Methods for extraction of molecules

Jacobsen, Simo

Publication date: 2020

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):
(51) International Patent Classification: G01N 27/447 (2006.01)

(21) International Application Number: PCT/EP2019/078363

(22) International Filing Date:
18 October 2019 (18.10.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
18201250.0 18 October 2018 (18.10.2018) EP


(72) Inventor: JACOBSEN, Simo, Abdessamad, Baalal; Drejegade 41, 3. tv., 2100 Copenhagen 0 (DK).

(74) Agent: ECKERT-BOULET BRAVO, Nadine et al.; Hoiberg P/S, Adelgade 12, 1304 Copenhagen K (DK).


Declarations under Rule 4.17:
— of inventorship (Rule 4.17(iv))

Published:
— with international search report (Art. 21(3))
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(54) Title: METHODS FOR EXTRACTION OF MOLECULES

(57) Abstract: The present invention relates to a method for the extraction of molecules having a positive or a negative charge such as nucleic acid molecules, from a sample such as a solution or separation matrix. The method is suited for high-throughput extraction. Also disclosed is a device for high-throughput extraction of charged molecules comprising a plurality of electrode pairs comprising a first electrode (4a) and a second electrode (4b) attached to a support frame (8), wherein the pairs are configured to be placed in a first compartment (2) and a second compartment (5). Also disclosed is a system comprising such a device and a first reservoir comprising a plurality of first compartments, wherein the spacing between the compartments matches the spacing between wells of a standard microplate, and uses thereof for the extraction of charged molecules.
Methods for extraction of molecules

Technical field

The present invention relates to a method for the extraction of molecules having a positive or a negative charge from a sample such as a solution or separation matrix. The method is suited for high-throughput extraction. Also disclosed is a device for high-throughput extraction of charged molecules. Also disclosed are systems comprising such a device and a first reservoir, and uses thereof for the extraction of charged molecules.

Background

Biological experimentation, in particular in the field of molecular biology, often requires separation of molecules, in particular macromolecules such as nucleic acid molecules, proteins, amino acids or carbohydrates, among others for analytical and experimental purposes. A common separation procedure involves electrophoresis, where molecules are separated by charge and/or size via mobility through a separating matrix, for example a gel, in the presence of an electrical field.

Gel separation matrices are typically made by pouring a liquid phase into a mold and letting the matrices solidify. In slab gel electrophoresis, outcroppings in plastic material form combs embedded in the top of the separating matrix. These allow the formation of sample loading wells when the combs are removed after solidification of the matrix. In order to separate the molecules, the gel matrix is placed in appropriate electrophoresis buffers, and an electrical field is generated.

The loading of samples suspected of containing molecules which it is desirable to separate and extract into the combs is a time consuming task, which it is difficult to perform in a high throughput manner. To prevent samples from mixing with the buffers used for electrophoresis, dense solutions are often added to the samples prior to loading into the wells.

After loading of the samples, an electrical field is generated by electrodes in electrical contact with the separation matrix. The field allows charged molecules to migrate towards the electrode of opposite charge.
Following electrophoresis, it may be of interest for many applications to recover, i.e. extract, molecules of interest from the separation matrix. This is difficult to do in a high-throughput manner. Current methods involve cutting a piece of the separation matrix, which is suspected to contain the molecules of interest, before extracting the molecules. The cutting is typically done manually, for instance with a scalpel. The piece is then transferred to a tube such as a microfuge tube, where it is processed to extract the molecules. Often, it is desirable to obtain a volume containing the molecules which is as small as possible, i.e. to obtain the molecules of interest as concentrated as possible.

The purified molecules of interest can then be used for other purposes, for example analytical purposes such as sequencing or structural studies, and/or experimental purposes.

There is a need for methods for extraction of charged molecules from a sample, which can be adapted for high-throughput.

**Summary**

The invention is as defined in the claims.

As detailed above, methods for extraction of charged molecules from a sample are needed, which can be adapted for high-throughput extraction.

Herein is thus provided a method for the extraction of molecules having a positive or a negative charge from a sample such as a solution or a separation matrix, comprising the steps of:

i) providing a sample (1) such as a solution or a separation matrix comprising the molecules,

ii) placing the sample in a first compartment (2), wherein the first compartment comprises a conductive fluid (3) such as an electrophoresis buffer, a first electrode (4a) and a second electrode (4b),

iii) generating a first electrical field in the conductive fluid by applying a first current to the first and second electrodes, such that the first electrode becomes charged with the same charge as the molecules and the second electrode becomes charged with a charge opposite of the charge of the molecules,
whereby the charged molecules are electrophoretically migrated to the second electrode, and optionally attached to the second electrode via a matrix such as a silica matrix, a nylon matrix, a calcium alginate matrix, or a polyvinylidene difluoride matrix such as a silica matrix, a nylon matrix, a calcium alginate matrix, a polyvinylidene difluoride matrix, an agarose matrix or an acrylamide-azlactone polymer matrix,

iv) removing the electrodes from the first compartment, and placing the electrodes in a second compartment (5), wherein the second compartment comprises a constricted volume (6) around at least the first electrode, and the second compartment comprises an elution buffer (7) in contact with the first and the second electrodes,

v) optionally generating a second electrical field in the elution buffer by applying a second current between the two electrodes, preferably such that the charges of the electrodes are reversed, whereby the charged molecules are electrophoretically migrated to the first electrode,

vi) optionally interrupting the second electrical field;

vii) and collecting the elution buffer from the second compartment, wherein the elution buffer comprises the molecules, whereby the molecules are extracted.

Also provided is a device (10) for high-throughput extraction of molecules having a positive or a negative charge from a plurality of samples, said device comprising a plurality of electrode pairs comprising a first electrode (4a) and a second electrode (4b) attached to a support frame (8), wherein the pairs are configured to be placed in a first and a second compartment, and optionally wherein the second electrodes are further configured to reversibly attach a column thereto.

Also provided is a system comprising a device as described herein and a first reservoir comprising a plurality of first compartments, said first reservoir being configured such that the first compartments each can receive one electrode pair of the device.

Also provided is the use of a device as described herein or of a system as described herein for the extraction of molecules having a positive or a negative charge from a sample such as a solution or a separation matrix.
Description of the drawings

Figure 1: The electrode pair with a first electrode (4a) and a second electrode (4b) connected to a support frame (8) is placed in a first compartment (2) comprising a conductive fluid (3) to extract molecules of interest (shown as hexagonal shapes) from a sample (1). The conductive fluid covers the conductive parts of both electrodes (in light grey). In this embodiment, the first electrode is longer than the second electrode.

Figure 2: After migration of the charged molecules to the second electrode, the electrode pair is transferred to a second compartment (5) comprising an elution buffer (7). The second compartment has a constricted volume (6), in this embodiment only around the first electrode. The elution buffer covers the conductive parts of both electrodes (in light grey). After reversal of the current, the charged molecules migrate to the first electrode and can be collected from the elution buffer when the current is interrupted.

Figure 3: An example of a device of the present disclosure. The device (10) comprises a plurality of electrode pairs comprising a first electrode (4a) and a second electrode (4b) attached to a support frame (8), and in this embodiment also comprises a plurality of holes (9) - here shown next to the second electrode.

Figure 4: A different view of the device (10) of figure 3.

Detailed description

Definitions

Charge The term as used herein refers to an electrical charge, in particular of a molecule or of an electrode. An electrical charge has an absolute value, which is the numerical value of the electrical charge, and a sign - i.e. the charge is either positive or negative.

Method for extraction of charged molecules

Herein is provided a method for the extraction of molecules having a positive or a negative charge from a sample such as a solution or a separation matrix, comprising the steps of:
i) providing a sample (1) such as a solution or a separation matrix comprising the molecules,

ii) placing the sample in a first compartment (2), wherein the first compartment comprises a conductive fluid (3) such as an electrophoresis buffer, a first electrode (4a) and a second electrode (4b),

iii) generating a first electrical field in the conductive fluid by applying a first current to the first and second electrodes, such that the first electrode becomes charged with the same charge as the molecules and the second electrode becomes charged with a charge opposite of the charge of the molecules, whereby the charged molecules are electrophoretically migrated to the second electrode, and optionally attached to the second electrode via a matrix such as a silica matrix, a nylon matrix, a calcium alginate matrix, or a polyvinylidene difluoride matrix such as a silica matrix, a nylon matrix, a calcium alginate matrix, a polyvinylidene difluoride matrix, an agarose matrix or an acrylamide-azlactone polymer matrix,

iv) removing the electrodes from the first compartment, and placing the electrodes in a second compartment (5), wherein the second compartment comprises a constricted volume (6) around at least the first electrode, and the second compartment comprises an elution buffer (7) in contact with the first and the second electrodes,

v) optionally generating a second electrical field in the elution buffer by applying a second current between the two electrodes, preferably such that the charges of the electrodes are reversed, whereby the charged molecules are electrophoretically migrated to the first electrode,

vi) optionally interrupting the second electrical field; and

vii) collecting the elution buffer from the second compartment, wherein the elution buffer comprises the molecules, whereby the molecules are extracted.

