

A POTENTIOMETRIC SENSOR BASED ON PROTOPORPHYRIN IX FOR THE ASSAY OF ACETYLCHOLINE

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A new potentiometric sensor based on protoporphyrin IX was developed and used for the assay of acetylcholine. The matrix used for the design of the sensor was graphite powder. The limit of determination obtained for acetylcholine using this sensor was 1×10^{-8} mol/L. The sensor based on protoporphyrin IX was reliably used for the analysis of acetylcholine in whole blood samples.

Keywords: acetylcholine, potentiometric sensor, biomedical analysis

1. Introduction

Acetylcholine is the first discovered neurotransmitter (by Henry Hallett Dale in 1915), and one the oldest neurotransmitters that occurred in the animal kingdom [1]. This neurotransmitter is involved in muscular contraction. It is also responsible for the functioning of the electric organs in some fish, like the electric eel (*Electrophorus electricus*) [2], and rays (Torpediniforms) [3]. The electric organs are made up of modified muscle or nerve cells, which became specialized in producing bioelectric fields stronger than those that normal nerves or muscles produced. It is also the main neurotransmitter in the autonomous nervous system and facilitates neuronal connections in the somatic nervous system, at the central, as well as the peripheral level. It is the only neurotransmitter which mediates somatic muscle contraction [1]. Acetylcholine changes the cell polarity by acting on specifying receptors, eventually opening ligand-gated channels.

The most common acetylcholine receptors are nicotinic and muscarinic receptors. Nicotine and muscarine are their agonists. Nicotinic receptors, present

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at the neuromuscular junctions, autonomic ganglia and in the central nervous system are ionotropic (permeable to ions like calcium, sodium, potassium). Muscarinic receptors are metabotropic, they stimulate a second messenger, but eventually open ion channels. They also appear in the peripheral and central nervous system. Although acetylcholine is essential 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 such as learning, memory, decision making, planning etc. The cholinergic mediation is altered in most psychiatric disorders.

In Alzheimer's disease, the only available effective treatment, capable of delaying the onset of full dementia for a few years, stimulates the cholinergic system via the acetylcholine (nicotinic) receptors or the enzymes involved in acetylcholine degradation (acetylcholinesterase). The treatment for myasthenia gravis, an autoimmune disorder in which the motor end plates are destroyed, is based upon a similar mechanism.

The analysis of acetylcholine is very important for clinical investigations and related research fields. Chromatography methods were applied for a long period of time [4-6], but more applications for the assay of this neurotransmitter are based on electrochemical sensors and biosensors [7-9], because these are cheaper tools and the time of analysis is short.

A potentiometric sensor based on graphite powder modified with a solution of protoporphyrin IX was developed and used for the analysis of acetylcholine in whole blood samples.

2. Experimental

2.1. Reagents

Acetylcholine (ACh), dopamine (DA), epinephrine (Epi), norepinephrine (NE), serotonin (5-HT), graphite powder (1-2 micron), protoporphyrin IX (PIX), tetrahydrofuran (THF), monosodium phosphate, and disodium phosphate were purchased from Sigma-Aldrich. Paraffin oil was supplied by Fluka. All solutions were buffered with a phosphate buffer solution pH=7.41. A stock solution (1×10^{-3} mol/L) of ACh was prepared by dissolving the necessary amount of ACh in buffer solution. The standard solutions between 1×10^{-10} - 1×10^{-3} mol/L were prepared from the stock solution (10^{-3} mol/L ACh), by serial dilutions. All solutions were freshly prepared before measurements.

2.2. Apparatus

A potentiostat/galvanostat PGSTAT 302N was used for all potentiometric measurements. An Ag/AgCl (0.1 mol/L KCl) electrode served as the reference electrode in the cell.

2.3. Sensor design

Protoporphyrin IX (PIX) was used as electroactive material in the design of the sensor. The matrix selected was graphite powder, in order to obtain a reliable design for the potentiometric sensor.

Paraffin oil and graphite powder was mixed in a ratio of 1:4 (w/w), followed by the addition of the electroactive material solution (10^{-3} mol/L in THF). 100 μ L of electroactive material solution were added to 100 mg graphite paste. The modified paste was placed into a plastic tube. The diameter of the active surface of the electrode was 300 μ m. Electric contact was obtained by inserting an silver wire. The surface of the sensor was wetted with deionised water and polished with alumina paper (polishing strips 30144-001, Orion), before use. When not in use, the electrode was stored in a dry state at room temperature.

