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## Smart Plastic Antibody Material for Hemoglobin Tailored by Silica Surface Imprinting and with Charged Binding Sites: Its use as Ionophore in Potentiometric Transduction

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Human hemoglobin (Hb) is a globular metalloprotein, present in the blood and involved in gas transport. Hb-associated disturbances are related to several diseases, such thalassemia, anemia, heart disease and leukemia, or to side-effects from other diseases, such as cancer. Overall, it is of great importance to know the concentration of Hb in the blood in many health-related conditions.

There are many methods described in the literature for determining Hb. Most of these rely on antibody/antigen interactions, due to the high selectivity of the affinity reaction taking place between these biomolecules. However, the use of antibodies for Hb determination in routine clinical use is very expensive, due to the high cost of the material, the need for special handling and storage, and the non-reusability. These constraints may be limited by replacing natural antibodies by plastic receptors, obtained by molecular imprinting procedures.

Thus, this work describes a novel smart plastic antibody material (SPAM) by surface imprinting technique for the detection of Hb and its application to design small, portable and low cost potentiometric devices. The SPAM material was obtained by linking Hb to silica nanoparticles and allowing its subsequent interaction with different vinyl monomers, of different chemical functions and ionic charges. Control materials were designed in parallel to assess the ability of establishing stereochemical recognition of Hb and the effect of the kind/charge of the monomers employed. Scanning Electron Microscopy analysis confirmed the surface modification of the silica material used for imprint.

All materials were mixed with PVC/plasticizer and applied as selective membranes in potentiometric transduction. Suitable emf variations were detected only for selective membranes having a SPAM material and a charged lipophilic anionic additive. All control materials were unable to produce a potentiometric response.

Overall, good features were obtained for SPAM-based selective membranes carrying an anionic lipophilic additive. In HEPES buffer of pH 5, limits of detection were 43.8 $\mu$ g/mL for a linear response after 83.8 $\mu$ g/mL with a cationic slope of +40.4mV/decade. Good selectivity was also observed against other coexisting biomolecules. The analytical application was conducted successfully, showing accurate and precise results.

**Keywords:** Surface molecular imprint; Hemoglobin; Potentiometry; Ion-selective electrodes.