TITLE OF THE INVENTION

SYSTEM FOR ELECTROCHEMICAL QUANTITATIVE ANALYSIS OF ANALYTES WITHIN A SOLID PHASE

BACKGROUND OF THE INVENTION

1. Field of the Invention - This invention is directed to a system, test method and test device which combines affinity-chromatographic test technique and electrochemical quantitative technique for analysis of one or more analytes in a test sample. More specifically, this invention is directed to an electrochemical quantitative analysis system for determination of an analyte (e.g. proteins, hormones or enzymes, small molecules, polysaccharides, antibodies, nucleic acids, drugs, toxins, viruses or virus particles, portions of a cell wall and other compounds which have specific or characteristic markers that permit their identification) within a solid phase (e.g. test strip) by measurement of electrochemical changes of label(s) associated with a labeled substance that can be correlated with the concentration of the analyte of interest. The test strip used in this analytical system includes a solid phase affinity chromatographic medium, having an electrochemical detection system associated therewith, within the fluid pathway through said medium. The inventions hereinafter set forth in detail are applicable to quantitative analysis of virtually any analyte, whose concentration can be correlated with an electroactive species of an electroactive substance that is capable of manifesting a change in electrical response within the electrochemical cell-like environment of the test device of this invention.

2. Description of Prior Art -

A. Immunodiagnostics - Immunochemical analysis for an analyte within a solid phase (test strip) generally involves an assay in which an analyte, in a liquid sample, and a labeled substance, interact with each other, or another reaction constituent. Within the context of the prior art, it is understood that a "labeled substance" can, thus, include (a) an analyte mimic that is labeled with an indicator, (b) a labeled ligand that is specific for interaction with the analyte of interest, or (c) a complex that is formed by interaction of (a) or (b) and yet another reaction constituent. The liquid containing the labeled substance and the analyte is then transferred to a porous film or membrane, where it is drawn by diffusion or capillary

action along a fluid pathway of the membrane, and encounters one or more companion test kit reagents (at least one of which is immobilized within a definitive area thereof). This labeled substance and/or the analyte is thereafter captured by an immobilized companion test kit reagent at a test site to form a complex. If the complex is present in sufficient concentration, it produces a discernible change at such site. Where the indicator is a pigment, such as a metal (e.g. colloidal gold), or alternatively, a colored latex particle, the discernible change is generally visible to the naked eye.

The following patents are regarded as representative of this type of immunochromatographic assay. These patents are arranged in chronological order and no significance is to be attached to their order of discussion as to the patentability of the invention described and claimed herein.

***Campbell et al*, US Patent 4,703,017 (assigned to Becton Dickinson)** describes a test method for detection of an analyte with a "direct" or "particulate" indicator, specifically an indicator that can be visually detected with the naked eye, thus, making such test method acceptable for home diagnostic use; or, alternatively, in a clinical setting devoid of sophisticated analytical equipment. The direct indicator of choice is a liposome containing a pigment or equivalent colorant. The claimed test method is described as suitable for urine analysis of analytes (e.g. pregnancy test); and, thus, capable of detection of an elevated level of analyte. Accordingly, such assay is regarded as a semi-quantitative determination of the analyte of interest. Because the test involves a single step (e.g. application of the sample to the test strip), the test has become characterized in the art as a "Rapid" test.

Rosenstein*, US Patent 5,591,645 (assigned to Becton Dickinson)** describes an immunochromatographic test method based upon the principles described in commonly assigned **US Patent 4,703,017 (to *Campbell et al*).** The ***Rosenstein test device incorporates all of the test kit reagent in the "same plane" of the test strip and contemplates applying the sample directly to the test strip to reconstitute the reagents contained therein and, thereby effect the desired analysis. The direct indicator of choice can be selected from a number of equivalent colorants, however, colloidal gold adsorbed to a binding protein (e.g. antibody or antigen) is generally preferred. The claimed test method is described as suitable for urine analysis of analytes (e.g. pregnancy test); and, thus, capable of detection of an elevated level of analyte or a

semi-quantitative determination of the analyte of interest. Because the test also involves a single step (e.g. application of the sample to the test strip), the test has become characterized in the art as a "Rapid" test.

***Swanson et al*, US Patent 5,073,484 (assigned to Abbott laboratories)** describes an immunochromatographic test method utilizing multiple test zones, with each zone arranged along a common linear fluid pathway. As the test sample and test kit reagents migrate along this fluid pathway, the analyte of interest (generally associated with a direct indicator) becomes bound to an immobilized companion reagent in each of the test zones. Each of the test zones is precalibrated in reference to a standard, and thus the appearance of color in specific test zone correlated with the concentration level of analyte in the sample. Accordingly, the concentration of the analyte in the sample can be estimated from the level of color development along the fluid pathway of the test device. Because the test also involves a single step (e.g. application of the sample to the test strip), the test has become characterized in the art as a "Rapid" test.

May et al*, US Patent 5,602,040 (assigned to Unilever Patent Holdings)** describes an immunochromatographic test method analogous to the test methods described in the ***Campbell et al and ***Rosenstein*** patents discussed above. One of principles differences identified and claimed by ***May et al***, is the lyophilization of a sugar, along with the direct indicator, in the sample receiving site. The sugar addition is reportedly required to effect reconstitution of the lyophilized indicator and thus permit its interaction with the analyte in the sample.

With the limited exception of the adaptation of the foregoing Rapid Tests to detection of an elevated level of analyte, these Rapid Tests are generally unsuitable for precise quantitative monitoring an analyte level, or for the quantitative detection and differentiation of more than one analyte within a single test environment.

