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Disposable Stochastic Sensors for the Simultaneous Assay of Acetylcholine and Dopamine in Whole Blood Samples

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ABSTRACT

Acetylcholine and dopamine are neurotransmitters important for aging and brain pathology. Their assay in whole blood is essential for fast and early detection of neurodegenerative disorders. Therefore, polymeric textile covered with a thin layer of Ag was used to provide stochastic sensors modified with maltodextrins presenting different dextrose equivalence: Maltodextrin I (dextrose equivalence 13.0–17.0), and Maltodextrin II (dextrose equivalence 16.5–19.5). These stochastic sensors were used reliable for both qualitative and quantitative analysis of acetylcholine and dopamine in whole blood samples. Their sensitivity and selectivity were high, and they were reliable for the assay of dopamine and acetylcholine in whole blood samples, with recoveries higher than 98.00%, and relative standard deviations (%) values lower than 1.00%.

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Acetylcholine; disposable stochastic sensors; dopamine

Introduction

Acetylcholine was the first discovered neurotransmitter (by Henry Hallett Dale in 1915), and one of the oldest neurotransmitters that occurred in the animal kingdom (Wessler, Kirkpatrick, and Racke 1999). Although acetylcholine is essential in mammals for muscle contraction and the functioning of the autonomous nervous system, it is also important for the functioning of the central nervous system, including the most phylogenetically new functions like learning, memory, decision making, and planning. The cholinergic mediation is also altered in most psychiatric disorders. Acetylcholine mediation seems to be affected by aging, first of all because of the loss of neuronal circuits. Many studies of humans and other mammals showed a deficit in acetylcholine mediation related to aging (Stanley and Fadel 2012). But the results of a study on *Aplysia,* an invertebrate, suggest a defect in cholinergic transmission during aging of the R15 neurons in the abdominal ganglia.

The oldest hypothesis of the appearance of Alzheimer's disease, on which most currently available drug therapies are based, is the cholinergic hypothesis (Francis et al. 1999) which proposes that Alzheimer's disease is caused by reduced synthesis of the neurotransmitter

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acetylcholine. Although the medication based upon this hypothesis can delay the full appearance of the symptoms of Alzheimer's disease for a few years, it have not been very effective. Dopamine is a well-known neurotransmitter which can be related to neurodegenerative diseases (because its concentration is decreasing with age), like Parkinson's disease (Shohamy and Wimmer 2013; Paladini and Roeper 2014). Screening of whole blood of old people for acetylcholine and dopamine can detect at a very early stage the associated neurodegenerative diaseases.

Electrochemical sensors have been proposed for the determination of acetylcholine and dopamine in different biological samples. To date, the most important results were obtained using biosensors for the assay of acetylcholine (Pandey et al. 2000; Sattarahmady, Heli, and Dehdari Vais 2013), and electrochemical sensors for the assay of dopamine (Chan et al. 2008; Zhang et al. 2014).

Stochastic sensors are well known for their ability to perform reliably both qualitative and quantitative analysis. Disposable sensors are always very much appreciated by the medical doctors and patients due to the avoidance of sample contamination. Building disposable stochastic sensors for screening of old people or people prone to develop neurodegenerative diseases may avoid the risk for these diseases which are not reversible in most of the cases.

Therefore, this paper proposed a screening method based on the utilization of stochastic sensors for fast detection and assay of two markers: acetylcholine and dopamine used in the diagnosis of neurodegenerative diseases. The sensors are based on plasma deposition of Ag on polymeric textiles, and its modification with nanostructured materials, namely maltodextrins of two dextrose equivalence: Maltodextrin I (dextrose equivalence 13.0–17.0), and Maltodextrin II (dextrose equivalence 16.5–19.5). The novelty of the paper is the design of the Ag-polymeric textile based disposible sensor used for the assay of acetylcholine and dopamine.

