A METHOD FOR SEPARATING BIOMOLECULES

FIELD OF THE DISCLOSURE

The present disclosure relates to the field of separation of biomolecules, and in particular is directed to a method for separating biomolecules from a cell culture or from a biological solution in a magnetic separator.

BACKGROUND OF THE DISCLOSURE

Separation of biomolecules by use of magnetic adsorbent beads is known in the art. For example, United States Patent US7506765 B2 describes a high-gradient magnetic separator for the selective separation of magnetic particles from a suspension which is conducted through a matrix of plate-like separation structures of a magnetic material which are disposed in a magnetic field. Further, United States Patent US6602422 B1, which is hereby incorporated by reference in its entirety, relates to a separation and release process for purifying biological material on a micro separation column. The method includes release of the biological material from magnetic carriers and elution from the micro separation column while the magnetic carriers are still magnetically retained by a matrix of ferromagnetic particles inside the micro separation column. The patent publication WO2018122246 describes a method for separating biomolecules from a cell culture, comprising binding the biomolecules to magnetic beads, separating the magnetic beads including bound biomolecules from the rest of the cell culture by use of a magnetic separator, forwarding the magnetic beads including bound biomolecules as a slurry with an added buffer to a separate elution cell, and eluting the biomolecules from the magnetic beads in the elution cell. Also, the patent publication WO2018122089 relates to magnetic beads comprising a porous matrix and one or more magnetic particles embedded in the matrix, and further comprising immunoglobulin-binding ligands covalently coupled to the porous matrix.

Magnetic bead processing is an effective way to purify a target biomolecule directly from crude feed cell stocks and this technology can replace clarification tools such as centrifugation, filtration and capture chromatography. The magnetic beads are functionalized with ligands which have affinity for the target biomolecule. The magnetic beads are added and mixed with the crude feed for a certain time to specifically bind all target biomolecules in the feed. After binding, the magnetic beads are trapped by a magnet while cells and impurities are decanted off. Wash buffer is added to the magnetic beads and the magnet is turned off and mixing is made with wash buffer and the magnetic beads. After mixing, the magnet is turned on to trap the beads and the wash buffer is decanted off. After several washings, elution buffer is added to the magnetic beads, in the same way as for the

wash buffer, and the target biomolecule is released from the magnetic beads using several batch elution steps. All washing steps and elution steps are made in batch mode and since it is required to perform several batch elution steps to obtain high elution yield, the target will be more diluted using this technique compared to column chromatography. It is also, using batch elution, more difficult to optimize elution conditions since the elution buffer needs to titrate the washing buffer to reach the correct elution conditions to release the target biomolecule from the magnetic beads and that requires different or large volumes of elution buffer for each elution step. One way to overcome this is to transfer the washed beads into a chromatography column for the elution step to decrease the volume elution buffer and collect the elution pool using fractionation with one elution buffer that is optimized. However, this needs additional equipment and column packing, which is time consuming.

Thus, there is a need in the art for improved methods for separation of biomolecules, comprising efficient capture and purification of biomolecules from crude feeds, while achieving a reduced process time and a more cost-efficient process.

SUMMARY OF THE DISCLOSURE

The above objective to provide improved methods for separation of biomolecules is achieved by the present disclosure, which relates to a method for separation of biomolecules by simultaneously applying flow elution and agitation in a magnetic separator. As shown herein, it is thereby possible to flow-elute the target biomolecule directly from the magnetic separator with a surprisingly high yield and purity by use of a small elution volume. The precision of the method equals the precision of column chromatography while requiring a substantially shorter process time.

More particularly, the present disclosure is directed to a method for separating a biomolecule from a cell culture or from a biological solution, comprising the steps of:

(a) providing magnetic particles comprising ligands capable of binding the biomolecule;

(b) contacting a cell culture or a biological solution comprising the biomolecule with the magnetic particles to obtain magnetic particles comprising the bound biomolecule;

(c) retaining the magnetic particles with a magnetic field in a magnetic separator;

(d) optionally washing the magnetic particles with a washing liquid;

(e) agitating the magnetic particles in at least one plane of the magnetic separator to form a fluidised bed of magnetic particles in the magnetic separator;

(f) providing a flow of an elution liquid in a direction essentially perpendicular to the at least one plane, to elute the bound biomolecule from the magnetic particles while retaining the magnetic particles with the magnetic field in the magnetic separator.

A further improvement realised by the present inventors is that the magnetic separation may be followed by membrane chromatography to purify the biomolecule further. Accordingly, the present disclosure provides a method for separating a biomolecule from a cell culture or from a biological solution, comprising the steps of:

(a) providing magnetic particles comprising ligands capable of binding the biomolecule;

(b) contacting a cell culture or a biological solution comprising the biomolecule with the magnetic particles to obtain magnetic particles comprising the bound biomolecule;

(c) retaining the magnetic particles with a magnetic field in a magnetic separator;

(d) optionally washing the magnetic particles with a washing liquid;

(e1) providing a flow of an elution liquid through the magnetic separator to elute the bound biomolecule from the magnetic particles while retaining the magnetic particles with the magnetic field in the magnetic separator;

(f1) forwarding the biomolecule eluted from the magnetic separator to a membrane chromatography device;

(g1) separating the biomolecule from impurities and/or contaminants by membrane chromatography.

Disclosed herein is a method for separating a biomolecule from a cell culture, comprising the steps of:

(a) providing magnetic particles comprising ligands capable of binding the biomolecule;

(b) contacting a cell culture comprising the biomolecule with the magnetic particles to obtain magnetic particles comprising the bound biomolecule;

(c) retaining the magnetic particles with a magnetic field in a magnetic separator;

(d) washing the magnetic particles with a washing liquid, comprising at least one washing step in continuous mode, comprising the steps of

(d1) agitating the magnetic particles in at least one plane to form a fluidised bed of magnetic particles, and

(d2) providing a flow of a washing liquid in a direction essentially perpendicular to the at least one plane to remove the cell culture while retaining the magnetic particles in the magnetic field;

(e) agitating the magnetic particles in at least one plane of the magnetic separator to form a fluidised bed of magnetic particles in the magnetic separator;

(f) providing a flow of an elution liquid in a direction essentially perpendicular to the at least one

plane, to elute the bound biomolecule from the magnetic particles while retaining the magnetic particles with the magnetic field in the magnetic separator;

wherein step (e) comprises agitating the magnetic particles by varying the magnitude of the magnetic field by applying an oscillating magnetic field.

Preferred aspects of the present disclosure are described below in the detailed description and in the dependent claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a flow chart of a method for separating a biomolecule according to the present disclosure.

Fig. 2 schematically shows a system suitable for use in the presently disclosed method.

Fig. 3 is a schematic drawing of a non-limiting embodiment of a magnetic separator suitable for use in the presently disclosed method.

Fig. 4 is a graph showing the elution curve from a polyclonal IgG experiment described in Example 1 below.

Figs. 5A, 5B, and 5C are graphs showing the elution curves from a monoclonal antibody (mAb) experiment described in Example 2 below.

Fig. 6 is a flow chart of an alternative method for separating a biomolecule according to the present disclosure.

Fig. 7 is a cross section of a membrane chromatography device which may be used according to one embodiment of the alternative method for separating a biomolecule according to the present disclosure.

Figs. 8A, 8B, 8C, and 8D are graphs showing the elution curves from a monoclonal antibody (mAb) experiment described in Example 3 below.

DETAILED DESCRIPTION OF THE DISCLOSURE

The present disclosure solves or at least mitigates the problems associated with existing methods for separating biomolecules by providing, as illustrated in Fig. 1, a method for separating a biomolecule from a cell culture or from a biological solution, comprising the steps of:

(a) providing magnetic particles comprising ligands capable of binding the biomolecule;

(b) contacting a cell culture or a biological solution comprising the biomolecule with the magnetic particles to obtain magnetic particles comprising the bound biomolecule;

(c) retaining the magnetic particles with a magnetic field in a magnetic separator;
(d) optionally washing the magnetic particles with a washing liquid;
(e) agitating the magnetic particles in at least one plane of the magnetic separator to form a fluidised bed of magnetic particles in the magnetic separator;
(f) providing a flow of an elution liquid in a direction essentially perpendicular to the at least one plane, to elute the bound biomolecule from the magnetic particles while retaining the magnetic particles with the magnetic field in the magnetic separator.

A significant advantage of the presently disclosed method is that the biomolecule is eluted with a surprisingly low total elution volume, more particularly an elution volume which is a maximum of 10 bed volumes of elution liquid, such as 9, 8, 7, 6, 5, 4, 3, 2 or 1 bed volumes; preferably a maximum of 4 bed volumes, more preferably a maximum of 3 bed volumes. Herein, a “bed volume” is to be understood as the volume of sedimented magnetic particles, normally expressed in millilitres (ml or mL) or in litres (L). The lower the total elution volume required to elute a biomolecule, the more concentrated and less dispersed is the sample of eluted biomolecule. A high concentration of biomolecule is desirable in any subsequent polishing steps, in order to avoid handling large volumes of sample.

As mentioned above, a further improvement of the presently disclosed method may involve additional purification of the biomolecule by membrane chromatography after the magnetic separation. Thus, the present disclosure provides, as illustrated in Fig. 6, a method for separating a biomolecule from a cell culture or a biological solution, comprising the steps of:
(a) providing magnetic particles comprising ligands capable of binding the biomolecule;
(b) contacting a cell culture or a biological solution comprising the biomolecule with the magnetic particles to obtain magnetic particles comprising the bound biomolecule;
(c) retaining the magnetic particles with a magnetic field in a magnetic separator;
(d) optionally washing the magnetic particles with a washing liquid;
(e1) providing a flow of an elution liquid through the magnetic separator to elute the bound biomolecule from the magnetic particles while retaining the magnetic particles with the magnetic field in the magnetic separator;
(f1) forwarding the biomolecule eluted from the magnetic separator to a membrane chromatography device;
(g1) separating the biomolecule from impurities and/or contaminants by membrane chromatography.

