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ABSTRACT

Hybrid nanocomposites based on siloxane sols, chitosan and poly(monomethyl itaconate) (PMMI) as organic polymer counterparts were prepared. Tetraethyl ortosilicate was used as inorganic network forming reagent that was first transformed into soluble polymeric species by acid catalyzed hydrolysis and condensation reactions. The siloxane sol was then mixed with different amounts of PMMI and chitosan at room temperature to form the hybrid material. These hybrid materials could be easily cast as transparent and flexible films. Atomic force microscopy study showed that the organic components are more or less homogeneously distributed at nanometer scale. The hybrid films were used as membranes for the construction of all-solid-state type potentiometric electrodes. The ion-sensing capacity of the nanocomposite was tested for different anions and the best results were obtained for NO_3^- . It was concluded that the $-\text{NH}_3^+\text{HCOO}^-$ groups of chitosan act as anion exchanger creating a potential difference that vary with ion concentration in solution in the concentration range of $1.5 \cdot 10^{-4} - 10^{-2} \text{ mol/dm}^3$.

INTRODUCTION

The synthesis and study of organic-inorganic hybrid materials, having the characteristic of being compatible at submicron level of the organic component with an inorganic network, is actually an expanding research area¹⁻³. This is due to the scientific interest of these materials with their multiple applications such as biomimetics, sensors and biosensors⁴⁻⁶.

Hybrid materials in which the organic component is a polymer are most interesting since materials with high functionality can be obtained. These materials are called polymeric hybrids presenting different properties than those of the starting materials but with the advantage of preserving the inherent property of each component.

The introduction of organic groups in the inorganic network originates new structural properties and therefore promotes new applications for the resulting composite material. For instance, the incorporation of an organic material can impart new electrical properties such as conductivity or redox by introducing a conducting polymer together with transition metal alkoxides⁷. The sol-gel process is an appropriate technique that allows the preparation of these materials. The low reaction temperature of sol-gel processing facilitates the incorporation of functional organic compounds into inorganic matrices. Alkoxysilane monomers are commonly used as inorganic network forming reagents that are first transformed into soluble polymeric species by controlled hydrolysis and condensation reactions catalyzed by acid or base. At this point, it is possible to mix the inorganic component with the organic one to obtain hybrid materials at room temperature. The resulting composites can be prepared with different physical forms such as films, powders and monoliths^{8,9} and they are attractive candidates for optical devices, separation media, catalyst supports, microelectronic coatings and sensing membrane materials¹⁰. Recently, hybrid materials containing chitosan has been used as encapsulating matrix in biosensors⁶. In this work we use hybrid films based in chitosan as sensor element in ion-selective electrodes (ISE).

Ion-selective electrodes are potentiometric transducers based on membranes that are responsible for potentiometric response and selectivity of these transducers. When compared to many other analytical techniques, ISEs are relatively inexpensive and simple to use and have

extremely wide range of applications and wide concentration range. ISEs are used for determining the concentrations of various ions in aqueous solutions. Some of the main areas in which ISEs have been used are: pollution monitoring (CN, F, S, Cl, NO_3^- in effluents and natural waters); agriculture (NO_3^- , Cl, NH_4^+ , K, Ca in soils and fertilizers); food processing (NO_3^- and NO_2^- in meat preservatives); explosives (F, Cl, NO_3^- in explosive materials and combustion products) etc. Normally, plasticized poly(vinyl chloride) (PVC) is used as membranes to construct ISEs. This implies the use of special plasticizers, which are highly toxic.

Chitosan is a natural polymer and its biodegradability, non-toxicity and biocompatibility makes it a promising compound for developing new materials. These properties prompted extensive investigations of chitosan in the past. For example, chitosan has been studied^{11,12} as a material for contact lens, and matrix for cell and enzyme immobilization⁵. However, despite its attractive properties chitosan has received only very limited attention as a sensor material for the design of electrodes.¹³⁻¹⁵

It is also known that the amino side group of chitosan is responsible for its polycationic character and the formation of well-known intermolecular complexes with carboxylic and polycarboxylic acids.^{16,17} In this work hybrid nanocomposites based on siloxane sols, chitosan and poly(monomethyl itaconate) (PMMI) as organic polymer counterparts were formed on the surface of electrodes. The deposited membranes were tested as sensor elements for different anions.

