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(54) Title: CONCENTRATED UNBOUND MAGNETIC PARTICLE ASSAY FOR BIOSENSORS

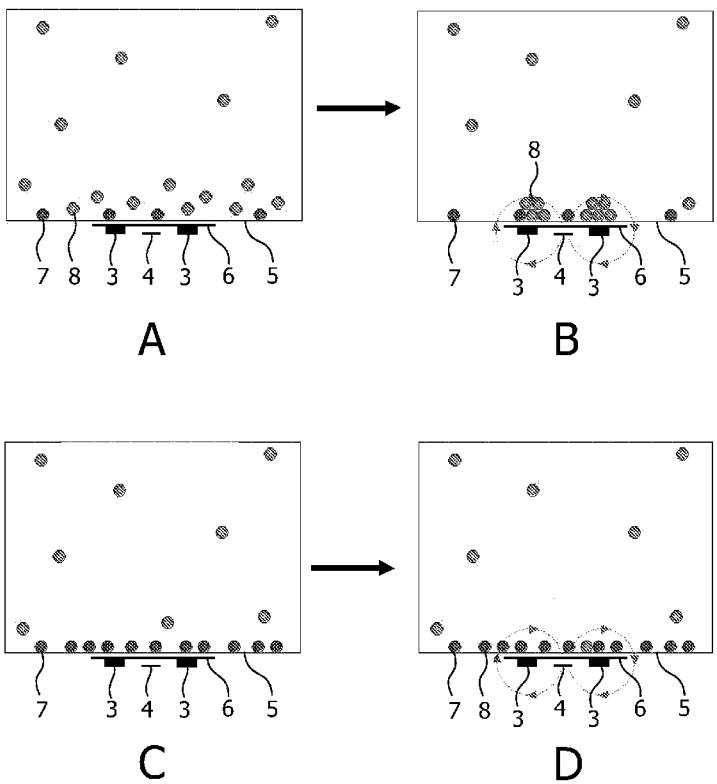

FIG. 2

(57) Abstract: A system (1) is provided comprising a reaction chamber (2) having a binding surface (5) and at least one detection region (6) within the binding surface and further comprising at least one means for generating one or more magnetic fields (3), wherein the at least one means for a generating one or more magnetic fields are placed such that one of the magnetic fields has a gradient with a component that is parallel to said binding surface (5) arranged to move unbound magnetic particles towards a detection region (6) within the binding surface.

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Concentrated unbound magnetic particle assay for biosensors

FIELD OF THE INVENTION

The present invention relates to systems, apparatus and methods for detecting and/or quantifying molecules in a sample using magnetic particles. The invention relates to disposable cartridges for use with such systems.

BACKGROUND OF THE INVENTION

The use of magnetic particles in bio-assays and biosensors has several attractive aspects. Magnetic particles with analytes can be stirred using a magnetic field which shortens the reaction-time between probes and analytes. Magnetic fields can be used to remove aspecific bound analytes from a probe, to concentrate magnetic particles in a part of a reaction chamber, or to move particles in and/or out of a reaction chamber. The magnetic properties of magnetic particles themselves can be used for detection purposes. For example, a GMR-type magneto-resistive sensor can measure the magnetic stray-field of bound magnetic beads. From this field the concentration of the analyte is calculated.

In such biosensors magnetic actuation is important in order to increase the performance of the magnetic biosensor in terms of point-of-care applications. Firstly, it allows up-concentrating the magnetic particles at the binding surface and speeding up the binding process of the magnetic particles at the binding surface. Secondly, magnetic washing can replace the traditional wet washing step, making the assay more accurate and reducing the number of operating actions.

The type of magnetic field gradients that are generally used are applied perpendicular or orthogonally to a binding surface to move particles to or from the binding surface. Also conical fields have been applied to concentrate particles to one particular point, e.g. the objective a microscope. Circular or random magnetic fields are used to create a stirring effect. Examples hereof are described in US5445971, US6548311, US6180418, Perrin *et al.* (1999), *J. Immun. Meth.* **224**, 77-87; Luxton *et al.* (2004) *Anal. Chem.* **76**, 1715-1719; Ferreira *et al.* (2005) *Appl. Phys Lett.* **87**, 013901.

Magnetic washing steps however are time- and energy-consuming, and complicate the design and increase the costs of reader and cartridge. Accordingly there is a

need for biosensor applications wherein washing steps are no longer needed but which still enable end-point detection.

WO2007129275 describes a device wherein a magnetic field is applied parallel to a binding surface such as to move magnetic particles over the binding surface towards a region outside the binding surface, so as not to interfere with detection at the binding surface. In these devices the interaction between the magnetically labeled analyte or probe and the binding surface occurs during the lateral movement, which may affect sensitivity of detection. More importantly however, these devices require reaction chambers, which comprise in addition to the binding surface a region outside the binding surface which does not interfere with detection.

Generally, only assays using kinetic measurements can be performed without washing steps. However, kinetic measurement methods cannot be used for all applications. Especially when short measuring times are used, the amount of data points gathered in that period becomes too low to perform kinetic measurements. Accordingly, devices which not only allow the different assay steps to be performed without washing steps, allowing a faster and more efficient detection and which allow a more simple design of the reaction chamber cartridge are highly desirable, more particularly in the context of high-throughput screening and on-site analysis.

SUMMARY OF THE INVENTION

The present invention is based on the observation that by concentrating and detecting unbound analyte or analyte-specific reagent using magnetic particles, devices can be developed whereby the reaction chamber has less constraints.

Accordingly, the present invention provides a sensing system for detection using magnetic particles, comprising a biochemically active binding surface with a probe, a detection region within this binding surface and means to generate a magnetic field that induces a movement of particles over the binding surface towards the detection region. Devices and methods described herein are particularly suitable for use in indirect detection methods, i.e. methods wherein unbound analyte or analyte-specific reagent is detected as a measure for the presence, and optionally the amount, of an analyte in a sample.

The systems, devices and methods of the present invention can be used in automated high-throughput testing devices for centralized laboratories. Reaction chambers are provided such as well plates or cuvettes, fitting into an automated instrument. When applied in e.g. immunoassays, a minimum number of fluid manipulation steps are needed, the

incubation occurs at high speed and washing steps are reduced to a minimum with a minimal fluid waste.

The present invention discloses devices and methods for biosensing using magnetic labels. The measuring time of assays is decreased by excluding the washing part of the actuation while performing end-point detection. Accordingly methods performed in accordance with the present invention are rapid, sensitive, and robust.

One aspect of the invention relates to methods for quantifying and/or detecting an analyte in a sample. In particular embodiments, methods for quantifying and/or detecting an analyte in a sample, comprise the steps of:

a) contacting the sample with an analyte labeled with a magnetic particle or with an analyte-specific probe labeled with a magnetic particle

b) applying the sample to a system (1) comprising

- a reaction chamber (2) having a binding surface (5) and at least one detection region (6) within the binding surface, whereby isolated analyte, analyte-analog or analyte-specific probe is bound to the binding surface (5) and

- at least one means for generating one or more magnetic fields (3), wherein the at least one means for a generating one or more magnetic fields are placed such that one of the magnetic fields has a gradient with a component that is parallel to the binding surface (5) and wherein the one or more magnetic fields are arranged to move unbound magnetic particles laterally over the binding surface (5) and to accumulate the particles in the detection region (6) within the binding surface;

c) allowing the analyte-specific probe to interact with the analyte or analyte analog on the binding surface.

d) generating the one or more magnetic fields; and

e) detecting the fraction of magnetic particles accumulated in the detection region.

In these methods, the steps (a) and (b) can be performed simultaneously or sequentially, whereby the contacting step (a) can also be performed after applying the sample to the system. The methods of the invention encompass different detection methods of analytes on a surface, including both direct detection with an analyte-specific probe and indirect (competitive) detection making use of an analyte or analyte-analog.

