SUMMARY ~~THE INVENTION~~

[0044] As such, the object described above is achieved according to the ~~invention~~disclosure by providing a method for purifying and enriching a target product selected from

- immunoglobulins or other proteins; or
- plasmid DNA, genomic DNA, RNA or other nucleic acids; or
- viruses,

comprising the following steps:

a. Providing a starting solution containing the target product;

b. Converting the starting solution into an aqueous two-phase system by adding at least one polymer and at least one salt in a suitable concentration, or by adding at least two polymers in a suitable concentration;

c. Mixing the aqueous two-phase system while maintaining a mass transfer, such that the target product is enriched in the light or heavy phase;

d. Allowing the phases to segregate in a separating device into a light phase, a heavy phase and, if an intermediate phase is present, an intermediate phase;

e. Continuously measuring electrical conductivity in at least one of the phases, in particular in the light and/or heavy phase, in the separating device to determine the position of the two phases and the phase boundary interface or, in the presence of an intermediate phase, the position of the intermediate phase and the two phase boundary interfaces;

f. Removing the phases from the separating device based on the continuously measured electrical conductivity of at least one of the phases in step e., in particular the light and/or heavy phase; and

g. Extracting the phase with the target product.

[0044a] In one aspect, the present disclosure provides a method for purifying and enriching a target product selected from the group consisting of

- immunoglobulins or other proteins; or
- plasmid DNA, genomic DNA, RNA or other nucleic acids; or
- viruses,

comprising the following steps:

a. Providing a starting solution containing the target product;

b. Converting the starting solution into an aqueous two-phase system by adding at least one polymer and at least one salt in a suitable concentration, or by adding at least two polymers in a suitable concentration;

c. Mixing of the aqueous two-phase system while maintaining a mass transfer, such that the target product is enriched in the light phase or in the heavy phase;

d. Allowing the phases to segregate in a separating device into a light phase, a heavy phase and, if an intermediate phase is present, an intermediate phase;

e. Continuously measuring the electrical conductivity in at least one of the phases in the separating device to determine the position of the two phases and the phase boundary interface or, in the presence of an intermediate phase, the position of the intermediate phase and the two phase boundary interfaces;

f. Removing each of the light phase, the heavy phase and, in the presence of an intermediate phase, the intermediate phase from the separating device based on the continuously measured electrical conductivity of at least one of the phases in step e.; and

g. Extracting the phase with the target product.

[0045] As such, the present ~~invention~~disclosure provides target product enrichment and separation and impurity removal simultaneously with the aid of an aqueous two-phase system. In steps a. to g. of the ~~invention~~disclosure, the conventional use of separators, in particular centrifugators, filtration and chromatography, is completely dispensed with.

[0046] According to one ~~embodiment~~example of the ~~invention~~disclosure, the method is used to purify and enrich a target product using as a starting solution a cell culture, a cell supernatant or a cell lysate, in which the target product is contained. In this case, the method of the present ~~invention~~disclosure is used for cell harvesting and/or isolation of the target product from the cells, cell culture and/or cell lysate. The method according to the ~~invention~~disclosure can therefore be used both for separating the cells and for processing the product produced by the cells, in which case the cells have already been separated. That is, the cells can be removed either beforehand, or only during the present method.

[0047] Instead of or in addition to electrical conductivity, turbidity can also be measured.

[0048] It is also an object of the ~~invention~~disclosure to provide a device for carrying out the method of the ~~invention~~disclosure, comprising
a separating device for separating the phases;
at least one outlet in the separating device;
at least one probe for measuring electrical conductivity and/or at least one probe for measuring turbidity in at least one of the phases in the separating device;
one or more removal device(s) and/or means for regulating and/or controlling the removal quantity, removal rate(s) and/or removal duration of the light phase, the heavy phase and, if present, the intermediate phase, based on the measured values of electrical conductivity (for the probe(s) for measuring electrical conductivity) or based on the measured values of turbidity (for the probe(s) for measuring turbidity) in at least one phase.