The addition of membrane chromatography after the magnetic separation results in a strikingly simple and fast process which results in a high purity of the biomolecule.

The term “biomolecule” has its conventional meaning in the field of bioprocessing, in which biomolecules are produced (often recombinantly) by cells in a cell culture and purified from the cell culture by any means of separation and purification. Alternatively, the biomolecules are present in a biological solution which does not necessarily originate from a cell culture. Non-limiting examples of biomolecules are biomacromolecules, which are large biological polymers that are made up of monomers linked together, e.g. peptides and proteins (which can be native or recombinant), including but not limited to enzymes, antibodies and antibody fragments, as well as carbohydrates, and nucleic acid sequences, such as DNA and RNA. Other non-limiting examples of biomolecules are plasmids and viruses. A biomolecule or a biomacromolecule may for example be a biopharmaceutical, i.e. a biological molecule, including but not limited to a biological macromolecule, which is intended for use as a pharmaceutical compound. Herein, a biomolecule to be separated from the rest of a cell culture or a biological solution by the presently disclosed method may alternatively be referred to as a “target biomolecule” or “target”. It is to be understood that “a biomolecule” is intended to mean a type of biomolecule and that the singular form of the term may encompass a large number of individual biomolecules.

Herein, the term “cell culture” refers to a culture of cells or a group of cells being cultivated, wherein the cells may be any type of cells, such as bacterial cells, viral cells, fungal cells, insect cells, or mammalian cells. A cell culture may be unclarified, i.e. comprising cells, or may be cell-depleted, i.e. a culture comprising no or few cells but comprising biomolecules released from the cells before removing the cells. Further, an unclarified cell culture as used in the presently disclosed method may comprise intact cells, disrupted cells, a cell homogenate, and/or a cell lysate.

The term “biological solution” is intended to mean a solution of biological origin, comprising a biomolecule or a mixture of several types of biomolecules. Examples of biological solutions are any type of bodily fluid originating from a human or an animal, such as plasma, blood, sputum, urine, and milk.

The term “magnetic particle” is defined herein as a particle which is able to be attracted by a magnetic field. At the same time, magnetic particles for use in the presently disclosed method shall not aggregate in the absence of a magnetic field. In other words, the magnetic particles shall behave like superparamagnetic particles. The particle may have any symmetric shape, such as a sphere or a cube, or any asymmetric shape. Spherical magnetic particles are often called magnetic beads. It is to be understood that the terms “magnetic particle”, “magnetic bead”, “Mag particle”, “Mag bead”,

“magparticle” and “magbead” may be used interchangeably herein, without limiting the scope to magnetic particles having a spherical shape.

Magnetic particles suitable for use in the presently disclosed method have been described in WO2018122089, which is hereby incorporated by reference in its entirety. In particular, magnetic particles may have a volume-weighted median diameter (d50,v) in a range of from 8 to 300 µm, such as 8, 9, 10, 15, 20, 25, 30, 35, 36, 37, 38, 39, 40, 50, , 60, 70, 80, 90, 95, 100, 105, 110, 150, 200, 250, or 300 µm, preferably in a range of from 20 to 200 µm, more preferably in a range of from 37 to 100 µm. Further, magnetic particles suitable for use in the presently disclosed method may have an average density of 1.05-1.20 g/ml sedimented magnetic particles.

A magnetic particle suitable for use in the presently disclosed method may comprise a porous matrix, preferably a porous polymer matrix, and one or more magnetic granules embedded in the porous matrix. A magnetic particle may suitably comprise about 5-15 wt. % of the magnetic granules. The magnetic granules embedded in the porous matrix of each magnetic particle may have a volume-weighted median diameter (d50,v) of from about 1 to about 5 µm. Further, a magnetic particle may suitably comprise a concentration of magnetic granules in a central region of the magnetic particle of at least 200% of the concentration in a surface region of the magnetic particle, wherein the central region is defined as having a distance of > 0.2 particle radii from the magnetic particle’s surface and the surface region is defined as having a distance of < 0.2 particle radii from the magnetic particle’s surface.

Magnetic particles for use in the presently disclosed method comprise ligands capable of binding the biomolecule. The ligands may be covalently coupled to the porous matrix of the magnetic particles. It is to be understood that the type of ligand and its affinity constant k_{off}/k_{on} towards the biomolecule is chosen based on the type of biomolecule which is to be separated from a cell culture or a biological solution. Further, it is to be understood that the concentration of ligand(s) per magnetic particle is dependent on or interrelated with, for example, the concentration of biomolecule in the cell culture or in the biological solution, the dimensions of the magnetic particles and/or the total volume of magnetic particles added to the magnetic separator.

In a currently preferred embodiment of the disclosure, the magnetic particles used in the presently disclosed method for separating a biomolecule are Mag Sepharose™ PrismA (GE Healthcare Bio-Sciences AB, art. no. 17550000).

Fig. 2 schematically shows a non-limiting example of a separation system **1**, which may be used to perform the method according to the present disclosure. The system **1** comprises a magnetic

separator **5**. The term “magnetic separator” has its conventional meaning in the field of separation processes, and refers to an apparatus which separates magnetic particles from a fluid. In a currently preferred embodiment of the present disclosure, the magnetic separator used in the method for separating a biomolecule is a high gradient magnetic separator, alternatively called a high gradient magnetic separation system (HGMS), as described in US7506765 B2, which is hereby incorporated by reference in its entirety. Parts of the separation system 1 shown in Fig. 2 are similar to parts of a system described in WO2018122246, which is hereby incorporated by reference in its entirety. The magnetic separator **5** comprises an inlet **5a** for receiving a feed from a cell culture **3** or a biological solution **3** comprising said biomolecule and for receiving magnetic particles comprising ligands capable of binding this biomolecule. The magnetic separator **5** is configured for separating said magnetic particles with said bound biomolecule from the rest of the feed. The magnetic separator **5** comprises parts of magnetic or magnetizable material inside the magnetic separator which parts attract the magnetic particles when a magnetic field is applied.

The separation system **1** shown in Fig. 2 comprises a capturing cell 9 which is connected to the inlet 5a of the magnetic separator **5**. The capturing cell **9** shown in Fig. 2 comprises a cell culture/biological solution inlet 9a for receiving a feed from a cell culture **3** and at least one magnetic particle inlet 9b for receiving magnetic particles. The capturing cell **9** is configured for mixing the feed from the cell culture or the biological solution and the magnetic particles thus allowing the target biomolecule to bind to the magnetic particles before forwarding it to the magnetic separator **5**. However, it is to be understood that the capturing cell **9** is an optional component of the system **1**. The cell culture **3**, or the biological solution **3**, could equally well function as a capturing cell if magnetic beads are added to the cell culture **3** or the biological solution, respectively, followed by providing a feed from the mixture of the cell culture and the magnetic particles, or from a mixture of the biological solution and the magnetic particles, respectively, comprising the bound biomolecule to the magnetic separator **5**. Another alternative would be to add magnetic beads directly to the magnetic separator **5** instead. Separate addition of cell culture or biological solution, respectively, and magnetic beads directly into the magnetic separator is possible for all the embodiments.

Accordingly, step (b) of the presently disclosed method may comprise:
(i) adding the magnetic particles to a magnetic separator, followed by providing a feed from the cell culture or the biological solution to the magnetic separator, or
(ii) providing a feed from a mixture of the cell culture and the magnetic particles, or from a mixture of the biological solution and the magnetic particles, comprising the bound biomolecule to the magnetic separator.

It is to be understood that after step (b), i.e. contacting the cell culture or the biological solution comprising the biomolecule with the magnetic particles to bind the biomolecule to the magnetic particles, the magnetic particles referred to in steps (c), (d) and (e) comprise the bound biomolecule.

According to step (d) mentioned above, the presently disclosed method may optionally comprise washing the magnetic particles with a washing liquid. For this purpose, the magnetic separator **5** is preferably connected to a washing arrangement **13** configured for washing out other components from the magnetic separator **5** than those magnetically bound to the parts of magnetic material. The washing arrangement **13** comprises at least one wash buffer providing arrangement **15** connected to a pump and to the inlet **5a** of the magnetic separator possibly via a capturing cell **9** and a wash buffer collection arrangement **17** connected to an outlet **5b** of the magnetic separator **5**. The washing arrangement **13** is configured for flowing washing buffer through the magnetic separator **5** for washing out other components of the feed than those bound to the magnetic parts.

According to the presently disclosed method, the magnetic beads may be washed in batch mode in the magnetic separator, which is configured for releasing the magnetic field when the magnetic particles are to be washed in batch mode. Alternatively, and advantageously, the above-described method comprises applying at least one washing step in continuous mode, in which case step (d) of the above-described method comprises the following substeps:
(d1) agitating the magnetic particles in at least one plane to form a fluidised bed of magnetic particles, and
(d2) providing a flow of a washing liquid in a direction essentially perpendicular to the at least one plane to remove the cell culture or the biological solution while retaining the magnetic particles in the magnetic field.

Herein, the phrase “essentially perpendicular to the at least one plane” is to be interpreted as meaning at an angle of 80-90° to the at least one plane.

Further, step (d) of the presently disclosed method may comprise the following substeps:
(i) removing the magnetic field;
(ii) resuspending the magnetic particles;
(iii) contacting the magnetic particles with a portion of washing liquid;
(iv) retaining the magnetic particles with a magnetic field; and
(v) removing the washing liquid from the retained magnetic particles.