B. Instrumental Analysis - Analysis of fluid samples has also traditionally involved the use of instrumentation and the measurement of changes in electrical potential and/or current at given site/electrode. Typically, the electrical measurement is referenced to a second electrode, and a number of measurements made over a given interval and/or defined range of conditions.

The following patents are regarded as representative of this type of electrochemical analysis. These patents are arranged in chronological order and no

significance is to attach to the order of discussion, or to their relative significance to the patentability of the invention described and claimed herein.

Wang* US Patent 5,292,423 (assigned to New Mexico State University Technology Transfer Corp.)** describes a method and apparatus for trace metal analysis of fluid samples (e.g. drinking water, blood, urine etc.) by means of initial adsorption of the trace metal from a fluid sample onto a screen printed carbon (working) electrode that had been pre-coated with mercury. The ***Wang test platform also employs at least one additional (reference) electrode. The electrode having the trace metal was, thereafter, subjected to analysis by either Anodic Stripping Voltammetry (ASV) or Potentiometric Stripping Analysis (PSA). The reported advantages of the ***Wang*** method and apparatus include the ability to adapt his invention to on-site field testing of sample fluids for suspected pollutants with a disposable testing device.

***Brooks* US Patent 5,753,517 (assigned to the University of British Columbia)** describes a quantitative immunochromatographic assay utilizing an apparatus for detection of a particulate latex indicator in accordance with the procedures associated with so called *RAMP*™ technology. According to *Brooks et al.*, the *RAMP*™ technology utilizes latex particles in a manner similar to enzymes in ELISA assays. In the *RAMP*™ technology based analysis, a test strip, having two distinct fluorescent labeled indicators, is placed in a cartridge, and a fluid sample applied to the test strip. The analyte of interest interacts with one of the indicators forming a fluorescent complex which is captured in the test zone of the test strip, and while the second indicator remains in tact within the test strip, (so as to provide an internal standard). The *RAMP*™ technology is, thus, capable of differentiation of the fluorescent complex from the internal standard, and the amount of analyte in the sample determined thereby.

Brooks et al. alternative system for performance of his analysis, contemplate the use of optical detection methods (light scattering) and measurement of changes in electrical conductivity or resistively. In one of the suggested alternatives, *Brooks et al.* contemplates ***(US 5,753,517 - col. 7, lines 7 - 19, inclusive)*** the quantitative measurement of the analyte of interest by the electrochemical detection of a released electroactive agents, such as bismuth, gallium or tellurium ions, from a complex associated with the analyte of interest. According to *Brooks et al* one of these

alternatives involves the use of a chelating agent-protein conjugate as an indicator for the analyte of interest, and detection thereof contemplates the addition of an acidic solution for release the metal label as ions for later quantitation by anodic stripping voltammetry (as described by *Hayes et al*, Anal. Chem. 66:1860-1865 (1994).

The following published technical literature is regarded as representative of this type of electrochemical analysis. These papers are arranged in chronological order and no significance is to attach to their order of discussion, or to their relative significance to the patentability of the invention described and claimed herein.

Electrochemical techniques have provided challenges associated with affinity-chromatographic reactions, because of their stable and sensitive signal, lower level of detection limit, simple operation and cost-effectiveness. Pioneering studies by Henieman et al.(Hayes, F.H. et al., Anal. Chem., 66, 1860-1865 (1994)) illustrated the use of metal ion labels for heterogeneous immunoassays with Anodic Stripping Voltammetric (ASV) detection. Such immunoassays involved covalently linking chelating agent to a protein to serve as a chelon for the metal label. Following competitive equilibrium between the labeled and unlabeled protein for the antibody (immobilized on the surface of a polyester tube), the metal label was released and transferred to an electrochemical cell for an ASV detection with a hanging mercury drop electrode (HMDE) and a deaerated solution. Similarly, *Wang et al.* (Wang J., Anal. Chem., 70, 1682-1685 (1998)) employed an antibody-coated, screen-printed sensor, performed the entire assay protocol directly on the surface of the disposable strip, and employed the highly sensitive potentiometric stripping mode for detecting the released metal ion label in microliter solutions. As desired for decentralized sensing applications, such an on-chip protocol offers several advantages compared to conventional ASV- based immunoassays, including simplified operation (e.g., the elimination of the separation and reagent volumes, elimination of toxic mercury drops, and a more sensitive stripping detection mode). The *Wang et al.* system requires relatively long incubation periods for pre-conditioning the sensor (prior to use) and for interaction with the sample; and, multiple washing steps, both of which make this method impractical and cumbersome.

OBJECTS OF THE INVENTION

It is the object of this invention to remedy the above and related deficiencies in the prior art.

More specifically, it is the principle object of this invention to adapt rapid affinity chromatographic electrochemical analysis to quantitative determination of analytes of interest.

It is another object of this invention to provide a system for electrochemical quantitative analysis of an analyte(s) within a solid phase test format that is comparable in ease of use to rapid immuchromatographic assays.

It is yet another object of this invention to provide a system for electrochemical quantitative analysis of an analyte(s) within a solid phase test format involving a simplified means for detection of labels by potentiostatic or potentiometric measurement of changes within the solid phase that are attributable to the concentration(s) of label(s) within the test site.

