تقديـــــر النيسروجولـــــين على ســـــــطح قطب الكربـــــــون المطبـــــــوع علـــــى شريحة من البلاستيك المعـــــدل بواســطة البلمرة الكهروكيميائية لمركب أورثو فينيل داي أمين .

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الملخص :

طريقة عملية لتطوير مستشعر حساس وانتقائي لمركب النيسروجولين Nicergolin (NIC) من خلال البلمرة الكهروكيميائية للمولنيمر أورثو فينيل داي أمين o–phenylenediamine في وجود (NIC) ومحلول مساعد $NaClO_4$ تركيزة 0.01 مولار باستخدام عدد15 دورة من تقنية الفولتاميترى الدائري(CV) فى المدى من 0 و حتى 0.8 فولت لتكوين بوليمر مطبوع فيه جزيئات (NIC) على سطح قطب الكربون المطبوع على شريحة من البلاستيك والذي يعرف بـ screen printed carbon electrode (SPCE) ، وبعد ذلك تم استخلاص (NIC) من البوليمر المتكون باستخدام 10 دورات CV من 0 و حتى 0.8 فولت فى وجود محلول الفوسفات المنظم ذو الأس الهيدروجينى 5.2 ، وعند استخلاص (NIC) من البوليمر المتكون يترك فراغات فى البوليمر لها نفس حجم وشكلNIC المستخلص تمثل بصمة له فقط وهو مايعرف بـmolecularly imprinted polymer (MIP) ، وبواسطته تم تحديد NIC في مصل الإنسان بإستخدام تقنية الفولتاميترى ذو النبض التفاضلى(DPV) ، حيث تم الحصول على أعلى إشارة أنودية لـ NIC في محلول فوسفات المنظم ذو الرقم الهيدروجيني 5.0. كان نطاق الاستجابة الخطية لـ 3.5×10^{-7} مولار إلى 3.5×10^{-5} مولار ، وكان حد الاكتشاف منخفضًا يصل إلى 3.5×10^{-8} مولار. تشير نتائج إلى أن مستشعرMIP كان مفيدًا لتحديد NIC مع انتقائية ممتازة وحساسية عالية وقابلية للتكرار، كما أوضحت التجارب على ذلك .أيضًا في بداية البحث تمت دراسة السلوك الكهروكيميائى لمركب NIC على SPCE باستخدام محلول الفوسفات المنظم 0.2 مولار عند قيم مختلفة من الأس الهيدروجينى تتراوح من 3 و حتى 10 وأظهرت نتائج القياسات أن عملية الأكسدة على SPCE كانت غير عكسية .

Determination of Nicergoline on an electropolymerized-molecularly imprinted Poly-o-phenylenediamine Polymer modified screen printed carbon electrode

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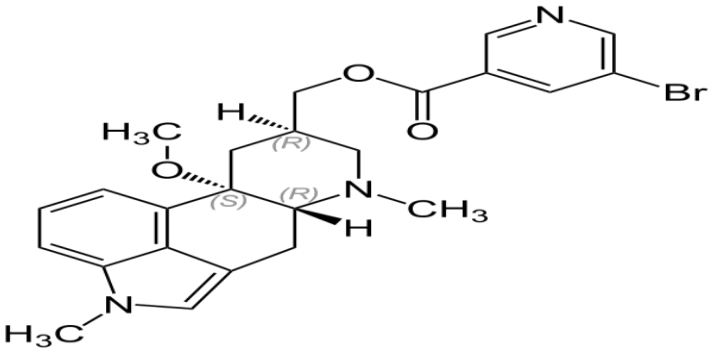
ABSTRACT

sensitive and selective sensor was successfully developed by integrating electropolymerization of molecularly imprinted polymer (MIP) on screen printed carbon electrode (SPCE) for the determination of Nicergoline (NIC) in human serum, The imprinted poly-o-phenylenediamine (o-PD), which was embedded in SPCE surface. functioned as a selective recognition element for NIC determination, The highest anodic signal of NIC was obtained in a phosphate buffer solution of pH 5.0. The linear response range for NIC was 3.5×10^{-7} to 5.0×10^{-5} M, and the limit of detection was as low as 2.5×10^{-8}M. The results of our investigation indicate that the MIP sensor was useful for the determination of NIC with excellent selectivity, high sensitivity, repeatability, and reproducibility.

