

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
31 August 2006 (31.08.2006)

PCT

(10) International Publication Number  
**WO 2006/089572 A1**

(51) International Patent Classification:  
**G01N 33/543** (2006.01) **G01N 33/68** (2006.01)  
**A61K 9/50** (2006.01)

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(21) International Application Number:  
PCT/EP2005/013025

(22) International Filing Date:  
5 December 2005 (05.12.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
10 2005 009 518.6  
28 February 2005 (28.02.2005) DE

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

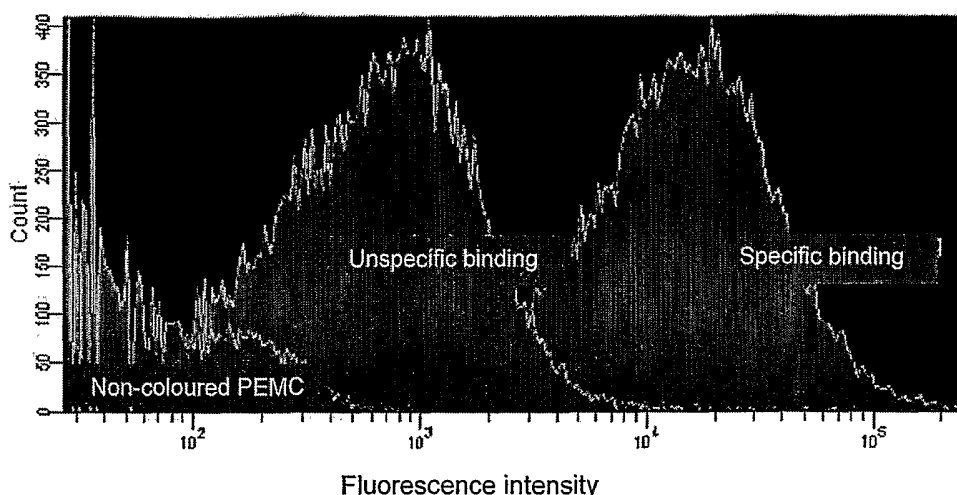
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: POLYELECTROLYTE CAPSULES FOR DETECTING ANTIGEN-ANTIBODY-REACTIONS



(57) Abstract: The present invention relates to a capsule with a shell of polyelectrolytes suitable to detect an antigen-antibody-reaction, the use of said capsule for detecting an antigen-antibody-reaction, and a method for detecting an antigen-antibody-reaction with said capsule.

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## 5 Polyelectrolyte capsules for detecting antigen-antibody-reactions

The present invention relates in general to the field of diagnostics, namely to a capsule suitable to detect an antigen-antibody-reaction. More precisely, the present invention relates to a capsule with a shell of polyelectrolytes suitable to detect an antigen-antibody-reaction using a gel matrix, the use of said capsule for detecting an antigen-antibody-reaction, and a method for detecting an antigen-antibody-reaction with said capsule.

## BACKGROUND OF THE INVENTION

15 In the last years many screening assays have been developed for detecting antibodies. These assays are either based on modified human or animal cells with a low lifetime or on spherical non-deformable particles. These spherical particles are not suitable for screening assays detecting antigen-antibody-reactions using a gel matrix. However, the development of the LBL-Technology® (the term "LBL" is derived from "layer-by-layer") provides a tool for making unique multifunctional capsules having a shell of polyelectrolytes, which differ from the known particles in respect to their permeability and deformability.

The detection and specification of different antibodies, for example, against antigens of erythrocytes, can be done with various methods, e.g. with a tube test. However, in this matter the use of a gel matrix for a so-called gel centrifugation test is of particular interest. In its use such a gel centrifugation test provides advantages compared to classical methods. The advantages are reasoned by a low consumption of cells and serum, a standardized evaluation and a higher sensitivity and specificity [Berber H., Lapierre Y., Hitzler W. et al.: Gel Centrifugation Test: A comparative study of a new method in red blood serology. First ISBT Regional Congress of the European Region, Lugano 7. – 10.5.1989, Arnette Verlag, 1989, 341-352; Lapierre Y., Rigal D. et al.: The gel test: A new way to detect red cell antigen-antibody reactions. Transfusion. 1990, 1: 109-133; Hitzler W.; Johanson C. et al.: Vergleichsstudie zur Antikörperidentifizierung im Gelzentrifugationstest (ID-Micro Typing System), Festphasen-Antiglobulintest (Solidscreen, Capture R., Ready ID) und Röhrchentest. Beitr. Infusionsther. 1992, 30: 370-374; Krethscmer V., Heuckeroth A. et al.: Superiority of gel centrifugation in antibody screening and identification. Infusion Therapy. 1992, 19: 226-