The methods described herein may all be adapted for high-throughput extraction of molecules from multiple samples, as will become evident from the below description.

**Sample**

The methods disclosed herein are suitable for the extraction of molecules having a positive or a negative charge from a sample such as a solution or a separation matrix.

The methods are particularly suitable for extracting molecules of interest, such as
nucleic acid molecules, for example DNA or RNA, proteins, amino acids, or carbohydrates such as monosaccharides or polysaccharides.

In a first step, a sample is provided comprising molecules to be extracted.

The sample in which the molecules are comprised may be in the form of a solution, or may be part of a separation matrix. For example, the sample is a piece of a separation matrix after molecules have been separated therein, so that the sample is suspected to comprise only molecules of interest. The separation matrix may in some embodiments be a portion of a gel cassette, which has for example been cut out after electrophoretic separation. The sample may also be in the form of a solution, containing or suspected of containing molecules of interest. The solution may originate from a separation matrix or a portion thereof, which has been solubilised as is known in the art prior to performing the present methods. The sample may also be a nucleic acid sample such as a whole genome nucleic acid sample, for example genomic DNA, exome or transcriptome sample, a total protein extract, such as a proteome, or a mixture thereof. The sample may be a body fluid sample, e.g. a blood sample, a serum sample or a urine sample. Such samples may be processed prior to purification as is known in the art.

The sample may aptly have a volume such that it can be placed in a first compartment such as a standard Eppendorf tube, for example a tube having a volume of 0.5 mL, 1 mL, 1.5 mL or 2.0 mL. The first compartment will be described in more detail herein below. Accordingly, the sample should preferably have a volume smaller than the volume of the first compartment. It is however also possible to divide the sample in several aliquots, to be placed in several first compartments.

**Molecules**

The molecules of interest have either a positive or a negative charge. In some cases, the charge is intrinsic to the nature of the molecule. For example, nucleic acid molecules are known to have a negative charge. Proteins may also be charged, either with a positive charge or with a negative charge. In other cases, the molecules are neutral, but can be charged if mixed with an appropriate compound, for example a charged stain or dye or buffer, for example prior to or during electrophoresis. In general, proteins are neutral at a pH equal to their isoelectric point (pi), positively
charged at a pH below their pi, and negatively charged at a pH above their pi. Thus, the use of appropriate buffers may confer a positive or a negative charge to proteins. The same holds for amino acids. As for monosaccharides or polysaccharides, borate ions may be added to the buffer resulting in formation of negatively charged sugar-borate complexes that can be separated based on their negative charge. The longer the sugar chain is, the more borate ions bind to it, and the more negatively charged the chain becomes. The skilled person knows which buffers, stains or dyes can be used in order to confer a charge to a protein, an amino acid or a monosaccharide or polysaccharide.

In some embodiments, the molecules of interest have a positive charge. In other embodiments, the molecules of interest have a negative charge. The absolute value of the charge is not important, as long as it is sufficient to allow the molecules to migrate according to their charge.

As used herein, the term “charged molecules” will be used interchangeably with the term “molecules having a positive or a negative charge”.

**First compartment**

In a second step, the sample is placed in a first compartment comprising a conductive fluid, a first electrode and a second electrode.

The first compartment is a fluid-tight container, such as a standard microcentrifuge tube, for example an Eppendorf tube, as is known in the art to be suitable for sample handling.

The first compartment may have various sizes and may have various maximal volume capacities, for example 0.1 ml, 0.2 ml, 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 5.0 ml, 10 ml, 15 ml, 15 mL or 50 mL, or more.

The first compartment may be equipped with a lid, or it may be devoid of lid.

**First and second electrodes**

The first compartment comprises a first electrode and a second electrode. These electrodes do not need to be attached to the first compartment, but may be placed
therein in such a way that the lower ends of both electrodes are below the upper end of the first compartment. In this way, both electrodes can be contacted with a conductive fluid comprised within the first compartment, in such a manner that an electrical field can be generated in the conductive fluid when an electrical current is applied to the electrodes.

In some embodiments, the first electrode is a cathode and the second electrode is an anode. In other embodiments, the first electrode is an anode and the second electrode is a cathode.

The electrodes may be indirectly connected, for example they are attached in their upper end via a support structure, which facilitates handling, particularly for embodiments where the first compartment has smaller volumes, in the range of some ml. Accordingly, in some embodiments, the first and second electrodes are attached in their upper end to a support frame. Each electrode may be divided in two parts: an upper part (or insulated part), which is insulated as is known in the art, for example by a plastic coating, and a lower part (or conductive part) which is the electrode as such and which may generate a field. At least the conductive parts of the first electrode and or the second electrode are in contact with the conductive fluid.

In preferred embodiments, the first electrode is longer than the second electrode. When placed in the first compartment, the first electrode will thus be closest to the bottom of the first compartment, while the second electrode will be farthest from the bottom of the first compartment.

In some embodiments, the second electrode may have a matrix attached thereto, which can facilitate extraction of the molecules, as will be detailed below. The matrix may be a silica matrix, which can be useful if the charged molecules are nucleic acid molecules or proteins. For proteins, the matrix may be a calcium alginate matrix or a polyvinylidene difluoride matrix. The matrix may be provided in the form of a column, as is known in the art, such as an immobilisation column or a capture column, comprising a material which will immobilise or capture the charged molecules. The column may in some embodiments comprise a volume of electrophoresis buffer. In some embodiments, the column may comprise a volume of binding buffer to facilitate binding of the charged molecules to the matrix, as is known in the art.
Preferably, the electrodes when placed in the first compartment are not in direct contact with its walls.

**Conductive fluid**

The conductive fluid is as known in the art, and may for example be a buffer such as an electrophoresis buffer, for example TE buffer, TAE buffer, TBE buffer, Tris Glycine SDS PAGE Buffer or Tris-Tricine-SDS PAGE Buffer. The volume of conductive fluid comprised in the first compartment is such that the first compartment when comprising the conductive fluid and the electrodes still can accommodate the sample. Accordingly, the volume of conductive fluid is smaller than the difference between the maximal volume capacity of the first compartment and the volume of the sample. It will often be desirable to have as big a volume of conductive fluid as possible, since this may facilitate extraction. At the very least, the volume of conductive fluid should be such that when the sample is placed therein, both electrodes are in direct contact with the conductive fluid, at least in their lower part - the conductive part, so that the electrodes can generate an electrical field in the conductive fluid when a current is applied to the electrodes.

**First electrical field**

In a third step of the method, once the sample and the electrodes have been placed in the first compartment comprising an appropriate volume of conductive fluid as described above, a first current is applied to the first and second electrodes to generate a first electrical field in the conductive fluid. The first electrical field is generated in such a way that the first electrode becomes charged with a charge of the same sign as the molecules to be extracted, and the second electrode becomes charged with a charge of the opposite sign. For example, if the molecules have a positive charge, the first electrode becomes positively charged and the second electrode becomes negatively charged. If the molecules have a negative charge, the first electrode becomes negatively charged, and the second electrode becomes positively charged. The absolute value of the charge is not essential, i.e. it is not a requirement that the charge of the first electrode is the same as the charge of the molecules - only that it has the same sign.

Once the electrodes are charged, the charged molecules will thus migrate to the second electrode, which has a charge opposite to the charge of the molecules. The
first current is thus applied to the electrodes for a time sufficient to allow migration of at least part of or all of the charged molecules. In some embodiments, a matrix is attached to the second electrode, which may facilitate extraction of the molecules from the sample since the molecules will attach thereto.

The skilled person will know how to design the first current so that an appropriate first electrical field is generated. For example, the first current may have the following parameters: voltage of 90 to 120 V, amperage of about 400 mA, and can be applied to the electrodes for 1 to 3 minutes.

In some embodiments, the first current may have a voltage between 50 and 200 V, such as between 60 and 190 V, such as between 70 and 180 V, such as between 80 and 170 V, such as between 90 and 160 V, such as between 90 and 150 V, such as between 90 and 140 V, such as between 90 and 130 V, such as between 90 and 120 V, such as 90 V, 100 V, 110 V or 120 V.

In some embodiments, the first current may have an amperage of between 100 and 700 mA, such as between 150 and 650 mA, such as between 200 and 600 mA, such as between 250 and 550 mA, such as between 300 and 500 mA, such as between 350 and 450 mA, such as 400 mA.

