

ENZYME IMMUNOASSAYS USING IMMOBILIZED REAGENTS IN A FLOWING STREAM

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a method and apparatus for the rapid quantitative determination of biochemical antigens contained in aqueous solutions, particularly in biochemical fluids such as blood serum, urine, and also in food preparations and medicaments.

Antigens, such as the anti-convulsants, cardiovascular medicines, and toxic drugs (such as heroine, cocaine, etc.), are generally quantitatively determined by known, complex and time consuming experimental procedures. For the quantitative determination of substances involved in biochemical processes, e.g. lower molecular weight substances, such as vitamins and steroids, or e.g. higher molecular weight substances, such as proteins or carbohydrates, it is often possible to employ the reaction products of these substances with specific binding partners, particularly proteins, for which they have chemical affinities. Therefore, it is possible to determine the concentration of drugs, such as steroids, heroine, cocaine, anticonvulsants, etc., by employing a protein capable of specifically binding with the particular drug. One example of a drug commonly assayed biochemically is insulin.

It is also possible to chemically link low molecular weight substances to a protein and to inject the complex into a test animal. The test animal reacts by producing an antibody to the low molecular weight substance. Alternatively, the low molecular weight substance, known as an antigen, may be injected directly into the test animal which will again produce an antibody to the antigen. Similarly, high molecular weight substances, such as proteins and carbohydrates, are capable of causing the production of antibodies when injected into test animals. Both low and high molecular weight antigens can cause the production of specific binding partners, always proteins, which are known as antibodies.

Immunoassays methodology has been shown to be extremely versatile in allowing for the quantification of both low and high molecular weight substances, even when a wide variety of other materials chemically similar to the desired unknown are present in the unknown sample. As stated above, immunoassay methodology relies upon the ability of antibodies to specifically combine with antigens. Various immunoassay methods currently are in use, e.g. radioimmunoassay, spin immunoassay, homogeneous enzyme immunoassay, and hemagglutination.

Radioimmunoassays require equipment and radioisotopes, which are relatively expensive.

The known enzyme immunoassays systems are extremely versatile in permitting both spectrophotometric and electrochemical determinations to be carried out. Immunoassay systems employ an enzyme, which is a catalytically active protein, to which there is complexed the antigen to be determined. The antigen-enzyme complex is bound to an antibody, thereby substantially reducing the enzyme activity of the complexed enzyme. Therefore, in a homogeneous system, by adding the unknown sample of the antigen, allowing the antigen to compete for binding sites on the antibody with the complexed enzyme, and tracing the rise in enzymatic activity, one may quantify the concentration

of the unknown antigen contained in the sample. Unfortunately, even enzyme immunoassays sometimes take long periods of time and involve the use of an expensive antibody which is frequently discarded as part of the immunoassay quantification procedure.

The present invention circumvents the time and expense of previous immunoassay methods.

2. Description of the Prior Art

Procedures are known where antigens are quantified using primarily two experimental methods. The first method involves the so-called homogeneous immunoassay technique, wherein an antigen or antibody, both in soluble form, are mixed to allow the specific binding reaction to proceed, and then detection proceeds in any of a variety of procedures. The second method for the detection of antigens is the so-called heterogeneous immunoassay method wherein one of the binding partners, either the antigen or antibody, is immobilized on a solid support and reacts with the other binding partner. Then, either the solid support or the liquid is separated from the other phase and subjected to analysis. Both homogeneous and heterogeneous immunoassays are commonly described in the art and are exemplified by U.S. Pat. Nos. Re. 29,169, 3,654,090 and 3,875,011.

U.S. Pat. No. Re. 29,169 discloses the reaction between a specific binding protein and its specific binding partner. One of the binding partners is immobilized and the assay is conducted by allowing the binding to take place, separating the solid phase from the reaction mixture containing the immobilized binding partner, reacting with an enzyme the solid phase which contains a determined amount of a coupling product of the substance to be determined, and subsequently performing an enzymatic assay on either the liquid or solid phase of the reaction mixture to obtain a quantitative indication of the amount of the specific binding partner.

U.S. Pat. No. 3,654,090 discloses an antigen-antibody reaction sequence wherein one component is immobilized and the other is covalently linked to an enzyme. By the addition of one component to the other, the amount of soluble or insoluble enzyme-labelled component may be determined in relatively small quantities. Similarly, U.S. Pat. No. 3,875,011 discloses a conjugated enzyme composition useful in homogeneous enzyme immunoassays. The drug to be determined is conjugated to glucose-6-phosphate dehydrogenase. The resulting product has a relatively high substrate turnover rate and provides enzyme magnification when employed in homogeneous immunoassays.

Many techniques are known in the prior art to determine the products of either a homogeneous or heterogeneous enzyme immunoassay procedure. For example, U.S. Pat. No. 3,876,504 discloses a method for determining antigens and antibodies in human body fluids, wherein one component of the reaction is immobilized on a glass microscope type slide, flooded with a test reagent containing the other component, and bonded to a color forming agent. By monitoring the colorimetric reaction which takes place, quantification of the antigens and antibodies is possible. U.S. Pat. No. 3,555,143 discloses a process wherein water insoluble polymers are used to insolubilize antibodies. The antigen is linked to a radioactive isotope and the antigen and antibody are allowed to react in a reaction medium. Subsequently, the antibody attached to the insoluble polymers are separated from the reaction medium and the radioactivity of the antigen isotope-antibody is deter-