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(54) **BIOSENSOR AND BLOOD COMPONENT ANALYZING METHOD**

BIOSENSOR UND VERFAHREN ZUM ANALYSIEREN VON BLUTKOMPONENTEN

BIOCAPTEUR ET PROCEDE D'ANALYSE DE COMPOSANTES DU SANG

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(73) Proprietor: **MATSUSHITA ELECTRIC INDUSTRIAL CO., LTD**
Kadoma-shi, Osaka 571-8501 (JP)

(72) Inventors:
- **TAKAHASHI, Mie**
  Niihama-shi, Ehime 792-0026 (JP)
- **NADAOKA, Masataka**
  Iyo-shi, Ehime 799-3113 (JP)
- **TANAKA, Hirotaka**
  Matsuyama-shi, Ehime 791-1102 (JP)
- **KITAWAKI, Fumihisa**
  Kadoma-shi,
  Osaka 571-0064 (JP)

(74) Representative: **Balsters, Robert et al**
Novagraaf International S.A.
25, avenue du Pailly
1220 Les Avanchets - Geneva (CH)

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Description

Technical Field

[0001] The present invention relates to a biosensor for analyzing blood components and a blood component analytical method and, more particularly, to a biosensor that is less affected by cell components such as hemocytes, as well as enables simple and quick measurement with high accuracy using a very small quantity of samples, and a blood component analytical method.

Background Art

[0002] Biochemical tests for blood are widely carried out as means for diagnosing the conditions of human's health. It is difficult to measure the kinds or concentrations of constitutive components in blood, such as metabolites, proteins, lipids, electrolytes, enzymes, antigens and antibodies, using whole blood, and thus they are normally measured using plasma or serum which is obtained by centrifuging whole blood as a sample.

[0003] The centrifugation requires times and efforts, and a centrifugation method requiring a centrifuge is unsuitable for cases where a particularly small quantity of samples are to be processed urgently or for on-site tests. Further, the quantity of serum or plasma obtained by the centrifugation is smaller than the quantity of blood which has been obtained by blood collection.

[0004] Thus, as blood component analytical methods which are not affected by cell components such as hemocytes even when whole blood is used as a sample, blood component analytical methods such as those disclosed in Japanese Published Patent Applications No. Sho.57-53661 and No. Hei.8-54387, employing a hemocyte separation method by which blood is exuded using glass fiber filter paper having an average diameter of 0.2-5 μm and a density of 0.1-0.5 g/cm^3, thereby separating plasma or serum, or a blood component analytical method such as one disclosed in Japanese Published Patent Application No. Hei.9-196908, employing a blood regulation method by which an aqueous solution of amino acid or inorganic salt is mixed with whole blood and thereafter hemocyte components are filtered, thereby avoiding clogging of filtered materials due to hemocytes, and a larger quantity of plasma or serum components are obtained using a smaller quantity of blood have been examined.

[0005] Further, a method as disclosed in Japanese Published Patent Application No. Hei.9-72904, by which blood is hemolyzed by a surfactant carried on a test strip and then a sample solution is developed on the test strip using a development solution has been examined.

[0006] It is true that the method employing the glass fiber filter paper of the prescribed density improves the hemocyte separation efficiency, but it takes quite a long time to separate hemocytes almost completely. Thus, the measurement cannot be performed quickly, and further not only a large quantity of blood is required to obtain a quantity of samples that is necessary for the test, but also the blood viscosities or hematocrit values differ among individuals so that the separation power has individual differences and thus the measurement accuracy is quite low. Further, it requires special filter paper, thereby being costly.

[0007] In the method by which an aqueous solution of the prescribed concentration of mineral salt or amino acid is added to whole blood and then hemocyte components are filtered, the efforts for hemocyte separation by the centrifugation are omitted, but the operation of previously adding an additive liquid to the blood to be processed is so complicated that it lack the simplicity as well as the measurement takes much time.

[0008] Further, in the method of previously hemolyzing blood, the basic principle of hemolysis is destroying a bilayer lipid membrane in the cell membrane of the hemocyte using a surfactant or a hemolyzer such as saponin, and crushing a hemocyte cell to pieces. In the case of a very small quantity of blood, the development solution is added after the hemolysis is performed, thereby enabling development on a chromatographic device. However, when the development solution is not used, the development layer is clogged with cell pieces, so that the collected blood sample cannot be developed without the development solution.

[0009] The present invention is made to solve the above-mentioned problems and has for its object to provide a one-step biosensor and a blood component analytical method, which can perform a high-accuracy blood component analytical method easily and quickly, with less expenses and without the need to previously carry out any processing for blood or the need for a development solution for developing a sample solution, even When a sample is whole blood.

[0010] EP-A-1 143 247, which is a prior art under Art. 54 (3) and (4) EPC, discloses an immunochromatographic specimen comprising a cell component destroying substance holding portion for destroying cell components between a sample addition portion and a marker holding portion, destroying cell components at analyzing a test target solution which includes cell components, i.e. at chromatographically developing, and thereby, enabling smooth penetration into a reaction layer.

[0011] EP-A-P 903 584 discloses an immunochromatographic test strip including a detergent, an ammonium salt and a buffer which act as a sample solubilizer, such as to facilitate flow of the sample through the strip.

Disclosure of the Invention

[0012] According to the present invention (Claim 1), there is provided a biosensor that is made of a single layer or plural layers of a porous material, having a reagent holding part for holding a reagent that labels an analysis target in the liquid specimen, and analyzing components in the liquid specimen by utilizing chromatography,

in which a cell shrinkage reagent which osmotically shrinks cells is carried on at least part of a region from a specimen application part on which the liquid specimen is applied to the reagent holding part, and a reaction layer is provided, on which the cells in the liquid specimen are shrunk by the cell shrinkage reagent, and the shrunk cell components are developed to chromatographically downstream side together with the liquid specimen.