In some embodiments, the first current may be applied for a duration of between 30 seconds and 5 minutes, such as between 1 minute and 4 minute, such as between 1 minute and 3 minutes, such as between 1.5 minute and 2.5 minute, such as 1 minute, 2 minutes or 3 minutes.

The parameters of the first current (voltage, amperage and duration) may depend on the nature of the charged molecules and of the sample in which they are comprised, and can be adjusted as is known to the person of skill in the art.

The methods may comprise an additional step of drying the first compartment, for example by applying air pressure thereto, after the charged molecules have migrated to the second electrode.
Second compartment

In a next step of the method, the electrode pair (i.e. the first and the second electrodes) are removed from the first compartment and placed in a second compartment; the second electrode has attached thereto at least part of the charged molecules. In embodiments where the second electrode is attached to a matrix, the matrix is transferred together with the electrode pair to the second compartment. In some embodiments, no current is applied to the electrode pair during transfer to the second compartment, i.e. the first current is interrupted. In other embodiments, the first current is maintained during transfer.

The second compartment comprises a constricted volume around at least the first electrode when the electrode pair is placed therein. The term “constricted volume” refers to the lower part of the second compartment, which a smaller diameter than the upper part of the first compartment. This will allow concentration of the molecules in a smaller volume than using a “normal” compartment, such as a standard microcentrifuge tube. Preferably, the constricted volume is not around the second electrode when the electrode pair is placed in the second compartment. The second electrode is thus preferably located in the upper part of the second compartment. The upper part of the second compartment should thus have dimensions such that it can accommodate the first and the second electrodes, and optionally the matrix if present.

In some embodiments the second compartment may be the same as the first compartment. In such embodiments, the first compartment thus comprises a constricted volume around at least the first electrode when the electrode pair is placed therein, as described for the second compartment. In such embodiments, the methods may advantageously comprise a step of drying the first compartment, e.g. by applying air pressure thereto, prior to the addition of elution buffer to the first compartment, which is now the second compartment.

The skilled person will know how to design the second compartment, and in particular how to choose the dimensions of the constricted volume, depending on his/her purpose.

In some embodiments, the height of the constricted volume is about half the total height of the second compartment. In some embodiments, the height of the constricted volume is a third of the total height of the second compartment.
In some embodiments, the ratio between the height of the constricted volume and the total height of the second compartment is between 1:10 and 3:4, such as between 1:9 and 2:3, such as between 1:8 and 1:2, such as between 1:7 and 1:3, such as between 1:6 and 1:4, such as about 1:5.

In some embodiments, the volume of the constricted volume is at the most 50% of the total volume of the second compartment, such as at the most 45%, such as at the most 40%, such as at the most 35%, such as at the most 30%, such as at the most 25%, such as at the most 20%, such as at the most 15%, such as at the most 10%, such as at the most 7.5%, such as at the most 5% of the total volume of the second compartment. The term “volume” here refers to the maximal capacity of the corresponding compartment or portion thereof, such as the second compartment or the constricted volume.

In some embodiments, the second compartment is a microfuge tube, such as a 2.0 mL, a 1.5 mL or a 1.0 mL tube, and the volume of the constricted volume is at the most 500 μL, such as at the most 400 μL, such as at the most 300 μL, such as at the most 250 μL, such as at the most 200 μL, such as at the most 150 μL, such as at the most 100 μL, such as at the most 75 μL, such as at the most 50 μL, such as at the most 40 μL, such as at the most 35 μL, such as at the most 30 μL, such as at the most 25 μL.

In some embodiments, the second compartment is a centrifuge tube, such as a 5 mL, a 10 mL, a 15 mL, a 25 mL, a 50 mL or a 100 mL tube, and the volume of the constricted volume is at the most 2.5 mL, such as at the most 2.0 mL, such as at the most 1.5 mL, such as at the most 1.0 mL, such as at the most 0.5 mL.

**Elution buffer**

The second compartment comprises an elution buffer. The buffer may already be present in the second compartment when the electrode pair is transferred thereto, or it may be added to the second compartment after transferring the electrode pair.

Suitable elution buffers are known in the art and the choice of buffer will largely be dictated by the nature of the charged molecules to be extracted. For example, TE buffer may be used for elution of DNA or RNA. The choice of buffer for the elution of
proteins or amino acids will depend on their charge, which may be different depending on the pH of the elution buffer, as is known in the art. For elution of monosaccharides or polysaccharides, boric acid-based buffers, for example comprising glycerol, may be suitable.

The volume of elution buffer needed to perform the present methods is such that both electrodes when placed in the second compartment are in contact with the elution buffer, at least in their conductive part.

In some embodiments, the volume of elution buffer is kept as little as possible. In other words, in some embodiments, the volume of elution buffer is such that both electrodes when placed in the second compartment are in contact therewith, and at least part of the conductive parts of each electrode is immersed in elution buffer so that a second electrical field can be generated as described below. Preferably, the entire conductive part of the first electrode and/or of the second electrode is immersed in elution buffer.

**Second electrical field**

In a fifth step of the method, a second electrical field is generated in the elution buffer by applying a second current to the two electrodes. This step is optional: in the absence of a second electrical field in the elution buffer, the molecules will also be released from the second electrode, at least to some extent. The use of a second electrical field may however be advantageous, for example to speed up release of the molecules from the second electrode, or in embodiments where a column is attached to the second electrode.

The second current preferably has a direction opposite to that of the first current, so that the second electrical field has a direction opposite to that of the first electrical field. In this way, the charges of the electrodes may be reversed: the first electrode now has a charge opposite to the charge it had under the first electrical field, and the second electrode now has a charge opposite to the charge it had under the second electrical field. As a consequence, the charged molecules are released from the second electrode and the matrix if present, and migrate electrophoretically to the first electrode, which now has a charge of the opposite sign as the charged molecules.
In some embodiments, for example for extraction of certain molecules such as proteins or amino acids, the charges are not reversed but the charge of the molecules is reversed, so that when the second current is applied said molecules are released from the second electrode and migrate to the first electrode. The charge of the molecules may be reversed by choosing an appropriate buffer, as is known in the art. In such embodiments, the charge of the first electrode in the second electrical field has the same sign as its charge in the first electrical field, and the charge of the second electrode in the second electrical field has the same sign as its charge in the first electrical field.

Accordingly, in some embodiments, the method comprises the steps of:

i) providing a sample (1) such as a solution or a separation matrix comprising the molecules,

ii) placing the sample in a first compartment (2), wherein the first compartment comprises a conductive fluid (3) such as an electrophoresis buffer, a first electrode (4a) and a second electrode (4b),

iii) generating a first electrical field in the conductive fluid by applying a first current to the first and second electrodes, such that the first electrode becomes charged with the same charge as the molecules and the second electrode becomes charged with a charge opposite of the charge of the molecules, whereby the charged molecules are electrophoretically migrated to the second electrode, and optionally attached to the second electrode via a matrix such as a silica matrix, a nylon matrix, a calcium alginate matrix, or a polyvinylidene difluoride matrix such as a silica matrix, a nylon matrix, a calcium alginate matrix, a polyvinylidene difluoride matrix, an agarose matrix or an acrylamide-azlactone polymer matrix,

iv) removing the electrodes from the first compartment, and placing the electrodes in a second compartment (5), wherein the second compartment comprises a constricted volume (6) around at least the first electrode, and the second compartment comprises an elution buffer (7) in contact with the first and the second electrodes, wherein the elution buffer confers a charge to the molecules of interest of opposite sign relative to the charge of the molecules in step i9, ii) and/or iii);

v) optionally generating a second electrical field in the elution buffer by applying a second current between the two electrodes, preferably such that the charges of
the electrodes are not reversed, whereby the charged molecules are
electrophoretically migrated to the first electrode,
vi) optionally interrupting the second electrical field; and
vii) collecting the elution buffer from the second compartment, wherein the elution
buffer comprises the molecules,
whereby the molecules are extracted, optionally wherein the molecules are nucleic acid
molecules, such as DNA molecules or RNA molecules, proteins, amino acids, or
carbohydrates such as monosaccharides or polysaccharides.