[0048a] In another aspect, the present disclosure provides a device for carrying out the method of the disclosure, comprising

a separating device for separating the phases;

at least one outlet in the separating device;

at least one probe for measuring electrical conductivity and/or at least one probe for measuring turbidity in at least one of the phases in the separating device;

one or more removal device(s) and/or means for regulating and/or controlling the removal quantity, removal rate and/or removal duration of the light phase, the heavy phase and, if present, the intermediate phase, based on the measured values of electrical conductivity and/or based on the measured values of turbidity in at least one phase.

[0049] A removal device is, for example, an immersion tube, the structure and function of which will be explained in detail later.

[0050] The ~~invention~~disclosure also relates to the use of the method or device according to the ~~invention~~disclosure for adjusting the position of the phases in a separating device and separately removing the phases from a separating device based on the

measured electrical conductivity values of the phases (for the probe(s) for measuring electrical conductivity) and/or based on the measured turbidity values of the phases (for the probe(s) for measuring turbidity).

[0050a] In another aspect, the present disclosure provides a use of the method of the disclosure or the device of the disclosure for adjusting the position of the phases in a separating device and separating the phases based on the measured electrical conductivity values of at least one of the phases and/or based on the measured turbidity values of at least one of the phases.

[0051] Additional features and advantages of the disclosure will be indicated in the detailed description that follows and, in part, will be readily apparent to the person skilled in the prior art from the description or will be recognized by putting the disclosure as described herein into practice, including the detailed description that follows, the claims along with the accompanying drawings. The claims set forth below form a part of this description and are incorporated herein directly and by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0052] Figures 1 to 22 are schematic illustrations of exemplary embodiments of the present disclosure. In the figures in detail:

[0053] Figure 1 shows a schematic side view in section of an exemplary embodiment of a separating device according to the invention, showing the course of an extraction of a batch at 3 points in time of the separation with a probe for measuring the electrical conductivity of the phases, plotted against time;

[0054] Figure 2 shows a schematic side view in section of an exemplary embodiment of a separating device according to the invention, representing a continuous extraction with two probes for measuring the electrical conductivity of the phases;

[0055] Figure 3 shows a schematic side view in section of an exemplary embodiment of a separating device according to the invention, representing a continuous extraction with two probes for measuring the electrical conductivity of the phases at a different point in time than Figure 2;

Claims

1. A method for purifying and enriching a target product selected from the group consisting of

- immunoglobulins or other proteins; or
- plasmid DNA, genomic DNA, RNA or other nucleic acids; or
- viruses,

comprising the following steps:

a. Providing a starting solution containing the target product;

b. Converting the starting solution into an aqueous two-phase system by adding at least one polymer and at least one salt in a suitable concentration, or by adding at least two polymers in a suitable concentration;

c. Mixing of the aqueous two-phase system while maintaining a mass transfer, such that the target product is enriched in the light phase ~~(20, 120)~~ or in the heavy phase ~~(40, 140)~~;

d. Allowing the phases to segregate in a separating device ~~(10, 100)~~ into a light phase ~~(20, 120)~~, a heavy phase ~~(40, 140)~~ and, if an intermediate phase is present, an intermediate phase ~~(30, 130)~~;

e. Continuously measuring the electrical conductivity in at least one of the phases in the separating device ~~(10, 100)~~ to determine the position of the two phases ~~(20, 120, 40, 140)~~ and the phase boundary interface or, in the presence of an intermediate phase ~~(30, 130)~~, the position of the intermediate phase ~~(30, 130)~~ and the two phase boundary interfaces;

f. Removing each of the light phase ~~(20, 120)~~, the heavy phase ~~(40, 140)~~ and, in the presence of an intermediate phase ~~(30, 130)~~, the intermediate phase from the separating device ~~(10, 100)~~ based on the continuously measured electrical conductivity of at least one of the phases in step e.; and

g. Extracting the phase with the target product.

