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A nonenzymatic Biosensor Based on Copper Nanoparticles Modified Electrode for Detection of Acetylcholine

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Abstract— **The electrocatalytic oxidation of acetylcholine (Ach) on copper microparticles modified carbon paste electrode (m-CPE) and copper nanoparticles-modified carbon paste electrode (n-CPE) was investigated. In the voltammogram recorded using m-CPE, a single anodic peak related to the oxidation of ACh was observed. Using n-CPE, however, two overlapped anodic peaks were observed. The amino acids oxidized on n-CPE at higher rates at lower potentials compared to m-CPE. A nonenzymatic amperometric biosensor was constructed and a simple analysis procedure was developed for the determination of ACh.**

I. Introduction

ACETYLCHOLINE (ACh) synthesized in nerve cells from choline (Ch) and acetyl coenzyme-A by choline acetyltransferase, plays an important role in the cholinergic synaptic neurotransmission. ACh and its metabolite Ch have received increased attention in the past decades since their brain levels seem related to various neural disorders such as Alzheimer's disease, progressive dementia [1-4] and schizophrenia [5,6].

Nanostuctured particles have attracted extensive scientific and industrial interest due to their unique electronic, optical, and catalytic properties [7-11]. Nanoparticles can display four unique advantages over macroelectrodes when used for electroanalysis: enhancement of mass transport, catalysis, high effective surface area and control over electrode microenvironment. [12]

Since 1990s, copper nanoparticles have attracted much attention of scientists. The primary application of copper nanoparticles is in catalysis, just like the bulk copper metal [13]. On the other hand, copper nanoparticles offer higher catalytic efficiency per gram than the bulk one due to their large surface-to volume ratios [14].

The electrochemical methods using chemically modified electrodes (CMEs) have been widely used as sensitive and selective analytical methods for the biosensing of the trace amounts of biologically important compounds [15-17] One of the most important properties of CMEs has been their ability to catalyze the electrode process via significant decreasing of overpotential respect to unmodified electrode. With respect to relatively selective interaction of the electron mediator with the target analyte in a coordination fashion, these electrodes are capable to considerably enhance the selectivity in the electroanalytical methods.

In the present work, nano-sized copper particles were used to prepare an enzyme-less nanobiosensor for the detection of acetylcholine by the amperometric method.

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II. Experimental

Electrochemical studies were carried out in a conventional glass cell incorporating three-electrode configuration containing 100 mM sodium hydroxide as running electrolyte, powered by an Autolab electrochemical analyzer (Eco-chemie, Utrecht, The Netherlands) equipped with a PGSTAT 30 potentiostat. The system was run by a PC through the FRA and GPES 4.9 softwares. An Ag/AgCl and a platinum plate were used as reference and counter electrodes, respectively.

Surface morphological studies were carried out using scanning electron microscopy (SEM), using a Philips instrument, Model X-30. The transmission electron microscopy (TEM) was performed using a CEM 902A ZEISS transmission electron microscope, with an accelerating voltage of 80 kV was used to obtain information about the morphology and size of particles.

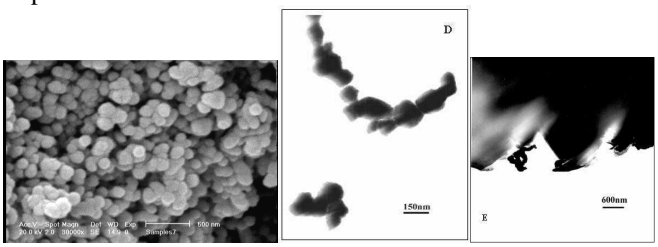
Copper nanoparticles were obtained from Aldrich and copper micro particles with the diameter of <63 μm were obtained from Merck.

The copper microparticles and copper nanoparticles modified carbon paste electrodes (denoted as m-CPE and n-CPE, respectively) were prepared by mixing carbon powder together with copper micro or nanoparticles at different ratios in an agate mortar until a uniform paste was obtained. The percentage (20% w/w) of copper informed throughout the text corresponds to the final percentage relative to the total paste composition.

III. Results and Discussion

Fig. 1A shows the scanning electron micrographs (SEM) of copper nanoparticles, 1B represents TEM images of copper nanoparticles and 1C shows TEM image for copper

nanoparticles-carbon microparticles mixture. Spherical nanoparticles were clearly observed for copper particles with an average size of 80 nm which some particles aggregations were observed. TEM images indicate that copper nanoparticles attached very well to the carbon microparticles to produce a blend.



(A) (B) (C)

Fig. 1. A) SEM of copper nanoparticles, B) TEM of copper nanoparticles and C) TEM of copper-carbon mixture.

Figure 2 shows the cyclic voltammograms of 9.1 mM ACh in 100 mM NaOH solution obtained using m-CPE and n-CPE in the potential range of 100 to 900 mV. ACh represents very weak oxidation signal on unmodified carbon paste electrode (u-CPE) (Figure 2, inset A). ACh oxidized on m-CPE and produces one anodic peak located at 700 mV. It is also oxidized on the n-CPE surface, however, via two overlapped anodic peaks located at around 620 and 750 mV.

