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The use of a gold disc microelectrode for the determination of copper in human sweat

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ABSTRACT

A novel approach of using a gold disc microelectrode to analyze sweat samples for copper ions by anodic square wave stripping voltammetry (SW stripping voltammetry) is described. Sweat was collected from the lower back of four subjects after physical exercise and the sample volume required for the determinations was 100 μL. Under the optimized conditions, the calibration plot was linear over the range 1–100 μmol L^{-1} Cu(II) with a limit of detection of 0.25 μmol L^{-1}. The precision was evaluated by carrying out five replicate measurements in a 1 μmol L^{-1} Cu(II) solution and the standard deviation was found to be 1.5%. Measurements were performed by inserting the microelectrode into sweat drops and Cu(II) concentrations in the analyzed samples ranged from 0.9 to 28 μmol L^{-1}. Values obtained by the proposed voltammetric method agreed well with those found using graphite furnace atomic absorption spectroscopy (GFAAS).

1. Introduction

The determination of chemical species in body fluids by non-invasive techniques has various advantages over blood sampling. Accordingly, alternative biological matrices such as saliva, tear, urine and sweat are receiving growing attention. Sweat is of particular interest as a consequence of the possible effect of sweat loss on the demands of the body for salt and vitamins, especially in hot environments. Moreover, the measurement of sweat chloride concentration remains a fundamental test for the diagnosis of cystic fibrosis [1–4], a common inherited disorder which does not allow chloride to be reabsorbed back into sweat duct cells. Hence, the amount of chloride ions in the duct increases and its concentration in sweat is elevated in individuals with cystic fibrosis. Sweat has also been used to measure other chemical species, including lactate [5,6], ammonium [6], cocaine [7], amphetamines [8], antigenic compounds [9] and trace metals [10–15].

Investigations on copper sweat concentration can aid clinical diagnosis of several diseases such as Wilson disease [16]. This is a genetic disorder responsible for excessive absorption of copper at the intestine and reduced excretion by the liver, causing an accumulation of the metal in the body. Copper is an important component of several metabolic enzymes and can be detected in sweat using atomic absorption spectrometry [10] or anodic SW stripping voltammetry [12,13].

The anodic SW stripping voltammetry is largely used for the detection of trace metals, including copper, since this technique allows the determination of low concentrations by preconcentrating the metal on the electrode surface [17–20].

Sweating is the mechanism to prevent rise in body temperature through to the evaporation of released water. Determination of chemical species in sweat for therapeutic and diagnosis purposes depends on stimulation for collection and further analysis. Sweat stimulation is usually performed by pilocarpine iontophoresis (introduction of a cholinergic drug into the skin using an electric potential gradient), physical exercises or thermal methods (sauna) [16]. As water evaporation occurs, the collection step is crucial since it can lead to inaccurate results. The collection methodology requires the sweat to be absorbed by materials impermeably sealed against a cleaned skin to avoid vapor losses. The collected sample is weighed, extracted and the residue is diluted for further analysis. A specific limitation of this methodology is that temporal information is not easily obtained. New strategies have been recently reported for real time measurements of sweat sodium [21] and pH [22].

Microelectrodes are devices with a critical dimension smaller than the thickness of the Nernst diffusion layer. Accordingly, they possess several attributes such as steady-state response for very short times. Electrochemistry can be performed at very small volumes when manufacturing procedures can ensure that both the sensing area and the non-conducting material are of very small size [23,24]. At these experimental conditions, the probe is localized very close to the assay site and the analytes or chemical reactions can be investigated in real time, as already demonstrated by our group in previous reports [25–27].

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This work represents an effort to develop a simple, rapid, reliable and low cost method for copper analysis in sweat samples. A sensing microelectrode structured as a small gold disc was used to measure the analyte by anodic SW stripping voltammetry. The proposed approach is an attractive alternative when compared to current sweat analysis techniques that require larger sample volumes, depend on sophisticated and expensive instrumentation and do not provide fast information.

2. Experimental

2.1. Chemicals and materials

All solid reagents were of analytical grade and were used without further purification. Solutions were prepared by dissolving the reagents in distilled, deionized water processed through a water purification system (Nanopure Infinity water purification system, Barnstead, Dubuque, IA).