It is still yet another object of this invention to provide a system for concurrent electrochemical quantitative analysis of a common test sample for multiple analytes within a solid phase test format involving a simplified means for detection of label(s) by stripping voltammetry

SUMMARY OF THE INVENTION

The above and related objects are achieved with the electrochemical quantitative analysis system, method and test strip of this invention for determination of the concentration of an analyte within a solid phase test environment. Initially, a test sample solution and a labeled substance are initially contacted, under assay conditions, within a solid phase test environment, and caused to migrate along a fluid pathway therein. Irrespective of the assay format (competitive assay, sandwich assay, etc.), the labeled substance is concentrated (bound) within a delimited area of the solid phase. Within the context of the system and analytical method of this invention, a "labeled substance" can include any suitable electroactive material that can be isolated within a delimited area of the solid phase, under assay conditions, or which can release an electroactive component within such delimited area, (also collectively hereinafter referred to as the "electroactive species"); and, such electroactive species thereafter, undergo an electrochemical transition (e.g. redox) incidental to

electrochemical quantitative analysis. The observed measurement (transition) of the electroactive species can be directly or inversely related, (e.g. by comparison to a standard curve), to the concentration of the analyte of interest in the test sample.

The two principal types of electroanalytical measurements for determination of the concentration of the analyte of interest are potentiometric and potentiostatic. In each of these analytical protocols, two electrodes are required/involved, and a contacting sample (electrolyte) solution, which together constitute an electrochemical cell-like environment within the test strip. The electrode surface is, thus, the junction between the ionic conductor (e.g. electrolytes from the aqueous fluid sample, test kit reagents, etc.) and an electronic conductor. Within this electrochemical cell-like environment of the test strip, the electrode that responds to the analyte of interest (or an electroactive substance that is indicative of the analyte of interest), is termed the "indicator" or "working" electrode, whereas the electrode that is maintained at a constant potential is termed the "reference" electrode (its response being independent of the sample solution).

In one of the embodiments of this invention, the immobilized labeled substance is contacted with a release reagent to initially cause the release/displacement of the label from the immobilized labeled substance within this delimited area. In the case of a metal label, for example, the released/displaced metal can be further interacted with the substance in the release reagent solution, to form an metal film (or metal surface-active complex) on the surface of the working electrode within the delimited area of the solid phase. Thereafter, the delimited area of the affinity chromatographic test strip is subjected to potentiostatic measurement, by anodic stripping voltammetry of the label from the metal film (or metal surface-active complex) on the working electrode, which causes the label, to undergo yet a second electrochemical transition, (conversion of the label from the reduced form to the oxidized state). This second electrochemical transition of the label (from the reduced to the oxidized state) has a characteristic fingerprint that can be monitored and which, when compared to a standard curve, can correlate directly with the concentration of the analyte in the sample.

The stripped label from the metal film (or metal surface-active complex) and, thus, the analyte of interest, can be quantified by measurement of changes within the delimited area of the test strip by potentiostatic electrochemical techniques. The

specifics of this electrochemical quantitative analysis, involve the application of an electrical potential to the delimited area, over a defined range of potentials, and the monitoring the rate of electron transfer (current) at each potential. The variation of electrical potential is analogous to the taking of a series of optical measurement of a colored indicator at varying the wavelengths. The electrical potential applied to the electrode drives (forces) the targeted electroactive species to gain or lose electrons (reduction or oxidation, respectively) at a given rate that is indicative of the concentration of such electroactive chemical species. Accordingly, the resultant current not only reflects the rate at which the electrons move across the electron/solution interface, but also (when compared to a standard), the concentration of the analyte in the test sample. These potentiostatic techniques can, thus, measure any chemical species that is electroactive, (e.g. that can be made to reduce or oxidize within the environment of an electrochemical cell)

One of the preferred designs of the test strip useful in the electrochemical quantitative analysis system of this invention has multiple components and multiple functional areas to (a) control the volume and rate of absorption of sample by the test strip; (b) accommodate the controlled interaction of the sample and a labeled substance with an immobilized binding substance within a delimited area of the test strip; (c) effect the separation of the labeled substance from the endogenous components of the sample; (d), the concentration of the labeled substance within a delimited area of the test strip for electrochemical processing; and, (e) the quantitative determination of the analyte of interest by electrochemical means.

In another of the preferred embodiments of the foregoing test strip design, the sample and the labeled substance can be combined within a bibulous pad (e.g., fiberglass) that is maintained in fluid communication with a solid phase affinity chromatographic test medium. Upon transfer of the sample thereto, the labeled substance is reconstituted, and the resultant labeled substance and/or a complex thereof which is formed with the analyte of interest, drawn into such test medium. As this fluid and its constituents, (e.g. sample, test kit reagent and reaction products thereof), diffuse into and within the affinity chromatographic test medium, the labeled substance becomes bound to a delimited area (also hereinafter "test site") along this fluid pathway. Typically, this delimited area can be defined by means of an immobilized binding substance that is specific for interaction with the analyte, an

analyte mimic or a complex of the analyte and/or a labeled ligand (e.g. by binding to an epitope on the analyte). As the analyte, analyte mimic or a complex of the analyte and/or a labeled ligand becomes increasingly concentrated at the test site, it can be measured and the amount thereof correlated with the analyte in the test sample.

The advantages of such controlled potential (potentiostatic) techniques include high sensitivity, selectively toward an electroactive species, a wide linear range, portable & low cost instrumentation and speciation capability.

