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15 Micro flow filtration system and flow filtration method for a fluid sample

20 The ~~invention~~ disclosure relates to a micro flow filtration system and a flow filtration method for increasing the concentration of a component contained in a fluid sample. The system comprises a fluid channel with a tangential flow filtration module (TFF-module) having a semipermeable membrane capable of separating the fluid sample into a retentate stream and a permeate stream upon passage of the fluid sample. The fluid can flow bidirectionally through the channel passing the TFF-module and thereby increasing the concentration of
25 the fluid.

30 Flow filtration systems for filtrating a fluid sample using a semipermeable membrane for purposes of purification or concentration are well known in the state of the art. These systems are used to remove particulate or molecular contaminants in the case of a purification or are used to increase the concentration of a component in a fluid for example for laboratory analysis. The membrane of such filtration systems can be located normal to the flow of a fluid sample which is characterized as a normal flow filtration, or the

having two ends. At both ends a piston pump or a syringe pump is located so that the fluid can be pumped bidirectionally through the TFF-module using the two pumps. The piston pumps have a reservoir for containing the fluid sample to be concentrated. The reservoir corresponds to the piston swept volume of the pump. So, the piston pumps are designed for a discrete volume range so that the pumps work optimally in a small volume range which is in the same range as the piston swept volume of the piston pump. The smaller the volume is to be handled, the smaller is the piston stroke length which is used to drive the fluid through the filtration system. This leads to the disadvantage that for a small fluid volume to be processed at the end of an filtration process the piston stroke length is also very small which leads to relative large deviations and a relative high inaccuracy.

So, especially in the pharamaceutic and biotechnology industry a strong demand still exists for a tangential flow filtration system being able to operate with small amounts of a fluid sample, preferably with less than 20 ml start volume and being able to concentrate a component of a solution by a large concentration factor. Such filtration systems are not commercially available at the time. ~~It is an object of the invention~~ Various embodiments seek to provide an optimized TFF-system being able both to handle small amounts of a fluid and to provide the requested concentration factor. Further, a clogging and fouling in the TFF-module should be avoided over a large time period during the filtration process.

~~The problem is solved by a micro flow filtration system for performing a fluidic process with the features according to claim 1. The object is also solved by a flow filtration method according to claim 9. According to a first aspect, there is provided a micro flow filtration system for performing a fluidic process, such as concentrating of components contained in a fluid sample or for exchanging the solvent of the sample fluid, the system (1) comprising:~~

~~- a fluid channel (2) having two ends (10,11) and being formed by conduits (4, 5) allowing a bidirectional flow of the fluid sample through the channel (2);~~

~~- a tangential flow filtration module (3) (TFF-module) having a first fluid port (14), a second fluid port (15), a permeate outlet (16) and a semipermeable membrane (17) capable of separating the fluid sample into a retentate stream flowing through one of the ports (14, 15) and a permeate stream upon passage of the fluid of the sample through the semipermeable membrane (17) and through the permeate outlet (16);~~

~~- at least two pairs (8, 9) of piston pumps (6) having each two piston pumps (6), each piston pump having a piston swept volume forming a reservoir (18, 19) being able to contain a fluid, the piston pumps (6) being capable to drive a fluid flow;~~

~~- the volume of the piston pumps (6) of one pair (8) of piston pumps (8a, 8b) is larger than the volume of the piston pumps (6) of the other pair (9) of piston pumps (9a, 9b),~~

~~- a control unit for controlling the piston pumps (6).~~

wherein

- the TFF-module (3) is located in the fluid channel (2) so that one conduit (4) is connected to the first fluid port (14) and the other conduit (5) is connected to the second fluid port (15) to allow a fluid flowing through the channel (2) to pass through the TFF-module (3);

- at each of the ends (10,11) of the channel (2) one piston pump (6) of each pair (8, 9) of piston pumps is arranged in such a manner that the piston pumps 6 are fluidically connected

- the control unit is arranged and adapted to control driving of the piston pumps of each pair (8, 9) of piston pumps (6) synchronously and/or to control the switching and/or the switching time for switching from one pair (8) of piston pumps (8a, 8b) to the other pair (9) of piston pumps (9a, 9b) and

- the system is arranged and adapted such that during the fluidic process at least for a period of time only one of the pairs (8, 9) of piston pumps (6) is used to drive the fluid flow through the fluid channel (2).