Experimental

Materials and reagents

All chemicals were of analytical grade. Dopamine, acetylcholine, and maltodextrins (Maltodextrin I (dextrose equivalence 13.0–17.0), and Matodextrin II (dextrose equivalence 16.5–19.5)) were obtained from Sigma Aldrich. Dopamine and acetylcholine solutions of different concentrations (10^{-3}–10^{-20} mol L^{-1}) were prepared in buffer solution (phosphate buffer solution, pH 7.4) using the serial dilution method.

Apparatus and methods

All measurements were performed with an AUTOLAB/PGSTAT 302N (Metrohm) connected to a personal computer with a GPES software, used to record the measurements. A disposable stochastic sensor was used as working electrode in the cell; the cell comprised of working electrode, Ag/AgCl as the reference electrode in the cell and a platinum wire as the counter electrode in the cell, respectively.

Design of Ag-polymeric textile based disposible sensor

Ag films were deposited on a polymeric textile using the anodic high voltage plasma - a low temperature plasma source working in vacuum of 2×10^{-5} Torr (Figure 1). Due to the

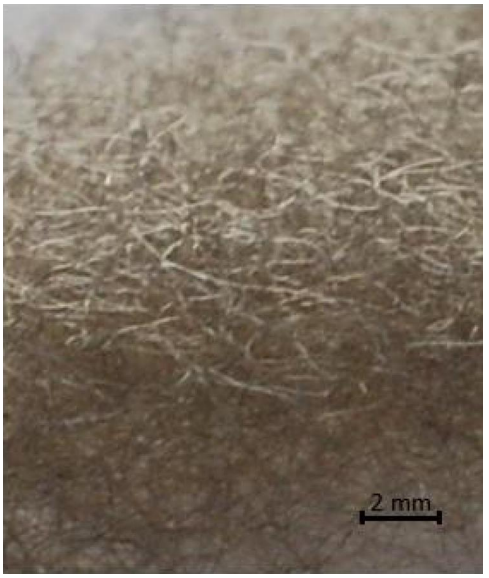

Figure 1. Topography of the silver film deposited on the polymeric texture by transmission electron microscope operated at 120 kV for the topography of the silver film.

small volume occupied by this plasma, heat sensitive substrates can be placed inside the vacuum chamber to be coated without being degraded, thanks to the thermal insulating nature of the vacuum. The operating principle of the plasma used in this work is based on creating ionized metal vapors by electron bombardment of the crucible containing the metal. The Ag plasma parameters used in this work were 1.4 A plasma current, 100 V plasma voltage and 21 min deposition time.

The modified polymeric textiles were immersed for 12 h in 10^{-3} mol L^{-1} solution of maltodextrin I, and Maltodextrin II and they were left to dry for 24 h. The area of the active side was 0.16 mm^2.

Stochastic mode

The three sensors were inserted in the whole blood solution and a potential of 50 mV versus Ag/AgCl was applied. The signatures of dopamine and acetylcholine ($time_{off}$ values) in Table 1 were used for their identification in the diagrams obtained from whole blood samples (Figure 2). Calibration equations: $1/time_{on} = a + b \times$ Concentration were recorded and used for the assay of the unknown concentration of dopamine and acetylcholine in the whole blood samples.

Samples

Twelve blood samples were collected from young and old patients from the University Hospital in Bucharest (Ethics committee approval no. 11/2013). Informed consent was obtained from all patients. No sampling process was performed before analysis.

Table 1. Response characteristics of stochastic sensors used for the assay of dopamine and acetylcholine.

