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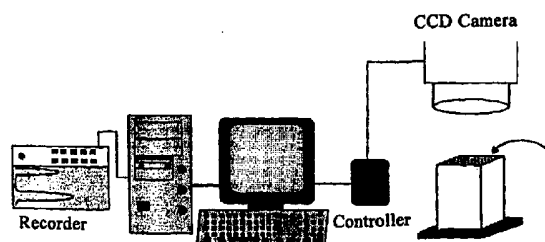
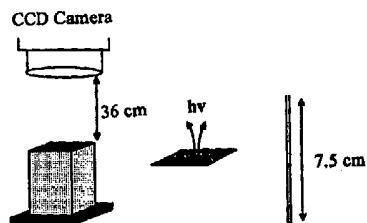
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(54) Title: CAPILLARY ASSAY METHOD

(57) Abstract

The invention concerns a method for determining the concentration of a constituent in an assay system within a capillary tube from which light is emitted, the light being proportional to the amount of the constituent to be assayed. The light from the system is detected and quantified, and the detection takes place from substantially the longitudinal direction of the capillary tube.



CAPILLARY ASSAY METHOD

5 The invention relates to a method of assaying an
unknown concentration of a substance in a sample. More
precisely, the invention refers to a method for determining
the concentration of a constituent in an assay system
within a capillary tube by detecting and quantifying light
10 emitted from the system, the light being proportional to
the amount of the constituent to be assayed in the system.

 There is an increasing demand for highly efficient
assay systems within the biotechnological field. Several
systems are based on light, e.g. from a luminescence
15 reaction, and have several advantages, such as speed and
absence of hazardous agents.

 Light emitting reactions have been used in some
immunoassays based on solid phase systems. These assays
relate both quantitative and qualitative information on
20 certain immunogenic species in a physiological sample, such
as blood or urine, and employ one or more specific
recognition molecules. At least one of the reacting species
is attached to the solid phase, while the other is in
contact with the liquid medium containing the sample. The
25 resulting immunological complex can be used as a method for
the determination of the extent of the reaction. The extent
of reaction is an indication of the amount of analyte in
unknown samples and can be employed in various modes.

 For example, an enzyme-linked immunosorbent assay
30 utilizes an enzyme-labelled immunoreactant (antigen or
antibody) and an immunosorbent (antigen or antibody bound
to a solid support). In this sensitive analytical technique
an enzyme is complexed to an antigen or an antibody. Excess
substances participating in the complex formation are
35 removed by washing, and a substrate is then added

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generating an activity which is directly proportional to the amount of binding and thus the concentration.

This technique can be carried out in several combinations, the most used process being to coat the wells of a microtiter plate with the antigen and reacting with an antibody conjugated to a suitable enzyme, e.g. horseradish peroxidase or alkaline phosphatase. Alternatively, the wells are coated with a monoclonal antibody followed by a reaction with the antigen. The antigen is subsequently reacted with another monoclonal antibody which is conjugated with a suitable enzyme. The former case is called a direct ELISA technique while the latter is referred to as a sandwich ELISA. In yet another format the wells are coated with the antigen followed by a reaction with a monoclonal antibody which is further allowed to react with another antibody-enzyme conjugate specific to the first antibody. In such assays the enzyme acts as a tag for the measurement of the extent of the reaction. For example, the number of enzyme molecules bound to the wells is an indication of the amount of antigen present in the wells.

In JP-A-62169054 a solid phase immunoassay is shown, in which small amounts of antigen solution are determined by adsorbing antigen onto the inner wall of a polymer tube and carrying out an antigen-antibody reaction in the sample solution.

US-A-5 624 850 depicts immunoassays in translucent capillary tubes, especially for detecting antibiotics in milk. A protein conjugate is used which is a hapten covalently bonded to a protein. Detection is accomplished by irradiating a specific binding pair member conjugated to a fluorescent label.

Similarly, a herbicide has been determined in a competitive immunoassay (Dzgoev et al., *Analytica Chimica Acta* 347 (1997) 87-93). Gold coated glass capillary tubes served as support in an imaging ELISA, bound conjugate of

herbicide/bovine serum albumin being determined by the quantification of the chemiluminescence emission from the enzymatic decomposition of a luminogenic substrate. The light emitted along the entire length of the capillary tubes complicated the interpretation of the data obtained.

Although ELISA is an analytical immunochemical method with high sensitivity for measuring the concentration of all the above proteins, there is still a demand for a more sensitive method. Physiologically important substances, such as acute phase proteins, have previously been measured within a range of down to about 10^{-7} M, and pesticides have been detected in concentrations down to 10^{-10} M.

Furthermore, existing assay systems, such as the cumbersome ELISA procedure, is expensive and rather time consuming. A cheaper and faster assay is thus required, in which the washing procedure of for example physiological samples is simplified. It is also desired to achieve an assay system which is robust and also can be used in the field.

The purpose of the invention is to achieve an assay method whereby the above-mentioned problems are eliminated, which method makes possible to more sensitively and rapidly detect light from smaller sample volumes of, for example, a physiological sample.

In order to achieve this purpose, the method according to the invention has been given the characterizing features of claim 1.

