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Ultra-thin porous glass membranes—An innovative material for the immobilization of active species for optical chemosensors

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ABSTRACT

In addition to polymers, porous glasses can be used for the immobilization of indicators, chromoionophores or enzymes. Advantages of these materials include, among others, the photochemical and thermal stability. Porous glass membranes (CPG) based on phase-separated alkali borosilicate glasses with thicknesses of 250–300 μm and dimensions of approximately 9–13 mm^2 were used in this work. The average pore diameter was found to be between 12 and 112 nm. Initially, the membrane permeability for water was determined. Furthermore, the absorption spectra for the water-soaked membranes were recorded optically. CPG membranes which are pH-sensitive were prepared based on the covalent immobilization of thymol blue and a derivative of styryl acridine. In each case, the absorption spectra of the immobilized indicators are shown. The t_{90}-times vary between 4 and 20 min and were determined for the thermodynamic equilibrium. The influence of the ionic strength on the characteristic curve is discussed and detailed results are given. After the storage time of about 900 days a pH-sensitivity for a CPG membrane styryl acridine derivative sample was still detectable.

1. Introduction

For the development of optical chemosensors, it is necessary to immobilize indicators, chromoionophores, enzymes or phase-transfer accelerators to a substrate or into a matrix [1,2]. Partially contradictory requirements are set for immobilization techniques of chromophores as optical chemosensors: On one hand, the chromophore must be accessible to ions, i.e., the immobilization matrix must have a high ion permeability. On the other hand, the leaching of the indicator to or away from the matrix is undesirable. Furthermore, the immobilization framework should have tailored optical properties, such as defined transparency or scattering properties.

Besides the application of polymers [3], porous glasses were used as a matrix for immobilizing [4,5] active components. Regarding the latter, one can distinguish between sol-gel membranes and porous glasses, produced by extraction of phase separated alkali borosilicate glasses (Porous VYCOR® Glass (PVG) or Controlled Pore Glass (CPG), see [6]). In this paper only controlled pore glass is considered.

In general, following differences between polymers as immobilization matrix and porous glasses as substrate for the immobilization are given [4]:

Organic carriers are photochemically and thermally unstable, which limits the application in sensor technology since excited states may react with the surroundings. Furthermore, polymeric networks are less rigid than glass. Reactions with internal impurities such as indicators, solvents and monomers can occur, especially at high temperatures. Polymers may swell and deform, while porous inorganic materials are more stable, have a higher surface area and chemical stability. However, the ranges of indicators which can be immobilized during the process without changes in their structure are limited.

The development of pH sensors on the basis of pH colorimetric indicators, immobilized onto porous glasses, was the subject of several studies. Baldini and Falai [7] proposed an optical-fibre pH sensor with methyl red as optical indicator. Methyl red was immobilized on CPG particles. In the first step, the surface of the CPG was modified with γ-aminopropyl-triethoxysilane. Afterwards, methyl red was covalently bound to the modified glass surface. Finally, the CPG particles were fixed on a plastic reflector. In comparison to former systems, the optical-fibre pH sensor showed several advantages. The working range of the sensor was quite broad. The authors observed a linear relationship from pH=3 up to pH=8. No leakage of the pH-sensitive dye from the CPG matrix was observed. The plastic reflector with the immobilized CPG particles could be replaced easily. Additionally, the sensor was characterized by a fast response time.

Several pH indicators, immobilized onto CPG particles with different pore diameters, were used as components of fibre-optic biosensors for the determination of different pesticides in fruits or vegetables [8,9]. The sensors are based on the inhibition of the

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Table 1
Physical properties of the appropriated CPG membranes.

	Specific surface area [m^2/g]	Specific pore volume [cm^3/g]	Porosity[a] [%]	Average pore diameter [nm]	Membrane thickness [μm]
CPG I	179*	0.56*	55*	12*	300
CPG II	66*	0.47**	51**	47**	300
CPG III	27*	0.45**	50**	66**	270
CPG IV	26*	0.56**	55**	112**	250

[a] Calculated with 2.2 g/cm^3 for skeletal density of the CPG-membranes (determined by helium pycnometry).
* Determined by low temperature nitrogen adsorption.
** Determined by mercury intrusion.