Microsensors based on polymeric textile and Ag film, modified with	Calibration equation* and correlation coefficient (r)	Linear concentration range (mol/L)	$Time_{off}$ (s)	Sensitivity (s^{-1}/g mL^{-1})	Limit of determination (mol/L)
Dopamine					
Maltodextrin I	$1/time_{on} = 0.03 + 7.52 \times 105 \times$ Concentrtaion $r = 0.9995$	1.0×10^{-10}–1.0×10^{-7}	1.6	7.52×105	1.0×10^{-10}
Maltodextrin II	$1/time_{on} = 0.03 + 1.00 \times 108 \times$ Concentration $r = 0.9877$	1.0×10^{-12}–1.12×10^{-10}	1.6	1.00×108	1.0×10^{-12}
Acetylcholine					
Maltodextrin I	$1/time_{on} = 0.03 + 2.00 \times 106 \times$ Concentration $r = 0.9999$	1.00×10^{-10}–1.00×10^{-8}	1.0	2.00×106	1.00×10^{-10}
Maltodextrin II	$1/time_{on} = 0.02 + 2.00 \times 106 \times$ Concentration $r = 0.9992$	1.00×10^{-11}–1.00×10^{-8}	1.1	2.00×106	1.00×10^{-11}

*$<1/time_{on}> = s^{-1}$, <Concentration> = mol/L.

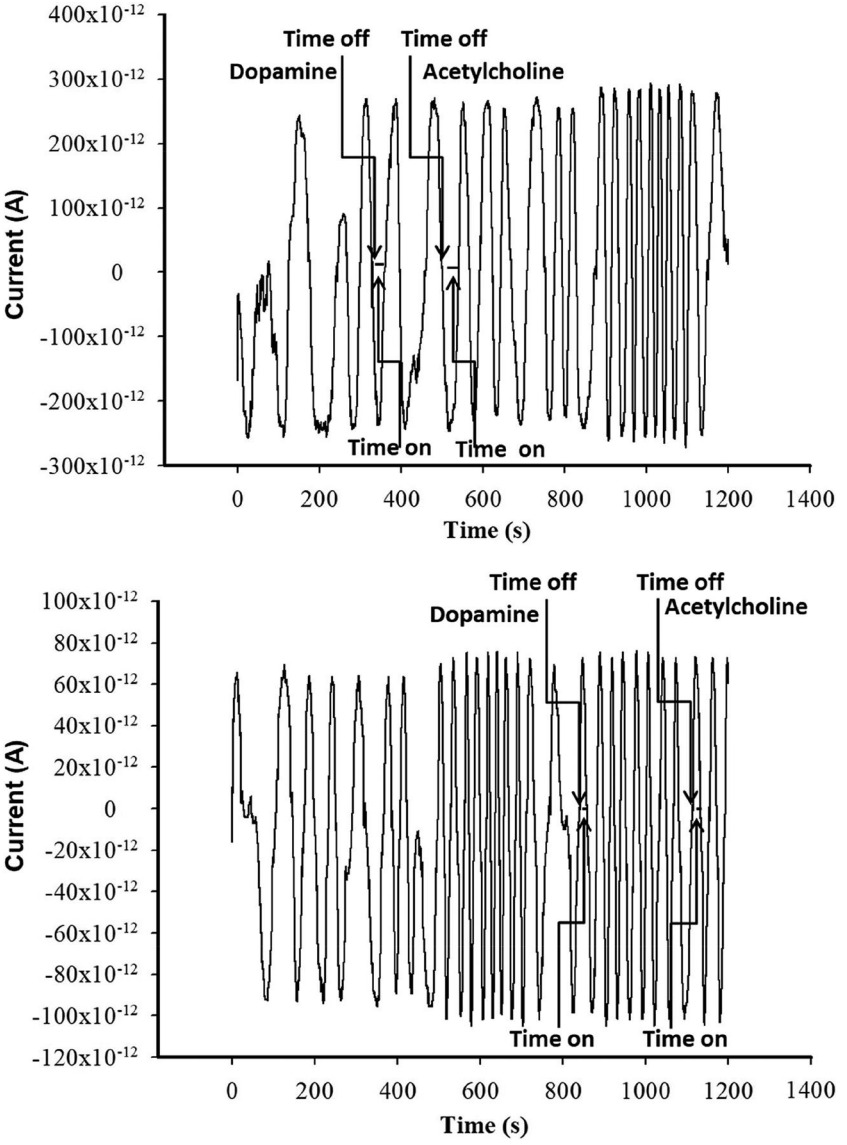

Figure 2. Diagrams obtained for the screening of whole blood using the sensors based on (a) Maltodextrin I, and (b) Maltodextrin II.