In order to accommodate multiple functional components within the integral device of the preferred test strip design of this invention, all of the components are preferably arranged/mounted on a common support or backing layer (typically an inert plastic, e.g. Mylar). The substrate of this test device can be pre-printed with a conductive material to afford electrical contact (direct or inductive coupling) between the affinity chromatographic test medium of the test strip and an electrochemical analyzer that is configured to measure subtle electrical changes within the delimited areas in the test strip. As more fully set forth in the description of the Figures, which follows, this support layer is typically imprinted at two or more locations with a conductive material or metal salt, to form what are referred to hereinafter as "electrodes". These electrodes are spatially arranged along the support layer to coincide with delimited areas of the solid phase medium. The electrode coincident with the delimited area of the test site is referred to as the "working electrode". Depending upon the electrochemical method of analysis, the test strip generally requires at least one additional (e.g. "reference" electrode) to form the plates of an electrochemical cell.

After having concentrated the labeled substance within the delimited area of the test site, it can be measured by a number of electrochemical techniques. As noted above, it may be desirable, preliminary to performing such measurement, to first isolate the label from the complex by "pre-concentration" thereof on the working electrode. This "pre-concentration" process can involve the release/displacement of the label from the complex and the capture of the label in a reduced form (e.g.-metal film (or metal surface-active complex)) on the working electrode where it is more electrochemically available or active. Upon completion of this pre-concentration process, the resultant metal film (or metal surface-active complex) can be subjected to potentiostatic electrochemical quantitative analysis by anodic stripping voltammetry.

In the context of this preferred analytical system (e.g. potentiostatic electrochemical quantitative analysis by anodic stripping voltammetry), the label is reoxidized, under electrochemical quantitative analytical conditions, and this electrochemical transition of the label (from the reduced to the oxidized state) is monitored. The current signal that is generated during this transition (peak and area) is dependent upon a number of system variables, the characteristics of the metal label and the electrode geometry. In each instance, however, these variables can be optimized by empirical adjustment; and, the analysis conditions tailored to correspond to standard curves which are used to correlate electrical signal response to analyte concentration. More specifically, Fig. ~~3~~ 4 is a graphical depiction of current signal response of a lead metal film on a carbon electrode to anodic stripping analysis from low to high concentration of lead. Similarly, Fig. 4 5 illustrates the variation in signal (current) intensity, as a function of the duration of "pre-concentration" interval, (prior to electrochemical analysis) in detection of bismuth. In the graphical depiction of this variable shown in Fig. 4 , 5 the longer the pre-concentration interval (which varied from 1 to 15 minutes) the greater the current signal intensity. Similar correlation/optimization in analytical process conditions is made for each of the labels that are selected and standard curves for each created, as appropriate, to correlate with the dynamic range of conditions likely to be encountered for a given analyte and over a range of concentration. Figure 6 shows the responses of multiple labels in a detecting window in one solution containing Indium, Lead, Copper and Bismuth. Using multiple labels in this invention, analytes of interest in one sample solution can be simultaneously quantified.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is an illustration, in perspective, of test strip component of the solid phase, electrochemical, quantitative analysis system of this invention (A: a two electrode system; B: a three electrode system).

Fig. 2 is a cross-section of the test strip of Fig. 1 along plane AA.

Fig. 3 is an illustration of the test device of this invention.

Fig. 4 graphically depicts a current signal response curve of a bismuth/mercury amalgam on a carbon electrode to anodic stripping analysis over a range of concentrations (1ng/ml to 50 ng/ml of pre-concentrated bismuth metal label).

Fig. 5 graphically depicts a current signal response curve illustrating the variation in signal (current) intensity, as a function of the duration of "pre-concentration" interval, (prior to electrochemical analysis) in detection of bismuth. In the graphical depiction of this variable shown in Fig. 4, the longer the pre-concentration interval (which varied from 1 to 15 minutes) the greater the current signal intensity.

Figure 6 graphically depicts a current signal response curve illustrating the responses of multiple labels in a detecting window in one solution containing Indium, Lead, Copper and Bismuth metal labels.

Fig. 7 graphically depicts a current signal response curve illustrating the electrochemical analysis of a standard Myoglobin antibody solutions at different concentration in accordance with the system, method and test strip (Figs. 1 & 2) of this invention.

Figure 8 graphically depicts a current signal response curve illustrating six repeat measurements of a 2.5 ng/ml HBsAg in serum sample using the system, method and test strip (Figs. 1 & 2) of this invention..

Figure 9 graphically depicts a current signal response curve illustrating the measurement of HBsAg in a serum sample, using a HBsAg antibody labeled by Lead, Copper and Bismuth ions.

Figure 10 graphically depicts a current signal response curve illustrating measurement of HCG (left), HBsAg (right) and control label(middle) in serum sample, using the system, method and test strip (Figs. 1 & 2) of this invention.

Figure 11 graphically depicts a current signal response curve illustrating competitive detection of human NPOR (25 mer oligonucleotide), A (upper) is the signal of blank solution (without human NPOR) and B(lower) is the signal of solution containing human NPOR.

DETAILED DESCRIPTION OF THE INVENTION INCLUDING PREFERRED EMBODIMENTS

One of the distinct advantages of the system, method and test strip of this invention, is the unique ability to perform concurrent electrochemical quantitative analysis for multiple analytes within a given test sample. Thus, it is possible to perform a drug of abuse panel of assays on a given sample by simply utilizing a different labeled substance for each of the analytes of interest within a given sample. Accordingly, it is understood that the discussion which follows is not intended to either limit the scope or application of the invention to analysis for a single analyte; and, that such description is only limited to analysis to a single analyte for ease of understanding and exemplification.

As noted above in summary fashion, and once again emphasized, the electrochemical, quantitative analysis system of this invention is based upon the initial concentration of a labeled substance within a delimited area of solid phase test environment; and, thereafter, causing an electronic transition in the form of the label, so as to produce a measurable electrical signal within the environment being monitored, which can be correlated with the quantity of the analyte within the test sample.

