking back into the vessel. FIG. 8 is a schematic drawing of the embodiment shown in FIG. 7 showing valve 34 between the extraction device and the vessel in a closed position.

[0064] FIG. 9 is a schematic drawing of the embodiment shown in FIG. 8 showing pump valve 28 between the vessel and pump 26 in an open position and the mixture flowing through the system from vessel 12 through conduit 18 to filtration device 20. As the mixture is filtered through filtration device 20, the filter waste, which in this example is plasma, is collected in waste filtration receptacle 22 as shown in FIG. 10. The system may be configured to continue recirculating the sample that passes through filtration device 20 until the volume of the sample recirculating through vessel 12 reaches a predetermined level. For example, a sensor may be used to optically monitor the concentration level of total nucleated cells (TNC) in vessel 12.

[0065] To clear filtration device 16, pump inlet valve 28 is closed relative to the vessel and opened relative to waste filtration receptacle 22 to allow the waste filtrate (plasma in this example) to recirculating through conduit 18 and filtration device 20 (FIG. 11). FIG. 12 is a schematic drawing of system 10 shown in FIG. 11 showing the waste filtrate pumped through the system until it has replaced a target retentate (e.g. TNCs) trapped in the fluid path.

[0066] The target retentate comprises one or more of the submaterials that intended to be separated from the biological material and collected in target retentate receptacle 24. In this example, the target retentate comprises TNC. Target retentate receptacle may be any receptacle suited for a given purposes such as collection bags for the various blood components. There may be a plurality of waste and target retentate receptacles depending on the materials being processed. Alternatively or in addition to a plurality of receptacles, the waste and target retentate receptacles may be interchangeable from one process to another and even during a single processing session when there are more than one submaterials that are desired to be collected. The system may also comprise a series of filtration devices and waste and target retentate receptacles, to capture and sort varying types of submaterials within a given starting material.

[0067] FIG. 13 shows system 10 following the step shown in FIG. 12, showing pump inlet valve 28 in a closed position relative to the waste filtrate receptacle and in an open position relative to the vessel, and pump outlet valve 30, between the pump and a target retentate receptacle, in an open position so that the target retentate is collected in the target retentate receptacle as shown in FIG. 14.

[0068] As shown in FIG. 15, even the small remaining amount of the target retentate at the bottom of vessel can be transported to the target retentate receptacle. However, a residual amount of target retentate may remain in the conduit between the vessel and the target retentate receptacle as shown in FIG. 16. To flush and collect this residual target retentate in the conduit, pump inlet valve 28 is opened relative to the waste filtrate receptacle (FIG. 17) to allow the waste filtrate to be pumped through conduit 18 until the waste filtrate has pushed the residual target retentate in the conduit into the target retentate receptacle (FIG. 18).

[0069] As a final flush of the system, the waste filtrate (e.g. plasma) may be flush through the system. As shown in FIG. 19, the pump outlet valve is closed relative to the target retentate receptacle and the waste filtrate is pumped through the system to flush out the entire system and to collect any remaining submaterials in the vessel. This collection of submaterials in the vessel may then be collected in an auxiliary filtrate receptacle that is interchangeable, or in addition to, target retentate receptacle 24 (FIG. 20).

[0070] The filtration device of the system shown in FIG. 1 is capable of isolating a cell fraction from a complex biological fluid such as peripheral blood, cord blood, and/or bone marrow. An example of a method for making the filtration device of system 10 is provided below.

[0071] System 10 may comprise other auxiliary components such as a memory storage device for storing information and data about the various materials, submaterials, and agents that may be processed through the system, and information about the mechanical and environmental variables to which the system may be adapted. The system may be programmed to intuitively adjust the mechanics and conditions of a given process in response to information and data collected by the sensors of the system. The memory storage device may comprise any suitable hard drive memory associated with the processor such as the ROM (read only memory), RAM (random access memory) or DRAM (dynamic random access memory) of a CPU (central processing unit), or any suitable disk drive memory device such as a DVD or CD, or a zip drive or memory card or stick. The memory storage device may be remotely located from the system and yet still be accessed through any suitable connection device or communications network including but not limited to local area networks, cable networks, satellite networks, and the Internet, regardless whether hard wired or wireless. The processor or CPU may comprise a microprocessor, microcontroller and a digital signal processor (DSP).

[0072] The system may further comprise an entry device and a display device to enable a user to input information into the system and to access and display information and data about a given process run or a plurality of runs, to compile information and data, and/or to generate reports. The display device may comprise any suitable device capable of displaying a digital image such as, but not limited to, devices that incorporate an LCD or CRT.

[0073] Unless otherwise indicated, the article "a" refers to one or more than one of the word modified by the article "a." Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, so forth used in the specification and claims are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

[0074] "Aggregating agent" is referred to herein as the molecules that help to facilitate aggregation of blood cells. Examples of aggregating agents include, but are not limited to, high molecular weight polymeric molecules such as certain proteins like fibrinogen or gamma globulin; gelatin, and certain polysaccharides like dextran, hetastarch, pentastarch, and poly ethylene glycol (PEG).

[0075] "Kit" is referred to herein as one or more reactants necessary for a given assay or test, set of directions to use the reactants present in the kit, any buffers necessary to maintain reaction conditions and other optional materials such as spin column or eppendorf tube.

[0076] The methods and kits of the invention to sediment blood cells generally comprise adding one or more non-toxic enhancers, such as sodium citrate or sodium succinate, to accelerate RBC sedimentation. Since, sodium citrate and sodium succinate have already been parenterally used in medical practices, the non-toxic enhancers of these methods and kits are safe for human in vivo applications and the recovered cells after sedimenting RBC may be used for therapeutic purposes.

[0077] One or more examples of the methods for enhancing sedimentation of red blood cells increase the recovery of highly purified cells such as TNCs having high cell viability that is desirable for various therapeutic applications.

[0078] The non-toxic enhancers used in one or more of the methods increase the rate of sedimentation. Non-limiting examples of non-toxic enhancers used in one or more of the methods are sodium citrate, sodium succinate and combinations thereof.

[0079] In some examples, the method to sediment cells comprises providing a sample comprising blood cells treated by adding an aggregating agent and a non-toxic enhancer in various concentration ranges. Examples of suitable concentration ranges include, but are not limited to 10 mM to 100 mM, 12.5mM to 75mM, 25mM to 75mM, and 50 mM to 75 mM.

[0080] In some examples, a method to sediment cells in a sample includes providing blood cells that are treated by addition of an aggregating agent and a non-toxic enhancer having a final concentration ranges from about 12.5 mM to about 100 mM, wherein the non-toxic enhancer is sodium citrate, sodium succinate or a combination thereof. In some embodiments, the aggregating agent comprises dextran and the non-toxic enhancer comprises sodium citrate, sodium succinate or a combination thereof.

[0081] One or more of the embodiments of the kit to sediment cells comprises an aggregating agent; and a non-toxic enhancer. One or more of the embodiments of the kit for aggregating cells comprises an aggregating agent, and a non-toxic enhancer wherein the non-toxic enhancers comprise sodium citrate or sodium succinate or a combination thereof. One or more of the embodiments of the kit for aggregating cells comprises an aggregating agent wherein the aggregating agent is dextran, and a non-toxic enhancer.

[0082] The methods of recovering cells with high purity and viability generally use an aggregating agent in combination with a non-toxic enhancer for sedimentation. For example, in one of the examples, a sample that includes red blood cells, is treated by adding an aggregating agent and a non-toxic enhancer, followed by incubation of the sample, and eventual recovery of the TNCs.

[0083] One or more of the methods of recovering a percentage of TNCs from a sample comprising red blood cells comprises adding an aggregating agent and a non-toxic enhancer at a predetermined concentration followed by incubation of the sample, and eventually recovering of

the total nucleated cells. In certain embodiments, the enhancer is sodium citrate or sodium succinate or a combination thereof.

EXAMPLES

[0084] Practice of the invention will be more fully understood from the following examples, which are presented herein for illustration only and should not be construed as limiting the invention in any way.

Example 1

[0085] Materials: Human peripheral blood was used for the experiments. The dextran T500 used in this example was obtained from Pharmacosmos A/s, Denmark; sodium citrate dihydrate was obtained from J T Baker; and sodium succinate was obtained from Sigma, St. Louise, Missouri.

[0086] The extent of red blood cell aggregation was measured in vitro in the presence of different biocompatible enhancers. A control sample, without an aggregation enhancer, was prepared by mixing 2.4 ml of a blood sample with 2.4 ml of phosphate buffered saline (PBS) containing 3% Dextran T500, and then incubated (the final concentration of dextran was 1.5%). Two test samples were also prepared. The first test sample was prepared by mixing 2.4 ml of the blood sample with 2.4 ml of PBS containing 3% Dextran T500 and 100mM sodium citrate, and then incubated (the final concentration of dextran was 1.5% and the final concentration of sodium citrate was 50mM). The second test sample was prepared by mixing 2.4 ml of the blood sample with 2.4 ml of PBS containing 3% Dextran T500 and 100mM sodium succinate, and then incubated (the final concentration of dextran was 1.5% and the final concentration of sodium succinate was 50mM). The incubation time for the control and test samples was about 20 minutes, at room temperature.

[0087] After sedimentation of red blood cells, the supernatant was recovered. The volume of the supernatant recovered was then measured. Each experiment was repeated three times (n=3) and the standard deviation for each set was calculated. The final data is presented as a bar graph in Fig.21. The higher value of standard deviation for the control (only dextran) is likely due to less compaction and the reduced recovery of supernatant.

[0088] Fig.21 depicts an example of the effect of sodium citrate and sodium succinate on red blood cell aggregation. The volume of supernatant recovered in the presence of dextran, serves

as a control, to which the volume of supernatant recovered in the presence of sodium citrate and dextran or in the presence of sodium succinate and dextran are compared. The extent of aggregation reflects the recovered supernatant volume. Increased compaction leads to better aggregation resulting in better supernatant recovery. The experiment was performed at room temperature and the incubation time for aggregation in this example was about 20 min.

Example 2

[0089] The efficiency of red blood cell aggregation was measured in vitro in the presence of varying concentration of non-toxic enhancer. A blood sample incubated with 1.5% Dextran T500, without an enhancer, served as a control. The control sample was prepared by mixing 2.0 ml of the blood sample with 2.0 ml of PBS containing 3% Dextran T500. Blood samples containing 1.5% Dextran T500 and 12.5 mM, 25mM, 50mM, 75mM and 100mM of sodium citrate as the enhancer served as the test samples. The test samples were prepared by mixing 2.0 ml of blood sample with 2.0 ml of PBS containing 3.0% Dextran T500 and 25mM, 50mM, 100mM and 150mM of sodium citrate, respectively, to reach final concentrations of 1.5% for dextran in each test sample, and 12.5mM, 25mM, 50mM and 75mM, for the respective test samples. The samples for control and test sets were incubated for 20 minutes, at room temperature.

[0090] After sedimentation of red blood cells, the fluid was recovered. The volume of the supernatant recovered was then measured. Each experiment was repeated three times (n=3) and the standard deviation for each set was calculated. The final data is presented as a bar graph in Fig. 22.

[0091] While only certain features of the invention have been illustrated and described herein, many modifications and changes will occur to those skilled in the art. It is, therefore, to be understood that the appended claims are intended to cover all such modifications and changes as fall within the true spirit of the invention.

CLAIMS:

1. An system for processing biological material comprising,
a vessel for containing and enabling the biological material to separate into two or more distinct submaterials;
an extraction device for removing at least one of the submaterials from the vessel;
a filtration device;
a conduit through which one or more submaterials are transported between the vessel and the filtration device; and
a control device for at least transporting one or more of the submaterials between the vessel and the filtration device via the conduit.

2. The system of claim 1, further comprising one or more receptacles for at least temporarily storing one or more filtrates.

3. The system of claim 2 or 3, wherein one of the receptacles is a waste filtrate receptacle.

4. The system of any preceding claim, wherein one of the receptacles is a target retentate receptacle.

5. The system of claim 1, further comprising a pump, in fluid communication with the conduit, for facilitating the transport of one or more submaterials between the vessel and the filtration device

6. The system of any preceding claim, further comprising a valve along the conduit for selectively directing target retentate into the target retentate receptacle.

7. The system of any preceding claim, further comprising a valve along the conduit for selectively recirculating the waste filtrate at least partially through the conduit.

8. The system of any preceding claim, wherein the vessel is adapted to separate the material into submaterials at least in part based on the relative weight of two or more submaterials.

9. The system of any preceding claim, wherein the vessel is adapted to separate the submaterials into sedimentary layers.

10. The system of claim 9, wherein the extraction device is adapted to draw off one or more of the sedimentary layers.

11. The system of claim 9 or 10, wherein the extraction device is adapted to draw off a lowermost layer within the vessel.

12. The system of any one of claims 9-11, wherein the extraction device is adapted to draw off an uppermost layer within the vessel.

13. The system of any preceding claim, further comprising a valve, in fluid communication with an agent receptacle, to selectively remove a determined amount of agent from the agent receptacle and introduce the determined amount of agent into the vessel.