2.2. Electrodes and instrumentation

A PalmSens portable electrochemical analyzer (Palmsens BV, Houten, The Netherlands) was used for electrochemical measurements. A graphite furnace atomic absorption spectrometer (GFAAS), model SIMAAS-6000, with a longitudinal Zeeman-effect background correction system, Echelle optical arrangement, solid state detector, and standard transversal heating graphite tube (THGA) with integrated pyrolytically coated platforms (Perkin-Elmer Life and Analytical Science, Shelton, CT, USA) was used in this work. A Cu hollow cathode lamp (324.8 nm, 10 mA, bandpass 0.7 nm) was used for Cu determinations. All Solutions were delivered into the graphite tube by means of an AS-72 autosampler (Perkin-Elmer Life and Analytical Science, Shelton, CT, USA). Argon 99.996% (v/v) (White Martins, São Paulo, SP, Brazil) was used as the purge gas.

2.3. Gold microelectrode fabrication

A gold 25 μm diameter disc microelectrode was fabricated following a conventional method. The gold fiber (Thornel) was cleaned by sonication in acetone, ethanol and distilled water, sequentially. Then, the fiber was dried using an IR lamp (Infraphil PAR38 150 W, Philips, Holland). Cleaned gold fibers were connected to a Ni/Cr wire with silver ink conductive paint (Joint Metal Comércio LTDA, São Paulo, Brazil), inserted into a glass capillary and vacuum-sealed with a P-97 Flaming/Brown Micropipette Puller (Sutter Instrument Company, USA). The surface of the microelectrode was polished with alumina powder (1, 0.3 and 0.05 μm, Alfa Aesar, MA, USA) on a microcloth polishing pad. Then, the electrode surface was rinsed with water and sonicated for 5 min in distilled water. The radius of the microelectrode was found to be 12.5 μm by measuring the steady-state current in a $K_3Fe(CN)_6$ solution containing KCl as supporting electrolyte.

2.4. Electroanalytical measurements

Copper analysis was carried out by inserting the working (gold microelectrode) and the reference (homemade $Ag/AgCl_{sat.\ KCl}$) electrodes into a sweat drop (100 μL). The whole system was supported on a gold substrate that was also used as counter electrode. Fig. 1a shows a representative scheme of the electrochemical cell and Fig. 1b shows a photograph of the fabricated gold microelectrode, taken with an Inverted microscope Observer D.1 (Carl Zeiss, Germany) equipped with a Ph2 63× objective and an AxioCam HSm digital camera (Carl Zeiss).

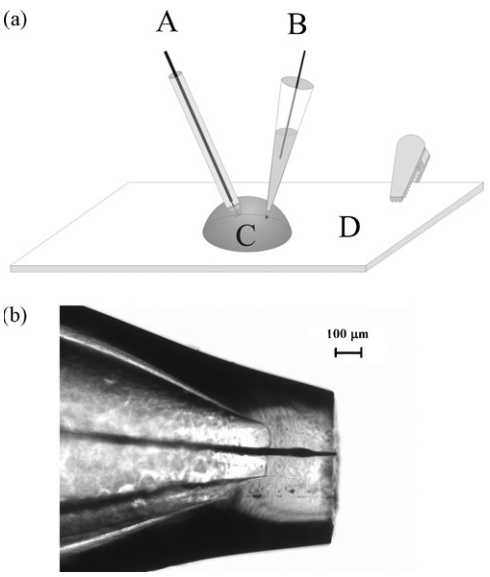

Fig. 1. (a) representative scheme of the electrochemical cell used in the sweat copper analysis. Gold microelectrode (A), reference electrode (B), sweat drop (C) and counter electrode (D). (b) Photograph of the fabricated gold microelectrode.