In certain instances, it may be appropriate (as an intermediate step in the analysis routine) to release/displace the label from the labeled substance, and thereby convert it, or cause it to react with another substance, that permits greater accessibility for electrochemical quantitative analysis. Where for example, the labeled substance is labeled with a metal, (e.g. labeled ligand), the isolation and concentration thereof within the delimited area of the test strip generally requires release and/or displacement thereof from its ligand constituents and the conversion, or reaction thereof, so as to cause/effect its transition to a form (e.g. readily oxidizable or reducible state), which is more readily available for electrochemical state to another. In one of the preferred embodiments of this invention, the labeled ligand is comprised of a functional group that forms a chelate-like coupling to the metal label. This coupling is, however, pH sensitive, and thus, the metal label can be released/displaced from the complex, after concentration within a delimited area of the solid phase, by simply contact thereof with an acidic salt solution.

Alternatively, where the labeled ligand is comprised of an indicator (e.g. metal) encapsulated within a liposome, such as described in ***Campbell, et al,*** US Patent 4,703,017 (previously discussed herein), that is in turn conjugated to a ligand, the integrity of liposome capsule can be readily compromised/dissolved with a number of common lipid solvents (e.g. detergents). The metal can, thus, be released from the ligand constituents of the labeled ligand, and made more readily accessible/available for quantitative analysis thereof.

Where the electrochemical quantitative analysis utilizes anodic stripping voltammetry, as the analytical method of choice, the released/displaced metal label can be reacted with mercuric ions within the acidic salt solution to form an amalgam on the working electrode contiguous with the delimited area of the solid phase medium.. The electrochemical reduction of the metal label is, thereafter, reversed under subsequent electrochemical quantitative analysis, and the subtle electrical changes monitored during such transition and correlated with the presence and concentration of analyte in the test sample.

In order to provide for ease of use and portability of the system and methods of this invention, the quantitative analysis system of this invention utilizes an affinity chromatographic test strip wherein a delimited area thereof is configured to concentrate the analyte of interest. The test devices illustrated in Figs. 1 & 2 are representative of a preferred embodiment of this test device.

The test device depicted in Fig. 1 & 2 is initially composed of an affinity-chromatographic test strip and an electrochemical detector system.

The affinity-chromatographic test strip includes a plurality of discrete areas; and, is preferably, a composite of at least two (2) and more preferably three (3) discrete components, in fluid communication with one another. As shown in Fig. 1, the test strip comprises a sample collection pad (12), having labeled substance (14) (e.g. labeled ligand or labeled analyte mimic) pre-disposed within the sample collection pad, either coincident with or contiguous with the sample collection pad (14), a membrane (22), having substance (18) (e.g., ligand or analyte mimic) pre-coated on the membrane, and a absorbent pad which is used to force migrating of sample solution. In the preferred embodiments of this invention, the labeled substance is lyophilized (to permit stabilization thereof prior to use); and, thereafter reconstituted upon contact with the liquid sample.

Within the context of the system and analytical method of this invention, a "labeled substance" can include any suitable electroactive material that can be isolated within a delimited area of the solid phase, under assay conditions, or which can release an electroactive component within such delimited area, (also collectively hereinafter referred to as the "electroactive species") and, thereafter, undergo an electrochemical transition (e.g. redox) incidental to electrochemical quantitative analysis. The observed measurement (transition) of the electroactive species can be directly or inversely related, (e.g. by comparison to a standard curve), to the concentration of the analyte of interest in the test sample.

In the preferred embodiments of this invention, the labeled test kit reagent is typically comprised of a protein or ligand associated with a metal label. Upon contact thereof with the sample, this metal labeled reagent interacts with the analyte of interest in the sample to form a complex. A labeled substance, which can be utilized in the affinity chromatographic isolation/concentration of the analyte of interest, comprises and a protein/ligand and a metal label that is preferable prepared in accordance with the procedures described in *US 4,732,974* and in the technical literature, Gary, et al, Covalent Attachment of Chelating Groups to Macromolecules, Biochem. And Biophys. Res. Comm., Vol. 77, No. 2(1977)pp581-585(which is herein incorporated by reference in its entirety). In brief, a metal label can be conjugated to the protein/ligand specific for the analyte, by initial modification of the protein/ligand by covalently binding thereto of an "exogenous chelating group" having an affinity for the metal label. The chelating groups specific for the metal indicator of interest, can be covalently coupled directly to the binding protein/ligand or indirectly coupled through an intermediate or linking group. The coupling site must of course be remote from the immuochemically active site that is specific for the analyte of interest. Metal labels that are particularly suitable for use in the synthesis of the labeled substances, that can be used as the test kit reagents in the systems and methods of this invention, include Mg, Al, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Sr, Y, Zr, Mo, Tc, Pd, Cd, In, Sn, Sb, Ba, Hf, W, Re, Hg, Tl, Pb, La, Ce, Rh, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Yd, Th, U, Pu and the isotopes thereof.

Another of the labeled substances that can be utilized in the affinity chromatographic isolation/concentration of the analyte of interest comprises an analyte mimic coupled to a liposome. The preparation of liposome labeled

immunoreagents, and their use in affinity chromatographic assays, is well-known and documented in the technical literature, ***Roberts, et al,*** Investigation of Liposome-Based Immunomigration Sensors for the Detection of Polychlorinated Biphenyl, *Anal. Chem*, Vol. 57, No 5 (February 1, 1995) pp. 482-491.