14. The system of any preceding claim, wherein the extracting device is further adapted to draw a determined amount of material from the vessel, into which the agent has previously been introduced, and then return the drawn material back into the vessel, to facilitate mixing of the material with the agent.

15. The system of any preceding claim, further comprising a sensing device for determining a location or level of at least one of the submaterials in the vessel.

16 The system of any preceding claim, wherein the transportation of one or more of the submaterials between the vessel and the filtration device is automated.

17 The system of any preceding claim, further comprising a sensor that determines the presence of one or more of the submaterials in the vessel.

18. A method for processing biological material comprising,
providing a biological material in a vessel;
adding an aggregating agent to the material in the vessel and allowing the material to separate into two or more distinct submaterials;
extracting one or more of the submaterials from the vessel;
automatically transporting one or more of the submaterials remaining in the vessel to a filtration device; and
collecting a resulting target retentate into a target retentate receptacle.

19. The method of claim 18, wherein the submaterials remaining in the vessel are transported to the filtration device via a conduit.

20. The method of any one of claims 18 or 19, further comprising sensing the presence of one or more of the submaterials in the vessel.

21. The method of any one of claims 18- 20, wherein one or more of the submaterials are extracted until a set point is reached.

22. The method of any one of claims 18-21, further comprising directing a resulting waste filtrate into a waste filtrate receptacle.

23. The method of claim 22, further comprising flushing the filtration device with the waste filtrate after the target retentate is collected in the target retentate receptacle.

24. The method of any one of claims 18-23, wherein the biological material comprises whole blood, cord blood or bone marrow.

25. The method of any one of claims 18-24, wherein the target retentate comprises nucleated cells.

26. The method of claim 25, wherein the nucleated cells comprise stem cells.

27. The method of any one of claims 18-25, further comprising adding an aggregation enhancing agent to the material in the vessel.

28. The method of claim 27, wherein the enhancing agent comprises citrate, succinate, a salt thereof, or a combination thereof.

29. A method to sediment cells in a sample comprising blood cells, comprising the steps of:
adding an aggregating agent; and
adding a non-toxic enhancer having a final concentration range from about 10 mM to about 100 mM.

30. The method of claim 29, wherein the non-toxic enhancer is sodium citrate or sodium succinate or a combination thereof.

31. The method of claim 29 or 30, wherein the concentration of the non-toxic enhancer ranges from about 12.5 mM to about 75 mM.

32. The method of claim 29 or 30, wherein the concentration of the non-toxic enhancer ranges from about 25 mM to about 75 mM.

33. The method of claim 29 or 30, wherein the concentration of the non-toxic enhancer ranges from about 50 mM to about 75 mM.

34. The method of any one of claims 29-33, wherein the aggregating agent is selected from the group consisting of dextran, hetastarch or gelatin and the non-toxic enhancer comprises sodium citrate or sodium succinate or a combination thereof.

35. A method to sediment cells in a sample comprising blood cells, comprising the steps of:
adding an aggregating agent, and
adding sodium citrate or sodium succinate or a combination thereof.

36. The method of claim 35, wherein the concentration of the non-toxic enhancer ranges from about 10mM to about 100mM.

37. The method of claim 35, wherein the concentration of the non-toxic enhancer ranges from about 12.5mM to about 75mM.

38. The method of claim 35, wherein the concentration of the non-toxic enhancer ranges from about 25mM to about 75mM.

39. The method of claim 35, wherein the concentration of the non-toxic enhancer ranges from about 50mM to about 75mM.

40. The method of any one of claims 35-39, wherein the aggregating agent is selected from the group consisting of dextran, hetastarch or gelatin and the non-toxic enhancer comprises sodium citrate or sodium succinate or a combination thereof.

41. A kit to sediment cells, in a sample comprising blood cells, comprising:
an aggregating agent; and

a non-toxic enhancer.

42. The kit of claim 41, wherein the non-toxic enhancer is sodium citrate or sodium succinate or a combination thereof.

43. The kit of claims 41 or 42, wherein the aggregating agent is dextran.

44. A kit to sediment cells in a sample comprising blood cells comprising:
an aggregating agent; and
a non-toxic enhancer, wherein the non-toxic enhancer is sodium citrate or sodium succinate or a combination thereof.

45. A kit for sedimenting cells in a sample comprising blood cells comprising:

an aggregating agent, wherein the aggregating agent is selected from the group consisting of dextran, hetastarch or gelatin, and
a non-toxic enhancer.

46. A method of recovering a percentage of total nucleated cells from a sample comprising red blood cells, comprising the steps of:
adding an aggregating agent and a non-toxic enhancer,
incubating the sample to allow a plurality of red blood cells to aggregate, and
recovering the total nucleated cells.

47. The method of claim 46, wherein the non-toxic enhancer is sodium citrate or sodium succinate or a combination thereof.

48. The method of claim 46 or 47, wherein the non-toxic enhancer has a final concentration range from about 10mM to about 100mM.

49. The method of claim 46 or 47, wherein the non-toxic enhancer has a final concentration range from about 12.5mM to about 75mM.

50. The method of claim 46 or 47, wherein the non-toxic enhancer has a final concentration range from about 25mM to about 75mM.

51. The method of claim 46 or 47, wherein the non-toxic enhancer has a final concentration range from about 50mM to about 75mM.