Alternatively or additionally, step (d) of the presently disclosed method may be repeated at least once, such as 1, 2, 3 or more times, before proceeding to step (e), or alternatively to step (e1).

The system **1** as shown in Fig. 2 further comprises a collection cell **7** for collecting eluate and waste. It is to be understood that in step (f) of the presently disclosed method, a flow of elution liquid is provided through the magnetic separator **5** by allowing the elution liquid to enter the magnetic separator **5** via the inlet **5a** and by allowing the elution liquid to exit the magnetic separator **5** via the outlet **5b**. The eluted biomolecule exits the magnetic separator **5** together with the elution liquid and may be collected in a collection cell **7** connected to the outlet **5b** of the magnetic separator **5**.

Consequently, the presently disclosed method may optionally further comprise a step (g) after step (f), comprising collecting the eluted biomolecule, which is exiting the magnetic separator with the elution liquid, in a collection cell, while retaining the magnetic particles with the magnetic field in the magnetic separator. The collection cell is connected to the outlet of the magnetic separator.

According to an alternative embodiment, instead of a collection cell **7**, the system **1** as shown in Fig. 2 comprises a membrane chromatography device **101** for further purification, also called polishing, of the biomolecule. In this embodiment, in step (e1) of the presently disclosed method, a flow of elution liquid is provided through the magnetic separator **5** by allowing the elution liquid to enter the magnetic separator **5** via the inlet **5a** and by allowing the elution liquid to exit the magnetic separator **5** via the outlet **5b**. The eluted biomolecule exits the magnetic separator **5** together with the elution liquid and may be further purified, by separation from impurities and/or contaminants, in the membrane chromatography device **101** connected to the outlet **5b** of the magnetic separator **5**.

It is to be understood that in the system **1** shown in Fig. 2, the cell culture **3** or the biological solution **3**, the magnetic separator **5** and the collection cell **7**, or alternatively the membrane chromatography device **101**, may be connected by pre-sterilized, flexible tubing and aseptic connectors. Furthermore, the collection cell **7**, or alternatively the membrane chromatography device **101**, can be pre-sterilized and disposable. A closed and sterile separation system for single use can hereby be provided.

The magnetic separator used to perform the presently disclosed method further comprises an agitating arrangement (not shown in Fig. 2; one non-limiting example shown in Fig. 3 as described further below). The agitating arrangement is configured to apply an agitating motion to the magnetic particles, to make the magnetic particles move around in a plane perpendicular to the direction of the flow through the magnetic separator. The agitating arrangement may comprise at least one component, such as a plurality of components, configured for varying the magnitude of the magnetic field, such as by applying an oscillating magnetic field, i.e. varying the magnitude in a regular manner around a central point or plane. Thus, step (e), or alternatively step (e1), of the presently disclosed method may comprise agitating the magnetic particles by varying the magnitude of the magnetic field in at least one plane of the magnetic separator, such as by applying an oscillating magnetic field

in at least one plane of the magnetic separator. Alternatively, the agitating arrangement may comprise at least one agitator, such as a plurality of agitators, in which case step (e), or alternatively step (e1), of the presently disclosed method comprises agitating the magnetic particles by applying, or switching on, the agitator(s). Herein, the term “agitator” refers to any type of device or substance/material capable of agitating, such as rotating or stirring, magnetic particles in the magnetic separator. Non-limiting examples of suitable agitators are devices such as rotatable separation structures, such as rotatable discs or rotatable blades, being attached to at least one fixed structure of the magnetic separator, such as being mounted on a central rotatable carrier shaft forming a rotor. The separation structures may for example consist of a wire mesh, a perforated metal foil or a perforated metal sheet.

Advantageously, the agitators may comprise magnetizable material, in which case the agitators may act as the parts of magnetic material inside the magnetic separator, which parts attract the magnetic particles when a magnetic field is applied.

Fig. 3 schematically shows a cross-sectional view of a non-limiting example of a magnetic separator **5** which may be used to perform the method according to the present disclosure. The arrows in Fig. 3 illustrate a flow of liquid (e.g. elution liquid) which may enter the magnetic separator **5** at the inlet **5a** and exiting the magnetic separator **5** at the outlet **5b**. The magnetic separator **5** comprises a housing **6**, and further comprises a hollow, cylindrical electro magnet **11** configured to create a magnetic field in the magnetic separator when the electro magnet is turned on. The magnetic separator further comprises an agitating arrangement **13** comprising a rotor **15** on which a plurality of rotatable discs **17** are mounted. The agitating arrangement **13** is configured for agitating the magnetic particles **21** in at least one plane of the magnetic separator **5** to form a fluidised bed of magnetic particles in the magnetic separator, in accordance with step (e), or alternatively step (e1), of the presently disclosed method. In Fig. 3, the magnetic separator **5** further comprises a plurality of stationary discs **19** mounted on the housing **6**. The rotatable discs **17** and the stationary discs **19** are arranged alternately in the housing **6**. The rotatable discs **17** and/or the stationary discs **19** may comprise magnetizable material. Further, the rotatable discs **17** and/or the stationary discs **19** may be perforated to allow magnetic particles and cell culture/biological solution to pass through the discs. It is to be understood that the configuration of the rotatable discs and/or the configuration of the stationary discs may be different from the configuration shown in Fig. 3. For example, the stationary discs **19** may be connected to the rotor **15**, e.g. by means of sealing material. Further, the rotatable discs may or may not be connected to the wall of the housing **6**. It is further to be understood that the agitating arrangement may comprise other components than those shown in Fig. 3. For example, instead of or in addition to having parts of magnetizable material being attached to a fixed structure

of the magnetic separator, it is contemplated to include parts of magnetic or magnetizable material inside the magnetic separator which are not attached to any fixed structure of the magnetic separator, such as a wad or flock of steel wool or wire wool, and which attract the magnetic particles when a magnetic field is applied and which have a capacity to retain the magnetic particles in the magnetic field in the magnetic separator.

Step (e), or alternatively step (e1a), of the presently disclosed method may comprise agitating the magnetic particles in at least one plane, such as in a plurality of essentially parallel or parallel planes, of a magnetic separator to form a plurality of fluidised beds of magnetic particles. Herein, “essentially parallel planes” is to be interpreted as planes being arranged at an angle of 0-10° in relation to each other. In the case of a plurality of essentially parallel or parallel planes, step (f), or alternatively step (e1b) comprises providing the flow of an elution liquid in a direction essentially perpendicular or perpendicular to the plurality of planes. Without wishing to be bound by theory, it is believed that if the agitating arrangement comprises a plurality of agitators in the form of rotatable components, arranged in parallel within the magnetic separator, and configured to agitate the magnetic particles in a plurality of parallel planes; it is possible to form a fluidised bed between every pair of agitators.

In the presently disclosed method, the speed of the agitator(s) in step (e), or alternatively in step (e1), may be in a range of from 15 to 1500 rpm, such as 15, 20, 30, 40, 50, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, or 1500, preferably in a range of from 50 to 300 rpm.

According to an alternative embodiment, the above-described steps (f) and (g), following step (e), are replaced by steps (f1) and (g1), following step (e1), comprising:
(f1) forwarding the biomolecule eluted from the magnetic separator to a membrane chromatography device;
(g1) separating the biomolecule from impurities and/or contaminants by membrane chromatography.

The location of the membrane chromatography device **101** in the system **1** according to Fig. 2 has been described above. Step (g1) may alternatively be called polishing of the biomolecule.

The term “membrane chromatography” has its conventional meaning in the field of bioprocessing. In membrane chromatography there is binding of components of a fluid, for example individual molecules, associates or particles, to the surface of a solid phase in contact with the fluid. The active surface of the solid phase is accessible for molecules by convective transport. The advantage of membrane adsorbers over packed chromatography columns is their suitability for being run with

much higher flow rates. This is also called convection-based chromatography. A convection-based chromatography matrix includes any matrix in which application of a hydraulic pressure difference between the inflow and outflow of the matrix forces perfusion of the matrix, achieving substantially convective transport of substance(s) into the matrix or out of the matrix, which is effected very rapidly at a high flow rate. Convection-based chromatography and membrane adsorbers are described in for example US20140296464A1, US20160288089A1, WO2018011600A1, WO2018037244A1, WO2013068741A1, WO2015052465A1, US7867784B2, hereby incorporated by reference in their entireties.

In the presently disclosed method, the membrane chromatography device may comprise a chromatography material comprising one or more electrospun polymer nanofibres which in use form a stationary phase comprising a plurality of pores through which a mobile phase can permeate. The stationary phase may be in the form of a membrane.

Alternatively, the membrane chromatography device may comprise at least one adsorptive membrane. The adsorptive membrane may comprise polymer nanofibres. Optionally, the membrane, e.g. an adsorptive membrane, may comprise a nonwoven web of polymer nanofibres.

The polymer nanofibers may have mean diameters from 10nm to 1000nm. For some applications, polymer nanofibers having mean diameters from 200nm to 800nm are appropriate. Polymer nanofibers having mean diameters from 200nm to 400nm may be appropriate for certain applications.

The chromatography material or the membrane, e.g. an adsorptive membrane, may comprise a polymer selected from the group consisting of polysulfones, polyamides, nylon, polyacrylic acid, polymethacrylic acid, polyacrylonitrile, polystyrene, and polyethylene oxide, and mixtures thereof. Alternatively, or additionally, the polymer may be a cellulosic polymer, such as selected from a group consisting of cellulose and a partial derivative of cellulose, particularly cellulose ester, e.g. cellulose acetate, cross-linked cellulose, grafted cellulose, or ligand-coupled cellulose.

