

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
 - 5 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
 - 10 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
 - 15 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
- 20 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
- 25 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.
- 30 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.

5 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;

10 (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).

15 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.

20 11. The method according to any one of the preceding claims, wherein the magnetic particles have a volume-weighted median diameter ($d_{50,v}$) 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.

25 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.

30 15. The method according to any one of claims 13-14, wherein the magnetic granules have a volume-weighted median diameter ($d_{50,v}$) 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.