In other embodiments, the method comprises the steps of:

i) providing a sample (1) such as a solution or a separation matrix comprising the
molecules,
ii) placing the sample in a first compartment (2), wherein the first compartment
comprises a conductive fluid (3) such as an electrophoresis buffer, a first
electrode (4a) and a second electrode (4b),
iii) generating a first electrical field in the conductive fluid by applying a first current
to the first and second electrodes, such that the first electrode becomes
charged with the same charge as the molecules and the second electrode
becomes charged with a charge opposite of the charge of the molecules,
whereby the charged molecules are electrophoretically migrated to the second
electrode, and optionally attached to the second electrode via a matrix such as
a silica matrix, a nylon matrix, a calcium alginate matrix, or a polyvinylidene
difluoride matrix such as a silica matrix, a nylon matrix, a calcium alginate
matrix, a polyvinylidene difluoride matrix, an agarose matrix or an acrylamide-
azlactone polymer matrix,
iv) removing the electrodes from the first compartment, and placing the electrodes
in a second compartment (5), wherein the second compartment comprises a
constricted volume (6) around at least the first electrode, and the second
compartment comprises an elution buffer (7) in contact with the first and the
second electrodes, wherein the elution buffer does not modify the sign of the
charge of the molecules of interest;
v) optionally generating a second electrical field in the elution buffer by applying a
second current between the two electrodes, preferably such that the charges of
the electrodes are reversed, whereby the charged molecules are
electrophoretically migrated to the first electrode,
vii) optionally interrupting the second electrical field; and
vii) collecting the elution buffer from the second compartment, wherein the elution buffer comprises the molecules, whereby the molecules are extracted, optionally wherein the molecules are nucleic acid molecules, such as DNA molecules or RNA molecules, proteins, amino acids, or carbohydrates such as monosaccharides or polysaccharides.

The absolute value of the charge of the electrodes is not essential, i.e. it is not a requirement that the charge of the second electrode is the same as the charge of the molecules - only that it has the same sign.

The skilled person will know how to design the second current so that an appropriate second electrical field is generated. For example, the second current may have the following parameters: voltage of 90 to 120 V, amperage of about 400 mA, and can be applied to the electrodes for 1 to 3 minutes.

In some embodiments, the second current may have a voltage between 50 and 200 V, such as between 60 and 190 V, such as between 70 and 180 V, such as between 80 and 170 V, such as between 90 and 160 V, such as between 90 and 150 V, such as between 90 and 140 V, such as between 90 and 130 V, such as between 90 and 120 V, such as 90 V, 100 V, 110 V or 120 V.

In some embodiments, the second current may have an amperage of between 100 and 700 mA, such as between 150 and 650 mA, such as between 200 and 600 mA, such as between 250 and 550 mA, such as between 300 and 500 mA, such as between 350 and 450 mA, such as 400 mA.

In some embodiments, the second current may be applied for a duration of between 30 seconds and 5 minutes, such as between 1 minute and 4 minute, such as between 1 minute and 3 minutes, such as between 1.5 minute and 2.5 minute, such as 1 minute, 2 minutes or 3 minutes.

The parameters of the second current (voltage, amperage and duration) may depend on the nature of the charged molecules and of the sample in which they are comprised, and can be adjusted as is known to the person of skill in the art.
Once the electrodes are charged, the charged molecules will thus migrate to the first electrode, which has a charge opposite to the charge of the molecules. The second current is thus applied to the electrodes for a time sufficient to allow migration of at least part of or all of the charged molecules.

In some embodiments, part of the elution buffer is removed from the second compartment after migration of the molecules to the first electrode. This may be desirable in embodiments where it is of importance to have a volume of eluant as small as possible. The elution buffer should however still be in contact with both electrodes, or at least with the conductive part of both electrodes.

Collection of the charged molecules

In a sixth step, the charged molecules are collected. This is done for example by interrupting the second current, and hence the second electrical field, and collecting the elution buffer from the second compartment. Because the electrodes are no longer charged when the second current is interrupted, the charged molecules are released from the first electrode and are now present in the elution buffer. The elution buffer comprising the charged molecules can then be collected. The charged molecules have thus been extracted.

In order to facilitate release of the charged molecules from the first electrode, it may be advantageous to generate a third electrical field by applying a third current to the first and second electrodes. Preferably, the third current is applied for a very short time, for example a pulse. The direction of the third current should be the reverse of the direction of the second current, so that the first electrode now has a charge of the same sign as the charged molecules, which it thus repels. The charged molecules are thus released in the elution buffer surrounding the first electrode. Provided that the third current is applied for a short period of time, the charged molecules will not have sufficient time to migrate back to the second electrode, but will remain in the elution buffer.

It may in some embodiments be practical to remove the electrode pair from the second compartment prior to collecting the elution buffer comprising the charged molecules. This is simply because the presence of the electrodes may hamper collection of the eluant due to physical hindrances.
Once the charged molecules have been released from the first electrode into the elution buffer, the eluant, i.e. the elution buffer comprising at least part of the charged molecules, can be collected. This can be done as is known to the person of skill in the art, for example by pipetting.

**Additional steps**

It will be understood that in addition to the steps described herein above, the methods of the present disclosure may comprise additional washing and/or drying steps. Washing steps imply the use of a washing solution. Ethanol solutions are examples of suitable washing solutions. Water may also be used as washing solution. The skilled person can easily determine which washing solutions may be suitable depending on the type of molecules to be extracted. The choice of washing solution may also be influenced by the presence of a column attached to the second electrode, and by its nature.

Drying steps may consist of applying air pressure to the first and/or the second compartment and/or the column when present before addition of a buffer, such as the elution buffer, or after the addition of a washing solution.

In some embodiments, as described above, a column such as a binding column may be attached to the second electrode. Suitable columns are known in the art. In such embodiments, the method may further comprise one or more steps of centrifuging and/or applying air pressure to the column to allow the buffer such as binding buffer or electrophoresis buffer comprised therein to run through the column and into the first compartment. Air pressure may also be applied in order to dry the column, for example before step iv) or v), preferably before step v). The skilled person will know which pressure to apply and for which duration. For example, the air pressure is applied for 1 minute at 0.2 to 0.4 bar. The air pressure may be applied at a pressure of 0.1 to 0.5 bar, such as 0.2 to 0.4 bar, such as 0.1 bar, 0.2 bar, 0.3 bar, 0.4 bar or 0.5 bar. In order to allow a buffer to run through the column, in some embodiments, the air pressure is applied for 10 seconds to 120 seconds, such as 20 seconds to 100 seconds, such as 30 seconds to 90 seconds, such as 40 seconds to 80 seconds, such as 50 seconds to 70 seconds, such as 60 seconds. In order to dry the column, longer times may be required. In some embodiments, the air pressure is thus applied for 1 minute or more,
such as 2 minutes or more, such as 3 minutes or more, such as 4 minutes, 5 minutes, 6 minutes, or more.

Washing solutions may also be applied to the column at the end of any step of the method. For example, ethanol solutions, methanol solutions or water solutions may be used.

In embodiments where a column is used, step iv) of the methods disclosed herein may comprise the addition of elution buffer to the column. The elution buffer may be run through the column and into the second compartment by applying air pressure to the column. Preferably, part of the elution buffer runs through the column and comes in contact with the elution buffer comprised in the second compartment.

**Electrophoresis**

In some embodiments, the method further comprises a step of performing electrophoresis prior to step i).

The electrophoresis step may be performed as is known in the art. Alternatively, the electrophoresis step may be as described in application "electrophoresis assembly", assigned to same applicant and having the same filing date as the present application.

In some embodiments, the electrophoresis step thus comprises the steps of:

1) Providing an electrophoresis assembly comprising two or more frames for holding a separation matrix, wherein each frame has at least one inner frame, and wherein the two or more frames are attached together;

2) Providing at least one separation matrix;

3) Loading the at least one separation matrix in the electrophoresis assembly;

4) Arranging the electrophoresis assembly in a first configuration wherein the two or more frames abut each other in a longitudinal direction;

5) Loading samples comprising molecules of interest onto the at least one separation matrix, optionally with a robot or a liquid handler;

6) Arranging the electrophoresis assembly in a second configuration wherein the two or more frames are in extension of each other;

7) Placing the electrophoresis assembly in a chamber comprising a conductive fluid such that the electrophoresis assembly is at least partially immersed in
said conductive fluid, said chamber further comprising at least one pair of electrodes at opposite ends of the chamber, said electrodes being capable of generating an electrophoretic electrical field in a first direction, wherein the electrophoresis assembly is placed in the chamber such that each separation matrix is in a direction parallel to the first direction, said chamber optionally further comprising at least one compartment configured to hold one or more detection means and/or at least one compartment configured to hold a light source;

8) Applying an electrophoretic current between the at least two electrodes, whereby migration of the samples through the separation matrix is enabled;

9) Retrieving one or more pieces of the separation matrix, wherein said one or more pieces contain or are suspected of containing charged molecules of interest,

Wherein the sample of step i) is the one or more pieces of 9).

