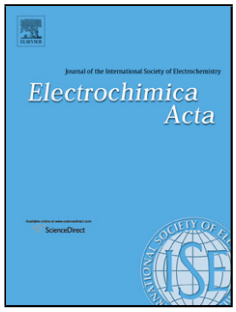

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PII: S0013-4686(17)30858-7
DOI: http://dx.doi.org/doi:10.1016/j.electacta.2017.04.100
Reference: EA 29358

To appear in: *Electrochimica Acta*

Received date: 12-1-2017
Revised date: 18-4-2017
Accepted date: 19-4-2017

Please cite this article as: M.Revenga-Parra, E.Martínez-Periñán, B.Moreno, F.Pariente, E.Lorenzo, Rapid taurine and lactate biomarkers determination with disposable electrochemical detectors, Electrochimica Actahttp://dx.doi.org/10.1016/j.electacta.2017.04.100

Rapid taurine and lactate biomarkers determination with disposable electrochemical detectors

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Abstract

Taurine and lactate are biomarkers used for the diagnosis of different pathologies. In this study, a simple analytical approach for the direct determination of taurine and lactate in tears and saliva is described. The method is based on the use of single wall carbon nanotubes screen printed electrodes modified with films derived from the electropolymerization of a new Schiff base metallate, as liquid chromatography electrochemical detector. The new Schiff base metallate is the complex of nickel(II) with the ligand N,N'-bis(2,4-dihydroxybenzylidene)-1,2-diaminobenzene ([Ni^{II}(2,4DHS)]), which is electropolymerized in alkaline solution to give electroactive films strongly adhered on the electrode surface. These films present a potent and stable electrocatalytic activity toward the oxidation of taurine and lactate. Hence, the resulting modified electrodes, coupled to a chromatographic system, allow the quantification of these biomarkers directly in biological samples without need of derivatization schemes or sample pre-treatment.

Keywords: Schiff base complexes; ; ; , biomarkers, taurine electrocatalysis, lactate electrocatalysis.

1. **Introduction**

The development of highly sensitive methods for accurate quantification of different biomarkers is receiving considerable attention in clinical diagnosis and biomedical research. Among known biomarkers, taurine and L-lactate are of great interest, since their presence in biological fluid or tissues are related to important diseases.

Taurine (2-aminoethane sulphonic acid) is a natural organic acid present in a great number of living organisms. This compound contains a sulphonic acid group, rather than the typical carboxilic acid moiety characteristic in the conventional amino acids. Because of this, taurine cannot be incorporated into the proteins and therefore it is probably the most abundant free amino acid present in a number of mammalian tissues including retina, skeletal, cardiac muscles and brain [1]. In healthy human adults, taurine is a product of methionine and cysteine catabolism. In addition, it can be absorbed from foods containing proteins from skeletal muscle. Although the best-known function of taurine is conjugation of bile acids, it also plays an important role in numerous biological and physiological processes [2, 3]. Among other metabolic actions attributed to taurine it may be included: modulation of the intracellular calcium ion homeostasis [4], protection against the ischemia reperfusion injury [5], it is a natural antioxidant [6] and depicts antiatheratogenic effects [7]. Indeed, deviations from normal taurine concentrations in tissues are correlated with several pathological disorders such as Alzheimer's disease, growth retardation, diabetes mellitus [8-9], epilepsy [10], sepsis [11] and several kinds of cancer [12-14] as well as different forms of psychosis including trauma [15], depression [16] and schizophrenia [17]. Recently, it has been found that high levels of taurine in tears also has a close relationship with the ocular surface disease and can be used to a biomarker to prevent this pathology [18]. Thus, the rapid and accurate determination of the level of this compound in various tissues and body fluids can be of great interest in the early diagnosis of several important pathologies and diseases.

The main problem for the development of a method for the determination of taurine is the lack of a powerful fluorophore or an electroactive group in its structure, that allows a direct spectrophotometric or electrochemical assay. This implies that usually its determination requires a prior derivatization step, which is complicated, increases the analysis time and involves a previous labor-intensive. Most of the methods described for the determination of taurine, use chromatography with spectrometric detection [8,19-23] and capillary electrophoresis (CE) [24-27]. However, electrochemical detectors have been less employed despite their high sensitivity, low cost, high reproducibility and possibility of miniaturization [28-32]. This is because the taurine

determination, in most of these cases, is based on its direct oxidation at the electrode surface, which requires high overpotentials and gives rise to reaction products that causes electrode fouling. Both things adversely affect the sensitivity and reproducibility of the determination. The use of suitable modified electrodes can overcome these drawbacks as it will be described below.

L-Lactate is a metabolite originated in the anaerobic metabolism and its presence causes an increase in the proton concentration inside the cells. In situations where the rate of lactate production is high enough that proton buffering capacity of cells may be exceeded, the cellular pH values decrease and this may result in cell acidosis [33]. Lactate levels in plasma reflect the equilibrium between the lactate production and lactate clearance systems [34]. Any disorder that causes an imbalance in this equilibrium may lead to lactic acidosis. This may occur in some conditions involving the anaerobic metabolism, such as an excessive exercise, hemorrhagic shock or pulmonary embolism [34]. Lactate levels also increase in several pathological conditions including: respiratory failure [35], liver disease, cardiogenic [36] or endotoxic [37] shocks, renal failure [38] and tissue hypoxia [39] and metastases in cancer [40]. Accordingly, high lactate levels may act as signal alarm for the diagnosis of the patient conditions in operating rooms, intensive care units or point of care stations [41]. Hence, the need of devices capable to determine lactate with rapid response, minimal or without sample preparation and low cost in samples where other potentially interfering compounds are present is evident.

Disposable and sensitive electrochemical detectors in HPLC systems, based on modified electrodes, could be a good alternative to develop methods meeting the above-mentioned characteristics, such as those based on metal/metal oxide modified electrodes [42-44]. Recently the incorporation of carbon nanostructures, such as graphene nanosheets and mostly carbon nanotubes, has improved the performance of these kind of modified electrode in the determination of carbohydrates, amino acids and related compounds [45-48].

In this work, we have developed a very effective taurine and lactate determination method based on the use of a new modified screen printed carbon electrode as electrochemical detector in HPLC systems. The screen printed electrode containing single wall carbon nanotubes was modified with electropolymerized films of a new Schiff base nickel(II) complex, formed with the ligand N,N'-bis(2,4-dihydroxybenzylidene)-1,2-diaminobenzene and Ni^{II}. The resulting Ni based modified electrode depicts a potent and persistent electrocatalytic activity towards the oxidation of taurine and lactate in alkaline medium allowing, coupled to

a chromatographic system, the quantification of these biomarkers in biological samples without derivatization schemes.

2. Experimental section

2.1 Reagents and Apparatus

1,2-diaminobenzene, 2,4-dihydroxybenzaldehyde, taurine, L-(+)-lactic acid lithium salt 97%, acetonitrile, ethanol and sodium hydroxide were obtained from Sigma-Aldrich Co. and were used as received. $Ni(NO_3)_2$ and acetone was obtained from Scharlau. Water was purified with a Millipore Milli-Q system and all the experiments were carried out at room temperature. Stock solutions containing taurine or lactate were freshly prepared daily in 0.1 M NaOH aqueous solution.