The labeled substance and/or complex of the labeled substance and the analyte, is carried by diffusion/capillary action from the sample application pad along the fluid pathway of the solid phase affinity chromatographic medium (16) to a downstream test site (18) where it is concentrated and immobilized. This can be accomplished in the traditional manner, by the use, for example, of a companion test kit reagent in the form of an immobilized binding protein or other expedient, that is specific for interaction with the analyte/metal complex. Thus, as the labeled substance and/or complex migrates/diffuses along the fluid pathway of the test device, it encounters the immobilized binding substance, and is thereupon captured so as to accumulate at the test site. After a suitable reaction period, sufficient analyte/labeled substance has accumulated in the delimited area of the test strip to provide the requisite amount to be detected and quantified by electrochemical analysis.

The electrochemical detector system (26) is prepared by printing two electrodes (for a two electrode system) or three electrodes (for a three electrode system) on a matrix material(e.g., Mylar, plastic or other material which is relatively electrically (non-conductive) insulating. A supporter (24) with double side adhesive is used to combine the affinity-chromatographic test strip and the electrochemical detector system. A hole in the supporter is between the electrochemical detector system and the test zone (18) in the membrane.

The detection and measurement of the quantity of the analyte in the sample is accomplished by means of equipment that can measure subtle electrochemical changes associated with an electroactive species, as for example, changes in current or voltage caused by the transition of such species form the reduced to an oxidized form. In practice, this can be accomplish by insertion of the affinity chromatographic test strip of this invention into a suitable measuring device and thereby electrically coupling the device, to electrodes (26) at the test site (18). The electrical coupling of the monitoring instrument to the affinity chromatographic test strip can be by direct (physical contact) or indirect (inductive) coupling of the instrument to the electrodes associated with the delimited area/test site of the test device. The test device is also

electrically concurrently coupled to the test strip at another location (28), to provide a point of reference or internal standard for comparison of readings at the test site. Thereafter, an electrical potential is applied to the test site to excite/convert the label from one form/state to another, and such transition monitored. This transition is manifest in a number of ways, depending upon the nature and/or magnitude of the electrical force applied to the test site, and other system variable relating to pre-concentration and the inherent characteristics of the label.

Another design for this test device is depicted in Fig. 3 wherein the affinity-chromatographic test strip and electrochemical detector system are placed within a housing which is composed of an isolator (30), a top section or cover (32) and a bottom section or base (34); the base being adapted for coupling to the cover, so as to seal and protect the affinity-chromatographic test strip and electrochemical detector system therein and, thus, provide a tamper resistant environment. The isolator (30) is funnel shaped and adapted to direct test kit reagents onto the test site and thereafter be inserted into the housing through a hole in the cover of the housing, whereby it punches out a defined area within detection zone (test site) of membrane so as to isolate a delimited area (volume) of membrane, in relation to the volume of the test kit reagents, for pre-concentration of the indicator.

EXAMPLES

The Examples that follow further define, describe and illustrate a number of the preferred embodiments of the test kit reagents and electrochemical methods of analysis of this invention. Apparatus and techniques used in the synthesis of the materials described/used in these Examples are standard, unless otherwise indicated. Parts and percentages appearing in such Examples are by weight, unless otherwise indicated.

EXAMPLE 1

1. Preparation of ions labeled proteins (e.g., antibody and antigen)

Diethylenetriamine pentaacetic acid and Triethylamine were dissolved in water with gentle heating. The resulting solution was lyophilized to yield a glassy residue and then such residue was dissolved in acetonitrile with gentle heating. This solution was added to isobutychloroformate in an ice bath during stirring for complete reaction. The resulting anhydride Diethylenetriamine pentaacetic acid was then

reacted with protein in a protection solution at a certain molar ratio at room temperature. The reaction mixture was then dialyzed against a citrate buffer. An excess of label solution was added to the dialyzed solution and incubated at room temperature. The labeled protein was dialyzed against phosphate buffer solution. The proteins (AntiHBsAg Ab, AntiHCG Ab, AntiMyoglobin Ab, AntiTroponine I Ab, G-SAG, IgG-Fc, HIV and HCV etc.) are well labeled by metal ions (e.g., Bismuth, Lead, Indium, Thallium and copper) using the method above in our Lab.. The labeled protein was used to prepare a solid phase & affinity chromatographic test strip.

2 *Preparation of affinity chromatography solid phase test device*

Two conductive deposits or areas (for a two electrode system), corresponding to the test site (carbon ink electrode), and the reference site (Ag/AgCl ink electrode) are ink jet printed on a polyester lamina. The size of each printed area is 4X4mm and approximately 5/1000 inch thick.

A membrane, having an immobilized ligand predisposed at the test site (detection zone), was laminated to the backing layer so as to align the test site over the carbon ink electrode system. A fiberglass pad (containing the label/ligand conjugate) and excess fluid absorbent medium are each positioned relative to each end of the laminate to form the assembled test strip. The test device fabricated in accordance with this Example is depicted in Figs. 1, 2 & 3.