Particular embodiments of the invention relate to methods for quantifying and/or detecting an analyte in a sample, comprising the steps of:

a) contacting the sample with an analyte-specific probe labeled with a magnetic particle,

b) applying the sample to a system (1) comprising

- a reaction chamber (2) having a binding surface (5) and at least one detection region (6) within the binding surface, whereby the analyte or analyte-analog is bound to the binding surface (5),and

- at least one means for generating one or more magnetic fields (3), wherein the at least one means for a generating one or more magnetic fields are placed such that one of the magnetic fields has a gradient with a component that is parallel to the binding surface (5) and wherein the one or more magnetic fields are arranged to move unbound magnetic particles laterally over the binding surface (5) and to accumulate the particles in the detection region (6) within the binding surface;

c) allowing the analyte-specific probe to interact with the analyte or analyte analog on the binding surface,

d) generating the one or more magnetic fields,

e) detecting the fraction of the magnetic particles accumulated at the detection region.

According to particular embodiments of methods described herein, the gradient of the one or more magnetic fields further has a component that is orthogonal to the binding surface, such that the one or more magnetic fields move magnetic particles towards the binding surface (5).

According to particular embodiments of methods described herein, the magnetic particles are labeled, and detection of the fraction of the magnetic particles accumulated at the detection region is performed based on the label.

Another aspect of the invention provides systems (1) comprising a reaction chamber (2) having a binding surface (5) and at least one detection region (6) within the binding surface and further comprising at least one means for generating one or more magnetic fields (3), wherein the at least one means for generating one or more magnetic fields are placed such that one of the magnetic fields has a gradient with a component that is parallel to the binding surface (5); and wherein the one or more magnetic fields are arranged to move unbound magnetic particles laterally over the binding surface (5) and to accumulate the particles in the detection region (6) within the binding surface.

According to particular embodiments of systems described herein, the one or more means for generating a magnetic field comprise at least one electric wire located below the binding surface and parallel to the binding surface.

Particular embodiments of systems described herein further comprise a detection means capable of detecting the magnetic particles in the detection zone. More particularly, the detection means is a means for detection of a magnetic property of at least one magnetic particle. Such magnetic-particle detection means is optionally suitable for detecting the magnetic field of the at least one particle present in the detection region and/or detecting the magnetizability of the at least one particle in the detection region when subjected to a magnetic detection field.

According to particular embodiments of systems described herein, the magnetic-particle detection means is a magneto-resistive sensor or a Hall sensor.

Additionally or alternatively, systems described herein comprise a detection means capable of detecting a label, such as but not limited to an optical label.

Particular embodiments of systems described herein comprise a bioactive molecule linked to the binding surface. More particularly, the binding surface comprises an analyte or analyte-analog bound to the binding surface (5). Additionally or alternatively, in certain embodiments of systems described herein, one or more analyte-specific probes are bound to the binding surface (5). Analyte-specific probes envisaged in this context include but are not limited to molecules selected from the group consisting of an oligonucleotide, an antibody or fragment thereof, a lectin, a pharmaceutical compound, a peptide or a protein.

A further aspect of the present invention provides cartridges comprising: a binding surface (5), at least one detection region (6) within the binding surface, and at least one physical carrier for generating one or more magnetic fields (3), whereby the physical carrier for generating magnetic fields is capable of generating a magnetic field which ensures the movement of unbound magnetic particles laterally over the binding surface (5) and/or ensures accumulation of the particles in the detection region (6) within the binding surface.

According to particular embodiments cartridges described herein, the at least one physical carrier for generating one or more magnetic fields (3) comprises at least one metal wire located below the binding surface and parallel to the binding surface.

According to further particular embodiments cartridges described herein comprise at least one physical carrier for generating one or more magnetic fields (3) which essentially consists of two parallel metal wires located below the binding surface and parallel to the binding surface.

The above and other characteristics, features and advantages of the present invention will become apparent from the following detailed description, taken in conjunction with the accompanying Figs., which illustrate, by way of example, the principles of the invention. This description is given for the sake of example only, without limiting the scope of the invention. The reference Figs. quoted below refer to the attached drawings:

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a schematic representation of a device (1) with a reaction chamber (2) in accordance with particular embodiments of the invention. The reaction chamber (2) comprises a binding surface (5) which comprises a probe specific for an analyte or an analyte-specific reagent. The binding surface (5) comprises a detection region (6). A magnetic field generator (3) ensures one or more magnetic fields, at least one of which comprises a component parallel with the binding surface. In particular embodiments of the use of device (1), a sample is contacted with a reagent labeled with a magnetic particle (or analyte within the sample is provided with a label) prior to entering the reaction chamber (2). A fraction (7) of the magnetically labeled reagents (or analyte) bind specifically to the binding surface. Application of the magnetic field causes the lateral movement of unbound magnetic particles (8) towards the detection region (6) where they are detected by a detection means (4).

Fig. 2 shows a particular embodiment of the methods of the present invention. Panels A and B represent the situation where the sample contains a limited number of particles capable of binding to the probe on the binding surface. Panel A demonstrates the contacting of the sample with the binding surface (5), whereby a limited number of magnetic particles (7) is specifically bound to the binding surface, and many unbound particles (8) remain on the binding surface. Upon application of the indicated magnetic field (Panel B) through the magnetic field generator(s)(3), the unbound particles (8) migrate to the detection region (6) where they are detected by the detection means (4). Panels C and D represent the situation where the sample contains a high number of particles capable of binding to the probe on the binding surface (5). Panel C demonstrates the contacting of the sample with the binding surface, whereby a high number of magnetic particles (7) is specifically bound to the binding surface, and only a limited number of unbound particles (8) remain on the binding surface. Upon application of the indicated magnetic field (Panel D), the unbound particles (8) migrate to the detection region (6).

Fig. 3 shows a dose-response curve of a competition assay for the detection of morphine in a sample in accordance with an embodiment of the methods of the present invention. Measurement is performed at 15 seconds after application of a magnetic field comprising a lateral component.

DETAILED DESCRIPTION OF THE EMBODIMENTS

The present invention will be described with respect to particular embodiments and with reference to certain drawings but the invention is not limited thereto but only by the claims. Any reference signs in the claims shall not be construed as limiting the scope. The drawings described are only schematic and are non-limiting. In the drawings, the size of some of the elements may be exaggerated and not drawn on scale for illustrative purposes.

Where the term "comprising" is used in the present description and claims, it does not exclude other elements or steps. Where an indefinite or definite article is used when referring to a singular noun e.g. "a" or "an", "the", this includes a plural of that noun unless something else is specifically stated.

Furthermore, the terms first, second, third and the like in the description and in the claims, are used for distinguishing between similar elements and not necessarily for describing a sequential or chronological order. It is to be understood that the terms so used are interchangeable under appropriate circumstances and that the embodiments of the invention described herein are capable of operation in other sequences than described or illustrated herein.

The terms or definitions used herein are provided solely to aid in the understanding of the invention.

DEFINITIONS

The term "**analyte**" as used herein refers to a compound in a sample of which the detection of presence and or/ concentration is desired.

The term "**analyte-specific probe**", as used herein, refers to a compound which is capable of specifically binding with the analyte, such as an antibody.

The term "**reaction chamber**" as used herein refers to a region within a device or a cartridge, where different reagents taking part in a reaction are contacted with each other.

The term "**binding surface**", as used herein, refers to the part of the reaction chamber, suitable for binding magnetically labeled analytes or analyte-specific probes.

The term **"detection region"** as used herein, refers to a region of a device or cartridge where detection is ensured by a detection means.

The terms **"(essentially) parallel"** or **"(essentially) orthogonal"** as used herein with respect to the orientation of a magnetic field with regard to the binding surface, refers to the movement of magnetic particles ensured by the magnetic field.