All electrochemical measurements were carried out using an Autolab potentiostat/galvanostat type PGSTAT 302N (Eco Chemie, Netherlands) and the software package GPES 4.9. Integrated single walled carbon nanotubes modified screen printed electrodes (SWCNTSPEs) from Dropsens, including a silver pseudoreference electrode and a carbon counter electrode were used. A wall jet flow cell from Dropsens (model FLWCL) was employed for on-line analysis. The supporting electrolyte was 0.1 M NaOH.

HPLC experiments were performed with a modular chromatograph (Jasco) coupled with an AUTOLAB potentiostast. Optimum chromatographic conditions were achieved using a Dionex AminoPac PA10 anion-exchange analytical column (4x250 mm) from Thermo Fisher with a mobile phase of 0.1 M NaOH plus 3% acetonitrile as organic modifier. The HPLC system was run isocratically at 0.75 mL min^{-1} flow rate.

2.2 Preparation of Ni^{II}-(N,N'-bis(2,4-dihydroxybenzylidene)-1,2-diaminobenzene) ([Ni^{II}(2,4DHS)])

The ligand, N,N'-bis(2,4-dihydroxybenzylidene)-1,2-diaminobenzene (2,4DHS), was synthetized according to the usual synthesis procedure of tetradentate Schiff base ligands [49]. 1,2-diaminobenzene (0.1080 g; 1.00 mmol) was mixed with 2,4-dihydroxybenzaldehyde (0.2762 g; 2.00 mmol) in refluxing ethanol for 3 h. The precipitate obtained was filtered and washed several times with ethanol and ether. Finally, the product was recrystallized by ethanol (0.2446 g, 70%). IR (KBr): $\nu_{C=N}$ 1610 cm^{-1}; $\nu_{Ar\text{-}O\text{-}H}$ 1308 cm^{-1}; $\nu_{C=C}$ 1481 cm^{-1}; $\nu_{Ar\text{-}H}$ 740 cm^{-1}; UV–vis in acetone: λ_{max} 362 nm.

2.0 mM solutions of the Ni complex [Ni^{II}(2,4DHS)] were prepared by addition of solid $Ni(NO_3)_2$ crystals into a 2.0 mM solution of 2,4DHS in acetone under stirring. The rapid change in the color of the solution from brightly yellow to deep orange evidenced the complex formation.

2.3 Preparation of [Ni^{II}(2,4DHS)]/SWCNT modified screen printed electrode

SWCNTSPEs were modified with [Ni^{II}(2,4DHS)] complex by immersing the electrode in 0.1 M NaOH solution containing 0.1 mM [Ni^{II}(2,4DHS)] and cycling the potential between +0.2 V and +0.8 V at a scan rate of 0.1 V s^{-1}. The resulting modified electrode was then conditioned in 0.1 M NaOH solution by potential cycling from +0.2 V to +0.8 V at a scan rate of 0.1 V s^{-1} for about 10 min (50 cycles of potential scans).

2.4 Determination of biomarkers in real samples

For taurine and lactate quantification in healthy volunteers' tears and saliva samples, respectively, standard addition method was used. Samples (0.10 mL of tears or saliva) were spiked with suitable amounts of the analyte, diluted in 0.50 mL of 0.1 M NaOH and injected into the HPLC system for analysis.

3. **Results and discussion**

3.1 Spectrophotometric characterization of [Ni^{II}(2,4DHS)]

[Ni^{II}(2,4DHS)] was prepared by mixing $Ni(NO_3)_2$ crystals with a solution of 2,4DHS in acetone as described in experimental section. The complex formation was corroborated by UV-vis spectroscopy. As can be observed in Fig. 1, the absorbance spectrum of 2,4DHS shows a maximum at 362 nm, which corresponds to the $\pi \rightarrow \pi^*$ transition of the electrons present in the azomethine group [50]. When [Ni^{II}(2,4DHS)] complex was formed, a red shift of this absorption band is observed up to 382 nm. In addition, a new broad absorption band due to the metal to ligand charge transfer transition appears. The stoichiometry and the formation constant of the complex were determined using the spectral data obtained from solutions containing a constant concentration of ligand and different concentrations of metal. From these results (data not shown), it was found a 1:1 stoichiometric ratio and a formation constant of 6.0×10^7.

Figure 1

3.2 Electropolymerization of [Ni^{II}(2,4DHS)] on SWCNTSPE

[Ni^{II}(2,4DHS)] was electropolymerized onto single walled carbon nanotubes modified screen printed electrodes (SWCNTSPEs) by cycling the potential from +0.2 to +0.8 V at 0.1 V s^{-1} in a solution 0.1 mM [Ni^{II}(2,4DHS)] in 0.1 M NaOH. Figure 2A shows the electroactive film growth pattern obtained under continuous potential cycling. The anodic and cathodic peaks observed are due to the Ni^{2+}/Ni^{3+} redox couple present in the polymeric film. The current increases gradually with the consecutive potential cycling suggesting the growth of the film. During the

electropolymerization process, the charge measured under the anodic voltammetric wave increases linearly with the continuous potential cycling during the first 50 cycles and then remains practically constant (inset Fig. 2A). Thus, 50 potential cycles were applied as optimal in the electrode modification step.

Figure 2

If after several potential cycles, the electrode is removed from the electrochemical cell, rinsed with water and placed in a 0.1 M NaOH containing no nickel-complex, the voltammetric response from +0.2 to +0.8 V depicts the typical response of the Ni^{2+}/Ni^{3+} redox couple, with well-defined anodic (at +0.39 V) and cathodic peaks (at +0.59 V) (see Fig. 2B). This fact confirms that the electrode surface is covered with a stable [Ni^{II}(2,4DHS)] electroactive film. A significant peak-to-peak separation (ΔEp) of 120 mV is observed. This behavior has been observed with other Ni based complex deposited on electrode surface [51] and suggests that counter-ion transport (OH^-) is the limiting step. The change of the Ni redox-state inside the film should involve the diffusional transport of the counter-ion into/out-of the film necessary to maintain electroneutrality [52]. A larger hysteresis in the voltammetry is indeed expected when the counter-ion diffusion becomes rate limiting. In fact, as one would expect when the charge transfer is limited by the diffusion of OH^- ions towards the film, peak currents present a linear dependence with the square root of the potential scan rate over the range of 0.01-0.40 V s^{-1} (Fig. 3B). If the potential is cycled at scan rates higher than 0.50 V s^{-1}, the peak-to-peak separation increases (Fig. 3C). This electrochemical behavior is indicative of serious limitations in the charge transfer kinetics under these conditions.