Results and discussion

Response characteristics of stochastic sensors

Stochastic sensors' response is based on channel conductivity: a potential of 125 mV versus Ag/AgCl was applied. The molecule enters the channel, making the intensity of current to become zero until is inside the channel (Figure 2). The time spent by the molecule to get into the channel is called signature of the molecule and is marked with $time_{off}$. The actual electrochemical processes are take place inside the channel. When the molecule first contacts the wall and redox processes are taking place, the time spend for these processes

is marked as $time_{on}$. A schematic representation of the sensor and setup of the measurement are presented in Figure 3.

First, the signatures of dopamine and acetylcholine were determined; the values are shown in Table 1. As it can be seen in Table 1, different signatures were obtained for the neurotransmitters dopamine and acetylcholine when the two sensors were used, proving that both analytes can be determined in one run by the same sensors.

Similar limits of determination were obtained for both neurotransmitters when Maltodextrin I and Maltodextrin II were used as modifiers. The highest sensitivity for the assay of dopamine was obtained when Maltodextrin II was used as modifier, while the highest sensitivity for the assay of acetylcholine was obtained when Maltodextrin I was used as modifier.

The proposed stochastic sensors showed good stability when used daily for measurements for more than 1 month. The relative standard deviation (%) values for the sensitivities varied by less than 1.00%.

The stability of the proposed stochastic sensors was measured when used for calibration every day for 30 days, and also during one day when 10 whole blood samples were screened. The results obtained showed that the relative standard deviation of the slope during the 30 days was less than 0.54%. Also, they could have been used successfully for the assay of dopamine and acetylcholine in whole blood samples, with high reliability (relative standard deviation values were less than 1.00%), showing a good stability of the proposed stochastic sensors.

Interferences were characterized for other neurotransmitters (epinephrine and norepinehrine) and the metal ions of Na, K, Ca, and Mg. For all of these substances, there were, different values for $time_{off}$, showing that these substances did not interfere with the determination of dopamine and acetylcholine.

Analytical applications

No sample preparation before the measurements were needed for the whole blood samples. In the recorded diagrams, $time_{off}$ and $time_{on}$ values were measured (Figure 2). The first

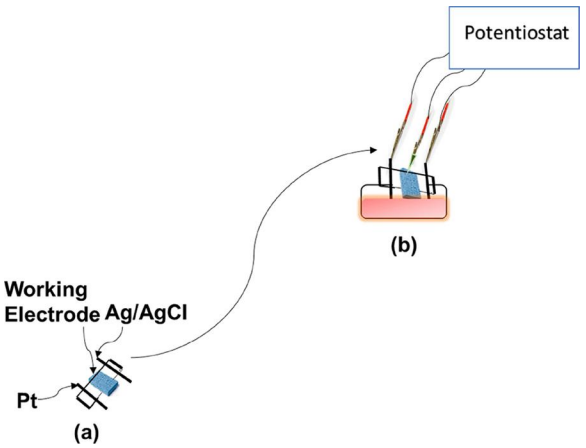

Figure 3. Schematic representation of the (a) disposable sensor and (b) setup of the measurement.

Table 2. Determination of dopamine and acetylcholine in whole blood samples.