In order to explain the invention in more detail illustrative embodiments thereof will be described with reference to the accompanying drawings in which

FIG 1 shows a typical result of a sandwich assay in capillary tubes for the retinol binding protein (RBP);

FIG 2 shows the validation of the results obtained from a sandwich assay in capillary tubes for the retinol binding protein;

FIG 3 shows the validation of the results obtained from a direct immunoassay in capillary tubes for the retinol binding protein; and

FIG 4 schematically illustrates the principles of a
5 set-up comprising a charge coupled device camera and a block with capillary tubes for the monitoring and quantification of an assay.

The method according to the invention has been developed for the assay of an unknown concentration of a
10 constituent in a sample, the constituent being assayed in an assay system within a capillary tube by detecting and quantifying light emitted from the assay system. The constituent can be a natural proteinous substance, or a molecule spontaneously binding to said substance.
15 Preferably, the constituent is a bioactive substance.

The light from the assay system is according to the invention detected from substantially the longitudinal direction of the capillary tube. In this connection the expression capillary tubes not only concerns individual
20 tubes but can also comprise bores in a unitary block.

Capillary tubes of different materials can be used in the method according to the invention. Single capillary tubes as well as the unitary block can be made of glass, plastic or metal, e.g. copper, on condition that photons
25 produced by the assay system within the capillary tube are reflected by the wall material. Preferably, capillary tubes made of glass or plastic are used.

The capillary tubes thus serve as reaction cells for an assay system. Multi-groove capillary blocks can be used
30 alone or single capillary tubes can be employed which are placed in such a block when the light emitted from the assay system is to be detected and quantified. Preferably, the capillary tubes are sealed at one end. In this way a multi-substance analysis can be performed, i.e. several
35 substances can be assayed at the same time.

The light emitted is then optically screened by using a detection device for photosensitive detection which is positioned at an optimal distance from the open end of the capillary tube, the emitted light from the capillary tube according to the invention being detected from substantially the longitudinal direction thereof. Such a light amplification system can for example comprise a photomultiplier tube, a photodiode, or an avalanche photodiode (APD).

10 A micrometer size beam from the capillary tube essentially implies the area of the sensitized tube presented an optical scanning mechanism having multi-analytic capability. In practice, the area of the capillary tube is preferably not greater than the dimensions of the 15 groove in a block for capillary tubes when single capillary tubes are used. The exit point of the capillary tube defines the point source of light to be detected and quantified since the bound light emitting entity is concentrated to a small region instead of the entire length 20 of the capillary tube. This is accomplished by the light emitted from capillary tube according to the invention being detected from substantially the longitudinal direction of the capillary tube. By this positioning of the capillary tube a maximum collection of light is obtained, 25 and the efficiency of the assay is dramatically increased in comparison with assay methods according to the state of the art.

The detection accomplished by the photosensitive device is then monitored, and the light proportional to the 30 amount of the constituent in the system is quantified by means of an optical scanning mechanism having multi-analytic capability and a image system. An example of a monitoring system is a CCD (charge coupled device) camera set-up which is based on light collection at a semi- 35 conductor interface.

The assay method according to the invention is suitable for light emitted from colorimetric, fluorescent as well as chemiluminescent systems. Thus, quantitative assays can be performed which generate an emitted signal, 5 for example from a chemiluminescence reaction, which is numerically monitored by means of an optical scanning mechanism having a multi-analytic capability. In must, however, be emphasized that such measurements are more reliable when performed on an immobilized solid phase in 10 comparison with a dispersed liquid phase.

Quantitative assay results can for example be obtained by using a system generating a signal from luminescence, the specific binding reagent being confined to a solid phase rather than distributed within the assay 15 medium. This can be accomplished if the signal generated from the solid phase in the capillary tube is recorded by means of a light sensitive device, for example a CCD camera. Thus, costly optical monitoring systems can be avoided, and the signal generated can be stored directly as 20 a time versus intensity profile. Analytical results are especially useful in this form when a large number of samples are screened. The coated capillary tubes will channel the light in their longitudinal direction and a stronger light intensity is provided for detection in this 25 direction than if the emitted light is detected perpendicular to the capillary tube. By this set-up a constituent in a sample can be assayed according to the method of the invention in concentrations as small as 10^{-18} M.

30 By using the method according to the invention robust and simple assays are provided which can be used for determining the concentration of a physiological analyte in an immunoassay. More precisely, an assay for a proteinous substance in a physiological sample is provided, the signal 35 used in the determination of the analyte being recorded

directly in a personal computer by using a suitable interface. The recorded light is derived from a light source within the capillary tube, which is channeled to a beam, the intensity of which is a measure of the analyte concentration.

Furthermore, by using the inventive method assays are provided for determining in a sample the presence of a natural protein or a molecule spontaneously binding to the same. An immunosorbent bound to the solid phase in an enzyme immunoassay can be for example be an antigenic protein or an antibody to the same. In order to work most efficiently, the solid support - on which the assay is performed - preferably comprises a porous matrix.

The method according to the invention can be used for determining the concentrations of a variety of proteinaceous substances in a physiological sample. An example is acute phase proteins which change in concentration when a patient is in "acute phase". Until now the standard method to measure acute phase proteins has been the erythrocyte sedimentation rate.