Figure 3

On the other hand, the peak current density of Ni^{2+} electrooxidation increases in an approximately linear way with the number of cycles. Thus, we used the peak current density (I_p) at 0.10 V s^{-1} as a measurement of the

film thickness. For 50 electrodeposition cycles, the value of I_p was found to be 1.3 ± 0.1 mA cm^{-2}. This value is about 2 times higher than that obtained when a screen-printed carbon electrode without carbon nanotubes was modified with the [Ni^{II}(2,4DHS)] following the same procedure. In addition, the reproducibility of I_p (evaluated from the response of 10 different electrodes) is much better than that obtained without carbon nanotubes. A relative standard deviation of 7% was calculated compared to 40% without nanotubes.

For HPLC electrochemical detector application, one of the properties to be evaluated for modified electrodes is their stability. In order to check the stability of the developed modified electrode, the potential was continuously cycled from +0.2 to +0.8 V at 0.1 V s^{-1} and the anodic I_p for 50 electrodeposition cycles was evaluated after each cycle. Figure 2B (inset) depicts the anodic I_p as a function of the number of potential cycles. As can be seen, after a few conditioning scans, the I_p remains practically constant, suggesting a strong adherence of the film to the electrode surface and therefore a great stability of the modified electrode. In fact, the electrochemical response of the [Ni^{II}(2,4DHS)]/SWCNTSPE remains constant for several weeks, if it is stored under dry conditions.

3.3 Electrocatalytic propierties of [Ni^{II}(2,4DHS)]/SWCNTSPE

Nickel metal centers have shown high electrocatalytic activity in basic media towards the oxidation of organic compounds containing hydroxyl groups and sulfur derivatives [53]. Hence, [Ni^{II}(2,4DHS)]/SWCNTSPE can be valuable in designing new electrocatalytic and sensing platforms. With this purpose in mind, the electrocatalytic activity of [Ni^{II}(2,4DHS)]/SWCNTSPE towards the oxidation of two important biomarkers, taurine and lactate, was evaluated (Fig. 4). In solutions where no biomarker was added, the well-defined voltammetric response of the Ni^{2+}/Ni^{3+} redox couple is evident (Fig. 4A and 4B, curve a). Upon addition of taurine, a dramatic increase in the anodic peak current concomitant with a decrease in the cathodic current is observed (Fig. 4A, curve b). This behavior is characteristic of an electrocatalytic effect and indicates that the film redox centers confined at the electrode surface participate in the oxidation of taurine, which causes a decrease in the number of Ni(III) centers and the consequent decrease in the cathodic current in the backward scan.

In the case of lactate, although it was also observed a strong electrocatalytic effect it is slightly different. As can be seen in Fig. 4B (curve b), in the presence of lactate there is also a dramatic increase in the anodic peak

current. However, at the reverse scan no reduction peak is observed. A decrease in current is also evident at potential above +0.65 V probably due to mass transport effects. At bare SWCNTSPE (insets in Fig. 4A and 4B) no oxidation peaks were observed neither for taurine nor for lactate.

The above results confirm that electropolymerization of [Ni^{II}(2,4DHS)] onto SWCNTSPE gives rise to films with a great density of redox centers, which results in a high electrocatalytic activity towards the oxidation of taurine and lactate.

Figure 4

It is well known that carbon nanotubes, besides increase the effective electroactive area, may play a key role on avoiding electrode fouling. To asses if this is the case, similar experiments to those described above were carried out with screen printed carbon electrodes without carbon nanotubes. In this case, on successive taurine measurements, a significant shift (±100 mV) in the catalytic peak potential is observed. This effect, which is not observed when carbon nanotubes are present, confirms the antifouling effect of CNTs.

The electrocatalytic responses for taurine and lactate at different scan rates (v: from 0.002 to 0.1 V s^{-1}) confirm that, in both cases, the plot of $I_{CAT}/v^{1/2}$ *vs* v shows the characteristic shape of an EC electrocatalytic process. In addition, the catalytic current increases linearly with the scan rate indicating that the process is controlled by the diffusion of the biomarker (Fig. 5).

Figure 5

The catalytic constants were estimated by chronoamperometry following the method of Galus [54]. This procedure is based on the equation:

$$\frac{I_{CAT}}{I_{RES}} = \gamma^{1/2}\left[erf(\gamma^{1/2}) + \frac{\exp(-\gamma)}{\gamma^{1/2}}\right]$$

Where I_{CAT} and I_{RES} are the currents at the modified electrode and in the presence and in the absence of biomarkers, respectively. γ is the argument of the error function described as: γ = kC*t, where C* is the bulk concentration of the biomarker in the solution and k is the catalytic rate constant. If γ exceeds 2, the error function is almost equal to one and the above equation can be simplified to:

$$\frac{I_{CAT}}{I_{RES}} = \pi^{1/2}\gamma^{1/2} = \left(\pi k C^{*} t\right)^{1/2}$$

Where t is the elapsed time in seconds. From this equation, the catalytic rate constant as a function of the biomarker concentration can be estimated by plotting I_{CAT}/I_{RES} *vs* $t^{1/2}$. A linear dependence with the square root of time was obtained for each concentration of taurine and lactate studied. From the slopes of these plots, values of k_{CAT} for taurine and lactate were found to be (6.2±0.5) $x10^2$ and (4.4±0.7) $x10^2$ M^{-1} s^{-1}, respectively.

3.4 [Ni^{II}(2,4DHS)]/SWCNTSPE as amperometric detector in flow systems

Considering the high electrocatalytic and reproducible response of the as prepared [Ni^{II}(2,4DHS)]/SWCNTSPE towards the oxidation of taurine and lactate, it can be employed as electrochemical detector in a FIA or HPLC system for the determination of both biomarkers in complex matrixes.

The operational potential for the [Ni^{II}(2,4DHS)]/SWCNTSPE towards the oxidation of taurine was evaluated from the hydrodynamic curve (Fig. S1). The maximum response was found to be around +0.6 V, which agrees well with the electrocatalytic behavior depicted in Fig.4. At potentials, higher than +0.6V the current response exhibits a noticeable decrease probably due to the competition reaction of the excess of hydroxide ions present at the electrode surface, with the subsequent oxygen evolution [55-57]. Thus, a value of +0.55 V, at which the catalytic /background current ratio is the highest, was chosen as operational potential for the following flow-through measurements. Similar behavior was observed for lactate.

Taurine and lactate FIA responses showed very good peak shapes (Fig. S2). Moreover, peak currents are dependent on the biomarker concentration in solution in a wide linear range, confirming the utility of the [Ni^{II}(2,4DHS)]/SWCNTSPE as a amperometric detector for both biomarkers. The detection and quantification limits, obtained according their usual definition, were found to be of 0.38 µM and 7.6 µM for taurine and lactate, respectively. Relative standard deviations less than 7.5% were obtained for the determination of 100 µM of each analyte with three different modified electrodes, which can be considered a good reproducibility (Table S1).

3.5 [Ni^{II}(2,4DHS)]/SWCNTSPE - HPLC for taurine and lactate determination in real samples.