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Up to now the gel centrifugation tests are performed with native erythrocytes. These erythrocytes have a durability of approximately 30 days after which they need to be replaced by fresh erythrocytes. For that reason before each test the erythrocytes need to be tested regarding their test criteria.

The German patent application 198 12 083 describes a process for producing capsules with a diameter of  $<10\text{ }\mu\text{m}$ , also called microcapsules, where several consecutive layers of oppositely charged polyelectrolyte molecules are applied to an aqueous dispersion of template particles. The template particles described in this connection are, in particular, partially crosslinked melamine-formaldehyde particles. After formation of the polyelectrolyte shell it is possible to disintegrate the melamine-formaldehyde particles by adjusting an acidic pH or by sulfonation.

Another German patent application (DE 19907552) discloses a process for producing capsules with a polyelectrolyte shell, where several consecutive layers of oppositely charged polyelectrolyte molecules are applied to a template selected from aggregates of biological or/and amphiphilic materials, and the template is subsequently disintegrated where appropriate. These so-called biological templates are selected from biological cells, aggregates of biological or/and amphiphilic materials such as, for example, erythrocytes, bacterial cells or lipid vesicles. These encapsulated template particles can be removed by subsequent solubilization or disintegration.

An object of the present invention was to provide means that allow a higher longevity of antigen-antibody tests, especially gel centrifugation tests. Furthermore, it has been an object of the present invention to increase the sensitivity of antigen-antibody test, especially gel centrifugation tests.

It has been found, surprisingly, that capsules having a shell of polyelectrolytes are able to perambulate a gel matrix, by e.g. using centrifugation. This gel matrix can be used for screening assays detecting antigen-antibody-reactions. In addition, it has been found that these capsules are still able to perambulate a gel matrix, even if they are loaded with an antigen. Further, it has been found that adding antibodies to the capsules loaded with antigens, these capsules lost their ability to perambulate the gel matrix. The adding of antibodies to the capsules loaded with antigens first leads to an antigen-antibody-reaction followed by the aggregation of the capsules. These aggregates may subsequently be detected to indicate an antigen-antibody reaction having taken place.

Since erythrocytes, as already mentioned, have a short term durability of approximately 30 days, capsules with a polyelectrolyte shell provide a new tool in antigen-antibody tests, e.g. gel centrifugation tests. These capsules have a considerably longer shelf life, and therefore there is no need to test them regarding their test criteria and their suitability for the test before performing such test. In addition, these capsules are deformable and have a significant major contact surface. Further, the use of these capsules for detecting antibodies using a gel centrifugation test provides a higher sensitivity than traditional antigen-antibody tests, more specifically gel centrifugation tests according to the prior art.

Thus, this invention relates to a capsule with a shell of polyelectrolytes suitable to detect an antigen-antibody-reaction using a gel matrix.

## SUMMARY OF THE INVENTION

In one embodiment the present invention concerns a capsule with a shell of polyelectrolytes, wherein the surface of the capsule is further modified with at least one substance suitable to detect an antigen-antibody-reaction.

In another embodiment the present invention relates to the use of a capsule according to the present invention for detecting an antigen-antibody-reaction.

In a further embodiment the present invention concerns a method for detecting an antigen-antibody-reaction comprising the following steps of:

- a) providing a plurality of capsules according to the present invention, preferably in the form of a suspension or solution, wherein the surface of the capsules comprise at least one antigen- and/or antibody;
- b) loading a portion of the capsules provided in a) on a first gel-matrix;
- 5 c) centrifuging the loaded first gel-matrix;
- d) incubating a portion of the capsules provided in a) with antibodies and/or antigens;
- e) loading the capsules provided in d) on the first or on a second gel-matrix;
- f) centrifuging the loaded first or the second gel-matrix; and

## 10 DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

Before the present invention is described in more detail below, it is to be understood that this invention is not limited to the particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the  
15 purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

20 Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integer or step.