2. The method according to claim 1,
characterized in that

the continuous measurement of the electrical conductivity in at least one of the phases in the separating device ~~(10, 100)~~ is carried out with one or more conductivity measuring devices in the form of one, two, three or more probes ~~(60, 160.1, 160.2, 162.1, 162.2, 162.3, 162.4, 163.1, 163.2, 163.3, 163.4)~~,

in particular, at least one probe ~~(160.1, 162.1, 163.1)~~ is used for continuously measuring the electrical conductivity of the heavy phase ~~(140)~~ and at least one probe ~~(160.2, 162.3, 162.4, 163.3, 163.4)~~ is used for continuously measuring the electrical conductivity of the light phase ~~(20, 120)~~ at a suitable position in the separating device ~~(100)~~.

3. The method according to one of the preceding claims 1 to 2,

characterized in that

an outlet ~~(50)~~ is provided in the separating device ~~(10)~~ and the removal of the phases ~~(10, 20, 30)~~ from the separating device ~~(10)~~ through the outlet ~~(50)~~ according to step f. is carried out by gravity or using positive pressure or negative pressure, generated with or without a pump, or a pump;

or

an upper outlet ~~(150.2)~~ is provided in the separating device ~~(100)~~, and the removal of the light phase ~~(120)~~ from the upper outlet ~~(150.2)~~ of the separating device ~~(100)~~ is carried out using gravity or positive pressure or negative pressure, generated with or without a pump, or a pump,

and

a lower outlet ~~(150.1)~~ is provided in the separating device ~~(100)~~, and the removal of the heavy phase ~~(140)~~ from a lower outlet ~~(150.1)~~ of the separating device ~~(100)~~ is carried out using gravity or positive pressure or negative pressure, generated with or without a pump, or a pump from the separating device ~~(100)~~.

4. The method according to one of the preceding claims 1 to 3,

characterized in that

the removal of a phase according to step f. is carried out in continuous operation by means of a height-adjustable immersion tube ~~(180, 182)~~ having an opening ~~(190, 192)~~ that is immersed in the phase to be removed, wherein

- the immersion tube (180) is connected to the lower outlet (150.2) in such a way that the phase can flow out under gravity to the outlet (150.1) through the opening (182) in the immersion tube (180) or

- the immersion tube (190) has an opening (192) through which the removal of the phase is carried out using positive pressure or negative pressure, generated with or without a pump, or a pump.

5. The method according to one of the preceding claims 1 to 4,

characterized in that

the height-adjustable immersion tube (180, 190) is used with an overtube (183, 193) extending from top to bottom,

wherein the immersion tube (180, 190) is formed of a height-adjustable inner tube (181, 191) having an opening (182, 192) for removing one of the phases, and an overtube (183, 193) extending from top to bottom, and the length of the overtube (183) is defined by an upper end arranged above the light phase (120) and a lower end (183a, 193a), wherein the diameter of the overtube (183, 193) is adjusted to be larger than the diameter of the inner tube (181, 191), such that an intermediate space between the inner tube (181, 191) and overtube (183, 193) is formed, and the length of the overtube (183) is selected such that the overtube (183, 193) projects beyond the opening (182, 192) of the inner tube (181, 191) and the lower end (183a, 193a) of the overtube (183, 193) is immersed in the heavy phase (140) or, if present, in the intermediate phase (130), such that the fluid of the phase into which the lower end (183a, 193a) of the overtube (183, 193) is immersed rises in the intermediate space and can exit through the opening (182, 192) in the inner tube (181, 191).

or

the height-adjustable immersion tube (180, 190) with an overtube extending from the bottom to the top is used,

wherein the height-adjustable immersion tube is formed of a height-adjustable inner tube (181, 191), which has an opening (182, 192) for removing one of the phases and an overtube extending from bottom to top, and the length of the overtube is defined by a lower end, which is arranged below the

heavy phase ~~(140)~~, and an upper end, wherein the diameter of the overtube is adjusted to be larger than the diameter of the inner tube, such that an intermediate space is formed between the inner tube ~~(181, 191)~~ and the overtube, and the length of the overtube is selected so that the overtube projects beyond the opening ~~(182, 192)~~ of the inner tube ~~(181, 191)~~ and the upper end of the overtube is immersed in the light phase ~~(120)~~ or, if present, in the intermediate phase ~~(130)~~, such that the fluid of the phase in which the upper end of the overtube is immersed flows into the intermediate space and can exit through the opening ~~(182, 192)~~ in the inner tube ~~(181, 191)~~.