In the last years, the importance of monitoring biomarkers in non-blood media is increasing in parallel with the demand for non-invasive analysis. Taurine and lactate are biomarkers, related to several pathologies, that can be present in abnormal levels in urine, saliva and tears, besides in blood. Therefore, the development of non-invasive analytical method for the determination of these biomarkers is a deal great of interest. Hence, we have developed a method for direct determination of taurine and lactate in tears and saliva, using the [Ni^{II}(2,4DHS)]/SWCNTSPE as amperometric detector in a HPLC system. We have optimized all the parameters, such as the influence of the mobile phase composition and flow rate, which is a crucial step on a good chromatographic separation. The optimization has been evaluated in terms of electrochemical signal and retention times. Best results were obtained when a 0.1 M NaOH solution containing acetonitrile (3%) as organic modifier and a flow rate of 0.75 mL min^{-1} were used. Fig. 6 shows the chromatogram of a mixture of taurine and lactate. As can be seen, two separated peaks, symmetric with minimal peak tailing, corresponding to the two analytes are observed. A summary of the analytical figures of merit obtained is presented in Table 1.

Figure 6

The detection limit, calculated from 3:1 signal-to-noise ratio of the standard deviation of the lowest concentration signal, was found to be of 0.97 µM and 4.74 µM for taurine and lactate, respectively. After injecting five replicates of a solution containing the two compounds, relative standard deviation values between 1.3-4.0% were obtained, demonstrating the good repeatability of [Ni^{II}(2,4DHS)]/SWCNTSPE and no evidence of fouling or deactivation. The reproducibility of the method was evaluated from chromatograms obtained for a mixture of 20 µM taurine and 50 µM lactate, using three different [Ni^{II}(2,4DHS)]/SWCNTSPEs as detectors. A relative standard deviation lower than 9% was calculated. These values demonstrate that the method is reliable and valuable to quantify both biomarkers.

The method developed compared to other reported in the bibliography for the determination of lactate or taurine shows in general improved detection limits (see Table 2S).

The applicability of the developed method was demonstrated by the direct determination of taurine and lactate in tears and saliva samples, respectively, from healthy volunteers. Prior to the determination, 0.10 mL of the sample were diluted in 0.50 mL of 0.1 M NaOH. A typical chromatogram for a sample of tears or saliva is

shown in Fig. 7A and 7B, respectively. Peaks at 8.8 min (Fig. 7A) and 5.1 min (Fig. 7B) were identified as taurine and lactate, respectively, by adding analyte standard solutions into the respective sample.

Figure 7

A recovery study of taurine and lactate was carried out to evaluate the accuracy of the method. For this purpose, the samples were spiked with a known amount of each analyte and analyzed in triplicate. The results are presented in Table 2. The good recoveries indicate that the developed method is adequate and accurate, providing a suitable detection method for the analysis of these biomarkers related to different pathologies in biological samples.

4. Conclusions

We have developed a simple analytical method for direct determination of two important biomarkers, taurine and lactate, in tears and saliva, respectively. A new Schiff base metallate is prepared just by addition of solid $Ni(NO_3)_2$ crystals into a solution of N,N'-bis(2,4-dihydroxybenzylidene)-1,2-diaminobenzene (2,4DHS), in acetone. The nickel complex is electropolymerized onto the single walled carbon nanotubes modified screen printed electrodes. The electrocatalytic effect of the resulting modified electrodes towards the oxidation of taurine and lactate is exploited to develop a chromatographic method to determine these biomarkers, using the developed modified electrodes as electrochemical detectors. The method represents a significant advance that provides an alternative and attractive solution to an important clinical problem, since determination of these biomarkers in biological fluids by non-invasive methods helps the rapid diagnosis of several pathologies.

Acknowledgments

The authors acknowledge Ministerio de Economía y Competitividad (project No. CTQ2014-53334-C2-1-R and CTQ2015-71955-REDT) and Comunidad de Madrid (NANOAVANSENS Program) for financial support. E.L. gratefully acknowledges the Fulbright scholarship-Salvador de Madariaga program from the Ministerio de Economía y Competitividad.

References

[1] H.C. Agrawal, W.A. Himwich, Amino acids, proteins, and monoamines of developing brain, in: W.A. Himwich (Ed.), Developmental Neurobiology, Vol. 3, Thomas, Springfield, 1970, pp. 287.

[2] R.J. Huxtable, Physiological actions of taurine. Physiol. Rev. 72 (1992) 101-163.

[3] J.G. Jacobsen, L.H. Smith, Biochemistry and physiology of taurine and taurine derivatives. Physiol. Rev. 48 (1968) 424–511.

[4] T.M. Foos, J.Y. Wu, The role of taurine in the central nervous system and the modulation of intracellular calcium homeostasis. Neurochem. Res. 27 (2002) 21–26.

[5] W. Oriyanhan, K. Yamazaki, S. Miwa, K. Takaba, T. Ikeda, M. Komeda, Taurine prevents myocardial ischemia/reperfusion-induced oxidative stress and apoptosis in prolonged hypothermic rat heart preservation. Heart Vessels 20 (2005) 278–285.

[6] S.W. Schaffer, J. Azuma, M. Mozaffari, Role of antioxidant activity of taurine in diabetes. Can. J. Physiol. Pharm. 87 (2009) 91–99.

[7] Y. Yamori, S. Murakami, K. Ikeda, Y. Nara, Fish and lifestyle-related disease prevention: experimental and epidemiological evidence for anti-atherogenic potential of taurine. Clin. Exp. Pharmacol. P. 31 (2004) S20-S23.

[8] S. Mou, X. Ding, Y. Liu, Separation methods for taurine analysis in biological samples. J. Chromatogr. B 781 (2002) 251–267.

[9] S.H. Hansen, The role of taurine in diabetes and the development of diabetic complications. Diabetes Metab. Res. Rev. 17 (2001) 330-346.

[10] H.O. Goodman, B.M. Connolly, W. McLean, M. Resnick, Taurine transport in epilepsy. Clin Chem. 26 (1980) 414-419.

[11] A.C. Woolf, J.P. Grovers, J.H. Moore, R.J. Duff, Arterial plasma amino acids in patients with serious postoperative infection and in patients with major fractures, Surgery 79 (1976) 283.

[12] G. E. Gray, A. M. Landel, M. M. Meguid, Taurine-supplemented total parenteral-nutrition and taurine status of malnourished cancer-patients. Nutrition 10 (1994) 11–15.

[13] O. Beckonert, J. Monnerjahn, U. Bonk, D. Leibfritz, Visualizing metabolic changes in breast-cancer tissue using 1H Quantitative NMR spectroscopy and self-organizing maps. NMR Biomed. 16 (2003) 1–11.

[14] E. De Micheli, A. Alfieri, G. Pinna, L. Bianchi, M.A. Colivicchi, A. Melani, F. Pedata, L. Della Corte, A. Bricolo, Extracellular levels of taurine in tumoral, peritumoral and normal brain tissue in patients with malignant glioma: an intraoperative microdialysis study. Adv. Exp. Med. Biol. 483 (2000) 621–625.

[15] M. Jeevanandam, D.H. Yuoung, L. Ramais, R.W. Schiller, Aminoaciduria of severe trauma. Am. J. Clin, Nutr . 49 (1989) 814-822.