According to a second aspect, there is provided a flow filtration method for concentrating a component contained in a fluid sample using a tangential flow filtration module (3), the method comprising the following steps:

- providing a micro flow filtration system (1) according to the first aspect comprising
 - a fluid channel (2) having two ends (10, 11) and being formed by two conduits (4, 5) allowing a bidirectional flow of the fluid sample through the channel (2);
 - a tangential flow filtration module (3) having a first fluid port (14), a second fluid port (15), a permeate outlet (16) and a semipermeable membrane (17) capable of separating the fluid sample;
 - at least two pairs (8, 9) of piston pumps (6), each piston pump (6) forming a reservoir (18, 19) being able to contain a fluid;
 - a control unit (80) for controlling movement of the pistons of the piston pumps (6);

wherein

- the TFF-module (3) is located in the fluid channel (2) so that one conduit (4) is connected to the first fluid port (14) and the other conduit 5 is connected to the second fluid port (15);

- at each of the ends (10, 11) of the channel (2) one piston pump (6) of each pair (8, 9) of piston pumps (6) is arranged in such a manner that the piston pumps (6) are connected fluidically;

- the piston pumps (6) of a first pair (8) of piston pumps have a larger volume than the piston pumps (6) of a second pair (9) of piston pumps (6);
- the control unit (80) is arranged and adapted to control of moving the pistons of the piston pumps of each pair (8, 9) of piston pumps synchronously and/or to control the switching and/or the switching time for switching from one pair (8) of piston pumps (8a, 8b) to the other pair (9) of piston pumps (9a, 9b),
- filling at least one piston pump (6) of the first pair (8) of piston pumps with a fluid;
- moving the piston of the first piston pump (8a) of the first pair (8) of piston pumps controlled by the control unit (80) in a first direction to reduce the piston swept volume to drive the fluid sample from the first piston pump (8a) through the fluid channel (2) and to pass the TFF-module (3) thereby increasing the concentration of the components in the fluid sample in the system (1) and withdrawing an amount of fluid in a permeate stream;
- moving the piston of the second piston pump (8b) of the first pair (8) of piston pumps controlled by the control unit (80) in the first direction to enhance the piston swept volume to allow fluid to enter the piston pump (8b) after passage through the fluid channel (2);
- moving the piston of the second piston pump (8b) and the piston of the first piston pump (8a) of the first pair (8) of piston pumps controlled by the control unit (80) in a second direction opposite to the first direction to drive back the fluid sample from the second piston pump (8b) through the TFF-module (3) into the first piston pump (8a);
- determining if the volume of the fluid sample in the system (1) has been reduced below a predetermined value or if an predetermined time interval after starting of the process has been reached;
- repeating the steps of moving the first and second piston pumps (8a, 8b) of the first pair of piston pumps (8) controlled by the control unit (80) in the first and second direction until the predetermined value or the predetermined time interval has been reached, thereby driving the fluid back and forth through the TFF-module (3); and
- if the volume of the fluid sample in the system (1) has been reduced below the predetermined value or if the predetermined time interval after starting of the process has been reached, using the piston pumps (9a, 9b) of the second pair (9) of piston pumps according to the steps above thereby establishing a continuous bidirectional flow of fluid in the channel (2).

| The micro tangential flow filtration system according to the ~~invention~~disclosure performing a fluidic process, like increasing the concentration of components contained in a fluid sample or exchanging the solvent of the sample solution by an exchange buffer, comprises a fluid channel with an integrated tangential flow filtration module. The fluid channel is formed by two conduits which are

one piston pump of the second pair of piston pumps. Alternatively, the piston pumps can be fluidically connected in series. The piston pumps of one pair of piston pumps are preferably equal in volume. So, there are similar piston pumps forming one pair of piston pumps.

[0015] In the context of the ~~invention~~disclosure a piston pump is understood as a pump having a piston which can be moved in a piston swept volume in such a manner that a fluid contained in the reservoir of the pump (piston swept volume) can be displaced at least partially, preferably completely. A preferred type of a piston pump is a syringe pump which allows to deplete the fluid completely out of the included reservoir. In the context of the ~~invention~~disclosure the phrase piston pump and syringe pump are used synonymously. Nevertheless, also a peristaltic pump can be used in the microfluidic filtration system to drive the fluid through the fluidic channel.

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The movement of the pistons of the piston pumps is controlled by the control unit. The control unit is arranged and adapted to control driving of the piston pumps of each pair of piston pumps synchronously. The control unit can further control the switching from one pair of piston pumps to the other pair of piston pumps. It also can control the switching time for switching.

