DISPOSABLE ELECTROCHEMICAL SENSOR FOR DETERMINATION OF HUMIC ACID IN SAPROPEL AND WATER SAMPLES

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The quantification of humic acid in sapropel, Techirghiol Lake, and Dead Sea water is essential due to its importance in therapeutics. An electrochemical sensor was designed by modifying a screen-printed sensor (based on carbon single-walled nanotubes) with a heptakis(2,3,6-tri-O-methyl)-beta-cyclodextrin solution (1.00×10^{-3} mol L^{-1}). The linear concentration range recorded for the evaluation of humic acid was between 10 fg $mL^{-1}$and 10 ng mL^{-1}, with a limit of detection of 3 fg mL^{-1}. Recoveries over 91.00 % with an RSD below 0.20 % were observed when the sensor was used to assay humic acid in sapropel, and Techirghiol Lake and Dead Sea water.

Keywords: humic acid, electrochemical sensor, sapropel

1. Introduction

Sapropel as well as Techirghiol Lake and the Dead Sea waters are well known for their cosmetic and therapeutic properties, most of these properties being associated with the existence of humic acid in their composition [1-3]. Determination of humic acid in sapropel and waters containing humic acid is needed before their utilization as therapeutic agents, as the pollutants may affect their concentration – a decrease in humic acid concentration was recorded in polluted sapropel and water samples [4-7].

To date, spectrometric methods of analysis [3,8,9], qualitative electrochemical methods of analysis [9], a stochastic method of analysis [10] as well as chromatographic methods of analysis [11] were proposed for the quantification of humic acid from sapropel and water samples. Except for the

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stochastic mode of analysis, all the other methods proposed to date needed a laborious processing of the sample before its analysis. Improving the limits of determination and detection as well as sensitivity and selectivity, are needed for the reliable evaluation of humic acid in sapropel and water samples. Utilization of electrochemical sensors having an active surface based on nanomaterials may facilitate decreased limits of detection and quantification as well as increased sensitivities [10, 12-14].

The novelty of this paper is the determination of humic acid by utilization of a disposable electrochemical sensor based on the modification of single-walled carbon nanotubes with heptakis(2,3,6-tri-O-methyl)-beta-cyclodextrin which increased the sensitivity of the measurements bringing the detection limit for the evaluation of humic acid to fg mL^{-1}.

2. Experimental

2.1. Materials and reagents

Sigma Aldrich (Milwaukee, USA) provided the humic acid, while Fluka provided the paraffin oil. Deionized water was acquired via a Millipore Direct-Q3. Heptakis(2,3,6-tri-O-methyl)-ß-cyclodextrin was supplied by CarboHyde Zrt. Screen-printed sensors DRP-110 SWCNT were bought from Metrohm (Herisau, Switzerland).

2.2. Apparatus

A Mini Potentiostat EmSTAT Pico (software PsTrace 5.9 PalmSens, Houten, Netherlands) was utilized for all measurements.

2.3. Design for the disposable electrochemical sensor

Screen-printed sensors DRP-110 SWCNT were modified with a solution of heptakis(2,3,6-tri-O-methyl)-beta-cyclodextrin (1.00×10^{-3} mol L^{-1}). 20 µL of this solution was dropped on the surface of the DRP-110 SWCNT and left for 3h at room temperature until utilization for measurements. Modified sensors were able to be kept at room temperature for more than one month.

2.4. Recommended procedure

All measurements were conducted using differential pulse voltammetry (DPV). The scan was done between 0 and 1.2 V with an E_{step} of 0.025 V, an E_{puls} of 0.2 V, and a scan rate of 0.05 V s^{-1}. Peaks developed at 789 mV $E_{1/2}$ potential were considered. In order to establish the working concentration range, samples with concentrations between 0.01 fg mL^{-1} and 100 ng mL^{-1} were scanned using DPV. The calibration equation parameters were statistically established by linear regression analysis. The current intensity was incorporated into the calibration graph for the analysis of humic acid concentration in sapropel and water samples.

2.5. Samples

Techirghiol Lake and the Dead Sea water/sapropel samples were chosen for their therapeutic properties. The sludge sample is a solid, fatty mass that cannot be absorbed and is therefore fluidized with seawater: one part salt water from the lake and two parts sludge. No water sample preparation was done before analysis.

3. Results and discussions

3.1. Response characteristics of the disposable electrochemical sensor

DPV was used for all measurements performed using the proposed disposable electrochemical sensor. The sensor response characteristics are presented in Table 1.