Introduction

Nicergoline (10α-methoxy-l,6-dimethylergoline-8β-methanol5-bromo nicotinic acid ester), (schem l) is used in cerebral metabolic vascular disorders, and dementia[1] , and has been registered in over fifty countries and has been used for more than three decades for the treatment of cognitive, affective, and behavioral disorders of older people [2].



Scheme 1 chemical structure ofNicergoline.

NIC is s a poorly water soluble (0.002 mg/mL) in its crystalline state and itis a potent blocking agent for "1-adrenoreceptors and thus has found effective clinical use in neurolog [3, 4] . Nicergoline is expected to degradate in bad storage conditions through the breakage of the ester linkage [5].

Several clinical studies have demonstrated that NIC was effective in lowering total peripheral resistance and normalized blood pressure without producing reflex tachycardia and concluded that the drug was useful in the early phases of acute myocardial infarction, due to its lowering of myocardial oxygen consumption [6].

Biotransformation of NIC both in man and animals demonstrated extensive metabolism in all species tested, the main metabolites being 1,6-dimethyl-8β-hydroxymethyl-l0-alphamethoxyergolineand8-β-hydroxyl meth-10-alpha-methoxy-6-methylergoline, as well as their glucuronides, all of which were excreted by the kidneys [7, 8].NIC is a derivative of semisynthetic ergot alkaloids, was first developed at Farmitalia Carlo Erba (Milan, Italy)[9, 10]. It shows vasodilating and α-receptor blocking activity[11], and has been used clinically in Japan for improving blood circulation and brain metabolism for three years.Theadsorption, distribution, metabolism and excretion of NIC in animals have been investigated with a tritium-labelledcompound [7]. A radioimmunoassay method for the determination of nicergoline in human plasma has beenreported[12], but the effect of immunological cross-reaction between the unchanged drug and its metabolites was not studied sufficiently, and the simultaneous determination of NIC and metabolites could not be performed. Recently, there has been interest in the use of high-performance

liquid chromatography-mass spectrometry (HPLC-MS) for the analysis of non-volatile organic compounds in various fields. Many groups have reported the determination of new drugs in biological fluids using HPLC-MS systems, including a thermospray method [13-16], a moving-belt method [17, 18] a vacuum nebulizing method[19] .

To our knowledge, analytical assays for NIC have appeared in the literature and Electrochemical methods have become very competitivefrom the analytical point of view because of sophisticated developments in electronics, three-electrodes systems, pulse voltammetry, ***etc.*** Modern polarography and voltammetry show important advantages in pharmaceutical analyses. Several reviews and papers related to the electrochemical determination of drugs in pharmaceutical forms have been published[20, 21] .

This paper reports the electrochemical behaviour of Nicergoline, and the development of a novel method to determine the nicergoline in serum, and comparative between use glass carbon electrode and carbon nano tube electrode . Carbon nanotube CNTcontinue to receive considerable attention in electrochemistry due to their low electrical resistance, high accessible surface area, chemical stability and enhanced sensing properties[22].

Primary efficacy measures were the cognitive portion of the Alzheimer's Disease Assessment

Experimental

Chemicals and reagents

Nicergoline, potassium dihydrogen phosphate, potassium hydrogen phosphate and were purchased from Sigma-Aldrich and other reagents were commercially available as analytical grade and used without further purification. O-phenylenediamine (98%, Sigma-Aldrich) . Stock solutions of nicergoline and buffer solutions were prepared by using ultra-pure deionized water.

Apparatus

Electrochemical measurements were performed with AU-TOLAB PGSTAT 302N potentiostat with FRA module for electrochemical

impedance (EIS) measurements (MetrohmAutolabb.v. , the Netherlands), using NOVA software.A three-electrode configuration composed of a working screen-printed carbon electrode (3.1mm diameter), printed from a carbon-based ink; a silver–silverchloride pseudo-reference electrode made from a silver-basedink, All pH-metric measurements were made on a CG 808 (Schott Gerate, Germany) digital pH-meter with glass combination electrode, which was previously standardized with buffers of known pHs.