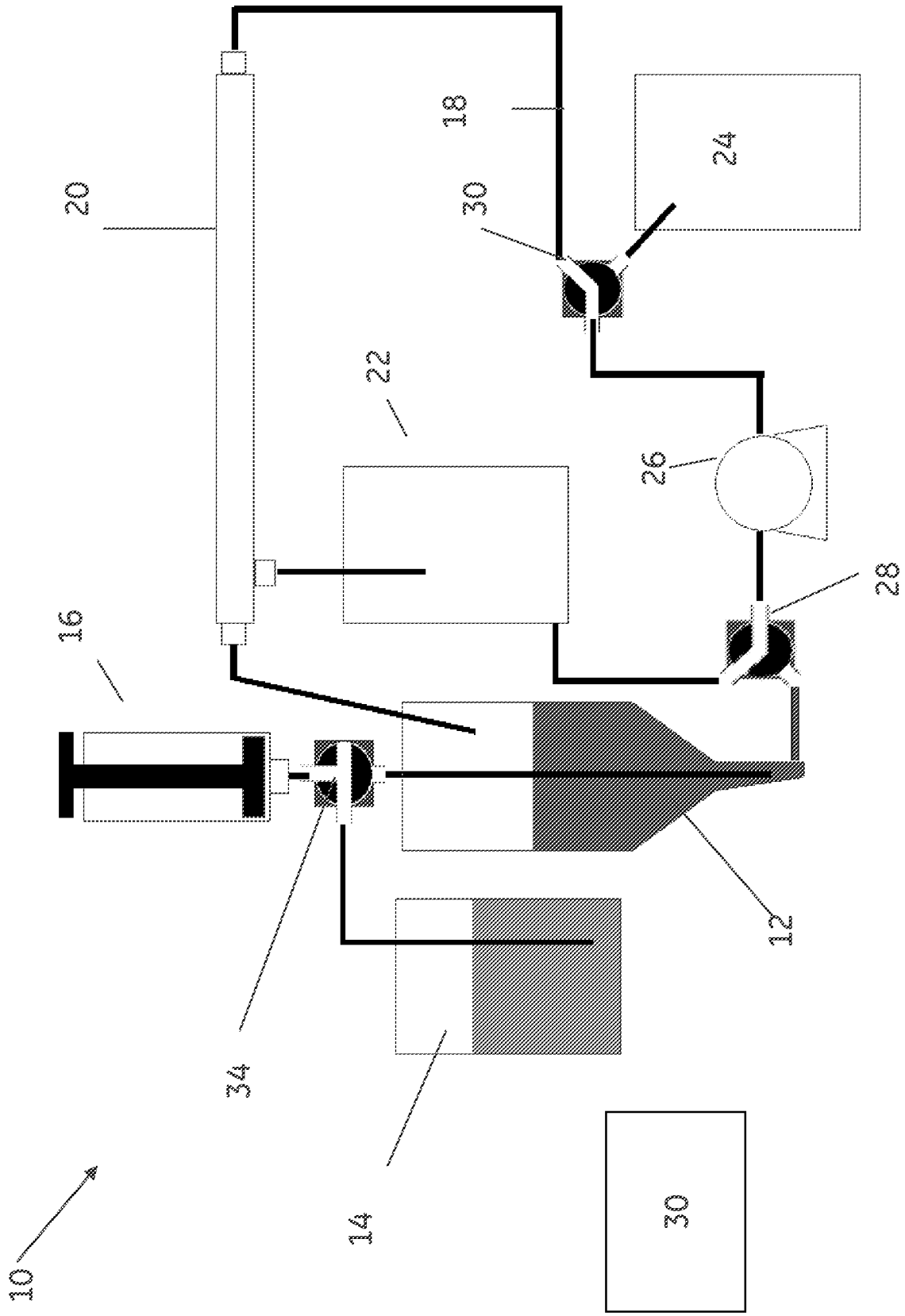


FIG. 1

FIG. 2

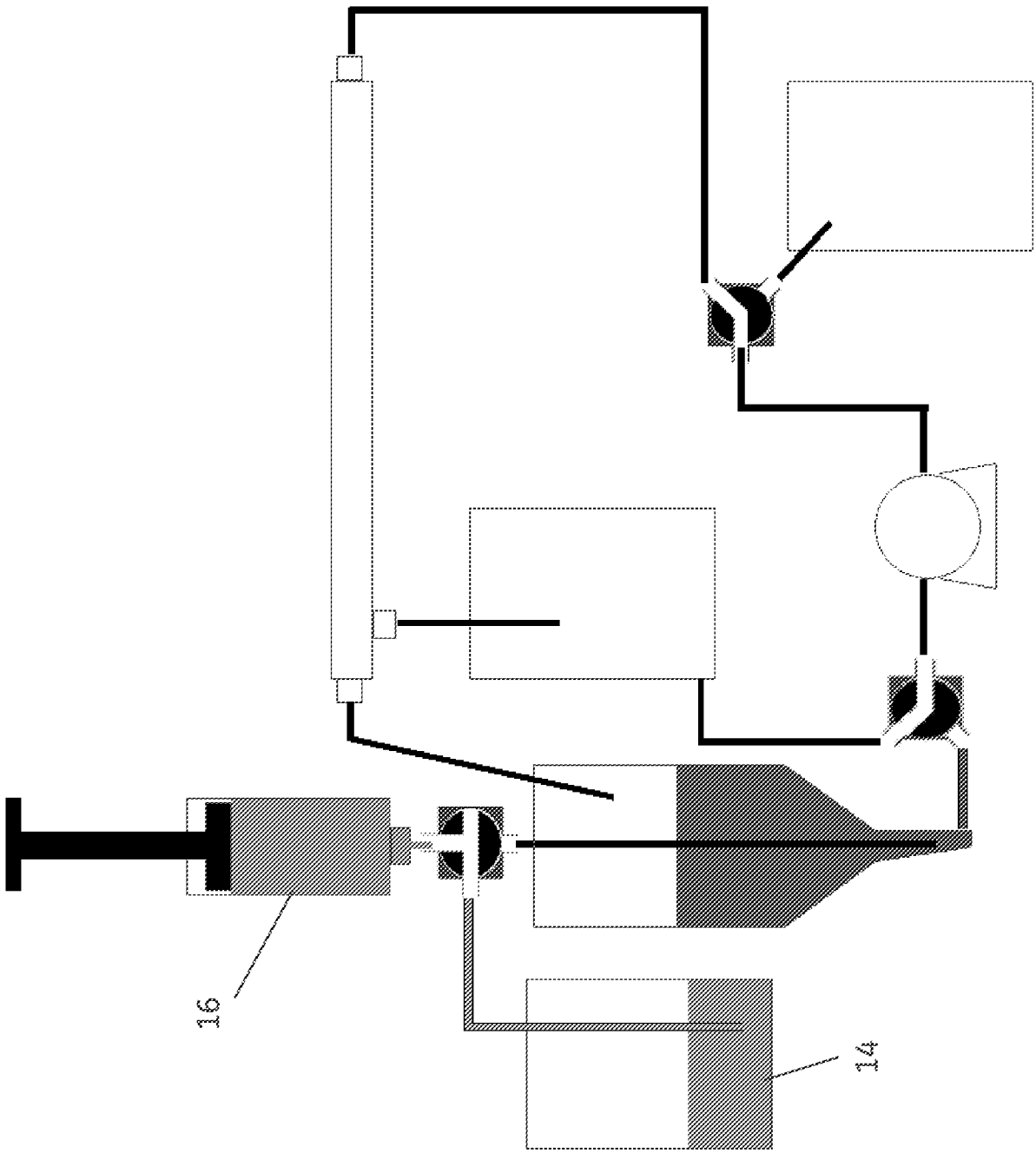


FIG. 3

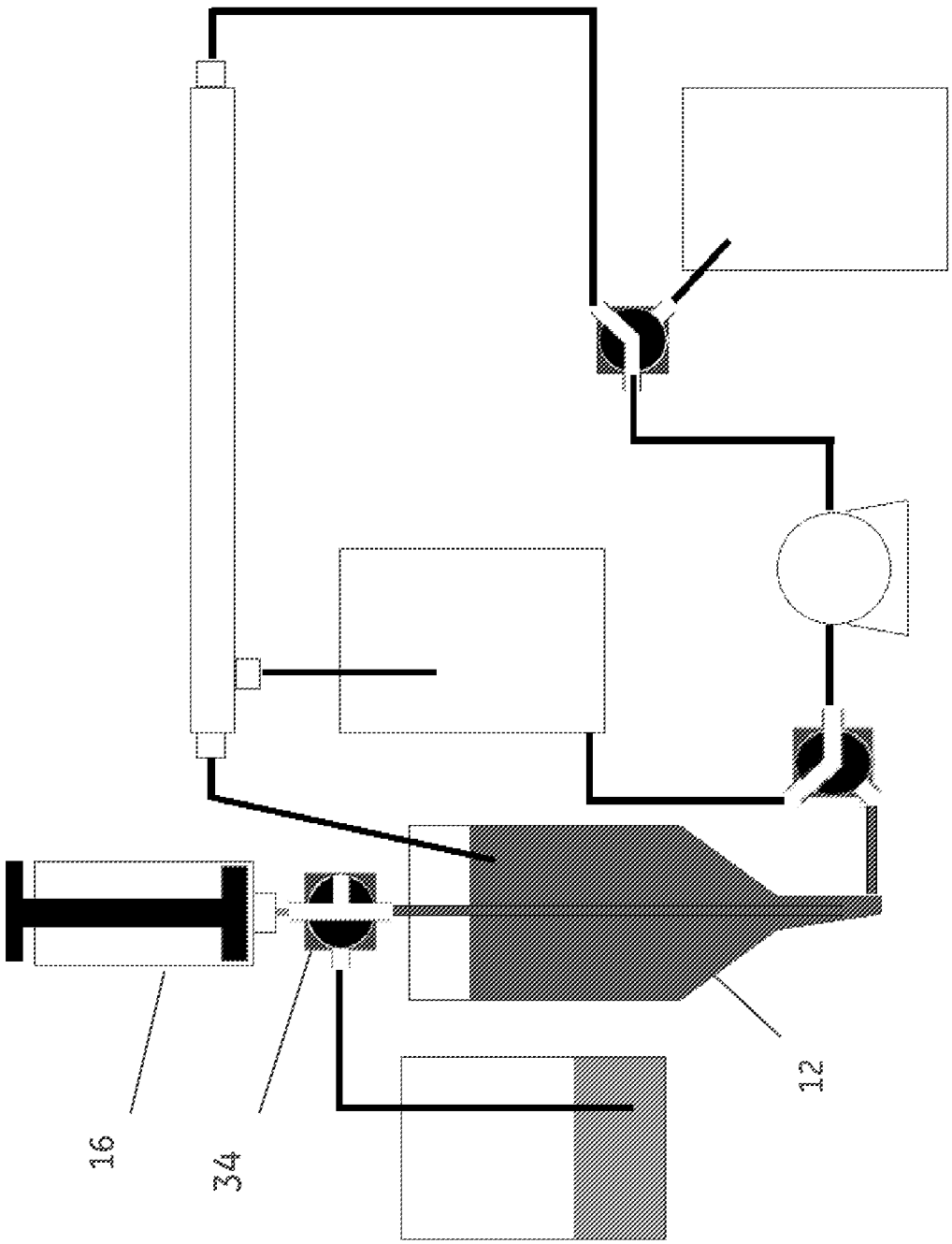


FIG. 4

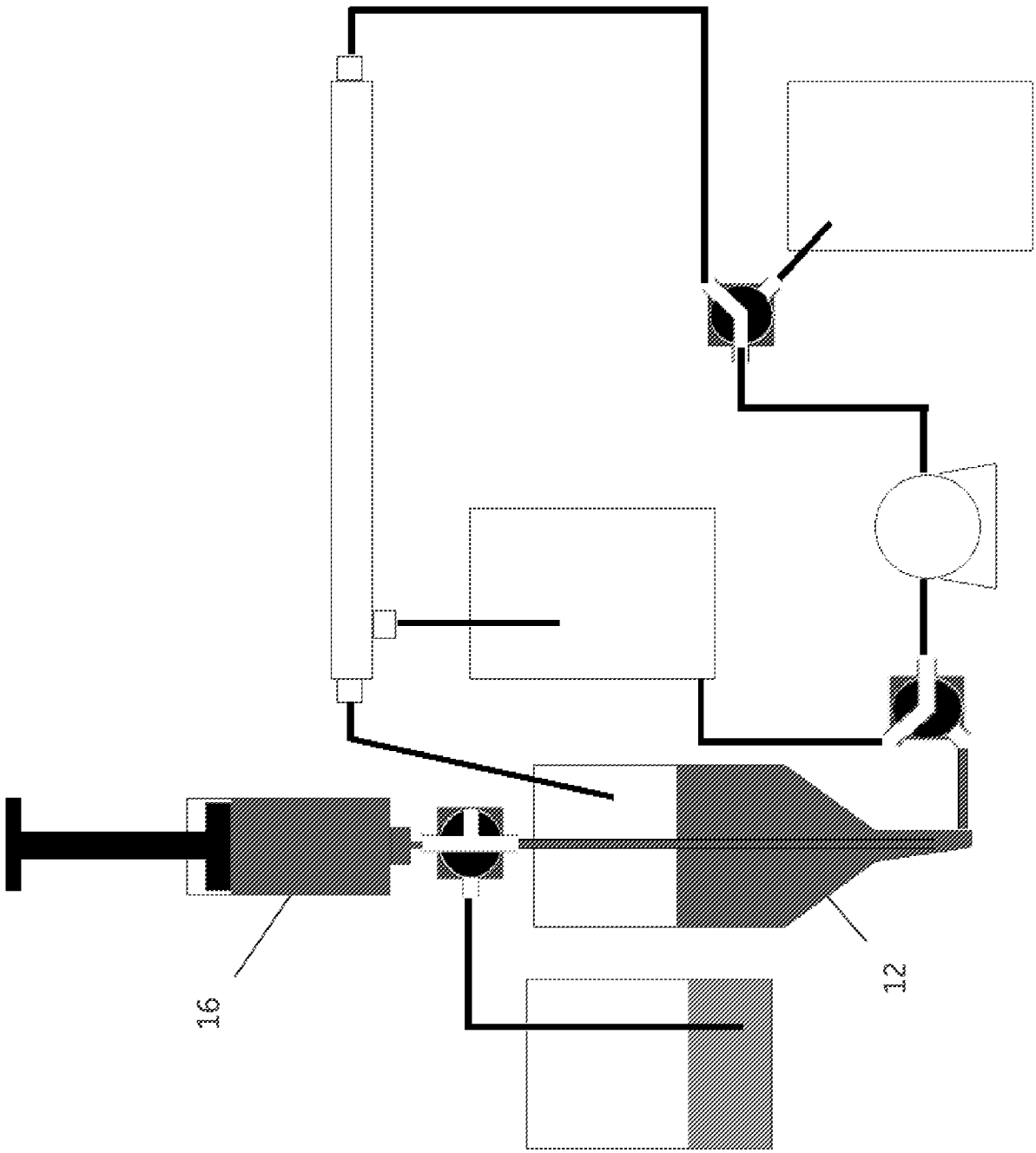


FIG. 5

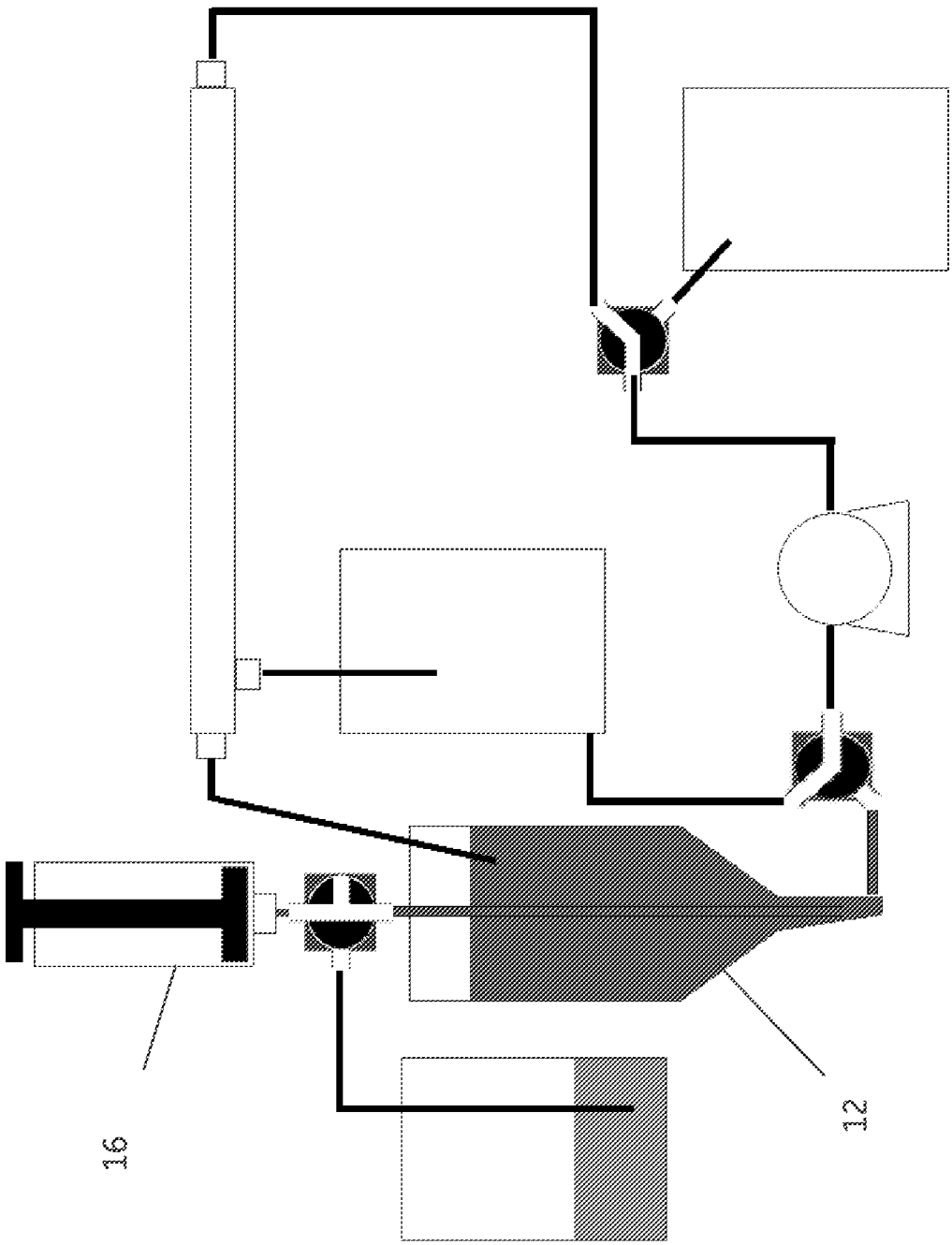


FIG. 6

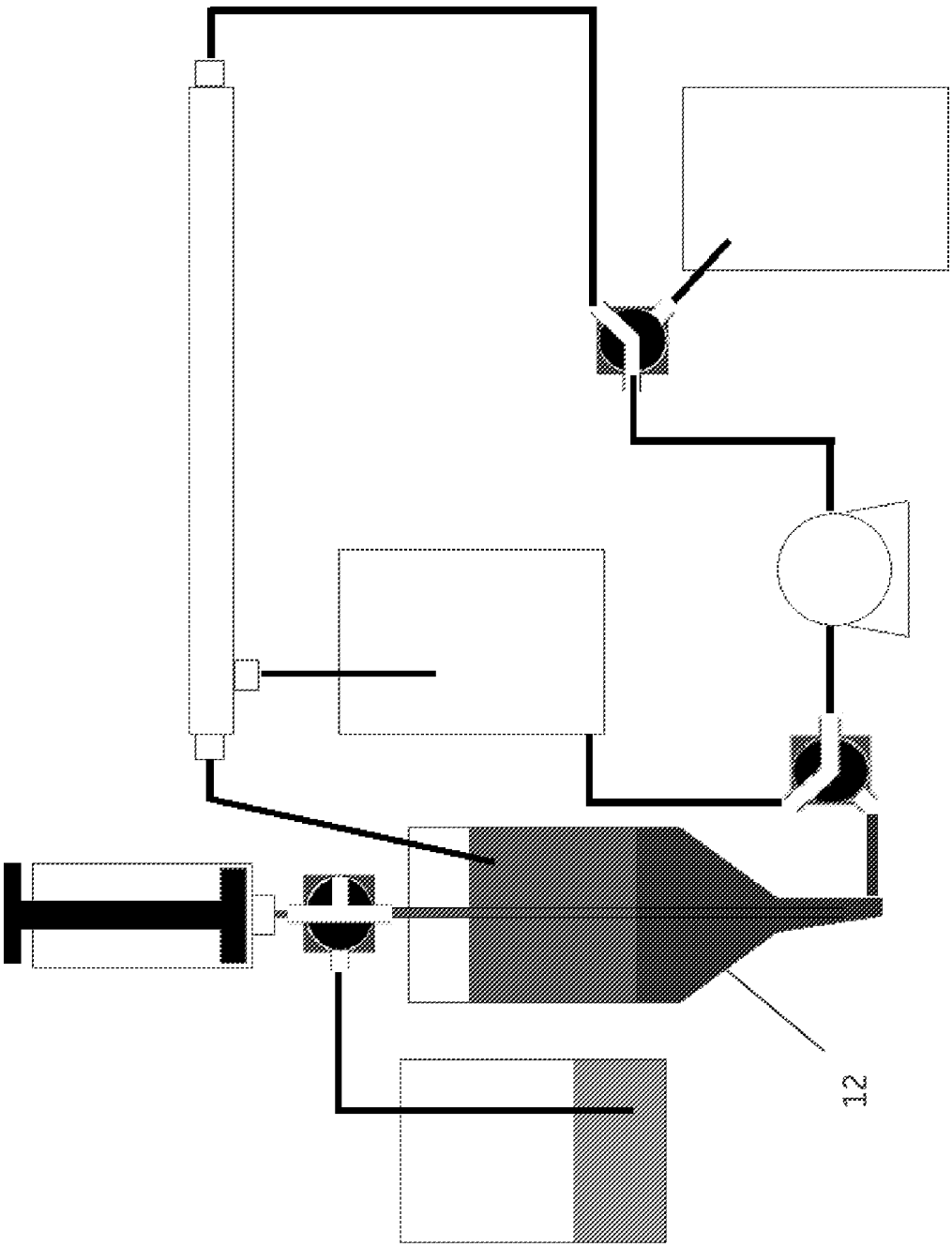


FIG. 7

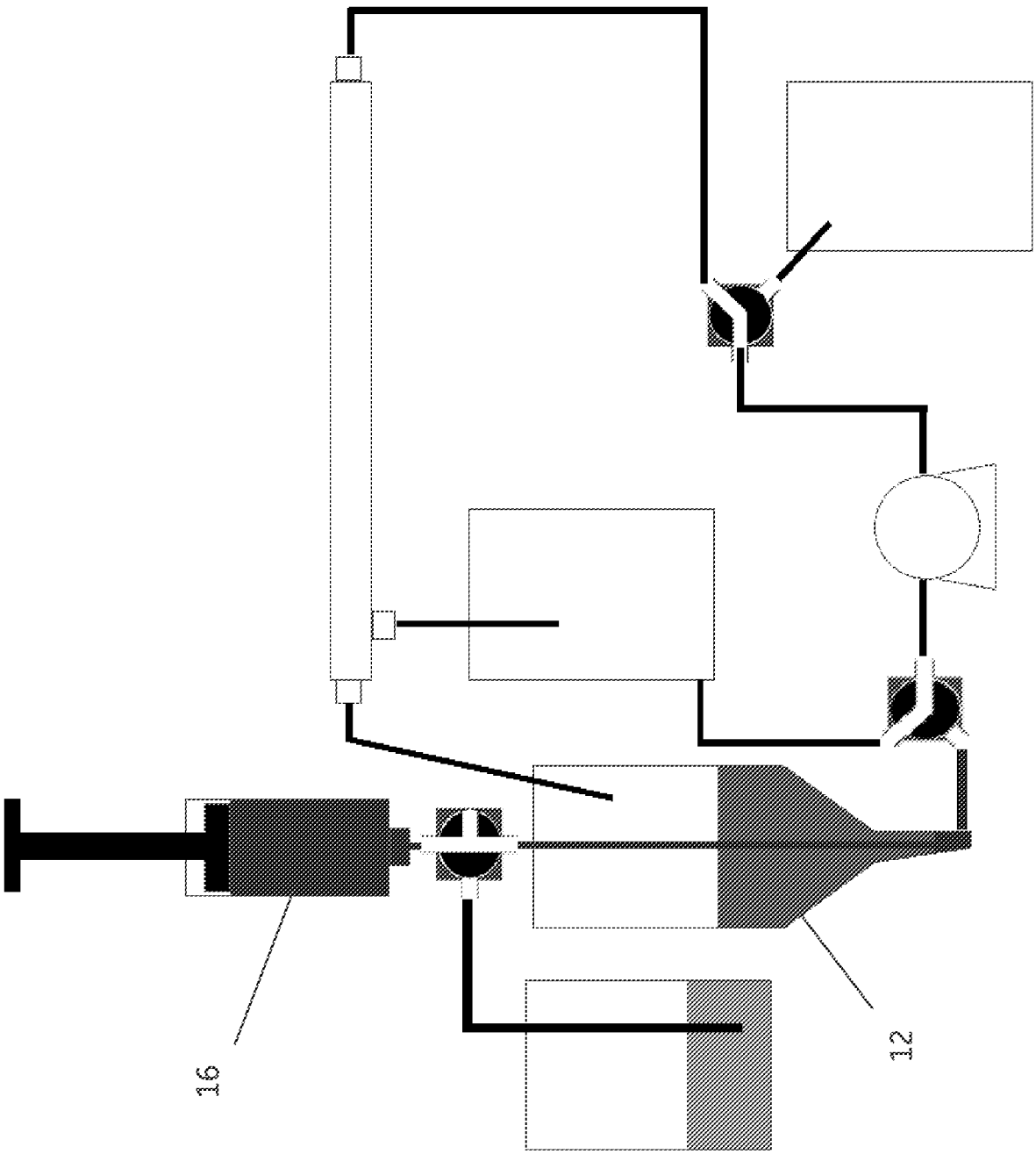


FIG. 8

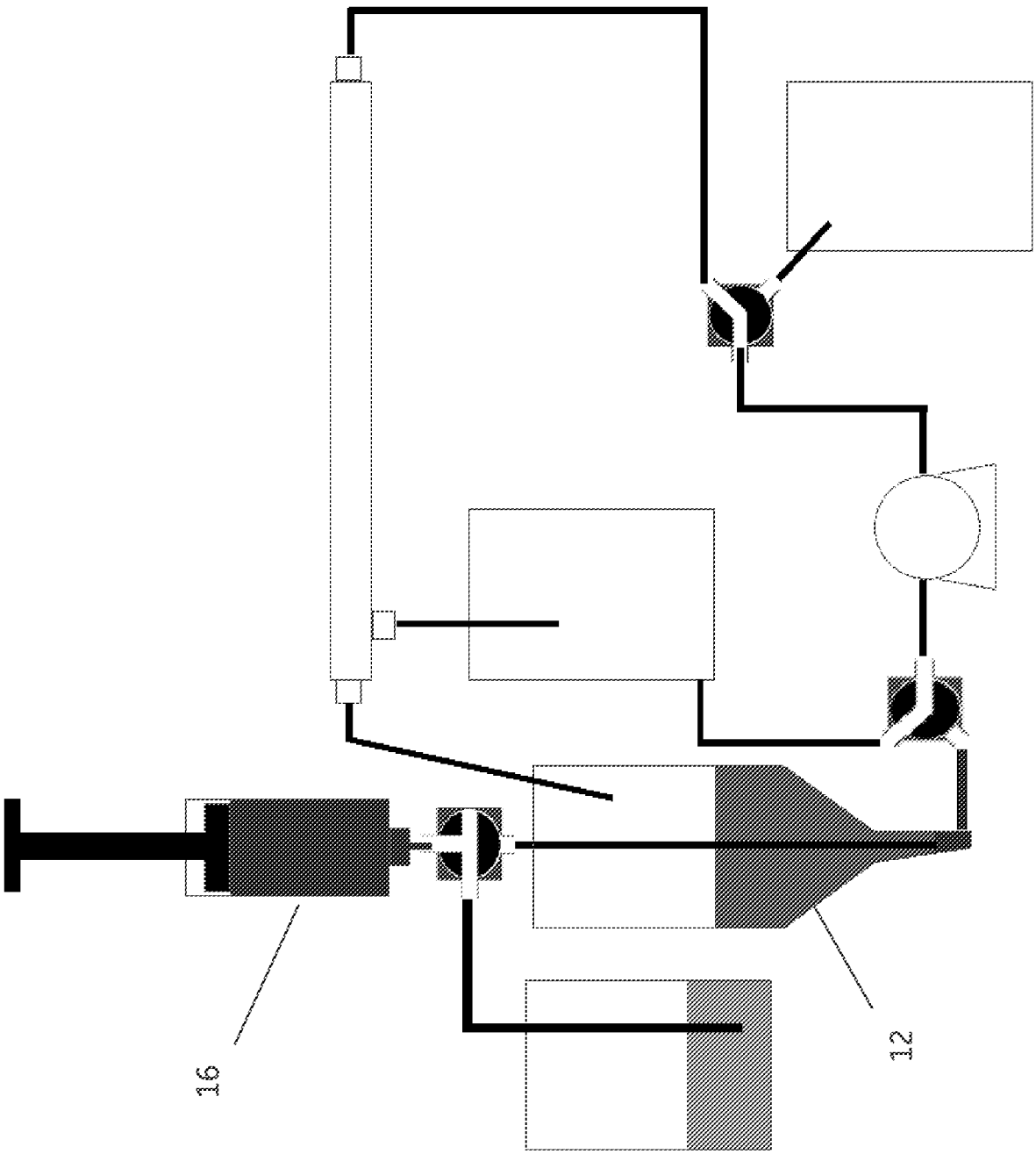


FIG. 9

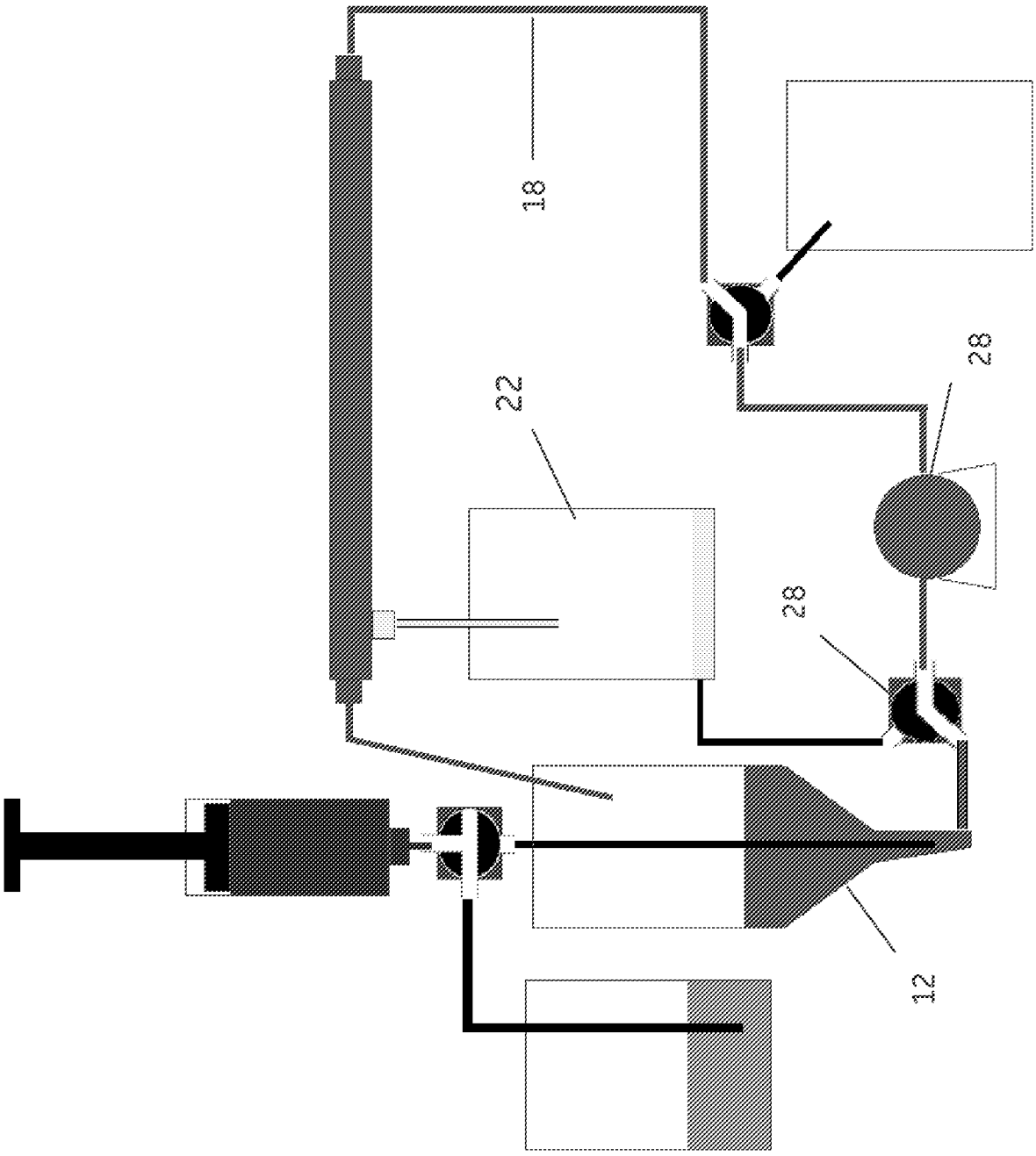


FIG. 10

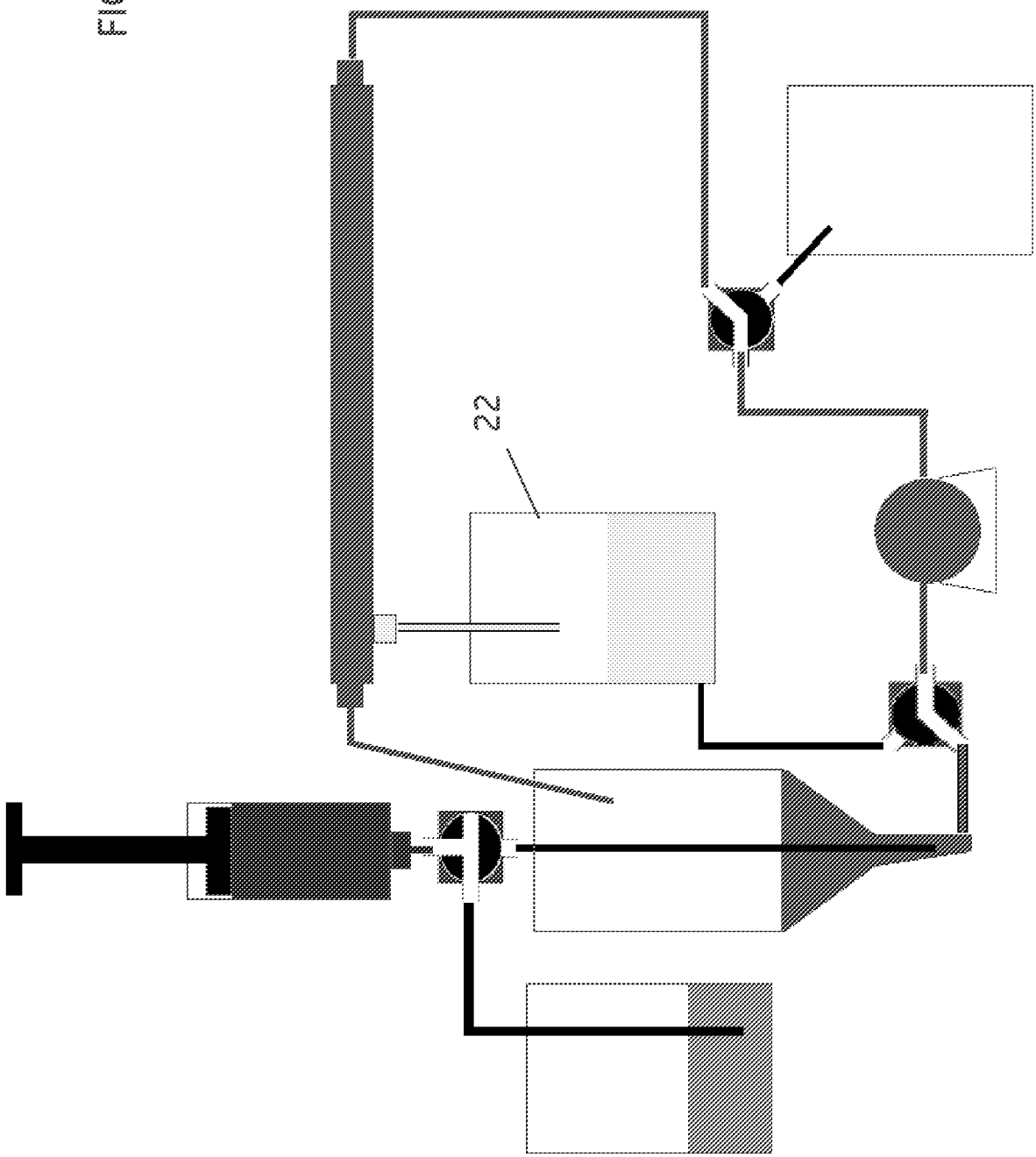


FIG.11

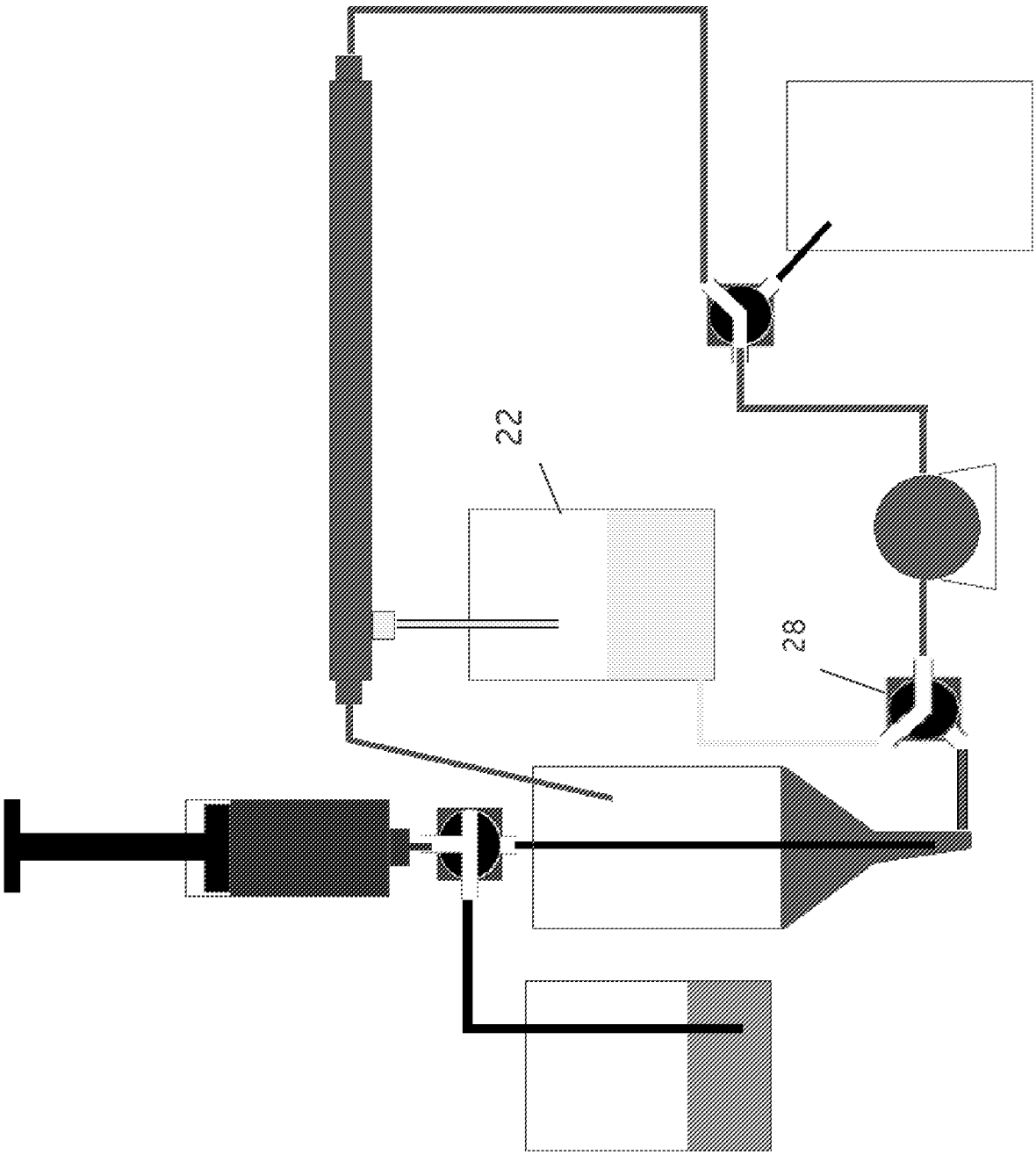


FIG. 12

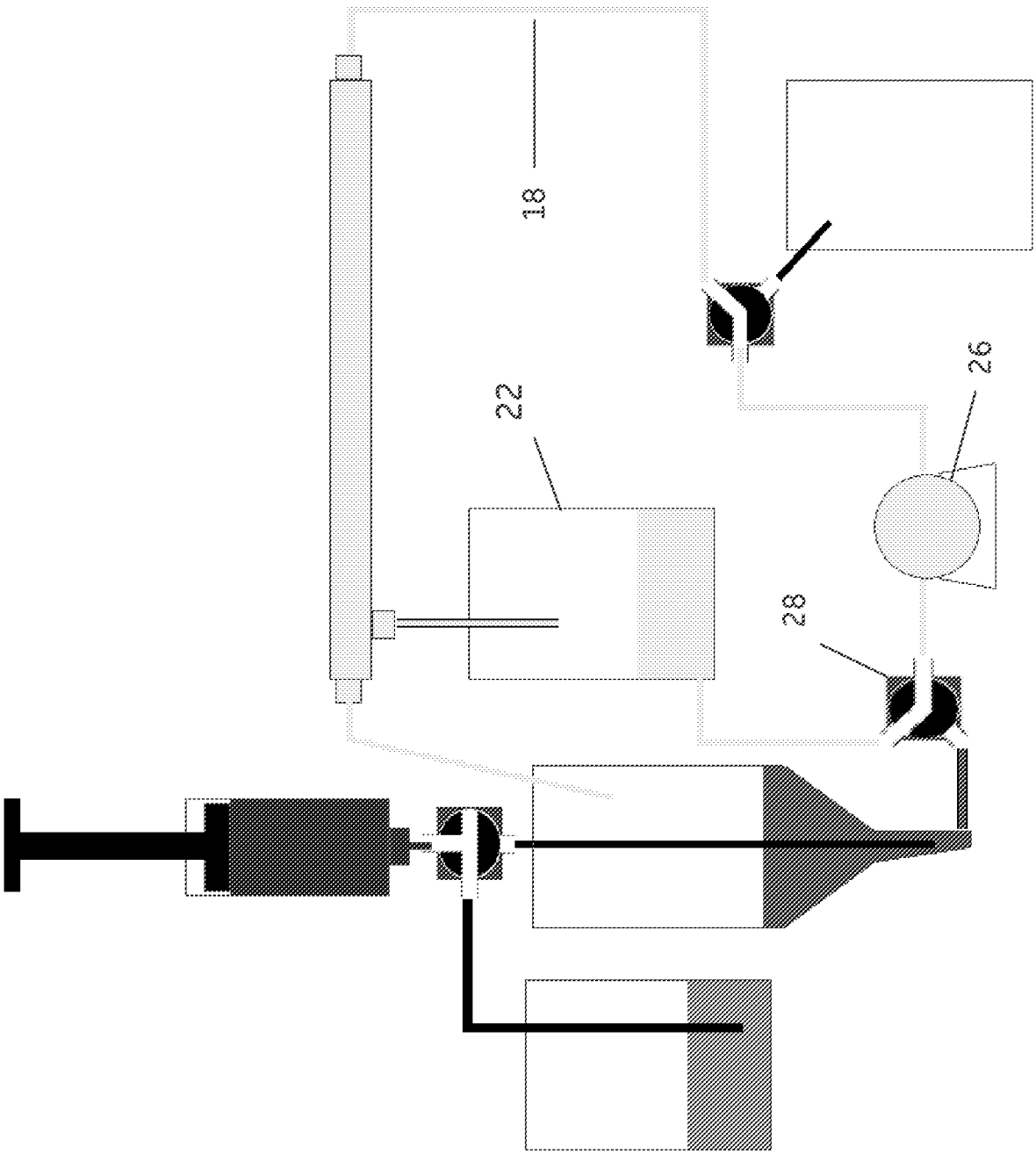


FIG. 13

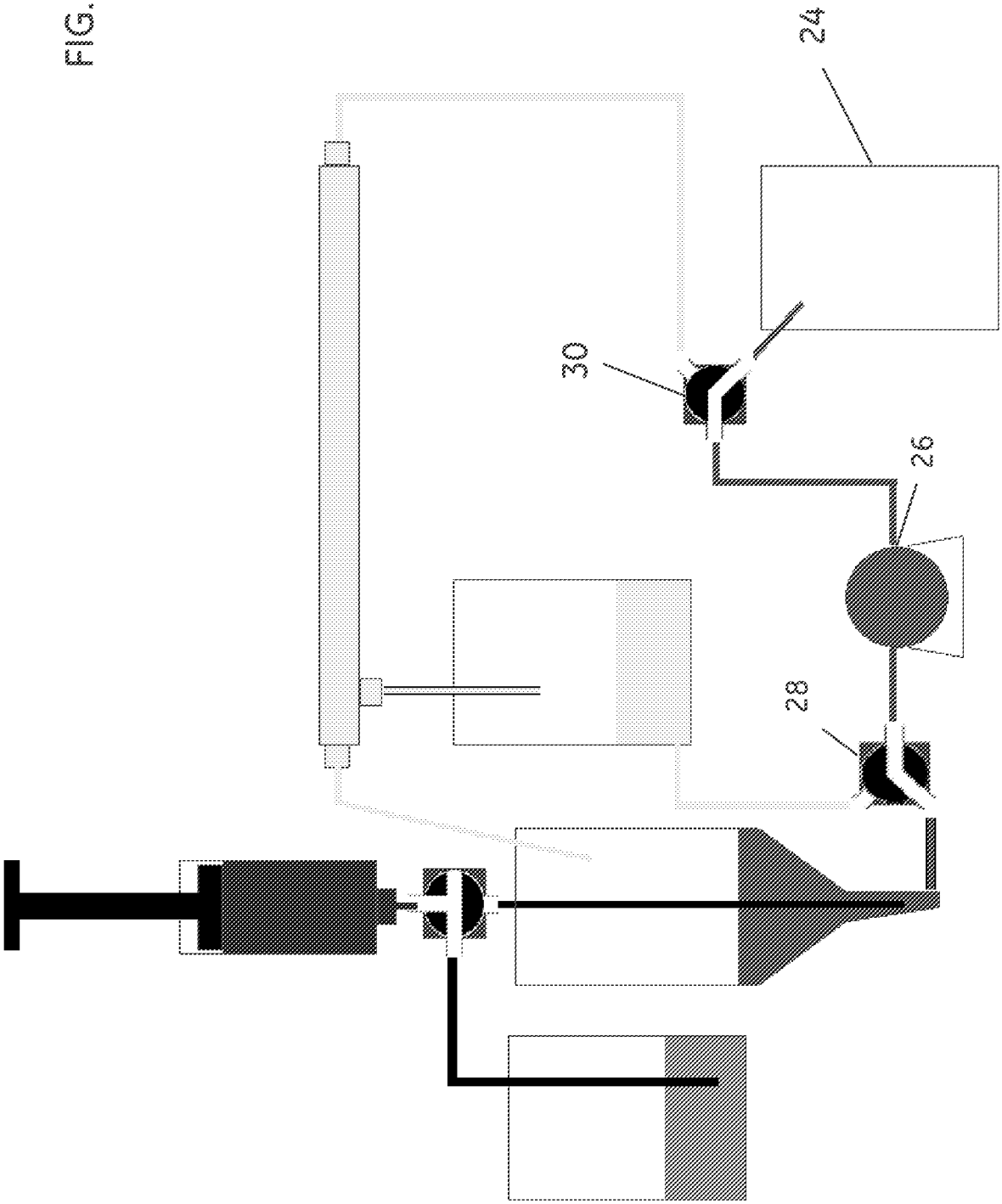


FIG. 14

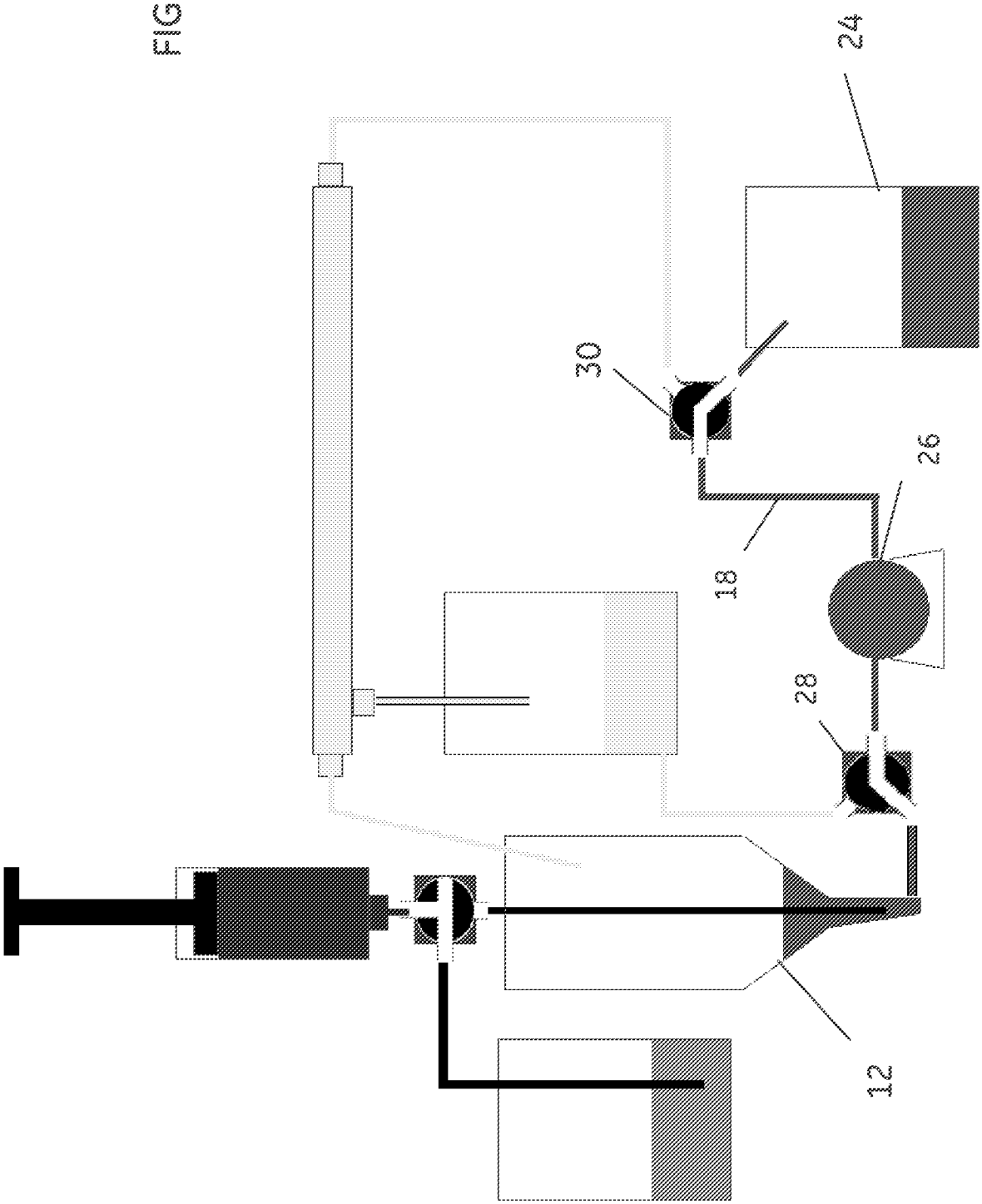


FIG.15

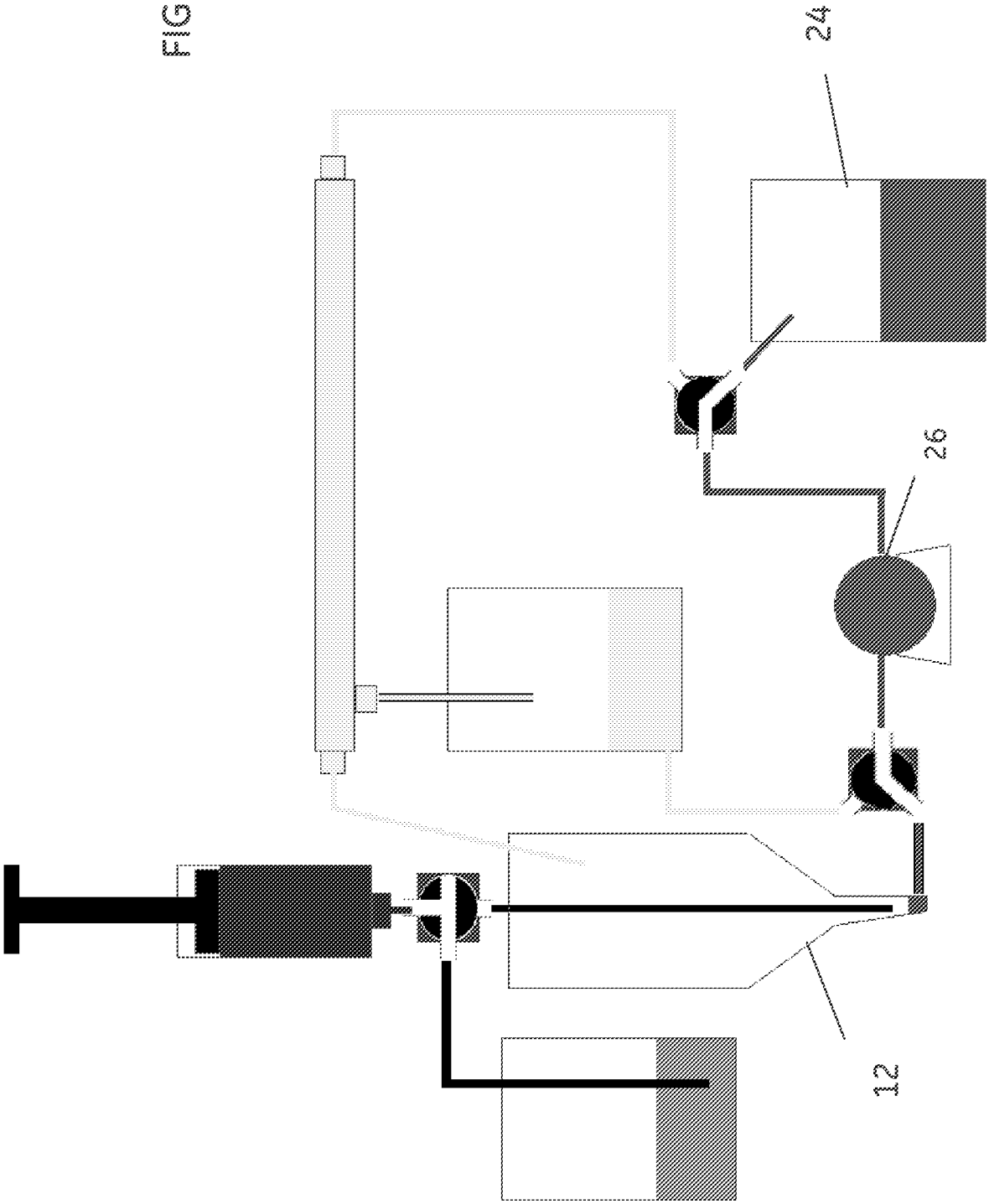


FIG. 16

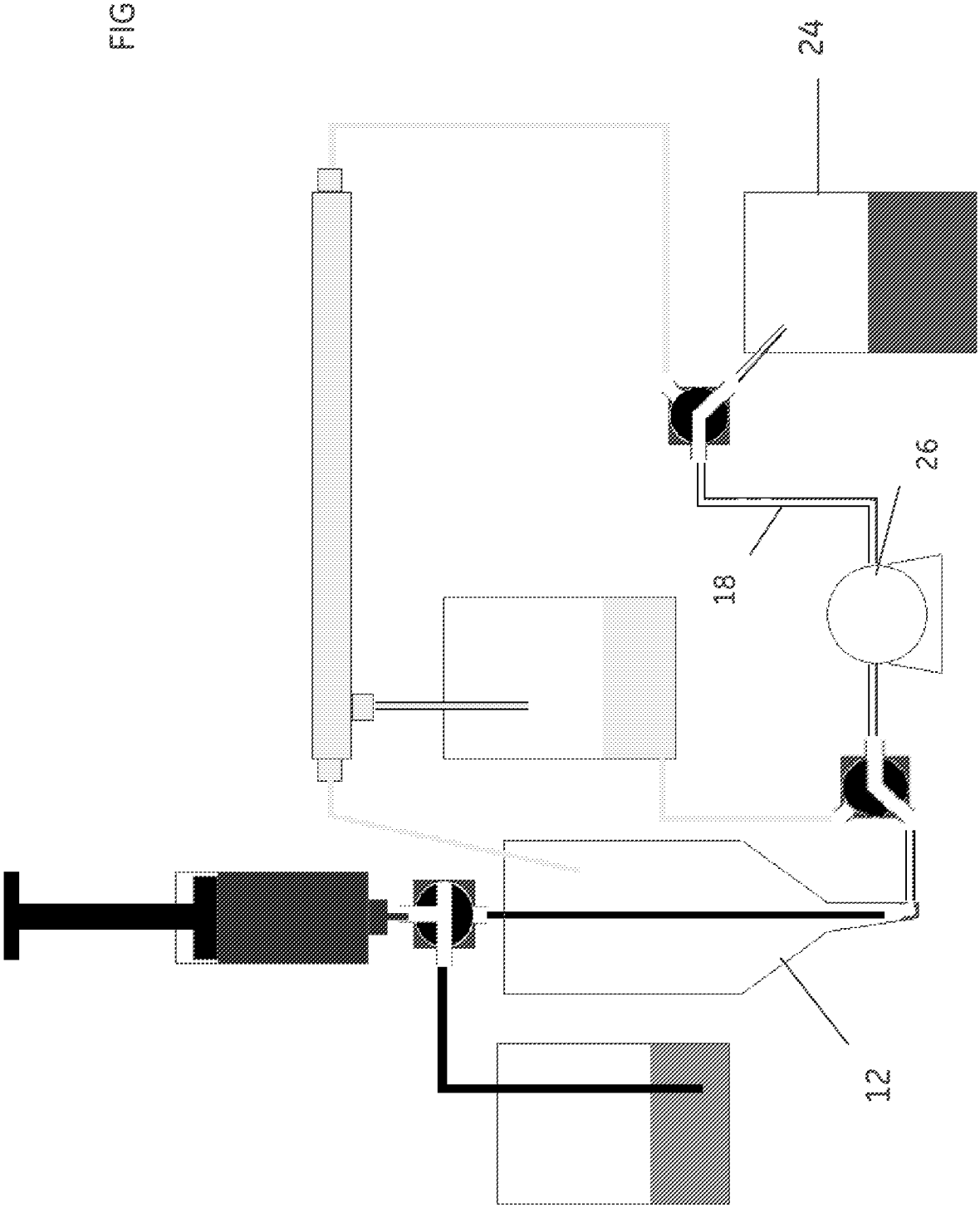


FIG. 17

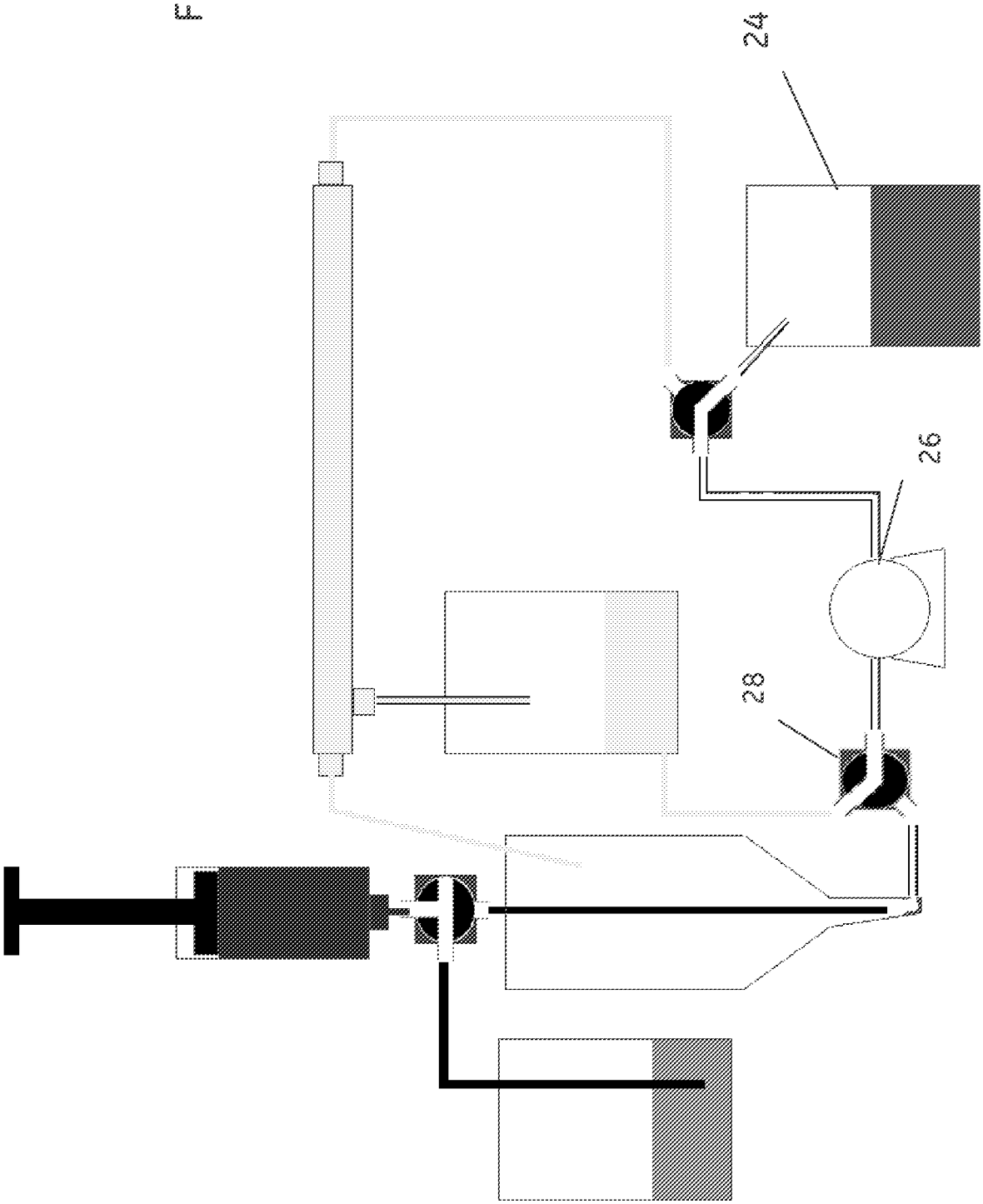


FIG. 18

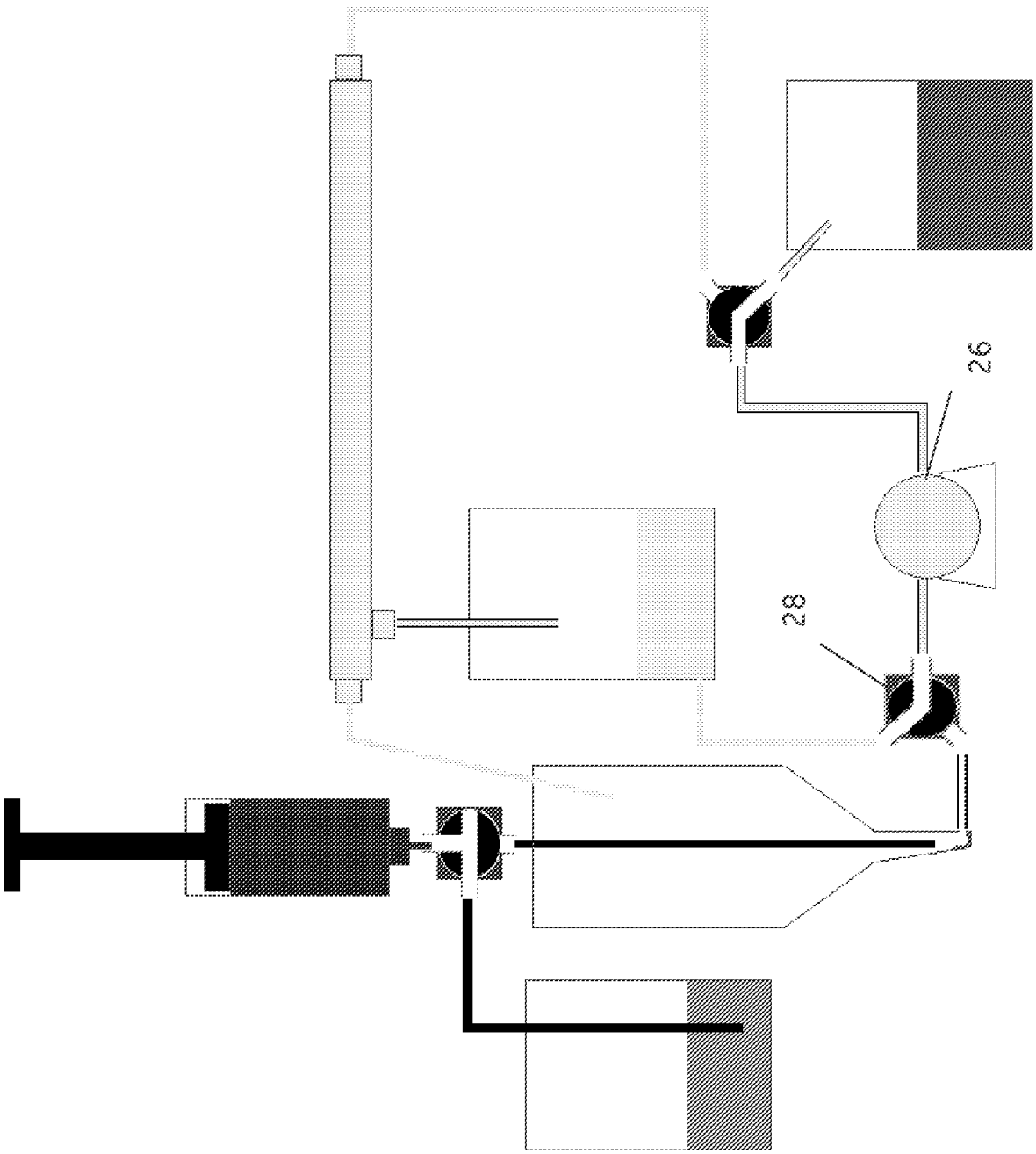


FIG. 19

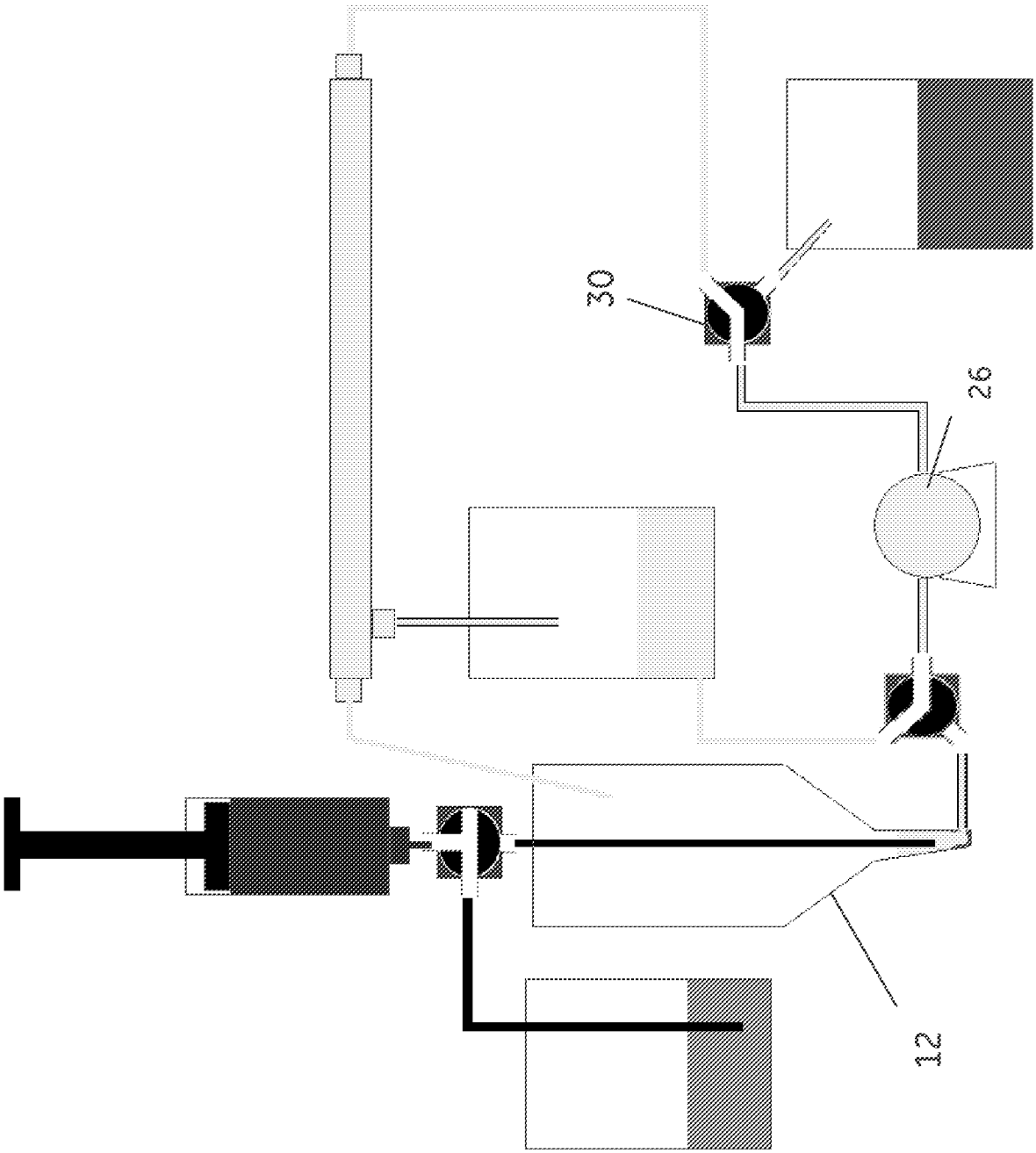


FIG. 20

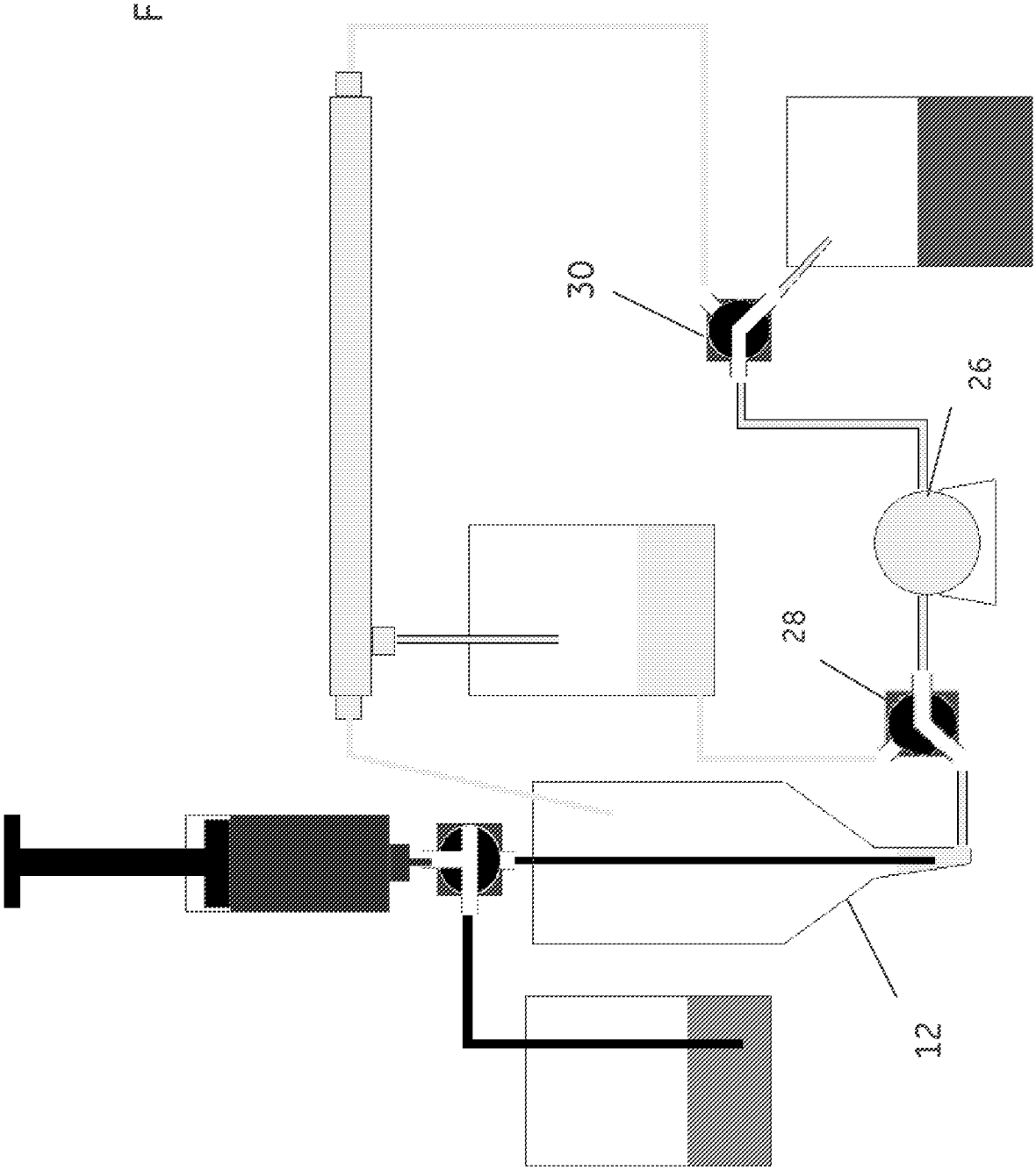
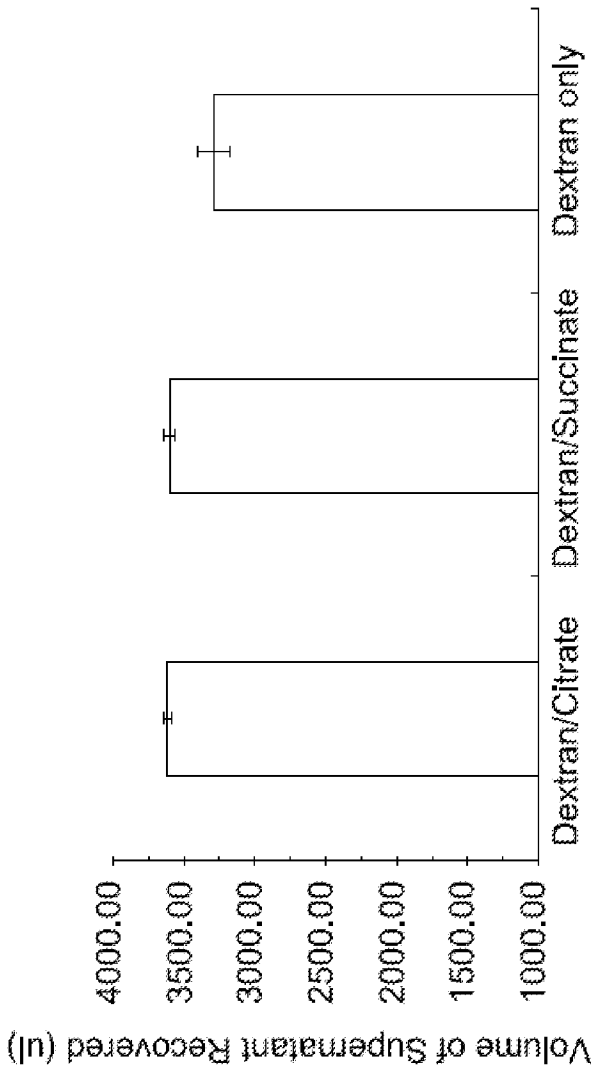
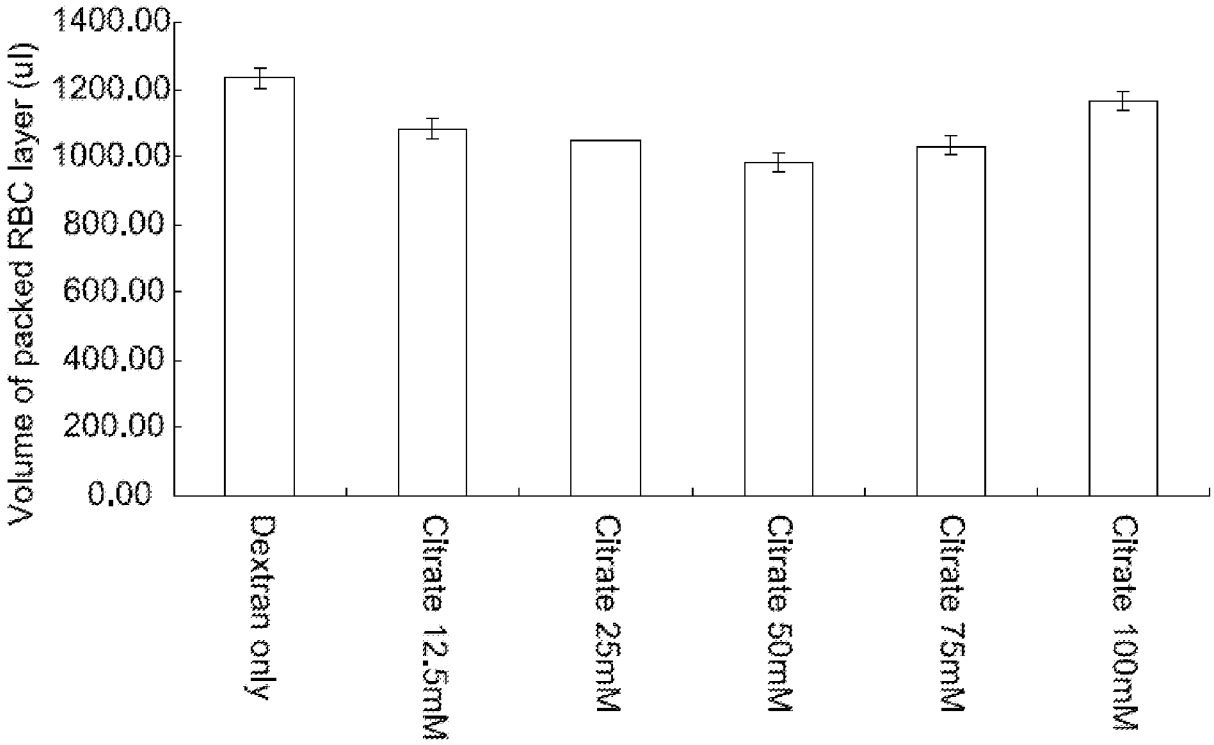


Fig. 21



ID	Sample	Volume of sup. recovered(microliter)	Mean vol	stdev
