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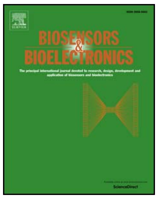

New sensor based on membranes with magnetic nano-inclusions for early diagnosis in periodontal disease

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ABSTRACT

A series of sodium selective membranes with magnetic nano-inclusions using p-tertbutyl calix[4]arene as ionophore and polymeric matrix (polyvinyl chloride) have been developed, and the corresponding sodium selective sensors were obtained for the first time. A linear range was registered between 3.1×10^{-5} and 10^{-1} mol dm^{-3} and near Nernstian electrochemical answer: 55.73 mV/decade has been recoreded for PVC (polyvinyl chloride) – based sodium selective sensor, with a response time of 45 s. Due to their small dimensions, sensors could be used for measuring ions from the gingival crevicular fluid directly into the peri-odontal pocket, avoiding the difficulties of collecting an appropriate amount of fluid for analysis. Alterations in the inorganic ions level could be evidenced with this new device, assisting the early diagnosis and prevention of periodontal disease.

1. Introduction

During the last decades, an important number of researchers focused on magnetic nanoparticles synthesis, on the study of their properties and adaptations performed for various applications (Tartaj et al., 2003; Thanh, 2012; Liu et al., 2013 Timonen et al., 2013). Using ferroferric nanoparticles several polymers could be reinforced, plasticized or transformed into sensing materials and used afterwards for solving important medical, industrial and research issues (Lin et al., 2006; Zhang et al., 2007).

The usage of polymeric coatings imposed an intensive control of the polymer compatibility with the matrix and the interaction strength. The covering could be either grafted or only strongly absorbed. Hydroxyl groups from nanoparticles surface could interact with a silane type coupling agent with various functionalities. A new sensing material could be obtained by modifying the initial structure of the magnetic nanoparticles by covering it with an appropriate reagent - $\omega - 10$-amino-acid (Nechifor et al., 2002), which could subsequently be functionalized with ionophores using cyanuric chloride as a spacer (Huthmacher and Most, 2005).

Lately, considerable attention was directed towards calix[*n*]arenes, an interesting type of ionophores. These compounds proved to be able to act as ionic and molecular binding hosts, being extensively treated as a new class of macrocyclic molecular receptors (Grady et al., 1996; Cram and Cram, 1997; Gutsche, 2008; Deska and Śliwa, 2010; Davis and Higson, 2011; Vicens and Böhmer, 2012). It was proved that calix[4]arene derivate have ionophoric properties towards ions of high biological interest such as sodium ions (Careri et al., 1993). Due to their selectivity for various ions, calix[*n*]arenes are used as ionophores in many polymeric ion selective membranes. Such membranes represent the key component of the ion selective electrodes (ISEs) with dynamic response given by the selective analyte complexation. Depending on the size and charge of the ion of interest, an ion selective membrane based electrode could select different sensory elements providing in this way a quick and powerful analytical sensing method. The synthetic macrocycles like calixarenes are of interest to transport across membranes, ion channels and slow release of drugs (Deska and Śliwa, 2010; Vicens and Böhmer, 2012). Concepts as sensors and implantable controlled drug delivery have also a continuous and tremendous development. Although they represent a great promise, there are pending matters related to the encapsulation/inactivation (Lieb and Stein, 1971; Saudek, 1997; Low et al., 2000; Zantner et al., 2007). Within biological

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systems, the exchange of ions between cells, tissues and devices represents a sort of physical-chemical communication and diffusion into the circulatory system (Lieb and Stein, 1971), therefore, the material used has to be biocompatible.

Periodontium, the support of maintaining teeth in function, consists in four main components functioning as a single unit but with distinct location, tissue architecture, biochemical and chemical composition: gingiva, periodontal ligament, cementum and alveolar bone (Fiorellini and Stathopoulou, 2012).

Periodontal disease, a chronic inflammation affecting nowadays many patients of all ages (Münchow et al., 2015), is one of the most aggressive pathology in dentistry, impairing the integrity of the periodontium and ultimately, tooth loss.

Up to now the diagnosis of periodontal disease was mostly based on a clinical and radiographic evaluation, not reflecting the underlying inflammatory response (Buduneli and Kinane, 2011; Baeza et al., 2015).

Gingival crevicular fluid (GCF), a plasmatic extravasate from the gingival sulcus, manly composed of serum as well as proximate periodontal tissue and bacteria, contents electrolytes, protein, glucose, enzymes, complement, leucocytes and exfoliated epithelial cells and reflects periodontal destructive processes, demonstrating a high diagnostic potential (Lamster and Ahlo, 2007). Therefore, designing innovative non-invasive methods for early detection, severity assessment, treatment efficacy as well as prognosis of disease progression is a great challenge nowadays.

Measuring electrolyte concentration in GCF, especially the concentration of sodium, potassium and calcium ions reflects the clinical status of the periodontal tissues, so that the estimation of these ions may be used as a potential diagnostic marker of an active disease status (Koregol et al., 2011).

The present study aims obtaining a new device able for local sensing, in periodontal pocket, the inorganic ions of interest utilizing membranes with magnetic nanoparticles and ionophores in different polymeric matrix. Implantable device failure is expected to be avoided by using magnetic properties of the nanoparticles included in the composition, due to the ability to be remote controlled based on its specific property, namely the magnetic behavior.