[16] K.H. Tachiki, H.C. Hendrie, J. Kellams, M.H. Aprison, A rapid column chromatographic procedure for the routine measurement of taurine in plasma of normals and depressed patients, Clin. Chim. Acta 75 (1977) 455-465.

[17] A. Agar, A. Pinsky, Comparative study of taurine, sulfur containing amino acids and phospholipids in schizophrenic and normal blood and urine. Isr. J. Chem. 2 (1964) 318-&.

[18] M. Nakatsukasa, C. Sotozono, K. Shimbo, N. Ono, H. Miyano, A. Okano, J. Hamuro, S. Kinoshita, Amino Acid Profiles in Human Tear Fluids Analyzed by High-Performance Liquid Chromatography and Electrospray Ionization Tandem Mass Spectrometry. Am. J. Ophthalmol. 151 (2011) 799–808.

[19] L.Q. Gu, X.F. Wang, Y.Y. Zhang, Y. Jiang, H. Lu, K.S. Bi, X.H. Chen, Determination of 12 potential nephrotoxicity biomarkers in rat serum and urine by liquid chromatography with mass spectrometry and its application to renal failure induced by Semen Strychni. J. Sep. Sci. 37 (2014) 1058–1066.

[20] M. A. Khalikova, D. Satinsky, P. Solich, A. A. Zinchenko, E. T. Zhilyakova, O. O. Novikov, A high-performance liquid chromatography method with pre-column derivatization for the simultaneous determination of reduced glutathione, carnosine and taurine. Anal. Meth. 6 (2014) 1475–1481.

[21] M. Aranda, G. Morlock, Simultaneous determination of riboflavin, pyridoxine, nicotinamide, caffeine and taurine in energy drinks by planar chromatography-multiple detection with confirmation by electrospray ionization mass spectrometry. J. Chromatogr. A 1131 (2006) 253–260.

[22] Y.Y. Yu, X.X. Zheng, T.T. Bian, Y.J. Li, X.W. Wu, D.Z. Yang, S.S. Jiang, D.Q. Tang, Development and application of a LC-MS/MS assay for the simultaneous quantification of edaravone and taurine in beagle plasma. J. Sep. Sci. 36 (2013) 3837–3844.

[23] D.L. Orth, HPLC Determination of Taurine in Sports Drinks. J. Chem. Edu. 78 (2001) 791-792.

[24] M.P. Lorenzo, A. Villasenor, A. Ramamoorthy, A. Garcia, Optimization and validation of a capillary electrophoresis laser-induced fluorescence method for amino acids determination in human plasma: Application to bipolar disorder study. Electrophoresis 34 (2013) 1701–1709.

[25] C.C. Klinker, M.T.Bowser, Monitoring Neurotransmitter Release from Isolated Retinas Using Online Microdialysis-Capillary Electrophoresis. Anal. Chem. 79 (2007) 8747–8754.

[26] Y. H. Deng, H. Wang, H. S. Zhang, Determination of amino acid neurotransmitters in human cerebrospinal fluid and saliva by capillary electrophoresis with laser-induced fluorescence detection. J. Sep. Sci. 31 (2008) 3088–3097.

[27] B. X. Shi, W. H. Huang, J. K. Cheng, Analysis of amino acids in human vascular endothelial (ECV-304) cells by microchip electrophoresis with fluorescence detection. J. Sep. Sci. 31 (2008) 1144–1150.

[28] W.P. King, P.T. Kissinger, Liquid-chromatography with amperometric reaction detection involving electrogenerated reagents-applications with insitu generated bromine. Clin. Chem. 26 (1980) 1484–1491.

[29] Y.H. Cao, X. Zhang, Q.C. Chu, Y.Z. Fang, J.N. Ye, Determination of taurine in Lycium Barbarum L. and other foods by capillary electrophoresis with electrochemical detection. Electroanalysis 15 (2003) 898–902.

[30] T.R.I. Cataldi, G. Telesca, G. Bianco, Improved determination of taurine by high-performance anion-exchange chromatography with integrated pulsed amperometric detection (HPAEC-IPAD). Anal. Bioanal. Chem, 378 (2004) 804-810.

[31] T.R.I. Cataldi, G. Telesca, G. Bianco, D. Nardiello, Quantitative determination of taurine in real samples by high-performance anion-exchange chromatography with integrated pulsed amperometric detection. Talanta 64 (2004) 626–630.

[32] F. Du, W. Zhao, S. Cao, Y. Fung, Determination of taurine in human tear fluid by capillary electrophoresis with indirect amperometric detection based on electrogenerated bromine. J. Sep. Sci. 38 (2015) 3271-3278.

[33] G. Kemp, Lactate accumulation, proton buffering, and pH change in ischemically exercising muscle. Am. J. Physiol. Regul. Integr. Comp. Physiol. 289 (2005) R895–R901.

[34] F. Valenza, G. Aletti, T. Fossali, G. Chevallard, F. Sacconi, M. Irace, L. Gattinoni, Lactate as a marker of energy failure in critically ill patients: hypothesis. Crit. Care 9 (2005) 588–593.

[35] D. DeBacker, J. Creteur, H.B. Zhang, M. Norrenberg, J.L.Vincent, Lactate production by the lungs in acute lung injury. Am. J. Respir. Crit. Care Med. 156 (1997) 1099–1104.

[36] J. Karlsson, J.T. Willerson, S.J. Leshin, C.B. Mullins, J.H. Mitchell, Skeletal-muscle metabolites in patients with cardiogenic-shock or severe congestive heart failure. Scand. J. Clin. Lab. Invest. 35 (1975) 73–79.

[37] M.M. Sayeed, P.N.A. Murthy, Adenine nucleotide and lactate metabolism in the lung in endotoxin shock. Circ. Shock 8 (1981) 657–666.

[38] R. Bellomo, Bench-to-bedside review: lactate and the kidney. Crit. Care. 6 (2002) 322–326.

[39] R. Rimachi, F. B. de Carvahlo, C. Orellano-Jimenez, F. Cotton, J.L. Vincent, D. De Backer, Lactate/pyruvate ratio as a marker of tissue hypoxia in circulatory and septic shock. Anaesth Intensive.Care 40 (2012) 427–432.

[40] S. Walenta, M. Wetterling, M. Lehrke, G. Schwickert, K. Sundfor, E.K. Rofstad, W. Mueller-Klieser, High lactate levels predict likelihood of metastases, tumor recurrence, and restricted patient survival in human cervical cancers. Cancer Res., 60 (2000) 916–921.

[41] I. Smith, P. Kumar, S. Molloy, A. Rhodes, P.J. Newman, R.M. Grounds, E.D. Bennett, Base excess and lactate as prognostic indicators for patients admitted to intensive care. Intensive Care Med. 27 (2001) 74–83.

[42] P. Luo, F. Zhang, R.P. Baldwin, Comparison of metallic electrodes for constant-potential amperometric detection of carbohydrates, amino acids and related compounds in flow systems, Anal. Chim. Acta, 244 (1991), 169-178.