In the presently disclosed method, the membrane chromatography device may comprise a chromatography material functionalised with (i) a positively charged group, such as a quaternary amino, quaternary ammonium, or amine group, or (ii) a negatively charged group, such as a sulfonate or carboxylate group. Alternatively, or additionally, the membrane chromatography device may comprise a chromatography material functionalised with a multimodal ligand selected from a group consisting of a multimodal anion exchange ligand and multimodal cation exchange ligand. The multimodal anion exchange ligand may be a N-benzyl-N-methyl ethanol amine ligand coupled to a

support, wherein said support is linked to the nitrogen atom of the ligand through a linker. Alternatively, or additionally, the membrane chromatography device may comprise a chromatography material functionalised with (i) an ion exchanger group, (ii) an affinity peptide/protein based ligand, (iii) a hydrophobic interaction ligand, (iv) an IMAC ligand, or (v) a DNA based ligand such as Oligo dT.

In order to increase surface area and capacity with convection based membrane adsorbers, the size of convective pores of the convective matrix may be reduced, such as by use of nanofibres as mentioned above. As a result, resistance to flow will increase, however. Therefore, high flow rates through a chromatography device comprising a convective matrix of high capacity will require a chromatography device and design which can withstand high operating pressures.

In an embodiment of the presently disclosed method involving membrane chromatography, one may use a membrane chromatography device as described in detail in the previously filed patent application IN 201911019289, internal reference no. 502800-IN-1, hereby incorporated by reference in its entirety.

As illustrated in Fig. 7, the membrane chromatography device **101** comprises a chromatography material unit **103** which is provided within a cassette **105**. The membrane chromatography device **101** further comprises a fluid distribution system **107** which is configured to distribute fluid into and out from the at least one chromatography material unit **103**. The chromatography material unit **103** is sandwiched between a distribution device **109a** and a collection device **109b** of the fluid distribution system **107** as described above. The distribution device **109a** and the collection device **109b** are identical in the embodiment shown in Fig. 7; however, this is not necessary. In the embodiment shown in Fig. 7, the cassette **105** comprises the chromatography material unit **103** and the fluid distribution system **107**, in the form of a distribution device **109a** and a collection device **109b**. In another embodiment, said fluid distribution system **107** can be provided separately from the cassette **105**, for example as a separate unit or integrated into a housing **113** of the chromatography device **101**.

The membrane chromatography device **101** further comprises a housing **113** in which the at least one chromatography material unit **3** and the cassette **105** are provided. The housing comprises a top plate **125** and a bottom plate **127**. An inlet **115** for receiving a fluid feed is provided in the top plate **125** and an outlet **119** for transferring a fluid outflow from the chromatography device is provided in the bottom plate **127**. The top plate **125** and the bottom plate **127** are connected to each other such that an inlet fluid channel **117** is connecting the inlet **115** with the chromatography material unit **103**

via the fluid distribution system **107** and an outlet fluid channel **121** is connecting the outlet **119** with the chromatography material unit **103** via the fluid distribution system **107**.

The distribution device **109a** of the fluid distribution system **107** may comprise a plate (not shown) which is provided abutting an inlet surface **153a** of the chromatography material unit **103**, wherein said plate comprises a number of openings for distributing a fluid feed provided from the inlet **115** of the chromatography device **101** to the chromatography material unit **103**, wherein a total area of said openings in the plate is smaller than the rest of the area of the plate or less than 20% or less than 10% of the rest of the area of the plate, wherein said openings are connected to a distribution device inlet **157a** via one or more fluid conduits (not shown) provided in the distribution device **109a**.

Further, the collection device **9b** of the fluid distribution system **107** may comprise a plate (not shown) which is provided abutting an outlet surface **153b** of the chromatography material unit **103**, wherein said plate comprises a number of openings for collecting a fluid from the chromatography material unit **103**, wherein a total area of said openings in the plate is smaller than the rest of the area of the plate or less than 20% or less than 10% of the rest of the area of the plate, wherein said openings are connected to a collection device outlet **157b** via one or more fluid conduits (not shown) provided in the collection device **109b**.

The at least one chromatography material unit **103** may comprise at least one adsorptive membrane, and/or any one or several of the chromatography materials described elsewhere herein. The least one chromatography material unit may comprise at least one adsorptive membrane sandwiched between at least one top spacer layer (not shown) and at least one bottom spacer layer (not shown), or may comprise at least two adsorptive membranes stacked above each other and interspaced with spacer layers (not shown) and sandwiched between at least one top spacer layer and at least one bottom spacer layer.

At least some parts of said chromatography device **101** may be sealed together, leaving at least the inlet **115** and the outlet **119** open. In some embodiments, at least some parts of said chromatography device **101** may be sealed together by plastic or elastomer. Alternatively, or additionally, at least some parts of said chromatography device **101** may be overmolded and sealed together. Further, the cassette **105** and/or the housing **113** may be overmolded. The overmolding is a process for creating a seal and for providing stability to the device, thereby providing a robust membrane chromatography device **101**. After the overmolding, the chromatography device **101** can withstand an operating pressure of at least 10 bar or at least 15 bar.

In step (f) or alternatively step (e1b), of the presently disclosed method, the linear flow rate in the direction essentially perpendicular to the at least one plane, or alternatively essentially perpendicular to the plurality of planes, is in a range of from 10 to 3000 cm/h, preferably in a range of from 50 to 600 cm/h. A similar flow rate may be applied where relevant in other steps of the presently disclosed method, i.e. a flow rate in a range of from 10 to 3000 cm/h, preferably in a range of from 50 to 600 cm/h, such as in, but not limited to, step (d), step (e) or alternatively step (e1), and/or step (g) or alternatively step (f1) and/or step (g1).

In step (g1) of the presently disclosed method, the residence time of the biomolecule in the membrane chromatography device may be in a range of from about 0.5 s to about 6 min, preferably from about 1 s to about 30 s. This equals that the residence time of the biomolecule in the membrane or chromatography material may be in a range of from about 0.5 s to about 6 min, preferably from about 1 s to about 30 s.

Consequently, the flow rate of elution liquid through the membrane chromatography device may be in a range which corresponds to a residence time in the membrane chromatography device in step (g1) which is in a range of from about 0.5 s to about 6 min, preferably from about 1s to about 30s.

The flow of elution liquid through the membrane or chromatography material may be a normal flow or a tangential flow.

Further, the membrane chromatography in step (g1) may be flow-through membrane chromatography.

The presently disclosed method may further comprise performing, between step (e1) and step (f1), an adjustment of the pH of the elution liquid, a dilution of the elution liquid, or an adjustment of the conductivity of the elution liquid. In the presently disclosed method, at least steps (c)-(f) or alternatively steps (c)-(e1), such as steps (b)-(f) or alternatively steps (b)-(e1), may be performed in the magnetic separator.

Alternatively, steps (b)-(f), or steps (b)-(e1), respectively, may be carried out in a system comprising a bioreactor vessel being fluidically connected to a contactor and the contactor being fluidically connected to the magnetic separator. The bioreactor vessel comprises the cell culture/biological solution before starting the separation of biomolecule from the cell culture/biological solution. The contacting of the magnetic particles with the cell culture/biological solution may be performed in the contactor, which may be a vessel to which the cell culture/biological solution and the magnetic particles are conveyed (e.g. pumped, entrained by a liquid stream or fed by gravity) and which may be agitated to some extent to provide rapid mass transport into the magnetic particles. The

bioreactor vessel or the contactor may e.g. be a flexible bag, such as a flexible plastic bag with one or more inlet and outlet ports.

The separation system used in the presently disclosed method may further comprise a pressurizing arrangement, for example a peristaltic pump, configured to create the flow through the magnetic separator. Alternatively, the flow through the magnetic separator may be created solely by gravity. In general, the pressure applied in the system to make the presently disclosed method work may be much lower than the pressure applied in a high-pressure liquid chromatography (HPLC) system. Advantages associated herewith include simpler construction and lower costs. A non-limiting example of a suitable range of pressures to be applied to the separation system is 0-0.5 bar, such as 0.01-0.5 bar.

In the presently disclosed method, the magnetic separator may be connected to a chromatography system (i.e. replacing a chromatography column in a chromatography system), such as an ÄKTA™ Pilot system (GE Healthcare). Although the pump pressure performance of the chromatography system is not needed, it may be convenient to use the buffer control features, including dual pumps for gradient generation, the valving and the detector of the chromatography system.

The system, and/or the magnetic separator and/or the membrane chromatography device therein, used in the presently disclosed method may be configured for automatic, semi-automatic or manual operation. It can be used in both pilot and manufacturing scale processes, with amounts of magnetic particles at least in the interval from 0.1 L to 10 L. It is applicable in particular to capture of biopharmaceuticals from non-clarified feeds such as cell cultures, biological solutions, or cell lysates.

Devices “comprising” one or more recited elements may also include other elements not specifically recited. The term “comprising” includes as a subset “consisting essentially of” which means that the device has the components listed without other features or components being present. Likewise, methods “comprising one or more recited steps may also include other steps not specifically recited.

The singular “a” and “an” shall be construed as including also the plural.

The magnetic separator used in the following examples is a High Gradient Magnetic Separation system (HGMS), more particularly a MES 100 RS (Andritz KMPT GmbH). However, it is to be understood that any type of magnetic separator being configured as described in detail above may be used for performing the presently disclosed method.

Equally, it is to be understood that any type of membrane chromatography device having a design which can withstand high operating pressures, as mentioned above, may be used for performing the

presently disclosed method, thus not being limited to a cellulose fiber chromatography (known as Fibro chromatography) cassette/unit, as used in some of the examples below. The Fibro chromatography (GE Healthcare Life Sciences) is an ultrafast chromatography purification for short process times and high productivity, which utilizes the high flow rates and high capacities of cellulose fiber (https://www.gelifesciences.com/en/us/solutions/bioprocessing/products-and-solutions/downstream-bioprocessing/fibro-chromatography).

