

**CHEMICAL LUMINESCENCE AMPLIFICATION  
SUBSTRATE SYSTEM FOR  
IMMUNOCHEMISTRY INVOLVING  
MICROENCAPSULATED FLUORESCER**

**CROSS REFERENCE TO RELATED  
APPLICATION**

This application is a continuation-in-part of applicants copending application Ser. No. 105,257, abandoned, filed Dec. 19, 1979, the priority of which is hereby claimed.

This invention relates to a system for the detection of a biological analyte of interest which comprises a microencapsulated fluorescer which has been conjugated to an immunological specie specific to the biological analyte of interest, a means for disrupting the microcapsule to free the fluorescer and an energy source other than electro-magnetic radiation which is capable of activating the fluorescer.

This invention also relates to a novel class of microencapsulated fluorescer materials which may be conjugated to an immunological specie specific to a biological analyte of interest to provide a means for the detection of such biological.

This invention also relates to novel microencapsulated fluorescer materials and conjugated microencapsulated fluorescer/immunological specie compositions useful in the detection of various biological analytes of interest.

This invention also relates to novel test kits for the detection of a biological analyte of interest employing the microencapsulated fluorescers described herein.

**BACKGROUND OF THE INVENTION**

The clinician is concerned with detecting the presence of, and quantitatively measuring, a variety of substances via the use of many different analytical techniques. The most commonly used techniques employ absorbometry, both at visible and ultraviolet wavelengths, however, emission, flame photometry and radioactivity are also commonly used. A novel technique, thus far relatively unexplored in chemistry, is that employing the phenomenon of luminescence.

Analyses based on the measurement of emitted light offer several distinct advantages over conventionally employed techniques, including high sensitivity, wide linear range, low cost per test, and relatively simple and inexpensive equipment.

It has been predicted that the phenomenon of luminescence, and more particularly chemiluminescence could have a major impact in two main areas of clinical analysis. First, it may have an important role as a replacement for conventional colorimetric or spectrophotometric indicator reactions in assays for substrates of oxidases and dehydrogenases. In this type of assay the sensitivity of the luminescence indicator reaction may be used to quantitate substrates not easily measured by conventional techniques (e.g., prostaglandins and vitamins).

The second major clinical application of luminescence must be in the utilization of luminescent molecules as replacements for radioactive or enzyme labels in immunoassay.

In each of these major clinical application areas, chemiluminescent reactions can provide a means to achieve a high level of analytical sensitivity.

Chemiluminescence may be simply defined as the chemical production of light. In the literature it is often confused with fluorescence. The difference between these two phenomena lies in the source of the energy which promotes molecules to an excited state. In chemiluminescence this source is the non-radiative energy yielded as the result of a chemical reaction. The subsequent decay of molecules from the excited state back to the ground state is accompanied by emission of light, which is called luminescence. In contrast, in fluorescence, incident radiation is the source of the energy which promotes molecules to an excited state.

From an analytical point of view, the types of luminescence that have engendered the most interest are chemiluminescence and bioluminescence. The latter being the name given to a special form of chemiluminescence found in biological systems, in which a catalytic protein increases the efficiency of the luminescent reaction. Bioluminescent reactions such as the enzymatic firefly process, have been very useful analytically and convert chemical energy to light with a quantum efficiency of 88%.

In contrast to bioluminescence with the longevity and efficiency of the firefly, the history of chemiluminescence (hereinafter referred to as CL), especially that occurring in the non-aqueous phase, is remarkably short. The important aqueous CL substances luminol and lucigenin were discovered in 1928 and 1935, respectively. A series of organic soluble CL materials were developed in the early 1960's based upon a study of the luminescent reactions of a number of oxalate compounds. A typical organic system useful for CL was disclosed by Bollyky et al., U.S. Pat. No. 3,597,362, and claimed to exhibit a quantum efficiency of about 23% compared with about 3% for the best known available aqueous systems.

Chemiluminescence has become increasingly attractive for its potential in the clinical laboratory, especially for use in the analysis of a number of biologically associated materials, and its known applications have been the subject of thorough reviews, see for example: Whitehead et al. (1979) Analytical Luminescence: Its potential In The Clinical Laboratory, *Clin. Chem.*, 25, 9 1531-1546; Gorus et al. (1979) Applications Of Bio- And Chemiluminescence In The Clinical Laboratory, *Clin. Chem.*, 25, 4 512-519; Isacson et al. (1974) Chemiluminescence In Analytical Chemistry, *Analytical Chimica Acta*, 68, 339-362.

With few exceptions, most published CL clinical analytical applications have made use of the less efficient but well known diacylhydrazides, acridinium salts, pyrogallol, or lophine structures. It is important to appreciate that due to the nature of the chemical composition of the above chemiluminescent structures in the presence of hydrogen-peroxide, or generators of H<sub>2</sub>O<sub>2</sub>, as compared to that of the oxidation reaction of diaryloxalate structures, the latter has over 20 times the quantum yield of chemiluminescence, although its requirement for hydrogen peroxide is greater than the former.

Hydrogen peroxide, an essential component in many chemiluminescent reactions, has usually been the species selected for use in detecting the analyte of interest. For example, in the determination of glucose-Auses et al. (1975), Chemiluminescent Enzyme Method For Glucose. *Analytical Chemistry*, 47, No. 2, 244-248 employed the oxidation of glucose in the presence of glucose oxidase as the source of H<sub>2</sub>O<sub>2</sub> which, in turn, was reacted