

## COLUMN METHOD OF IMMUNOASSAY EMPLOYING AN IMMOBILIZED BINDING PARTNER

### BACKGROUND OF THE INVENTION

Specific binding substances are those substances which interact with specific binding partners to form a bond therebetween to the exclusion of other substances. The bond formed is generally a physical bond, but in some instances it may be termed a physicochemical bond or even a chemical bond. It may be said that a specific binding substance and its specific binding partner have an affinity to bind or react with one another. Specific binding substances are generally either peptides, proteins, carbohydrates, glycoproteins, or steroids. Some examples of specific binding pairs, which comprise a specific binding substance and its specific binding partner, are antigens and their antibodies, haptens and their antibodies, enzymes and their substrates, hormones and their receptors, and vitamins and their receptors.

As used herein, the term specific binding substance may refer either to the substance being determined or its specific binding partner; the term specific binding pair refers to a specific binding substance and a specific binding partner therefor; the term unknown or unknown substance refers to the substance being determined; and the term labeled component refers to a labeled form of either the substance being determined or a specific binding partner.

Assay methods for determining specific binding substances are based on the ability of such substances to react with their specific binding partners in labeled form in a manner similar to that in which they react in their unlabeled form. There are basically three approaches to the quantitative determination of specific binding substances using such an assay method. The equilibrium method is founded on the competition between the unknown and the labeled form of the substance being determined for a limited quantity of specific binding partners. The saturation method has its basis in the saturation of a portion of a quantity of binding partners with the unknown, followed by the reaction of the remaining unbound partners with a quantity of the labeled form of the substance being determined. The direct method is based on adding the unknown to a quantity of specific binding partners, followed by the addition of excess specific binding partners in a labeled form in order to bind with all of the bound unknown. All of these methods provide quantitative determinations through calculations based on the extent of binding of the labeled component. Specific binding assays may be termed immunoassays where antigens, haptens, or antibodies are involved, and may be termed radioimmunoassays where the same are involved and where the label is a radioactive label.

The introduction of the two-phase system, consisting of a fluid phase and a solid phase, to specific binding assays has greatly simplified these assays. In such two-phase systems, the solid phase consists of the binding partners in an insolubilized form. The utilization of techniques involving adsorption, chemical coupling, and physical entrapment provides means of insolubilizing the binding partners. The advantage of the two-phase system lies primarily in the ease of separation of bound labeled component from unbound labeled component.

Specific binding assays find utility in a vast number of areas. The fields of research, medicine and industry all find use in such an advantageous technique. These methods may be applied to the wide range of substances possessing a specific binding affinity for a binding partner and provide determinations which are specific, sensitive, precise and reliable compared to alternative methods.

### DESCRIPTION OF THE PRIOR ART

The separation of the labeled component which is bound to its binding partner from that which is unbound is critical to specific binding assays. In general, the rate and reliability with which this separation is accomplished bears directly upon the rate and reliability of the assay as a whole. Various methods based on a two-phase system employing insolubilized binding partners have been developed aimed at quick, reliable determinations. The techniques employed in insolubilizing the binding partners in conjunction with specific binding assays include pre-precipitation of the binding partner, sometimes referred to as a double antibody method where the binding partner is an antibody, entrapment of the binding partner within an acrylamide gel, and polymerization of the binding partner. The techniques of chemically binding or coupling the binding partner to an insoluble polymer or absorbing it to the inner surface of an assay test tube are more commonly used. A vast number of known procedures for coupling binding partners to a variety of polymers are available for use in conjunction with a two-phase specific binding assay system.

At the present time, the known specific binding assays which utilize insolubilized binding partners employ a closed or non-flow through system such as test tubes containing the insolubilized binding partners in a loose particle form, test tubes having the binding partners adsorbed to their inner surfaces, or syringes containing the binding partners in a gel entrapped form. Since samples of unknown are generally highly dilute and since the use of a closed system limits sample size, incubation times must be long in order to obtain reliable results. Recommended incubation times range from between 6 hours to 2 days with some much longer. Separation of the bound and unbound labeled component, even through the use of insolubilized binding partners, still requires excessive manipulation of the system such as centrifuging and extensive washing.

Flow-through systems utilizing insolubilized binding partners are limited in the prior art to techniques such as purification and concentration of dilute unknowns. In relation to specific binding assays, such systems have served only as preparatory steps to the actual assay in the prior art. Conventional specific binding assays are then performed on the purified, concentrated unknown, which assays remain time consuming and require much system manipulation.

In the context of this disclosure, determination of a substance in a fluid sample implies either the determination of the concentration or the absolute amount of that substance in the fluid sample.

### SUMMARY OF THE INVENTION

It has now been found that by utilizing a two-phase, flow-through assay system in conjunction with either an equilibrium, a direct, or a saturation assay technique, a highly advantageous, rapid and reliable method and device is provided for quantitatively determining one of