25 Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, etc.), whether supra or infra, are hereby incorporated by reference in their entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

30

As outlined above there is a need in the prior art to provide new capsules applicable for an screening assay using a gel matrix for detecting an antigen-antibody-reaction. There is also a need in the art for tests for detecting an antigen-antibody reaction, which tests have a longer shelf-life and a higher sensitivity than tests from the prior art.

In a first aspect the present invention provides a capsule with a shell of polyelectrolytes, wherein the surface of the capsule is further modified with at least one substance suitable to detect an antigen-antibody-reaction.

5

In one embodiment of the capsule according to the present invention the substance suitable to detect an antigen-antibody-reaction is at least one antigen and/or antibody.

10

In a preferred embodiment according to the present invention the antibody and/or antigen is labelled with fluorescence dyes, radioactive isotopes, or nanoparticles, such as nanomagnets. Preferably these fluorescence dyes are selected from the group comprising 7-aminoactinomycine d (7-AAD), acridine orange, alexa dyes, amino methylcoumarine-acetate (AMCA), Cy-dyes, e.g. Cy2, Cy3, Cy5, Cy7, 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI), fluorescein isothiocyanate (FITC), Hoechst-dyes, e.g., Hoechst 33342, Hoechst 15 33258, "blue" fluorescence, 5,5',6,6'-Tetrachloro-1,1',3,3'-tetraethyl-benzimidazolylcarbocyanine jodide (JC-1), peridinin-chlorophyll-protein (PerCP), propidium jodide, rhodamine Red-X, phycoerythrin (R-PE), Aminomethylcoumarin acetate sulfonyl chloride rhodamine -derivate (Texas-Red), and Tetramethyl rhodamine isothiocyanate (TRITC).

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In another embodiment of the capsule according to the present invention the antibody is monoclonal or polyclonal.

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In a preferred embodiment of the capsule according to the present invention the shell consists of alternating layers of oppositely charged polyelectrolytes. Herein polyelectrolytes mean in general polymers with groups which are capable of ionic dissociation and may be a constituent or substituent of the polymer chain. The number of these groups capable of ionic dissociation in polyelectrolytes is normally so large that the polymers are water-soluble in the dissociated form (also called polyions). In this connection, the term poly-electrolytes also 30 means ionomers in which the concentration of ionic groups is insufficient for water solubility but which have sufficient charges to enter into self-assembly. The shell preferably comprises "true" polyelectrolytes. Depending on the nature of the groups capable of dissociation, polyelectrolytes are divided into polyacids and polybases.

On dissociation of polyacids there is formation of polyanions, with elimination of protons, which can be both inorganic and organic polymers. Examples of polyacids are polyphosphoric acid, polyvinylsulfuric acid, polyvinylsulfonic acid, polyvinylphosphonic acid and polyacrylic acid. Examples of the corresponding salts, which are also referred to as polysalts, are

5 polyphosphate, polysulfate, polysulfonate, polyphosphonate and polyacrylate

Polybases contain groups able to take up protons, for example by reaction with acids to form salts. Examples of polybases with groups capable of dissociation located on the chains or laterally are polyallylamine, polyethyleneimine, polyvinylamine and polyvinylpyridine.

10 Polybases form polycations by taking up protons

Polyelectrolytes suitable according to the present invention are both biopolymers such as, for example, alginic acid, gum arabic, nucleic acids, pectins, proteins and others, and chemically modified biopolymers such as, for example carboxymethylcellulose and ligninsulfonates, and

15 synthetic polymers such as, for example, polymethacrylic acid, polyvinylsulfonic acid, polyvinylphosphonic acid and polyethyleneimine.