2.5. Sweat collection

The sweat samples were collected from four individuals of both sexes aged between 23 and 26 years old. The sampling procedure must avoid any evaporation and minimize epidermic contamination. Hence, the skin of the individuals was carefully washed with soap and water, rinsed with distilled water and then wiped twice with a cotton swab immersed in 50% ethanol. After the cleaning procedure the area was isolated from the external environmental with a plastic film in order to reduce sweat evaporation and contamination. Samples were collected directly from the lower back of individuals following moderate exercise for 30 min (exhaustive walk). Sweat samples were stored in air tight plastic containers and kept at 4 °C.

2.6. Absorption atomic spectroscopy analysis

The GFAAS heating program consisted of five steps (temperature/°C, ramp/°C s^{-1}, hold/s): 1 (100, 5, 10), 2 (140, 5, 10), 3 (1100, 10, 30), 4 (2200, 0, 5), and 5 (2400, 1, 3). 10 μL aliquots of samples or standard solutions were introduced into the graphite furnace with 10 μL of the chemical modifier prepared using suprapure solutions of 10 g L^{-1} of Pd ($Pd(NO_3)_2$) and 10 g L^{-1} of Mg ($Mg(NO_3)_2$) in 15% (v/v) HNO_3 (Merck, Darmstadt, Germany). The analytical reference solutions containing 10–50 μg L^{-1} Cu(II) were obtained by successive dilution of 1000 mg L^{-1} ($Cu(NO_3)_2$) (Titrisol, Merck, Darmstadt, Germany) with 0.1% (v/v) HNO_3.

150 μL aliquots of sweat samples were diluted with 1350 μL of ultrapure water. The standard and sample solutions were directly prepared in the autosampler cups (volume = 1500 μL) and the homogenization was done using a micropipette. All measurements had at least three replicates, based on integrated absorbance.

3. Results and discussion

3.1. SW stripping voltammetry parameters optimization

The association of microelectrodes with SW stripping voltammetry permits to determine low concentrations of metals without sample composition changes. Among the stripping waveforms, the square wave modulation combines high sensitivity with high speed

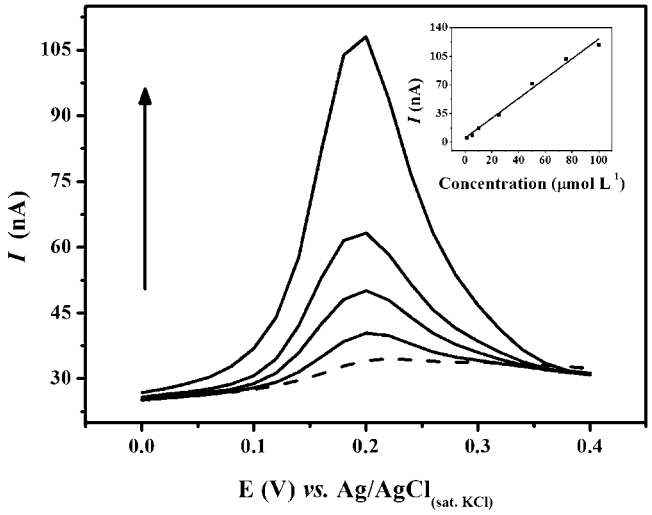

Fig. 2. Representative SW voltammograms recorded with a gold microelectrode in a drop of solution containing 0.1 mol L^{-1} NaCl, 0.1 mol L^{-1} acetate buffer (pH 6.0) before (dashed line) and after addition of a Cu(II) solution to give concentrations varying from 1 to 85 μmol L^{-1} (full line). Inset: calibration plot.

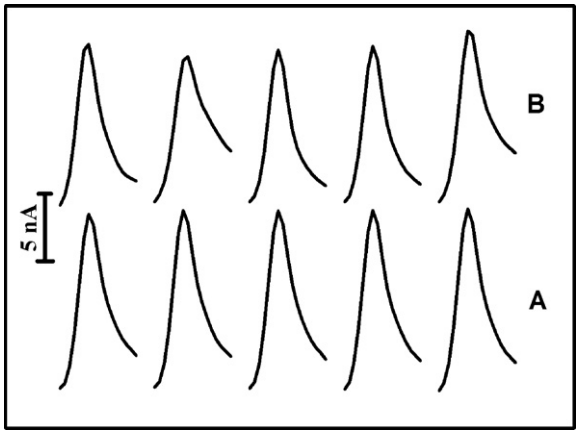

Fig. 3. SW voltammograms recorded with a gold microelectrode in a solution containing 0.1 mol L^{-1} NaCl, 0.1 mol L^{-1} acetate buffer (pH 6.0) and 1 μmol L^{-1} Cu(II) to investigate the repeatability (A) and the reproducibility (B) of the determinations.