Examples of classical acute phase proteins are ceruloplasmin, complement C3 and C4, orosomucoid, α_1 -antitrypsin, α_1 -antichymotrypsin, haptoglobin, fibrinogen, C-reactive protein, and serum amyloid A. Most recently disclosed acute phase proteins can also be assayed by means of the method according to the invention. Examples of these proteins are the retinol-binding protein (RBP) and the mannose-binding protein (MBP). Similarly, the plasminogen activator inhibitor type 1 (PAI-1) has been assayed with excellent results by means of the inventive method. Assays based on the method according to the invention have for example been performed on the retinol binding protein (RBP) or retinol in a sample by means of specific binding to a solid phase containing an immobilized monoclonal antibody having specificity for RBP, the enzyme-labelled immuno-

reactant participating in either a direct, sandwich or a competitive type of reaction. Assay procedures can also be performed wherein a modified form of retinol is employed for the detection of unknown concentrations of retinol by using RBP as a specific binding reagent. The enzyme-labelled immunoreactant can be an antibody towards the protein in question or a molecule spontaneously binding to the same.

The enzymic label is preferably involved in a luminescent reaction, most preferably in a chemiluminescent reaction. The emitted light is used as a means of determining the extent of complex formation between immunosorbent and enzyme-labelled reactant. The binding of the labelled reagent to the solid phase is then detected by a CCD camera monitoring the chemiluminescent signal, and the amplified electronic signal is recorded in for example a personal computer. The extent of the reaction is accordingly monitored as intensity units generated from the point source of the capillary block at a suitable distance from the camera.

Typically, capillary tubes uniformly coated with a specific binding reagent demonstrate in a sandwich configuration a linearity with varying analyte concentrations when subjected to the method according to the invention. The external aperture of the capillary tube defines the point source obtained from a much larger region of light-emitting label. In order to achieve suitable quantitative assay results, the embodiment in itself ensures that the light sensitivity of the CCD camera is optimized in terms of the dark current, the distance of the point source of light from the detector, the overlapping of the pixels on the generated image due to fuzzy effects from adjacent capillary tubes in the block etc.

The dimensions of the capillary tubes can of course vary in dependence of the specific assay applications. The

diameter can vary from about 0.5 mm to about 2 mm. In order to be efficiently treated and/or handled the length of a capillary tube should be at least 2 cm up to 10 cm or more. Individual capillary tubes preferably have a diameter of 1
5 mm, a length of 7.5 cm, and a wall thickness of 0.1 mm. The distance between the bores in a block depends on the photosensitive device used for detecting the emitted light.

In a typical set-up conventional single capillary tubes are used which have an internal diameter at the top
10 of about 0.8 mm and the length is approximately 7.5 cm. The distance between the open top end of the capillary tube placed in the block and the camera depends on the focusing lens used. The man skilled in the art will understand that in any arrangement simple experiments will have to be
15 conducted before the optimum values of these parameters are ascertained. A subtle advantage of the present design of the set-up is that the aperture of the capillary tube defines the point source and hence the point source is independent of the actual body of the block with capillary
20 tubes. Moreover, the block can be chosen so as to act as a screen for prevention of any extraneous light generated by the unbound label to contribute to a background signal.

EXAMPLES

25 The embodiments of the invention shown in the examples below have been selected and described in order to explain the principles of the invention.

In order to clearly exemplify the invention assay procedures are shown which have been adapted to the
30 specific detection of retinol and the retinol binding protein by using a modified ELISA procedure in glass capillary tubes, the detection being monitored by using a sensitive CCD (charge coupled device) camera operated on a PMIS (Photometric Imaging System) software in a PC-windows
35 environment.

The retinol binding protein (RBP) is an example of an acute phase protein, i.e. a plasma protein which drastically increases in concentration shortly after a tissue damage. RBP is involved in the transportation of retinol (the inactive form of vitamin A) and the importance of retinol to daily life and living is well known. It is involved in the visual cycle and its deficiency leads to several forms of visual disorders. However, as retinol is insoluble in water and plasma (containing 90% water) it can be transported within our body only as a complex with RBP.

The normal level of RBP in human plasma is about 40-50 µg/ml. Immunochemical determination of RBP in normal biological fluids provide the following average values: serum 46 mg/L; urine 0.11 mg/24 h volume, cerebrospinal fluid, 0.35 mg/L. A patient with tubular proteinuria secretes up to 150 mg of RBP per 24 h into his urine.

The assay of retinol by using the modified immunoassay performed in capillary tubes can also be applied to retinoids as a substitute for retinol. Retinoids represent numerous synthetic and naturally occurring compounds similar in structure to retinol, whose diverse pharmacological and antitumour activities are now well documented. Due to their high hydrophobicity the natural retinoids need specific carrier proteins which solubilize and transport the retinoids through the body fluids and to the target cells. Thus, RBP also exhibits affinity to these natural retinoids. However RBP also binds strongly to synthetic retinoids such as for example Fenretinide™ and Accutane™. In addition, RBP is also found to associate with other molecules such as all-trans-retinol, β-carotene, retinyl acetate, retinal, retinoic acid, cholesterol and phytol, which may lead to a decrease in the transport of retinol leading to pathological symptoms of decreased RBP levels. Thus, from the clinical point of view the measurement of RBP and molecules spontaneously binding to RBP

forms a generic basis for the diagnosis of corresponding disorders. Other proteins or proteinous substances of clinical interest to be assayed according to the invention are bioactive peptides, such as hormones, catalytic peptides, and glycoproteins, for example the mannose binding protein.