It is possible to employ linear or branched polyelectrolytes. The use of branched polyelectrolytes leads to less compact polyelectrolyte multifilms with a higher degree of

20 porosity of the walls. To increase the capsule stability it is possible to crosslink polyelectrolyte molecules within and/or between the individual layers, for example by crosslinking amino groups with aldehydes. A further possibility is to employ amphiphilic polyelectrolytes, for example amphiphilic block or random copolymers with partial polyelectrolyte characteristics to reduce the permeability to small polar molecules. Such

25 amphiphilic copolymers consist of units differing in functionality, for example acidic or basic units on the one hand, and hydrophobic units on the other hand, such as styrenes, dienes or siloxanes etc., which can be arranged as blocks or randomly distributed over the polymer. It is possible by using copolymers which change their structure as a function of the external conditions to control the permeability or other properties of the capsule walls in a defined

30 manner. Suitable examples thereof are copolymers with a poly-(N-isopropylacrylamide) content, for example poly-(N-isopropylacrylamide-acrylic acid), which change their water solubility as a function of the temperature, via the hydrogen bonding equilibrium, which is associated with swelling.

There are in principle no restrictions on the polyelectrolytes or ionomers to be used as long as the molecules used have a sufficiently high charge or/and have the ability to enter into a linkage with the underlying layer via other interactions such as, for example, hydrogen bonding and/or hydrophobic interactions

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Suitable polyelectrolytes are thus both low molecular weight polyelectrolytes or polyions and macromolecular polyelectrolytes, for example also polyelectrolytes of biological origin.

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In a preferred embodiment of the capsule according to the present invention the shell comprises at least three layers.

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In one preferred embodiment according to the present invention at least one layer comprises a substance suitable to detect an antigen-antibody-reaction, preferably a plurality number of layers comprise a substance suitable to detect to provide a multiplex detection system.

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In one embodiment of the capsule according to the present invention the capsule further comprises a template of a material selected from inorganic, biological and/or amphiphilic materials, wherein said template preferably being located in the interior of said shell and/or being covered by said shell.

25

In a preferred embodiment of the capsule according to the present invention the biological and/or amphiphilic materials are selected from the group comprising biological cells, biological cell aggregates, biological subcellular particles, virus particles, aggregates of biomolecules, vesicles, micelles, and lipid aggregates. Examples of biological or/and amphiphilic materials which can be used as a template are biological cells, for example eukaryotic cells, such as, for example, mammalian erythrocytes or plant cells, single-cell organisms such as, for example, yeasts, bacterial cells such as, for example, E.coli cells, cell aggregates, subcellular particles such as, for example, cell organelles, pollen, membrane preparations or cell nuclei, virus particles and aggregates of biomolecules, for example protein aggregates such as, for example, immune complexes, condensed nucleic acids, ligand-receptor complexes etc. This process is also suitable for encapsulating living biological cells and organisms. Likewise suitable as templates are aggregates of amphiphilic materials, in particular membrane structures such as, for example, vesicles, for example liposomes or micelles, and other lipid aggregates.

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In a preferred embodiment of the capsule according to the present invention the inorganic materials are selected from the group comprising ceramic particles, e.g. oxidic ceramic particles, such as silicon dioxide, titanium dioxide, zirconium dioxide optionally doped with  
5 other metal oxides, magnetic particles such as iron oxide-containing particles, magneto-optical particles, nitridic ceramic particles, e.g.  $\text{Si}_3\text{N}_4$ , carbidic ceramic particles, metallic particles, e.g. gold, silver, palladium and sulfur or selenide-containing particles such as cadmium sulfide, cadmium selenide etc. Especially preferred are oxidic ceramic particles such as silicon dioxide.

10 In a further embodiment of the capsule according to the present invention the template has been used for fabricating said capsule but has subsequently been removed to create a cavity within said capsule. For the application of the polyelectrolyte layers to the template there is preferably firstly production of a dispersion of the template particles in an aqueous solution. A  
15 polyelectrolyte species with the same or the opposite charge as the surface of the template particle is then added to this dispersion. After removal of any excess polyelectrolyte molecules present, the oppositely charged polyelectrolyte species used to build up the second layer is added. Subsequently there are further alternate applications of oppositely charged layers of polyelectrolyte molecules, it being possible to choose for each layer with the same  
20 charge identical or different polyelectrolyte species or mixtures of polyelectrolyte species.