3. Measurement of the analyte

In the course of performance of an assay for HCG, HBsAg, Myoglobin or multiple analytes utilizing the foregoing metal ion labeled ligand(s) and affinity chromatographic solid phase test device, a liquid sample is first applied to the sample application region followed by the migration of the liquid sample by capillary action along the affinity chromatographic strip towards the other end of the affinity chromatographic strip. As the liquid migrates within the sample application pad, and reconstitutes the labeled ligand(s) contained therein, analyte(s) in the sample react with the labeled ligand(s) forming a complex(es) which is transferred to and drawn into the affinity chromatographic test medium. When the complex reaches the detection region (test site), the analytes in the complex react with the immobilized ligand(s) in the detection region forming a labeled ligand-analyte-ligand complex(es),

while the unbound constituents and fluid fraction continue to be drawn and flow into the absorbent medium at the opposite end of the device. The formation of the complex in the detection region is then registered and the labeler metal ions quantified by the Square Wave Stripping Voltammetry (SWSV). As described above, this stripping procedures involves preconcentration (or formation adsorptive accumulation of a surface-active complex) and determination of the metal ions.

The foregoing analysis is particularly advantageous where the test sample is a turbid solution that can interfere with more traditional optical measurement techniques, such as a blood or a urea sample solution Fig. 7 graphically illustrates an electrochemical analysis of a standard Myoglobin antibody solution in different concentration in accordance with the system, method and test device (Fig. 3) of this invention. The low detection limits and sensitivity of this assay due, in part, to the high molar labeling ratio of lead ions relative to the anti-Myoblobin antibody, and the duration of the interval of pre-concentration of the lead on working electrode. Figure 8 shows repeat measurements of 2.5 ng/ml HBsAg in serum sample in accordance with the system, method and test device (Fig. 3) of this invention. A reproducible response is observed over six repeat measurements. Also, simultaneous detection for multiple analytes of interest is available with the system, method and test device (Fig. 3) of this invention. Figure 9 is the response of HBsAg in serum sample, using a HBsAg antibody labeled by Lead, Copper and Bismuth ions, different ion can be seen in the response curve, which shows that complexes(Anti-HBsAg antibody-HBsAg antigen-Anti-HBsAg antibody-Anti-HBsAg antibody labeled different metal ion complexes) were formed and detected in the test zone. Figure 10 is a simultaneous detection for HCG(left) and HBsAg(right) in serum sample, using the system, method and test device (Fig. 3) of this invention, the peak at middle is a control test which tells that the device is work.

EXAMPLE 2

Nucleic acids (Oligonucleatides, DNA and RNA) sensor

1. Preparation of ions labeled oligonucleatide

Diethylenetriamine pentaacetic acid and Triethylamine were dissolved in water with gentle heating. The resulting solution was lyophilized to yield a glassy residue and then such residue was dissolved in acetonitrile with gentle heating. This

solution was added to isobutychloroformate in an ice bath during stirring for complete reaction. The resulting anhydride Diethylenetriamine pentaacetic acid was then reacted with oligonuleatides at a certain molar ratio at room temperature. The reaction mixture was then dialyzed against a tris buffer. A excess of label solution was added to the dialyzed solution and incubated at room temperature. The labeled oligonucleotide was dialyzed against tris buffer solution. The labeled oligonucleotide was used to prepare a solid phase & affinity test sensor.

2 Preparation of affinity chromatography solid phase test device

Two conductive deposits or areas (for a two electrode system), corresponding to the test site (carbon ink electrode), and the reference site (Ag/AgCl ink electrode) are ink jet printed on a polyester lamina. The size of each printed area is ~ 4X4mm and approximately 5/1000 inch thick.

A membrane, having an immobilized ligand predisposed at the test site (detection zone), was laminated to the backing layer so as to align the test site over the carbon ink electrode system. A fiberglass pad (containing the label/oligonucleatide conjugate) and excess fluid absorbent medium are each position relative to each end of the laminate to form the assembled test strip. The test device fabricated in accordance with this Example is depicted in Figs. 1, 2 & 3.

3. Measurement of the human NPOR

In the course of performance of an assay for human NPOR utilizing the foregoing metal ion labeled oligonucleatide and affinity chromatographic solid phase test device, a liquid sample contain the oligonucleatide of interest is first applied to the sample application region followed by the migration of the liquid sample by capillary action along the affinity chromatographic strip towards the other end of the affinity chromatographic strip. As the liquid migrates within the sample application pad, the labeled oligonucleatide imbedded in the contact pad is carried out and interacts competitively with the complement oligonucleatide coated at the test zone to the oligonucleatide of interest in sample solution, forming a labeled oligonucleatide-complement oligonucleatide complex and oligonucleatide-complement oligonucleatide complex, while the unbound constituents and fluid fraction continue to be drawn and flow into the absorbent medium at the opposite end of the device. The formation of the complexes in the detection region is then registered and the

labeler metal ions quantified by the Square Wave Stripping Voltammetry (SWSV). As described above, this stripping procedures involves preconcentration (or formation adsorptive accumulation of a surface-active complex) and determination of the metal ions. Figure 11 shows competitive detection of human NPOR (25 mer oligonucleotide), using the system, method and test device (Fig. 3) of this invention. A(upper) is the signal of blank solution (without human NPOR) and B (lower) is the signal of solution containing human NPOR. Such result offers a method to detect and sequence DNA..