The present invention provides methods and tools for detecting analytes using magnetic particles. Typically, in detection and/or quantification methods and in systems making use of magnetic particles, the magnetic particles are bound as a label to an analyte (either the analyte in the sample or isolated analyte serving as competitor) or to an analyte-specific probe, and detection is based on the reaction of the labeled analyte or probe with a probe or analyte, respectively, which is bound to the binding surface. The present invention provides methods and systems wherein unbound magnetic particles (linked as a label to a probe or analyte) are moved within a reaction chamber, based on the application of one or more magnetic fields. In particular embodiments, the magnetic particles are moved essentially parallel to a binding surface.

The force exerted on a magnetic particle by a magnetic field is given by the gradient of the potential energy of the particle in the magnetic field. The magnetic potential energy can be calculated by taking the integral of the magnetization and the field (see for example J.D. Jackson, Classical Electrodynamics, John Wiley & Sons, Inc., 1999). As a result, the force on a magnetic particle relates to the gradient of the magnetic field. In other words, a magnetic particle has a tendency to move from a region of lower to a region of higher magnitude of the magnetic field.

In methods and devices described herein, at least one magnetic field is provided with a gradient which is essentially parallel to the binding surface or has at least a component parallel to the binding surface. In addition, the at least one magnetic field ensures the movement towards a region within the binding surface which is the detection region. Accordingly, the one or more magnetic fields allow the movement of magnetic particles (which are present in the vicinity of the binding surface but not bound to the binding surface) across the binding surface, from an area outside the detection region within the binding surface, towards a detection region.

The methods and tools of the present invention provide the advantage that the detection region can be provided as a region within the binding surface. The configuration of the detection region present within the binding surface and the means for generating one or

more magnetic fields is such that the detection region is formed by the gradient of the one or more magnetic fields. In particular embodiments the one or more magnetic fields are essentially parallel to the binding surface and the detection region is formed by the one or more borders of the magnetic field on the binding surface. This ensures a flow of particles which is essentially parallel to the binding surface towards (and up until) the border of the one or more magnetic fields. In particular embodiments, two or more fields are generated whereby both fields are positioned such as to ensure the movement of particles towards the same detection region. The detection region within the binding surface is accordingly defined by the position of the magnetic field generators.

As the movement of magnetic particles is determined by magnetic field gradients, there is often a relationship between the flow and the magnetic field gradient orientation. In particular embodiments, the lateral flow (or flow essentially parallel) to the binding surface corresponds to flow orientations with an angle of less than 45 °, or less than 20, less than 10 or less than 5° from the binding surface. With respect to magnetic fields or magnetic field gradients lateral (or essentially parallel) magnetic fields generally relates to a component of the field or gradient which has an orientation corresponding to an angle of less than 45°, or less than 20, less than 10 or less than 5° with the binding surface. Notwithstanding the above, the magnetic field gradient, may make other angles with the substrate or the binding surface, e.g. higher than 45° such as 85° or higher.

The one or more magnetic fields ensure the accumulation of unbound magnetic particles present on or near the binding surface in the detection region, where they can be detected. The presence of binding surface in the detection region causes some background signal from the particles which bind specifically to the binding surface within the detection region. However, this background signal does not interfere with quantitative detection of the unbound particles.

In particular embodiments of methods and devices described herein, in addition to (or integrated in) the magnetic field comprising a lateral component, a force such as a magnetic field is generated that has a gradient component orthogonal to the binding surface and directed towards the binding surface. This force ensures the movement of unbound magnetic particles within the reaction chamber towards the binding surface. In this way the number of magnetic particles that are contacted with the binding surface is increased. In more particular embodiments the force orthogonal to the binding surface is a magnetic force.

As for a lateral magnetic force, with an orthogonal magnetic force the movement of magnetic particles is determined by magnetic field gradients and there is a relationship between the orientation of the flow and the magnetic field gradient orientation. With respect to flow, particular embodiments of orthogonal flow are flow orientations corresponding to an angle of more than 45°, or more than 70°, more than 80°, or more than 85°, most particularly 90° with the binding surface. With respect to magnetic fields or magnetic field gradients the term orthogonal relates to the presence of a component of the field or gradient which has an orientation corresponding to an angle of more than 45°, or more than 70°, more than 80° or more than 85°, most particularly 90° with the binding surface.

In particular embodiments, a magnetic field comprising an orthogonal component is combined with the magnetic field comprising a lateral component, i.e. as one magnetic field with both a lateral and an orthogonal component (resulting in a circular movement perpendicular to the plane of the binding surface).

In particular embodiments of methods and devices described therein, in addition to or as an alternative to an orthogonal magnetic force, other means are provided which ensure the movement of magnetic particles to the binding surface. This can be a physical attribute of the reaction chamber, e.g. funnel-shaped towards the binding surface. Additionally or alternatively other forces are used such as fluid flow or acoustic or ultrasonic fluid excitation.

Methods, systems and devices described herein make use of magnetic particles. The nature of the magnetic particle used in the context of the present invention is not critical. Suitable magnetic particles include completely inorganic particles and particles which are a mixture of an inorganic and an organic material (e.g. a polymer).

Magnetic particles are widely used in biological analysis, e. g. in high-throughput clinical immunoassay instruments, sample purification, cell extraction, etc. Several diagnostic companies (Roche, Bayer, Johnson & Johnson, Abbott, BioMerieux, etc.) fabricate and sell reagents with magnetic particles, e.g. for immunoassays, nucleic-acid extraction, and sample purification.

Attachment of the analytes or probes to the surface of magnetic particles can be performed by methods described in the art. For instance, the particles may carry functional groups such as hydroxyl, carboxyl, aldehyde or amino groups. These may in general be provided, for example, by treating uncoated monodisperse, superparamagnetic particles, to provide a surface coating of a polymer carrying one of such functional groups, e. g.

polyurethane together with a polyglycol to provide hydroxyl groups, or a cellulose derivative to provide hydroxyl groups, a polymer or copolymer of acrylic acid or methacrylic acid to provide carboxyl groups or an aminoalkylated polymer to provide amino groups. US Patent 4654267 describes the introduction of many such surface coatings. Other coated particles may be prepared by modification of the particles according to the US Patents 4336173, 4459378 and 4654267. For example, macroreticular porous polymer particles, prepared from styrene-divinylbenzene and with a diameter of 3.15 μm were treated with HNO_3 to introduce-NO_2 groups at the surface of the pores. Then the particles were dispersed in an aqueous solution of Fe. The Fe^{2+} is oxidized by the NO_2 groups, which leads to precipitation of insoluble iron oxy- hydroxy compounds inside the pores. After heating the iron exists as finely divided grains of magnetic iron oxides throughout the volume of the porous particles The NO_2 groups are reduced by the reaction with Fe to NH_2 groups. To fill up the pores and to introduce the desired functional groups at the surface s, different monomers are caused to polymerize in the pores and at the surface. In the case of a preferred type of particle, the surface carries-OH groups connected to the polymeric backbone through $(CH_2CH_2O)_{8-10}$ linkages. Other preferred carry -COOH groups obtained through polymerization of methacrylic acid. For example, the NH_2 groups initially present in the particles may be reacted with a diepoxide as described in US Patent 4654267 followed by reaction with methacrylic acid to provide a terminal vinyl grouping. Solution copolymerization with methacrylic acid yields a polymeric coating carrying terminal carboxyl groups. Similarly, amino groups can be introduced by reacting a diamine with the above product of the reaction with a diepoxide, while reaction with a hydroxylamine such as aminoglycerol introduces hydroxy groups. The coupling of a bio active molecule to a particle can be irreversible but can also be reversible by the use of a linker molecule for the crosslinking between particle and bioactive molecule. Examples of such linkers include peptides with a certain proteolytic recognition site, oligonucleotide sequences with a recognition site for a certain restriction enzyme, or chemical reversible crosslinking groups as those comprising a reducible disulfide group. A variety of reversible crosslinking groups can be obtained from Pierce Biotechnology Inc. (Rockford, IL, USA).