1	Dextran/Citrate	3650		
2	Dextran/Citrate	3620		
3	Dextran/Citrate	3600	3623.33	25.17
4	Dextran/Succinate	3600		
5	Dextran/Succinate	3650		
6	Dextran/Succinate	3580	3610.00	36.06
7	Dextran only	3170		
8	Dextran only	3300		
9	Dextran only	3400	3290.00	115.33



ID	Sample	RBC vol (ul)	Mean	stdev
1	Dextran only	1200.00		
2	Dextran only	1250.00		
3	Dextran only	1250.00	1233.33	28.87
4	Citrate 12.5mM	1100.00		
5	Citrate 12.5mM	1050.00		
6	Citrate 12.5mM	1100.00	1083.33	28.87
7	Citrate 25mM	1050.00		
8	Citrate 25mM	1050.00		
9	Citrate 25mM	1050.00	1050.00	0.00
10	Citrate 50mM	1000.00		
11	Citrate 50mM	1000.00		
12	Citrate 50mM	950.00	983.33	28.87
13	Citrate 75mM	1050.00		
14	Citrate 75mM	1000.00		
15	Citrate 75mM	1050.00	1033.33	28.87
16	Citrate 100mM	1200.00		
17	Citrate 100mM	1150.00		
18	Citrate 100mM	1150.00	1166.67	28.87

Fig. 22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2009/051327

A. CLASSIFICATION OF SUBJECT MATTER

IPC: see extra sheet
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)

IPC: A61M, B01D, G01N

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

SE,DK,FI,NO classes as above

