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New molecularly-imprinted polymer for carnitine and its application as ionophore in potentiometric selective membranes

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ABSTRACT

Carnitine (CRT) is a biological metabolite found in urine that contributes in assessingseveral disease conditions, including cancer. Novel quick screening procedures for CRT are therefore fundamental. This work proposes a novel potentiometric device where molecularly imprinted polymers (MIPs) were used as ionophores. The host-tailored sites were imprinted on a polymeric network assembled by radical polymerization of methacrylic acid (MAA) and trimethylpropane trimethacrylate (TRIM). Non-imprinted polymers (NIPs) were produced as control by removing the template from the reaction media. The selective membrane was prepared by dispersing MIP or NIP particles in plasticizer and poly(vinyl chloride), PVC, and casting this mixture over a solid contact support made of graphite.
The composition of the selective membrane was investigated with regard to kind/amount of sensory material (MIP or NIP), and the need for a lipophilic additive. Overall, MIP sensors with additive exhibited the best performance, with near-*Nernstian* response down to ~1×10^{-4} mol L^{-1}, at pH 5, and a detection limitof ~8×10^{-5} mol L^{-1}. Suitable selectivity was found for all membranes, assessed by the matched potential method against some of the most common species in urine (urea, sodium, creatinine, sulfate, fructose and hemoglobin). CRT selective membranes including MIP materials were applied successfully to the potentiometric determination of CRT in urine samples.

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1. Introduction

Cancer diseases are responsible for about 20% of deaths in the European Region, most of these are attributed to breast, cervical or colorectal cancer [1,2]. Screening programs and early diagnostic will be more effective once they rely on non- or minimally-invasive methods with high sensitivity and specificity, easily available and cost effective. These may be reached by the early detection of biomarker proteins that are found early in the body and that have predictive value for specific diseases.

Carnitine (CRT) is a quaternary ammonium compound biosynthesized from the amino acids lysine and methionine and is responsible in living cells for the transport of long-chain fatty acids into mitochondria where β-oxidation takes place [3]. CRT is a potential biomarker of ovarian cancer, it was identified as a metabolite in the normal ovary and transformed in primary and metastatic ovarian cancer [4,5]. It is however widely recognized by its antioxidant properties, by which it may become an efficient tool at the prevention and protection of oxidative stress related diseases, including heart failure [6], lipid peroxidation [7], Down syndrome [8], aging [9] and HIV [10]. Furthermore, CRT has also correlated to the inhibition of carcinogenesis and cisplatin-induced injury of kidney and intestine [11,12], and to antidiabetic activity since the long-chain free fatty acid transport by CRT is associated withthe glucose metabolism [13,14]. There is also a close correlation between L-CRT supplementation and the beneficial effects in the treatment of varicocele, a major cause of male infertility [15].

Several analytical methods have been reported in the literature to analyze CRT in pharmaceutical formulations and in biological samples such as chromatographic techniques [16–19], capillary zone electrophoresis [20–22], fluorimetry[23], spectrophotometry[24], and electrochemical assays with potentiometric [25–27], amperometric [28] or quartz-crystal microbalance transduction [29]. Typically, separative methods, such as chromatographic and capillary zone electrophoresis, are accurate, precise and robust, but are unsuitable for routine applications for requiring high sample running times, organic solvents, pretreating sample procedures, expensive equipment, and high purity requirements for reagents. Optical methods such as fluorimetry and spectrophotometry are typically much less expensive but may lack the desired selectivity and the ability to handle easily turbid and colored samples. Other methods reported in the literature include potentiometric titrations, as an indirect measure of CRT that is not suitable for the direct analysis of complex samples. Other methods make use of antibody/

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antigen reactions [28,29], providing specific and sensitive responses but requiring long time and expensive reagents for routine analytical readings.

Ion selective electrodes (ISEs) may turn out an advantageous alternative for CRT determination in complex samples. CRT bears a positive charge from a quaternary ammonium group and has one carboxylic acid function that may lead to one negative charge, meaning that its detection as an ionic species is feasible. The use of ISEs also provides a highly specific and sensitive measurement and offers high precision and rapidity, low cost of analysis, and enhanced selectivity and sensitivity over a wide range of concentrations [30–32]. In this, an ionophore is immobilized in a plasticized membrane, acting as an electroactive material.

Conventional ionophores are ion exchangers and neutral macrocyclic compounds [25–27], but it has been shown that new materials that are complementary to the size and charge of a particular ion can lead to very selective interactions, thus enhancing the selectivity of the final device. This is the case of molecularly imprinted polymers (MIPs), by which polymeric materials are easily tailored with suitable selectivity for a guest compound [33,34]. There are various successful applications of MIPs, including chromatography [35,36], artificial antibodies [37–39], chemical sensors [40,41], and solid-phase extraction (SPE) [42,43], as well as potentiometric ionophores[44,45].

The present work describes the development of CRT MIP-based ISEs. The imprinted material is synthesized by cross-linking the polymerization of methacrylic acid (MAA) with trimethylolpropane trimethacrylate (TRIM) within the template molecule. The sensing materials are dispersed in a PVC matrix plasticized with *o*-nitrophenyl octyl ether (*o*NPOE). Main analytical features, selectivity properties and practical application to urine analysis are presented.