The electrophoresis step may be a step of dry or semi-dry electrophoresis. The present methods may thus also be performed in an electrophoresis chamber devoid of conductive fluid, e.g. in a process of dry or semi-dry electrophoresis. Separation matrices typically contain a high percentage of conductive fluid, which may be sufficient for separating the molecules in the samples without actually submerging the separation matrix in conductive fluid. In such embodiments, the electrodes used for separating the samples are brought into contact with the upper and the lower part of the separation matrix.

In embodiments where the chamber comprises a conductive fluid, contact may be established between the electrodes via the conductive fluid, by placing the electrodes in the conductive fluid in which the electrophoresis assembly is at least partly immersed.

Thus in some embodiments, the electrophoresis step comprises the steps of:

i) Providing an electrophoresis assembly (1) comprising two or more frames (2) for holding a separation matrix (4), wherein each frame has at least one inner frame (3), and wherein the two or more frames are attached together;

ii) Providing at least one separation matrix (4);

iii) Loading the at least one separation matrix in the electrophoresis assembly;
iv) Arranging the electrophoresis assembly in a first configuration wherein the two or more frames abut each other in a longitudinal direction;
v) Loading samples comprising molecules of interest onto the at least one separation matrix, optionally with a robot or a liquid handler;
vi) Arranging the electrophoresis assembly in a second configuration wherein the two or more frames are in extension of each other;
vii) Placing the electrophoresis assembly in a chamber (5) comprising at least a first pair of electrodes (6) at opposite ends of the chamber, wherein the electrophoresis assembly is contacted with said electrodes, said electrodes being capable of generating a first electrical field in a first direction, wherein the electrophoresis assembly is placed in the chamber such that each separation matrix is in a direction parallel to the first direction, said chamber optionally further comprising at least one compartment configured to hold one or more detection means (7) and/or at least one compartment (8) configured to hold a light source (9);
viii) applying a current between the at least two electrodes, whereby migration of the samples through the separation matrix is enabled.

The term “abutting each other in a longitudinal direction” in relation to two frames refers herein to the fact that two frames abut each other on their biggest lateral side, i.e. the side that defines the biggest plane of the frame. In some embodiments, each frame can hold a single separation matrix. In some embodiments, each frame has an opening on an upper side for a separation matrix to be inserted therein. In some embodiments, each frame has a hinge or a slidable connection which can be used to attach two frames to one another.

Above-mentioned application describes electrophoresis assemblies suitable for performing electrophoresis as described above. The electrophoresis assemblies and the methods described in said application can be used for high-throughput electrophoresis and optionally also for high-throughput recovery of pieces of separation matrices containing or suspected of containing the charged molecules of interest.

Accordingly, in one embodiment, the present method if for the extraction of molecules having a positive or a negative charge from a sample such as a solution or a separation matrix, comprising the steps of:
1) Providing an electrophoresis assembly comprising two or more frames for
holding a separation matrix, wherein each frame has at least one inner frame,
and wherein the two or more frames are attached together;

2) Providing at least one separation matrix;

3) Loading the at least one separation matrix in the electrophoresis assembly;

4) Arranging the electrophoresis assembly in a first configuration wherein the two
or more frames abut each other in a longitudinal direction;

5) Loading samples comprising molecules of interest onto the at least one
separation matrix, optionally with a robot or a liquid handler;

6) Arranging the electrophoresis assembly in a second configuration wherein the
two or more frames are in extension of each other;

7) Placing the electrophoresis assembly in a chamber comprising a conductive
fluid such that the electrophoresis assembly is at least partially immersed in
said conductive fluid, said chamber further comprising at least one pair of
electrodes at opposite ends of the chamber, said electrodes being capable of
generating an electrophoretic electrical field in a first direction, wherein the
electrophoresis assembly is placed in the chamber such that each separation
matrix is in a direction parallel to the first direction, said chamber optionally
further comprising at least one compartment configured to hold one or more
detection means and/or at least one compartment configured to hold a light
source;

8) Applying an electrophoretic current between the at least two electrodes,
whereby migration of the samples through the separation matrix is enabled;

9) Retrieving one or more pieces of the separation matrix, wherein said one or
more pieces contain or are suspected of containing charged molecules of
interest,

i) Thereby providing a sample (1) comprising charged molecules,

ii) placing the sample in a first compartment (2), wherein the first compartment
comprises a conductive fluid (3) such as an electrophoresis buffer, a first
electrode (4a) and a second electrode (4b),

iii) generating a first electrical field in the conductive fluid by applying a first current
to the first and second electrodes, such that the first electrode becomes
charged with the same charge as the molecules and the second electrode
becomes charged with a charge opposite of the charge of the molecules,

whereby the charged molecules are electrophoretically migrated to the second
electrode, and optionally attached to the second electrode via a matrix such as
a silica matrix, a nylon matrix, a calcium alginate matrix, or a polyvinylidene difluoride matrix such as a silica matrix, a nylon matrix, a calcium alginate matrix, a polyvinylidene difluoride matrix, an agarose matrix or an acrylamide-azlactone polymer matrix,

iv) removing the electrodes from the first compartment, and placing the electrodes in a second compartment (5), wherein the second compartment comprises a constricted volume (6) around at least the first electrode, and the second compartment comprises an elution buffer (7) in contact with the first and the second electrodes,

v) optionally generating a second electrical field in the elution buffer by applying a second current between the two electrodes, preferably such that the charges of the electrodes are reversed, whereby the charged molecules are electrophoretically migrated to the first electrode,

vi) optionally interrupting the second electrical field; and

vii) collecting the elution buffer from the second compartment, wherein the elution buffer comprises the molecules, whereby the charged molecules are extracted.

As detailed above, the step of electrophoresis may be a step of dry or semi-dry electrophoresis.

Accordingly, in one embodiment, the present method if for the extraction of molecules having a positive or a negative charge from a sample such as a solution or a separation matrix, comprising the steps of:

1) Providing an electrophoresis assembly comprising two or more frames for holding a separation matrix, wherein each frame has at least one inner frame, and wherein the two or more frames are attached together;

2) Providing at least one separation matrix;

3) Loading the at least one separation matrix in the electrophoresis assembly;

4) Arranging the electrophoresis assembly in a first configuration wherein the two or more frames abut each other in a longitudinal direction;

5) Loading samples comprising molecules of interest onto the at least one separation matrix, optionally with a robot or a liquid handler;

6) Arranging the electrophoresis assembly in a second configuration wherein the two or more frames are in extension of each other;
7) Placing the electrophoresis assembly in a chamber comprising at least one pair of electrodes at opposite ends of the chamber, said electrodes being capable of generating an electrophoretic electrical field in a first direction, wherein the electrophoresis assembly is placed in the chamber such that each separation matrix is in a direction parallel to the first direction and wherein the electrophoresis assembly is contacted with said electrodes, said chamber optionally further comprising at least one compartment configured to hold one or more detection means and/or at least one compartment configured to hold a light source;

8) Applying an electrophoretic current between the at least two electrodes, whereby migration of the samples through the separation matrix is enabled;

9) Retrieving one or more pieces of the separation matrix, wherein said one or more pieces contain or are suspected of containing charged molecules of interest,

i) Thereby providing a sample (1) comprising charged molecules,

ii) placing the sample in a first compartment (2), wherein the first compartment comprises a conductive fluid (3) such as an electrophoresis buffer, a first electrode (4a) and a second electrode (4b),

iii) generating a first electrical field in the conductive fluid by applying a first current to the first and second electrodes, such that the first electrode becomes charged with the same charge as the molecules and the second electrode becomes charged with a charge opposite of the charge of the molecules, whereby the charged molecules are electrophoretically migrated to the second electrode, and optionally attached to the second electrode via a matrix such as a silica matrix, a nylon matrix, a calcium alginate matrix, or a polyvinylidene difluoride matrix such as a silica matrix, a nylon matrix, a calcium alginate matrix, a polyvinylidene difluoride matrix, an agarose matrix or an acrylamide-azlactone polymer matrix,

iv) removing the electrodes from the first compartment, and placing the electrodes in a second compartment (5), wherein the second compartment comprises a constricted volume (6) around at least the first electrode, and the second compartment comprises an elution buffer (7) in contact with the first and the second electrodes,

v) optionally generating a second electrical field in the elution buffer by applying a second current between the two electrodes, preferably such that the charges of
the electrodes are reversed, whereby the charged molecules are electrophoretically migrated to the first electrode, vi) optionally interrupting the second electrical field; and vii) collecting the elution buffer from the second compartment, wherein the elution buffer comprises the molecules, whereby the charged molecules are extracted.

In some embodiments, each frame can hold a single separation matrix. In some embodiments, each frame has an opening on an upper side for a separation matrix to be inserted therein. In some embodiments, each frame has a hinge or a slidable connection which can be used to attach two frames to one another.

