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## Novel phenothiazine enhancers in chemiluminescent enzyme immunoassay

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Commonly in chemiluminescent enzyme immunoassay (CL-EIA) a light is formed upon the oxidation of luminol with hydrogen peroxide catalyzed by peroxidase used as a label of immunoreagents. Since a peroxidase is poor catalyst in this reaction, enhancers are added to increase CL intensity. For long time the most popular enhancer was 4-iodophenol. In this work we analyzed some phenothiazine derivatives and showed that phenothiazines carrying groups with negative charge are potent primary enhancers in peroxidase-catalyzed CL, whereas phenothiazines with positive charge have no enhancing ability. The most efficient primary enhancers, whose the enhancing activity is higher many times than that of p-iodophenol, are 3-(10<sup>7</sup>-phenothiazinyl)propane-1-sulfonate (SPTZ) and 3-(10<sup>7</sup>-phenothiazinyl)propionic acid (PPA). Some pyridine derivatives are secondary enhancers increasing the enhancing ability of phenothiazines. Screening of some pyridines showed that N-morpholinopyridine (MORPH) is the most active secondary enhancer. The mechanism of its enhancing action was proposed. The conditions of the performance of the enhanced CL reaction using SPTZ or PPA in combination with MORPH as primary and secondary enhancers were optimized. The detection systems with SPTZ/PPA and MORPH were applied successfully in construction of ultrasensitive EIA kit for determination of human thyroglobulin and methylglyoxal-modified low density lipoprotein. The obtained results open good perspectives for use of ECR with phenothiazines/MORPH in the development of ultra-sensitive immunoassay kits.

### Biography

Ivan Yu Sakharov is a Leading Scientific Researcher at the Department of Chemistry, Lomonosov Moscow State University (Russia). He has graduated with PhD degree in Kinetics and Catalysis in 1982 and DSc degree in Biotechnology in 1992. Presently his work focuses on the development of novel sensitive chemiluminescent methods for their use in analytical practice. He has published more than 147 papers in peer-reviewed journals.

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