SURFACE CHARACTERISTICS OF CHEMICAL SENSORS

G. Gabor and G. Frishman
Israel Institute for Biological Research
Ness-Ziona 70450, Israel

ABSTRACT
The role of various surface structure features of optical sensors (optrodes) is related to their efficiencies. Various \(0\) and pH optrode models, in which the support-reagent distance, the polarity and pore size are varied are presented and their contribution to enhance sensitivity and reduce response times is discussed. For a given support - e.g. spherical silica beads with 200\(\mu\) diameter (Daltosils) optimal surface to pore diameter ratio, allowing sufficient concentrations of the reagent and the analyte respectively, yields the most efficient sensor (re sensitivity, response time etc). Increasing the support reagent distance results in the enhancement of the sensitivity. For \(pO_2\) sensors, the sensitivity expressed as \(R\), increases from 39 to 131 (a factor over 3) upon the introduction of 5 more C-C bonds. The sensitivity of the pH sensors, in which the distance is increased by 8 chemical bonds, is increased by a factor of over 4 from 1.74 to 7.4 for HPTS, or from 1.52 to 5.6 for 7-hydroxy-coumarin-3-acetic acid.

INTRODUCTION
The design of extremely small devices (sensors) that permit in situ on line measurements in biological and industrial systems are of major interest[1-10]. An efficient optical sensor is based on a fast chemical reagent between the analyte and the reagent immobilized at the sensor tip[3,5,8-10]. The analyte is imposed by need, while the reagent is selected, keeping in mind that it should have the following properties:
1) Easily measurable optical signal (absorption or fluorescence) resulting from its interaction with the analyte - meaning high sensitivity.
2) Fast reaction with the analyte - i.e. fast response
3) It should be immobilizable
In this paper we will discuss the region of immobilization, that includes the surface of the support and the reagent phase and its possible contribution to the efficiency in several ways:
a) The reagent-surface distance, ruled by the absence/presence (and length) of a spacer between the reagent and the surface, 1) exposes the reagent to the analyte stream; 2) determines its orientation relative to the surface - by imposing rigidity or permitting free rotation.
b) The polarity of the reaction site is determined by the vicinal groups, on the surface[11,12] or the spacer. When highly polar silica surfaces - CPG or Daltosi[13] are employed for oxygen sensors, they must be modified to facilitate the approach of the
none polar $O_2$ molecule - the analyte - to the bound reagent. A non polar spacer might serve a double purpose.

c) The support's pore size controls the approach of the analyte to the surface bond reagent, whose quantity is ruled by the surface area. This is a trade off.

To demonstrate these features $pO_2$ and pH optrode models were used.

**EXPERIMENTAL**

**Materials**

CPG (Controlled Pore Glass) from Fluka, Switzerland and spherical CPG, Datitosil, with various known pore parameters and sizes from Serva, Germany - were used as supports (or sensor heads). CPG 170 and CPG 550 are 75-125 $\mu$m beads with pore diameters of 170 and 550Å respectively. The daltosils had 100-200 $\mu$m diameters and pore diameters of 200, 600, 1000 and 2500 Å [13].

Pyrenbutyric Acid (PBA) aminopropyltriethoxysilane (APTES), 6-amino-caproicacid (6ACA) and hexamethylenediamine (HMDA) were from Aldrich, USA. Pyrennonanoicacid (PNA); the coumarin derivatives: umbelliferrone-3-aceticacid (3AU), umbelliferrone-4-aceticacid (4AU) and umbelliferrone-3-carboxylicacid (3CU) as well as hydroxypyrene-trisulfonicacid (HPTS) and its acetate ester were from Molecular Probes, USA.

**Measurements**

All measurements were carried out by an SLM-500 Spectrofluorometer. CPG or (Daltosil) beads carrying the immobilized reagents were held in front of a fiber bundle in a polyethylene tube. The diameter of the tube was about the same or slightly bigger than the ending of the fiber bundle; it was in fact a sleevelike tube extension, that permitted the injection of the various buffers onto the sensor heads in it. The fiber bundle, purchased from Volpi A.G., Switzerland, contained 100 optical fibers of 200 $\mu$m diameter, 50 connected to the light source and 50 to the PMT of the SLM-500.

The fluorescence 1) of the pyrene derivatives was excited at 342 nm, and recorded at 395 nm and 460 nm; 2) of the coumarin derivatives were excited at 370-395 nm and recorded at 445-465 nm; 3) and of HPTS was excited at 470 nm and recorded in the 490-600 nm range.

The quenching efficiency $R_I$ is defined as the ratio of the fluorescence intensities at 0% and 100% oxygen - $I/I_{O_2}$. The relative quantities of monomers and dimers (excimers) is expressed by $R_M$. $R_M$ is the ratio of the fluorescence intensities in the monomer band-$I_M$ to that in the excimer band-$I_{EX}$ of pyrene monitored at 395 nm and 460 nm respectively $R_M = I_M/I_{EX}$

**Immobilization of the reagents**

All reagents were immobilized - chemically bond - to the various silica beads "the sensor heads" (supports): silanization of the beads applying APTES according to the Whelall method[14] was followed by DCC coupling of the various reagents (except HPTS)[15].

To vary the distance between the fluorescent entity and the silica surface pyrenbutyric and pyrennonanoic acids were used for the $pO_2$ sensor heads, yielding a distance difference of 5 C-C bonds between