The pair of electrodes comprised in the chamber is different from the pairs of electrodes used for the extraction of charged molecules otherwise described herein.

Support frame
The electrodes may be indirectly connected, for example they are attached in their upper end via a support structure, which facilitates handling, particularly for embodiments where the first compartment has smaller volumes, in the range of some mL. The presence of such a support structure, for example a support frame, may greatly facilitate performing the present methods in a high-throughput manner. Accordingly, in some embodiments, the first and second electrodes are attached in their upper end to a support frame.

In some embodiments, the support frame comprises at least one hole located next to the upper end of the first electrode and/or the upper end of the second electrode, said hole being configured to allow a solution to flow through.

In some embodiments, the hole is a valve of which the opening and closing can be controlled. For example, the valve is a solenoid valve.

Device for high-throughput extraction of charged molecules
As detailed above, the present methods are particularly advantageous for high-throughput extraction of molecules having a positive or a negative charge.
Accordingly, herein is provided a device (10) for high-throughput extraction of molecules having a positive or a negative charge from a plurality of samples, said device comprising a plurality of electrode pairs comprising a first electrode (4a) and a second electrode (4b) attached to a support frame (8), wherein the pairs are configured to be placed in a first and a second compartment, and optionally wherein the second electrodes are further configured to reversibly attach a column thereto.

Said device is preferably suitable for performing the methods described herein in a high-throughput manner.

The samples may be as described herein. Preferably the plurality of samples is at least two samples, such as 3 samples or more, such as 4 samples or more, such as 5 samples or more, such as 8 samples or more, such as 12 samples or more, such as 16 samples or more, such as 24 samples or more, such as 32 samples or more, such as 36 samples or more, such as 40 samples or more, such as 48 samples or more, such as 96 samples or more, such as 192 samples or more, such as 384 samples or more, such as 768 samples or more, such as 1536 samples or more.

Support frame

In some embodiments, the support frame to which the plurality of electrode pairs is attached has dimensions such that the electrode pairs attached thereto have a spacing matching the spacings between the wells of a standard plate or deep-well plate. This means that the spacings between the first compartments and second compartments in which the plurality of electrode pairs is to be placed also match the spacings between the wells of a standard microplate or deep-well plate. This allows the device to be used in high-throughput methods, since it will enable the use of automated or manual liquid handlers which are routinely used in the art. In some embodiments, the electrode pairs are placed to match a 96-well microplate, a 192-well microplate, a 384-well microplate, a 768-well microplate or a 1536-well microplate.

In some embodiments, the support frame comprises at least one hole located next to the upper end of each of the first electrodes and/or the upper end of each the second electrodes of the plurality of electrodes, said hole being configured to allow a solution to flow through.
In some embodiments, the hole is a valve of which the opening and closing can be controlled. For example, the valve is a solenoid valve.

The support frame thus preferably comprises a plurality of holes, and at least one hole per electrode pair. The holes are in some embodiments configured to allow air pressure to be applied to the first and/or second compartment. The holes may also or alternatively be configured to allow a solution to flow into the first and/or second compartments.

In some embodiments, the device further comprises a column plate, i.e. a plurality of columns, such that each column can be attached to each second electrode and surround it. In some embodiments, the column is a plurality of columns which can be reversibly attached to the second electrode, for example by being “clicked” thereto.

In some embodiments, the device is useful for the extraction of molecules selected from nucleic acid molecules, such as DNA or RNA molecules, proteins, amino acids, or carbohydrates such as monosaccharides or polysaccharides.

System

Also provided herein is a system comprising a device as described herein above, and a first reservoir comprising a plurality of first compartments, where the first reservoir is configured such that the first compartments each can receive one electrode pair of the device. As explained above, the spacings between the first compartments may aptly match the spacings between wells of a standard microplate or deep-well plate.

In some embodiments, the system further comprises a second reservoir comprising a plurality of second compartments, wherein the second reservoir is configured such that the second compartments each can receive one electrode pair of the device. In some embodiments, the second compartments comprise a constricted volume in their lower part, said constricted volume being configured to receive at least one first electrode, as described herein above.

Kit of parts

Also provided herein is a kit of parts, comprising:
a) a second compartment comprising a constricted volume, as described herein;
b) optionally a first compartment as described herein;
c) optionally a conductive fluid;
d) optionally an elution buffer;
e) optionally a washing solution; and
f) instructions for use.

The above elements comprised in the kit of parts may be as described herein.

Items

1. A method for the extraction of molecules having a positive or a negative charge from a sample such as a solution or a separation matrix, comprising the steps of:
   i) providing a sample (1) such as a solution or a separation matrix comprising the molecules,
   ii) placing the sample in a first compartment (2), wherein the first compartment comprises a conductive fluid (3) such as an electrophoresis buffer, a first electrode (4a) and a second electrode (4b),
   iii) generating a first electrical field in the conductive fluid by applying a first current to the first and second electrodes, such that the first electrode becomes charged with the same charge as the molecules and the second electrode becomes charged with a charge opposite of the charge of the molecules, whereby the charged molecules are electrophoretically migrated to the second electrode, and optionally attached to the second electrode via a matrix such as a silica matrix, a nylon matrix, a calcium alginate matrix, or a polyvinylidene difluoride matrix such as a silica matrix, a nylon matrix, a calcium alginate matrix, a polyvinylidene difluoride matrix, an agarose matrix or an acrylamide-azlactone polymer matrix,
   iv) removing the electrodes from the first compartment, and placing the electrodes in a second compartment (5), wherein the second compartment comprises a constricted volume (6) around at least the first electrode, and the second compartment comprises an elution buffer (7) in contact with the first and the second electrodes,
   v) optionally generating a second electrical field in the elution buffer by applying a second current between the two electrodes, preferably such that the charges of
the electrodes are reversed, whereby the charged molecules are
electrophoretically migrated to the first electrode,
vi) optionally interrupting the second electrical field; and
vii) collecting the elution buffer from the second compartment, wherein the elution
buffer comprises the molecules,
whereby the molecules are extracted.

2. The method according to item 1, wherein the molecules are nucleic acid
molecules, such as DNA molecules or RNA molecules, proteins, amino acids,
or carbohydrates such as monosaccharides or polysaccharides.

3. The method according to any one of the preceding items, comprising step v) of
generating the second electrical field in the elution buffer.

4. The method according to item 3, wherein the charges of the electrodes are
reversed.

5. The method according to any one of the preceding items, wherein step v)
further comprises removing part of the elution buffer from the second
compartment after the charged molecules have migrated to the first electrode.

6. The method according to any one of the preceding items, wherein step vi)
further comprises a step of generating a third electrical field in the elution buffer
by applying a short-term current between the two electrodes such that the first
electrode becomes charged with the same charge as the molecules and the
second electrode becomes charged with a charge opposite of the charge of the
molecules, said third electrical field facilitating release of the molecules from the
first electrode immediately prior to collecting the elution buffer.

7. The method according to any one of the preceding items, wherein a column is
attached to the second electrode, said column optionally comprising a matrix for
binding the molecules, such as a silica matrix, and/or optionally comprising a
volume of electrophoresis buffer.

8. The method according to any one of the preceding items, wherein a binding
buffer is added to the column before step iv).
9. The method according to any one of the preceding items, wherein air pressure is applied to the column to run the binding buffer and optionally the electrophoresis buffer through the column and into the first compartment.

10. The method according to any one of the preceding items, further comprising the step of running a washing solution through the column, such as an ethanol solution.

11. The method according to any one of the preceding items, wherein the first compartment is emptied, preferably by applying air pressure to the first compartment.

12. The method according to any one of the preceding items, further comprising a step of drying the column before step iv) or step v), preferably before step iv).

13. The method according to any one of the preceding items, wherein step iv) further comprises adding elution buffer to the column.

14. The method according to any one of the preceding items, wherein step iv) further comprises applying air pressure to the column so that part of the elution buffer runs through the column and comes in contact with the elution buffer comprised in the second compartment.

15. The method according to any one of the preceding items, wherein the first electrode (4a) is longer than the second electrode (4b).

16. The method according to any one of the preceding items, wherein the method is high-throughput.

17. The method according to any one of the preceding items, wherein the first and second electrodes are attached in an upper end to a support frame (8).

18. The method according to item 17, wherein the support frame comprises at least one hole (9) located next to the upper end of the first electrode and/or the upper
end of the second electrode, said hole being configured to allow a solution to flow through.

19. The method according to item 18, wherein the hole is a valve of which the opening and closing can be controlled, such as a solenoid valve.