[0013] According to the biosensor that is constituted as described above, cells in an added liquid specimen shrink without being destroyed by contact with the cell shrinkage reagent, and the shrunk cells are developed together with the liquid specimen toward chromatographically downstream. Further, the cells can penetrate a chromatographic carrier efficiently and sufficiently without adding a development solution, whereby the quantity of liquid flowing chromatographically downstream is increased. Accordingly, even when whole blood or a bacteria solution is a sample, a high-accuracy analysis can be performed easily and quickly with less cost. The cell shrinkage described here refers to a state where the cell shrinks by the action of osmotic pressure of the cell under a condition in which a substance that can pass through the cell membrane is in high concentration, utilizing the property of membrane equilibrium of the cell. It is favorable that the cell shrinkage reagent is a substance having the effect of making the cell shrink by the action of osmotic pressure.

[0014] According to the present invention (Claim 2), in the biosensor defined in Claim 1, a liquid specimen is whole blood.

[0015] According to the biosensor that is constituted as described above, there is no need for an operation of previously eliminating hemocyte components in the whole blood, whereby a high-accuracy blood component analysis can be performed easily and quickly, with less cost.

[0016] According to the present invention (Claim 3), in the biosensor of Claim 1, a liquid specimen is a solution including bacteria.

[0017] According to the biosensor that is constituted as described above, there is no need for an operation of previously eliminating or crushing cell components in the bacteria solution, where a high-accuracy analysis can be performed easily and quickly, with less costs.

[0018] According to the present invention (Claim 4), in the biosensor of Claim 1, the cell shrinkage reagent is inorganic salt.

[0019] According to the present invention (Claim 5), in the biosensor of Claim 1, the cell shrinkage reagent has a concentration of 0.1 - 5.0 M.

[0020] According to the biosensor that is constituted as described above, the cells in an added liquid specimen shrink without being destroyed by contact with the inorganic salt, whereby the liquid specimen in which the shrunk cells are mixed can penetrate a chromatographic carrier efficiently and sufficiently without adding a development solution. Accordingly, the quantity of the liquid flowing chromatographically downstream is increased, whereby even when whole blood or a bacteria solution is a sample, a high-accuracy analysis can be performed easily and quickly, with less cost.

[0021] According to the present invention (Claim 6), in the biosensor of Claim 1, the cell shrinkage reagent is amino acid or saccharide.

[0022] According to the biosensor that is constituted as described above, cells in an added liquid specimen shrink by contact with amino acid, and the liquid specimen in which the shrunk cell components are mixed can penetrate the chromatographic carrier efficiently and sufficiently without adding a development solution, whereby the quantity of the liquid flowing chromatographically downstream is increased. Therefore, even when whole blood or a bacteria solution is a sample, a high-accuracy analysis can be performed easily and quickly, with less cost.

[0023] According to the present invention (Claim 7), in the biosensor of Claim 1, a carrier that carries the cell shrinkage reagent is dried naturally or dried by air-drying.

[0024] According to the biosensor that is constituted as described above, denaturation of the cell shrinkage reagent and the like is reduced, whereby the cells can shrink efficiently.

[0025] According to the present invention (Claim 8), in the biosensor of Claim 1, a carrier that carries the cell shrinkage reagent is dried by freeze-drying.

[0026] According to the biosensor that is constituted as described above, the crystals of the cell shrinkage reagent are fine grained and easily dissolved, whereby the cells can shrink in a shorter time.

[0027] According to the present invention (Claim 9), in the biosensor of Claim 1, a carrier that carries the cell shrinkage reagent is dried by heat drying.

[0028] According to the biosensor that is constituted as described above, the cell shrinkage reagent can be dried in a shorter time, thereby simplifying manufacturing steps.

[0029] According to the present invention (Claim 10), in the biosensor of Claim 1, the biosensor is a one-step immunochromatographic test strip.

[0030] According to the biosensor that is constituted as described above, there is no need to previously preprocess a liquid specimen including cell components such as whole blood, and measurement targets in wide fields can be measured by obtaining an antibody or an antigen for the measurement target, utilizing an immune reaction. Therefore, in cases where blood is employed, easy and quick measurement can be performed. Here, one step refers to an operation which, in its measurement operation, does not require preprocessing of the liquid specimen including cell components such as the whole blood, and only requires spotting and sticking of the liquid specimen to a test strip but does not require the need to use a development solution that is different from the liquid specimen for the test strip before and after spotting and sticking of the liquid specimen or to carry out a washing

operation for the purpose of B/F separation. The immunochromatographic test strip refers to a sensor for detecting a target substance in the liquid specimen utilizing the antigen-antibody reaction, on the carrier on which chromatographic development is performed.

[0031] According to the present invention (Claim 11), in the biosensor of Claim 1, the biosensor is a dry analytical element.

[0032] According to the biosensor that is constituted as described above, the entire biosensor is a dry carrier, so that it is not only easily portable but also there is no need for strict preservation environments or preservation conditions, whereby the biosensor that can be easily handled and preserved for a long time under all conditions can be obtained. The dry analytical element described here refers to one in which all members constituting the biosensor and a carried reagent are in dry states.

[0033] According to the present invention (Claim 12), there is provided a blood component analytical method using a biosensor that is made of a single layer or plural layers of a porous material, having a reagent holding part for holding a reagent that labels an analysis target in the liquid specimen, and analyzing components in the liquid specimen by utilizing chromatography, wherein :

> a cell shrinkage reagent which osmotically shrinks cells is carried on at least part of a region from a specimen application part on which the liquid specimen is applied to the reagent holding part,
> a blood specimen is applied to the specimen application part, and the cell shrinkage reagent is dissolved from the region where the cell shrinkage reagent is carried, by penetration of the applied blood specimen, and
> the cells included in the liquid specimen are shrunk by the dissolved cell shrinkage reagent, the shrunk cells are developed on a reaction layer to chromatographically downstream side together with the liquid specimen, thereby analyzing the components in the liquid specimen.