2. EXPERIMENTAL

Chitosan (high molecular weight) purchased from Aldrich, (Milwaukee, USA), was purified by extraction with acetone in a Soxhlet for 24 h and dried under vacuum at room temperature to constant weight. The degree of deacetylation and molecular weight were determined according to the procedures described by Rinaudo et al.¹⁸. The degree of deacetylation was estimated as 83% and the weight average molecular weight was determined as $3.55 \times 10^5 \text{ g mol}^{-1}$ by combined viscosity-light scattering measurements. CHI was used as a 1% (w/v) solution in dilute formic acid (5% v/v). In order to obtain a siloxane sol, a solution of Tetraethoxysilane (TEOS) (Aldrich) in ethanol (Aldrich) was hy-

dolyzed with an aqueous solution of formic acid diluted in ethanol in order to have a molar composition TEOS: H₂O: Formic acid: EtOH = 1: 1: 0.01: 10. This solution was kept at 50°C in an open container during one day.

Monomethyl itaconate was prepared by reaction of itaconic acid with methanol under fairly acidic conditions by the method described by Baker et al. for lower monomers¹⁹. PMMI was obtained by radical polymerization in bulk under N₂ at 75 °C in the presence of 0.2 mol % of azobisisobutyronitrile as radical initiator. The apparent number and weight average molecular weight ($= 1.1 \times 10^5 \text{ g mol}^{-1}$, $= 1.6 \times 10^5 \text{ g mol}^{-1}$) was determined by gel permeation chromatography (GPC).

Film preparation and deposition on graphite surface: Siloxane sol and PMMI ethanol solutions were mixed in the appropriate ratios and stirred for 30 min. Then a CHI solution was incorporated and the mixture stirred for 24 hours. The SiO₂/CHI/PMMI molar ratio of the samples was calculated from the molecular weight of the monomeric unit of CHI (168.1 g mol⁻¹ considering the acetylation degree of 17%) and the molecular weight of the PMMI repeating unit (144 g mol⁻¹). Films of 15-20 μm thickness were obtained by deposition of the precursory solution on the surface of a conductive epoxy resin prepared by mixing Araldite with hardener and graphite which is used to construct an all-solid-state type electrode. The method for the construction of all-solid-state type electrode has been described previously²⁰.

Electromotive force (EMF) measurements: Electrochemical potentials were measured with the following galvanic cell: Ag/AgCl(s), KCl (1M)/bridge electrolyte/sample solution/ion-selective membrane/graphite epoxy resin. The sample solutions for potentiometric measurements consisted of sodium salts of the given anion. The ISEs were conditioned for at least 1 day in 10⁻² molar solution of the ions being investigated and then kept in the same solution when not in use. EMF values were measured with a pH/mV meter Hanna model pH 211 at ambient temperature (~22 °C). The measured anion concentrations were changed by injection of know concentration of the solution while stirring with a magnetic bar. Selectivity coefficients were calculated by the mixed solution method.²¹ Activities were calculated according to the Debye-Huckel procedure.²²

Calibration curves were used to calculate parameters such as slope, detection limit and the range of linear response. This was done through data adjustment by linear regression method following the Nernst law.

Characterization of the hybrid films: The films were analyzed in an infrared Fourier transform spectrophotometer, Nicolet 520 FT-IR, in the region from 4000 to 400 cm⁻¹. A commercial atomic force microscopy (AFM) (Nanoscope III, Digital Instruments) was used to perform the surface analysis of the hybrid films operating in tapping mode. For the purpose of comparison, the root mean square roughness (RMS) was chosen to characterize the topography of the films.

Swelling experiments: The swelling behavior was determined by immersing pre-weighed dry samples of about 200-250 mg in pH 4 and pH 9 solutions at 37 °C. The water uptake was calculated by measuring the weight gain of the samples at a given time interval. Excess surface water was blotted out with filter paper before weighing. The weight degree of swelling (W_{sd}) was calculated using the following equation:

$$W_{sd} (\%) = \frac{W_s - W_d}{W_d} \cdot 100$$

Where, W_s and W_d denote the weight of the swollen and dry samples, respectively.

3. RESULTS AND DISCUSSION

Sol-gel derived glasses are often fabricated using tetraethoxysilane (TEOS) as starting alkoxysilane. Despite the many advantages of a silica sol-gel matrix, the brittleness of these materials is a mayor obstacle because makes them unsuitable for ion-sensing membranes due to the mechanical strength problems. The capacity of chitosan to form transparent and flexible hybrid films with silica has been studied in our previous work²³. We found that these films disintegrated easily in aqueous solutions. However, the ternary nanocomposites CHI/SiO₂/PMMI prepared as self-supporting films were stable in aqueous solutions and they remained unaltered at all time. Moreover, the flexibility of the ternary films was found to depend on the SiO₂ and PMMI content.