After application of the required number of layers, the enveloped template particles can--if desired--be disintegrated. The disintegration can take place by adding lytic reagents. Lytic reagents suitable in this case are those able to disintegrate biological materials such as  
25 proteins or/and lipids. The lytic reagents preferably contain a deproteinizing agent, for example peroxo compounds such as, for example,  $\text{H}_2\text{O}_2$  or/and hypochlorite compounds such as, for example, sodium or potassium hypochlorite. The disintegration of the template particles surprisingly takes place within a short incubation time, for example 1 min to 1 h at room temperature. The disintegration of the template particles is substantially complete  
30 because even on examination of the remaining shells under the electron microscope there are no longer any residues of the particles detectable. On incorporation of biological polyelectrolytes into the shell it is also possible to produce empty layers within the polyelectrolyte shell.

In one embodiment of the capsule according to the present invention in dependence of the used gel matrix the capsule has a diameter of between 0.1  $\mu\text{m}$  and 15  $\mu\text{m}$ , preferably of between 1  $\mu\text{m}$  and 10  $\mu\text{m}$ .

- 5 In another embodiment of the capsule according to the present invention the surface of the capsule is further modified with additional substances selected from the group comprising enzymes, nanoparticles, nanomagnets, colloidal nanoparticles, molecule complexes and aggregates, emulsions, liposomes, vesicles, layer-by-layer-particles, and cell constituents.
- 10 In a further embodiment of the capsule according to the present invention the capsule further comprises a coating.

In a preferred embodiment of the capsule according to the present invention, the coating comprises polyelectrolytes or lipids or both of them.

15

In a preferred embodiment of the capsule according to the present invention the substance suitable to detect an antigen-antibody-reaction is at least one antibody.

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In another preferred embodiment of the capsule according to the present invention the substance suitable to detect an antigen-antibody-reaction is at least one antigen.

25

In a further embodiment of the capsule according to the present invention the substance suitable to detect an antigen-antibody-reaction is at least one antibody and at least one antigen.

In one embodiment of the capsule according to the present invention the surface of the capsule is further modified by staining with a substance selected from the group comprising dyes of different arts, isotopes and magnetic substances.

30

In a preferred embodiment the dyes of different arts are fluorescence dyes selected from the group comprising 7-aminoactinomycine d (7-AAD), acridine orange, alexa dyes, amino methylcoumarine-acetate (AMCA), Cy-dyes, e.g. Cy2, Cy3, Cy5, Cy7, 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI), fluorescein isothiocyanate (FITC), Hoechst-dyes, e.g., Hoechst 33342, Hoechst 33258, "blue" fluorescence, 5,5',6,6'-Tetrachloro-1,1',3,3'-tetraethyl-

benzimidazolylcarbocyanine iodide (JC-1), peridinin-chlorophyll-protein (PerCP), propidium iodide, rhodamine Red-X, phycoerythrin (R-PE), Aminomethylcoumarin acetate sulfonyl chloride rhodamine -derivate (Texas-Red), and Tetramethyl rhodamine isothiocyanate (TRITC).

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In a preferred embodiment of the capsule according to the present invention the density of polyelectrolytes in the shell of the capsule is modified by a substance selected from the group comprising macromolecules, charged or un-charged polymers, and nanoparticles, wherein the nanoparticles having a diameter of between 5 to 100 nm.

10

In a second aspect the present invention relates to the use of a capsule according to the present invention for detecting an antigen-antibody-reaction.

15

In one embodiment of the use of said capsule according to the present invention the capsule is loaded onto a gel-matrix.

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In another embodiment of the use of said capsule according to the present invention the capsule is centrifuged through a gel-matrix. In this context, the term "centrifugation" is meant to include and signify any process which allows a directional movement of said capsule through said gel matrix, including but not limited to processes wherein an external force is applied to said capsule, such as centrifugation by spinning, application of gravitational forces, application of an electrical field, and application of an magnetic field.

25

In one embodiment of the use of said capsule according to the present invention the capsule is used for any test suitable to detect the agglutination of particles, e.g., micro titer plates or light scatter methods. Such agglutinated particles may e.g. be agglutinated antigen-antibody complexes.