6. The method according to claim 4 or 5,

characterized in that

the length of the overtube ~~(183, 193)~~ extending from top to bottom and its diameter are selected in such a way that the entry of suspended matter, flocs and other solid or semi-solid components, into the intermediate space between the inner tube ~~(181, 191)~~ and the overtube ~~(183, 193)~~ is prevented by the overtube ~~(183, 193)~~;

or

the length of the overtube extending from bottom to top and its diameter are selected in such a way that the entry of suspended matter, flocs and other solid or semi-solid components into the intermediate space between the inner tube ~~(181, 191)~~ and the overtube is prevented by the overtube.

7. The method according to one of the preceding claims 1 to 6,

characterized in that

the probe(s) ~~(160.2, 162.3, 162.4, 163.3, 163.4)~~ for the light phase ~~(120)~~ is (are) positioned lower than the upper outlet ~~(150.2)~~ in the separating device ~~(100)~~ and the probe(s) ~~(160.1, 162.1, 163.1)~~ for the heavy phase ~~(140)~~ is (are) positioned higher than the lower outlet ~~(150.1)~~ in the separating device ~~(100)~~;

and/or

an immersion tube ~~(180, 190)~~ with or without an overtube ~~(183, 193)~~ is used,

wherein, when using an immersion tube ~~(180, 190)~~ without an overtube ~~(183, 193)~~, the opening ~~(182, 192)~~ of the immersion tube ~~(180, 190)~~ for removing the heavy phase ~~(140)~~ is located lower than the probe(s) ~~(160.1, 162.1, 163.1)~~ in the heavy phase ~~(140)~~ and wherein the opening ~~(182, 192)~~ of an immersion tube ~~(180, 190)~~ for removing the light phase ~~(120)~~ is located higher than the probe(s) ~~(160.2, 162.3, 162.4, 163.3, 163.4)~~ in the light phase ~~(120)~~; and

wherein, when using an immersion tube ~~(180, 190)~~ with an overtube ~~(183, 193)~~ extending from top to bottom, the lower end ~~(183a, 193a)~~ of the overtube ~~(183, 193)~~ is located lower than the probe(s) ~~(160.1, 162.1, 163.1)~~ in the heavy phase ~~(140)~~ for removing the heavy phase ~~(140)~~ when the heavy phase ~~(140)~~ is removed through the opening ~~(182, 192)~~ of the immersion tube ~~(180, 190)~~; and

wherein, when using an immersion tube with an overtube extending from the bottom to the top, when the light phase ~~(120)~~ is removed through the opening ~~(182, 192)~~ of the immersion tube, the upper end of the overtube for removing the light phase ~~(120)~~ is located higher than the probe(s) ~~(160.2, 162.3, 162.4, 163.3, 163.4)~~ in the light phase ~~(120)~~.

8. The method according to one of the preceding claims 1 to 7,

characterized in that

continuously measuring the electrical conductivity of the light phase ~~(120)~~ in the separating device ~~(100)~~ in step e. for one or more probes ~~(160.2, 162.3, 162.4, 163.3, 163.4)~~ in each case produces a value for electrical conductivity, which is compared with a target value range for the electrical conductivity of the light phase ~~(120)~~ and, if the target value range is exceeded, heavy phase ~~(140)~~ is removed, wherein the removal quantity, removal rate and/or removal duration for the heavy phase ~~(140)~~ are controlled in such a way that the measured conductivity of the light phase ~~(120)~~ is again in the target value range;

or

continuously measuring the electrical conductivity of the heavy phase ~~(140)~~ in the separating device ~~(100)~~ in step e. for one or more probes ~~(160.1, 162.1, 163.1)~~ in each case produces a value for electrical conductivity, which is compared with a target value range for the electrical conductivity of the heavy

phase and, if the electrical conductivity falls below the target value range, less heavy phase ~~(140)~~ is removed, wherein the removal quantity, removal rate and/or removal duration for the heavy phase ~~(140)~~ are controlled in such a way that the measured conductivity of the light phase ~~(120)~~ is again in the target value range.