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

EPO-INTERNAL, WPI DATA, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 20030134416 A1 (YAMANISHI ET AL), 17 July 2003 (17.07.2003), claims 36-38,150,158-161,167-197; paragraphs (0121)-(0124),(0193)	1-28
A		29-51
	--	
X	US 6444471 B1 (JOHNSON), 3 Sept 2002 (03.09.2002), column 6, line 29 - line 42; column 7, line 22 - line 60; column 13, line 44 - line 48	29-51
A		1-28
	--	
X	US 5482829 A (KASS ET AL), 9 January 1996 (09.01.1996), figure 1, abstract	41,43,44,45
	--	

[X] Further documents are listed in the continuation of Box C. [X] 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	Date of mailing of the international search report
7 January 2010	08-01-2010
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Agneta Seidel / JA A Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2009/051327

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

The following separate inventions were identified:

I: Claims 1-26, 27-28 (part) and 46-51 (part) directed to an automated method to separate submaterials in a blood sample by sedimentation without centrifugation followed by filtration.

.../...

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Box III

The invention includes a device with a pump, valves, conduits and a syringe type extraction device.

II: Claims 27-28 (part), 29-45 and 46-51 (part) directed to using an optimum amount of a non-toxic enhancer for minimum red blood cell volume in a sediment.

The present application has been considered to contain 2 inventions which are not linked such that they form a single general inventive concept, as required by Rule 13 PCT for the following reasons:

Invention I relates to the problem that centrifugation-based methods for blood-cell processing have limited flexibility and portability. This problem appears to be solved by using sedimentation without centrifugation, followed by separation and filtration.

Invention II relates to the problems of obtaining a small volume of red blood cells that is needed in blood cell banking applications and that known sedimentation enhancing agents are potentially toxic. These problem are solved by using an optimum concentration of 10-100 mM of sodium citrate of sodium succinate as an enhancer.