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The control unit controls the movement of the pistons of one pair of piston pumps so that they can perform a synchronous movement for moving the fluid through the TFF-module with a constant flow speed and a constant pressure rate. Therefore, the stroke of the pistons in the piston swept volume of each piston pump is synchronized or adapted. Because during the concentration process an amount of fluid is withdrawn as a permeate stream in the TFF-module the stroke of the piston of the piston pumps has to be adapted accordingly. Also, if the fluid amount in one pair of piston pumps decreases to a predetermind rate a switching from one pair of piston pumps to the other pair of piston pumps has to be performed. For controlling the switching and/or the switching time the control unit can evaluate information from measurement and control elements which can be a sensor, for example

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pumping and flowing of the fluid through the TFF-module from one piston pump to the other piston pump of the same pair of piston pumps.

5 In an alternative embodiment the switch from the first pair of piston pumps to the second pair of piston pumps can be performed after a predetermined time after starting of the fluidic process has been reached. The predetermined time interval criterion is preferably used if a filtration process is performed which is highly reproducible and well defined. This criterion can be used if a switching time can be determined from other preliminary tests or can be calculated using
10 theoretical equations based on known system parameters. In this case an online determination of the actual volume can be avoided. The control unit can therefore comprise a timer or the like.

The volume level, at which the switch between the two pairs of piston pumps
15 is conducted, can be monitored using one of a couple of ways. For example, the amount of fluid withdrawn as permeate stream can be measured using a balance so that the amount is weighted and the volume is calculated. Therewith the actual volume in the fluid channel can be determined. Further, the volume can be determined using the distinct stroke length of one piston
20 and monitoring the increments of the motor necessary to perform this distinct stroke length until a change in the systems pressure, e. g. the transmembrane pressure of the filtration module is detected. In each case the sensor information is provided to the control unit for controlling the piston pumps respectively.

| In the micro flow filtration system according to the ~~invention~~ disclosure, preferably two pairs of piston pumps are used, each pair containing piston pumps for a discrete volume range. The volume of the piston pumps of one pair of piston pumps is larger than the volume of the piston pumps of the other pair of piston
30 pumps. In practical use the first pair of piston pumps comprises piston pumps with a volume which is larger than the volume of the piston pumps of the second pair of piston pumps. This allows to handle a relatively large volume using the first pair of piston pumps having the larger volume. If the volume is

reduced to a smaller amount (e.g. if a predetermined fluid volume within the fluidic system is reached) the system switches from the first pair to the second pair of piston pumps to handle the smaller (rest-)volume of the fluid. So, the systems always works with a volume optimized pair of piston pumps with respect to the current fluid volume. This allows an optimized dosing of the fluid and an optimized moving of the piston pumps at each stage of the fluid process.

Preferably the piston pumps of the first pair have a volume which is at least three times larger than the volume of the piston pumps of the second pair, preferably at least five times or ten times larger and particularly preferably at least 25 times larger. In some preferred embodiments the volume of the piston pumps of the first pair are at least 100 times larger than the volume of the piston pumps of the second pair. So, a wide range of volume can be covered with such combination of piston pumps. Preferably the piston pumps of one pair are substantially larger than the piston pumps of the other pair. To achieve an advantageous and positive effect with the combination of the piston pump pairs, the volume of the piston pump pairs has to be different in any case.

So, using a micro flow filtration system according to the invention disclosure allows to operate with fluid samples having a volume of at most 100 ml, preferably at most 25 ml and particularly preferably at most 10 ml or 1 ml. Due to the fact that the concentration rate which is desired and the start volume of the fluid sample are influenced by the minimal working volume of the system, a minimal working volume which is as small as possible is desired. Preferably the minimal working volume of the system is at most 1 ml, preferably at most 700 μ l, further preferably at most 500 μ l, particularly preferably at most 200 μ l and also particularly preferably at most 100 μ l.