9. The method according to one of the preceding claims 1 to 8,
characterized in that
the electrical conductivity values are used to vary the position of the phase(s) and phase boundary interface(s) in continuous operation by monitoring and controlling one or more of the following:

- the removal rate for the heavy phase being increased or decreased;
- the removal rate for the light phase being increased or decreased;
- the removal quantity for the heavy phase being increased or decreased;
- the removal quantity for the light phase being increased or decreased;
- the removal duration for the heavy phase being increased or decreased;
- the removal duration for the light phase being increased or decreased;
- the removal of the light or heavy phase being interrupted;
- the feed quantity of the aqueous two-phase system flowing into the separating device ~~(100)~~, with or without the target product, being increased or decreased, wherein the aqueous two-phase system without the target product is a flushing solution;
- the feed rate of the aqueous two-phase system flowing into the separating device, with or without a target product, being increased or decreased, wherein the aqueous two-phase system without a target product is a flushing solution;

and/or

- the immersion tube being driven up or down to allow to flow out or suck away one or two phases and, if necessary, repositioned upward or downward;

wherein the one or more removal devices and/or means may be used to carry out the preceding measures:

- gravity;
- positive pressure;
- negative pressure;
- one or more pumps;

and/or

- removal devices for controlled draining or interrupting the removal of phases at the outlet, selected from continuously adjustable valves with different degrees of opening, adjustable hose pinch clamps, adjustable shut-off valves or adjustable flow limiters.

10. The method according to one of the preceding claims 1 to 9, characterized in that

for continuously measuring the electrical conductivity of the phases in the separating device ~~(10, 100)~~, two, three, four or more probes ~~(160.1, 160.2, 162.1, 162.2, 162.3, 162.4, 163.1, 163.2, 163.3, 163.4)~~ are each inserted into and used in an insertion opening ~~(157.1, 157.2, 158.1, 158.2, 158.3, 158.4, 159.1, 159.2, 159.3, 159.4)~~ in the wall ~~(155)~~ of the separating device ~~(100)~~, wherein the insertion openings ~~(157.1, 157.2, 158.1, 158.2, 158.3, 158.4, 159.1, 159.2, 159.3, 159.4)~~ have an arrangement that is selected from:

- an axial arrangement of the insertion openings ~~(157.1, 157.2)~~, wherein the insertion openings ~~(157.1, 157.2)~~ are arranged on a straight connecting line;
- a radial arrangement of the insertion openings ~~(158.1, 158.2, 158.3, 158.4)~~, wherein the insertion openings ~~(158.1, 158.2, 158.3, 158.4)~~ are arranged in an arc; or

- a radially offset arrangement of the insertion openings ~~(159.1, 159.2, 159.3, 159.4)~~, wherein the insertion openings ~~(159.1, 159.2, 159.3, 159.4)~~ are arranged in an arc and additionally shifted in a preferred direction.

11. The method according to one of the preceding claims 1 to 10, characterized in that

- the mixing of the aqueous two-phase system is carried out by using active mixing and/or static mixing.