EXAMPLE 1. Treatment of capillary tubes prior to immobilization.

10 The glass capillary tubes used were commercially available with the dimensions 7.5 cm in length, 1 mm in diameter and a wall thickness of 0.1 mm. The capillary tubes were washed with dilute HNO_3 followed by rinsing with abundant amounts of deionized water and then dried at

15 100 °C for 1 h.

The nature of the treatment of the capillary tubes prior to immobilization of the specific reagents is important. In an initial approach the solid phase in the form of a glass capillary tube was pre-treated with

20 specific reagents for removal of interfering molecules followed by the silanization of the surface with silane based compounds. Especially, the nature of the silane is of specific interest due to the effects on the binding of the reagents to the solid phase support. Two separate

25 approaches can be employed. Firstly, the treatment can be restricted to one form of silane which preferably forms a sol-gel type of coating on the capillary tubes, and secondly mixed silanes from shorter preparation times are used to form a uniform matrix on the surface of the solid

30 phase.

However, the mode of treatment was also found to be important for filling a capillary tube with the silane. A rocking/spinning motion or a coating under a continuous flow of the silane solution by using a peristaltic pump was

35 found to drastically affect the performance of the supports for the chemiluminescent assays. For example, a continuous

flow of silane solution of about 1 ml/min or less was found to be suitable for the uniform silanization of the solid support and resulted in a much enhanced reaction efficiency.

- 5 The silane solution for the sol-gel coating was prepared by mixing 5 ml tetramethoxysilane (TMOS), 5 ml 3-glycidylpropoxytrimethoxysilane (GPTMOS), 90 ml deionized water, and 100 μ L 0.1 M HCl. The pH of the silane solution was adjusted to 4.0 with a 10 % acetic acid solution, and
- 10 the solution was stirred overnight at 4 °C and 200 rpm in an airtight container for hydrolysis of the silane to yield the sol solution. The clear solution obtained was used for the sol-gel coating process. Glass capillaries tubes having a total volume of 45 μ L were rinsed in 10 % nitric acid for
- 15 5 minutes and boiled in hot water for 1 h. They were further rinsed with deionized water until a neutral pH was obtained and were then allowed to dry at room temperature. Before use, the capillary tubes were dried at 90 °C for 1 h in a hot air oven. The sol-gel coating of the glass
- 20 capillary tubes were carried out using three different approaches, i.e. static, shaking or continuous flow. In the "static" technique the capillary tubes were filled with the sol solution and left on the bench. In the "shaking" technique the capillary tubes were filled with the sol
- 25 solution, and the unsealed ends were strapped to a rocker agitated with a rocking speed of 30 rpm. The effects due to evaporation at the unsealed ends of the capillary were negligible. In the "continuous flow" method the sol solution was continuously pumped through the capillary tube
- 30 in the upward direction at a flow rate of 1 ml/min. The sol-gel coating was generally performed at room temperature for 90 min. In the case of continuous flow, however, the coating was also performed for varying times, i.e. 15, 30, 60, 90, 120, and 240 min.

After the silanization procedure each capillary tube should preferably have a minimum aperture of about 0.5 mm. In addition, it is an important aspect of the invention that the solid support used for immobilization is a porous matrix coated on the walls of the capillary. Preferably, a xero-gel is used which is a specific kind of sol-gel formed after heating of the same.

EXAMPLE 2. Detection systems.

The extent of the reaction can also be determined after the addition of an additional compounds to the assay mixture. Of particular interest are compounds which can be made to luminesce by means of photochemical, chemical, or electrochemical means. In photoluminescence the compound is induced to luminesce in a process, in which it absorbs electromagnetic radiation, for example in fluorescence or phosphorescence. In chemiluminescence a luminescent species is generated by the chemical transfer of energy to the compound in question.

When such a compound is excited into a luminescent state by chemical means a high energy derivative is obtained, e.g. by means of chemical oxidation. Upon oxidation, the chemiluminescent species emits a photon. Some compounds which can be used in luminescence-based methods, such as luminol, are not repetitive in their nature of the detectable event but produce a photon only once per molecule, and such compounds are especially suitable to use in connection with the invention. Luminol is particularly preferred as a chemiluminescent agent. However, a range of alternative compounds can be utilized, for example isoluminol, luciferin and other acridinium esters.

Thus, an effective detection system is provided for example with a label of horseradish peroxidase together with luminol and a peroxide (such as H_2O_2) in the reaction medium.

Generally, chemiluminescent reactions have short life-times and result in time constraints in the experimental procedures. This can be overcome by the use of enhancers which improve the effective duration of the light emission. Such reagents prolong the emission for a suitable period of time and enable an appropriate measurement.