Further, the membrane chromatography material to be used in the membrane chromatography device is not limited to the so-called Fibro adhere material, as used in some of the following examples. The Fibro adhere (GE Healthcare Life Sciences) is a material comprising cellulose nanofibres, which may be derivatised with a strong ion exchange multimodal ligand. Another non-limiting example of a suitable membrane chromatography material is Capto™ adhere (GE Healthcare Life Sciences), which is based on a rigid agarose matrix that allows high fluid velocities to be used. The agarose matrix is derivatised with a multimodal anion exchange ligand, N-benzyl-N-methylethanolamine.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

Example 1

Purpose

The purpose of this study was to bind and elute IgG using Mag Sepharose™ PrismA and a High Gradient Magnetic Separator (HGMS) system, MES 100 RS (Andritz KMPT GmbH). The purpose was to see if it was possible to elute IgG from the HGMS system using flow elution.

Materials

Polyclonal IgG (Gammanorm™), Octapharma
NaH_2PO4 H_2O, pa, Merck
Na_2HPO4 2 H_2O, pa, Merck
Na-acetate trihydrate, pa, Merck
Acetic acid, pa, Merck
Tween™ 20, Merck

Tris base, pa, Merck

Mag Sepharose™ PrismA, GE Healthcare

Equipment

HGMS Equipment, MES 100 RS, Andritz KMPT GmbH

25 L plastic buffer trays

Mixing rotor, RW20, Janke & Kunkel

125 mL sterile plastic bottles, Nalgene

Spectrophotometer, GENESYS™ 10S UV-Vis, Thermo Scientific

Glass filter, G3, ~700 mL, Scott Duran

Centrifuge, 5810R, Eppendorff

Buffers

<u>20 mM Na-Phosphate + 0.15 M NaCl pH 7.4</u>

11.35 g Na $NaH_2PO_4 \cdot H_2O$, 74.3 g Na_2HPO_4 and 219.2 g NaCl was diluted and mixed with 25 L dH_2O in a 25 L buffer container.

<u>100 mM NaOAc pH 3.2</u>

140 mL acetic acid and 7.4 g $NaOAc \cdot 3 \cdot H_2O$ was diluted and mixed with 25 L dH_2O in a 25 L buffer container.

<u>1 M NaOH</u>

40 g NaOH was diluted and mixed with 1000 mL milliQ water.

<u>PBS with 0.05% Tween</u>

Two tablets of PBS from Medicago was mixed with 2000 mL MilliQ water. 1 mL Tween 20 was pipetted and mixed with the solution.

<u>100 mM NaOAc pH 2.9</u>

5.7 mL Acetic acid and 0.02 g NaOAc $3H_2O$ was mixed with 1000 mL milli-Q water.

Protocol

Preparation of Mag Sepharose PrismA

200 mL Mag Sepharose PrismA was washed with 5 cv PBS on a glass filter (G3 pore size) prior use.

Preparation of IgG, 2mg/ml in 6 L

12 g (73 ml of 165mg/ml) Human IgG, Gammanorm was diluted in 6L of 20 mM Na-Phosphate + 0.15 M NaCl pH 7.4. The total volume was 6080 ml and the IgG concentration was analyzed with UV280 to 1,9 mg/ml.

Incubation of Mag Sepharose PrismA 37-100 with IgG

The 400 mL of the washed beads were transferred into a bucket with the 6 L solution with IgG g and mixed for one hour.

HGMS process

The cooling water, air pressure and distilled water was turned on the MES 100 RS system. The peristaltic pump of the system was first calibrated with water to fill 1 L volumetric flask at room temperature to wash the tubes and remove air from the system, and then with the prepared buffers.

Table 1: Calibration of peristaltic pump

% speed of pump	Flow (L/min)
5	0,14
10	0,29
20	0,58
30	0,87
40	1,16
50	1,45
60	1,73
70	2,02
80	2,31
90	2,6
100	2,89

After incubation, the following program was used on the magnetic separator:

2 GE-IgG-purif:

Main Step 1: Load magnetic particles (MP)

Sub1: load: stop all before start, step time 250 sec, magnet on, pump set value +50%, open valve XV01 and XV14.

The remaining feed was pumped in manually using PBS buffer to wash the tube.

Main Step 2: recirculate

Sub1: recirculate: Step time 30 sec, Magnet on, Pump set value +30 %, open valve XV08 and XV13.

Main Step 3: wash buffer

Sub1: feed buffer 1: Step time 60 sec, Magnet on, Pump set value +70 %, open valve XV02 and XV14.

Sub2: mix buffer: Stop all before start, step time 20 sec, Mixer set value 60%

Sub3: trap: Step time 10 sec, Magnet on,

Sub4: recapture: Step time 60 sec, Magnet on, Pump set value +30 %, open valve XV08 and XV13.

Sub5: transition: Step time 1 sec, Magnet on, open valve XV02, XV08 and XV13 and XV14.

Main Step 4: wash H2O

Sub1: feed buffer 2: Step time 60 sec, Magnet on, Pump set value +70 %, open valve XV03 and XV14.

Sub2: mix buffer 2: Stop all before start, step time 20 sec, Mixer set value 60%

Sub3: trap: Step time 10 sec, Magnet on,

Sub4: recapture: Step time 60 sec, Magnet on, Pump set value +30 %, open valve XV08 and XV13.

Sub5: transition: Step time 1 sec, Magnet on, open valve XV02, XV08 and XV13 and XV14.

Main Step 5: elution

Sub1: trap: Step time 10 sec, Magnet on

Sub2: recap: Step time 60 sec, Magnet on, Pump set value +30 %, open valve XV08 and XV13.

Sub3: transition: Step time 1 sec, Magnet on, open valve XV07, XV08 and XV13 and XV16.

Sub4: feed eluate: Step time 4200 sec, Magnet on, Mixer set value 10%, Pump set value +5 %, open valve XV07 and XV16.

The eluate was collected in ~100 ml fractions by hand in pre-weight 125 ml plastic bottles. After elution each bottle was weighed and the actual elution volume in each bottle was determined by subtracting the total volume with the empty bottle volume (51.5 mg).

Analysis

The eluates were neutralized to pH 5 with 2 M tris base. Titer was determined in all fractions by UV280 using a Spectrophotometer, GENESYS from ThermoFisher.

Results, analysis and conclusions

The concentration was calculated by dividing the UV280 value in a 1cm cuvette with the extinction coefficient 1.36 to obtain the concentration in mg/ml of IgG. The samples were diluted if the UV280 value was above 1. The concentration and cumulative yield were plotted against the cumulative volume of the elution.

The graph in Fig. 4 shows a distinct elution peak with a yield of 98% with 2.7 x CV (the total magnetic bead volume volume of 0.4 L).

Conclusion

The mixing and flowrate was set such that no magbeads were coming out from the magnetic chamber during the elution. The mixing while having the magnet switched on at the same time will cause the magbeads to be suspended in the magnetic chamber allowing a continuous elution instead of batch elution of the IgG.

This experiment shows that it is possible to elute IgG in a continuous mode wile mixing in the magnetic the magnetic chamber with Mag Sepharose Prisma 37-100 µm, resulting in a 98% yield in 2.7 volumes of Mag Sepharose beads.

Example 2

Purpose

The purpose of this study was to clarify and purify a CHO cell culture with an IgG1 monoclonal antibody using Mag Sepharose PrismA (37-100 µm) and a High Gradient Magnetic Separator (HGMS) system, MES 100 RS (Andritz KMPT GmbH). The purpose was to see if it was possible to elute the mAb from the HGMS system using flow elution without losing any Mag Sepharose.

Materials

CHO cell culture, GE Healthcare
NaH_2PO_4 H_2O, pa, Merck
Na_2HPO_4 2 H_2O, pa, Merck
Na-acetate trihydrate, pa, Merck
Acetic acid, pa, Merck
Tween 20, Merck
Tris base, pa, Merck
Mag Sepharose PrismA, 37-100 µm, GE Healthcare
mAb standard, 8.82 mg/mL, GE Healthcare

Equipment

HGMS Equipment, MES 100 RS, Andritz KMPT GmbH
25 L plastic buffer trays
Mixing rotor, RW20, Janke & Kunkel
125 mL sterile plastic bottles, Nalgene
HPLC, 1260 Infinity, Agilent Technologies
Glass filter, G3 pore size, ~700 mL, Scott Duran
Centrifuge, 5810R, Eppendorff
pH meter, 913 pH meter, Metrohm
MabSelect SuRe HiTrap, 29-0491-04, GE Healthcare
Superdex 200 Increase 10/300 GL, , GE Healthcare
0.2 µm syringe filters, Sterivex HV, Millipore
10-100 µL pipette, , Mettler Toledo
100-1000 µL pipette, , Eppendorff
Cellbag™ 10 L, BC11, Basic, GE Healthcare

Conditions and Observations

Preparation of solutions:

<u>20 mM Na-Phosphate + 0.15 M NaCl pH 7.4</u>

11.35 g Na $NaH_2PO_4 \cdot H_2O$, 74.3 g Na_2HPO_4 and 219.2 g NaCl was diluted and mixed with 25 L dH_2O in a 25 L buffer container.

<u>100 mM NaOAc pH 3.2</u>

140 mL acetic acid and 7.4 g $NaOAc \cdot 3 \cdot H_2O$ was diluted and mixed with 25 L dH_2O in a 25 L buffer container.

<u>1 M NaOH</u>

40 g NaOH was diluted and mixed with 1000 mL milliQ water.