[43] Y.G. Zhao, X.F. Fang, Y.S. Gu, X.Q. Yan, Z. Kang, X. Zheng, P. Lin, L.C. Zhao, Y. Zhang, Gold nanoparticles coated zinc oxide nanorods as the matrix for enhanced L-lactate sensing, Colloids Surf. B, 126 (2015), 476-480.

[44] G.C. Sedenho, P.T. Lee, H.S. Toh, C. Salter, C. Johnston, N.R. Stradiotto, R.G. Compton, Nanoelectrocatalytic Oxidation of Lactic Acid Using Nickel Nanoparticles, J. Phys. Chem. C, 119 (2015), 6896–6905.

[45] A. Arvinte, A.M. Sesay, V. Virtanen, Carbohydrates electrocatalytic oxidation using CNT-NiCo-oxide modified electrode, Talanta 84 (2011) 180-186.

[46] R.P. Deo, N.S. Lawrence, J. Wang, Electrochemical detection of amino acids at carbon nanotube and nickel-carbon nanotube modified electrodes, Analyst 129 (2004) 1076-1081.

[47] B. Rafiee, A.R. Fakhari, Electrocatalytic oxidation and determination of insulin at nickel oxide nanoparticles-multiwalled carbon nanotube modified screen printed electrode, Biosens. Bioelectron. 46 (2013) 130-135.

[48] W. Guo, H. Li, M. Li, W. Dai, Z. Shao, X. Wu, B. Yang, Synthesis of nickel nanosheet/graphene composites for biosensor applications, Carbon 79 (2014) 636-645.

[49] R.H. Bailes, M. Calvin, Oxygen-carrying synthetic chelate compounds.VII. Preparation, J. Am. Chem. Soc. 69 (1947) 1886–1893.

[50] G.L. Estiú, A. H. Jubert, J. Costamagna, J. Vargas, Quantum chemical calculations of the structures and electronic properties N,N'-bis(3,5-dibromosalicylidene)-1,2-diaminobenzene and Its cobalt (II) complex. Origin of the redox activity of the cobalt complex. Inorg. Chem. 35 (1996) 263-266.

[51] M. S. Ureta-Zañartu, T. González, F. Fernández, D. Báez, C. Berríos, C. Gutiérrez, Electro-Oxidation of Benzyl and Aliphatic Alcohols on polyNiTSPc- and Ni(OH)2-Modified Glassy-Carbon and Gold Electrodes. Int. J. Electrochem. Sci. 7 (2012) 8794-8812.

[52] M. S. Ureta-Zañartu, A. Alarcón, C. Berrios, G. I. Cárdenas-Jirón, J. H. Zagal, C. Gutiérrez, Electropreparation and characterization of polyNiTSPc films. An EQCM study. J. Electroanal. Chem. 580 (2005) 94-104.

[53] M. Fleischmann, K. Korinek, D. Pletcher, J. The oxidation of organic compounds at a nickel anode in alkaline solution. Electroanal. Chem. 31 (1971) 39-49.

[54] Z. Galus, Fundamentals of Electrochemical Analysis. Ellis Horwood, New York (1994).

[55] K. Sato, J. Jin, T. Takeuchi, T. Miwa, Y. Takekoshi, S. Kanno, S. Kawase, Nickel–titanium alloy electrodes for stable amperometric detection of underivatized amino acids in anion-exchange chromatography, Talanta 53 (2001) 1037-1044.

[56] I.G. Casella, M. Gatta, T.R.I. Cataldi, Amperometric determination of underivatized amino acids at a nickel-modified gold electrode by anion-exchange chromatography, J. Chrom. A 878 (2000) 57-67.

[57] I.G. Casella, M. Contursi, Isocratic ion chromatographic determination of underivatized amino acids by electrochemical detection, Anal. Chim. Acta 478 (2003) 179-189.

[58] D. L. P. da Silva, H. H. Ruettinger, Y. Mrestani, W. F. Baum, R. H. H. Neubert, Development of capillary electrophoresis methods for quantitative determination of taurine in vehicle system and biological media, Electrophoresis 27 (2006) 2330– 2337.

[59] F. Qu, Z. Qi, K. Liu, S. Mou, Ion chromatographic determination of taurine in medicine, nutrient capsule and human urine with electrochemical detection. J. Chromatogr. B 730 (1999) 161-166.

[60] P. Luo, F. Zhang, R. P. Baldwin, comparison of metallic electrodes for constant-potential amperometric detection of carbohydrates, amino acids and related compounds in flow systems, Anal. Chim. Acta 244 (1991) 169-178.

Figure captions

Fig. 1. Absorption spectra of a solution containing 30 µM 2,4DHS (line a), 2.0 mM $Ni(NO_3)_2$ (line b) and 30 µM [Ni^{II}(2,4DHS)] (structure inset) (line c) in acetone.

Fig. 2. (A) Cyclic voltammograms of 0.1 mM of [Ni^{II}(2,4DHS)] complex at SWCNTSP electrode in 0.1 M NaOH during modification of the electrode surface. The inset shows the plot of the anodic charge *vs* the number of electropolymerizing scans. (B) Cyclic voltammogram of [Ni^{II}(2,4DHS)] /SWCNTSPE obtained after 50 electropolymerizing scans in 0.1 M NaOH. The inset shows the anodic peak current density dependence on the number of continuous cyclic potential scans for a film derived from [Ni^{II}(2,4DHS)]. Scan rate 0.1 V s^{-1}.

Fig. 3. (A) Cyclic voltammetric response of [Ni^{II}(2,4DHS)]/SWCNTSPE in 0.1 M NaOH at different scan rates. The inset (B) shows the dependence of the anodic and cathodic peak current on the square root of the scan rate. The inset (C) shows the dependence of the peak potential on the logarithm of scan rate.

Fig. 4. Cyclic voltammograms of [Ni^{II}(2,4DHS)]/SWCNTSPE in 0.1 M NaOH in the absence (curve a) or in the presence (curve b) of 5.00 mM of taurine (A) or 0.1 M of lactate (B). In the insets, cyclic voltammograms of bare SWCNTSPE in 0.1 M NaOH in the presence of 5.00 mM of taurine (A) or 0.1 M of lactate (B). Scan rate 0.005 V s^{-1}.

Fig. 5. Variation of the electrocatalytic current (I_{CAT}) with the square root of the scan rate (A) and variation of the scan rate-normalized current (I_{CAT} /$v^{1/2}$) with the scan rate (B) for [Ni^{II}(2,4DHS)]/SWCNTSPE in 0.1 M NaOH containing taurine, as an example.

Fig. 6. Typical chromatograms obtained using [Ni^{II}(2,4DHS)]/SWCNTSPE as electrochemical detector for standard mixture solutions of taurine and lactate at different concentrations. Chromatographic conditions: AminoPac PA10 column; 0.1 M NaOH and 3% acetonitrile mobile phase; 0.75 mL min^{-1} flow rate; 20 µL injection volume; +0.55 V detection potential.