Thus, by the addition of a suitable enhancer to the reaction medium, which is sufficiently chemical inert and is not affected by the peroxide reaction, the light from the medium is provided with an appropriate wavelength which is sufficiently high to enable the enhancement in the signal after the reaction. Preferably, the enhancement of the chemiluminescent reactions is accomplished by using compounds which essentially are substituted phenols, e.g. p-iodophenol. Thus, a suitable chemiluminescent cocktail comprises a solution of 0.5 mM luminol, 0.01 mM 4-iodophenol, and 50% hydrogen peroxide.

EXAMPLE 3. Incorporation of a fluorescent material.

It is advantageous to incorporate in the reaction mixture a fluorescent system which can absorb the chemiluminescent light and emit light at a different wavelength. Such a system helps to screen out the effect of light generated in the bulk sample, especially if the emitted light is viewed through an appropriate filter. For example, blue light from luminol can be absorbed by coumarin, which will emit a yellow/green light. The fluorescing agent is located on or near the signal sensing means. Alternatively, the fluorescer can be used as a marker distributed within the bulk sample so that only the locally generated chemiluminescence will be detected.

EXAMPLE 4 Sandwich immunoassay.

The silanized capillary tubes were initially coated with a first specific reagent (anti-protein antibody), incubated at 37 °C for 1 h, blocked with bovine serum

albumin and reacted with a standard protein. A second specific reagent having an enzyme label was added. The light generated from the peroxide reaction with luminol is a measure of the extent of the reaction, which was
5 monitored on a CCD camera and quantified.

In a well designed sandwich immunoassay, which is preferred, a first specific reagent (a monoclonal antibody) with a specific reactivity for the analyte (e.g. a protein) is immobilized on the solid phase. In this connection a
10 specific antibody means an antibody which has been selected from several similar suitable antibodies.

The assay medium, which is generally aqueous, contains a second binding reagent a having specificity for the analyte and an attached label. In the absence of
15 analyte no coupling will occur with the first reagent and hence no detectable signal will be generated. In the sandwich configuration the analyte essentially acts as a linking molecule between the unlabelled (first) and the labelled (second) specific reagent, and the extent of
20 coupling provides a measure of the analyte concentration in the sample.

A typical result of a sandwich assay for a protein, i.e. RBP, in a glass capillary tube is shown in FIG 1. The validation of the results obtained with accompanying
25 statistical details is depicted in FIG 2. The intensity of the chemiluminescent signal at 492 nm (O.P.) was determined.

EXAMPLE 5 Direct immunoassay.

30 A typical direct immunoassay is generally performed in an aqueous medium in contact with the solid phase, which contains the immobilized specific molecule (i.e. a known amount of the analyte to be assayed) having a certain specificity for the recognition molecule (i.e. a monoclonal
35 antibody) being determined. The assay medium consists - as in Example 3 - of a surplus quantity of the labelled

monoclonal antibody (or analogues thereof possessing identical recognition sites) which is allowed to react with a varying quantity of the immobilized analyte, and in this way a calibration curve is obtained. The unknown quantity of the analyte is determined by immobilizing the analyte on the walls of the capillary tube and filling up unreacted sites of the solid phase with an inert protein (for example a gelatin fraction). The analyte is then allowed to react with the specific and labelled reagent. The extent of the reaction is determined by the signal from the labelled reagent and is monitored by using an enzymatic assay generating either a detectable emitted light. This light can for example fluorescence or chemiluminescence which is monitored by using suitable detectors. The emitted light is compared with the standard results, i.e. the results obtained in the absence of the analyte, and a measure of the analyte concentration in the sample is obtained. The validation of the results obtained in a typical direct immunoassay is shown in FIG 3 with accompanying statistical details.

EXAMPLE 6 Competitive immunoassay.

In a competitive immunoassay, which is generally performed with the aqueous phase in contact with the solid phase containing the immobilized specific monoclonal antibody, the analyte is in the liquid phase. The competition takes place between labelled and unlabelled analyte. If the analyte is present in a sample, a suitable ratio of the labelled analyte is mixed with the sample and is allowed to react with the specific reagent of the immobilized solid phase. A control is obtained by the labelled analyte being allowed to react with the immobilized solid phase and is a measure of the total reaction. The presence of unlabelled analyte in the sample results in the loss of a certain percent of the signal, which is thus a measure of the unlabelled analyte in the sample. Usually, a dilution

of the sample determines how the original concentration of the analyte in the sample should be calculated.

EXAMPLE 7 Detection of emitted signal.

5 When incubated with a sample and a soluble labelled specific binding reagent a porous solid phase material with an immobilized specific binding agent will be localized within a liquid medium adjacent to a light sensitive detection camera, which enables the light to be detected
10 from a point source, i.e. from a unitary block or a block (c.f. FIG 4), in which the capillary tubes are placed, for the monitoring and quantification of the assay. In this connection the point source is the point, from which the light to be detected exits the capillary tube. The label is
15 capable of participating in a chemical reaction which results in the generation of light, and the liquid medium contains some additional reagents with the ability of enhancing the light.

A commercially available CCD camera was used with a
20 water jacket maintaining the temperature at the chip of -40 °C. A typical set-up is schematically shown in FIG 4.