2. Experimental

2.1. Materials

The materials used for membrane preparation were: a) polymeric matrix: PVC (Polyvinyl chloride) high molecular mass (Breon, BP Chemicals, UK) b) plasticizers: 2-nitro phenyl octyl ether, o-NPOE (Fluka, Germany); c) ionophore: in lab prepared p-tert-butyl calix[4] arene from p-*tert*-butylphenol (Sigma-Aldrich, Germany), 37% formaldehyde solution (Fisher Chemical Company), sodium hydroxide, ethyl acetate, acetic acid, nitrogen, acetone, toluene, diphenyl ether, phenol, all purchased from Sigma-Aldrich, Germany; d) lipophilic anionic site: potassium tetrakis(4-chlorophenyl)borate, KTpClPB (Fluka, Germany); e) solvent: tetrahydrofuran, THF (Fluka, Germany) freshly distilled; f) analytical grade chlorides of sodium, potassium, calcium, magnesium and ammonium (Fluka, Germany); g) materials for obtaining covered functionalized nanoparticles: $(NH_4)_2SO_4 \cdot FeSO_4 \cdot 6H_2O$, $Fe_2(NH_4)_3 \cdot (NH_4)_2SO_4$ and KOH, cyclohexanol from Sigma-Aldrich, Germany, iso-propanol and pyridine (Merck, Germany), deionized water, acetone, $CHCl_3$ (Merck, Germany), cyanuric chloride (Sigma-Aldrich, Germany) and $\omega-10$-amino-acid (Merck, Germany); h) polyimide (PI) of electronic use (PI-2555, Hitachi DuPont MicroSystems, LLC, Japan) for sensor encapsulation.

2.2. Methods

2.2.1. Magnetic nanoparticles synthesis

The functionalized magnetic nanoparticles have been synthetized according to the modified co-precipitation method proposed by (Nechifor et al. 2010; Massart, 1981). Respecting the ratio $[Fe^{2+}]/[Fe^{3+}] = 1$ and the requested basic pH value, all the chemicals: $(NH_4)_2SO_4 \cdot FeSO_4 \cdot 6H_2O$, $Fe_2(NH_4)_3 \cdot (NH_4)_2SO_4$, KOH have been thermostatically mixed up (30 min at 60 °C). The obtained Fe_3O_4 precipitate was washed with deionized water and dispersed in chloroform immediately after its collecting from the reaction mass with a permanent magnet (Carpenter, 2001). Subsequently, the magnetic nanoparticles have been functionalized (Berry and Curtis, 2003) inside the colloidal mill vat (500 rotations/min) being covered with $\omega-10$-amino-acid (in acetone media for 12 h) and then cyanuric chloride has been added (in acetone media and pyridine for avoiding the reaction reversibility). Finally, the functionalized nanoparticles have been washed with acetone and deionized water.

The obtained nanoparticles were characterized before and also after the functionalization by SEM, FTIR and TGA analysis. The detailed aspects of these characterizations are presented elsewhere (Nechifor et al., 2010).

2.2.2. Procedure for obtaining p-tert-butyl calix[4]arene

The desired ionophore, p-tert-butyl calix[4]arene was obtained as indicated by Gutsche (Gutsche et al., 1986), form a mixture of p-*tert*-butylphenol, 37% formaldehyde solution and sodium hydroxide (Meier and Detellier, 1998; Zantner et al., 2007) heated for 2 h at 120 °C in a water separator. The obtained yellowish product was allowed to cool down at room temperature and was taken out the flask, triturated and dissolved in chloroform, then washed with aqueous HCl solution for remaining NaOH removal. For the final washing stage, deionized water was used. After chloroform evaporation, the intermediate compound obtained was put in diphenyl ether refluxed for 2–2.5 h (under N_2). Ethyl acetate has been added before filtration of the synthetized compound and a white crystalline reaction product was obtained. This compound was used for its high affinity for Na^+. The poor solubility of p-tert-butyl calix[4]arene (de Namor et al., 1995,1986) was overcome by binding to the magnetic nanoparticles and subsequently homogeneously dispersing it into the polymeric matrix.

2.2.3. Procedure for obtaining polymeric ion-selective membranes

p-tert-butyl calix[4]arene (TBC4A), was mixed with the prepared functionalized magnetic nanoparticles (fMNp) for obtaining a complex active element (CAE) within the selective membrane.

Various PVC based sodium selective membranes were prepared from the typical composition: 33% PVC, 66% plasticizer (o-NPOE), 1% CAE and lipophilic agent. The cocktail of CAE, plasticizer and PVC was dissolved in freshly distilled tetrahydrofuran and then mechanically and magnetically stirred at room temperature. The selective membrane composition has been optimized, using ac impedance results, by varying the proportions (% in weight) of the components as following: PVC solution in THF between 32.0–33.5%, the plasticizer, oNPOE, between 64.0–66.5%, CAE between 1.0 – 1.4% and the lipophilic salt from 0.0% to 0.4%. For obtaining CAE, a ratio of fMNp: TBC4A equal to 1:2 was applied. The membrane compositions obtained were cast onto special PTFE molds with mobile support allowing membrane thickness control − 0.2 mm (Totu et al., 2006) - and then the solvent was left to evaporate for 24 h in a desiccator.

2.3. Structural characterization

The selective membranes obtained were structural characterized taking as reference a TBC4A, PVC membrane without magnetic nanoparticles.

2.3.1. FT-IR spectrophotometric analysis

The ATR - FTIR spectra of the samples were recorded at room temperature using a Perkin–Elmer Spectrum Two IR spectrometer (PerkinElmer, UK). Attenuated total internal reflection FTIR