Magnetic particles are commercially available in various sizes, ranging from nanometers to micrometers. Magnetic particles of different sizes (such as but not limited to sizes of 10 nm to 5μm, typically between 50 nm and 1 μm) are envisaged to be suitable for use in the context of the invention, provided that they can be moved by the magnetic field and allow sensitive detection. Similarly, the shape of the particles (spheres, spheroids, rods)

is not critical. It is envisaged that different types of magnetic particles, e.g. with different magnetic and/or optical properties can be used simultaneously within one reaction chamber (magnetic particle multiplexing).

Methods, devices and tools provided in the present invention envisage the movement and detection of magnetic particles. In particular embodiments, the magnetic particles are detected in a detection region based on their magnetic properties. In this regard, the use of particles with different types of magnetic properties (magnetic, paramagnetic, superparamagnetic, ferromagnetic, i.e. any form of magnetism which has a magnetic dipole in a magnetic field, either permanently or temporarily) is envisaged.

Additionally or alternatively it is envisaged that magnetic particles can be detected based on the presence of a label either directly attached to the magnetic particle or indirectly bound to the particle through an analyte.

Accordingly, in particular embodiments, the magnetic particle used in methods and devices described herein are labeled. Labels can be attached to magnetic particles via the inorganic or via the organic component at the outside or can be incorporated into the particle.

In further particular embodiments, the compound (i.e. analyte, analyte-analog or analyte-specific probe) which comprises the magnetic particle as a label in the context of the present invention further comprises a non-magnetic label.

Suitable labels in the context of the present invention are those labels which are classically used in *in vitro* assays such as, but not limited to, chromophoric groups, radioactive labels, electroluminescent, chemiluminescent, phosphorescent, fluorescent or reflecting labels.

A further aspect of the invention provides systems or devices comprising a reaction chamber having a binding surface and at least one detection region within the binding surface and further comprising one or more means for applying one or more magnetic fields, which is/are placed such that they ensure a movement of magnetic particles present on (or close to) the binding surface essentially parallel to the binding surface. More particularly the one or more magnetic fields that can be generated span the distance between the detection region and the outer boundaries of the binding surface, such as to ensure movement of particles from all regions of the binding surface towards the detection surface.

Accordingly, systems are provided wherein one or more magnetic fields can be generated by one or more magnetic field generating means. Different types of magnetic field generating means are envisaged in the context of the present invention, such as permanent magnets, electromagnets, coils and/or wires. The strength of the magnetic force on

the particles is such that the induced travel distance is larger than the distance traveled without magnetic fields, i.e. the magnetic forces should be dominant over translational Brownian motion. Most particularly, the magnetic field generated by the magnetic field generating means ensure that the travel distance of the magnetic particles towards the detection region (upon activation) of the magnetic field generating means is larger than the distance towards the detection region as measured on the binding surface.

According to the present invention, magnetic field(s) generated by the one or more magnetic field generating means can be constant, pulsating, or can vary in strength. Moreover, where more than one magnetic field is generated, their exact orientation may be fixed or may vary, provided that the field gradient is essentially parallel to the detection surface or has at least a component parallel to the binding surface.

In particular embodiments a magnetic field comprising a lateral component is generated by providing a permanent magnet and moving either the magnetic field generating means or the binding surface relative to each other.

According to particular embodiments, the magnetic field generating means comprises an electromagnet or one or more electric wires. This makes it possible to avoid mechanical moving of parts in the device. According to particular embodiments, a magnetic field generating means is placed below the binding surface and generates an essentially out-of-plane field with smaller in-plane components.

According to particular embodiments systems are provided which contain two parallel electric wires each generating a magnetic field. An example hereof is shown in Fig. 2. In order to allow the manipulation of the magnetic particles from an area on the binding surface (5) away from the detection region towards the detection region (6), which is located in the centre of the binding surface, the wires are placed next to each other beneath the binding surface at the edges of the detection region. In this embodiment the two parallel wires and the magnetic field generated thereby define a detection region which is essentially rectangular in shape (the long sides being defined by the length of the wires and the corresponding magnetic field, the short sides generally being defined by the edges of the binding surface. Generation of a current within these wires ensures the generation of a magnetic field around each of these wires, whereby the magnetic field at the binding surface ensures movement of particles towards the detection region. In more particular embodiments, the current in the wires is generated in the same direction such that two magnetic fields are created which transport magnetic particles along the binding surface towards the detection

region (6). In particular embodiments, the detection region is located on the binding surface in a zone defined by the two wires.

In particular embodiments, the size and shape of the detection region is modified by applying the magnetic field generators in a configuration, which results in, for example a square, triangular, pentagonal, hexagonal or circular detection region. A reduction of the surface of the detection region allows an increase in sensitivity and reduces the background. In particular embodiments, the magnetic field generator comprises one or more electric wires and the shape of the one or more wires ensures the shape of the detection region.

As described above, the present invention provides systems which ensure movement of magnetic particles essentially parallel to the binding surface by provision of a magnetic field gradient that has at least a component parallel to the binding surface.

In particular embodiments, in addition to the one or more magnetic fields ensuring a lateral movement of unbound magnetic particles, means for generating other forces for moving/immobilizing magnetic particles are provided. Examples of other forces envisaged in this context are other (non-parallel) magnetic fields, electrical fields, acoustic forces, hydrodynamic forces, gravitational forces...etc. Thus, according to particular embodiments, devices are provided which comprise a magnetic field generating device which, in addition to the magnetic field gradient which is essentially parallel to the binding surface allowing a lateral movement of the particles over the binding surface, generate magnetic fields that cause movements of the magnetic particles orthogonal to (and optionally from) the binding surface, which can be alternated with the lateral movement. This can be of interest in the context of stirring (i.e. ensuring movement of magnetic particles over the binding surface) and/or binding (i.e. ensuring the binding of particles to the binding surface). Examples of the use of magnetic fields in the context of stirring are described in US5445971, US6548311, US6180418, Perrin et al. (1999), *J. Immun. Meth.* **224**, 77-87; Luxton *et al.* (2004) *Anal. Chem.* **76**, 1715-1719; and Ferreira et al. (2005) *Appl. Phys Lett.* **87**, 013901. Using magnetic field generating devices, e.g. such as those known in the art, different methods are available to ensure specific movements of magnetic particles within a reaction chamber such as to achieve a maximal interaction of the magnetic particles with probes on the binding surface.

The binding surface present in systems and devices according to the invention is typically a specially derivatised surface to which molecules, more particularly probes can be bound. Examples of suitable surface include, glass, metal, plastic, an organic crystal or an

inorganic crystal (e. g. silicon), an amorphous organic or an amorphous inorganic material (e. g. silicon nitride, silicon oxide, silicon oxinitride, aluminum oxide). Suitable surface materials and linking chemistries are known to the person skilled in the art, and are described for instance in "Diagnostic Biosensor Polymers", by A. M. Usmani and N. Akmal, American Chemical Society, 1994 Symposium Book Series 556, Washington DC, USA, 1994, in "Protein Architecture, Interfacing Molecular Assemblies and Immobilization Biotechnology", edited by Y. Lvov and H.Mhwald (Marcel Dekker, New York, 2000), in "The Immunoassay Handbook" by David Wild (Nature Publishing Group, London, 2001, ISBN 1 -56159-270-6) or "Handbook of Biosensors and Electronic Noses. Medicine, Food and the Environment" by Kress-Rogers (ISBN 0-8493-8905-4). Supports for coupling proteins to coated and uncoated plastic and glass supports are disclosed in Angenendt et al. (2002; *Anal Biochem.* **309**, 253-260). Dufva (2005; *Biomol Eng* **22**, 173-184), review the methodology to attach oligonucleotides and factors influencing this process.