The light generated from the bottom of the capillary tube will be channeled through the area of the immobilization matrix and most of the light will be propagated
25 in the longitudinal direction of the capillary tube to reach the silicon chip in the camera. In this connection the distance between block and camera has been optimized to achieve maximum light collection efficiency. A maximum collection efficiency is thus achieved by the light being
30 propagated to one end of the capillary tube, maximum light intensity being obtained.

A comparison was performed with previous measurements according to the state of the art, the light from the capillary tubes being monitored from a direction
35 perpendicular to the same. In a typical assay according to the invention a 6 fold increase in sensibility of the assay

was obtained when the capillary tubes were monitored from a vertical direction instead of the horizontal direction.

By positioning the capillary tubes in a vertical position in the block relative to the camera a plurality of
5 assays can be examined simultaneously, i.e. one single exposure can be used for monitoring the results of several individual capillary tubes placed in the capillary block.

The CCD camera can be used in the binned mode and the full camera mode, a sensitive assay with good detection
10 limit being obtained. For instance, 96 capillary tubes can be measured simultaneously. Thus, an assay procedure is provided, wherein multiple samples (i.e. 100 samples) can be detected in a single exposure and then computerized in order to quantify unknown amounts of the substance to be
15 assayed.

EXAMPLE 8 Method for assaying RBP and retinol.

A method for assaying RBP and retinol is provided, which method comprises of the steps of:

20 (I) immobilizing or localizing the porous immobilization matrix on the solid support of glass capillary tubes of a suitable dimension and in a suitable mode i.e. static, rocking or a continuous flow system.

(II) preparing an aqueous assay medium containing (a) a
25 porous solid phase carrier material, within which the monoclonal antibody specific for RBP is immobilized and suspended in the aqueous assay medium but is readily localized on the solid support (b) a sample containing the analyte i.e. RBP or retinol to be determined, usually in an
30 aqueous environment (c) a labelled reagent soluble or dispersible in an assay medium, which is either a second specific binding reagent participating in a reaction with the analyte or the first specific binding agent in a sandwich configuration, in which the analyte itself or an
35 analogue thereof can be detected, and the label is involved in generating a chemiluminescent signal;

(III) incubating the assay medium to enable the labelled second reagent to bind to the particulate solid phase which usually is in the porous state and containing suitable groups for the efficient coupling to the matrix;

- 5 (IV) diluting the assay medium by using a fluid with such additional reactants that may be essential to enhance the signal, especially the increase in the intensity of a chemiluminescent signal, as well as the stability of such a signal for a longer time during the measurement period; and
- 10 (V) detecting the light by using a sensitive CCD camera for measuring the chemiluminescence emitted from the point source comprising the label bound to the solid phase matrix on the glass capillary tube support.

15 EXAMPLE 9. Modification of the procedure for assaying RBP and retinol.

In another embodiment of the invention a modification of the above-mentioned procedures was carried out with a two step incubation, which involved an initial incubation

20 with the first specific reagent localized on the solid support and the sample in the absence of the labelled reagent, wherein at least the bulk of the analyte from the sample is linked to the first specific reagent. Then the incubation with the second specific reagent with the label

25 yielding a chemiluminescent signal was performed.

A localization step is usually inserted between the two incubation steps before the bulk of the sample is removed.

As a further or alternative modification, the

30 addition of one or more - or indeed all - the reagents necessary to cause the chemiluminescent reaction can take place earlier in the procedure, e.g. during stage (a) or when the labelled reagent is added in a two-stage incubation.

35

EXAMPLE 10. Use of the solid support coated with a suitable porous matrix.

The solid support of the capillary tube was coated with a porous matrix and then employed in the reaction. In this embodiment of the invention it is not necessary inject the reagents into the capillary tube. In all the applications with the various modes of direct, sandwich and competitive immunoassay, respectively, the reagent was sucked into the capillary tube directly by the capillary force. This is an unique advantage since it is economical with reference to time as well as reagents.

After the filling the capillary tube its bottom end is sealed, preferably by using a ceramic pad. A leakproof seal is thus provided for the measurement of light from the upper end of the capillary tube, which is free for detection of the generated light.

The volume of the capillary tube, approximately 40 μ l, is an extremely small volume to be assayed in comparison with conventional immunoassays which employ a few hundred microlitres of reagent volume. Thus, this assay format provides for an efficient chemiluminescent response in a minimum volume and within 90 seconds of exposure. If necessary, the risk of interfering light from any unbound label remaining in the bulk of the sample can be minimized by incorporating in the assay medium of an attenuator which blocks such non-specific binding to the porous matrix within the solid support of the capillary tube.

EXAMPLE 11. Labeling a specific binding component for the continuous generation of a chemiluminescent signal.

In this example an immunoassay is provided which utilizes a specific binding partner labelled in such a way that the label continuously generates a chemiluminescent signal in the reaction medium, usually a buffer. The signal is masked or quenched or otherwise suppressed while the

labelled binding partner is uniformly distributed within the reaction medium. The binding results in a local light flux which provides for a detectable chemiluminescent signal, the magnitude of which being dependent on the extent of binding, and the label generates light continuously while in the reaction medium. The label is washed away by a washing medium, and only specifically bound reagent is monitored within the assay format. A detectable signal is produced only when the second specific reagent with the label - or the label directly coupled to the analyte - is reacted with the first specific reagent on the support matrix.

