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Fluorescent Biosensors

# Polydiacetylene/Anti-HBs Complexes for Visible and Fluorescent Detection of Hepatitis B Surface Antigen on a Nitrocellulose Membrane

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**Abstract:** The immunochromatographic assay (ICA) using a nitrocellulose (NC) membrane offers several advantages. This technique is a rapid and straightforward method in contrast to other immunoassays. Polydiacetylene (PDA) vesicles have unique optical properties, displaying red color and red fluorescence at the same time. In this system, red-phase PDA vesicles are used as a fluorescent dye as well as a surface for immobilized hepatitis B surface antibody (HBsAb). PDA has a remarkable stability compared with other fluorescent dyes. In this study, the most suitable PDA/HBsAb complexes are introduced for detecting hepatitis B surface antigen (HBsAg). Then, the PDA/HBsAb complexes affixed antibody is attached to NC membrane, which has two lines to confirm detection of HBsAg. The main advantage of this system is that the detection of HBsAg can be observed in both visible and fluorescent images due to the optical properties of polydiacetylene. Detection of HBsAg is observed up to 0.1 ng mL$^{-1}$ by fluorescent analysis and confirmed by red line on the NC membrane up to 1 ng mL$^{-1}$ (HBsAg) using the naked eye. Consequently, these results show that PDA/HBsAb complexes were successfully applied to ICA for the diagnosis of hepatitis B.

Hepatitis B is an infectious disease caused by the hepatitis B virus (HBV). This disease, classified by the World Health Organization as one of the 10 top leading causes of death, is very common and presents other clinical complications such as hepatocirrhosis, hepatic insufficiency and liver cancer prior to the passing of an individual.[1] Hepatitis B has a high prevalence, with a worldwide infected population of 2 billion people, mainly occurring in Asia, India, Eastern Europe and the Middle East. Patients may not experience any pain in the initial stages of the disease until it further develops into chronic cancers over years if not treated, earning it the name "the disease of silence". Thus, the importance of an early diagnosis cannot be overemphasized. Such a diagnosis will involve the detection of hepatitis B surface antigen (HBsAg), which is the antigen expressed in the human body on infection with HBV.

Immunoassay is the method of detection for a small amount of protein in specimens using characteristic of antigen-antibody reactions. Some of the immunoassays for detecting HBsAg that have been reported include enzyme immunoassay (EIA),[2]and radioimmunoassay (RIA).[3] EIA is a complex and a technically detailed procedure, requiring analysis by professionals. Implementing RIA also has the disadvantages of high cost and radiation exposure. In recent years, the use of immunochromatographic assay (ICA) on a nitrocellulose (NC) membrane for hepatitis B diagnosis have extensively investigated.[4,5]

ICA is a combination of two methods, that is, immunochemistry using antibody-antigen specific reactions and a chromatographic assay utilizing the capillarity of porous membranes. NC membranes consist of a test line with fixed antibodies and a control line with fixed antigens. Therefore, when the HBsAg-combined sample is loaded on an NC membrane, the test line and control line become visible due to the binding of antigens and antibodies. In ICA, the reaction time is relatively short (ca. 20 min), providing a rapid reading time and an economically feasible alternative. Experts as well as other expensive state-of-the-art devices, as with other methods, are not needed.[6–8]

PDA vesicles display unique optical properties, which is evidenced by the blue-to-red color transition on stress perturbation. The red phase simultaneously exhibits both visible red color and red fluorescence. Self-assembly of diacetylene monomers occurs by irradiation with 254 nm UV light via 1,4-addition reaction and polydiacetylenes are polymerized. This polymerized PDA is visibly blue but non-fluorescent.[9,10] When blue-phased PDA is stimulated by heat,[11–13] organic solvent,[14,15] pH,[16]mechanical stress[9] and ligand-receptor interaction.[17] PDA undergo a blue-to-red chromatic transition and appear fluorescent.[18] For this reason, we used a red-phase of PDA as fluorescent dye to detect HBsAg.[19]

In this study, we introduce an ICA employing polydiacetylene, which can detect HBsAg on an NC membrane. Hepatitis B surface antibody (HBsAb) was immobilized on polydiacetylene

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