In particular embodiments, systems and devices are provided wherein the binding surface is coated by an analyte, analyte-analog or analyte-specific probe. In particular embodiments, binding surfaces are provided, which are coated over their entire surface with probe or analyte (including in the detection region), thereby simplifying the manufacture of the reaction chamber and devices comprising the reaction chamber. As described above, use of devices whereby the detection region is a part of the binding surface will result in some background detection in the detection region. Indeed, upon contacting the surface with a sample comprising magnetic beads, magnetic beads bound by the specific analyte or probe on the binding surface will also be present within the detection region. Thus the detection region will contain both specifically bound magnetic particles and unbound magnetic particles, whereby the latter accumulate in the detection region by application of a lateral magnetic field. Nevertheless, the Example below demonstrates that the sensitivity of an assay is sufficiently high to determine the contribution of unbound particles amongst the bound magnetic particles.

In particular embodiments a binding surface is provided which ensures that non-specific sticking of particles to the binding surface is avoided, e.g. by optimizing the properties of the coating of the binding surface.

In alternative embodiments, of systems and devices provided herein, the detection region is a portion which is located physically within the binding surface, but which is not modified with probe or analyte and as such is not part of the binding surface. Using this

type of detection region, only unbound magnetic particles will accumulate at the detection region.

The systems and devices of the present invention are based on the detection of unbound magnetic particles after accumulation thereof in a detection region. As detailed above the magnetic fields generated by the one or more magnetic field generators define the boundaries of the detection region. Detection within the detection region is ensured by a detection means (4) which can be integrated into the device or can be adapted to be placed on the device to ensure detection.

Detection means suitable for use in the systems and devices provided herein are capable of detecting the presence of magnetic particles on the detection region. In particular embodiments, detection of the unbound magnetic particles in the detection region is performed based on the magnetic properties of the magnetic particles. Additionally or alternatively, magnetic particles in the detection region are detected base on the presence of a label. The label can be attached to the magnetic particles, or can be bound to or incorporated into the unbound analyte or analyte-specific probe.

Accordingly, detection means present in or used in combination with systems and devices of the present invention are detection means capable of detecting the relevant signal such as, but not limited, to a magnetic signal, magnetoresistance, a Hall effect, an optic signal (reflection, absorption, scattering, fluorescence, chemiluminescence, RAMAN, FTIR, etc.), an acoustical signal (quartz crystal microbalance (QCM), surface acoustic waves (SAW), Bulk Acoustic Wave (BAW) etc.). These may be generated by liposomes, micelles, bubbles, microbubbles, microspheres, lipid-, or polymer coated bubbles, microbubbles and/or microspheres, microballoons, aerogels, clathrate bound vesicles, and the like. Such vesicles may be filled with a liquid, a gas, a gaseous precursor, and/or a solid or solute material. In particular embodiments, a detection means is provided which is a magnetoresistance element.

Further particular embodiments of systems and devices described herein comprise, in addition to the components described above, one or more of the following components. The system of the invention will usually comprise one or more inlet means for introducing sample, magnetic particles and/or reagents into the reaction chamber, and optionally an outlet means for removing reagents, reaction waste, and/or optionally, magnetic particles, from the reaction chamber. These can optionally be coupled to sources comprising each of the reagents.

A further aspect of the present invention provides cartridges comprising a reaction chamber which comprises a binding surface which comprises a detection region and a physical carrier for generating one or more magnetic fields within the reaction chamber. Most particularly cartridges are provided which comprise a binding surface, and beneath said binding surface two parallel electrical wires which, when provided with a current, ensure a magnetic field on the binding surface. In further particular embodiments, the binding surface is coated with an analyte, analyte-analog or analyte-specific probe.

In further particular embodiments, the cartridge is a disposable cartridge.

According to particular embodiments, a sensor for detecting magnetic particles in the detection region is integrated into the reaction chamber (e.g. magnetoresistive sensor is integrated). Alternatively, the sensor is provided as separate part from the reaction chamber (e.g. optical unit). In this embodiment, the reaction chamber optionally comprises a detection window, which allows the detection magnetic particles in the detection region. The location of the detection window is determined by the location of the detection region within the binding surface. Most particularly, the detection window is centrally located as a spot or longitudinal region above or below the detection region, corresponding with the detection region.

In particular embodiments, detection is ensured by a sensor below the binding surface in the detection region. Optionally, a detection window is provided in the material supporting the detection surface.

Where the detection is based on magnetic field or optical methods, the provision of a specific detection window may be superfluous, e.g. where all or part of the reaction chamber and/or binding surface material is transparent for the signal to be detected.

In particular embodiments, systems are provided which are single-chamber (bio)sensors, with low reagent use and small required sample volume. (Bio)sensors in accordance with the present invention can be operated with a minimum of equipment, washing steps and buffers.

Particular embodiments of the present invention provide optionally disposable cartridges comprising a binding surface and at least one detection region within the binding surface. Disposable cartridges can further comprise magnetic particles integrated therein or these can be provided separately. The material of the cartridge is such that magnetic fields can be generated therein. For example, the cartridge is made of glass or a synthetic material, such as plexiglass [poly(methy)methacrylate] or clear PVC (polyvinyl chloride) or PC (polycarbonate) or COP (e.g. Zeonex) or PS (polystyrene).

Cartridges envisaged in the context of the present invention further optionally comprise at least one physical carrier for generating a magnetic field gradient, whereby generation of a magnetic field gradient in said physical carrier results in a gradient essentially parallel to the binding surface or having at least a component parallel to the binding surface.

The devices described in the present invention can be used as rapid, robust, and easy to use point-of-care biosensors for small sample volumes. As detailed above, the reaction chamber (or the binding surface alone) can be a disposable item to be used with a compact reader, containing one or more magnetic field generating means and one or more detection means.

According to yet a further aspect, the invention provides methods whereby the systems and/or cartridges of the present invention are used for the detection and/or quantification of analytes.

In particular embodiments methods are provided which comprise the steps of applying a sample to a system (1) comprising

- a reaction chamber (2) having a binding surface (5) and at least one detection region (6) within said binding surface, and
- at least one means for generating one or more magnetic fields (3), wherein the at least one means for a generating one or more magnetic fields are placed such that one of said magnetic fields has a gradient with a component that is parallel to said binding surface (5) and wherein the one or more magnetic fields are arranged to move unbound magnetic particles laterally over the binding surface (5) and to accumulate said particles in the detection region (6) within the binding surface; wherein said analyte or analyte-analog is bound to said binding surface (5)

More particularly, methods according to the present invention encompass generating a magnetic field within the reaction chamber such as to move unbound magnetic particles laterally over the binding surface (5) and to accumulate said particles in the detection region (6), where they can be detected.

Methods envisaged in the context of the present invention include the detection of different types of molecules, more particularly biomolecules such as DNA, RNA, proteins, carbohydrates, lipids and organic anabolites or metabolites. The nature of the sample comprising the analyte to be detected is not critical and can be for instance any sample of a living or dead organism (body fluid such as, but not limited to blood or urine, hair, stool, etc.), an environmental sample (water, soil, plant material), food or feed products or products used in the manufacturing thereof, a sample of a chemical reaction process etc..

The detection can be performed on a sample which is the result of a pre-processing step such as a semi-purification, purification, semi-purification and/or amplification of the analyte. According to a particular embodiment, the analyte is a single stranded nucleotide sequence, which has been amplified using PCR.

Methods of the present invention provide for qualitative and/or quantitative detection of an analyte based on the reaction/binding of the analyte with an analyte-specific probe. Typical specific interactions include DNA/DNA or DNA/RNA binding, protein/protein, protein/DNA and protein/carbohydrate interactions, antibody/ antigen interactions, and receptor/ligand binding. Also synthetic molecules can be used to detect an analyte (e.g. enzyme inhibitors, pharmaceutical compounds, lead compounds isolated from library screenings). Accordingly, examples of analyte-specific probes include but are not limited to oligonucleotides, antibodies, enzyme substrates, receptor ligands, lectins etc.... In particular embodiments of the present invention interactions occurring at the binding surface are antigen-antibody interactions e.g. antibody probes linked to the binding surface which specifically bind antigens in a sample or antigens attached as probe to the binding surface which bind antibodies present in a sample.

