

scale filtration process is installed using only small amounts and volumes of the solution and the samples. This is especially important if the components are proteins or the like which are sensitive of damages due to shear forces and other forces existing in large-scale devices. For example biotechnologically produced proteins which have to be concentrated in a solution are extremely costly and on the other side extremely sensitive of damages due to the forces which arises in industrial scale process devices. Therefore, the risk to concentrate such proteins in a solution in industrial scale devices is high. Further, these proteins are often not available in a larger amount.

So, there is a strong demand in the industrial process development for a flow filtration device being able to concentrate a component of a solution preferably by the factor of more than 10 and using a start volume that is at most 20 ml.

~~It is an object of the invention~~Various embodiments seek to provide an optimized TFF-system being able to handle a start volume of a solution of at most 20 ml, preferably at most 10 ml, and to concentrate the components of the solution. The resulting volume of the concentrated solution at the end of the concentration process should preferably be at most 2 ml, particularly preferably at most 1 ml, particularly preferably at most 100 μ l. The filtration process should be fast and economical, repeatable and accurate.

The problem is solved with a micro fluidic flow filtration system for increasing the concentration of components contained in a fluid sample with the features ~~according to claim 1 as disclosed herein~~. The ~~object~~problem is also solved with an integrated microfluidic element ~~device according to claim 11 as disclosed herein~~.

According to a first aspect, there is provided a microfluidic flow filtration system having a fluid circuitry (1).

the fluid circuitry (1) comprising the following elements:

- a tangential flow filtration element (7) having a feed inlet (12), a retentate outlet (13), a permeate outlet (14) and a membrane (15) capable for separating the fluid sample into a retentate stream and a permeate stream upon passage of the fluid sample into the tangential flow filtration element (7) through the feed inlet (12).
- an element for pumping for creating and driving a fluid flow of the fluid sample through the fluid circuitry (1) and the tangential flow filtration element (7).
- at least two elements for obtaining information about the properties of the fluid sample within the circuitry (1);and

a plurality of conduits (24) connecting the elements to the fluid circuitry (1) through which a fluid stream of the fluid sample is conducted;

wherein

- a minimal working volume of the circuitry (1) being defined by the minimal fluid volume retained in the elements and the conduits (24), such that the fluid can be recirculated in the circuitry (1) without pumping air through the circuitry (1),
- the minimal working volume of the circuitry (1) is at most 5 ml,
- the functionality of at least two elements of the group of elements of the circuitry (1) is integrated in an integrated microfluidic device (20),
- the one integrated microfluidic element (20) defines a volume element having a volume which is at most one fourth of the minimal working volume of the fluid circuitry (1).

According to a second aspect, there is provided integrated microfluidic element (20) for a microfluidic flow filtration system with a fluid circuitry (1) having a minimal working volume of at most 5 ml, for a system (100) according to the first aspect

characterized in that the integrated microfluidic element (20)

defines a volume element having a volume which is at most one fourth of the minimal working volume of the fluid circuitry of the micro flow filtration system, and

provides the functionality of at least two elements of the group of elements of the fluid circuitry (1),

wherein

the group of elements comprises a tangential flow filtration element (7), an element for pumping suitable for creating and driving a fluid flow and at least two elements for obtaining information about the properties of the fluid sample within the circuitry.

The micro flow filtration system according to the ~~invention~~disclosure for increasing the concentration of components contained in a fluid sample has a fluid circuitry in form of a loop in which the fluid sample or solution is recirculated. The fluid circuitry comprises a tangential flow filtration element (TFF-element), an element for pumping which is suitable for increasing and driving a fluid flow of the fluid sample through the fluid circuitry and through the tangential flow filtration

element, at least two elements for obtaining information about the properties of the fluid sample within the circuitry, and a plurality of conduits connecting the elements to the fluid circuitry through which the fluid stream of the fluid sample is conducted. Optionally, the fluid circuitry comprises also a regulator
5 element for regulation the pressure and/ or the flow in the circuitry.