As both the problems and solutions are technically different, no single general concept can be formulated based on the technical features of the inventions. Consequently, the requirements of Rule 13.1 PCT are not met.

It was investigated under Rule 13.2 if any further feature, either in the claims or derivable from the description, could be considered as a same or corresponding feature, and could be considered a special technical feature establishing a technical link between the two inventions.

No such features were identified.

Consequently, the two inventions are not so linked as to form a single general inventive concept as required by Rule 13.1 PCT.

Box III

However, a search has been carried out which relates to both inventions and all claims. It was considered that the complete search involved only little additional effort and did not justify a request for an additional fee.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2009/051327

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4663058 A (WELLS ET AL), 5 May 1987 (05.05.1987), claims 1,6,7	1-6,8-9, 18-19,24
A	claims 1,6, abstract	7,10-17, 20-23,25-51
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X	WO 2007049286 A1 (TATA MEMORIAL CENTRE), 3 May 2007 (03.05.2007), page 39, line 7 - line 18, figure 11B	1-2,4-6, 8-10,12,16, 18,19,24
A		3,7,11, 13-15,17, 20-23,25-51
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X	US 5670060 A (MATKOVICH ET AL), 23 Sept 1997 (23.09.1997), column 3, line 16 - line 39; column 4, line 17 - line 22; column 6, line 16 - line 26, figure 2, abstract	1-2,4-6, 8-10,12,16
A		3,7,11, 13-15,17-51
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X	DE 102004018347 A1 (SCHMOLZ, MANFRED), 27 October 2005 (27.10.2005), claims 7-9,12	1-6,8-10,12, 18-20,22,24, 27
A		7,11,13-17, 21,23,25,26, 28-51
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P,X	WO 2009002849 A2 (MILLENNIUM MEDICAL TECHNOLOGIES, INC.), 31 December 2008 (31.12.2008), claims 21, 64,70	1-28
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International patent classification (IPC)

A61M 1/36 (2006.01)
A61M 1/38 (2006.01)
B01D 36/04 (2006.01)
G01N 33/50 (2006.01)

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Cited literature, if any, will be enclosed in paper form.

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