Fig. 7. Chromatograms showing the profile of spiked tears (A) and saliva (B) sample using [Ni^{II}(2,4DHS)]/SWCNTSPE for electrochemical detection. Other conditions as in Fig. 4.

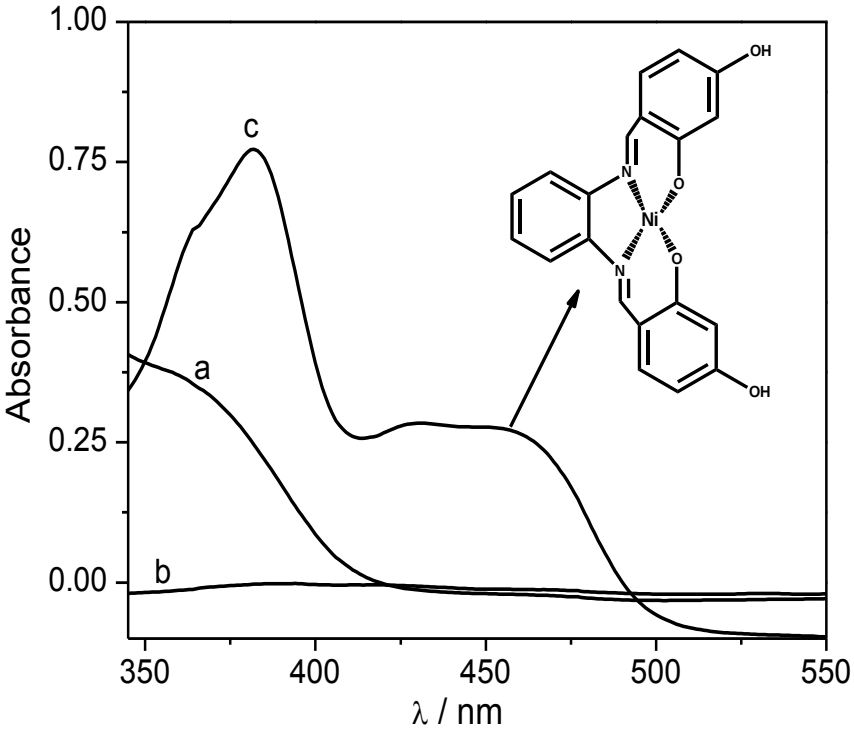

Figure 1

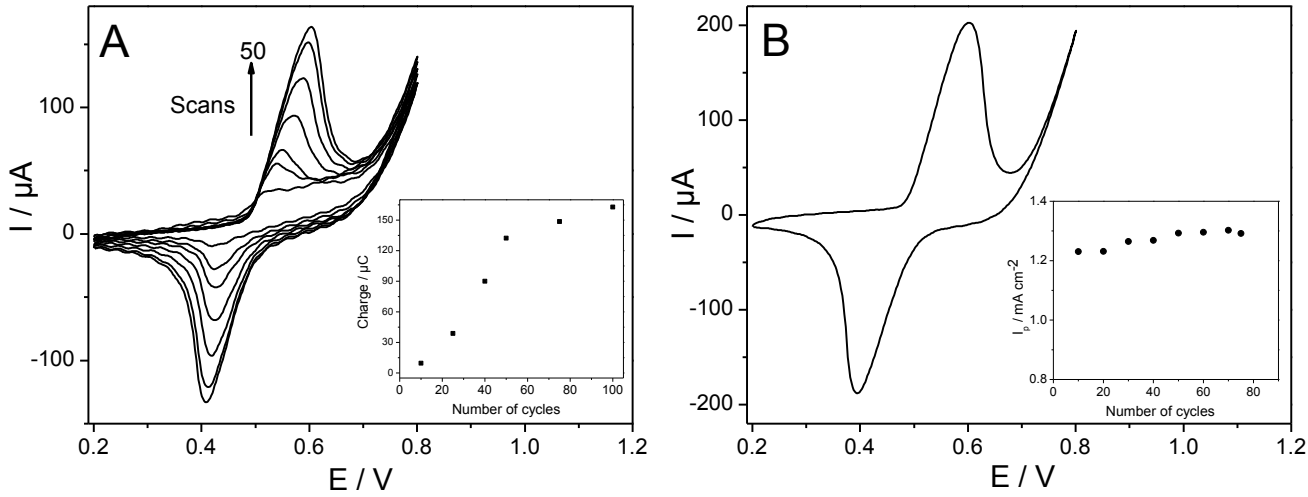

Figure 2

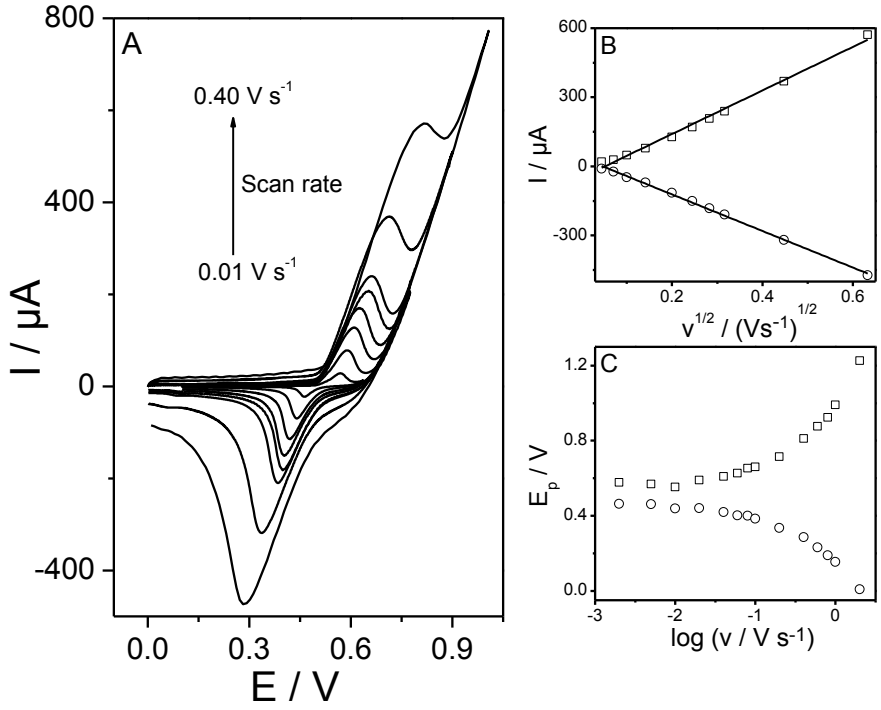

Figure 3

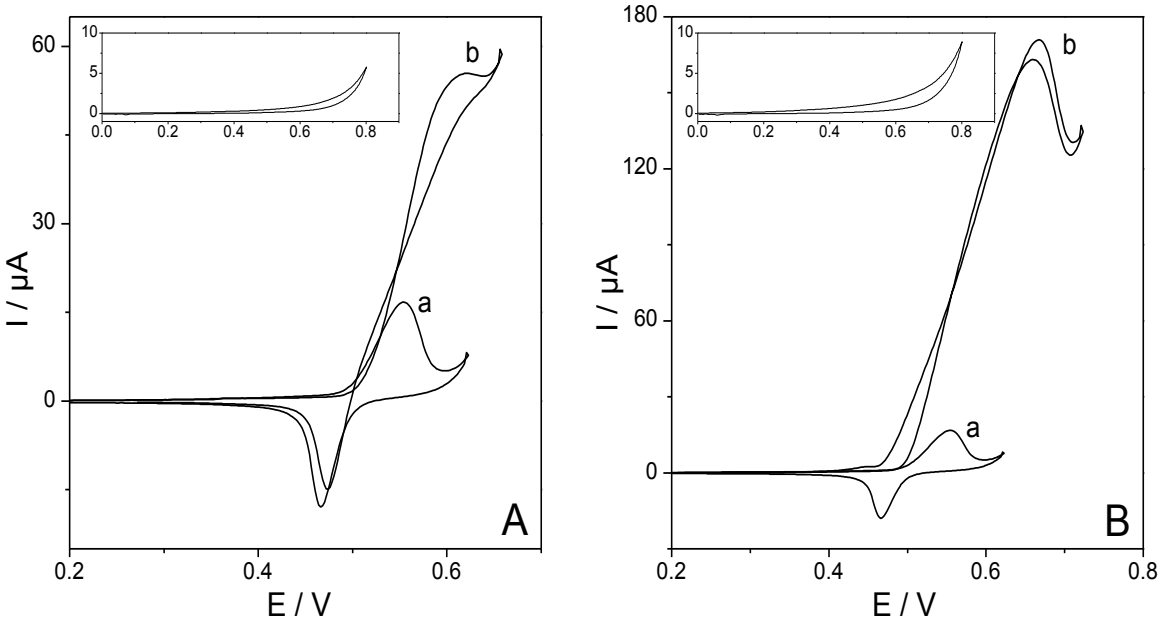

Figure 4

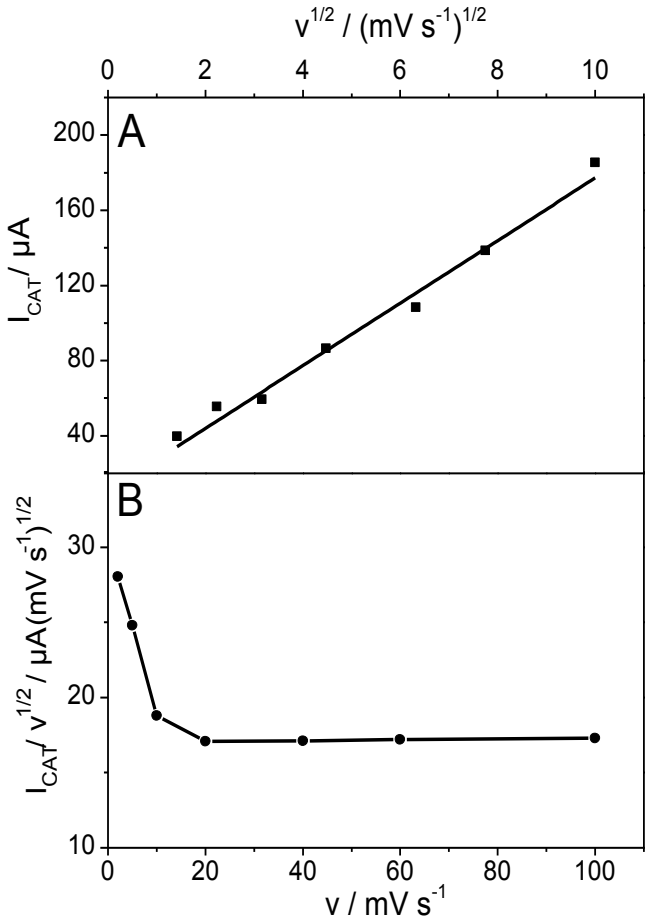

Figure 5

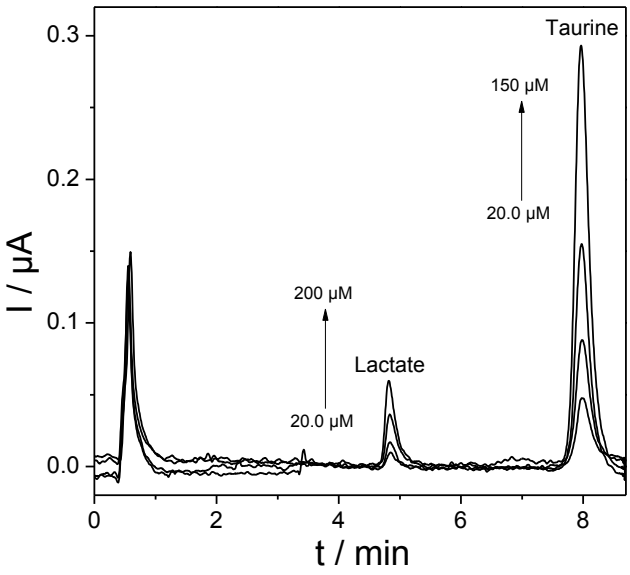

Figure 6

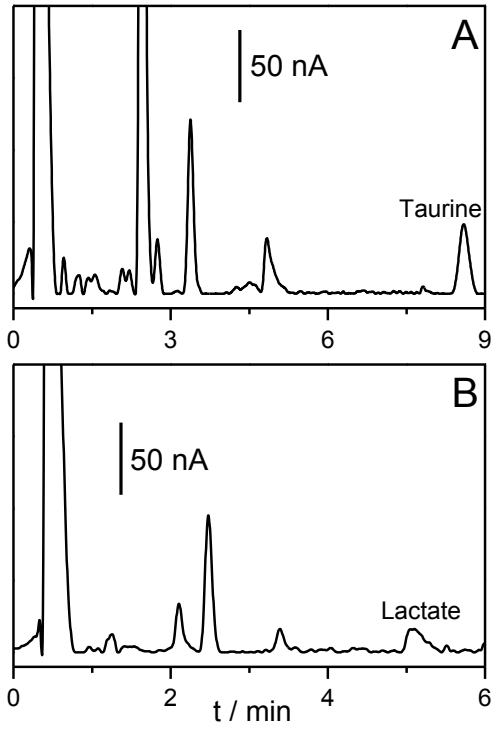

Figure 7

Table 1. Analytical parameters for the sequential determination of taurine and lactate using the [Ni^{II}(2,4DHS)]/SWCNTSPE as amperometric detector in HPLC

Biomarker	Sensitivity ($\mu C\ mM^{-1}\ cm^{-2}$)	Detection Limit (μM)	Quantification Limit (μM)	Linear range (μM)
Taurine	106±9	0.97	3.22	up to 160
Lactate	15±2	4.74	15.8	up to 210

Table 2. Determination of biomarkers in real samples

	Recovery % ± SD*	
	Taurine	Lactate
Sample		
Tears	112±5	-
Saliva	-	94±7

*SD: Standard deviation of three determinations