According to particular embodiments, an analyte-specific probe is used which is an analyte-specific oligonucleotide, i.e. an oligonucleotide comprising a sequence which is complementary to a sequence specific for the analyte.

Different assay principles are envisaged in the detection methods of the invention. In particular embodiments detection is be based on competitive binding of the analyte to an analyte-specific probe.

In particular embodiments of such competitive assays, when the amount of analyte in the sample is low, the number of unbound particles is relative high and visa versa. By detecting the number of unbound particles, the signal becomes high if the amount of analyte in the sample is low and the signal becomes low if the amount of analyte in a sample is high.

For example, methods are envisaged whereby the binding surface is coated with an analyte-like compound. Analyte-specific probes labeled with magnetic particles are contacted with the binding surface and bind weakly to the analyte-like compound. Upon contacting with a sample comprising the analyte, the analyte binds strongly to the analyte-specific probe, which is displaced from the binding surface. The accumulation of magnetic particles (bound to the analyte-specific probe) in the detection region is proportionate with the concentration of analyte in the sample.

In alternative embodiments of a competitive assay, an isolated form of the analyte is bound to the binding surface, and a magnetically labeled analyte-specific probe (e.g. antibody) is mixed with the sample prior to contacting with the binding surface. Magnetically labeled antibodies which are not bound to the analyte in the sample, bind to the analyte on the binding surface. Magnetically labeled antibodies which bind the analyte in the sample can not bind to the analyte on the binding surface and are moved towards the detection surface upon application of the magnetic field(s). Accordingly, the more analyte is present in the sample, the more particles will accumulate at the detection region.

In further alternative embodiments of a competitive assay, analyte-specific probes are bound to the binding surface and magnetically labeled analyte-analog or analyte are added to the sample prior to contacting the sample with the binding surface.

In alternative embodiments, the detection and/or quantification method used is a direct detection method. For example, the analyte present in the sample is bound to magnetic particles (e.g. incorporation by PCR amplification) and an analyte-specific probe is linked to the binding surface. Optionally, unlabeled analyte is added to the sample. The signal is generated by (excess) labeled analyte which does not bind to the binding surface and accumulates at the detection region. This signal is proportionate to the presence (and/or amount) of analyte present in the sample. However, such methods require exact calibration of the amount of analyte-specific probe bound to the binding surface or of the amount of unlabeled analyte added and may be more cumbersome.

In particular embodiments, detection of the analyte is envisaged to require the addition of further reagents such as secondary antibodies, labels, substrates etc... More specifically for specific analytes (large molecules, e.g. proteins, possessing at least two epitopes) sandwich assays can be envisaged. In a sandwich assay, molecules of interest (proteins) from an applied sample fluid are trapped ('sandwiched') between a probe (a first antibody) on a biologically active binding surface and a biologically active molecule (a second antibody) with a label (a magnetic particle).

It will be clear to the skilled person that the above-described embodiments are not limitative to the invention and that further variations on the assays described above are envisaged.

In particular embodiments, methods according to the present invention comprise applying the sample to a system (1) comprising:

- a reaction chamber (2) having a binding surface (5) and at least one detection region (6) within the binding surface, whereby a first bioactive agent is bound to the binding surface (5) and
- at least one means for generating one or more magnetic fields (3), wherein the at least one means for a generating one or more magnetic fields are placed such that one of the magnetic fields has a gradient with a component that is parallel to the binding surface (5) and wherein the one or more magnetic fields are arranged to move unbound magnetic particles laterally over the binding surface (5) and to accumulate the particles in the detection region (6) within the binding surface;

Depending on the nature of the assay, a further method step involves contacting the sample and/or binding surface with a second bioactive agent, which is bound to a magnetic particle. This contacting step can take place prior to or simultaneously with the contacting of the sample with the system.

Further methods steps include allowing the sample, first and second bioactive agent to interact, generating the one or more magnetic fields to ensure movement of the fraction of magnetic particles not bound to the surface and detecting the fraction of magnetic particles accumulated in the detection region.

The nature of the first (and optional second) bioactive agent is determined by the type of detection to be performed. In particular embodiments, the first and second bioactive agents are analyte-specific probes, such that upon interaction of the sample with the first and second analyte-specific probes, the analyte present in the sample is sandwiched at the surface.

In more particular embodiments however, the detection is performed indirectly, based on competitive binding of the analyte in the sample with an analyte-specific probe. Accordingly, embodiments are envisaged wherein the first bioactive agent bound to the surface is an analyte-specific probe and labeled analyte is added to the sample. Alternatively, analyte or analyte analog is bound to the surface (first bioactive agent), labeled analyte-specific probe is contacted with the surface and sample is added thereto. Displaced labeled analyte-specific probe is moved towards the detection region when the magnetic field is generated.

Accordingly, particular embodiments of methods for detecting an analyte in a sample comprise:

a) Contacting the sample with an analyte-specific probe labeled with a magnetic particle

b) Applying the sample to a system (1) comprising:

- a reaction chamber (2) having a binding surface (5) and at least one detection region (6) within the binding surface, wherein said analyte or analyte-analog is bound to said binding surface (5)and

- at least one means for generating one or more magnetic fields (3), wherein the at least one means for a generating one or more magnetic fields are placed such that one of said magnetic fields has a gradient with a component that is parallel to said binding surface (5) and wherein the one or more magnetic fields are arranged to move unbound magnetic particles laterally over the binding surface (5) and to accumulate said particles in the detection region (6) within the binding surface;

c) allowing said analyte-specific probe to interact with the analyte or analyte analog on said binding surface

d) generating said one or more magnetic fields, and

e) detecting the fraction of said magnetic particles accumulated to the detection region.

The particles which are present at the detection region comprise those particles which did not bind to the bioactive agent on the binding surface and which are accumulated at the detection region as a result of the lateral movement.

It is noted that, as the detection region is comprised within the binding surface (as a part thereof), the detection region also comprises particles which bind to the bioactive agent present on the surface in the detection region. This however generates a background signal which can be taken into account.

The fraction of particles within the total number of particles detected in the detection region, which are unbound can be determined using calibration standards.

In methods described herein, instead of washing away unbound magnetic particles, unbound magnetic particles are used to determine the presence (and quantity) of the analyte.

Methods of the present invention involve the contacting of sample with magnetic particles (e.g. magnetically labeled analyte-specific probes or magnetically labeled analyte or analyte-analog) and the movement of particles within a reaction chamber. Magnetic particles can be part of a fluid reagent or of a dry reagent. Besides magnetic particles, the reagent can for example contain buffer salts, detergents, biomolecules that assist in the biological interactions, etc. These steps can be performed in a liquid, which is any liquid compatible with the reagents used (i.e. analyte, analyte-specific probe, label), such as

standard buffers, or minimally pre-treated or even pure sample (e.g. blood or saliva). Liquid can be introduced in the reaction chamber for rinsing purposes. Alternatively, the methods are performed with a minimum amount of liquid at the detection surface. In principle as a result of the application of a magnetic field magnetic particles present in the sample or on the binding surface are divided into a fraction bound specifically to the binding surface and a fraction which is moved towards the detection region.

Methods of the present invention further comprise a detection step, which allows determining qualitatively or quantitatively the magnetic particles present in the detection region, which is a (direct or indirect) measure for the amount of analyte in the sample. The detection step is ensured using one or more detection means as described above.

In particular embodiments, the magnetic properties of the magnetic particles are used to detect the presence and or quantity of magnetic particles accumulated in the detection region. In addition or alternatively, the detection of magnetic particles is performed visually based on optical properties of the particle or of labels, which are attached directly or indirectly to the magnetic particles (see above).