EXAMPLE 12. Adaptation to the medium used.

Preferably, a chemiluminescent label is utilized in the inventive method, which participates by reacting with the other components of the medium. However, the medium used is often not fully transparent for the light generated by the label, and the presence of opaque or translucent medium would result in an insufficient amount of light being transferred to the detector. Consequently, the reaction medium may contain a chemical species that interacts with a component of the light generating system and enhances the production of light from the light-generating system. Subsequent immobilization of the first specific component to a matrix followed by its coupling to the analyte molecule results in a localized optical signal having a sufficient intensity to be detected.

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The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in Australia.

5 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
10 the exclusion of any other integer or step or group of integers or steps.



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CLAIMS

1. Method for determining the concentration of a stationary constituent in a light emitting assay performed
5 within capillary tubes by simultaneously detecting and quantifying the light emitted and reflected by the wall material within the capillary tubes, the light being proportional to the amount of the constituent assayed and quantified by means of an optical scanning mechanism having
10 multi-analytic capability, wherein the capillary tubes comprise multiple bores in a unitary block and the light emitted in the assay is detected from substantially the longitudinal direction thereof by means of a photosensitive device positioned at an optimal distance from the block for
15 maximum collection of light.
2. Method as claimed in claim 1, wherein the bores comprise individual capillary tubes sealed at one end.
- 20 3. Method as claimed in claim 1 or 2, wherein the wall material of the bores are made of glass, plastic or metal.
4. Method as claimed in claim 3, wherein the metal is copper.
- 25 5. Method as claimed in any preceding claim, wherein the unitary block is provided with up to 100 bores.
6. Method as claimed in any preceding claim, wherein the
30 photosensitive device is a photomultiplier tube, a photodiode, or an avalanche photodiode (APD).
7. Method as claimed in any preceding claim, wherein the detected light is quantified by means of an optical scanning mechanism having multi-analytic capability.



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8. Method as claimed in claim 7, wherein the optical scanning mechanism is a charge coupled device (CCD) camera.

9. Method as claimed in any preceding claim, wherein the assay is an enzyme-linked immunosorbent assay (ELISA) carried out on a solid support on the inside of the bore, the constituent being proportional to the amount of product formed in the assay, and an additional light emitting compound is added to the system.

10

10. Method as claimed in claim 9, wherein the constituent is obtained from a physiological sample and is a natural proteinous substance, or a molecule spontaneously binding to the same.

15

11. Method as claimed in claim 9 or 10, wherein the solid support comprises a porous matrix, on which said assay is performed.

20

12. Method as claimed in claim 10 or 11, wherein the natural proteinous substance is an acute phase protein, a bioactive peptide or a catalytic protein.

25

13. Method as claimed in claim 12, wherein acute phase protein is the retinol binding protein (RBP).

30

14. Method as claimed in any one of claims 10 to 13, wherein the molecule spontaneously binding to the natural proteinous substance is a retenoid or retinol.

15. Method as claimed in any one of claims 11 to 14, wherein the porous matrix is silane or modified silane.

16. Method as claimed in claim 15, wherein the modified silane is a xero-gel formed after heating of a silane of the type sol-gel.



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17. Method as claimed in any one of claims 12 to 15,
wherein the natural proteinous substance is bound directly
to the porous matrix and competitively assayed, the enzyme-
labelled reactant being a specific antibody to the natural
5 proteinous substance, a retenoid or retinol.

18. Method as claimed in any one of claims 12 to 15,
wherein the natural proteinous substance is bound to the
porous matrix via a first specific antibody to the same and
10 assayed in a sandwich assay, in which the enzyme-labelled
reactant is a second specific enzyme-labelled antibody to
the same.

19. Method as claimed in any one of claims 12 to 15,
15 wherein the natural proteinous substance is bound to the
porous matrix via a specific antibody to the same and the
natural binding molecule is assayed in an indirect ELISA, in
which the enzyme-labelled reactant is the natural binding
molecule.