20. The method according to any one of the preceding items, further comprising a step of performing electrophoresis prior to step i), said step comprising the steps of:

1) Providing an electrophoresis assembly comprising two or more frames for holding a separation matrix, wherein each frame has at least one inner frame, and wherein the two or more frames are attached together;

2) Providing at least one separation matrix;

3) Loading the at least one separation matrix in the electrophoresis assembly;

4) Arranging the electrophoresis assembly in a first configuration wherein the two or more frames abut each other in a longitudinal direction;

5) Loading samples comprising molecules of interest onto the at least one separation matrix, optionally with a robot or a liquid handler;

6) Arranging the electrophoresis assembly in a second configuration wherein the two or more frames are in extension of each other;

7) Placing the electrophoresis assembly in a chamber comprising a conductive fluid such that the electrophoresis assembly is at least partially immersed in said conductive fluid, said chamber further comprising at least one pair of electrodes at opposite ends of the chamber, said electrodes being capable of generating an electrophoretic electrical field in a first direction, wherein the electrophoresis assembly is placed in the chamber such that each separation matrix is in a direction parallel to the first direction, said chamber optionally further comprising at least one compartment configured to hold one or more detection means and/or at least one compartment configured to hold a light source;

8) Applying an electrophoretic current between the at least two electrodes, whereby migration of the samples through the separation matrix is enabled;

9) Retrieving one or more pieces of the separation matrix, wherein said one or more pieces contain or are suspected of containing charged molecules of interest,

wherein the sample of step i) is the one or more pieces of 9).
21. A device (10) for high-throughput extraction of molecules having a positive or a negative charge from a plurality of samples, said device comprising a plurality of electrode pairs comprising a first electrode (4a) and a second electrode (4b) attached to a support frame (8), wherein the pairs are configured to be placed in a first and a second compartment, and optionally wherein the second electrodes are further configured to reversibly attach a column thereto.

22. The device according to item 21, wherein the device is suitable for performing the method according to any one of items 1 to 19 in a high-throughput manner.

23. The device according to any one of items 21 to 22, wherein the support frame comprises a plurality of holes (9) located next to the upper end of each the first or the second electrodes, said hole being configured to allow air pressure to be applied to and/or a solution to flow into said first and/or second compartments.

24. The device according to any one of items 21 to 23, wherein the plurality of holes is a plurality of valves of which the opening and closing can be controlled, such as a plurality of solenoid valves.

25. The device according to any one of items 21 to 24, wherein the column is configured to surround the second electrode but not the first electrode.

26. The device according to any one of items 21 to 25, wherein the molecules are nucleic acid molecules, such as DNA molecules or RNA molecules, proteins, amino acids, or carbohydrates such as monosaccharides or polysaccharides.

27. The device according to any one of items 21 to 26, wherein the electrode pairs are placed to match a microplate standard, such as a 96-well microplate, a 192-well microplate, a 384-well microplate, a 768-well microplate or a 1536-well microplate, or a deep well plate standard.

28. A system comprising a device according to any one of items 21 to 27 and a first reservoir comprising a plurality of first compartments, said first reservoir being configured such that the first compartments each can receive one electrode pair of the device.
29. The system according to item 28, further comprising a second reservoir comprising a plurality of second compartments configured such that the second compartments each can receive one electrode pair of the device, wherein the second compartments optionally comprise a constricted volume in their lower part, said constricted volume being configured to receive at least one first electrode.

30. Use of a device according to any one of items 21 to 27 or of a system according to any of items 28 to 29 for the extraction of molecules having a positive or a negative charge from a sample such as a solution or a separation matrix.

31. A kit of parts, comprising:
   a) a second compartment comprising a constricted volume as defined in any one of the preceding items;
   b) optionally a first compartment as defined in any one of the preceding items;
   c) optionally a conductive fluid as defined in any one of the preceding items;
   d) optionally an elution buffer as defined in any one of the preceding items;
   e) optionally a washing solution as defined in any one of the preceding items; and
   f) instructions for use.
Claims

1. A method for the extraction of molecules having a positive or a negative charge from a separation matrix, comprising the steps of:
   i) providing a sample (1) comprising the separation matrix comprising the molecules,
   ii) placing the sample in a first compartment (2), wherein the first compartment comprises a conductive fluid (3) such as an electrophoresis buffer, a first electrode (4a) and a second electrode (4b), wherein the first electrode (4a) is longer than the second electrode (4b),
   iii) generating a first electrical field in the conductive fluid by applying a first current to the first and second electrodes, such that the first electrode becomes charged with the same charge as the molecules and the second electrode becomes charged with a charge opposite of the charge of the molecules, whereby the charged molecules are electrophoretically migrated to the second electrode, and optionally attached to the second electrode via a matrix such as a silica matrix, a nylon matrix, a calcium alginate matrix, or a polyvinylidene difluoride matrix such as a silica matrix, a nylon matrix, a calcium alginate matrix, a polyvinylidene difluoride matrix, an agarose matrix or an acrylamide-azlactone polymer matrix,
   iv) removing the electrodes from the first compartment, and placing the electrodes in a second compartment (5), wherein the second compartment comprises a constricted volume (6) around at least the first electrode, and the second compartment comprises an elution buffer (7) in contact with the first and the second electrodes,
   v) optionally generating a second electrical field in the elution buffer by applying a second current between the two electrodes, preferably such that the charges of the electrodes are reversed, whereby the charged molecules are electrophoretically migrated to the first electrode,
   vi) optionally interrupting the second electrical field; and
   vii) collecting the elution buffer from the second compartment, wherein the elution buffer comprises the molecules, whereby the molecules are extracted, optionally wherein the molecules are nucleic acid molecules, such as DNA molecules or RNA molecules, proteins, amino acids, or carbohydrates such as monosaccharides or polysaccharides.
2. The method according to claim 1, wherein step v) further comprises removing part of the elution buffer from the second compartment after the charged molecules have migrated to the first electrode.

3. The method according to any one of the preceding claims, wherein step vi) further comprises a step of generating a third electrical field in the elution buffer by applying a short-term current between the two electrodes such that the first electrode becomes charged with the same charge as the molecules and the second electrode becomes charged with a charge opposite of the charge of the molecules, said third electrical field facilitating release of the molecules from the first electrode immediately prior to collecting the elution buffer.

4. The method according to any one of the preceding claims, wherein a column is attached to the second electrode, said column optionally comprising a matrix for binding the molecules, such as a silica matrix, and/or optionally comprising a volume of electrophoresis buffer.

5. The method according to any one of the preceding claims, wherein a binding buffer is added to the column before step iv).

6. The method according to any one of the preceding claims, wherein air pressure is applied to the column to run the binding buffer and optionally the electrophoresis buffer through the column and into the first compartment.

7. The method according to any one of the preceding claims, further comprising the step of running a washing solution through the column, such as an ethanol solution.

8. The method according to any one of the preceding claims, further comprising a step of drying the column before step iv) or step v), preferably before step iv).

9. The method according to any one of the preceding claims, wherein step iv) further comprises adding elution buffer to the column.

10. The method according to any one of the preceding claims, wherein step iv) further comprises applying air pressure to the column so that part of the elution
buffer runs through the column and comes in contact with the elution buffer comprised in the second compartment.

11. The method according to any one of the preceding claims, wherein the method is high-throughput.

12. The method according to any one of the preceding claims, wherein the first and second electrodes are attached in an upper end to a support frame (8).

13. The method according to claim 12, wherein the support frame comprises at least one hole (9) located next to the upper end of the first electrode and/or the upper end of the second electrode, said hole being configured to allow a solution to flow through, optionally wherein the hole is a valve of which the opening and closing can be controlled, such as a solenoid valve.

14. The method according to any one of the preceding claims, further comprising a step of performing electrophoresis prior to step i), said step comprising the steps of:

1) Providing an electrophoresis assembly comprising two or more frames for holding a separation matrix, wherein each frame has at least one inner frame and optionally at least one opening in an upper end for inserting the separation matrix therein, and wherein the two or more frames are attached together;

2) Providing at least one separation matrix;

3) Loading the at least one separation matrix in the electrophoresis assembly;

4) Arranging the electrophoresis assembly in a first configuration wherein the two or more frames abut each other in a longitudinal direction;

5) Loading samples comprising molecules of interest onto the at least one separation matrix, optionally with a robot or a liquid handler;

6) Arranging the electrophoresis assembly in a second configuration wherein the two or more frames are in extension of each other;

7) Placing the electrophoresis assembly in a chamber comprising at least one pair of electrodes at opposite ends of the chamber, said electrodes being capable of generating an electrophoretic electrical field in a first direction, wherein the electrophoresis assembly is placed in the chamber such that each separation matrix is in a direction parallel to the first direction and
wherein the electrophoresis assembly is contacted with said electrodes, said chamber optionally further comprising at least one compartment configured to hold one or more detection means and/or at least one compartment configured to hold a light source;

5 8) Applying an electrophoretic current between the at least two electrodes, whereby migration of the samples through the separation matrix is enabled;

9) Retrieving one or more pieces of the separation matrix, wherein said one or more pieces contain or are suspected of containing charged molecules of interest,

10 wherein the sample of step i) is the one or more pieces of 9).