<u>PBS with 0.05% Tween</u>

Two tablets of PBS from Medicago was mixed with 2000 mL MilliQ water. 1 mL Tween 20 was pipetted and mixed with the solution.

<u>100 mM NaOAc pH 2.9</u>

5.7 mL Acetic acid and 0.02 g NaOAc $3H_2O$ was mixed with 1000 mL milli-Q water.

Preparation of mAb feed

An amount of ~7 L of CHO cells containing mAb was used. Cell density was 23.81 MVC/mL, Viability: 66.4% and mAb titer 2.7 mg/mL. The cells were pumped into a 10 L cell bag (basic).

Preparation of Titer analysis method, MabSelect SuRe HiTrap bind and elute

A MabSelect SuRe HiTrap™ column was coupled onto the HPLC system. A standard curve with mAb (mAb titer 8.82 mg/mL) was prepared in 250 µL plastic HPLC vials according to the table below.

Table 2: Preparation of standard curve mAb

STD conc (mg/mL)	STD volume (µL)	PBS volume (µL)
1	22.7	177.3
2	45.4	154.6
3	68.0	132.0
4	90.7	109.3
5	113.3	86.7

Description, MabSelect SuRe Hitrap method (titer with HPLC):

Injection volume: 50 µL

Flow: 1 mL/min

Detection: 280 nm

A-buffer: PBS+0.05% Tween pH 7.4

B-buffer: 100 mM NaOAc pH 2.9

Table 3: Time scheme for HPLC method

Time (min)	%A-buffer
0	100
3	100
13	30
14	30
16	0
16.01	100

18	100
23	100

Titer determination of cell culture

~10 mL cell suspension was centrifuged at 3000 rpm for 5 minutes and the volume cell debris (solids) was determined to 5% by monitoring the total volume cell suspension and total volume solids. Supernatant was filtrated through a 0.2 µm filter into a 1 mL HPLC vial. Titer of the supernatant was determined according to the titer method described above.

Table 4: Standard curve mAb

STD mAb (mg/mL)	**Response (mAUs)**
0	0
1	3993
2	8545
3	13293
4	17301
5	22187

Titer mAb cell supernatant:

Response= 11924 mAUs

Titer= 11924/4383.7= 2.72 mg/mL

Preparation of Mag Sepharose PrismA

400 mL Mag Sepharose PrismA was washed with 5 cv PBS on a glass filter (G3 pore size) prior use.

Incubation of Mag Sepharose PrismA with cell culture

The 400 mL washed beads were transferred into a bottle with 6250 g CHO cell suspension. The magnetic beads were mixed with the CHO cell suspension for one hour. One mL of sample was centrifuged and analysed with the mAbSelect SuRe Hitrap method (see above) to investigate remaining mAb in the feed (not bound to the Mag Sepharose PrismA).

HGMS process

The cooling water, air pressure and distilled water was turned on the MES 100 RS system. The peristaltic pump of the system was calibrated with water to fill 1 L volumetric flask at room temperature.

The inlet tubes and valves to be used XV01, XV02, XV 03, XV 07 were primed with dH2O. Air bubbles in the magnetic separator was removed by priming the system and at the same time turn on the mixer at 50%, until all air is removed. The hose between circulation valves XV08 and XV013 was filled until no air was present using back-flow in the system.

After water priming the system was primed with actual running buffers.

XV01= Mag bead mixture load (primed with 20 mM Na-phosphate + 0.15 M NaCl pH 7.4)

XV02= 20 mM Na-phosphate + 0.15 M NaCl pH 7.4

XV03= dH_2O

XV 07= 100 mM Na-acetate pH3.2

After incubation the following program was used on the magnetic separator.

Step 1 Load mixture of magnetic particles and feed

The pumping time was set to 250 seconds to pump in 6 L feed mixture. Remaining feed was pumped in manually using PBS buffer to wash the inlet tube of the mixture.

The system was set to recirculation to trap all magnetic beads on the magnetizable discs in the separation unit.

Step 2 Wash with 20 mM Na-phosphate + 0.15 M NaCl, repeated 4 cycles

Sub step 1. Feed buffer for 60 s using 70% pump speed (2 L buffer).

Sub step 2. Mix buffer and beads for 20 s and 60% rotor speed, turning magnet and valves off.

Sub step 3. Trap the beads by turning on the magnet.

Sub step 4. Recapture. Recirculate to trap all beads using pump rate at 30%.

Sub step 5. Transition. Short (1s) valves opening to avoid back pressure.

Step 3. Wash with water, one cycle

Same substeps were used at water wash as for the washings with 20 mM Na-Phosphate + 0.15 M NaCl, except that water was pumped from valve XV03.

Step 4. Elution

In this step we flow-eluted the mAb using mixer on and magnet on.

Sub step 1. Trap. Turned magnet on.

Sub step 2. Recap. Recirculate for 60 sec using 30% pump speed.

Sub step 3. Transition. Opening of valves for short moment (1s) to avoid back pressure.

Sub step 4. Feed eluate: Elution with 100 mM NaOAc pH 3.2, valve XV07. Magnet on, pump speed at 5% (140 mL/min), mixer set at 10%. Elution was made for 4286 s.

The eluted material was collected in ~100 mL fractions by hand in pre-weight plastic 150 mL bottles.

After elution each bottle was weighed and the actual elution volume in each bottle was determined by subtracting the total volume with the empty bottle volume. See table 5 below.

Table 5: Volumes of elution fractions

Fraction	Volume (g, mL)
4	115,51
5	111,22
6	89,7
7	102,61
8	97,7
9	92,7
10	99,25
11	107,55
12	115,19
13	109,14
14	112,26
15	109,22
16	125,24
17	114,47
18	121,98
19	113,73
20	112,8
21	115,34
22	121,87
23	121,87
24	111,03
25	162,53

The eluate fractions were light yellow and clear.

The eluates were neutralized to pH 5 with 2 M tris base and after that they were 0.2 µm filtrated. Actual volume of 2 M Tris base was included in the weight of the eluates above. Titer was determined in all fractions by the MagSelect SuRe HiTrap bind and elute assay, see above.

Finally Fractions 7-18 were pooled and 1 mL of this pool were diluted 20x with 200 mM Na-Phosphate pH 6.8 in a 1.5 mL HPLC vial and analysed with analytical size exclusion chromatography

(SEC) on the HPLC system and compared with the mAb standard (that was diluted 8x with same buffer).

The Mag Sepharose in the separator was released and pumped back into a vessel. The resin was washed with 2 cv 1 M NaOH for 30 minutes on a G3 glass filter, it was equilibrated with 5 cv PBS and finally it was washed with 2 cv 20% Ethanol as preservation solution and the resin was transferred back into a plastic bottle with 20% EtOH for storage.

Purity by SEC method

Injection volume: 10 µL
Flow: 0.8 mL/min
Column: Superdex 200 Increase 10/300 GL
Stop time: 26 min
Detection at 214 nm.

HCP analysis

In the Start and pool eluate samples, 50µL preservation solution was added to 450 µL sample prior to the host cell protein (HCP) analysis.

Analysis was performed using a 3rd generation CHO-HCP ELISA kit (Cygnus) on a Gyrolab workstation (Gyros Protein Technologies) with software package 5.3.0.

Results, analysis and conclusions

Fig. 5A shows the elution profile of the mAb from the magnetic separator. The pooled fraction, fractions 7-18, corresponding to 1.2 L eluate shows that 87% mAb yield can be obtained with 3 bed volumes (one bed volume = 400 mL) elution buffer. The concentration in the pooled eluate was 10.7 mg/mL which is a 4x concentration. The binding yield in the incubation step was 94% and the total elution yield was 91%.

The pooled sample was analysed by SEC and HCP assay and compared with a pure mAb standard. The results are shown in Fig. 5B (smaller scale) and Fig. 5C (larger scale; zooming in on the base of the peaks). The solid line is the pooled mAb eluate and the dotted line is the pure mAb standard. The chromatogram is normalized. The aggregate peak, main peak and peak after main peak was integrated and the purity was determined to 98.5%.

Example 3

Purpose

The purpose of this study was to investigate which mixing speed (rotor speed) and flow can be used for the flow-elution of IgG from Mag Sepharose PrismA, using magnetic fluidized beads without migration of beads. It was also investigated how the IgG elution profile was affected by different mixing speed, volume of beads and bead sizes.

Equipment

HGMS Separator, MES100RS serial no 400213239, Andritz Gmbh

Mixer at incubation, IL82274-3, Janke-Kunkel

UV reader, SpectraMax™ plus, Molecular Devices

UV reading plate, 96 well, 3635 lot 33918007, Corning

Balance, PG 5002 DeltaRange, Mettler

Plastic bottles 130 mL

25 L plastic buffer bottles

Mag Sepharose prismA 0-37 µm Lot LS-033447, GE Healthcare

Mag Sepharose PrismA 37-100 µm LS-32871, GE Healthcare

Materials

Human IgG, Gammanorm 165 mg/mL, Octapharma

$NaH_2PO_4 \cdot H_2O$, pa, Merck

$Na_2HPO_4 \cdot 2\ H_2O$ pa, Merck

NaCl, pa, Merck

Acetic acid, pa, Merck

Na-acetate $\cdot$ 3 H_2O, pa, Merck

Conditions and Observations

Preparation of solutions:

<u>A-buffer, 20 mM PO_4 + 0.15 M NaCl pH 7.5</u>

Buffer salt amount (g)	Component	Mw (g/mol)
11,356	NaH2PO4 (x 1 H2O)	137,99
74,347	Na2HPO4 (x 2 H2O)	177,99
219,15	NaCl	58,44
25 liter	Final volume with water	

B-buffer, 0.1 M Na-acetate pH 3.2

Buffer salt amount (g)	Component	Mw (g/mol)
139,853 ml	Acetic acid	60,05
7,431	Na-acetate (x 3 H2O)	136,08
25 liter	Final volume with water	

IgG 2 mg/mL

73 mL of IgG (Gammanorm) was diluted to 6 L A-buffer to obtain an IgG concentration of ~2.0 mg/mL.