30

In a third aspect the present invention concerns a method for detecting an antigen-antibody-reaction comprising the following steps of:

- a) providing a plurality of capsules according to the present invention, preferably in the form of a suspension or solution, wherein the surface of the capsules comprise at least one antigen- and/or antibody;
- b) loading a portion of the capsules provided in a) on a first gel-matrix;

- c) centrifuging the loaded first gel-matrix;
- d) incubating a portion of the capsules provided in a) with antibodies and/or antigens;
- e) loading the capsules provided in d) on said first or on a second gel-matrix;
- f) centrifuging said loaded first or said second gel-matrix; and
- 5 g) comparing the centrifuged gel matrices of c) and f).

The term "centrifuging", as used herein, is used in the same manner as outlined previously.

10 In one embodiment of the method according to the present invention, the method comprises an additional step h) determining whether an antigen-antibody-reaction has taken place by comparing the location of the capsules on the gel matrix after step c) with the location of the capsules on the gel matrix after step f).

15 In another embodiment of the method according to the present invention, the antibodies and/or antigens in step d) are labelled with fluorescence dyes, radioactive isotopes, or nanoparticles, such as nanomagnets. Preferably these fluorescence dyes are selected from the group comprising 7-aminoactinomycine d (7-AAD), acridine orange, alexa dyes, amino methylcoumarine-acetate (AMCA), Cy-dyes, e.g. Cy2, Cy3, Cy5, Cy7, 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI), fluorescein isothiocyanate (FITC), Hoechst-dyes, e.g.,  
20 Hoechst 33342, Hoechst 33258, "blue" fluorescence, 5,5',6,6'-Tetrachloro-1,1',3,3'-tetraethyl-benzimidazolylcarbocyanine jodide (JC-1), peridinin-chlorophyll-protein (PerCP), propidium jodide, rhodamine Red-X, phycoerythrin (R-PE), Aminomethylcoumarin acetate sulfonyl chloride rhodamine -derivate (Texas-Red), and Tetramethyl rhodamine isothiocyanate (TRITC).

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In a preferred embodiment of the method according to the present invention the gel matrices in steps b) and e) are provided in one or several devices suitable to be centrifuged, preferably a tube(s).

30 The following example illustrate the present invention without, however, limiting the same thereto.

Furthermore, reference is made to the figures wherein:

**Figure 1:** Shows the fluorescence signals of capsules loaded only with antigens (unspecific binding) and capsules having undergone an antigen-antibody-reaction (specific binding). Unlabelled capsules are free of antigens and antibodies, respectively.

5 **Figure 2:** Shows the location of the capsules loaded with antigens after centrifugation through a gel matrix, here on a so-called gel test card. Left: The capsules only loaded with antigens pass the gel matrix unhamperedly and accumulate on the bottom. Right: Capsules having an antigen-antibody-reaction forming aggregates. Since these aggregates are not soluble during centrifugation they cannot pass the gel matrix, thus, they accumulate on top of the gel matrix  
10 or in upper parts thereof.

### EXAMPLE

#### a) Loading of a capsule having a shell of polyelectrolytes with antigens

15 A suspension of a plurality of capsules having a shell of polyelectrolytes were washed and centrifuged in a physiological phosphate buffer (PBS, Sigma). The supernatant was removed. Bovine IgG (0.6 mg/ml) was added to said capsules and incubated for 20 minutes at 4°C. Then the capsules loaded with IgG were washed three times in PBS. Single capsules loaded with antigen were available (Figure 1: unspecific binding).

#### b) Centrifuging of the gel matrix

20 A portion of the capsules loaded with antigens provided in step a) were centrifuged through a gel matrix and accumulated at the lower region of said gel matrix (Figure 2: left column).

#### c) Antigen-antibody-reaction at a capsule having a shell of polyelectrolytes

25 Anti-IgG (0.3 mg/ml) labelled with FITC was incubated for 20 minutes on ice without any light exposure with a portion of the capsules provided in step a). Aggregates were formed by the specific binding of the antibodies to the antigens. The binding of the antibody was proved with FACSCanto by the company BectonDickson (Figure 1: specific binding)

#### d) Centrifuging of the capsules having aggregates of antigens and antibodies in a gel matrix

30 Since the antigen-antibody-reaction leads to the formation of aggregates of the capsules, centrifuging of said capsules resulted in the accumulation of said capsules on the top of the gel matrix (Figure 2: right).