The fluid circuitry has a minimal working volume which is also called minimal recirculation volume. This volume is the amount of fluid which has to be retained in the fluid circuitry such that the fluid can be recirculated in the circuitry
10 without pumping air through the circuitry. In other words, the elements of the fluid circuitry and the conduits connecting these elements have to be filled with a fluid in such a manner that a continuous fluid flow in the circuitry can be arranged. The minimal working volume depends on the elements used in the circuitry. For example, if tubings for transporting the fluid are installed in the
15 circuitry, these tubings have also a non-negligible contribution to the minimal working volume. The minimal working volume of the circuitry of the micro fluid filtration system according to the inventiondisclosure is at most 5 ml. Preferably, the minimal working volume of the circuitry is even smaller, e.g. preferably at most 2 ml, further preferably at most 1 ml, particularly preferably at most 100 μ l.

According to the inventiondisclosure the fluid circuitry contains also an integrated micro-
fluidic element in which the functionality of at least two elements of the group of elements of the circuitry is integrated. So, the integrated microfluidic element is an element or a component of the circuitry in which the function of at
25 least two of the above mentioned elements of the circuitry is implemented. So, instead of the two separate elements, which are substituted, only one integrated microfluidic element is arranged in the circuitry. Preferably, the integrated microfluidic element is arranged in the retentate circuitry of the fluid circuitry and not in the permeate circuitry. Especially in a concentration mode
30 such positioning of the microfluidic element is advantageous because the functionality of two elements of the circuitry is provided in only one element and the working volume of the system is decreased or reduced.

reservoir element is integrated into the fluid circuitry loop. The reservoir element has a reservoir inlet and a reservoir outlet both in connection to the circuitry. Preferably the inlet and the outlet of the reservoir are arranged at the bottom of the reservoir which can also be a tank or the like. This assures that the contribution of the reservoir to the minimal working volume of the circuitry is relatively small.

Preferably the volume of the reservoir is at most 20 ml, in particular at most 10 ml. During the ~~invention~~disclosure it was recognized that the size of the reservoir can be further reduced to a maximum volume of the reservoir of 5 ml. In a preferred embodiment the reservoir has a volume of at most 2 ml, very preferably of at most 1 ml. If very small solution volumes have to be processed the volume of the reservoir can be further reduced to a volume of at most 0,7 ml or preferably of at most 0,5 ml. Especially if fluid batches of approximately 0,5 ml or less have to be processed in the microfluidic filtration system, the integration of the functionality of at least two elements of the circuitry into an integrated microfluidic element plays an important role.

In a preferred embodiment the circuitry of the filtration system comprises an optical measuring element for obtaining information about the concentration of components contained in the fluid solution. This optical measuring element preferably comprises a cuvette or the like through which the fluid sample flows during the concentration process and which is transparent in such a manner that an optical measuring of parameters related to the concentration of one or more components contained in the fluid solution can be performed.

In another preferred embodiment the circuitry comprises a measuring element for obtaining information about the viscosity of the fluid sample and/or at least a valve element and/or a capillary channel element and/or a hollow fiber element wherein these elements are also members of the group of the elements of the circuitry. The measuring element for obtaining information about the viscosity of the fluid sample usually consists of at least two pressure sensors whose results can be used to determine the viscosity of the fluid sample using

the Hagen-Poiseuille equation. During the development of the invention disclosure investigations have been performed which show that there is a linear dependency between the pressure difference at two points in the fluid circuitry and the viscosity of the solution flowing through the circuitry.

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The tangential flow filtration element of the circuitry comprises a feed inlet, a retentate outlet, a permeate outlet and a semipermeable membrane which is capable of separating the fluid sample into a retentate stream and a permeate stream upon passage of the fluid sample into the tangential flow filtration element through the feed inlet. For the concentration process tangential flow filtration elements can be used which are commercially available from a couple of manufacturers. An important criterion for choosing a fluid filtration element for a microfluidic flow filtration system to process even small volumes of a solution is the contribution of the tangential flow filtration element to the minimal working volume. So, the surface area of the membrane and the volume of the tangential flow filtration element are important. The contribution to the minimal working volume of the flow filtration element should be as small as possible, preferably the minimal working volume of the TFF-element should be at most 1000 μl , preferably at most 500 μl or further preferably at most 100 μl , particularly preferably at most 20 μl .