Test of rotor speed and flow to investigate bead losses

100 mL, 300 mL or 400 mL (wet sedimented resin) Mag Sepharose Prisma 37-100 µm or 0-37 µm was pumped into the HGMS system with the magnet turned on. The rotor was turned on at different speeds and the flow pump with A-buffer was also varied to investigate at which rotor speed and flow rate the magnetic beads start to migrate (visually) from the HGMS chamber.

The results are shown in tables 6-10 below.

Loading 100 mL Mag Sepharose 0-37 µm: Beads were trapped up to 2100 mL/min with a rotor speed of 150 rpm. Beads start to migrate out of the HGMS at 225 rpm rotor speed.

Loading 300 mL Mag Sepharose 0-37 µm: Beads were trapped up to 560 mL/min with a rotor speed of 75 rpm.

Loading 100 mL Mag Sepharose 37-100 µm: Beads were trapped up to 2100 mL/min with a rotor speed of 150 rpm.

Loading 300 mL Mag Sepharose 37-100 µm: Beads were trapped up to 560 mL/min with a rotor speed of 150 rpm. The beads are also still trapped at 300 rpm rotor speed at 140 mL/min.

Loading 400 mL Mag Sepharose 37-100 µm: Beads were trapped up to 1400 mL/min with a rotor speed of 75 rpm.

It was possible to run higher flow rate for all loadings at lower rotor speed than the rotor speed where beads start to migrate out from the HGMS system, see tables.

Table 6: Magnetic bead migration study on 100 mL Mag Sepharose 0-37 µm

100 mL Mag Sepharose 0-37 µm						
Flow rates		Rotor speed (%, rpm)				
Flow rate settings (%)	Flow rate (mL/min)	0%, 0 rpm	5 %, 75 rpm	10 %, 150 rpm	15 % 225 rpm	20 %, 300 rpm
5	140	Not tested	Not tested	X	O	O
10	280	Not tested	Not tested	X	Not tested	O
20	560	Not tested	Not tested	X	Not tested	Not tested
30	840	Not tested	Not tested	Not tested	Not tested	Not tested
40	1120	Not tested	Not tested	Not tested	Not tested	Not tested
50	1400	X	Not tested	X	Not tested	Not tested
60	1680	Not tested	Not tested	Not tested	Not tested	Not tested
70	1960	Not tested	Not tested	Not tested	Not tested	Not tested
75	2100	X	Not tested	X	Not tested	Not tested
80	2240	Not tested	Not tested	Not tested	Not tested	Not tested
100	2800	Not tested	Not tested	Not tested	Not tested	Not tested
		X= No particle leakage, O= particle migrates from separation chamber				

Table 7: Magnetic bead migration study on 300 mL Mag Sepharose 0-37 µm.

300 mL Mag Sepharose 0-37 µm						
Flow rates		Rotor speed (%, rpm)				
Flow rate settings (%)	Flow rate (mL/min)	0%, 0 rpm	5 %, 75 rpm	10 %, 150 rpm	15 % 225 rpm	20 %, 300 rpm
5	140	Not tested	X	O	Not tested	Not tested
10	280	Not tested	X	O	Not tested	Not tested
20	560	Not tested	X	Not tested	Not tested	Not tested
30	840	Not tested	O	Not tested	Not tested	Not tested
40	1120	Not tested	Not tested	Not tested	Not tested	Not tested
50	1400	X	O	Not tested	Not tested	Not tested
60	1680	Not tested	Not tested	Not tested	Not tested	Not tested
70	1960	Not tested	Not tested	Not tested	Not tested	Not tested
75	2100	X	Not tested	Not tested	Not tested	Not tested
80	2240	Not tested	Not tested	Not tested	Not tested	Not tested
100	2800	Not tested	Not tested	Not tested	Not tested	Not tested
		X= No particle leakage, O= particle migrates from separation chamber				

Table 8: Magnetic bead migration study on 100 mL Mag Sepharose 37-100 µm.

100 mL Mag Sepharose 37-100 µm						
Flow rates		Rotor speed (%, rpm)				
Flow rate settings (%)	Flow rate (mL/min)	0%, 0 rpm	5 %, 75 rpm	10 %, 150 rpm	15 % 225 rpm	20 %, 300 rpm
5	140	Not tested	X	X	Not tested	O
10	280	X	Not tested	X	Not tested	O
20	560	Not tested	X	X	Not tested	O
30	840	Not tested	Not tested	Not tested	Not tested	Not tested
40	1120	Not tested	Not tested	Not tested	Not tested	Not tested
50	1400	X	Not tested	X	Not tested	Not tested
60	1680	Not tested	Not tested	Not tested	Not tested	Not tested
70	1960	Not tested	Not tested	Not tested	Not tested	Not tested
75	2100	X	Not tested	X	Not tested	Not tested
80	2240	Not tested	Not tested	Not tested	Not tested	Not tested
100	2800	Not tested	Not tested	Not tested	Not tested	Not tested
		X= No particle leakage, O= particle migrates from separation chamber				

Table 9: Magnetic bead migration study on 300 mL Mag Sepharose 37-100 µm.

300 mL Mag Sepharose 37-100 µm						
Flow rates		Rotor speed (%, rpm)				
Flow rate settings (%)	Flow rate (mL/min)	0%, 0 rpm	5 %, 75 rpm	10 %, 150 rpm	15 % 225 rpm	20 %, 300 rpm
5	140	Not tested	Not tested	X	Not tested	X
10	280	Not tested	Not tested	X	Not tested	O
20	560	X	Not tested	X	Not tested	O
30	840	X	Not tested	Not tested	Not tested	Not tested
40	1120	Not tested	Not tested	Not tested	Not tested	Not tested
50	1400	X	Not tested	O	Not tested	Not tested
60	1680	X	Not tested	Not tested	Not tested	Not tested
70	1960	Not tested	Not tested	Not tested	Not tested	Not tested
75	2100	Not tested	Not tested	O	Not tested	Not tested
80	2240	X	Not tested	Not tested	Not tested	Not tested
100	2800	Not tested	Not tested	Not tested	Not tested	Not tested
		X= No particle leakage, O= particle migrates from separation chamber				

Table 10: Magnetic bead migration study on 400 mL Mag Sepharose 37-100 µm.

400 mL Mag Sepharose 37-100 µm						
Flow rates		Rotor speed (%, rpm)				
Flow rate settings (%)	Flow rate (mL/min)	0%, 0 rpm	5 %, 75 rpm	10 %, 150 rpm	15 % 225 rpm	20 %, 300 rpm
5	140	Not tested	X	O	O	O
10	280	X	X	O	Not tested	Not tested
20	560	X	X	Not tested	Not tested	Not tested
30	840	X	X	Not tested	Not tested	Not tested
40	1120	Not tested	Not tested	Not tested	Not tested	Not tested
50	1400	X	X	Not tested	Not tested	Not tested
60	1680	Not tested	Not tested	Not tested	Not tested	Not tested
70	1960	Not tested	Not tested	Not tested	Not tested	Not tested
75	2100	X	Not tested	Not tested	Not tested	Not tested
80	2240	Not tested	Not tested	Not tested	Not tested	Not tested
100	2800	Not tested	Not tested	Not tested	Not tested	Not tested
		X= No particle leakage, O= particle migrates from separation chamber				

Flow elution of IgG study

400 mL of each resin prototype was incubated for >1 h with 6 L of the IgG 2 mg/mL sample.

100 or 400 mL (1.6 L or 6.4 L of IgG resin mixture) of the IgG adsorbed Mag Sepharose PrismA was pumped into the system and the magnetic beads were trapped by the magnet and the beads were washed three times with 1 L A-buffer.

Elution was performed at different rotor speed at 140 mL/min to investigate how bead volume and rotor speed affect elution performance.

At elution, ~100-2100 mL fractions were collected and the absorbance was determined for each fraction using a UV plate reader, and also the exact volume in each fraction was determined using weighing. The UV for each fraction was plotted against the cumulative elution volume. See figures 1-4.

In general, 0% rotor rotation showed insufficient elution of the IgG since after several column volumes of elution buffer, > 4000 mL, high concentration of IgG was still present in the eluate and the elution profile showed no tendency to decline in concentration.

For the <37 µm beads, less volume of beads leads to less dependence of rotor speed. The shape of the elution peak for 75 and 150 rpm rotor speed for 100 mL beads were similar (Fig. 8A). For 400 mL beads the shape of the elution peaks differed more significantly between rotor speeds, with the most effective elution of IgG using 150 rpm (Fig. 8C).

For the 37-100 µm beads, less volume of beads leads to less dependence of rotor speed. For 100 mL beads, the elution peak for 75 rpm rotor speed and 150 rpm rotor speed were similar (Fig. 8B). For 400 mL beads the shape of the elution peak differed dramatically between 150 rpm rotation and 0 rpm rotation. At 150 rpm rotation the IgG was eluted off in a symmetric elution peak while at 0 rpm rotation the IgG was still eluting off after 6500 mL elution buffer (Fig. 8D).

Comparing 0-37 µm beads with 37-100 µm beads, the elution peak shapes are similar using 100 mL beads (see Fig. 8A and Fig. 8B, respectively). At 400 mL beads and 150 rpm rotation, the larger beads, 37-100 µm, showed much sharper elution peak than the elution peak for 400 mL <37 µm beads. Using the larger beads, a majority of IgG seems to be eluted off before 2000 mL elution buffer while for the <37 µm beads the elution peak has not reached down to a stable baseline at 4000 mL (see Fig. 8C and Fig. 8D, respectively).

