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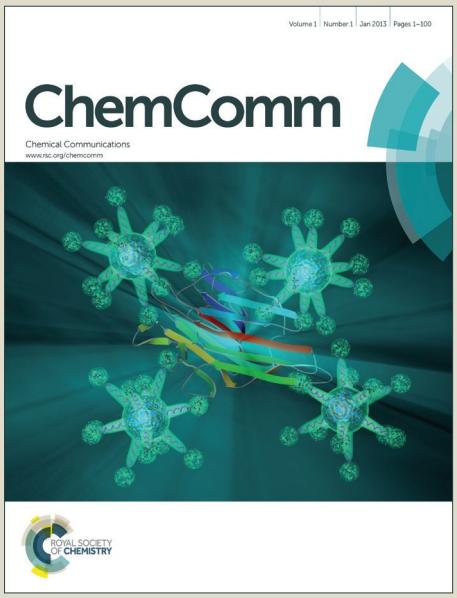

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Synthesis and deposition of Tröger base polymer on electrode surface for potentiometric detection of neuroblastoma tumor marker metabolite

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The development of novel diagnostic tools is a primary goal in bioanalytical chemistry. Here we report the synthesis of a Tröger base functionalized with amino- and coumarin-units designed as a monomeric unit for the development of an electrochemical cancer sensor. The synthesized receptor was deposited onto a conducting support using electrochemical polymerization, characterized spectroscopically and tested potentiometrically towards metabolites used as tumor markers of neuroblastoma.

The development of detection methods for diseases with high mortality represents a crucial target of contemporary science. At present, the classical methodology for cancer detection is based on biopolymer analysis. [1–5] A modern innovative approach is based on low-molecular weight non-classical tumor markers (TMs) and cancer cell metabolite identification [6,7,8]. Despite the potential significance to detect low-molecular weight non-classical TMs, there is no yet reports concerning of their determination using supramolecular analytical chemistry, especially potentiometry.[9] Neuroblastoma, a neuroblastic tumor derived from the perimordial neural crest, remains the leading cause of disease in infancy. A promising screening test of neuroblastoma could rely on the monitoring of urine catecholamine metabolites, namely homovanillic acid (HVA) and vanillylmandelic acid (VMA) (Fig. 1) which serve as TMs. [10]

Ultrasensitive diagnostic devices based on electrochemical principles could be one quite simple approach to screening the low-molecular-weight non-classical TMs of neuroblastoma. Recently, Nemiroski et al. have proposed universal mobile and highly effective electrochemical devices for the detection of (i) glucose in the blood for personal health, (ii) sodium in urine for clinical analysis, and (iii) a malarial antigen for clinical research.[11] The compatibility of similar devices with any mobile phone or wearable electronics guarantees that sophisticated diagnostic testing can be performed by users with a broad spectrum of needs, resources, and levels of technical expertise. A few reports have been related to voltammetric determination of HVA and VMA without using supramolecular receptors. [12]

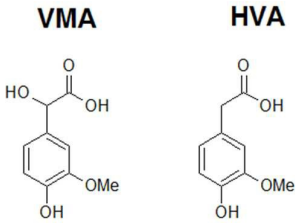

Fig. 1 The structures of the tested metabolites for screening neuroblastoma

Since a number of biologically relevant molecules are electroactive, the selectivity of many electrochemical sensors based on electroactivity is limited. Therefore, a promising way to avoid overlapping electrochemical signals and increase selectivity is the development of a potentiometric sensor derived from a recognition receptor selective towards the low-molecular-weight target metabolite. We are pioneered in potentiometric determination of the metabolites for neuroblastoma screening.

A Tröger's base is a molecule containing two arenes connected to the *b* and *f* sides of 1,5-methano-1,5-diazocine.[13,14] Due to its unique structural features (C_2-symmetry and rigid V-shape geometry) and the possibility of functionalization, a Tröger's base is an attractive receptor for the development of optical and spectroscopic sensors.[13–16] The sensor technology assume immobilization of a supramolecular