15. The method according to any one of the preceding claims, further comprising a step of performing electrophoresis prior to step i), said step comprising the steps of:

15 1) Providing an electrophoresis assembly comprising two or more frames for holding a separation matrix, wherein each frame has at least one inner frame and optionally at least one opening in an upper end for inserting the separation matrix therein, and wherein the two or more frames are attached together;

20 2) Providing at least one separation matrix;

3) Loading the at least one separation matrix in the electrophoresis assembly;

4) Arranging the electrophoresis assembly in a first configuration wherein the two or more frames abut each other in a longitudinal direction;

5) Loading samples comprising molecules of interest onto the at least one separation matrix, optionally with a robot or a liquid handler;

25 6) Arranging the electrophoresis assembly in a second configuration wherein the two or more frames are in extension of each other;

7) Placing the electrophoresis assembly in a chamber comprising a conductive fluid such that the electrophoresis assembly is at least partially immersed in said conductive fluid, said chamber further comprising at least one pair of electrodes at opposite ends of the chamber, said electrodes being capable of generating an electrophoretic electrical field in a first direction, wherein the electrophoresis assembly is placed in the chamber such that each separation matrix is in a direction parallel to the first direction, said chamber optionally further comprising at least one compartment configured to hold
one or more detection means and/or at least one compartment configured to
hold a light source;

8) Applying an electrophoretic current between the at least two electrodes,
whereby migration of the samples through the separation matrix is enabled;

9) Retrieving one or more pieces of the separation matrix, wherein said one or
more pieces contain or are suspected of containing charged molecules of
interest,
wherein the sample of step i) is the one or more pieces of 9).

16. A device (10) for high-throughput extraction of molecules having a positive or a
negative charge from a plurality of separation matrices, said device comprising
a plurality of electrode pairs comprising a first electrode (4a) and a second
electrode (4b) attached to a support frame (8), wherein the first electrode (4a) is
longer than the second electrode (4b), and wherein the pairs are configured to
be placed in a first and a second compartment, and optionally wherein the
second electrodes are further configured to reversibly attach a column thereto.

17. The device according to claim 16, wherein the device is suitable for performing
the method according to any one of claims 1 to 22 in a high-throughput manner.

18. The device according to any one of claims 16 to 17, wherein the support frame
comprises a plurality of holes (9) located next to the upper end of each the first
or the second electrodes, said holes being configured to allow air pressure to
be applied to and/or a solution to flow into said first and/or second
compartments, optionally wherein the plurality of holes is a plurality of valves of
which the opening and closing can be controlled, such as a plurality of solenoid
valves.

19. The device according to any one of claims 16 to 18, wherein the column is
configured to surround the second electrode but not the first electrode.

20. The device according to any one of claims 16 to 19, wherein the molecules are
nucleic acid molecules, such as DNA molecules or RNA molecules, proteins,
amino acids, or carbohydrates such as monosaccharides or polysaccharides.
21. The device according to any one of claims 16 to 20, wherein the electrode pairs are placed to match a microplate standard, such as a 96-well microplate, a 192-well microplate, a 384-well microplate, a 768-well microplate or a 1536-well microplate, or a deep well plate standard.

22. A system comprising a device according to any one of claims 16 to 19 and a first reservoir comprising a plurality of first compartments, said first reservoir being configured such that the first compartments each can receive one electrode pair of the device, wherein the electrode pair consists of a first and a second electrode, wherein the first electrode is longer than the second electrode, optionally wherein the system further comprises a second reservoir comprising a plurality of second compartments configured such that the second compartments each can receive one electrode pair of the device, wherein the second compartments optionally comprise a constricted volume in their lower part, said constricted volume being configured to receive at least one first electrode.

23. Use of a device according to any one of claims 16 to 19 or of a system according to claim 22 for the extraction of molecules having a positive or a negative charge from a separation matrix.

24. A kit of parts, comprising:
   a) a second compartment comprising a constricted volume as defined in any one of the preceding claims;
   b) optionally a first compartment as defined in any one of the preceding claims;
   c) optionally a conductive fluid as defined in any one of the preceding claims;
   d) optionally an elution buffer as defined in any one of the preceding claims;
   e) optionally a washing solution as defined in any one of the preceding claims; and
   f) instructions for use.
**INTERNATIONAL SEARCH REPORT**

**International application No**
PCT/EP2019/078363

---

**A. CLASSIFICATION OF SUBJECT MATTER**

G01N 27/447

---

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

G01N C12N B01L

---

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO - INTELLIGENT INVENTION SERVICES (INSPEC)

---

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>HANNU AHOKAS: &quot;Electroelution of nucleic acid in microcentrifuge tube&quot;, NUCLEIC ACIDS RESEARCH ADVANCE ACCESS, vol. 15, no. 16, 1 January 1987 (1987-01-01), pages 6759-6759, XP055655452, GB</td>
<td>1</td>
</tr>
<tr>
<td>A</td>
<td>US 4 859 302 A (ALFENITO MARK R [US]) 22 August 1989 (1989-08-22) column 1, line 4 - line 6 column 2, line 58 - line 68</td>
<td>1-15</td>
</tr>
</tbody>
</table>

---

**Date of the actual completion of the international search**

13 January 2020

**Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-5040, Fax. (+31-70) 340-3016**

---

**Date of mailing of the international search report**

19/03/2020

**Authorized officer**

Müller, Thomas

---

Further documents are listed in the continuation of Box C. See patent family annex.
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>WO 98/58251 A1 (BILATEC GES ZUR ENTWICKLUNG BI [DE]; LANGE HANS [DE]) 23 December 1998 (1998-12-23) page 17, line 17 - line 21 page 20, line 31 - page 22, line 2 page 23, line 18 - page 34, line 15 -----</td>
<td>1-15</td>
</tr>
<tr>
<td>A</td>
<td>WO 01/07452 A1 (DNA RES INSTR LTD [GB]; BAKER MATTHEW JOHN [GB]) 1 February 2001 (2001-02-01) the whole document -----</td>
<td>1-15</td>
</tr>
</tbody>
</table>
# INTERNATIONAL SEARCH REPORT

**Box No. II**  
**Observations where certain claims were found unsearchable (Continuation of Item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. [ ] Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III**  
**Observations where unity of invention is lacking (Continuation of Item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

> see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [x] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
   1-15

**Remark on Protest**

- [ ] The additional search fees were accompanied by the applicant’s protest and, where applicable, the payment of a protest fee.

- [ ] The additional search fees were accompanied by the applicant’s protest but the applicable protest fee was not paid within the time limit specified in the invitation.

- [ ] No protest accompanied the payment of additional search fees.
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-15

   Method for extracting molecules having a positive or a negative charge from a separation matrix, comprising steps of placing first and second electrodes into first and second compartments, comprising an electrophoresis buffer and an elution buffer respectively, and collecting the elution buffer from the second compartment.

   ---

2. claims: 16-23

   Device for the extraction of molecules having a positive or a negative charge from a plurality of separation matrices comprising a plurality of electrode pairs attached to a support frame wherein the electrodes are configured to be placed in a first and a second compartment.

   ---

3. claim: 24

   Kit comprising a second compartment having a constricted volume

   ---
<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 4859302 A</td>
<td>22-08-1989</td>
<td>NONE</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 0988536 A1</td>
<td>29-03-2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2002504232 A</td>
<td>05-02-2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 6264814 B1</td>
<td>24-07-2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 9858251 A1</td>
<td>23-12-1998</td>
</tr>
<tr>
<td>WO 0107452 A1</td>
<td>01-02-2001</td>
<td>AU 6297800 A</td>
<td>13-02-2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 0107452 A1</td>
<td>01-02-2001</td>
</tr>
<tr>
<td>US 2003038032 A1</td>
<td>27-02-2003</td>
<td>AT 483967 T</td>
<td>15-10-2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2456684 A1</td>
<td>06-03-2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 1419376 A1</td>
<td>19-05-2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 2187200 A2</td>
<td>19-05-2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 2295956 A2</td>
<td>16-03-2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2005501251 A</td>
<td>13-01-2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2007178437 A</td>
<td>12-07-2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2003038032 A1</td>
<td>27-02-2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2006124463 A1</td>
<td>15-06-2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2010032298 A1</td>
<td>11-02-2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 03019164 A1</td>
<td>06-03-2003</td>
</tr>
</tbody>
</table>