and is quite insensitivity to dissolved oxygen [28]. Preliminary experiments involved the optimization of SW stripping voltammetry parameters for copper analysis using the apparatus shown in Fig. 1. Data were obtained using a 0.1 mol L^{-1} NaCl + 0.1 mol L^{-1} acetate buffer (pH 6.0) + 1 μmol L^{-1} Cu(II) solution. The supporting electrolyte composition was chosen as an attempt to simulate the human sweat [15] and will be referred in this paper as synthetic sweat. From the Handbook of Chemical Equilibria in Analytical Chemistry [29] it is known that the stability constants involving copper complexes with acetate are rather low (as illustrated below), which means that the formation of such complexes does not influence the Cu(II) electrochemical behaviour to a significant extent. The SW stripping voltammetry parameters were optimized choosing the highest current value obtained for each parameter in order to obtain best sensitivity. The parameters studied were $E_{deposition}$, E_{step}, $E_{amplitude}$, $E_{cleaning}$, frequency, $t_{deposition}$ and $t_{cleaning}$ and the optimum value chosen were −1.2 V, 20 mV, 65 mV, 0.7 V, 70 Hz, 200 s and 30 s respectively.

3.2. Analytical aspects

In order to investigate the relationship between Cu(II) concentration and SWV response, voltammograms were recorded in a drop of synthetic sweat after addition of 10 μL aliquots of a 0.1 mmol L^{-1} copper solution. Some of the voltammograms recorded at the optimized conditions and the calibration plot are shown in Fig. 2. Linearity is noticed from 1 to 100 μmol L^{-1} and the straight line obeys the equation I (nA) = $5.3 \times 10^{-9} + 1.2 \times 10^{-3}$ C/μmol L^{-1}. The limits of detection (3σ/s, σ being the standard deviation of 10 replicates of the blank and s the slope of the calibration plot) and quantification (10σ/s) were found to be 0.25 and 0.80 μmol L^{-1}, respectively [30–32].

By looking at the voltammograms shown in Fig. 2, it is possible to observe that peak potentials corresponding to the anodic oxidation of Cu^0 to Cu(II) appear at about 0.2 V vs Ag/AgCl (KCl conc.). This electrochemical process depends largely on the concentration of chloride ions in the solution, as the stabilization of Cu(II) by chloride shifts the potential to less positive values [33].

The agreement between independent results obtained under the same conditions by the same operator, measured as the repeatability of the method, was investigated by carrying out experiments with a synthetic sweat solution containing 1 μmol L^{-1} Cu (II). The voltammograms obtained in these experiments are shown in Fig. 3A and the standard deviation of the signals was found to be less than 1.5%. The reproducibility of the method was also investigated by recording SW voltammograms under the same conditions, but by different operators. Fig. 3B depicts the results obtained in this experiment and the standard deviation of the peak measurements was found to be 7.8%. The rate of mass-transport is greatly enhanced at electrodes with very small size. Hence, the deleterious influence of irregular solution stirring is avoided when microelectrodes are used in stripping voltammetric methods, as data can be obtained in quiescent conditions. This particular feature of microelectrodes may explain the good precision of the proposed method.

3.3. Sweat analysis

To demonstrate the applicability of the proposed method to a practical microanalytical problem, sweat samples were studied for their Cu(II) content. Because of the relatively high amount of Cu(II) in some of the samples, a peak distortion was noticeable in the recorded voltammograms probably because of intermetallic compound formation at these experimental conditions or because of the complex nature of the matrix [34]. To overcome this problem, experiments were performed at a much shorter deposition time than the one found in the optimization study, i.e. 15 s instead of 200 s.