Other arrangements for embodying the invention will be obvious for those skilled in the art.

It is to be understood that although preferred embodiments, specific constructions and configurations, as well as materials, have been discussed herein for devices and methods according to the present invention, various changes or modifications in form and detail may be made without departing from the scope and spirit of this invention. The invention is illustrated by the Examples provided below which are to be considered for illustrative purposes only and the invention is not limited to the specific embodiments described therein.

EXAMPLES

Example 1: competition assay

A pilot experiment was performed using morphine as an analyte. Morphine is a small molecule, with only one epitope, so a competitive assay has to be performed to indicate the amount of morphine in a sample. On a polystyrene surface (96 wells titre plate), 1 µl of BSA-morphine (1 mg/ml in phosphate buffered saline (PBS)) was applied homogeneously on a well and dried [morphine-3-glucoronide was coupled in excess BSA via its lysine residues]. After coating, the wells were blocked with 10mg/ml BSA + 0.65%

Tween-20 in PBS for 1 hour. Then, the blocking solution was discarded and 10 mg/ml BSA + 0.65% Tween-20 in PBS containing anti-morphine Ab coated magnetic particles (200nm Protein G coated magnetic particles) were applied to the wells (1:10 dilution of magnetic particles, total amount of solution was 50 µl. The titer plate was placed on a magnet. After binding of the antibodies, a well of the plate was placed above two parallel electric wires. Through these wires an electric current was applied. After 15 seconds the presence of magnetic particles between the wires was detected with a GMR. In the absence of morphine all the magnetic particles with the antibody bind to the morphine on the plate.

When morphine (1 to 20 ng/ml in a microtiter well) was added to the antibody-coated magnetic particles prior to the application to the titre plate, a fraction of the antibodies were saturated with morphine and did not bind to the analyte. Upon application of the electric current through the wires, these magnetic particles accumulated between the wires where they were detected.

Example 2: Quantitative detection of morphine in a competition assay with lateral magnetic field.

Using the principle and device as described in Example 1, a dose-response curve was measured, where the concentration of morphine had been varied between 1 and 20 ng/ ml.

The results of this experiment are shown in Fig. 3. As can be seen in this Fig., even in the absence of morphine analyte in the sample, the presence of magnetic particles is measured, because a number of antibodies are bound to the morphine which is present at the binding region within the binding surface.

This background however does not interfere with the detection of small amount of analyte in the sample. Fig. 3 shows a clear difference in signal between 0 and 1 ng/ml of morphine in the sample. The accumulation of the beads with morphine from the sample bound to the antibodies, is sufficiently high to give a detectable signal on top of that of the antibodies bound to the binding region within the binding surface.

CLAIMS:

1. A method for quantifying and/or detecting an analyte in a sample, comprising the steps of:

a) contacting the sample with an analyte labeled with a magnetic particle or with an analyte-specific probe labeled with a magnetic particle,

b) applying the sample to a system (1) comprising

- a reaction chamber (2) having a binding surface (5) and at least one detection region (6) within the binding surface, whereby isolated analyte, analyte-analog or analyte-specific probe is bound to the binding surface (5) and

- at least one means for generating one or more magnetic fields (3), wherein the at least one means for generating one or more magnetic fields are placed such that one of the magnetic fields has a gradient with a component that is parallel to the binding surface (5) and wherein the one or more magnetic fields are arranged to move unbound magnetic particles laterally over the binding surface (5) and to accumulate the particles in the detection region (6) within the binding surface;

c) allowing the analyte-specific probe to interact with the analyte or analyte analog on the binding surface.

d) generating the one or more magnetic fields

e) detecting the fraction of magnetic particles accumulated in the detection region.

2. The method according to claim 1, wherein the gradient of said one or more magnetic fields further has a component that is orthogonal to said binding surface, such that said one or more magnetic fields move magnetic particles towards said binding surface (5).

3. The method according to 1 or 2, wherein detection step (e) comprises detecting a magnetic property of magnetic particles accumulated in the detection region.

4. The method according to claim 1 or 2, wherein said magnetic particles are labeled and detection step (e) comprises detecting the label of magnetic particles accumulated in the detection region.

5. The method according to any one of claims 1 to 4, wherein said analyte-specific probe is selected from the group consisting of an oligonucleotide, an antibody or fragment thereof, a lectin, a pharmaceutical compound, a peptide or a protein.

6. A system (1) comprising a reaction chamber (2) having a binding surface (5) and at least one detection region (6) within said binding surface and further comprising at least one means for generating one or more magnetic fields (3), wherein the at least one means for generating one or more magnetic fields are placed such that one of said magnetic fields has a gradient with a component that is parallel to said binding surface (5) and wherein the one or more magnetic fields are arranged to move unbound magnetic particles laterally over the binding surface (5) and to accumulate said particles in the detection region (6) within the binding surface.

7. The system according to claim 6, wherein said one or more means for generating a magnetic field comprise one or more electric wires located below the binding surface and parallel to the binding surface.

8. The system according to claim 6 or 7, further comprising a detection means capable of detecting said magnetic particles in said detection zone.

9. The system according to claim 8, wherein said detection means is a means for detection of a magnetic property of at least one magnetic particle.

10. The system according to claim 8, wherein said detection means is a means for detection of a label.

11. A cartridge comprising:

- a binding surface (5) and at least one detection region (6) within said binding surface, and

- at least one physical carrier for generating one or more magnetic fields (3), which is capable of generating a magnetic field which ensured the movement of unbound magnetic particles laterally over the binding surface (5) and accumulation of said particles in the detection region (6) within the binding surface.

12. The cartridge of claim 11, wherein said at least one physical carrier for generating one or more magnetic fields (3) comprises a metal wire located below the binding surface and parallel to the binding surface.

13. The cartridge of claim 11, wherein said at least one physical carrier for generating one or more magnetic fields (3) consists of two parallel metal wires located below the binding surface and parallel to the binding surface.