[0034] According to the blood component analytical method that is constructed as described above, the erythrocyte in blood is shrunk without being destroyed, whereby blood containing the shrunk erythrocyte can penetrate a chromatographic carrier sufficiently without causing clogging and sufficiently without addition of a development solution, whereby the quantity of the liquid flowing chromatographically downstream is increased. Therefore, even when whole blood is used as a sample, a high-accuracy blood component analysis is performed easily and quickly with less cost.

[0035] According to the present invention (Claim 13), in the blood component analytical method of Claim 12, a blood' specimen to be added is whole blood.

[0036] According to the blood component analytical method that is constructed as described above, there is no need to previously perform an operation of eliminating hemocyte components in the whole blood, whereby a high-accuracy blood component analysis can be performed easily and quickly, with less cost.

[0037] According to the present invention (Claim 14), in the blood component analytical method of Claim 12, the cell shrinkage reagent is inorganic salt.

[0038] According to the present invention (Claim 15), in the blood component analytical method of Claim 12, the cell shrinkage reagent has a concentration of 0.1 - 5.0 M.

[0039] According to the blood component analytical method that is constructed as described above, the erythrocyte in blood is shrunk without being destroyed, whereby blood containing the shrunk erythrocyte can penetrate a chromatographic carrier efficiently without causing clogging and sufficiently without addition of a development solution, and the quantity of the liquid flowing the chromatographically downstream is increased. Therefore, even when whole blood is a sample, a high-accuracy blood component analysis can be performed easily and quickly with less cost.

[0040] According to the present invention (Claim 16), in the blood component analytical method of Claim 12, the cell shrinkage reagent is amino acid or saccharide.

[0041] According to the blood component analytical method that is constructed as described above, the erythrocyte in blood is shrunk without being destroyed, whereby blood containing the shrunk erythrocyte can penetrate a chromatographic carrier efficiently without causing clogging and sufficiently without addition of a development solution, whereby the quantity of the liquid flowing the chromatographically downstream is increased. Therefore, even when whole blood is used as a sample, a high-accuracy blood component analysis can be performed easily and quickly, with less cost.

[0042] According to the present invention (Claim 17), in the blood component analytical method of Claim 12, the biosensor is a one-step immunochromatographic test strip.

[0043] According to the blood component analytical method that is constructed as described above, there is no need to previously carry out preprocessing such as centrifugation to eliminate cell components in whole blood, and measurement targets in wide fields can be measured by obtaining an antigen or an antibody for the measurement target, utilizing an immuno reaction. Therefore, a blood component analytical method can be obtained in which, in cases where a liquid sample including cell components such as whole blood is employed, an easy and quick measurement can be performed.

[0044] According to the present invention (Claim 18), in the blood component analytical method of Claim 12, the biosensor is a dry analytical element.

[0045] According to the blood component analytical method that is constructed as described above, the entire biosensor is a dry carrier, so that it is not only easily portable but also there is no need for strict preservation environments or preservation conditions, whereby a blood

component analytical method that is easily handled can be obtained.

Brief Description of the Drawings

[0046]

Figure 1 is a diagram illustrating a lateral flow-type biosensor utilizing chromatography according to a first embodiment of the present invention.

Figure 2 is a diagram illustrating the lateral flow-type biosensor according to the first embodiment of the present invention, with a specimen addition part being omitted and a shrinkage reagent holding part serving also as a specimen addition part.

Figure 3 is a diagram showing the rates of solution penetration onto a reaction layer of the lateral flow-type biosensor according to the first embodiment of the present invention with changes in the concentration of a shrinkage reagent.

Best Mode for Carrying out the Invention

[0047] Hereinafter, embodiments of the present invention will be described with reference to the drawings. The embodiments described here are only examples and the present invention is not limited to these embodiments.

(Embodiment 1)

[0048] Figure 1 is a diagram illustrating a lateral flow-type biosensor utilizing chromatography according to a first embodiment.

[0049] As shown in figure 1, the biosensor according to the first embodiment includes a carrier support 1 that is made of plastic or the like, and a specimen addition part 2 that is constituted by fabric or glass fiber filter paper having a high water absorbing property, to which part a liquid specimen is added or applied, a shrinkage reagent holding part 8 where a cell shrinkage reagent having an ability of contracting cell components is solubly held on the fabric or the glass fiber filter paper, a marker reagent holding part 3 in which a marker reagent which produces some reaction with an analysis target is solubly held on the fabric or the glass fiber filter paper, a reaction layer 4 that is made of nitrocellulose or the like, a specific protein immobilization part 5 in which a specific protein is immobilized on an area of the reaction layer 4, and a water absorbing part 6 which finally absorbs the liquid specimen, which are formed on the carrier support 1.

[0050] The cell shrinkage reagent held by the shrinkage reagent holding part 8 is for example inorganic salt, amino acid, or saccharide. Here, the inorganic salt refers to inorganic compounds including salt, such as sodium chloride, potassium chloride, and sodium phosphate. The amino acid refers to compounds having the carboxyl group and the amino group in the same molecule, such as glycin and glutamic acid, and includes also imino acid such as proline and hydroxylprolin. The saccharide refers to sugar such as glucose, sucrose and trehalose, or sugar alcohol such as glucitol.

[0051] The reaction between the analysis target and the marker reagent refers to a specific bonding reaction between a ligand and a receptor such as an antigen-antibody reaction, or an arbitrary specific reaction such as an enzyme reaction.

[0052] The marker reagent held by the marker reagent holding part 3 refers to metallic sols such as gold colloid, nonmetallic sols, dye sols, colored particles, coloring matters, enzymes, proteins or the like.

[0053] Next, a blood component analytical method using the biosensor according to the first embodiment will be described.