WHAT IS CLAIMED IS:

1. In a system for the quantitative determination of an analyte in a solid phase test environment which utilize (a) an affinity chromatographic medium having one or more delimited areas specific for binding and/or capture of an affinity substance associated with the analyte of interest, and (b) a labeled ligand test kit reagent specific for interaction with the analyte of interest, the improvement comprising:

A. a solid phase test strip comprising a solid phase, affinity chromatographic medium having a fluid pathway defined therein, a sample collection medium in fluid communication with said medium and a means for establishing electrical contact with one or more delimited areas of said affinity chromatographic medium,

(1) said affinity chromatographic medium being further characterized as having at least one delimited area wherein an immobilized binding substance is present therein, the immobilized binding substance being specific for an affinity chemical interaction with a component of a labeled substance selected from the group consisting of an analyte mimic labeled with an indicator, a ligand labeled with an indicator or a mixture thereof,

(2) said sample collection medium being further characterized as having disposed therein at least one labeled substance, each of said labeled substance being characterized as having a different label and being further characterized as specific for interaction with only one analyte of interest;

B. at least one labeled substance comprising a label, which is electroactive under electrochemical analytical conditions, and capable of undergoing an electrochemical transition in the form of the label, and thereby causing a discernible electrical signal, characteristic of said label, that can be correlated with a standard for determination of the presence and quantity of each analyte of interest in the test sample; and

C. electrochemical monitoring means, including means for (1) establishing electrical contact, under electrochemical analytical conditions, between said delimited area of said affinity chromatographic medium and an electrochemical

sensor, (2) sensing an electrical signal from an electrochemical sensor, and (3) means for interpretation and/or correlation of the signal from said electrochemical sensor in respect to an analyte of interest.

2. The improvement of Claim 1, wherein the affinity chromatographic medium is further characterized as having a second delimited area and a second means for establishing electrical contact therewith under assay conditions, and means for comparison or reference thereof to said electrochemical changes, characteristic of each label.

3. The improvement of Claim 1, wherein the label is a metal selected from the group consisting of Mg, Al, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Sr, Y, Zr, Mo, Tc, Pd, Cd, In, Sn, Sb, Ba, Hf, W, Re, Hg, Tl, Pb, La, Ce, Rh, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Yd, Th, U, Pu and the isotopes thereof.

4. The improvement of Claim 1, wherein label comprises a labeled ligand or liposome containing a substance that is electroactive under electrochemical analytical conditions.

5. The improvement of Claim 1, wherein the electrochemical analytical conditions comprise potentiostatic measurement of an electroactive substance indicative of the analyte of interest.

6. The improvement of Claim 1, wherein the electrochemical analytical conditions comprise potentiometric measurement of an electroactive substance indicative of the analyte of interest.

7 In a method for quantitative determination of an analyte in a solid phase test environment which utilize (a) an affinity chromatographic medium having one or more delimited areas specific for binding and/or capture of an affinity substance associated with the analyte of interest, and (b) a labeled ligand test kit reagent specific for interaction with the analyte of interest, the improvement comprising:

A. Providing,

(1) a solid phase test strip comprising a solid phase, affinity chromatographic medium having a linear pathway defined therein, a sample collection medium in fluid communication with said medium and a means for establishing electrical contact with one or more delimited areas of said affinity chromatographic medium,

(a) said affinity chromatographic medium being further characterized as having at least one delimited area wherein an immobilized binding substance is present therein, the immobilized binding substance being specific for immunochemical interaction with said analyte or the analyte component of a complex formed between said analyte and a labeled ligand test kit reagent,

(b) said sample collection medium being further characterized as having disposed therein at least one labeled ligand test kit reagent, each of said test kit reagent being characterized as having a different label and being specific for interaction only one analyte of interest;

(2). at least one label ligand test kit reagent comprising a label, which upon release or dissociation from said ligand, is capable of undergoing an electrochemical transition in the form of the label, and thereby causing a discernible electrical signal, characteristic of said label, that can be correlated with a standard for determination of the presence and quantity of each analyte of interest in the test sample; and

(3) electrochemical monitoring means, including means for (a) establishing electrical contact, under electrochemical analytical conditions, between said delimited area of said affinity chromatographic medium and an electrochemical sensor, (b) sensing an electrical signal, and (c) means for interpretation and/or correlation of the signal from said sensor electrochemical sensor in respect to an analyte of interest.

B. Applying a sample to said solid phase test strip, under assay conditions, so as to initiate analysis thereof; and

C. Monitoring said test strip, under quantitative analysis conditions, for electrochemical changes within said delimited area of said test strip.

8. The improvement of Claim 7, wherein the affinity chromatographic medium is further characterized as having a second delimited area and a second means for establishing electrical contact therewith under assay conditions, and means for comparison or reference thereof to said electrochemical changes, characteristic of each label.

9. The improvement of Claim 7, wherein the label is a metal selected from the group consisting of Mg, Al, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Sr, Y, Zr, Mo, Tc, Pd, Cd, In, Sn, Sb, Ba, Hf, W, Re, Hg, Tl, Pb, La, Ce, Rh, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Yd, Th, U, Pu and the isotopes thereof.

10. The improvement of Claim 7, wherein label comprises a liposome containing a substance that is electroactive under electrochemical analytical conditions.

11. The improvement of Claim 7, wherein the electrochemical analytical conditions comprise potentiostatic measurement of an electroactive substance indicative of the analyte of interest.

12. The improvement of Claim 7, wherein the electrochemical analytical conditions comprise potentiometric measurement of an electroactive substance indicative of the analyte of interest.

13. In an affinity chromatographic test strip having a fluid pathway defined therein, and at one delimited area thereof specific for capture/immobilization of a component of a reaction of a sample solution and a labeled substance, the improvement comprising:

an affinity chromatographic medium, having a fluid pathway therein, that is characterized by at least one delimited area comprising an immobilized binding substance along said fluid pathway,

a first means for establishing electrical contact, under assay conditions, between each of said delimited areas of said affinity chromatographic medium, and an external source of electrical energy, and

a second means for establishing an electrical reference contact, under assay conditions, relative to each of said delimited areas of said affinity chromatographic medium, and an at least one other area of said affinity chromatographic medium.