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Novel biomimetic composite material for potentiometric screening of acetylcholine, a neurotransmitter in Alzheimer's disease

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ABSTRACT

This work describes a novel approach to produce an antibody-like biomimetic material. It includes preparing composite imprinted material never presented before, with highly conductive support nanostructures and assembling a high conductivity polymeric layer at low temperature. Overall, such highly conductive material may enhance the final features of electrically-based devices. Acetylcholine (ACh) was selected as target analyte, a neurotransmitter of importance in Alzheimer's disease. Potentiometric transduction was preferred, allowing quick responses and future adaptation to point-of-care requirements.

The biomimetic material was obtained by bulk polymerization, where ACh was placed in a composite matrix of multiwalled carbon nanotubes (MWCNTs) and aniline (ANI). Subsequent polymerization, initiated by radical species, yielded a polymeric structure of polyaniline (PANI) acting as physical support of the composite. A non-imprinted material (NIM) having only PANI/MWCNT (without ACh) has been prepared for comparison of the biomimetic-imprinted material (BIM). RAMAN and Fourier Transform Infrared spectroscopy (FTIR), Transmission Electron microscopy (TEM), and Scanning Electron microscope (SEM) analysis characterized the structures of the materials.

The ability of this biomaterial to rebind ACh was confirmed by including it as electroactive compound in a PVC/plasticizer mixture. The membranes with imprinted material and anionic additive presented the best analytical characteristics, with a sensitivity of 83.86 mV decade^{-1} and limit of detection (LOD) of 3.45 × 10^{-5} mol/L in HEPES buffer pH 4.0.

Good selectivity was observed against creatinine, creatine, glucose, cysteine and urea. The electrodes were also applied on synthetic serum samples and seemed a reliable tool for screening ACh in synthetic serum samples. The overall performance showed fast response, reusability, simplicity and low price.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease associated to dementia, and until now there are no definitive treatments or prophylactic agents. This disease is related to the presence of two types of abnormal deposits, senile plaques and neurofibrillary tangles, and extensive neuronal loss [1]. It is linked to cognitive and memory deterioration, a variety of neuropsychiatric symptoms, behavioural disturbances, and progressive impairment of daily life activities. Despite its great social and economical impact, there is no current test capable of providing an accurate diagnosis of AD, which is essential to give the appropriate therapy and follow-up to each patient. Research activities targeting such possibility include the identification of AD biomarkers in several biological fluids.

Cholinergic abnormalities are also present in AD brains [2,3]. It has been known that the level of acetylcholine (ACh) receptors is reduced in AD episodes [4] and that dysfunction of cholinergic signal transmission could be responsible for the symptoms of AD. In addition, anti-cholinergic drugs, used for the treatment of Parkinson's disease, lead to tempt amnesia, which clinically looks like the symptoms of AD [5]. However, the amnesia induced by anti-cholinergic drugs can be reversed by withdrawal of the drugs. This phenomenon implies that augmentation of the concentration of ACh within the surviving synaptic clefts could counter the amnesic symptoms found in AD.

Acetylcholine is a neurotransmitter and can be found in both peripheral and central nervous systems (PNS and CNS) in mammals including humans [6]. In the PNS, ACh binds to Acetylcholine receptors (AChR) and regulates muscle contraction. In the CNS, ACh plays a crucial role in the processes related to behavioural activities, arousal, attention, learning, memory, etc. ACh is synthesized in neurons from choline by means of choline acetyltransferase and acetyl coenzyme A [7]. The dysfunctional ACh regulation in the brain causes neuropsychiatric disorders

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such as Parkinson disease, AD, and myasthenia gravis. An accurate, fast, inexpensive and reliable method to monitor ACh in biological fluids is therefore crucial.

The literature contains several analytical procedures for ACh detection and quantification. It includes mostly separative techniques, including capillary electrophoresis [8–11] and liquid-chromatography [12–16]. These techniques are unsuitable for routine control procedures because each assay may take several days, being conducted within proper laboratorial facilities. Other methods report enzymatic [17–20] assays. These methods offer high selectivity but the overall procedure is time-consuming and too expensive for routine analytical measurements. Colorimetric methods are also reported [21–25], some of which combine enzymes [22,23] and metal oxide nanomaterials [22]. In general, colorimetric assays hold advantages over other instrumental analysis due of their low cost and simplicity. Electrochemical biosensors have also been employed in ACh detection. Some approaches reported in the literature make use of enzymatic systems [26,27] [28] or aptamers [29] as biorecognition element of the binding event. Thus, other methods enabling with expedite procedures and highly specific/sensitive measurements are still appreciated.

Ion-selective electrodes (ISEs) with polymeric selective membranes and solid-contact have been used for long [30,31], replacing in many occasions other wet-based analytical methods. ISEs offer high precision and rapidity, low cost of analysis, selectivity and sensitivity over a wide range of concentrations [32–35]. The most relevant feature of the potentiometric response is its high selectivity for a target species. In general, an ionophore compound is at the core of the selective response. It is mostly governed by ion-exchange constants to the target analyte as well as on the standard free energies of the respective ions in the aqueous and organic phases [36–39]. While the lipophilic environment is dictated by the plasticizer, the selection of ligands that strongly bind the preferred ion and only weakly all the others [39] tend to favour the selectivity.