Figure 1 shows the infrared spectra for the pure components and the hybrid film CHI/SiO₂/PMMI with molar ratio (r) 0.6:1:0.3 (CSP1). For comparison purposes, a chitosan film was prepared by casting the precursor solution. The region from 2000 to 350 cm⁻¹ is shown in detail.

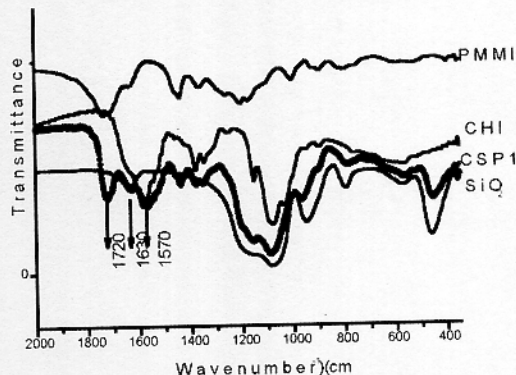


Fig. 1. Infrared spectra for CHI, PMMI, SiO₂ and hybrid film CHI/SiO₂/PMMI (0.6:1:0.3)

The main absorption bands of PMMI, CHI and SiO₂ are present in the spectra of hybrid films. The most important change is the displacement of the carbonyl absorption band of PMMI (1734 cm⁻¹) to lower frequencies ($\approx 15 \text{ cm}^{-1}$). This could indicate the role of the carboxyl groups in the compatibilization of the system components probably through hydrogen bonding. The characteristic signals of SiO₂ appear at 1089, 967 and 786 cm⁻¹. These IR peaks confirm the formation of structural units of Si-O-Si in the hybrid films. Another important change is the band at 1634 cm⁻¹ that it is not present in the spectra of the starting polymers or even in their physical mixture. This peak suggests the existence of a specific interaction between chitosan and PMMI what is indicative of complexation between the amino groups of chitosan and the carboxyl groups of PMMI.²⁴

Atomic force microscopy (AFM): The surface analysis of the hybrid films was performed by AFM. A typical AFM height image of the samples is presented in figure 2 top. The surface appears to be free of visible defects and is very smooth. For samples with molar ratios CHI/SiO₂/PMMI 0,6:1:0,3 and 0,3:1:0,3 average roughness value (RMS) of 0.436 nm and 0.443 nm were observed.

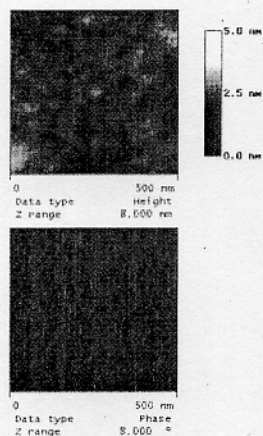


Fig. 2. AFM image of the surface of hybrid film CSP5. Top: Surface topology. Bottom: Phase contrast.

The organic components are more or less homogeneously distributed as is apparent by the film transparency and in addition, the included silica particles must be dispersed on length scales below that of the visible light wavelength. The phase contrast picture (Figure 2 bottom) shows a homogeneous surface indicating either that the silica particles are located in the interior of the film or that the surface of the film is composed of silica to a significant part. Hence, no localized areas of soft and hard material could be observed.

Ion selective electrodes based on CHI/SiO₂/PMMI hybrid films.

Studies carried out on the ternary hybrid systems has permitted us to obtain films with determined properties which makes them attractive to be employed as sensing membranes in ion-selective electrodes. We found that these hybrid films adhere strongly to the glass and graphite surfaces. Moreover, they do not disintegrate in water, likely due to the polyelectrolyte complex formation between CHI and PMMI. On the other hand, the method used for their preparation permitted us to deposit them on the surface of a conducting resin that is the method used for the construction of all-solid-state type electrodes. The construction of all-solid-state electrodes implies the deposition of a sensing membrane on the surface of a polymeric substrate mixed previously with a conducting material such as graphite which act as internal electrical solid contact.