Procedure

Electrochemical behavior of nicergoline

(Fig.1) shows typical cyclic voltammograms of 5.0×10^{-5} M NIC in PBS pH 4.0 using SPCE. NIC is oxidized, yielding two oxidation peak. The oxidation process involved is irreversible, as no cathodic peak was found at scan rates between 10 and 100 mVs^{-1}. A positive shift in the peak potential was observed with increasing scan rate, which confirms the irreversible nature of the oxidation process. The relationship between the oxidation peak potentials and scan rates ca be described as following: $E_{pa} = 0.0542 \log \upsilon + 0.9107$, $r = 0.9949$. According to Laviron's theory [23], the slope was equal to $2.303RT/\alpha nF$. Then the value of αn_a was calculated as 0.56. As for a totally irreversible electrode reaction process, α was assumed as 0.5. On the basis of the above discussion, the n_a was calculated to be 1.0 which indicated that one electron was involved in the oxidation process of NIC at the SPCE. The diffusion control of the processes was evident from the linear relationship between current and the square root of the scan rate. This evidence is confirmed by plotting the logarithm of peak current ($\log i_{pa}$) versus the logarithm of the scan rate ($\log \nu$). The plots yielded a straight lines with slopes close to the theoretical value of 0.50, which is expected for an ideal reaction condition for diffusion-controlled electrode process[24].

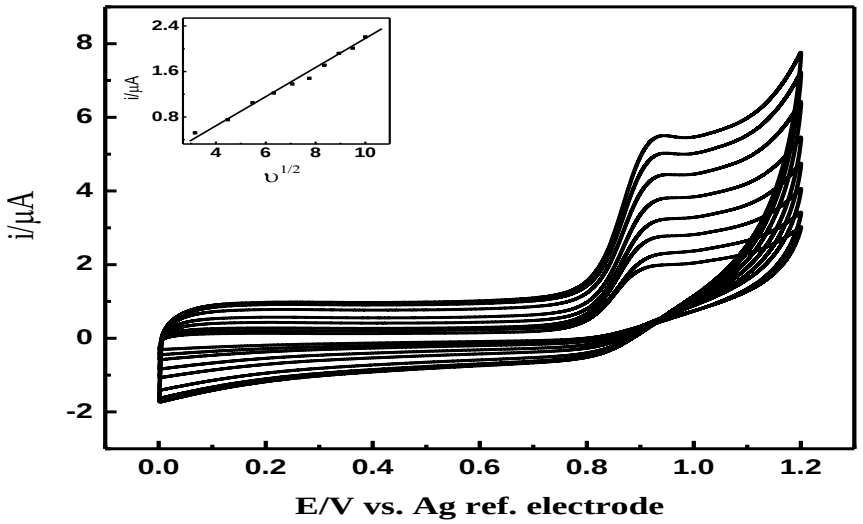


Fig. 1:Cyclic voltammograms obtained for 5.0×10^{-5}MNIC at SPCE in 0.2 M PBS pH 4.0 at υ= 20 (a), 30 (b), 40(c), 5(d), 60(e),70(f), 80(g) and 90(h) mVs^{-1}.Inset$_{iP}$–$\upsilon^{1/2}$plot.

The electrochemical behavior of NIC was initially studied over the pH interval of pH 3.0-10.0 in phosphate buffer solutions as supporting electrolytes by DPV on SPCE. DPV was also used to investigate the effect of pH on the electrochemical oxidation of 5.0 $\times10^{-5}$M NIC in aqueous supporting electrolytes over a pH range from 3.0 to 10.0 (Fig. 2) as already found by CV. The peak potential of anodic peak of NIC is shifted linearly towards more negative values and peak current also increased up to pH = 4.0 and afterwards decreased with increasing pH values.butpeak potential of pH 4.0 showed two peaks separated by.