Determination of Cu(II) in the sweat samples was also done by the standard addition method as shown by the results in Fig. 4. Sweat samples were collected from four individuals of both sexes aged between 23 and 26 years old. Concentration of Cu(II) ranged from 0.9 to 28.0 μmol L^{-1} Cu(II) and the results are consistent with earlier findings reported in the literature, where the Cu(II) concentration varied between 0.4 and 33.2 μmol L^{-1} [13,16]. This wide concentration range in sweat can be explained by differences in dairy ingested food, medication and beverages, among other environmental aspects.

The t-test was applied to compare the results obtained by SW stripping voltammetry with those found using a reference method. The paired t-test indicated that there was no significant difference between the results obtained with both methods at a 95% confidence level. Hence, it can be conclude that the proposed strategy is reliable and can be successfully applied for field determination of copper ions in sweat drops. Table 1 shows the results obtained with both methods.

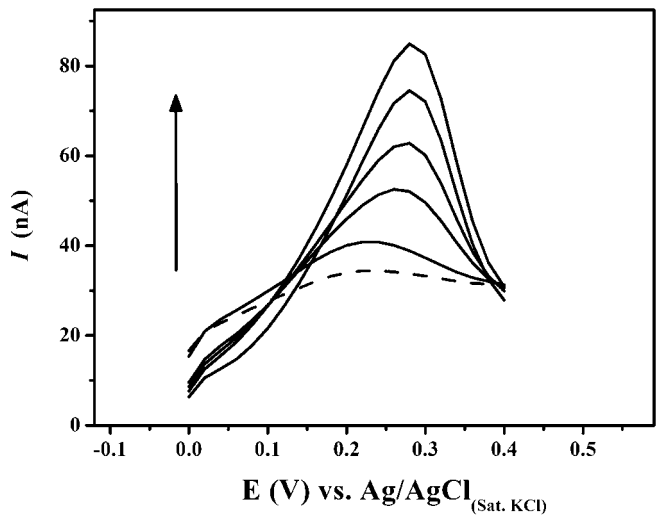

Fig. 4. SW voltammograms recorded with a gold microelectrode in a sample of human sweat before (dashed line) and after (solid lines) successive increments of Cu(II) to give final concentrations in the range 10–50 μmol L^{-1}.

Table 1
Comparison of the results for Cu(II) content in four sweat samples using the proposed SW voltammetric procedure and a reference method.

Sample	Electroanalytical measurement (μmol L^{-1})	RSD (%)	Spectrophotometric measurement (μmol L^{-1})	RSD (%)
1	28.0	3.2	28.7	4.0
2	8.0	2.5	7.9	3.0
3	2.1	4.8	2.2	2.4
4	0.9	6.0	0.8	1.6

4. Conclusions

In the present work we have demonstrated that a gold disc microelectrode can be successfully used as a sensor for measuring copper in sweat samples by anodic SW stripping voltammetry. Because the volume sample required for the analysis is very small (only a few microliters) and the linear concentration range is suitable for performing the measurements at very low deposition times, the approach offers rapid, precise and straightforward detection. The copper concentration in the analyzed sweat samples was found to fall within the range of values reported in the literature.