2. Experimental

2.1. Apparatus and chemicals

Practical potentials were measured in a pH meter, Crison, GLP 21 ($\pm$0.1 mV sensitivity), at room temperature, and under constant stirring. A home-made commutation unit with one six-way out and one-way in was employed to enable the simultaneous reading of six ISEs. The reference electrode was Ag/AgCl, of double junction from Crison, 52–40. The selective electrode was constructed as described elsewhere [46], holding conventional configuration, no internal reference solution and an epoxy-graphite conductive support. The potentiometric cell was assembled as: conductive graphite | CRT selective membrane | buffered sample solution (MES, 1.0×10^{-2} mol L^{-1}, pH 5) || electrolyte solution, KCl | AgCl(s) | Ag. All pH measurements used a combined glass electrode from Crison.

2.2. Reagents

All chemicals were of analytical grade and de-ionized water (conductivity < 0.1 μS cm^{-1}) was employed throughout. CRT, potassium *tetrakis*(4-chlorophenyl)borate (TpClTPB), *o*NPOE, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), poly(vinyl chloride) (PVC) of high molecular weight, trimethylpropane trimethacrylate (TRIM), hemoglobin and methacrylic acid (MAA) were purchased from Fluka. Benzoyl peroxide (BPO), acetonitrile (ACN), magnesium chloride and tetrahydrofuran (THF) were from Riedel–deHäen, urea from Fagron, fructose and sodium chloride from Pancreac and sodium sulfate from Normapur.

2.3. Synthesis of imprinted-ionophore

MIP materials were obtained by dissolving the template (CRT, 1.0 mmol) with the functional monomer (5.0 mmol, MAA), the cross-linker (TRIM, 20.0 mmol) and the radical initiator (BPO, 0.32 mmol), in 3.5 mL acetonitrile. The mixture was sonicated, degassed with nitrogen for 10 min, and cured at 70 °C for 1 h and30 min. Non-imprinted polymers (NIPs) were prepared in parallel, repeating this procedure without the template. The resulting polymers were then crushed dried to small-size particles. Template, exceeding reactants and small size by-products were extracted from the particles by thorough washing with acetonitrile/acetic acid (5:1, v/v). The absence of CRT within the acetonitrile/acetic acid MIP material washing extracts was confirmed by UV/Vis spectrophotometry. Finally, the particles were washed with water and dried, at room temperature, for 12 h, and kept in a dessicator.

2.4. Surface analysis of the host-tailored polymers

FTIR analysis was conducted in a Nicolet 6700 FTIR spectrometer (Thermo) coupled to a diamond ATR accessory of the same brand. Infrared spectra were collected under room temperature control after background correction. The number of scans was 32 for both sample and background. The collected spectra plotted wavenumber (525 to 4000 cm^{-1}) against % transmittance. Resolution was 4000.

Raman microscopy was made in Raman microscope, equipped with 532 nm laser. The spectrum was measured using 50 μm pinhole aperture and 50× lens.

2.5. Preparation of CRT-selective electrodes

The sensing membranes were prepared by mixing 0.21 g of PVC, 0.35 g of plasticizer *o*NPOE and 0.015 g or 0.00375 g of the sensing polymer (Table 1) in 3.5 mL THF. Some membranes also included 0.007 g or 0.0007 g of TpClTPB, acting as lipophilic additive.

The resulting membrane solutions were drop-casted over the conductive support of the conventional electrodes. Membranes were let dry for 24 h and conditioned in water. The electrodes were kept in this solution when not in use.

2.6. Potentiometric procedures

All measurements were made at room temperature. Calibration curves were done in constant ionic strength and pH conditions, using 1.0×10^{-2} mol L^{-1} HEPES buffer solution as background, adjusted to the desired pH with NaOH or HCl solution. The selective membrane of the electrodes was incubated in 50.00 mL buffer, where increasing aliquots of a 1.0×10^{-2} mol L^{-1} stock solution of CRT (0.0200–5.00 mL) were added. Emf readings were recorded after stabilization to $\pm$0.2 mV and the values was plotted as a function of logarithm CRT concentration.

Potentiometric selectivity coefficients were assessed by the matched potential method (MPM). For this purpose, a known amount of the primary ion (*aA*) in solution was added of a known amount of primary ion (*aA′*), and the corresponding potential change (ΔE) is recorded. Then, another solution of the same amount of primary ion (*aA*) is added of interfering ion (*aB*) until the same potential change (ΔE) is met.

$$K_{A,B}^{POT} = \frac{(aA\ddot{E} - aA)}{aB}. \quad (1)$$

The initial concentration of primary ion was set to 9.9×10^{-5} mol/L (*aA*) and the concentration of primary ion solution added into this was 4.8×10^{-4} mol L^{-1} (*aA′*). The corresponding potential change depended on the composition of the selective membrane, being ~20 mV on MIP/additive devices. The interference of creatinine, urea, sodium, potassium, calcium and sulfate was assessed by adding small aliquots of the corresponding solutions into the primary ion solution of *aA*. This addition was made consecutively until one of the following conditions was met: the potential change equaled the one observed with the main ion addition for the same electrode; or the initial concentration of primary ion was altered by more than 5% as aresult of dilution.

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