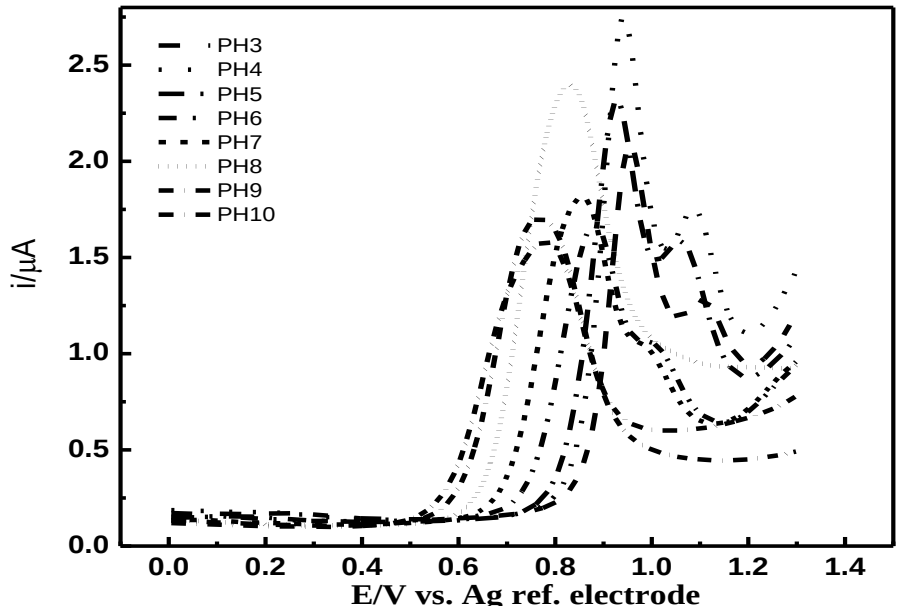


Fig. 2: Effect of pH on determination of 5.0×10^{-5} M NIC using DPVs at the screen printed carbon electrode in 0.2 M phosphate buffer solution.

Electropolymerization of *o*-PD in the presence of nicergoline

Fig. (3A) demonstrates CV of electropolymerization of o- phenylene diamine in the presence of nicergoline . The oxidation peak of nicergoline (template) can be seen easily in this figure. This oxidation peak indicates that the template is becoming part of the polymeric chain. nicergoline molecules diffuse towards the surface of the SPCE during the electropolymerization process and trapped into the polymer matrix. The creation of the molecular imprints is favored (MIP) by the diffusion of the electroactive template, generating a far higher number of recognition sites during the electrodeposition of the polymer. The nicergoline template molecules are trapped in the polymer matrix as a result of the ability of these molecules to interact with the O-phenylenediamine units. Hydrogen bonding could occur between the nitrogen atom of the N–H group of o-phenylenediamine and The oxygen atom in nicergoline structure . This imprinting process creates a microenvironment for the recognition of nicergoline molecule based on shape selection and positioning of the functional groups, and non-imprinted polymer electrode (NIP) was prepared in every case under the same

experimental conditions but without adding nicergoline to check the reliability of the measurements[25].

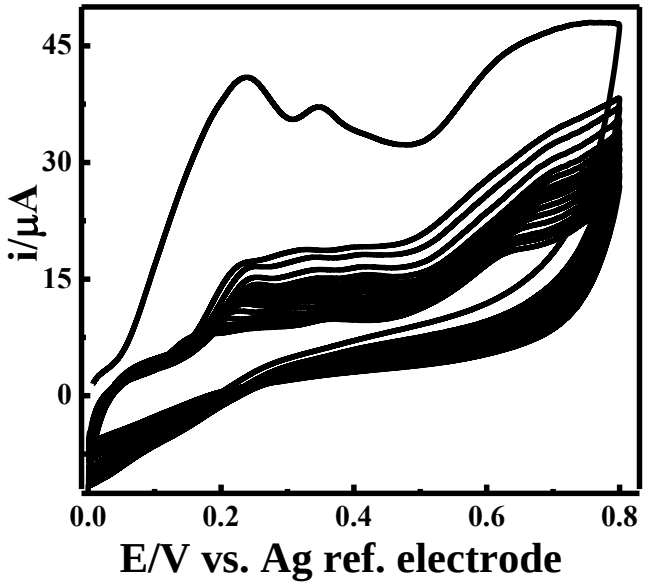


Fig. 3: Cyclic voltammograms taken during the electropolymerization of 5 mM O-phenylenediamine in 0.2 M PBS supporting electrolyte (pH 5.2) in the presence of 1.0 mM Nicergoline at SPCE. ; scan rate: 50 mV s^{-1}.

Results and discussion:

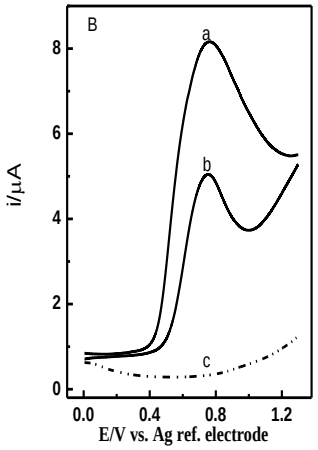


Fig. 4: DPV on SPCE of 5.0×10^{-5}M NIC in 0.2 M phosphate buff er solution of pH 5.0 at (a) MIP electrode , (b) bare electrode, and (c) non-imprinted electrode (NIP).