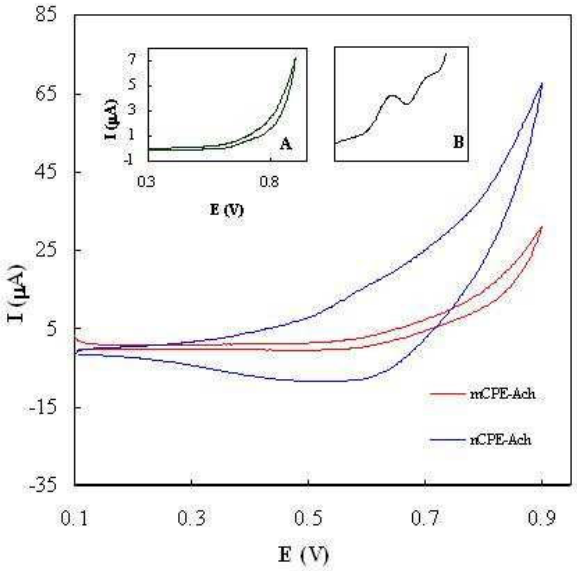


Fig. 2. Cyclic voltammograms at the surface of m-CPE and n-CPE in absence and presence of 9.1 mM Ach. A) Cyclic voltammograms at the surface of u-CPE in absence and presence Ach. B) Derivative voltammogram of the electrocatalytic oxidation of Ach at the surface of n-CPE

The anodic charge passed just before the onset of oxidation wall of the voltammogram in the absence of Ach was related to the Cu(II)/Cu(III) couple [18-21]. The electrochemical processes which occurred at the anodic edge of the voltammogram can expressed as:

$$Cu(OH)_2 \rightarrow Cu(III) \qquad (5)$$

As for the nature of Cu(III) entities, species ranging from copper oxy-hydroxide, CuOOH, to Cu(III) radical depicted as CuOO•H have been proposed [22,23]. It is well known that copper can oxidized the organic as well as biological compounds by chemical reaction between those substrates and Cu(III) active species via a redox mediation electron transfer process at the anodic edge of the voltammograms in alkaline solutions [22-30]. Based on this explanation, it can be deduced that the electrooxidation of ACh on investigated copper-based electrodes occurred via the active Cu(III) species. In the voltammogram of ACh solution using n-CPE (Figure 2, blue curve) two anodic overlapped peaks appeared in the potential range of Cu(III) generation, following by enormous decreasing the cathodic peak of Cu(III) reduction in the reverse sweep. This indicates that ACh oxidized electrocatalytically in two steps on n-CPE with respect to m-CPE. The fine tunable steps of electrocatalytic oxidation of ACh on nanoparticles of copper can be better visualized when the derivative voltammogram is looked at, as shown in the inset B of Figure 3. From the data presented in Figure 2, it is also witnessed that the onset potential of ACh elelctrooxidation on n-CPE shifts in negative direction with respect to the case of m-CPE. Nanoparticles of copper caused the oxidation process to be occurred at thermodynamically favorable potentials and also to increase the current, i.e. kinetically enhanced the reaction rate.

Oxidation of the ACh at thermodynamically favourable potentials with higher catalytic rates can be related to nanometer dimensions of nanoparticles of copper which are stably distributed on the carbon surface which is fully and easily accessible for the substrates, and consequently can be readily and completely used as an electrochemical sensing unit. Moreover, nanoparticles are often irregularly shaped objects, and hence there are some defect sites, such as steps that separate planar atomic terraces, or kinks where a step advances or recedes, exposing corner or edge atoms on a plane.

In order to develop an enzyme-less biosensor for the analysis of ACh, amperometry technique was employed. Typical amperometric signals obtained during successive increments of ACh to the solution using n-CPE are depicted in Figure 3. Similar amperograms recorded using m-CPE and is also represented in Figure 3. Gentle stirring for a few seconds was needed to promote solution homogenization after each injection. The electrode response is quite rapid and proportional to the ACh concentration with the highest sensitivity using n-CPE. The determined parameters for calibration curves of Ach, LOD and LOQ are obtained 3.9×10^{-5} and 1.3×10^{-4} respectivly and the slope of calibration curves as were obtained.

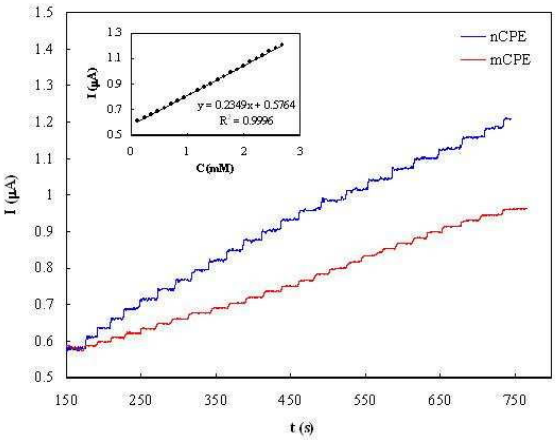


Fig. 3. Current signal as a function of time in 100 mM NaOH solution during repetitive injections of Ach at the surface of n-CPE and m-CPE. E=640 mV. Inset: Dependency of the peak current on Ach concentration.

I. Conclusion

A carbon paste electrode modified with copper nanoparticles was employed for the investigation of the electrocatalytic oxidation and determination of Ach without enzyme. The Ach oxidized on copper-based electrodes via mediation of Cu(III) active species. In the course of electrooxidation of the Ach in the cyclic voltammograms two anodic diffusion-controlled peaks appeared when a blend of copper nanoparticles/carbon powder was used, while other micro structured electrode exhibit only one anodic peak. The appearance of the two anodic peaks at lower potentials with higher corresponding currents with respect to the micro structured copper was related to the two steps of electrooxidation of the Ach in a manner that the nanoparticles of copper can resolve the two fine-steps of the electrooxidation process. An amperometric procedure was successfully applied using the nanoparticles for the biosnsing of Ach with a high sensitivity.

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