It is quite clear for a person skilled in the art that the element for pumping is a pump which is integrated in the circuitry. Here the known pumps can be used. For example, the pumping element can comprise a syringe or two or more syringes to assure a continuous flow of the fluid through the circuitry, alternatively piston pumps or peristaltic pumps or gear pumps can be used.

Preferably the element for obtaining information about the properties of the fluid is an element for obtaining information about the pressure and the flow within the circuitry particularly preferably one or more pressure sensors.

To arrange a micro flow filtration system with small minimal working volume the internal diameter of the conduits used in the circuitry is at most 1,5 mm, preferably at most 1 mm. For minimal working volumes of less than 200 μ l
5 conduits which have an internal diameter of at most 0,7 mm or particularly preferably of at most 0,3 mm are used. The conduits are preferably channels or passages in an element of the circuitry. They can also be a pipe or a tubing. These conduits connect the integrated microfluidic element of the circuitry with the other elements of the circuitry. Therefore, a rigid pipe as a conduit can be
10 used.

In a preferred embodiment of the flow filtration system according to the invention disclosure the system comprises a second reservoir which is located outside the circuitry but which is in fluid connection with the circuitry. The second reservoir 15 has a volume which is substantially larger than the volume of the circuitry itself or of a first reservoir element in the circuitry if present. So, the second external reservoir would be the main reservoir for the fluid solution containing the component to be concentrated. Preferably this second reservoir outside the circuitry has a volume which is at least 10 times larger than the volume of the reservoir element of the circuitry or of the circuitry itself, particularly preferably at least 20 times larger.

The system according to the invention disclosure can be used for concentrating a component contained in a fluid sample like a protein or a molecule or the like. Op-
25 tionally the system can also be used in a diafiltration mode. In this case the external second reservoir contains a solution with a buffer that should be used to exchange a buffer in the solution contained in the circuitry which also contains the desired component. So, an exchange of the buffer can easily be performed. It is evident that a diafiltration mode can also be supplied using only
30 one reservoir in the circuitry. In such case, the circuitry is filled with the fluid sample and the reservoir is filled with the buffer solution.

In the field of biotechnology and process technology there are a lot of applications which require the determination of the concentration of components of the fluid sample, the formation of aggregates within the fluid sample and the viscosity of the fluid sample using a flow through method. A typical example is the requirement that the concentration of proteins contained in the fluid sample should be measured online during the concentration process. So, the actual fluid parameters, i.e. viscosity, concentration, and aggregate formation have to be determined during the process. Based on these online-measured parameters, the system can be controlled respectively. Essential process parameters for the concentration and purification process will change during the process. This leads to the resulting knowledge that the concentration and the rate of aggregate formation of the proteins and the viscosity of the solution have to be measured in real time so that an optimal concentration process can be performed by adjusting the relevant process parameters.

15 In the development of the subject matter of the inventiondisclosure it has been identified that for the determination of the concentration using photometric methods can be applied. For this photometry technique preferably a cuvette is used through which the fluid sample flows without diluting the sample. For an optical measurement of the absorption a light source is used which is transmitting light through the cuvette. In a preferred embodiment light crosses the cuvette perpendicular to the flow direction. Performing the photometric measurement using the cuvette preferably an ultraviolet radiation is used to determine the concentration of compounds contained in the sample (e.g. of proteins). For this online determination of the concentration good results could be achieved using a radiation of light with a wave length of $280 \text{ nm} \pm 10 \text{ nm}$.

For measuring the appearance of aggregates in the sample fluid, the ratio between the absorption values at the wavelength of $280 \pm 10 \text{ nm}$ and at a wave length larger than 290 nm (e.g. $320 - 330 \text{ nm}$) is preferred.

In addition, the viscosity of the fluid sample can be measured using a pressure sensing element comprising two pressure sensors at the inlet and at the outlet

of the capillary, respectively. These pressure sensors detect a difference of pressures between the pressure sensor at the inlet and the pressure sensor at the outlet during the flow of the sample through the cuvette. This measurement is performed using a known geometry of the cuvette and a predetermined (constant) flux or flow through rate. Based on the change of the pressure during the flow through the cuvette, the viscosity of the fluid sample can be calculated based on well-known equations like the Hagen-Poiseuille equation. The viscosity is proportional to the measured pressure difference.