Fig. 4 show the electrochemical behavior of 5.0× 10^{-5}M NIC in 0.2 Mphosphate buffer of pH 5.0 at MIP-SPCE sensor (curve a), bare – electrode (curve b), the NIP sensor (curve c) There is no oxidation current recorded using the non-imprinted electrode as the poly-o-phenylenediamine is less conductive. In principle; the current response of the MIP sensor is basically due to the electron transfer of the accumulated molecules in the cavities close to the electrode surface,The entrapment of NIC molecules in the small volume of the polymer enhances the pre-concentration within the polymer; accordingly one expects a higher current response for the MIP sensor in comparison with the bare electrode.

Electrochemical Characterization of the MIP Electrode

To confirm the electrochemical properties of the different electrodes, CV experiments were carried out after each kind of electrode was prepared. Figure 5 shows the CV of five different electrodes in PBSpH 5.0 containing 1 mmol/L $[Fe(CN)6]^{3-}$/$[Fe(CN)6]^{4-}$at room temperature. Curve a shows a pair of typical redox peaks of ferricyanide occurring on the surface of the bare SPCE. The difference in the peak potentials between the oxidative and reductive reactions was 0.5 V, and the ratio of the peak currents was about 1:1, respectively. this indicated that the bare SPCE electrode was activated successfully. Curves b, c ,d and e show the cyclic voltammograms with no peak appearing for the electrode modified with imprinted or non imprinted films and SPCE -MIP after the template removal or SPCE -MIP after incubating respectively. In addition to this, we observed that the response of the modified electrode was Much less than the bare SPCE. The results indicate that a non conductivepoly(o-PD) film was successfully formed on the electrode surface and hindered ferricyanide from getting access to the surface of the SPCE [26].

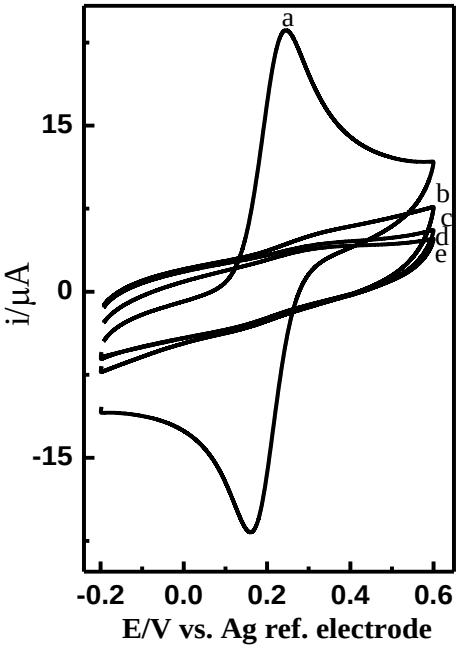


fig.5:(A) EIS recorded CVs and (B) CVs in a 0.01 M $[Fe(CN)6]^{3-}$ $[Fe(CN)6]^{4-}$solution for bare SPCE (a), SPCE -MIP NIC (b), SPCE -NIP (c), and SPCE -MIP after extracting NIC (d) and SPCE -MIP after incubating in 1.0×10^{-5} M NIC solution (e)

Optimization of electrochemical measurement conditions

Effect of pH

On the basis of the determination of NIC on the poly(o-PD) film, the current response of MIP electrode is largely affected by the pH value of the test solution. To research the influence of pH on the MIP sensor, DPV tests were carried out in PBS atdifferent pH values. Because the NIC molecule has been proved unstable in alkalinity solution[27], When pH was less than 5, the peak current decreased with increasing solution pH. On the contrary, the peak current responses of the MIP electrode increased gradually when the pH was greater than 4.Therefore, the test solution with a pH of 5 was chosen for all further experiments.

Effect of Incubation Time

The adsorption kinetics of NIC was investigated by varying the adsorption time from 1 min to 10 min, and the initial concentration of NIC kept constant at 1.0×10^{-5} M. The peak current increased rapidly with the incubation time and then leveled off after 2 min, presumably resulting from reaching the absorption balance between the sample solution and surface of MIPs-SPCE. The result reveals rapid response equilibrium of NIC molecules to poly(o-PD) - SPCE , which might be due to the surface binding sites of SPCE-MIP through π–π stacking between aromatic rings and hydrogen bonds between and nitrogen and oxygen-containing groups of the o-PD units and NIC. Thus, the absorption time of 2 min was selected as an optimum for further experiments.