[0054] Initially, a liquid specimen including cell components, such as whole blood and bacteria solution, is added to the specimen addition part 2. When the added liquid specimen reaches the shrinkage reagent holding part 8, the shrinkage reagent held by the shrinkage reagent holding part 8 is dissolved by the penetration of the liquid specimen, thereby contracting the cells. Accordingly, the liquid specimen penetrates chromatographically downstream without causing clogging, even under a state where the cell components are mixed.

[0055] Next, when the liquid specimen in which the contracted cells are mixed reaches the marker reagent holding part 3, the marker reagent held on the marker reagent holding part 3 is dissolved by the penetration of the liquid specimen and then penetrates the reaction layer 4.

[0056] Then, in the specific protein immobilization part 5 on an area of the reaction layer 4, a reaction is produced with the marker reagent eluted from the marker reagent holding part 3. At this time, when the liquid specimen includes the analysis target, some color reaction is seen in the specific protein immobilization part 5.

[0057] Finally, the liquid specimen is absorbed by the water absorbing part 6, thereby completing the reaction.

[0058] As described above, according to the biosensor and the blood component analytical method of the first embodiment, the cell shrinkage reagent held on the shrinkage reagent holding part makes the cells in the liquid specimen shrink without being destroyed. Therefore, the liquid specimen in which the shrunk cells are mixed, penetrates chromatographically downstream without causing clogging. Accordingly, even when whole blood or bacteria solution is the sample, it can be developed without previous preprocessing of the sample. Therefore, the development solution is not required to develop the sample solution chromatographically downstream, whereby the high-accuracy blood component analysis can be performed easily and quickly with reduced costs.

[0059] In the biosensor of the first embodiment, the specimen addition part can be omitted and the specimen can be directly added to the reaction layer.

[0060] Figure 2 is a diagram illustrating the lateral flow-

type biosensor of the first embodiment, with the specimen addition part being omitted and the shrinkage reagent holding part serving also as a specimen addition part.

[0061] When the shrinkage reagent holding part serves also as the specimen addition part as shown in figure 2, the number of members and the number of steps can be reduced.

[0062] While the biosensor of the first embodiment is constituted by plural members, the biosensor can be constituted by a single-layer member that has a shrinkage reagent holding area on which the shrinkage reagent is solubly held, a marker reagent holding area on which the marker reagent is solubly held, and a specific protein immobilization part on which the specific protein is immobilized, all of which are formed on a reaction layer that is made of a porous material such as nitrocellulose.

[0063] Further, the biosensor of the present invention may be a dry analytical element. The dry analytical element described here refers to one in which all members constituting the biosensor and a carried reagent are in dry states. When the test strip is in a dry state, the entire biosensor is a dry carrier, so it is not only easily portable, but also strict preservation environments or preservation conditions are not required, whereby it can be easily handled and the long preservation under all conditions is enabled.

[0064] Further, the biosensor of the present invention may have a one-step immunochromatographic test strip. Here, the one-step refers to operations, in its measurement operation, requiring no preprocessing of a liquid specimen including cell components such as whole blood, and requiring only spotting and sticking of the liquid specimen onto a test strip, without the need to use a development solution that is different from the liquid specimen on the test strip before and after the spotting and sticking of the liquid specimen or the need for washing operation. The immunochromatographic teat strip refers to a sensor for detecting a substance to be tested in the liquid specimen utilizing an antigen-antibody reaction on a carrier which is chromatographically developed. When the test strip is a one-step immunochromatographic test strip as described above, the need to previously carry out preprocessing of the liquid specimen including cell components such as whole blood is not required, and a measurement target in wide fields can be measured by obtaining an antigen or an antibody for the measurement target, using the immune reaction. Therefore, in cases where the liquid specimen including cell components such as whole blood is used, a simple and quick measurement is enabled.

(Examples)

[0065] The method for implementing the present invention will be described in more detail, with reference to following examples. Here, the present invention is not limited to the following examples.

Example 1.

(Qualitative analysis of hCG in whole blood by lateral flow-type biosensor utilizing chromatography)

[0066] An immuno biosensor including an anti hCG-β antibody immobilized line, and a wide band of a complex of an anti hCG-α antibody and a gold colloid in a nitrocellulose membrane was manufactured. This biosensor is a lateral flow-type biosensor having a configuration as shown in figure 1 and was manufactured as follows:

a) Preparation of biosensor

[0067] An anti hCG-β antibody solution which was diluted by a phosphate buffered solution to regulate its concentration was prepared. This antibody solution was applied onto a nitrocellulose membrane using a solution discharge device. Thereby, a detecting antibody immobilized line was obtained on the nitrocellulose membrane. After dried, this nitrocellulose membrane was immersed into a Tris-HCl buffer solution containing 1-% skim milk, and shaken gently. Then, 30 minutes later, the membrane was moved into a Tris-HCl buffer solution tank, and shaken gently for 10 minutes. Thereafter, the membrane was shaken gently in another Tris-HCl buffer solution tank for another 10 minutes, thereby washing the membrane. After the twice washing, the membrane was picked up from the liquid tank and dried at room temperature.

[0068] The gold colloid was prepared by adding a 1-% citric acid solution to a circulating 100°C solution of 0.01% chloroauric acid. After the circulation was continued for 30 minutes, the gold colloid was set aside at room temperature to be cooled. An anti hCG-α antibody was added to the gold colloid which had been adjusted to pH9 with a 0.2M potassium carbonate solution and then agitated for several minutes. Thereafter, a quantity of 10% BSA (bovine serum albumin) solution of pH9 was added so as to finally obtain a 1% solution, and then agitated, whereby an antibody-gold colloid complex (marker antibody) was prepared. The marker antibody solution was centrifuged at 4°C and 20000G for 50 minutes, thereby isolating the marker antibody. Then the isolated marker antibody was suspended into a washing buffer (1% BSA-phosphate buffer) and thereafter centrifuged, thereby washing and isolating the marker antibody. This marker antibody was suspended by a washing buffer, filtered by a 0.8-μm filter, then regulated to reduce the quantity to one tenth the quantity of the initial gold colloid solution, and an obtained solution was stored at 4°C.

