Application of covalently immobilized horseradish peroxidase for the determination of anions

***Yablotskiy* K.V.**

M.V. Lomonosov Moscow State University, Chemistry Department

E-mail: yakos@list.ru

Horseradish peroxidase (HRP) is widely used in chemical analysis owing to its high catalytic activity, rather wide substrate specificity, and accessibility. Numerous methods for covalent HRP immobilization, which provides the enhancement of its stability, are known from literature. Silica gels belong to the widespread insoluble bearers for enzymes immobilization due to their environmental purity, mechanic and biologic stability, simplicity of recycling, and low cost.

In our investigation silica gels modified with different functional groups (amino, epoxy, cycloepoxy, isocyanate, thiocyanate) were used for the covalent immobilization of HRP. The catalytic activity and stability of the obtained enzyme preparations were studied using the reaction of *o*-dianisidine oxidation with hydrogen peroxide as an indicator. The main kinetic parameters (K_m, V_{max}) were calculated for this reaction in the presence of each enzyme preparation under the revealed optimum conditions. It was found that HRP immobilized on silica gels modified with amino and thiocyanate groups were the most stable: only these preparations lost less than 10% of their initial activity after 3 months of storage. Besides, HRP immobilized on thiocyano-silica showed the highest catalytic activity.

That is why HRP covalently immobilized on silica gel modified with thiocyanate groups was used for the development of the highly sensitive, rather selective, rapid, and simple procedures for the determination of inorganic anions such as fluoride, cyanide, and thiocyanate (with the limits of detection 2, 1 nM, and 0.5 μM respectively). The determination of the indicated anions was based on their inhibitory effect on the enzyme as the ligands capable to form stable complexes with Fe(III) - a cofactor of HRP.

The proposed procedures were successfully applied for the determination of F^- in mineral and drinking waters, and CN^- and SCN^- - in biological fluids (blood and saliva) of smokers and nonsmokers. The results of anions determination in real samples obtained with the proposed techniques correlated well enough with the results of their determination by alternative methods - potentiometry and ion chromatography with fluorescent detector. At the same time the enzymatic procedures are more rapid and exact; besides, they use less complicated equipment.