10 In a preferred embodiment, the integrated microfluidic element according to the ~~invention~~disclosure comprises and combines these two functionalities of the two pressure sensors and the cuvette. So, the integrated microfluidic element can be used to determine the viscosity, the concentration of the compound, and the formation of aggregates in the fluid sample in real time and online in one
15 element. In addition to this advantage the minimal working volume of this integrated microfluidic element is substantially smaller than using three separate components and conduits for connecting these components.

| In the context of the ~~invention~~disclosure it was recognized that measuring the viscosity
20 and the concentration of the sample fluid in the fluidic circuitry in parallel and within the same microfluidic element leads to a couple of difficulties. To determine the viscosity a pressure drop along a capillary has to be detected. In typical lab-scale systems or industrial-production-scale-systems such measurements are not possible due to the large volumes of the fluid samples to be
25 transported through the fluidic system and due to the large diameters of the tubes and fluid conduits. So, the overall pressure and the pressure differences within such systems are too low to allow a precise viscosity determination
| based on pressure drop measurements. During the ~~invention~~disclosure it was recognized that for a precise viscosity measurement the capillary has to be very
30 small in diameter (smaller than 0.5 mm) and preferably with a relatively short length (less than 200 mm), as the diameter has the largest influence on the pressure drop. Therefore, variances in the channel diameter have a significant impact on the precision of the measurement of the pressure drop. To deter-

with the two pressure sensors, can be used in an integrated microfluidic element.

In another alternative or cumulative preferred embodiment, the integrated microfluidic element according to the ~~invention~~disclosure comprises and combines the functionality of the micro tangential flow filtration element (TFF-element) and at least one element for obtaining information about the properties of the fluid sample within the circuitry. Preferably, elements for obtaining information about the properties of the fluid sample within the circuitry are pressure sensors to measure the pressure of the fluid sample in the circuitry and, based thereon, can be used to determine the viscosity of the fluid sample. So, in this preferred embodiment a micro TFF-element is combined with at least one pressure sensor, further preferred with two pressure sensors.

15 Preferably the TFF-element comprises a TFF-housing which can be a filter cassette. The TFF-housing is part of the housing of the integrated microfluidic element. In this case the TFF-housing is integrated in the housing of the microfluidic element. Preferably the membrane of the TFF-element is disposable. The membrane can be exchanged in the case that the membrane is clogged-up. If the efficiency of the TFF-element is dropped to a predetermined threshold value an exchange of the membrane becomes necessary. To design the TFF-element with a disposable membrane has the advantage that the filter cassette itself (TFF-housing) can remain unchanged in the fluid circuitry so that the connections to the connected conduits do not have to be touched especially not to be opened. Further, if the TFF-element is integrated in the microfluidic element together with two pressure sensors, the sensors remain at their location, especially in their position in the housing of the microfluidic element. So, neither the sensors themselves nor their electrical connections are influenced if only the membrane of the TFF-element is exchanged in case of clogging. Further the size and especially the volume of the fluidic channels of a TFF-element comprising pressure sensors can be reduced because the channels connecting the filter cassette and the pressure sensors can be short.

In a further preferred embodiment this integrated microfluidic element also contains the functionality of the pressure regulator element so that in addition the pressure in the circuitry can be controlled and adjusted. So, not only the functionality of two elements is integrated within the integrated microfluidic element according to the inventiondisclosure, but the functionality of three (or even more) elements.

In another preferred embodiment of the integrated microfluidic element according to the inventiondisclosure, it comprises the functionality of a capillary or a capillary channel and of the two pressure sensors.

The inventiondisclosure 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 inventiondisclosure. The described embodiments do not represent any limitation of the inventiondisclosure defined in its generality.

- Fig. 1 shows a schematic view of a filtration system;
- 20 Fig. 2 shows a flow filtration circuitry according to the invention with an integrated microfluidic element;
- Fig. 3 shows another schematic view of a fluidic circuitry for the determination of viscosity according to the invention;
- 25 Fig. 4 shows an integrated microfluidic element comprising the functionality of two pressure sensors and a measuring element for detecting the concentration and viscosity;
- 30 Fig. 5a, b shows two embodiments of another microfluidic element comprising the functionality of measuring the pressure in the circuitry and filtering the fluid sample.