Example 4

Purpose

The purpose of this study was to evaluate the effect of adding a membrane chromatography step for polishing of a biomolecule after magnetic separation. More particularly, the study comprised polishing of an IgG1 monoclonal antibody (mAb) by membrane chromatography with Fibro adhere unit prototypes after purification with Mag Sepharose PrismA by use of a High Gradient Magnetic Separator system.

Materials

Start sample: IgG1 monoclonal antibody from an XDR-10 cultivation in CHO cells, viability 66.4%, concentration of 2,7 g/L
Superdex™ 200 Increase 10/300 GL, GE Healthcare
MagSepharose PrismA 37-100 µm, 400 ml, GE Healthcare
Fibro Adhere unit prototypes, 0.4 ml:

- Fibro Adhere 1: The material was crosslinked with divinylsulfone (DVS) after the addition of allyl glycidyl ether (AGE), with an Adhere reaction concentration of 167g/Ltr. Titration of 563 umol/g.
- Fibro Adhere 2: The material was crosslinked with DVS before the AGE was added, with an Adhere reaction concentration of 167g/Ltr. Titration of 212 umol/g.
- Fibro Adhere 3: The material was crosslinked with DVS before the AGE was added with an Adhere reaction concentration of 500g/Ltr. Titration of 478 umol/g.

Equipment

ÄKTA™ Pure 25, GE Healthcare

HPLC Infinity 1200, Agilent technologies

Buffers

25 mM Na-Phosphate + 0.15 M NaCl pH 6,3

4.88 g Na $NaH_2PO_4 \cdot H_2O$, 2.6 g Na_2HPO_4 and 17.5 g NaCl was diluted and mixed with 2 L dH_2O

25 mM Na-Phosphate pH 7

1.16 g Na $NaH_2PO_4 \cdot H_2O$, and 2.367 g Na_2HPO_4 was diluted and mixed with 1 L dH_2O

25 mM Na-Phosphate + 1M NaCl pH 7

0.464 g Na $NaH_2PO_4 \cdot H_2O$, 3.851 g Na_2HPO_4 and 58.44 g NaCl was diluted and mixed with 1 L dH_2O

25 mM Na-Phosphate pH 7.5

0.73 g Na $NaH_2PO_4 \cdot H_2O$, and 3.508 g Na_2HPO_4 was diluted and mixed with 1 L dH_2O

100 mM Acetic acid pH 3.0

6 ml Glacial Acetic acid was diluted and mixed with 1 L dH_2O

Conditions and Observations

The eluate from MagSepharose PrismA using the High Gradient Magnetic Separator system, as described in Example 2, was used for further polishing by membrane chromatography with Fibro adhere unit prototypes.

Fibro Adhere units were connected to an ÄKTA Pure 25 system and three different 25 mM Na-phosphate buffer conditions (pH and conductivity) were evaluated, see table 11. The equilibration was performed at a flowrate of 16ml/min for 50 unit volumes (20 ml) before 4 ml of the mAb sample was applied at 16 ml/min and the flowthrough collected. The Fibro Adhere unit was washed with 20 unit volumes (8 ml) and cleaned with 100 mM Acetic acid pH 3.0 for 25 unit volumes (10 ml) at a flowrate of 16 ml/min before re-equilibration for 50 unit volumes (20 ml). The UV was monitored at all times at 280 nm.

Table 11: Buffer conditions of mAb polishing with Fibro Adhere.

Buffer	pH	Conductivity
1	6.3	18mS/cm
2	7.0	10 mS/cm
3	7.5	3.5 mS/cm

Results, analysis and conclusions

The chromatograms from mAb polishing with Fibro Adhere at the varying buffer conditions showed a high UV value during the sample application at all three buffer conditions. The flowthrough of sample during sample application was collected and further analyzed, see table 12. The concentration of mAb and percentage of aggregates were analysed by SEC-HPLC, Superdex 200 increase 10/300 GL at a flowrate of 0.8 ml/min for 26 min per sample with 0.2 M Na phosphate buffer. The host cell protein level in the flow-through fractions were analysed with Cygnus 3rd generation CHO HCP ELISA kit.

Table 12: Results from evaluation of mAb polishing with Fibro Adhere prototypes in flowthough mode at different buffer conditions. The start material was mAb purified from MagSepharose PrismA with a titer of ~ 3 mg/ml and HCP of 160 ppm.

Buffer conditions	pH 6.6 18mS/cm		pH 7.0 10 mS/cm		pH 7.5 3.5mS/cm	
Prototypes	Yield (%)	HCP ppm	Yield (%)	HCP ppm	Yield (%)	HCP ppm
Fibro Adhere 1	97	111	99	54	99	26
Fibro Adhere 2	104	135	102	79	98	32
Fibro Adhere 3	90	172	102	57	93	25

In conclusion, the polishing of mAb resulted in high yields, 93-100%, and in a substantial reduction of host cell proteins (HCP). The highest HCP reduction was with pH 7.5 and at a conductivity of 3.5 mS/cm, which resulted in a 93-99% yield, and the HCP reduction was highest with the Fibro Adhere prototypes with the highest ligand densities.

It is to be understood that the present disclosure is not restricted to the above-described exemplifying embodiments thereof and that several conceivable modifications of the present disclosure are possible within the scope of the following claims.

CLAIMS

1. A method for separating a biomolecule from a cell culture, comprising the steps of:
(a) providing magnetic particles comprising ligands capable of binding the biomolecule;
(b) contacting a cell culture comprising the biomolecule with the magnetic particles to obtain magnetic particles comprising the bound biomolecule;
(c) retaining the magnetic particles with a magnetic field in a magnetic separator;
(d) washing the magnetic particles with a washing liquid, comprising at least one washing step in continuous mode, comprising the steps of

(d1) agitating the magnetic particles in at least one plane to form a fluidised bed of magnetic particles, and
(d2) providing a flow of a washing liquid in a direction essentially perpendicular to the at least one plane to remove the cell culture while retaining the magnetic particles in the magnetic field;

(e) agitating the magnetic particles in at least one plane of the magnetic separator to form a fluidised bed of magnetic particles in the magnetic separator;
(f) providing a flow of an elution liquid in a direction essentially perpendicular to the at least one plane, to elute the bound biomolecule from the magnetic particles while retaining the magnetic particles with the magnetic field in the magnetic separator;

wherein step (e) comprises agitating the magnetic particles by varying the magnitude of the magnetic field by applying an oscillating magnetic field.

2. The method according to claim 1, wherein step (e) comprises agitating the magnetic particles in a plurality of essentially parallel planes of the magnetic separator to form a plurality of fluidised beds of magnetic particles, and wherein step (f) comprises providing the flow of the elution liquid in a direction essentially perpendicular to the plurality of planes.

3. The method according to any one of the preceding claims, wherein the biomolecule is eluted with a maximum of 10 bed volumes of elution liquid, such as 9, 8, 7, 6, 5, 4, 3, 2 or 1 bed volumes; preferably a maximum of 4 bed volumes, more preferably a maximum of 3 bed volumes.

4. The method according to any one of the preceding claims, wherein at least steps (c)-(f) , such as steps (b)-(f), are performed in the magnetic separator, preferably wherein the magnetic separator is a high gradient magnetic separation system.

5. The method according to any one of the preceding claims, wherein step (b) comprises:
(i) adding the magnetic particles to a magnetic separator, followed by providing a feed

from the cell culture to the magnetic separator, or
(ii) providing a feed from a mixture of the cell culture and the magnetic particles comprising the bound biomolecule to a magnetic separator.

6. The method according to any one of the preceding claims, wherein steps (b)-(f) are carried out in a combined bioreactor vessel/contactor and magnetic separator.

7. The method according to any one of the preceding claims, wherein step (d) comprises the following substeps:
(i) removing the magnetic field;
(ii) resuspending the magnetic particles;
(iii) contacting the magnetic particles with a portion of washing liquid;
(iv) retaining the magnetic particles with a magnetic field; and
(v) removing the washing liquid from the retained magnetic particles.

8. The method according to any one of the preceding claims, wherein step (d) is repeated at least once before proceeding to step (e).

9. The method according to any one of the preceding claims, wherein the linear flow rate of the elution liquid in step (f) is in a range of from 10 to 3000 cm/h in step (f), preferably in a range of from 50 to 600 cm/h.

10. The method according to any one of the preceding claims, wherein the magnetic separator is connected to a chromatography system.

11. The method according to any one of the preceding claims, wherein the magnetic particles have a volume-weighted median diameter (d50,v) in a range of from 8 to 300 µm, preferably in a range of from 37 to 100 µm.

12. The method according to any one of the preceding claims, wherein the magnetic particles have an average density of 1.05-1.20 g/ml sedimented particles.

13. The method according to any one of the preceding claims, wherein each of the magnetic particles comprises a porous polymer matrix and one or more magnetic granules embedded in the porous polymer matrix.

14. The method according to claim 13, wherein each of the magnetic particles comprises 5-15 wt. % of the magnetic granules.

15. The method according to any one of claims 13-14, wherein the magnetic granules have a volume-weighted median diameter (d50,v) of from 1 to 5 µm.

16. The method according to any one of claims 13-15, wherein each of the magnetic particles comprises a concentration of magnetic granules in a central region of the particle of at least 200% of the concentration in a surface region of the particle, wherein the central region is defined as having a distance of > 0.2 particle radii from the particle surface and the surface region is defined as having a distance of < 0.2 particle radii from the particle surface.