

ing or anti-analyte layers prior to exposure to the analyte. Such a modified quartz crystal microbalance is referred to herein as a biologically modified quartz crystal microbalance, or BMQCM. Once the conjugate is bound to the BMQCM-bound analyte, a substrate specific for the enzyme is added to the system. The enzyme catalyzes a reaction in which the substrate is converted to a product which either (1) accumulates on the surface of the BMQCM; (2) reacts with and is subsequently incorporated into the BMQCM, either electrostatically, covalently or by simple adsorption; or (3) reacts with the BMQCM, but results in incorporation of a species other than the enzymatic reaction product. The resulting mass changes produce corresponding changes in the resonant frequency of the quartz crystal, as measured by an external oscillator circuit and frequency meter

In another aspect, the present invention is a process for measuring an analyte utilizing a reaction chamber in which a quartz crystal microbalance is placed opposite and in close proximity to a surface having capture reagent adsorbed thereon. Upon exposure to the sample, analyte is bound by the capture reagent. The resulting bound complex is then reacted with a conjugate comprising an enzyme and either an anti-analyte reagent or the analyte. A substrate is then introduced. The enzyme catalyzes a reaction in which the substrate is converted into a product which accumulates on the surface of the quartz crystal microbalance, thereby changing its mass and resonant frequency. The accumulation of product on the microbalance can be mediated by a reactive layer on the microbalance. The reactive layer can be chosen to mediate mass accumulation by, for example, physical adsorption, ion complexation or covalent attachment of the catalysis product. Alternatively, the reactive layer can be chosen so that the catalysis product causes a change in the reactive layer that results in simple adsorption, ion exchange or covalent attachment of another reagent in the reaction medium.

BRIEF DESCRIPTION OF THE DRAWING

The drawing consists of eight figures.

FIGS. 1 through 7 depict various modes of carrying out the present invention.

FIG. 8 depicts suitable circuitry for measuring the resonant frequency of the BMQCM.

DETAILED DESCRIPTION OF THE INVENTION

The invention may be understood by reference to the Drawing wherein like reference numerals are used to indicate like elements.

Referring now to FIG. 1, there is seen a biologically modified quartz crystal microbalance (BMQCM) indicated generally by the reference numeral 10. The BMQCM comprises a quartz crystal wafer 12 sandwiched between two electrodes 14, 16. Adsorbed to one surface 18 of the electrode 16 is capture reagent 20.

Upon exposure of the BMQCM having capture reagent bound thereto to a solution (not shown) containing analyte 22, the analyte 22 will be bound by the adsorbed capture reagent 20, thus forming a bound complex. After a suitable incubation period, unbound analyte is washed away.

The QCM is then contacted with a conjugate 24 comprising anti-analyte reagent and an enzyme, designated generally by E. After a suitable incubation period, unbound conjugate is washed away.

The QCM, having the conjugate bound thereto, is then contacted with a solution containing a substrate, designated generally by S, which is specific for the enzyme E. The enzyme will then catalyze a reaction in which the substrate is converted to a product P. The enzyme and substrate system is chosen such that the product P is insoluble and precipitable on the BMQCM surface. The product P will accumulate on the surface 18, thereby leading to a change in mass and hence a change in resonant frequency, as measured by an external circuit 26.

Suitable anti-analyte reagents and capture reagents include those reagents which are capable of participating in complexation reaction with the analyte. Preferred reagents include antibodies, lectins, chelating agents, binding proteins, DNA and RNA polynucleic acid probes, and cell receptors. The choice of reagent will depend on the analyte to be measured. The anti-analyte reagent and capture reagent may be the same or different chemically.

Suitable analytes include proteins, hormones, enzymes, antibodies, drugs, carbohydrates, nucleic acids, etc.

Examples of enzyme/substrate systems which are capable of producing an insoluble product which is capable of accumulating on the surface of the BMQCM include alkaline phosphatase and 5-bromo-4-chloro-3-indolylphosphate (BCIP). The enzymatically catalyzed hydrolysis of BCIP produces an insoluble dimer which precipitates on the surface of the BMQCM. Other analogous substrates having the phosphate moiety replaced with such hydrolytically cleavable functionalities as galactose, glucose, fatty acids, fatty acid esters and amino acids can be used with their complementary enzymes.

Other enzyme/substrate systems include peroxidase enzymes, for example horseradish peroxidase (HRP) or myeloperoxidase, and one of the following: benzidine, benzidine dihydrochloride, diaminobenzidine, o-tolidene, o-dianisidine and tetramethylbenzidine, carbazoles, particularly 3-amino-9-ethylcarbazole, all of which have been reported to form precipitates upon reaction with peroxidases. Also, oxidases such as aldehyde oxidase, glucose oxidase, L-amino acid oxidase and xanthine oxidase can be used with oxidizable substrate systems such as a phenazine methosulfate-nitroblue tetrazolium mixture.

Referring now to FIG. 2, there is seen an alternative embodiment of the BMQCM shown in FIG. 1. Specifically, the surface 18 has been modified by coating it with a layer 28. The layer 28 can serve as a "priming" layer, which enhances attachment of the capture reagent 20. The layer 28 can also serve to enhance mass accumulation on the BMQCM by (1) specific reaction between product P and the layer 28, (2) ion exchange between P and the layer 28 or (3) simple absorption of P into the layer 28.

Illustrative surfaces 28 are polymer films and silane reagents that serve to enhance the binding of the capture reagent during equilibration by either hydrophobic interactions or covalent interactions. An example of a polymer film is polystyrene, which, itself, can be applied by conventional methods, such as spin coating. Higher surface area coatings for greater capture reagent coverages can be achieved by fabrication of irregular and three dimensionally shaped surfaces, such as by aerosol application which deposits minute droplets of polymer. Suitable silanes include the general class of