Sample	Neurotransmitter	Sensor based on Maltodextrin I	Sensor based on Matodextrin II
1	Dopamine, pg/mL	82.90 ± 0.12	80.34 ± 0.22
	Acetylcholine, μmol/L	2.52 ± 0.07	2.73 ± 0.09
2	Dopamine, pg/mL	70.20 ± 0.15	70.55 ± 0.13
	Acetylcholine, μmol/L	1.42 ± 0.03	1.09 ± 0.02
3	Dopamine, pg/mL	25.60 ± 0.23	28.60 ± 0.18
	Acetylcholine, μmol/L	5.87 ± 0.19	5.56 ± 0.06
4	Dopamine, pg/mL	47.30 ± 0.12	47.21 ± 0.13
	Acetylcholine, μmol/L	1.20 ± 0.07	1.78 ± 0.07
5	Dopamine, pg/mL	16.70 ± 0.16	16.90 ± 0.11
	Acetylcholine, μmol/L	2.54 ± 0.09	3.14 ± 0.08
6	Dopamine, pg/mL	14.70 ± 0.13	11.98 ± 0.12
	Acetylcholine, μmol/L	2.68 ± 0.08	2.82 ± 0.05
7	Dopamine, pg/mL	21.00 ± 0.12	21.19 ± 0.09
	Acetylcholine, μmol/L	7.84 ± 0.10	7.75 ± 0.11
8	Dopamine, pg/mL	2.37 ± 0.04	2.24 ± 0.04
	Acetylcholine, μmol/L	9.28 ± 0.02	9.08 ± 0.02
9	Dopamine, pg/mL	21.29 ± 0.15	21.58 ± 0.17
	Acetylcholine, μmol/L	11.00 ± 0.11	11.15 ± 0.10
10	Dopamine, pg/mL	26.30 ± 0.07	27.90 ± 0.08
	Acetylcholine, μmol/L	1.74 ± 0.02	1.86 ± 0.01
11	Dopamine, pg/mL	48.70 ± 0.15	48.01 ± 0.17
	Acetylcholine, μmol/L	1.43 ± 0.03	1.86 ± 0.03
12	Dopamine, pg/mL	60.40 ± 0.17	60.84 ± 0.15
	Acetylcholine, μmol/L	3.20 ± 0.02	3.60 ± 0.02

step was to identify – accordingly with their signatures, dopamine and acetylcholine on the diagram; this step was followed by the assay of $time_{on}$ value and use it for the determination of the concentrations of dopamine and acetylcholine. Recovery tests of dopamine and acetylcholine in whole blood samples were performed by the addition of a known concentration of the two neurotransmitters in whole blood samples, after measuring the initial concentrations of dopamine and acetylcholine. For the sensor where the Maltodextrin I was used as modifier, the following values for recovery were recorded: dopamine: 98.97 ± 0.12%; and for acetylcholine: 98.22 ± 0.02%, while for the sensor based on Maltodextrin II, the values for recovery obtained were: for dopamine: 99.03 ± 0.09%, and for acetylcholine: 98.98 ± 0.02%.

The results obtained for the assay of dopamine and acetylcholine in whole blood samples are shown in Table 2. Paired *t*-tests at 99.00% confidence level were performed for the results obtained for the assay of dopamine and acetylcholine in whole blood samples (Table 2). The values for pair-*t* test at the 99.00% confidence level were 2.160 for dopamine, and 2.765 for acetylcholine. Both values were less than the tabulated theoretical value: 4.032. Accordingly, there is no statistically significant difference between the results obtained using the proposed sensors at 99.00% confidence level for the assay of dopamine and acetylcholine in whole blood samples.

Compared with the other methods of analysis, the proposed method is more reliable: both qualitative and quantitative analysis can be performed, it is inexpensive and fast and can be used for multiple analyte detection in whole blood.

Conclusion

Two stochastic sensors based on maltodextrins with different dextrose equivalents and Ag film deposited on polymeric textile were designed, characterized, and used for the

determination of dopamine and acetylcholine. The best of the two proposed stochastic sensors was the one based on Maltodextrin II, due to its high sensitivity and its lower determination limit; regarding selectivity and recovery, both produced comparable results. The sensors can be used for the monitoring of patients prone to develop neurodegenerative disorders.

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