For a preliminary evaluation, CHI/SiO₂/PMMI film with composition 0,6:1:0,3 was selected as sensing membrane for the ion selective electrodes. The constructed electrodes with these membranes were activated in 10⁻² mol/dm³ solutions of the anions to be studied. The potentiometric response of the electrodes was evaluated by plotting E (mv) values against log a, as already described in the experimental section. The slope (S) obtained from these plots indicated that the electrodes responded adequately. Figure 3 shows the calibration curve obtained for NO₃⁻, Cl⁻ and Ac⁻ and the characteristics of the potentiometric response toward these anions are listed in table 1.

Table 1. Characteristics of the potentiometric response towards different ions for electrodes with hybrid membranes CHI/SiO₂/PMMI (CSP1) (0,6:1:0,3).

Conditioning solution	Slope (mV/decade)	r*	Linear range mol/dm ³	Detection limit mol/dm ³
NaNO ₃	-59	0.9980	1.5 10 ⁻⁴ – 10 ⁻²	1.0 10 ⁻⁴
Na ₂ HAsO ₄	-40	0.9776	2.0 10 ⁻⁴ – 10 ⁻²	1,6 10 ⁻⁴
NaCl	-55	0.995	2.5 10 ⁻⁴ – 10 ⁻²	2.0 10 ⁻⁴
NaAc	-27	0.9960	7.5 10 ⁻⁴ – 10 ⁻²	7,5 10 ⁻⁴
Na ₂ SO ₄	-31	0.9944	3.1 10 ⁻⁴ – 10 ⁻²	2.5 10 ⁻⁴

* Correlation coefficient

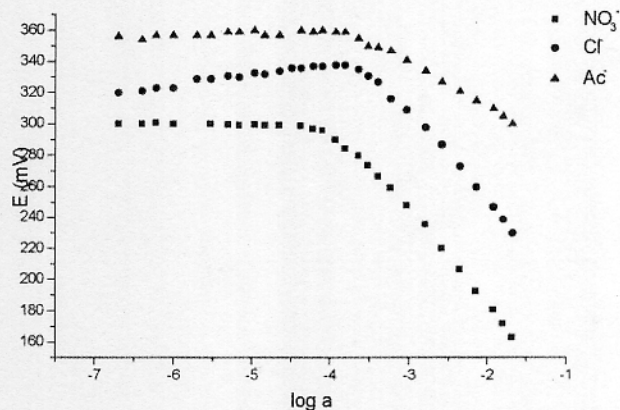


Fig. 3. Potentiometric responses of electrodes based on CHI/SiO₂/PMMI (0,6:1:0,3) membrane conditioned in the corresponding anion solution.

From the results it can be appreciated that the best parameters obtained are for the nitrate ion since the value of slope is quite near to that predicted by the Nernst law. On the other hand, the detection limit of the electrode is lowest in this case as compared to those obtained for other anions studied. Therefore, new electrodes were constructed and were tested for nitrate ion detection.

In order to study the effect of the membrane composition on the response of the electrode to nitrate, membranes with lower chitosan concentration as well as binary membranes consisting of CHI/PMMI and CHI/SiO₂ were prepared and their performance as ISE for nitrate ion detection was studied. Membranes obtained from binary systems CHI/SiO₂ were not stable. They were easily disintegrated in water and therefore were not studied further. The results are shown in table 2.

Table 2. Effect of membrane composition on the response of electrodes for NO_3^- .

Membrane	CHI:SiO ₂ :PMMI	Slope (S) (mV/decade)	r*	Linear range mol/dm ³
CSP2	0.3:1:0.3	-72	0.9934	4.1 10 ⁻⁴ -7.110 ⁻³
CP	1:0:0.5	-67	0.9699	4.4 10 ⁻⁴ - 10 ⁻²
CS	0.5:1:0	-63	0.9955	5.910 ⁻⁴ - 10 ⁻²

* Correlation coefficient

It was observed that by varying the composition of the membranes their response did not improve, on the contrary, higher slope values were obtained indicating unsatisfactory response of the electrodes. These results also confirm that in the films studied, chitosan is responsible for the response of the electrode for nitrate ion detection.

In the preparation of the membranes, chitosan was first dissolved in dilute formic acid. The resulting solution had a pH of 1.2. This means that all amine groups of chitosan ($\text{pK}_a=6.3$)¹¹ are protonated at this pH, forming a quaternized ammonium salt. The $-\text{NH}_3^+\text{HCOO}^-$ groups of chitosan can act as anion exchanger between the membrane and solution interface and this creates a potential difference that vary with ion concentration in solution.

It is worth to mention that the electrodes had a response time of 20-25 sec. The fast response of these electrodes could be explained by considering their water sorption behavior.