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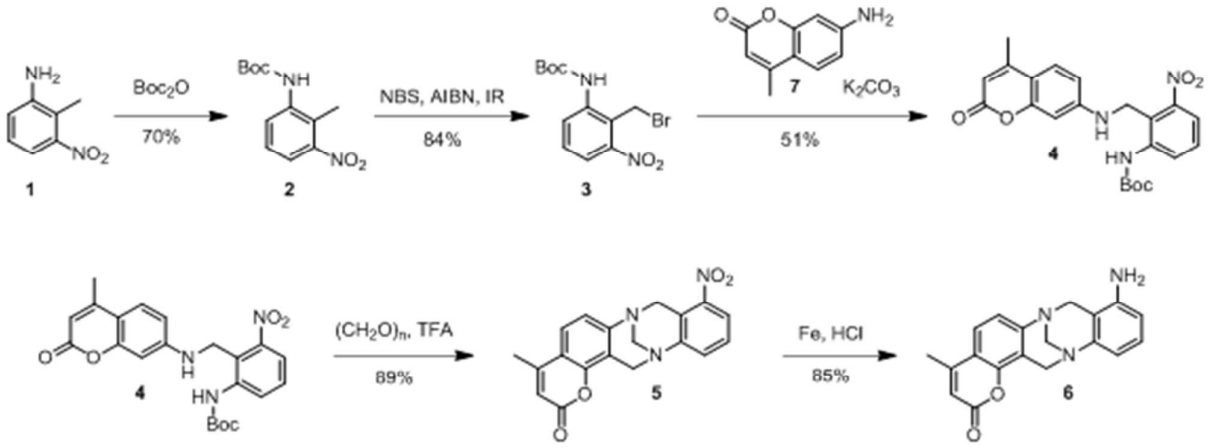

Scheme 1 Stepwise preparation protocol of Tröger base functionalized with amino- and coumarin-units **6**.

receptor onto the electrode interface between transducer and measured sample. The practically applicable approach is to immobilize receptor into a suitable matrix. However, a non-covalent immobilized monomer can leach out from the matrix as result of desorption and other physical effects occurring on the electrode surface contacting with sample. In contrary, covalent immobilization of receptor allows to limit the leaching out from sensor surface and to construct microsensors for practical application. In this paper, a Tröger's base derivative **6** (Scheme 1) was designed to allow us to deposit it electrochemically on the electrode surface and to detect the metabolite in question potentiometrically. The success of the supramolecular design was proven using spectroscopic and nanoscopic experiments, comprising both optical and mechanical measurements. Scheme 1 shows the synthesis of receptor **6**. †

We should note that this is not a case of preparation of a molecularly imprinted polymer. TMs are not present in the electropolymerization mixture. † A cyclic voltammogram of **6** on a Pt electrode (Fig. 2) exhibits two anodic peaks at ca. 0.55 and 1.02 V (from the 1st potential scan) and the reduction peak at ca. 0.47 V. The current intensity increased from 1 up to 4 cycles indicating the polymer growth (Fig. 2). In the subsequent scans, the current decreased indicating the growth of the non-conducting polymeric film. The presence of the anodic peak at 1.02 V is related to the oxidation of the monomer and initiating the electrosynthesis of the polymeric film.[17] A couple (peaks **Ia** and **Ib**) is evident at a potential of ca. 0.55 V, corresponding to the reduction–oxidation of an adsorbed product.

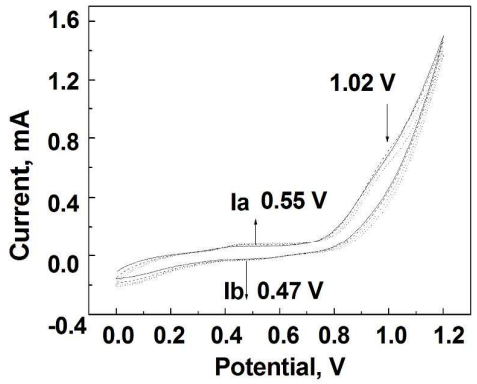

Fig. 2 Cyclic voltammogram of a Tröger's base derivative **6** in CH_3CN/ TBAFB/HCl on a Pt electrode. The electrode potential was swept repeatedly between 0.0 and 1.2 V vs. Ag/AgCl at a scan rate of 50mV s^{-1}, number of scans are 4. †

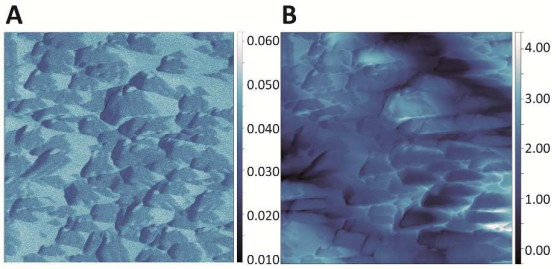

Fig. 3 SNIM images 5x5 µm of the Au electrode surface. Mechanical amplitude signal (A) and optical signal (B).

