

All solutions were prepared in aqueous buffer (TBS) containing 0.02M Tris, 0.15M NaCl, and 0.01% (w/v) NaN₃ (pH 7.4).

The immunospecific reactivity of liposomes non-covalently derivatized with B-Ab was examined by measuring changes in intensity of scattered light as liposomes aggregated in the presence of multivalent antigen. Immunospecific aggregation data are shown in FIGS. 8 and 9. FIG. 8 shows that the amount of aggregation induced by the presence of antibody in 10 l of ascites fluid increased as the molar ratio of B-PE in liposomes increased, although significant non-specific aggregation occurred above 0.5% mol % B-PE. In FIG. 9, increasing amounts of B-Ab were added to 0.1 mol % B-PE liposomes which were first incubated with 0.4 nmol of avidin. Immunospecific aggregation continued to increase even at a molar ratio of B-Ab to avidin of 3.5.

Based on the above data, an optimized formulation was chosen for measuring a concentration-dependent immunospecific response. Liposomes were prepared containing 0.1 mol % B-PE, since this preparation seemed adequately resistant to nonspecific aggregation. Avidin was added to a small volume of liposomes at a concentration to provide a B-PE:avidin molar ratio of 5, and after 2 min, B-Ab was added at a B-Ab:avidin molar ratio of 3. After 10 min, varying amounts of purified mouse IgG were added. Samples were allowed to react at room temperature for 1 hr., at which time they were diluted with 2 ml TBS. The calibration curve is shown in FIG. 9. Scatter intensity increased linearly as a function of IgG concentration between 1 and 10 pmol IgG. The curve was nonlinear above and below these concentrations.

In another embodiment of the present invention, a sample kit for qualitatively testing the presence of an analyte in a sample is provided. The kit comprises a capillary tube or receptor cartridge having immobilized receptor packed therein. The receptor may be an antibody or antigen analog or the like. A mixture is formed by adding a sample to be tested for presence of an analyte to liposomes each having a surface membrane and an interior aqueous phase, the surface membrane having a binding agent incorporated therein, the aqueous phase including a color dye. The capillary tube is inserted, preferably vertically, into the mixture, and through capillary action, mixture is drawn upward within the capillary tube. Detection of bound liposomes can be enhanced by blotting the capillary tube onto absorbent material to absorb the aqueous phase and thus concentrate the dye on the absorbent material leaving a color spot to facilitate detection. The liposomes and the analyte compete for binding to the receptor. The resulting color intensity of the tube or the absorbent material is indicative of the presence of analyte in the sample, greater color intensity indicating greater liposome binding and therefore lower analyte concentration. The immobilized receptor cartridge can be reusable after disrupting liposomes and washing out reactants.

Although the present invention has been described in connection with preferred embodiments, it will be appreciated by those skilled in the art that additions, modifications, substitutions and deletions not specifically described may be made without departing from the spirit and scope of the invention defined in the appended claims.

What is claimed is:

1. A flow injection immunoassay method for detecting or quantifying an analyte in a test sample, said method comprising:

- (a) providing a stationary solid phase support having bonded thereto a receptor for competitively binding thereto said analyte and sensitized liposomes;
- (b) forming said sensitized liposomes having a surface membrane and an interior aqueous phase, said surface membrane having a binding agent incorporated therein that binds to said receptor in competition with said analyte and said liposomes including a marker compound, wherein said sensitized liposomes will not disrupt as a result of any bonding between said binding agent and said receptor;
- (c) flowing a sample which may contain said analyte past said solid phase support in a moving, non-segmented carrier stream;
- (d) flowing said liposomes past said solid phase support in said stream, whereby flow separates liposomes bound to said solid phase support from liposomes unbound to said solid phase support; and
- (e) detecting said marker compound or a reaction product of said marker compound of either the bound liposomes or the unbound liposomes to determine the presence or amount of analyte in said test sample.

2. A flow injection immunoassay method for detecting or quantifying an analyte in a test sample, said method comprising:

- (a) providing a stationary solid phase support having bonded thereto a receptor for only one of said analyte and sensitized liposomes;
- (b) forming said sensitized liposomes having a surface membrane and an interior aqueous phase, said surface membrane having a binding agent incorporated therein that binds to at least one of said receptor and said analyte and said liposomes including a marker compound, wherein said sensitized liposomes will not disrupt as a result of any bonding between said binding agent and said receptor or said binding agent and said analyte;
- (c) flowing a sample which may contain said analyte past said solid phase support in moving, non-segmented carrier stream;
- (d) flowing said liposomes past said solid phase support in said stream, whereby flow separates liposomes bound to said solid phase support from liposomes unbound to said solid phase support; and
- (e) detecting said marker compound or a reaction product of said marker compound of either the bound liposomes or the unbound liposomes to determine the presence or amount of analyte in said test sample.

3. The immunoassay of claim 1, wherein said liposomes are sensitized with binding agents via a noncovalent bridge between biotinyl liposomal lipid and biotinyl binding agent with the protein avidin.

4. The immunoassay of claim 1, wherein said marker compound comprises unquenched fluorophores which are detected while within said interior phase of said bound or unbound liposomes.

5. The immunoassay of claim 1, wherein said marker compound is an electroactive marker.

6. The immunoassay of claim 1, wherein said marker compound is an enzyme and an enzyme reaction product is detected.