Table 1

The response characteristics of the disposable electrochemical sensor

Linear concentration range (g mL^{-1})	1.00×10^{-14} - 1.00×10^{-8}
Calibration equation (<I>=μA, <C_{HA}>=g mL^{-1})	$I = 110.08(\pm3.4) + 5.31(\pm1.14)\times10^{-11} \times C_{HA}$
Correlation coefficient	0.9976
Sensitivity (μA g^{-1} mL)	$5.31(\pm1.14)\times10^{-11}$
Quantification limit (g mL^{-1})	1.00×10^{-14}
Detection limit (g mL^{-1})	3.00×10^{-15}
Number of data points	7

A wide working concentration range (Fig. 1) was recorded for the assay of humic acid, making possible its assay without a preconcentration step. The limit of quantification was resoluted accordingly with IUPAC references [15], as the lower concentration from the working concentration range; the quantification limit is 10 fg mL^{-1} – lower than those reported earlier [3-9]. The determined limit of detection was 3 fg mL^{-1} – far lower than the reported limits of detection [3-9] to date.

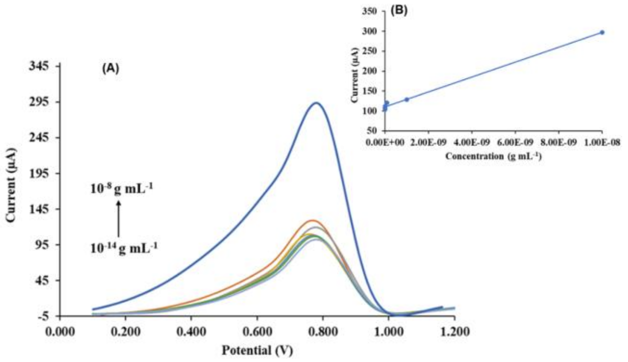

Fig. 1. **(A)** Differential pulse voltammograms obtained for the calibration of the sensor (scan rate of 0.05 V s^{-1}); **(B)** calibration graph of the disposable sensor utilized for the assessment of humic acid

Reproducibility of the sensors' design was determined as follows: ten sensors were manufactured according to the procedure described above; for each of the sensors was determined the sensitivity and the relative standard deviation (RSD) vs. the average value was reported. The sensor's design demonstrated good reproducibility with an RSD of 1.74 %. The sensitivity was assessed over a 30-day period, yielding an RSD of 2.15 %, therefore demonstrating the sensor's commendable temporal stability.

3.2. Selectivity of the disposable electrochemical sensor

The selectivity of the disposable electrochemical sensor was assessed using the mixed solution method: measurements of solutions containing both the humic acid and the possible interferent will be compared with those obtained for a solution containing the humic acid; in both solutions, humic acid will have the same concentration, while the concentration of the interferent will be tenfold higher than the concentration of humic acid. Estradiol, NH_4^+, and Fe^{2+} were studied as possible interferents. Amperometric selectivity constants were determined; their values are given in Table 2.

Table 2.

Amperometric selectivity coefficients

K_{sel}^{amp}		
Estradiol	NH_4^+	Fe^{2+}
1.20×10^{-4}	1.00×10^{-4}	1.03×10^{-4}

All recorded values for the amperometric selectivity coefficient were of the order of 10^{-4}, demonstrating that the suggested disposable sensor is selective towards the investigated chemicals.

3.3. Validation of the disposable electrochemical sensor for the quantification of humic acid in sapropel and water samples

Samples of water and sapropel from Techirghiol Lake and the Dead Sea were used for the method validation. The humic acid concentration values from the samples were compared with those derived from the previously proposed HPLC method by Brondi et al. [11] (Table 3).

Table 3.

Quantification of humic acid in sediment and aqueous samples (N=10)

Sample	**Humic acid**	
	Disposable sensor	HPLC [11]
Water sample*	31.12±0.13	31.97±1.32
Sapropel sample 1**	484.97±0.21	484.02±1.76
Sapropel sample 2**	742.98±0.35	743.20±1.95

*pg/mL; **pg humic acid/g mud

DPV voltammograms obtained for the quantification of humic acid in sapropel and aqueous samples are shown in Figure 2.

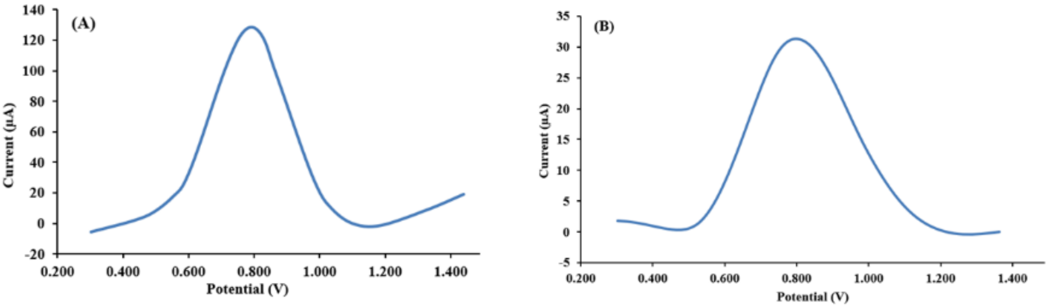

Fig. 2. Differential pulse voltammograms obtained for the (**A**) water samples, and (**B**) sapropel

A strong correlation exists between the data produced from the suggested method, which employs a disposable electrochemical sensor, and the HPLC method, according to the results introduced in Table 3.