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References

[1] A. Lynch, D. Diamond, M. Leader, Analyst 125 (2000) 2264.
[2] M. Seia, L. Costantino, V. Paracchini, L. Porcaro, P. Capasso, D. Coviello, C. Corbetta, E. Torresani, D. Magazzu, V. Consalvo, A. Monti, D. Costantini, C. Colombo, Clinical Biochemistry 42 (2009) 611.
[3] J. Coakley, S. Scott, R. Mackay, R. Greaves, L. Jolly, J. Massie, A. Mishra, A. Bransden, J.C.G. Doery, A. Chiriano, H. Robins, Annals of Clinical Biochemistry 46 (2009) 332.
[4] R. Jayaraj, P.V. Barton, P. Newland, R. Mountford, N.J. Shaw, E. McCarthy, D.M. Isherwood, K.W. Southern, Annals of Clinical Biochemistry 46 (2009) 73.
[5] J.M. Green, R.C. Pritchett, T.R. Crews, J.R. McLester, D.C. Tucker, European Journal of Applied Physiology 91 (2004) 1.
[6] F. Meyer, O. Laitano, O. Bar-Or, D. McDougall, G.J.F. Heingenhauser, Brazilian Journal of Medical and Biological Research 40 (2007) 135.
[7] S.L. Kacinko, A.J. Barnes, E.W. Schwilke, E.J. Cone, E.T. Moolchan, M.A. Huestis, Clinical Chemistry 51 (2005) 2085.
[8] B.S. De Martinis, Current Pharmaceutical Analysis 4 (2008) 274.
[9] V. Drapel, A. Becue, C. Champod, P. Margot, Forensic Science International 184 (2009) 47.
[10] D.C. Hohnadel, Sunderma, M.W. Fw, M.D. Nechay, McNeely, Clinical Chemistry 19 (1973) 1288.
[11] J.R. Cohn, E.A. Emmett, Annals of Clinical and Laboratory Science 8 (1978) 270.
[12] J.L. Stauber, T.M. Florence, Science of the Total Environment 60 (1987) 263.
[13] J.L. Stauber, T.M. Florence, Science of the Total Environment 74 (1988) 235.
[14] S.J. Montain, S.N. Cheuvront, H.C. Lukaski, International Journal of Sport Nutrition and Exercise Metabolism 17 (2007) 574.
[15] A. Crew, D.C. Cowell, J.P. Hart, Talanta 75 (2008) 1221.
[16] M. Schaefer, M. Schellenberg, U. Merle, K.H. Weiss, W. Stremmel, BMC Gastroenterology 8 (2008) 9.
[17] B.K. Jena, C.R. Raj, Analytical Chemistry 80 (2008) 4836.
[18] R.M. Takeuchi, A.L. Santos, M.J. Medeiros, N.R. Stradiotto, Microchimica Acta 164 (2009) 101.
[19] J.F. Huang, B.T. Lin, Analyst 134 (2009) 2306.
[20] P. Salaun, C.M.G. van den Berg, Analytical Chemistry 78 (2006) 5052.
[21] D. Morris, S. Coyle, Y.Z. Wu, K.T. Lau, G. Wallace, D. Diamond, Sensors and Actuators B-Chemical 139 (2009) 231.
[22] B. Schazmann, D. Morris, C. Slater, S. Beirne, C. Fay, R. Reuveny, N. Moyna, D. Diamond, Analytical Methods 2 (2010) 342.
[23] R. Kashyap, M. Gratzl, Analytical Chemistry 70 (1998) 1468.
[24] B.A. Grzybowski, R. Haag, N. Bowden, G.M. Whitesides, Analytical Chemistry 70 (1998) 4645.
[25] D. Lowinsohn, H.E.M. Peres, L. Kosminsky, T. Paixao, T.L. Ferreira, F.J. Ramirez-Fernandez, M. Bertotti, Sensors and Actuators B-Chemical 113 (2006) 80.
[26] T. Paixao, D. Lowinsohn, M. Bertotti, Journal of Agricultural and Food Chemistry 54 (2006) 3072.
[27] T. Paixao, L.F. Barbosa, M.T. Carri, M.H.G. Medeiros, M. Bertotti, Analyst 133 (2008) 1605.
[28] J. Wang, Stripping Analysis, VCH, Deerfield Beach, 1985.
[29] S. Kotrlý, L. Šúcha, Handbook of Chemical Equilibria in Analytical Chemistry, Ellis Horwood Limited, New York, 1985.
[30] A. Abcd, Analyst 112 (1987) 199.
[31] D.A. Skoog, D.M. West, F.J. Holler, S.R. Crouch, Fundamentals of Analytical Chemistry, Pioneer Thomson, USA, 2004.
[32] D.A. Skoog, F.J. Holler, T.N. Nieman, Principles of Instrumental Analysis, Brooks Cole, USA, 2006.
[33] R.D. Riso, M. Waeles, P. Monbet, C.J. Chaumery, Analytica Chimica Acta 410 (2000) 97.
[34] M.A. Baldo, C. Bragato, S. Daniele, Analyst 122 (1997) 1.