12. The method according to one of the preceding claims 1 to 11, characterized in that at least one of the following conditions is satisfied:

- the method is carried out continuously or discontinuously;
- a cell culture, cell culture supernatant or cell lysate containing the target product is used as the starting solution;
- an aqueous two-phase system is prepared with the starting solution, with which a phase ratio of the phase containing the target product to the counterphase of > 1.1 is present;
- a density ratio of light phase ~~(20, 120)~~ to heavy phase ~~(40, 140)~~ of at least 1.06 is used;
- in the aqueous two-phase system, polyethylene glycol (PEG), polypropylene glycol (PPG), dextran, PEG-PPG-PEG copolymer (EOPO) or mixtures of these are used as the polymer or as one of the polymers;
- in the aqueous two-phase system, polyethylene glycol with a molecular weight between 200 and 8000 g/mol is used as the polymer;
- in the aqueous two-phase system, at least one polymer is used in a concentration of from 5 to 35 wt.%, based on the total quantity of aqueous two-phase system;
- in the aqueous two-phase system, a phosphate salt, ammonium salt, potassium salts, acetate salts, sodium salts or citrate salt or mixtures of these are used as salt or as one of the salts;
- instead of a salt, an acid selected from citric acid or acetic acid is used;

- stabilizing components selected from amino acids, sugars or other stabilizing additives are added to the aqueous two-phase system;
- the method is carried out continuously, but steps a. and/or d. are carried out semi-continuously.

13. The method according to one of the preceding claims 1 to 12, characterized in that

instead of or in addition to the continuous measurement of the electrical conductivity, the turbidity in at least one of the phases in the separating device ~~(10, 100)~~ is continuously measured, wherein the turbidity can be carried out with one or more turbidity measuring devices in the form of one, two, three or more probes.

14. The method according to one of the preceding claims 1 to 13, characterized in that

at the end of the extraction method for the residual discharge of a light phase ~~(120)~~ containing the target product, a flushing solution ~~(115)~~ is added to the separating device ~~(100)~~, which displaces the light phase ~~(120)~~ containing the target product from the separating device ~~(100)~~ and the latter can thus be removed through the outlet ~~(150.2)~~ arranged at the top,

and/or

at the end of the extraction method for the residual discharge of a light phase ~~(120)~~ containing the target product, no more heavy phase ~~(140)~~ is removed, the latter thereby accumulates and displaces the light phase ~~(120)~~ upward, such that the light phase ~~(120)~~ can be removed through the outlet ~~(150.2)~~ arranged at the top;

or

at the end of the extraction method for the residual discharge of a heavy phase ~~(140)~~ containing the target product, a flushing solution ~~(115)~~ is added to the separating device ~~(100)~~, which displaces the heavy phase ~~(140)~~ containing the target product from the separating device ~~(100)~~ and the latter can thus be removed through the outlet ~~(150.1, 150.3)~~ arranged at the bottom,

and/or

at the end of the extraction method for residual discharge of a heavy phase ~~(140)~~ containing the target product, no more light phase ~~(120)~~ is removed, the latter thereby accumulates and displaces the heavy phase ~~(140)~~ downward, such that the heavy phase ~~(140)~~ can be removed through the outlet ~~(150.1, 150.3)~~ arranged at the bottom.

15. A device for carrying out the method according to one of the claims 1 to 14, comprising

a separating device ~~(10, 100)~~ for separating the phases;

at least one outlet ~~(50, 150.1, 150.2)~~ in the separating device ~~(10, 100)~~;

at least one probe ~~(60, 160.1, 160.2, 162.1, 162.2, 162.3, 162.4, 163.1, 163.2, 163.3, 163.4)~~ for measuring electrical conductivity and/or at least one probe for measuring turbidity in at least one of the phases in the separating device ~~(10, 100)~~;

one or more removal device(s) and/or means for regulating and/or controlling the removal quantity, removal rate and/or removal duration of the light phase ~~(20, 120)~~, the heavy phase ~~(40, 140)~~ and, if present, the intermediate phase ~~(30, 130)~~, based on the measured values of electrical conductivity and/or based on the measured values of turbidity in at least one phase.

16. A use of the method according to one of the claims 1 to 14 or the device according to claim 15 for adjusting the position of the phases in a separating device ~~(10, 100)~~ and separating the phases based on the measured electrical conductivity values of at least one of the phases and/or based on the measured turbidity values of at least one of the phases.