4. Conclusions

The disposable electrochemical sensor demonstrated efficacy in assaying humic acid across an extensive linear concentration range, with high sensitivity and high selectivity in sapropel and water samples. A rapid and reliable assessment of humic acid content is possible using this sensor.

The primary function of the disposable electrochemical sensor is its application in the on-site quality assessment of sapropel and water samples, which will be used for therapeutic and cosmetic purposes.

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REFERENCES

[1]. *G.P. Alexandrova, G. Dolmaa, U. Enkhbadral, G.L. Grishenko, Sh. Tserenpil, B.G. Sukhov, D. Regdel, B.A. Trofimov,* A new humic acid remedy with addition of silver nanoparticles, Mongolian J Chem, Vol. **13**, 2012, pp. 7-11.

[2]. *B.A. Gomes de Melo, F. Lopes Motta, M.H.A. Santana*, Humic acid: structural properties and multiple functionalities for novel technological developments, Mater. Sci. Eng. C Mater. Biol. Appl., Vol. **62**, 2016, pp. 967-974.

[3]. *M. Hoteteu, C. Munteanu, E.V. Ionescu, E.R. Tucmeanu*, Bioactive substances of the Techirghiol therapeutic mud, Balneo Res. J., Vol. **9**, Iss. 1, 2018, pp. 5-10.

[4]. *T.W. Lee, C. Chen*, Humic acid changes effect of naturally occurring oxidants on the environmental transformation of molybdenum disulfide nanosheets, J. Environ. Manage., Vol. **368**, 2024, 122190.

[5]. *M. Kamalakannan, J. Thomas, N. Chandrasekaran*, Humic acid alleviates the toxicity of polystyrene nanoplastics in combination with their copper nanoparticle co-pollutants in *Artemia salina*, Environ. Sci.: Nano, Vol. **11**, 2024, pp. 4074-4088.

[6]. *S. Wang, Z. Liang, Y. Wang, H. Jin, S. Li, R. Guan*, Humic acid as eco-friendly corrosion inhibitor for EH40 ship plate steel, Surf. Interface Anal., Vol. **56**, Iss. 9, 2024, pp. 654-665.

[7]. *J. Chen, B. Zhang, C. Wang, P. Wang, G. Cui, H. Gao, B. Feng, J. Zhang*, Insight into the enhancement effect of humic acid on microbial degradation of triclosan in anaerobic sediments, J. Hazard Mater., Vol. **461**, 2024, 132549.

[8]. *L. Jarukas, L. Ivanauskas, G. Kasparaviciene, J. Baranauskaite, M. Marksa, J. Bernatoniene*, Determination of organic compounds, fulvic acid, humic acid, and humin in Peat and sapropel alkaline extracts, Molecules, Vol. **26**, Iss. 10, 2021, 2995.

[9]. *A. Ukalska-Jaruga, R. Bejger, G. Debaene, B. Smreczak*, Characterization of soil organic matter individual fractions (fulvic acids, humic acids, and huminus) by spectroscopic and electrochemical techniques in agricultural soils, Agronomy, Vol. **11**, Iss. 6, 2021, 1067.

[10]. *R.I. Stefan-van Staden, A.E. Sandu-Dorneanu, C. Cioates Negut, G. Stanciu*, Fast analysis of humic acid in mud and water samples, J. Electrochem. Soc., Vol. **171**, 2024, DOI 10.1149/1945-7111/ad8fd6.

[11]. *A.M. Brondi, J. Daniel, V.X. Monteiro de Castro, A. Bertoli, J.S. Garcia, M.G. Trevisan*, Quantification of humic and fulvic acids, macro- and micronutrients and C/N ratio in organic fertilizers, Comm. Soil Sci. Plant Anal., Vol. **47**, Iss. 22, 2016, pp. 2506-2513.

[12]. *E. DeVoe, S. Andreescu*, Review – catalytic electrochemical biosensors for dopamine: design, performance, and healthcare applications, ECS Sens. Plus, Vol. **3**, Iss. 2, 2024, 020601.

[13]. *C.J. Weber, M.D. Whisonant, O. Clay, O. Simoska*, Perspective – Surface – display techniques in electrochemical biosensor designs for health monitoring, ECS Sens. Plus, Vol. **3**, Iss. 2, 2024, 020603.

[14]. *R.I. Stefan-van Staden*, Perspective - Challenges in Biomedical Analysis: From Classical Sensors to Stochastic Sensors, ECS Sens. Plus, Vol. **1**, Iss. 1, 2022, 011603.

[15]. *D.B. Hibbert, E.H. Korte, U. Örnemark*, Metrological and quality concepts in analytical chemistry (IUPAC Recommendations 2021), Pure Appl. Chem., Vol. **93**, Iss. 9, 2021, pp. 997–1048.