The binding event within the potentiometric response depends mostly of the stereo-chemical interactions between target and ionophore, where the host-guest complexation leads to molecular recognition effects at the interface of the modified electrode and the aqueous solution [40,41]. Only few potentiometric sensors for ACh detection have been described in the literature. These include enzyme (Acetilcolinesterase) [42], plastic antibody [43] or Heptakis(2,3,6-tri-*o*-methyl)-β-cyclodextrin [44] as a biorecognition elements.

Trying to improve such host-guest complexation event in potentiometric-based response, host-tailored chemistry has been tested as conventional ionophores in recent years [45]. In this, a biomimetic material designed by molecular imprinted polymer (MIP) technology may be assembled according to the specific analyte being targeted, thereby generating an increased selectivity [39,46].

Several research work have been reported in the literature with the integration of the biomimetic materials and potentiometric transduction for proteins [47,48], antibiotics [39,49,50], pesticides [51] and others compounds [52] and only one work for ACh detection [43].

Different immobilization strategies have been reported so far to integrate MIP biomimetic materials in electrochemical sensing devices. These include bulk polymerization, [53] epitope approach [54] or surface imprinting [55,56], having the polymerization process initiated by chemical or UV stimulus. Bulk imprinting is herein a standard technique for the successful imprinting of small-molecular-weight MIPs. In this, 3-D binding sites are formed for the entire template and the polymerization can be done at environmental temperature.

In general, some low-cost and stable materials such poly(3,4-ethylenedioxythiophene) [34], polyaniline (ANI) [57] and polypyrrole [58] can be employed to drive bulk polymerization processes and could be used as ionophore material in PVC polymeric matrix. These materials show excellent proprieties in terms of easy synthesis and stability, which are expected to contribute to the improvement of the operational features of the resulting devices. They can work as polyionic carriers facilitating the access of the analyte to the sensory material across the membrane. Their combination in composite structures with other highly conductivity materials, such as graphene or carbon nanotubes, is also expected to yield response.

Therefore, the present work describes for the first time the development of ACh based ISEs where the ionophore is a biomimetic material. This material is prepared within a highly conductive polymeric matrix, by combining monomers multiwall carbon nanotubes (MWCNT) and ANI in bulk imprinting. The resulting materials are further dispersed in poly(vinyl chloride) (PVC) membranes plasticized with *Orto*-nitrophenyl octyl ether (*o*NPOE). To effect of ACh imprinting upon the potentiometric response is evaluated by comparing the response with non-imprinted polymeric materials (NIP), where the ACh is absent in the polymerization stage. The analytical performance of all ISEs prepared is evaluated and the best devices applied to the analysis of synthetic serum samples.

2. Experimental section

2.1. Apparatus

Emf was measured in a Crison μpH 2002 decimilivoltammeter (±0.1 mV sensitivity), at room temperature (25 °C), and under constant stirring by means of a Crison, micro ST 2038. The output signal linked to a commutation point with six ways out, enabling the simultaneous reading of six selective electrodes.

The potentiometric cell was assembled as conductive contact of epoxy-graphite|ACh selective membrane|buffered sample solution (HEPES, 1.0×10^{-2} mol/L, pH 3.0, 4.0, 6.0 and 8.5)||electrolyte solution, KCl|AgCl(s)|Ag. The reference was an Ag/AgCl double-junction electrode (Orion 90-02-00).

Fourier transform infrared spectroscopy (FTIR) measurements used a Nicolet 6700 FTIR spectrometer, coupled to a diamond-based ATR (attenuated total reflectance) accessory. Raman spectroscopy measurements were made in a Thermo Scientific DXR Raman, equipped with a 532 nm laser. The RAMAN and FTIR were fabricated in Japan, Tokyo.

Scanning Electron microscopy (SEM) images were acquired using a Quanta 650 FEG (FEI) at a working distance of approximately 10 mm, an acceleration voltage of 30 kV and spot size 3. For Transmission Electron microscopy (TEM) assays, aTEM from JEOL, JEM 2100, with an accelerating voltage of 200 kV, was used. The thermal behaviour of MIP and NIP was evaluated in the thermogravimetry (TG)/differential thermal analyzer (DTA) Exstar TG/DTA 7200 from Hitachi.

2.2. Reagents

All chemicals were of analytical grade and de-ionized water (conductivity < 0.1 μS/cm) was employed. In this work, 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), ACh, Glucose (Glu), MWCNT and ANI were obtained from Sigma; Creatinine (Crea) from Aldrich; PVC of high molecular weight, *o*NPOE, Ammonium persulphate (APS), and Tetrahydrofuran (THF) were obtained from Riedel-deHäen. *Tetrakis*(4-chlorophenyl)borate (TpClPB) and Tetraoctylammonium bromide (TOB) from Acros; Hydrochloric acid (HCl) from Panreac; Sodium hydroxide (NaOH) from Scharlau; Cysteine (Cys) from Merck; and Urea from Fragon.

2.3. Solutions

Stock solutions of 1.00×10^{-2} mol/L ACh were prepared in buffer. Less concentrated ACh standards were prepared by accurate dilution of the previous solution in buffer. Buffer solutions were 1.00×10^{-2} mol/L HEPES. The pH of this solution was altered by adding suitable volumes of either concentrated hydrochloric acid or saturated sodium hydroxide solutions, freshly prepared.