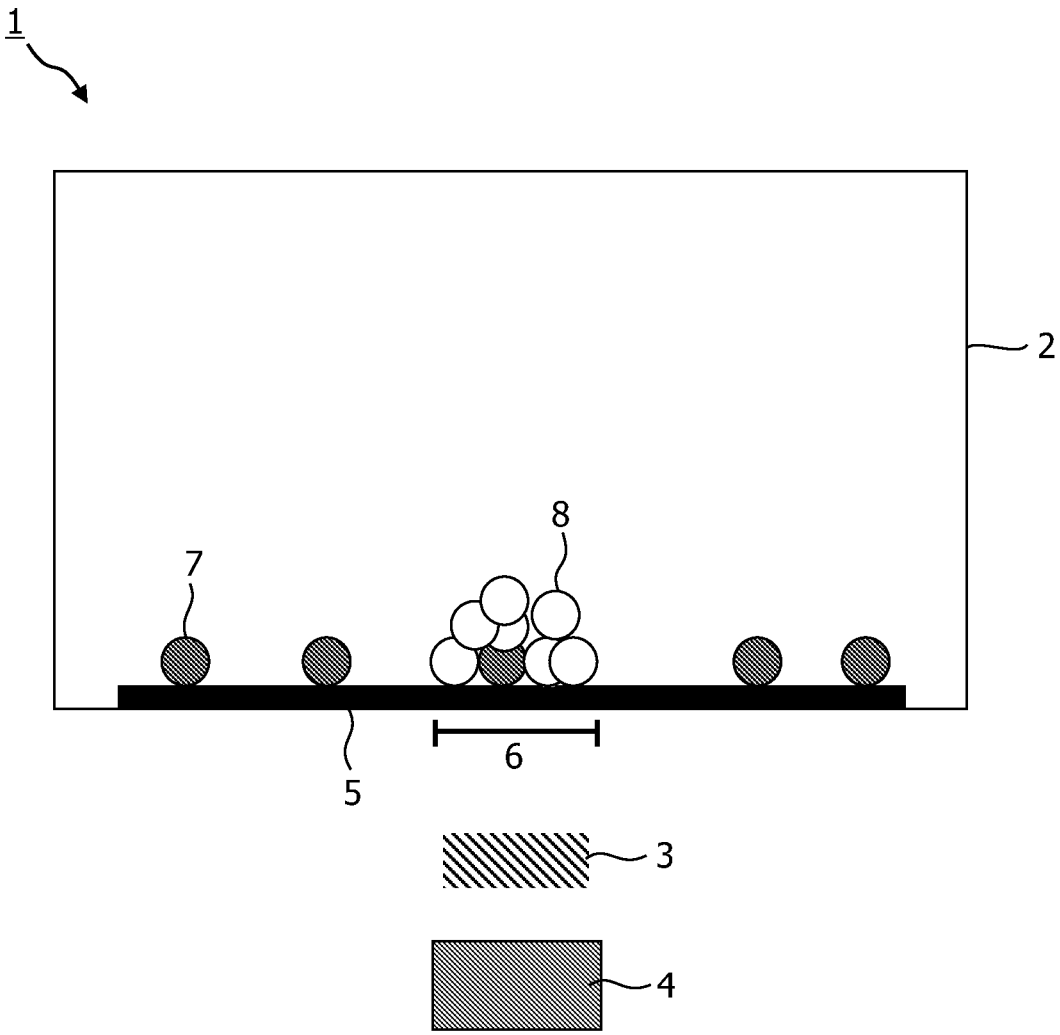

FIG. 1

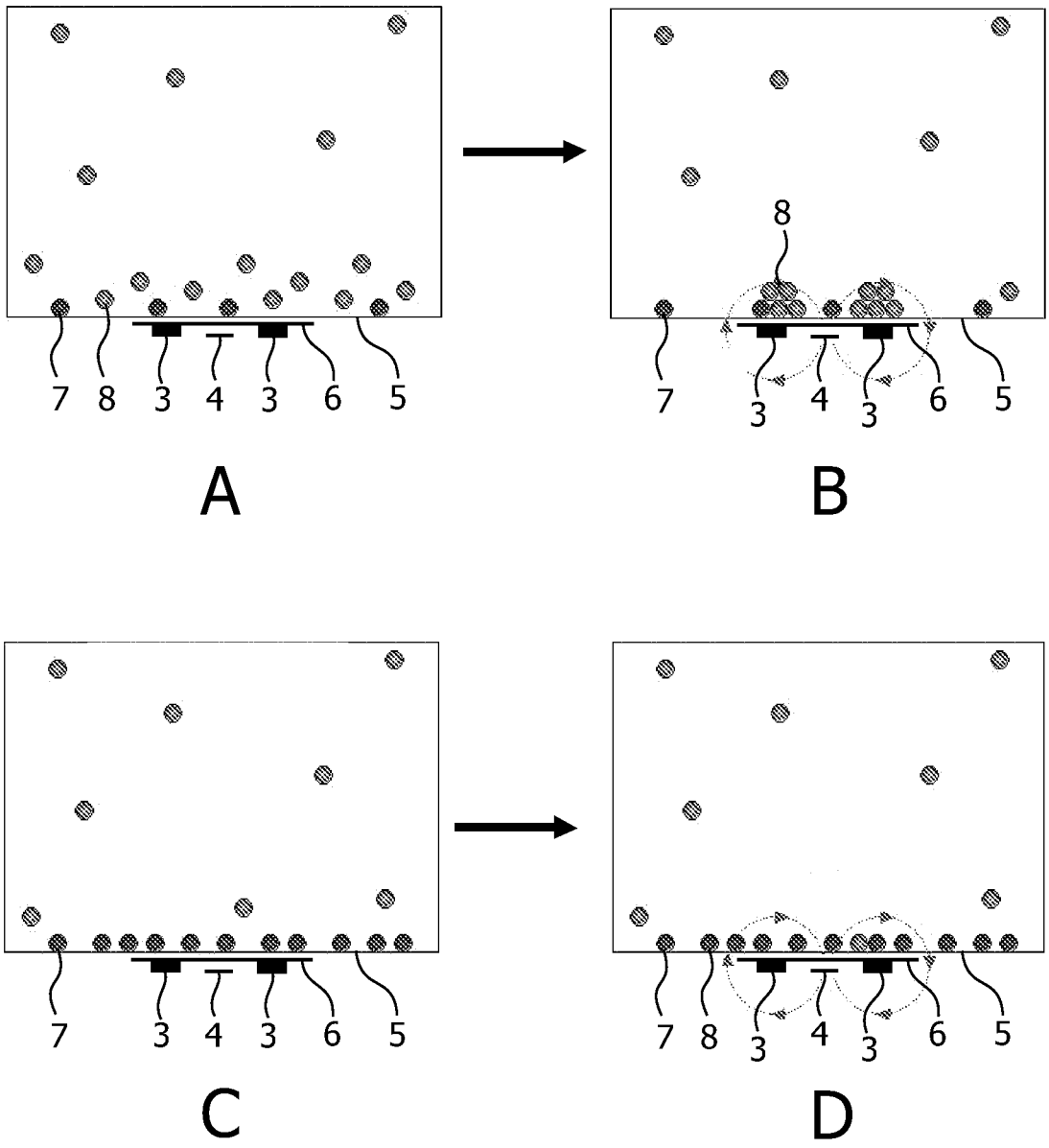

FIG. 2

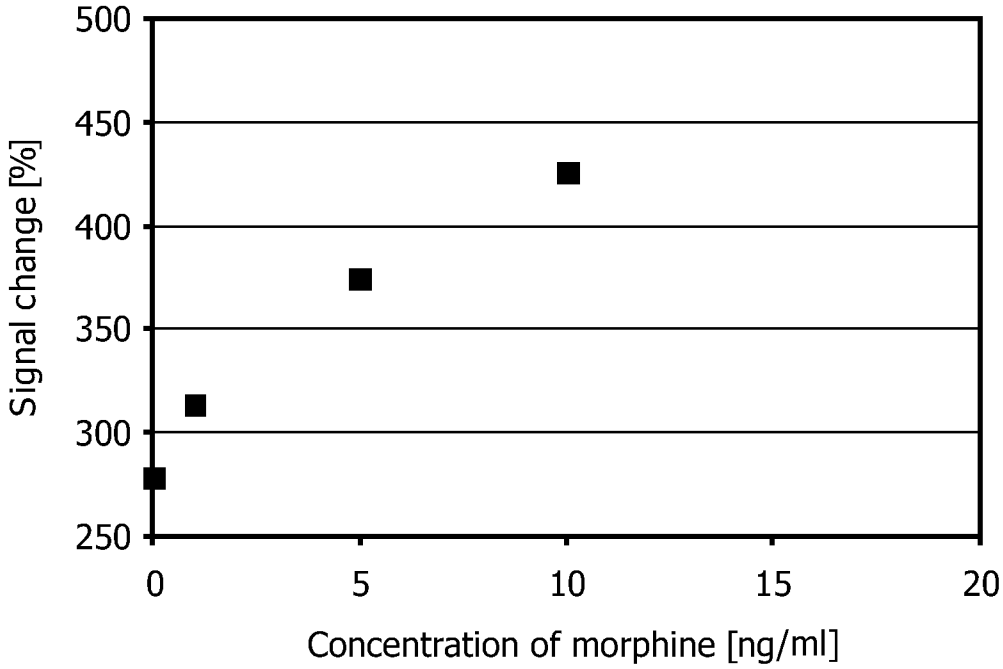
500
450
400
350
300
250
Signal change [%]
0
5
10
15
20
Concentration of morphine [ng/ml]

FIG. 3