2.4. Procedure

Direct potentiometry was used for the measurements of the potential of each standard solution (10^{-10} – 10^{-3} mol/L). The electrode was placed in the standard solution, while the potential was recorded, and graphs of E(mV) versus pACh, (pACh = $-\lg[C_{\text{ACh}}]$) were plotted. The unknown concentrations were determined from the calibration graph.

2.5. Sample preparation for whole blood

Whole blood samples were taken from different patients, according with the Ethics Committee Approval no. 11/2013, obtained from the University of Medicine and Pharmacy "Carol Davila", Bucharest. The apparatus cell was filled with the whole blood and the potential developed were measured. The unknown concentrations were determined according with the procedure described above.

3. Results and discussion

3.1. Potentiometric sensor response

The sensor based on graphite paste modified with PIX was tested using direct potentiometric method for the assay of ACh. The sensor showed a linear and near-Nernstian response (58.87mV/decade of concentration) for a concentration range between 10^{-8} – 10^{-6} mol/L ACh. Table 1 shows the response characteristics of the sensor.

Table 1

Response characteristics of the potentiometric sensor used for the analysis of ACh.

Potentiometric sensor based on	E* (mV)	Slope (mV/decade of concentration)	Working concentration range (mol/L)	Limit of detection (mol/L)
PIX/graphite	-250.85	58.87	10^{-8} – 10^{-6}	5×10^{-9}

The response time was 2 minutes for the concentrations within the linear concentration range.

3.2. Selectivity of the proposed sensor

Dopamine (DA), epinephrine (Epi), norepinephrine (NE) and serotonin (5-HT) were considered for the selectivity tests of the sensor proposed for the assay of ACh. The mixed solution method was applied for the determination of the potentiometric selectivity coefficients. The ratio between the analyte and interferent was 1:10 (mol:mol) in the mixed solution.

Potentiometric selectivity coefficients were calculated using the equation [10]:

$$K_{pot} = (10^{\frac{\Delta E}{S}} - 1) \times \frac{C_i}{C_j^{z_i/z_j}} \quad (1)$$

where ΔE is the difference between the potential recorded for the mixed solution ($E_{i,j}$) and for the solution that contains only the main ion (E_i), $\Delta E = E_{i,j} - E_i$ (all recorded in mV); S is the slope of the electrode deduced from the equation of calibration (mV/ decade of concentration); C_i is the concentration of the main ion i , C_j is the concentration of the interfering species j ; z_i is the charge of the main ion i , z_j is the charge of the interfering species j .

Results for the potentiometric selectivity coefficients are shown in Table 2. The values obtained for the potentiometric selectivity coefficients presented in Table 2 have shown that DA and 5HT slightly interfere with the assay of ACh, while no interference is expected from Epi and NE.

Table 2

Potentiometric selectivity coefficients for the potentiometric sensor designed for the assay of ACh.

Interferent	K_{sel}^{pot}
DA	6.1×10^{-3}
Epi	7.8×10^{-4}
NE	9.3×10^{-4}
5-HT	3.3×10^{-3}

3.3. Analytical applications

The proposed electrode was used for the assay of ACh in real whole blood samples. The recovery test was performed for the assay of ACh in whole blood samples by spiking the samples with different quantities of ACh. The results have shown a recovery of 93.40% with an RSD(%) less than 1.00%.

(Table 3)

Sample nr.	Determination of Ach in whole blood samples	
	Ach (nmol/L)	
	Potentiometric sensor	Standard method
1	32.60	31.46
2	38.80	38.68
3	63.70	62.94
4	57.60	58.00

Four samples of whole blood were used for the assay of ACh. The results obtained (Table 3) are in good agreement with those obtained using the standard method of analysis performed in the accredited clinical laboratory.

4. Conclusions

A new potentiometric sensor based on protoporphyrin IX was designed by its physical immobilization on graphite paste. The sensor presented a near-Nernstian response on the linear concentration range between 10^{-8} and 10^{-6} mol/L. Its selectivity was tested versus other neurotransmitters like dopamine, epinephrine, norepinephrine, and serotonin. Dopamine and serotonin were slightly interfering, while epinephrine and norepinephrine did not interfere in the measurements. The acetylcholine was reliably assayed from whole blood samples with recoveries higher than 93.00%.

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