[0069] The marker antibody solution was set in the solution discharge device, applied to a position which is at a distance from the antibody immobilized position on the anti hCG-β antibody immobilized dry membrane, and then the membrane was dried. Thereby, a marker reagent holding part was obtained on the immobilized membrane.

[0070] A potassium chloride aqueous solution that was adjusted to 0.15M was spotted and stuck to fabric, by 0.1 ml per a unit area, then immediately frozen by liquid nitrogen, thereby performing freeze-drying. Thus, a shrinkage reagent holding member that was impregnated with potassium chloride was obtained.

[0071] The antibody immobilized membrane including the marker reagent holding part that was prepared as described above was affixed on the carrier support, then the shrinkage reagent holding member, fabric as the specimen addition part, and glass fiber filter paper as the water absorbing part were added thereto, thereafter cut into small pieces of 0.5-cm width, thereby preparing the biosensor.

b) Preparation of specimen

[0072] Human blood to which heparin was added as an anticoagulant was adjusted to have a hematocrit value of 45%. Then, hCG solutions of known concentrations of were added to this blood, whereby hCG solutions of various known concentrations were prepared.

c) Measurement of coloration degree on biosensor

[0073] whole blood including hCG was added to the specimen addition part on the biosensor by about 150μm, developed in the direction of the water absorbing part, and made cause an antigen-antibody reaction, thereby producing a color reaction in the antibody immobilized part. Then the coloration state five minutes after the specimen addition to the biosensor was checked visually. Further, the time which it took the blood to penetrate the reaction layer by 2 cm on the biosensor to the chromatographically downstream side was measured.

[0074] Figure 3 is a diagram showing the rates of solution penetration onto the reaction layer with changes in the concentration of the shrinkage reagent in the lateral flow-type biosensor according to the first example. More specifically, this figure shows the time which it takes the blood to penetrate the reaction layer by 2 cm when the KCl concentration is changed to 0.01M, 0.1M, 0.15H or 5M, the shrinkage reagent holding part 3 carrying the KCl solution of each concentration is incorporated into the sensor, and then the blood having a 45% hematocrit value is added. Here, it can be seen that when no KC1 is included, it takes about one hour to penetrate, while as the KCl concentration increases as 0.01M, 0.1M, and 0.15M, the penetration time is reduced more.

[0075] From the above-mentioned result, it is understood that the area that holds the cell shrinkage reagent, provided in the lateral flow-type biosensor according to this first example, is greatly related to the reduction in the penetration rate on the reaction layer.

[0076] Here, when the concentration of the cell shrinkage reagent is set at 0.1M - 5.0M, the cell components can shrink to optimal sizes. Consequently, the whole blood can be made penetrate quickly without causing clogging on the carrier.

[0077] In this first example, the carrier that carries the cell shrinkage reagent (a potassium chloride aqueous solution in this first example) is dried by freeze-drying. Therefore, the crystals of the cell shrinkage reagent are fine grained and easily dissolved, whereby the cell components can shrink in a shorter time.

[0078] While in this first example the carrier which carries the cell shrinkage reagent is dried by freeze-drying, it may be dried naturally or dried by air-drying. Thereby, the denaturation of the cell shrinkage reagent and the like is reduced, whereby the cell components can shrink efficiently. Further, the carrier that carries the cell shrinkage reagent may be dried by heat drying. Thereby, the drying of the cell shrinkage reagent in a shorter time is enabled, thereby simplifying manufacturing processes.

[0079] Further, in the biosensor of the first example, the chromatography material that is constituted by an arbitrary porous carrier, such as nitrocellulose and glass fiber filter paper is employed, and generally the biosensor that is constituted by such material has the capability of analyzing and detecting a specific substance using an arbitrary specific measurement principle such as an antigen-antibody reaction, and qualitatively or quantitatively analyzing the substance. In this first example, the case of an antigen-antibody reaction using the marker has been described, while any thing such as enzymes may be used as long as some changes occur before and after the reaction.

[0080] Further, the test strip that is constituted by plural porous carriers including fabric as the specimen addition part and glass fiber filter paper as the water absorbing part has been described as an example. However, the test strip may have a single layer structure from which the fabric and the glass fiber filter paper is eliminated and which is provided with the antibody immobilized part which is immobilized on the porous carriers, the marker reagent holding part on which a marker reagent is elutably held, and the shrinkage reagent holding part on which a cell shrinkage reagent is elutably held.

Industrial Availability

[0081] According to the biosensor and the blood component analytical method of the present invention, even when whole blood is used as a sample, a high-accuracy blood component analysis can be carried out easily and quickly, with less expenses, and without the need to previously carrying out some processing for the blood or the need for a development solution for developing a sample solution, and the present invention is especially suitable for a one-step biosensor and blood component analytical method.

Claims

1. A biosensor that is made of a single layer or plural

layers of a porous material, said biosensor having a reagent holding part for holding a reagent that labels an analysis target in the liquid specimen, and analyzing components in the liquid specimen by utilizing chromatography, wherein
a cell shrinkage reagent which osmotically shrinks cells is carried on at least part of a region from a specimen application part on which the liquid specimen is applied to the reagent holding part, and
a reaction layer is provided, on which the cells in the liquid specimen are shrunk by the cell shrinkage reagent, and the shrunk cells are developed to chromatographically downstream side together with the liquid specimen.

2. The biosensor of Claim 1 wherein a liquid specimen is whole blood.

3. The biosensor of Claim 1 wherein a liquid specimen is a solution including bacteria.

4. The biosensor of Claim 1 wherein the cell shrinkage reagent is inorganic salt.

5. The biosensor of Claim 1, wherein the concentration of the cell shrinkage reagent is 0.1 - 5.0 M.

6. The biosensor of Claim 1 wherein the cell shrinkage reagent is amino acid or saccharide.

7. The biosensor of Claim 1 wherein a carrier that carries the cell shrinkage reagent is dried naturally or dried by air-drying.