Analytical performance

The DPV responses of the MIP sensors modified electrode after incubation in the NIC solution were increased linearly with the concentration in the range of 3.5×10^{-7} to 5.0×10^{-5} M of NIC. A linear calibration graph has been constructed by plotting the corresponding absolute value of voltammetric peak current versus NIC concentration[28]. The linear regression equation was expressed as I (μA) = 0.051[NIC] + 0.001.

Detection limit at MIPs based electrochemical sensor was evaluated according to the 3Sb/m criteria, where Sb is the standard deviation of the blank and m is the slope of the linear calibration curve 28. The LOD was found to be 2.5×10^{-8} mol L^{-1}.

Reproducibility, repeatability, and storage stability of the MIP sensor

To test the reproducibility of the proposed technique, three MIP sensors were constructed under identical experimental conditions. The current change was obtained for 1.0×10^{-6} M NIC, by using each of the MIP sensors. The relative standard deviation (RSD) was 3.5 %. The repeatability of one electrode was also examined and the calculated RSD was about 4.2 % (n=5). The sensor can retain 90 % of its original response after the electrode was stored for 1 month in air at room temperature, suggesting acceptable storage stability.

Sample Analysis

To application of the proposed sensor for the determination of NIC in the real samples conditions such as urine and serum was studied . For the preparation of human serum sample, 5.0 mL of blood sample was kept at 37 ◦C for 5 min, and then the sample was centrifuged (5 min with 3000 rpm) after the addition of 2.0 mL methanol. The obtained deproteinized human serum was diluted to 20.0 mL by doubly distilled water. The samples were measured by DPV as shown in Fig. 6, the results were listed in Table 1 with the recoveries between 97.40 % and 106.25 % with RSDs of 2.2 – 5.4%. These results indicated that the SPCE - MIP sensor could be successfully applied for the determination of NIC in the real samples

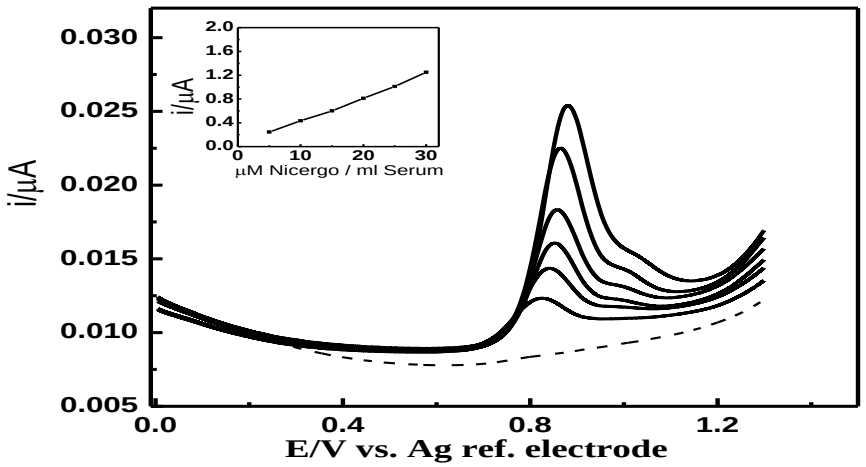


Fig 5: DPVs for the determination of NIC µg in 1ml serum samples spiked with 1.0 -2.0 -3.0 -4.0 - 5.0 and 6.0 µg Nicer per 1mL serum with 0.2 M phosphate buff er solution of pH 5.0 at MIP; the dotted lines (...) represent the blank; inset: calibration curve of NIC in serum at MIP. Step potential 6 mV, modulation amplitude 50 mV and scan rate 50 mV/s[29]

Table 1. Recovery tests of NIC in serum samples.

Added (µM)	Found(µM)	Recovery(%)	RSD[a] (%)
5.00	4.83	97.40	2.7
10.00	9.66	103.30	2.5
15.00	15.32	102.13	3.1
20.00	21.25	106.25	4.2
25.00	24.70	98.80	5.3

[a]RSD value reported is for n=5.

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