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Scanning near-field infrared microscopy (SNIM) combined with nanomechanical patterning shows that the polymeric film on the electrode surface is nanostructured and consists of "hills" and "valleys" with a maximal height profile of 0.5 µm (Fig. 3A). The amplitude of the infrared signal assigned to aromatic C–C stretching mode suggests that the adhering film is porous, with grains located on the edges of both "hills" and "valleys" (Fig. 3B).

To confirm the formation of the polymer derived from **6**, Fourier transform infrared (FTIR) spectra of the monomer and polymer prepared by chemical polymerization in CH_3CN were compared (Fig. 4). The polymer spectrum differs substantially from that of the monomer between 3400 – 3200 cm^{-1} and under 1600 cm^{-1}. In contrast to the monomer spectrum, which exhibits bands at ~3350 cm^{-1} and ~3237 cm^{-1} assigned to a primary amino group, the polymer spectrum exhibits the band at ~3217 cm^{-1} of the secondary amino group.[18] The intensity

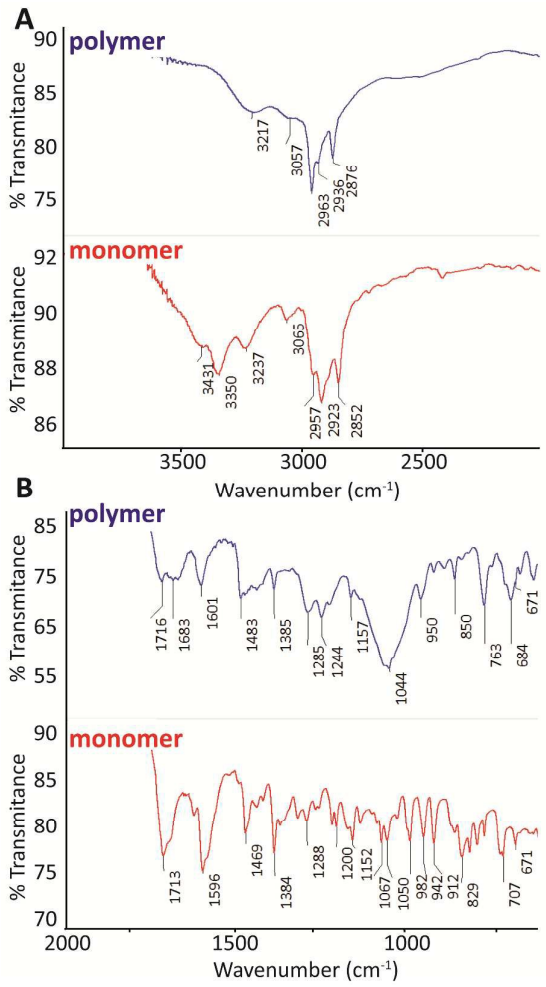

Fig. 4 FTIR spectra of polymer (blue) and monomer (red) from 4000 to 2000 cm^{-1} (A) and from 1900 to 600 cm^{-1} (B). Each spectrum was averaged from 256 scans with 4-cm^{-1} resolution.

ratio of bands at ca. 2960 cm^{-1} and 2930 cm^{-1} of asymmetric stretching vibrations of CH_3 and CH_2 groups, respectively, is reversed. These changes may be rationalized as follows: in the methylcoumarin unit, the possible delocalization of electrons in the lactone ring taking place during polymerization leads to the change in the dipole moment of CH_3 vibrations and to the conformation changes. The bands at 1713 and 1716 cm^{-1} of the C=O vibration of the coumarin unit are observed in both the monomer and polymer spectra, respectively. After polymerization, the intensity of the coupled N–H and C–C vibrations band at 1683 cm^{-1} is remarkably increased to a comparable intensity to the C=O vibration.[19] The aromatic ring modes are shown as a set of bands under 1600 cm^{-1} (1596, 1469, 1288, 1152, 1067, 1050, 942, 829 and 707 cm^{-1}) in both spectra with different intensities. We note that the C=N stretching vibration at 1483 cm^{-1} assigned to the benzenoid units[18] characteristic for polyaniline is observed in the polymer spectrum. The intense band at 1044 cm^{-1} results from the sulfate anion as a product of aniline oxidation by persulfate and somewhat overlaps with the band at ca. 1140 cm^{-1} assigned to protonated polyaniline.[18] Hence the FTIR spectra reveal the polymerization of **6** via its amino group according to the mechanism of aniline polymerization.