8. The biosensor of Claim 1 wherein a carrier that carries the cell shrinkage reagent is dried by freeze-drying.

9. The biosensor of Claim 1 wherein a carrier that carries the cell shrinkage reagent is dried by heat drying.

10. The biosensor of Claim 1 wherein the biosensor is a one-step immunochromatographic test strip.

11. The biosensor of Claim 1 wherein the biosensor is a dry analytical element.

12. A blood component analytical method using a biosensor that is made of a single layer or plural layers of a porous material, said biosensor having a reagent holding part for holding a reagent that labels an analysis target in the liquid specimen, and analyzing components in the liquid specimen by utilizing chromatography, wherein :

a cell shrinkage reagent which osmotically shrinks cells is carried on at least part of a region from a specimen application part on which the liquid specimen is applied to the reagent holding part,
a blood specimen is applied to the specimen application part, and the cell shrinkage reagent is dissolved from the region where the cell shrinkage reagent is carried, by penetration of the applied blood specimen, and
the cells included in the liquid specimen are shrunk by the dissolved cell shrinkage reagent, the shrunk cells are developed on a reaction layer to chromatographically downstream side together with the liquid specimen, thereby analyzing the components in the liquid specimen.

13. The blood component analytical method of Claim 12 wherein a blood specimen to be added is whole blood.

14. The blood component analytical method of Claim 12 wherein the cell shrinkage reagent is inorganic salt.

15. The blood component analytical method of claim 12, wherein the concentration of the cell shrinkage reagent is 0.1 - 5.0 M.

16. The blood component analytical method of Claim 12 wherein the cell shrinkage reagent is amino acid or saccharide.

17. The blood component analytical method of Claim 12 wherein the biosensor is a one-step immunochromatographic test strip.

18. The blood component analytical method of Claim 12 wherein the biosensor is a dry analytical element.

Patentansprüche

1. Aus einer einzelnen Schicht oder aus mehreren Schichten eines porösen Materials bestehender Biosensor, der einen Reagenshalteteil zum Halten eines Reagens hat, das ein Analysentarget in der flüssigen Probe markiert, und der Komponenten in der flüssigen Probe mittels Chromatographie analysiert, **dadurch gekennzeichnet, dass**
ein Zellschrumpfungsreagens, das Zellen osmotisch schrumpft, in zumindest einem Teil eines Bereichs von einem Probenaufbringungsteil, an dem die flüssige Probe aufgebracht wird, zu dem Reagenshalteteil übertragen wird und
eine Reaktionsschicht vorgesehen ist, auf der die Zellen in der flüssigen Probe mit dem Zellschrumpfungsreagens geschrumpft werden, und die geschrumpften Zellen zusammen mit der flüssigen Probe chromatographisch vorwärts entwickelt werden.

2. Biosensor nach Anspruch 1, **dadurch gekennzeichnet, dass** eine flüssige Probe Vollblut ist.

3. Biosensor nach Anspruch 1, **dadurch gekennzeichnet, dass** eine flüssige Probe eine Lösung mit Bakterien ist.

4. Biosensor nach Anspruch 1, **dadurch gekennzeichnet, dass** das Zellschrumpfungsreagens anorganisches Salz ist.

5. Biosensor nach Anspruch 1, **dadurch gekennzeichnet, dass** die Konzentration des Zellschrumpfungsreagens 0,1 - 5,0 M beträgt.

6. Biosensor nach Anspruch 1, **dadurch gekennzeichnet, dass** das Zellschrumpfungsreagens Aminosäure oder Saccharid ist.

7. Biosensor nach Anspruch 1, **dadurch gekennzeichnet, dass** ein Träger, der das Zellschrumpfungsreagens trägt, natürlich oder durch Lufttrocknung getrocknet wird.

8. Biosensor nach Anspruch 1, **dadurch gekennzeichnet, dass** ein Träger, der das Zellschrumpfungsreagens trägt, durch Gefriertrocknung getrocknet wird.

9. Biosensor nach Anspruch 1, **dadurch gekennzeichnet, dass** ein Träger, der das Zellschrumpfungsreagens trägt, durch Wärmetrocknung getrocknet wird.

10. Biosensor nach Anspruch 1, **dadurch gekennzeichnet, dass** der Biosensor ein Einschritt-Immunchromatographie-Teststreifen ist.

11. Biosensor nach Anspruch 1, **dadurch gekennzeichnet, dass** der Biosensor ein Trockenanalyse-Element ist.

12. Blutkomponenten-Analysenverfahren, das einen aus einer einzelnen Schicht oder aus mehreren Schichten eines porösen Materials bestehenden Biosensor verwendet, der einen Reagenshalteteil zum Halten eines Reagens hat, das ein Analysentarget in der flüssigen Probe markiert, und der Komponenten in der flüssigen Probe mittels Chromatographie analysiert,
dadurch gekennzeichnet, dass
ein Zellschrumpfungsreagens, das Zellen osmotisch schrumpft, in zumindest einem Teil eines Bereichs von einem Probenaufbringungsteil, an dem die flüssige Probe aufgebracht wird, zu dem Reagenshalteteil übertragen wird,
eine Blutprobe auf den Probenaufbringungsteil aufgebracht wird und das Zellschrumpfungsreagens durch Penetration der aufgebrachten Blutprobe aus dem Bereich, in dem das Zellschrumpfungsreagens übertragen wird, freigesetzt wird und
Zellen, die in der flüssigen Probe enthalten sind, von dem freigesetzten Zellschrumpfungsreagens geschrumpft werden und die geschrumpften Zellen auf einer Reaktionsschicht zusammen mit der flüssigen Probe chromatographisch vorwärts entwickelt werden, wodurch die Komponenten in der flüssigen Probe analysiert werden.

13. Blutkomponenten-Analysenverfahren nach Anspruch 12, **dadurch gekennzeichnet, dass** eine zuzugebende Blutprobe Vollblut ist.