immobilized enzyme acetylcholinesterase by various pesticides. The enzyme-catalyzed substrate degradation is used for the detection of the enzyme activity. In the absence of pesticides, the enzyme is more active and therefore the hydrolysis of the substrate occurs faster. The formed acetic acid decreases the pH in the solution, which is detected by the pH-sensitive indicator. In the presence of different amounts of pesticide, the enzyme activity is reduced. Under comparable conditions it results in a less decreasing pH value. The concentration of the pesticide is proportional to the changes in the pH value. In this sensor concept, the immobilized pH indicator acts as optical transducer of the enzyme's inhibition by the corresponding pesticide. Xavier et al. [8] immobilized chlorophenol red onto diazotized CPG particles with 70 nm pore diameter. The modified CPG was placed at the common end of a bifurcated fibre optic bundle, which was then integrated in a flow-through cell. The CPG/indicator component showed a broad working range between pH=4 and pH=9.5. This was sufficient to detect the pH changes through the enzyme-catalyzed reaction. In an earlier study, Andres and Narayanaswamy [9] covalently immobilized thymol blue onto aminopropyl CPG beads with 50 nm pore size through a condensation reaction with formaldehyde. The bioactive component of the sensor consisted of a mixture of CPG beads loaded with immobilized acetylcholinesterase or thymol blue. As thin layer the glass bead mixture was packed at the tip of a bifurcated fibre optic sensor head, which was again placed in a flow-through cell. The working range of the CPG/indicator component was not specified.

In this work, porous glass membranes, prepared by extraction of phase-separated alkali borosilicate glasses (CPG membranes), were tested as a matrix for the immobilization. Compared to granular particles, membranes show the following advantages:

- favorable optical and handling properties due to plane parallel shape
- best reproducibility of the indicator–membrane-complex's properties
- simple combination of multiple functional layers in a sensor
- possibility of changing individual membranes in order to regenerate the functional species or to practice a disposable concept.

The suitability of the CPG membranes as immobilization matrix for dyes will be demonstrated exemplary using the immobilization of thymol blue and a styryl acridine derivative. Porous membranes with pore diameters in the range of 12 to 112 nm were used and characterized.

2. Experimental part

2.1. Materials

2.1.1. CPG membranes

In comparison to sol–gel membranes or layers, which are preferably used as components of optical chemosensors, porous glass membranes based on phase-separated alkali borosilicate glasses show several advantages:

- narrow pore size distribution,
- controllable pore size in a broad range between 2 and 200 nm,
- good reproducibility in the textural properties (pore structure),
- high hydrolytic stability.

However, porous glass membranes are commercially available only as Porous VYCOR® Glass (PVG) with a pore size of about 4 nm. This limits the use of PVG membranes in many sensor applications (bulky functional species, accessibility for the analyte, response time). To overcome this limitation, porous glass membranes with a controlled pore size (CPG) in the range between 12 and 112 nm were used. In this work porous glass membranes (thickness d=250–300 μm) were used, for the first time, as hydrophilic carrier for the immobilization of pH-sensitive dyes whereas the membranes themselves act as mechanical support. The amount of immobilized indicator as well as the accessibility of the functional species could be optimized by variation the textural material properties. The properties of the different porous glass (CPG) membranes are summarized in Table 1.

The manufacturing process of the CPG membranes is described in [10]. Initial glass blocks with a composition of 70 wt% SiO_2, 23 wt% B_2O_3 and 7 wt% Na_2O and a lateral block size of 9 × 13 mm^2 were used for the preparation of the CPG membranes. A typical composition of this porous network was 94.5 wt% SiO_2, 5.3 wt% B_2O_3 and 0.2 wt% Na_2O. The texture properties of the CPG membranes were determined by mercury intrusion and low temperature nitrogen adsorption.

2.1.2. Used chemicals

The silane (γ-aminopropyltriethoxysilane) and dimethylformamide (DMF) were purchased from Fluka (Steinheim) in p.a.-quality. Thymol Blue, 2-(4-morpholino)ethanesulfonic acid; (MES; biological buffer solution, pH=6.1) and formaldehyde (CH_2O; 37 wt% in H_2O) in p.a.-quality were received from Sigma (Neu-Ulm). Different buffer solutions where used to analyze the pH-sensitive behavior of the membrane/indicator complex and the influence of the ionic strength. The buffer solutions were prepared according to Coch Frugoni [11]. The styryl acridine derivative is described in Section 2.2.4. All chemicals used but not mentioned in this chapter were of analytical grade.

2.2. Characterization and modification of CPG membranes

2.2.1. Water permeability

The permeability of distilled water at 25 °C was calculated after Darcy (Eq. (1)) for some selected CPG membranes. That parameter is significant for flow through membrane sensors [12]. The membrane