

whereby tracer is bound to the analyte which is bound to the supported binder.

If the assay is an inhibition type of assay, then the supported binder is specific for only the tracer, and the tracer is also specific for the analyte. In such an assay, the presence of analyte inhibits binding of tracer to the supported binder.

Thus, the tracer when bound to the solid support is either directly bound to the binder on the support or is bound to analyte which is bound to binder on the solid support.

The type of binder which is used in the assay is dependent upon the analyte to be assayed, as well as the specific assay procedure. As known in the art, the binder which is supported may be an antibody including monoclonal antibodies, an antigen, a protein specific for the material to be bound or a naturally occurring binder. Thus, for example, in a competitive type of assay for an antigen or hapten, the binder may be an antibody or a naturally occurring substance which is specific for the tracer and the antigen or hapten. If the assay is for an antibody, then the binder may be, for example, an antigen or an antibody which is specific for the antibody to be assayed. In a "sandwich" type of assay wherein the analyte is an antibody, the supported binder may be an antigen for the antibody, or a protein, such as protein A which selectively binds Fc fragments of certain antibodies. In a "sandwich" type of assay, if the analyte is an antigen (an antigen having more than one determinant site), then the binder may be an antibody or naturally occurring binder which is specific for the antigen to be assayed.

The selection of a suitable binder for support on the solid substrate is deemed to be within the scope of those skilled in the art from the teachings herein.

The ligand which is labeled for use as a tracer in the assay of the present invention is also dependent upon the analyte to be assayed, as well as the assay procedure. Thus, for example, if a competitive assay is employed for determining antigen or hapten, the ligand employed in producing the tracer would be either the analyte or appropriate analog thereof. (The term "appropriate analog" means that the analog of the analyte is bound by the binder for the analyte.)

If the assay is a "sandwich" type of assay for an antibody, then the ligand employed in producing the tracer would be a ligand which is specific for the analyte to be assayed, such as, for example, an antibody elicited in response to the antibody or antigen to be assayed. The selection of a suitable ligand for producing the tracer is deemed to be within the scope of those skilled in the art from the teachings herein.

As hereinabove indicated, in producing the tracer the ligand is labeled with a particulate label. A preferred particulate label is a sac, which includes a detectable marker which is not visible.

The sac which is used to label the ligand for producing a tracer may be any one of a wide variety of sacs, including but not limited to intact erythrocytes, erythrocyte ghosts, liposomes (single walled [sometimes called vesicles] or multilamellar), polymer microcapsules (for example, those made by coascervation, or interfacial polymerization), etc.

Erythrocyte ghosts are known in the art and are prepared by suspending erythrocyte cells in a solution of substantially lower osmolarity. The ghosts are "re-sealed" in an aqueous solution including the marker whereby the ghosts include the marker in the interior

thereof. Such procedures are known in the art and the resealing solution of appropriate osmolarity generally includes, in addition to the marker, alkali and alkaline earth metal halides and a coenzyme; e.g., adenosine triphosphate. The preparation of ghosts, as sacs, is disclosed, for example, by D'Orazio et al, *Analytical Chemistry*, Vol. 49, No. 13, pages 2083-86 (Nov. 1977).

Polymer microcapsules are also produced by procedures known in the art except that the solution in which the microcapsules are formed also includes the marker whereby the interior of the polymer microcapsule includes the marker. The preparation of such microcapsules is disclosed for example in *Microencapsulation Processes and Applications*, edited by Jan E. Vandegger (Plenum Press 1974).

As known in the art, liposomes can be prepared from a wide variety of lipids, including phospholipids, glycolipids, steroids, relatively long chain alkyl esters; e.g., alkyl phosphates, fatty acid esters; e.g., lecithin, fatty amines and the like. A mixture of fatty materials may be employed, such as a combination of neutral steroid, a charged amphiphile and a phospholipid. As illustrative examples of phospholipids, there may be mentioned lecithin, sphingomyelin, dipalmitoyl, lecithin, and the like. As representative steroids there may be mentioned cholesterol, cholestanol, lanesterol, and the like. As representative examples of charged amphiphilic compounds, which generally contain from 12 to 30 carbon atoms, there may be mentioned mono- or dialkyl phosphate ester or an alkylamine; e.g., dicetyl phosphate, stearyl amine, hexadecyl amine, dilauryl phosphate, and the like.

The liposome sacs are prepared in an aqueous solution including the marker whereby the sacs will include the marker in the interior thereof. The liposome sacs are easily prepared by vigorous agitation in the solution, followed by removal of marker from the exterior of the sac.

Further details with respect to the preparation of liposomes are set forth in U.S. Pat. No. 4,342,826 and PCT International Publication No. WO80/01515, both of which are hereby incorporated by reference.

As representative examples of detectable markers which may be included in the sac, there may be mentioned: fluorescent materials, radioisotopes; enzymes, spin labels, chemiluminescent materials, etc.

The ligand may be labeled with the particulate label so as to produce a tracer for use in the invention by procedures generally known in the art, with the procedures which is used being dependent upon the ligand and the particulate label which is employed. Such techniques include adsorption, covalent coupling, derivatization, coactivation, the the like. In producing a tracer wherein the ligand is labeled with a sac, the sac may be produced from a component which has been derivatized with a ligand, whereby the sac, when produced, is sensitized with the ligand. In another procedure, the sac including the marker may be initially formed, followed by sensitizing the sac with ligand by procedures known in the art.

Thus, the tracer is comprised of a ligand and a particulate label (solid or solid-like, as opposed to non-solid labels, such as radioisotopes, enzymes and various fluorescent materials), and the particulate label includes the detectable marker.

The solid substrate employed in the assay is preferably in sheet form, with the substrate, in sheet form, generally being in the form of a card, a test strip or