### Claims

1. A capsule with a shell of polyelectrolytes, wherein the surface of said capsule is further modified with at least one substance suitable to detect an antigen-antibody-reaction.
2. Capsule according to claim 1, wherein said substance suitable to detect an antigen-antibody-reaction is at least one antigen and/or antibody.
3. Capsule according to claim 2, wherein said antibody and/or antigen is labelled with fluorescence dyes, radioactive isotopes, or nanoparticles.
4. Capsule according to claim 2 or 3, wherein said antibody is monoclonal or polyclonal.
5. Capsule according to any of claims 1 to 4, wherein said shell consists of alternating layers of oppositely charged polyelectrolytes.
6. Capsule according to claim 5, wherein said polyelectrolytes are polyacids or polybases.
7. Capsule according to claim 5 or 6, wherein said shell comprises at least three layers of polyelectrolytes.
8. Capsule according to any of claims 1 to 7, wherein at least one layer comprises a substance suitable to detect an antibody-antigen-reaction.
9. Capsule according to any of claims 1 to 8, wherein said capsule further comprises a template of a material selected from inorganic, biological and amphiphilic materials, wherein said template preferably being located in the interior of said shell and/or being covered by said shell.
10. Capsule according to claim 9, wherein said biological and/or amphiphilic materials are selected from the group comprising biological cells, biological cell aggregates, bio-

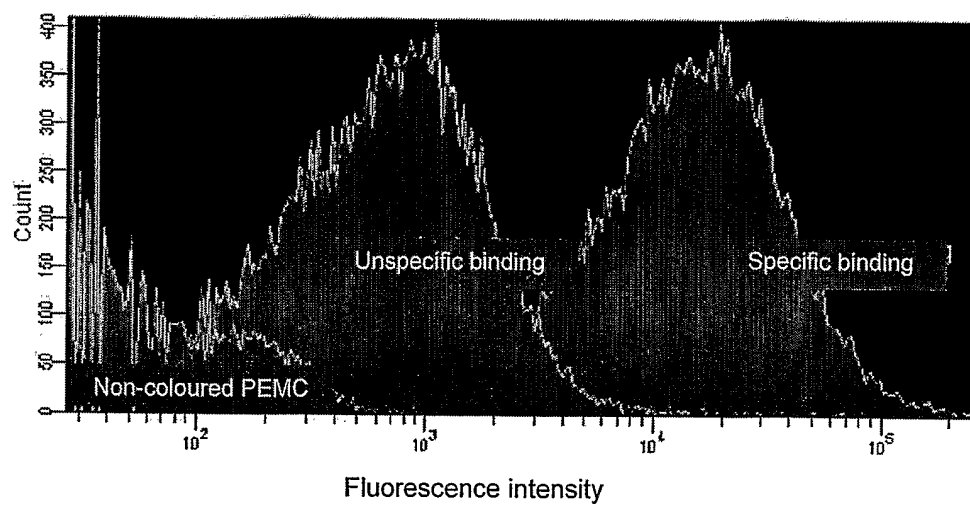
logical subcellular particles, virus particles, aggregates of biomolecules, vesicles, micelles, and lipid aggregates.

11. Capsule according to claim 9, wherein said inorganic materials are selected from the group comprising ceramic and metal particles.
12. Capsule according to any of claims 9 to 11, wherein said template has been used for fabricating said capsule but has subsequently been removed to create a cavity within said capsule.
13. Capsule according to any of claims 1 to 12, wherein said capsule has a diameter of between 0.1  $\mu\text{m}$  and 15  $\mu\text{m}$ .
14. Capsule according to any of claims 1 to 13, wherein the surface of said capsule is further modified with additional substances selected from the group comprising enzymes, nanoparticles, nanomagnets, colloidal nanoparticles, molecule complexes and aggregates, emulsions, liposomes, vesicles, layer-by-layer-particles, and cell constituents.
15. Capsule according to any of claims 1 to 14, wherein said shell further comprises a coating.
16. Capsule according to claim 15, wherein said coating comprises polyelectrolytes, lipids or both.
17. Capsule according to any of claims 1 to 16, wherein said substance suitable to detect an antigen-antibody-reaction is at least one antibody.
18. Capsule according to any of claims 1 to 16, wherein said substance suitable to detect an antigen-antibody-reaction is at least one antigen.
19. Capsule according to any of claims 1 to 16, wherein said substance suitable to detect an antigen-antibody-reaction is at least one antibody and at least one antigen.
20. Capsule according to any of claims 1 to 19, wherein the surface of said capsule is fur-