14. Blutkomponenten-Analysenverfahren nach Anspruch 12, **dadurch gekennzeichnet, dass** das Zellschrumpfungsreagens anorganisches Salz ist.

15. Blutkomponenten-Analysenverfahren nach Anspruch 12, **dadurch gekennzeichnet, dass** die Konzentration des Zellschrumpfungsreagens 0,1 - 5,0 M beträgt.

16. Blutkomponenten-Analysenverfahren nach Anspruch 12, **dadurch gekennzeichnet, dass** das Zellschrumpfungsreagens Aminosäure oder Saccharid ist.

17. Blutkomponenten-Analysenverfahren nach Anspruch 12, **dadurch gekennzeichnet, dass** der Biosensor ein Einschritt-Immunchromatographie-Teststreifen ist.

18. Blutkomponenten-Analysenverfahren nach Anspruch 12, **dadurch gekennzeichnet, dass** der Biosensor ein Trockenanalyse-Element ist.

Revendications

1. Biocapteur qui est fait d'une seule couche ou d'une pluralité de couches d'un matériau poreux, ledit biocapteur ayant une partie de maintien de réactif pour maintenir un réactif qui marque une cible d'analyse dans le spécimen liquide, et analyser des composants dans le spécimen liquide en utilisant une chromatographie, dans lequel
un réactif de contraction de cellules qui contracte des cellules osmotiquement est porté sur au moins une partie d'une région d'une partie d'application de spécimen sur laquelle le spécimen liquide est appliqué à la partie de maintien de réactif, et
une couche de réaction est pourvue, sur laquelle les cellules dans le spécimen liquide sont contractées par le réactif de contraction de cellules, et les cellules contractées sont développées vers le côté chromatographiquement en aval en même temps que le

spécimen liquide.

2. Biocapteur de la revendication 1 dans lequel un spécimen liquide est du sang complet.

3. Biocapteur de la revendication 1 dans lequel un spécimen liquide est une solution incluant des bactéries.

4. Biocapteur de la revendication 1 dans lequel le réactif de contraction de cellules est un sel inorganique.

5. Biocapteur de la revendication 1 dans lequel la concentration du réactif de contraction de cellules est de 0,1 - 5,0 M.

6. Biocapteur de la revendication 1 dans lequel le réactif de contraction de cellules est un acide aminé ou un saccharide.

7. Biocapteur de la revendication 1 dans lequel un porteur qui porte le réactif de contraction de cellules est séché naturellement ou séché par le biais d'un séchage à l'air.

8. Biocapteur de la revendication 1 dans lequel un porteur qui porte le réactif de contraction de cellules est séché par lyophilisation.

9. Biocapteur de la revendication 1, dans lequel un porteur qui porte le réactif de contraction de cellules est séché par un séchage à la chaleur.

10. Biocapteur de la revendication 1, dans lequel le biocapteur est une bandelette de test immuno-chromatographique à une étape.

11. Biocapteur de la revendication 1, dans lequel le biocapteur est un élément analytique sec.

12. Procédé d'analyse de composants sanguins utilisant un biocapteur qui est fait d'une seule couche ou d'une pluralité de couches d'un matériau poreux, ledit biocapteur ayant une partie de maintien de réactif pour maintenir un réactif qui marque une cible d'analyse dans le spécimen liquide, et analyser des composants dans le spécimen liquide en utilisant une chromatographie, où:

un réactif de contraction de cellules qui contracte des cellules osmotiquement est porté sur au moins une partie d'une région depuis une partie d'application de spécimen sur laquelle le spécimen liquide est appliqué à la partie de maintien de réactifs,
un spécimen sanguin est appliqué à la partie d'application de spécimen, et le réactif de contraction de cellules est dissous de la région où le réactif de contraction de cellules est porté, par une pénétration du spécimen sanguin appliqué, et
les cellules incluses dans le spécimen liquide sont contractées par le réactif de contraction de cellules dissous, les cellules contractées sont développées sur une couche de réaction vers le côté chromatographiquement en aval en même temps que le spécimen liquide, analysant ainsi les composants dans le spécimen liquide.

13. Procédé d'analyse de composants sanguins de la revendication 12 dans lequel un spécimen sanguin devant être ajouté est du sang complet.

14. Procédé d'analyse de composants sanguins de la revendication 12 dans lequel le réactif de contraction de cellules est un sel inorganique.

15. Procédé d'analyse de composants sanguins de la revendication 12, dans lequel la concentration du réactif de contraction de cellules est de 0,1 - 5,0 M.

16. Procédé d'analyse de composants sanguins de la revendication 12, dans lequel le réactif de contraction de cellules est un acide aminé ou un saccharide.

17. Procédé d'analyse de composants sanguins de la revendication 12, dans lequel le biocapteur est une bandelette de test immuno-chromatographique à une étape.

18. Procédé d'analyse de composants sanguins de la revendication 12, dans lequel le biocapteur est un élément analytique sec.

