

0.1 M potassium phosphate buffer of pH=9.0. 4 ml of anti-human gamma-globulin (protein concentration of 20 mg/ml) was then added to the fluorescein phosphate solution. The mixture was continuously stirred for one hour at 4° C. and allowed to stand at the same temperature for another 24 hours. Excess fluorescein was removed by extensive dialysis against 0.1 M potassium phosphate buffer of pH=7.2. During dialysis, 100 ml of buffer each time was used, and the buffer was changed every 2 hours for 5 times.

Gamma-globulin coated porous glass was prepared as follows: 50 mg of epoxy-glass (3000 (Å) pore size) was prepared in the same way as described in Example IV. 2.5 mg m-sodium periodate was dissolved in 5 ml of deionized water. Glass was then added to this solution and stirred at room temperature for 2 hours. The glass was washed thoroughly with deionized water and then with 10 ml 0.1 M potassium phosphate pH=9.0 buffer and kept for one hour. The glass was then filtered and ready for coupling. 5 ml human gamma-globulin (protein concentration of 30 mg/ml) was diluted with 5 ml of 0.1 M, pH=9.0 phosphate buffer. The activated glass was then added to this solution and was stirred at 4° C. for 2 hours before being allowed to stand overnight at the same temperature. After reaction was completed, the glass was washed extensively with 0.1 M potassium phosphate buffer of pH=7.2 and then filtered for immediate use. 30 mg of human gamma-globulin coated porous glass was added to 0.5 ml of fluorescein-antihuman gamma-globulin conjugate. The slurry was incubated on 24 cycles of agitation/settling (60/90 seconds ratio). Excess antibody solution was decanted and the glass was washed with 0.01 M potassium phosphate buffer of pH=7.2 until no light was detected by testing the decanted buffer in oxalate/peroxide system.

The glass was then washed with 5 ml t-butanol and excess butanol was withdrawn. Green color light was observed on glass particles upon addition of oxalate and peroxide.

#### EXAMPLES XVIII-XXI

##### USE OF ENCAPSULATED FLUORESCER AS A LABEL IN CHEMILUMINESCENT IMMUNOASSAYS

The following Examples are given to illustrate the preparation and use of encapsulated fluoroscres as labels, and are in no way intended to limit the scope of the invention described herein.

#### EXAMPLE XVIII

##### Chemiluminescence and entrapment of hydrophilic fluorescer (Rhodamine B) in liposome

A chloroform solution of phosphatidylcholine (egg) cholesterol and phosphatidylethanoamine in molar ratio of 6:2:2 (total concentration about 26 mg) was placed into a 50 ml round bottom flask and the solvent evaporated on a rotary vacuum evaporator at room temperature. The lipid film was then purged with N<sub>2</sub>. 2.0 ml of 0.01 M borate buffer, pH 8.5 containing Rhodamine B was added to the lipid film, the film was dislodged from the walls of the flask by vigorous shaking. The resulting emulsion liposome suspension was removed and was sonically treated for 5 minutes in ice bath employing an ultrasound generator.

The liposome solution was passed through a sepharose 6B column to separate the uniform single compartment liposomes from the multilamellar ones. The uniform single compartment liposome fraction was

again passed through G-75 sephadex column to remove free Rhodamine B.

25 μl 0.0144 M to TCPO (2,4,5-Trichlorophenyl oxalate) in Glyme and 25 μl of 1.23 M H<sub>2</sub>O<sub>2</sub> were pipetted into a 6×50 mm test tube. The liposome enclosed Rhodamine B solution was first treated with Triton X-100 (to release the Rhodamine B), 50 μl of this solution was then injected to the H<sub>2</sub>O<sub>2</sub>/oxalate mixture, light was detected by Pico-Lite luminometer with a red filter.

#### EXAMPLE XIX

##### Chemiluminescence and entrapment/embedding of hydrophobic fluorescer DAET (Dianisylethynyltetracene) in liposome

A chloroform solution containing gangliosides, phosphatidylcholine, cholesterol (10:45:45 mole ratio) and 200 mM DAET was evaporated in rotary vacuum evaporator at room temperature. The lipid film was purged with N<sub>2</sub> and flooded with 2.0 ml of 0.010 M borate buffer pH 8.5. The lipid emulsion solution was then sonicated for five (5) minutes in an ice bath.

The liposome solution was first passed through Sepharose 6B column and again G-75 Sephadex column to separate undispersed lipids, multilamellar liposomes and free fluorescer.

The liposome solution was treated with Triton X-100 to ensure the rupture of the lipid membrane. 25 μl of 0.0144 M TCPO and 25 μl of 1.23 M H<sub>2</sub>O<sub>2</sub> were pipetted into a 6×50 mm test tube and the tube was loaded into the analyzer of Pico-Lite luminometer. A 50 μl of the Triton X-100 treated liposome solution was injected into the test tube and the emission of light was detected by Pico-Lite luminometer with a red filter.

#### EXAMPLE XX

##### Chemiluminescence and entrapment of fluorescer (hydrophilic or hydrophobic) bound onto silica sol

0.5 ml silica sol of 5 nm size was diluted with 0.5 ml of 0.01 M borate buffer pH 12.5. 25 μl of γ-aminopropyltriethoxysilane was then added to the sol solution. The mixture was then vigorously shaken to disperse the sole particles. The sol solution was then dialyzed extensively against deionized water pH=9.0. LRSC (lissamine Rhodamine sulfonyl chloride) 5 mg in 1.0 ml 0.01 M borate pH=12.5 was then added to the sol solution and incubated overnight. The Rh-B/sol was then separated from free fluorescer by either dialysis or gel filtration. The fluorescent sol was then dispersed in borate buffer and enclosed by liposome as indicated in Example XIX.

The entrapped Rh-B/sol was treated with Triton X-100, and the solution was then injected to a mixture of TCPO/H<sub>2</sub>O<sub>2</sub>. Light was detected from Pico-Lite luminometer with a red filter.

#### EXAMPLE XXI

##### Chemiluminescent immunoassay utilizing liposome entrapped fluorescer as a label

20 μl of 25% glutaraldehyde was added to 2 ml liposome containing Rhodamine B as prepared in Example XVIII and incubated at 20° C. for 10 minutes. Excess glutaraldehyde was dialyzed against 1 liter of 0.145 M NaCl for 1 hour and again 1 liter of borate buffer for another hour at room temperature. The activated liposome was then incubated with Anti-Hb<sub>2</sub>Ag solution at 4° C. overnight.