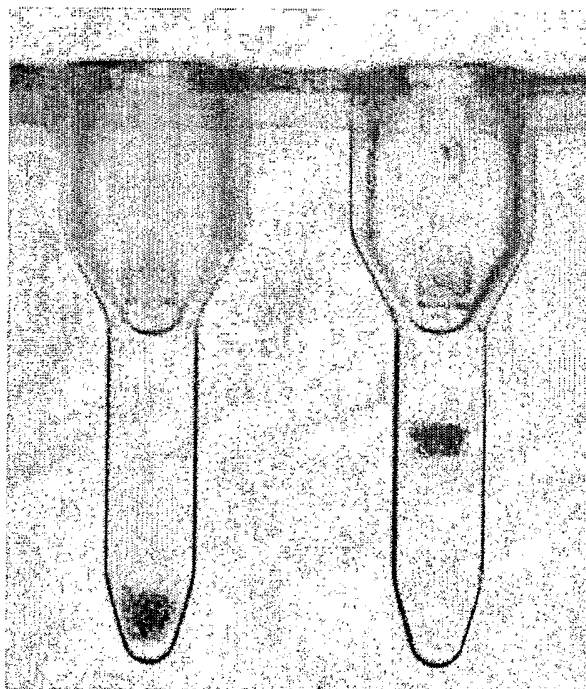
ther modified by staining with a substance selected from the group comprising dyes of different arts, isotopes and magnetic substances.

21. Capsule according to any of claims 1 to 20, wherein the density of polyelectrolytes in said shell of said capsule is modified by a substance selected from the group comprising macromolecules, charged or un-charged polymers, and nanoparticles, wherein said nanoparticles have a diameter of between 5 to 100 nm.
22. Use of a capsule according to any of claims 1 to 21 for detecting an antigen-antibody-reaction.
23. Use according to claim 22, wherein said capsule is loaded onto a gel-matrix.
24. Use according to any of claims 22 - 23, wherein said capsule is centrifuged through a gel-matrix.
25. Use of a capsule according to any of claims 1 to 21 for any test suitable to detect the agglutination of particles, such as agglutinated antigen-antibody-complexes.
26. Method for detecting an antigen-antibody-reaction comprising the following steps of:
  - a) providing a plurality of capsules according to any of claims 1 to 21, preferably in the form of a suspension or solution, wherein the surface of said capsules comprise at least one antigen- and/or antibody;
  - b) loading a portion of the capsules provided in a) on a first gel-matrix;
  - c) centrifuging the loaded first gel-matrix;
  - d) incubating a portion of the capsules provided in a) with antibodies and/or antigens;
  - e) loading the capsules provided in d) on said first or on a second gel-matrix;
  - f) centrifuging said loaded first or said second gel-matrix; and
  - g) comparing the centrifuged gel matrices of c) and f).
27. Method according to claim 26, comprising an additional step h) determining whether an antigen-antibody-reaction has taken place by comparing the location of the capsules on the gel matrix after step c) with the location of the capsules on the gel matrix after step f).

28. Method according to claims 26 or 27, wherein the antibodies and/or antigens in step d) are labelled with fluorescence dyes, radioactive isotopes, or nanoparticles.
29. Method according to any of claims 22 to 28, wherein the gel matrices in steps b) and e) are provided in one or several devices suitable to be centrifuged, preferably a tube(s).

**Figure 1**

**Figure 2**



## INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2005/013025

## A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/543 A61K9/50 G01N33/68

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 A61K

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 practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, INSPEC

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                    | Relevant to claim No. |
|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
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☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

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Date of the actual completion of the international search

18 April 2006

Date of mailing of the international search report

12/05/2006

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## C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                       | Relevant to claim No. |
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Information on patent family members

International application No

PCT/EP2005/013025

| Patent document<br>cited in search report |   | Publication<br>date | Patent family<br>member(s)                                                                                                                                          | Publication<br>date                                                                                                                      |
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