Fig.1

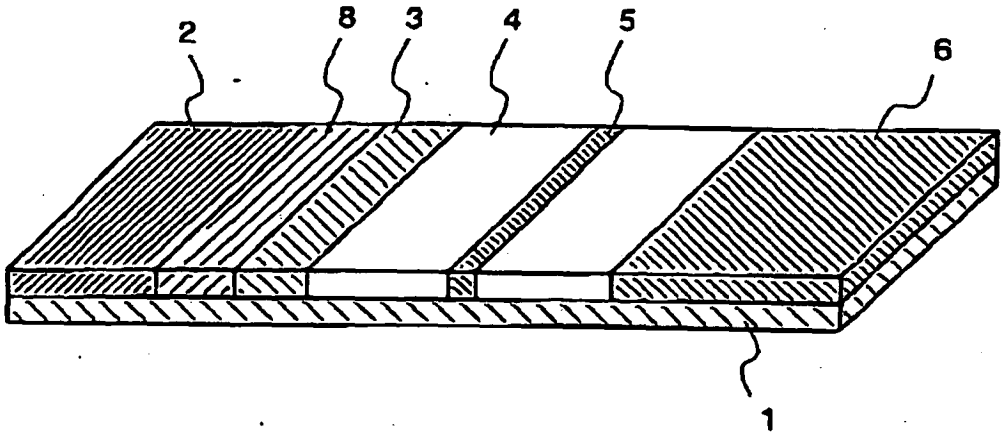


Fig.2

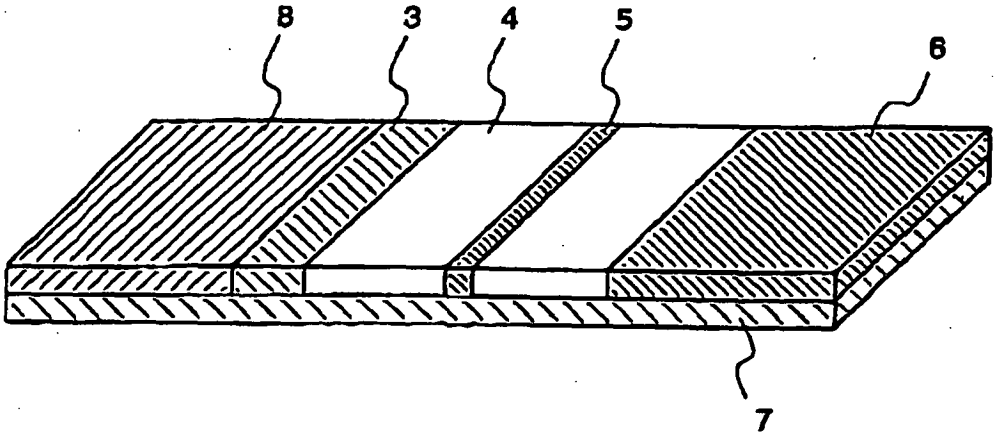
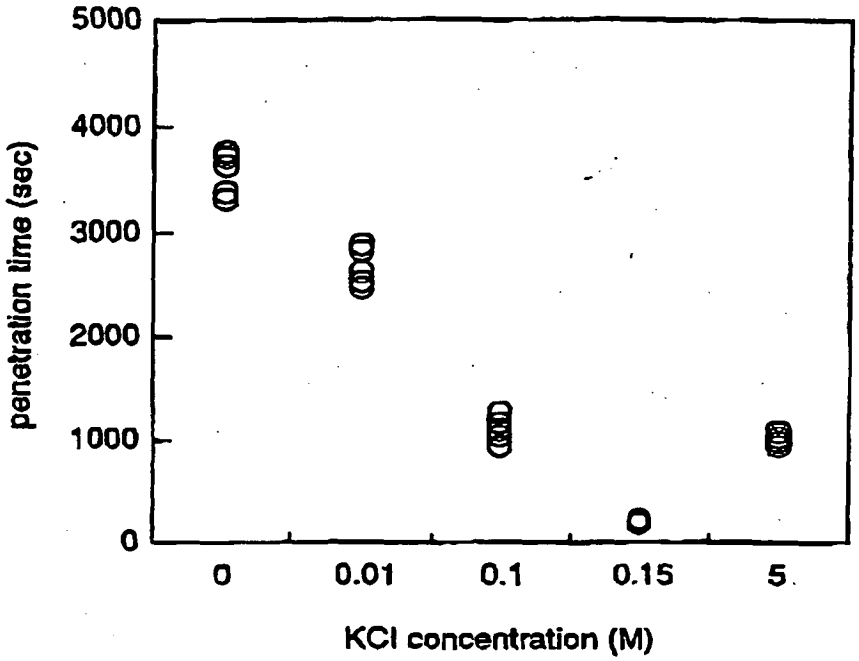


Fig.3


5000
4000
3000
2000
1000
0
penetration time (sec)
0
0.01
0.1
0.15
5
KCl concentration (M)

REFERENCES CITED IN THE DESCRIPTION

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- JP 8054387 A **[0004]**
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- EP 1143247 A **[0010]**
- EP 903584 A **[0011]**

专利名称(译)	生物传感器和血液成分分析方法		
公开(公告)号	EP1202061B1	公开(公告)日	2008-05-14
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申请(专利权)人(译)	松下电器产业有限公司.		
当前申请(专利权)人(译)	松下电器产业有限公司		
[标]发明人	TAKAHASHI MIE NADAOKA MASATAKA TANAKA HIROTAKA KITAWAKI FUMIHISA		
发明人	TAKAHASHI, MIE NADAOKA, MASATAKA TANAKA, HIROTAKA KITAWAKI, FUMIHISA		
IPC分类号	G01N33/543 G01N31/22 G01N30/88 G01N33/53 G01N30/90 G01N33/50 G01N33/558		
CPC分类号	G01N30/90 G01N33/5002 G01N33/5005 G01N33/558 Y10S436/808 Y10S436/81		
优先权	2000164990 2000-06-01 JP		
其他公开文献	EP1202061A4 EP1202061A1		
外部链接	Espacenet		

摘要(译)

根据本发明的生物传感器和血液成分分析方法，在由单层或多层多孔材料制成的生物传感器中，如图1所示，具有试剂保持部分并利用色谱法，细胞收缩试剂保持在试剂保持部分的至少一部分上，或者至少部分在试剂保持部分上游的色谱显影部分上。根据具有上述结构的生物传感器和血液成分分析方法，即使全血是样品，也可以以较低的成本容易且快速地进行高精度的血液成分分析。

Fig.1

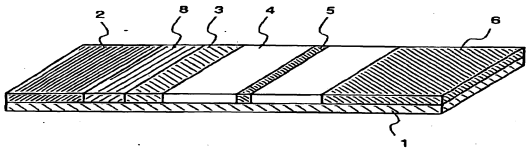

Fig.2