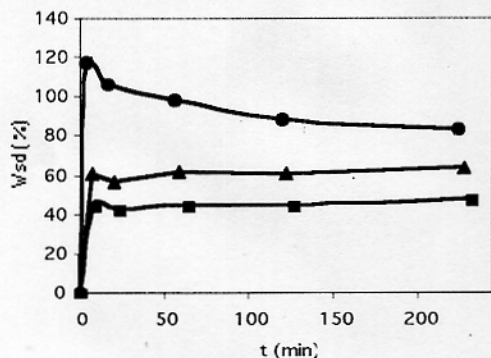


Fig. 4. Water sorption isotherms for CSP-2 (■), CSP-1 (▲) and CP (●) at pH 4 and 37 °C.

Figure 4 shows the variation of the water uptake (W_{sd}) as a function of time at pH 4 for hybrid samples of different compositions, including the sample without SiO₂ (CP). The water sorption kinetics shows that the water content at equilibrium was reached in the first minute, which indicates a fast ion interchange capacity of the membrane. As expected, the hybrid films show a lower swelling due to the more rigid structure associated with the SiO₂ matrix (see figure 4).

The reproducibility in the response of the electrodes with time was studied. The results are shown in figure 5, where the response of the same electrode is compared as function of the time of the usage of the electrode. As can be seen from this figure, the values of measured potentials for the same activity of NO_3^- ion, for different time of the use of the electrode, is relatively close to each other.

A better reproducibility was observed with the increase in activity.

This fact is easy to understand by considering that working with low activity levels needs a series of precaution to be taken since the presence of other ionic species affects the reproducibility of the measurement, independent from the quality of the electrode used.

The constructed electrodes had a lifetime of 15 days without divergence in their response as was evidenced from the fact that values of the slope did not decrease during this period.

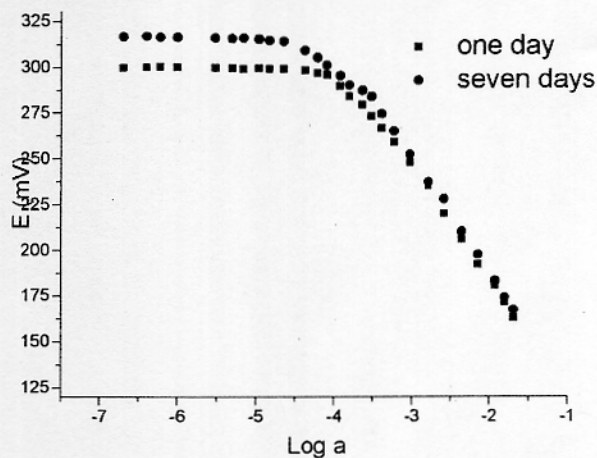


Fig. 5. Calibration curves for study the electrode response reproducibility with time.

The quantification of the selectivity or the degree of interference of some anions on the response of the electrodes was studied by determining the corresponding potentiometric selectivity coefficient (K_{AB}^{Pot}). The procedure consists in calibration of the electrodes in the presence of a fixed concentration of an interfering anion. The results are shown in table 3.

Table 3. K_{AB}^{Pot} Values for different interfering anions.

Interfering anion	$K_{NO_3^-, B^-}^{Pot}$
Ac ⁻	9.1 10 ⁻²
Cl ⁻	1.1 10 ⁻¹
SO ₄ ²⁻	1.1 10 ⁻²

The values obtained for SO₄²⁻ ions are lower than those obtained for monovalent ions. This means that the electrodes showed higher selectivity for NO₃⁻ over SO₄²⁻ while monovalent ions presented mayor interference. The values of detection limits found for the electrodes studied here are comparable with those reported recently in the literature²⁵ for other electrodes for nitrate, while the values of selectivity coefficients are higher. However, these electrodes could be used for nitrate ion determination in their range of nernstian response.

CONCLUSIONS

We have described the ion sensing properties of hybrid films based on chitosan, PMMI and SiO₂. The hybrid membranes can be obtained as self-supported homogeneous and transparent films or can be cast on the surface of a graphite electrode due to its good adhering and mechanical properties. This allows the construction of robust electrodes based solely on low environmental impact materials. Also it's possible to control the concentration of amino and carboxylic groups in the films and thereafter change its ionic properties by varying the composition of the organic components. The -NH₃⁺HCOO⁻ groups can act as anion exchanger for the detection of anions. This allowed the hybrid material to be used in the development of electrodes for the potentiometric determination of NO₃⁻ anions in a fast and easy way.

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