The electrochemically modified electrodes were tested for the potentiometric detection of TMs (Fig. 5). † A potentiometric response of –39 mV $decade^{-1}$ towards VMA was found in the concentration range from 9.9×10^{-6} up to 3.3×10^{-3} mol L^{-1} (squared correlation coefficient was equaled 0,9914). The under-Nernstian behavior could be caused by the roughness of the polymeric film and the effect of surface morphology of supporting metallic material. In the case of HVA, the potentiometric response of the prepared electrode was insignificant (not shown). The tested TMs are structurally closely related (Fig. 1) and differ by the hydroxy

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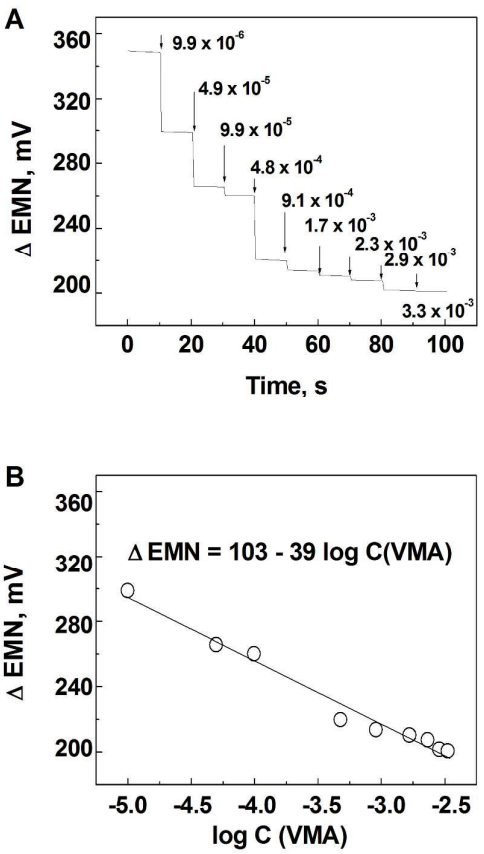

Fig. 5 (A) Potentiometric response and (B) calibration graph of electrode modified with polymer based on **6** towards VMA in 0.1 mol L^{-1} phosphate buffer at pH=7.

group present in VMA. The potentiometric findings indicate that the electrode based on the polymer derived from **6** enable the selective detection of VMA in the presence of HVA (log $K^{Pot.}_{VMA, HVA}$=–0.9). † Fact that a polymer film prepared from Tröger base prefers VMA to HVA may be result of both its spatial arrangement and accessibility of binding sites for tested TMs into the polymeric film onto the electrode surface. Actually, our spectroscopic findings confirmed that the conformation changes are taking place during electropolymerization. The hydroxyl groups of VMA (Figure 1) may reinforce hydrogen bonding between VMA and the polymeric film. [21] For infants (0-1 year old) and children (1-13 years old), the level of the analyzed TMs is (1.0 – 5.4) × 10^{-5} mol L^{-1} in urine. [22] The content of TMs exceeding the above-mentioned range 10 times is signal of worry. [23] Experiments were conducted to measure the concentration of VMA added to an artificial urine. [24] It was found (6 ± 2) × 10^{-5} mol L^{-1} (n=3) VMA when was introduced 5 × 10^{-5} mol L^{-1} VMA. These results indicated that the constituents of the prepared urine sample do not interfere the VMA detection and proposed electrode based on the Troger's base polymer provide an alternative device for VMA determination.

In conclusion, we have presented an innovative detection of neuroblastoma TM which is based on specific and selective interaction with a polymer film (prepared from **6)** and VMA. The detection was purely potentiometric. The geometry of electrochemically polymerized receptor **6** enabled it to interact specifically with VMA in contrast to HVA.

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