The minimal working volume is the volume of fluid which can be operated in a flow filtration system without pumping air through the system. So, the components, elements, and conduits like the fluidic channel and the TFF-

module have to be filled by an amount of fluid which avoids an air cavitation in the liquid solution, in other words, which avoids the entrance of air into the system. Preferably the volume of the fluid contained in the microfluidic filtration system is equal to the volume of one piston pump of the larger pairs of pumps. The fluid volume contained in the system can also be as large as the sum of the volumes of all piston pumps contained in the system. It is also possible that an external fluid reservoir is connected to the system, e.g. to one of the conduits of the fluidic channel, so that the fluid being withdrawn in the TFF-module is refilled from the external reservoir. A unidirectional fluid flow from the reservoir to the fluidic channel has to be established. This is performed by means known in the state of the art. Preferably the control unit for controlling the filtration process and especially for controlling the movement of the pistons is adapted to synchronize the stroke of the pistons of each pair of piston pumps. This is especially valuable if a pair of of piston pumps consists of equal piston pumps so that the piston pumps have an equal swept volume and a equal diameter. So, the same stroke of the piston (the way that the piston is moved) of each piston leads to the same volume. In other words, the control unit contrails the strokes of the pistons such that the piston swept volume of each piston pumps is synchronized accordingly. A control has to be performed in such a manner that pumping of air in the fluid circuit and in the system is avoided.

In a preferred embodiment of the filtration system according to the inventiondisclosure, the system comprises a balance to weight the permeate withdrawn from the fluid channel. Using the weight of the withdrawn fluid mass, the volume of the withdrawn permeate can be calculated. Knowing the start volume of the concentration process allows calculating the actual overall volume which is contained in the system.

pump moves out of its chamber so that the desired transmembrane pressure can be adjusted and controlled.

5 If the actual amount of fluid falls below a predetermined level, the pistons of the second pair of piston pumps are moved instead of those of the first piston pumps. A transmission from the first pair of piston pumps to the second pair of piston pumps can be arranged. Such transmission (switching) is preferably controlled by a control unit. Then, the process steps continue using the second pair of piston pumps.

15 In a preferred embodiment of the system according of the ~~invention~~disclosure the piston pumps are motor driven. Preferably the system comprises one motor for each piston pump for driving the piston of the pump. The operation of each piston pump using a separate motor allows a very exact adjustment and an easy control of the piston pumps so that the process parameters can be adapted easily and with only a few regulating steps. Controlling the motors is performed by one single unit or by one control unit for each motor. The same control unit that controls one or several motors or an additional control device can be used to control, monitor and address the motor control units.

20 Preferably the used motors are stepping motors. So, the motor can be easily and exactly controlled using incremental steps to drive the motor. The fluid to be pumped through the channels and the desired pressure in the channel can be adjusted in the simple way.

25 In a further preferred embodiment the system comprises an optical-measuring device for determining the concentration and aggregate formation of the components contained in the fluid sample. The optical-measuring device preferably comprises a cuvette which is integrated in the fluidic channel. Using the cuvette an optical measurement of the concentration can be performed.
30 So, a real time measurement is possible. The acquired measuring data correlating to the concentration can be used to online control the concentration process. Preferably the cuvette has a defined cross-section area surrounded

by two pressure sensors measuring a pressure difference to calculate the fluids viscosity according to the Hagen-Poiseuille equation. The measuring data (sensor information) can also be used by the control unit to control the pistons of the piston pumps and so to control the whole filtration process.

It is evident that the system according to the ~~invention~~disclosure and also the inventive method can be used for performing a fluidic process such as concentrating of components contained in a fluid sample or for exchanging the solvent of the sample fluid. So, not only a concentration or purification can be performed but also a diafiltration. In the case of diafiltration the piston pump of the pair of piston pumps, respectively, with the smaller volume is filled with the fluid sample. The piston pump of the pair having the larger volume is filled with the exchange buffer or solvent which should be exchanged. At the beginning of the diafiltration process the pumps with the smaller volume drive the fluid sample through the fluid channel and the TFF-module so that permeate is withdrawn from the system. The piston pumps with the larger volume containing the buffer solution are also working to refill the amount of fluid which is withdrawn of the system via the TFF-module. This can take place continuously which may have the disadvantage in special embodiments that the refill of fluid or exchange buffer using the pumps with the large volume may be imprecise. In such cases it may be advantageous if the pumps containing the exchange buffer (having the larger volume) only starts working if a predetermined amount of fluid has been withdrawn from the system. So, it is also possible to perform a diafiltration with fluid samples of a very small amount of less than 10 ml.

The ~~invention~~disclosure is illustrated in more detail hereafter based on particular embodiments shown in the figures. The technical features shown therein can be used individually or in combination to create preferred embodiments of the ~~invention~~disclosure. The described embodiments do not represent any limitation of the ~~invention~~disclosure defined in its generality by the claims.