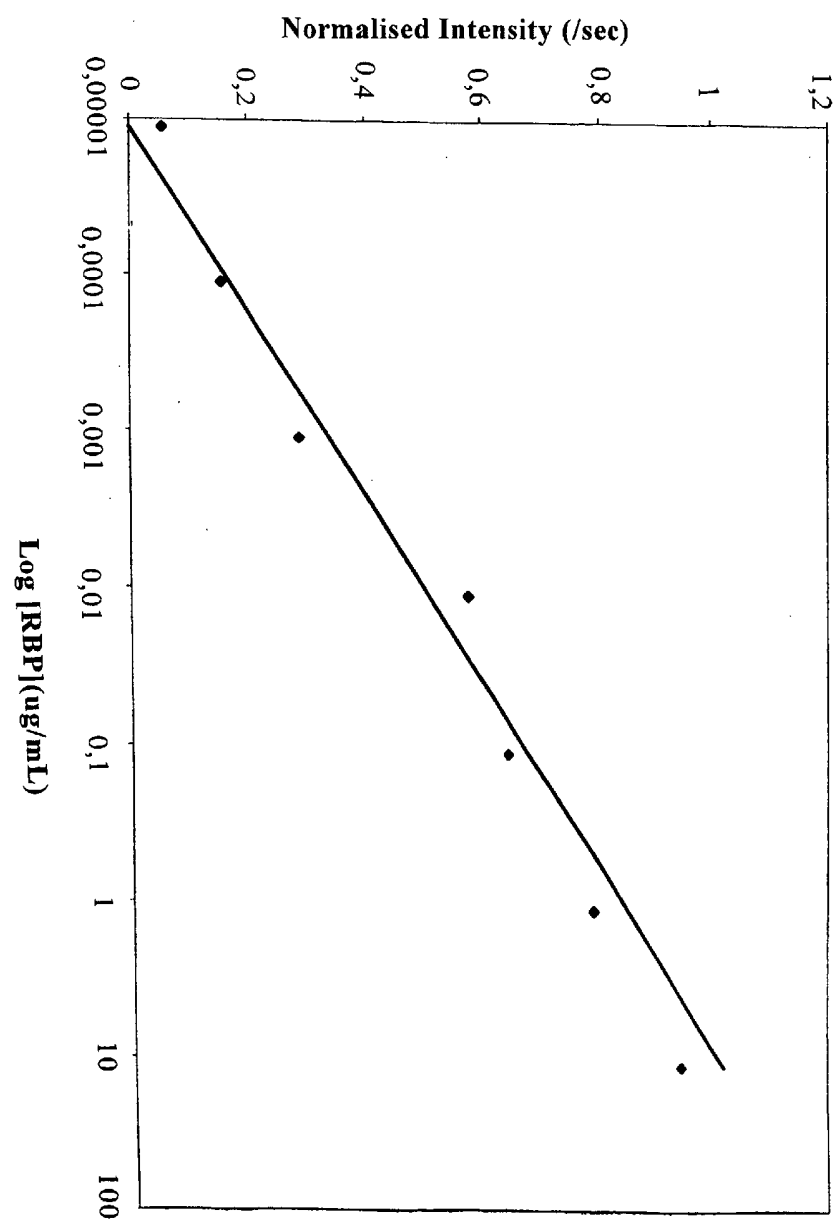
20. Method for determining the concentration of a
stationary constituent in a light emitting assay
substantially as herein described with reference to the
examples herein.

25
DATED this 10th day of April, 2002
Prolight Diagnostics AB
by DAVIES COLLISON CAVE
Patent Attorneys for the Applicant



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Fig. 1



SUBSTITUTE SHEET (RULE 26)

Fig. 2

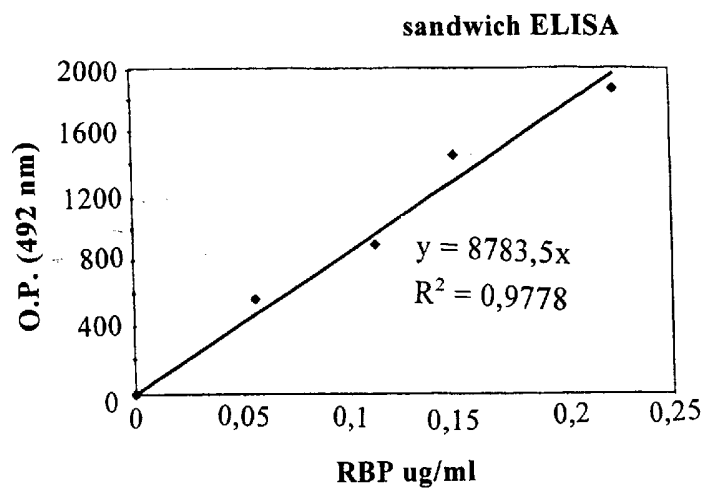
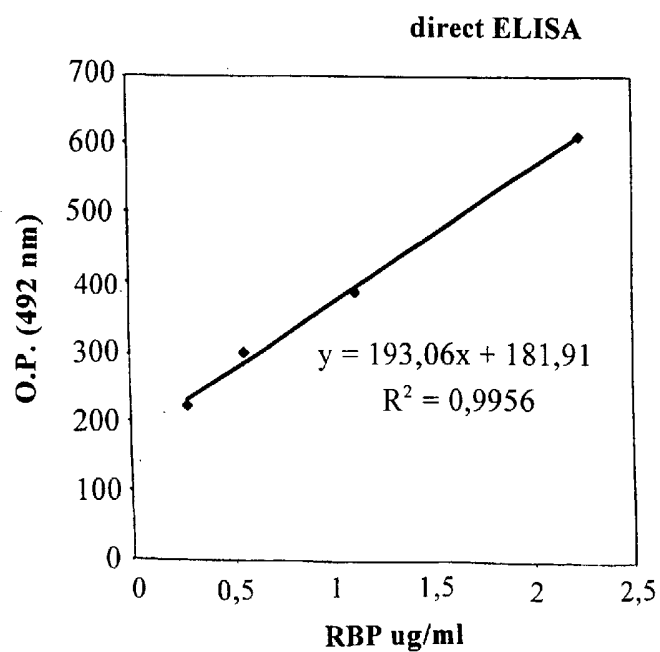


Fig. 3



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Fig. 4

