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Preparation of Ag/NaA zeolite modified carbon paste electrode as a DNA biosensor

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ABSTRACT

In this research, we reported a method for synthesis of NaA zeolite. NaA zeolite has been successfully prepared within 30 min by microwave heating without using any conventional hydrothermal treatment and due to the exchange process the silver-loaded NaA microsized zeolites was produced. The synthesized zeolite was characterized using X-ray diffraction, scanning electronic microscopy and FT-IR techniques. The modified carbon paste electrode was prepared by incorporation of Ag(I) zeolite in the carbon paste matrix. Differential pulse voltammetry (DPV) was employed for development of electrochemical DNA hybridization biosensors based on Ag/NaA zeolite modified carbon paste electrode (Ag/ZMCPE) and methylene blue (MB) as electroactive label was used. The sensors rely on immobilization of a 15-mer single stranded oligonucleotide probe for detection of target DNA, as a model. The hybridization event was evaluated by DPV. Moreover, Ag/ZMCPE showed better advantages than NaA zeolite modified carbon paste electrode (ZMCPE). Under optimized experimental conditions, limit of detection was calculated 4.00×10^{-12} M.

1. Introduction

Zeolites are an important group of crystalline aluminosilicates currently available for various fields of applications. These minerals are widely used as sorbents, ion exchangers, catalysts and catalyst supports [1,2]. The catalytic nature and activity, and other properties of zeolites can be greatly improved by cation exchange [3]. Zeolite-modified electrodes (ZMEs) form a subcategory of the so-called “chemically modified electrodes” (CMEs), which were largely studied and promoted by Murray and coworkers [4,5]. ZME can be exploited as electrochemical sensors in relation with zeolite’s properties, e.g., ion exchange capacity, molecular selectivity, and catalyst-assisted reactivity. Moreover, metal ion-doped zeolites allow exploitation of ion-exchange capacity of zeolite for the development of electrochemical sensors for the sensing non-electroactive inorganic or organic species [6]. In recent years, there has been considerable interest in developing electrochemical DNA biosensors for the rapid and inexpensive diagnosis of genetic diseases, forensic analysis and other applications [7,8]. DNA biosensors consist of a biological recognition layer, usually single stranded DNA and a transducer converting the recognition event into a measurable signal. Optical, piezoelectric or electrochemical instruments are often used in DNA biosensors as transducers [9–11]. Electrochemical methods, in particular, provide sensitive, cost effective and rapid way of analysis [12,13]. The detection is accomplished by immobilization of single stranded DNA onto electrode surface and hybridization of a target DNA sequence present in the sample. The method is very efficient and specific, because DNA sensor can detect an analyte even in the presence of a mixture of many different nucleic acid fragments [14]. Methylene blue (MB) has been widely used as an electroactive label to monitor the DNA hybridization reaction [15–20] because less MB can bind to double strand DNA (dsDNA) compared to single strand DNA (ssDNA) [18]. The single mismatches in oligonucleotides were investigated by Kelly et al. [21] based on charge transport from the intercalated MB through self-assembled oligonucleotide. Erdem et al. studied the interaction between MB and ssDNA and dsDNA immobilized on gold electrode [22,23] and on carbon paste electrode [16–18]. The electrochemical detection of hybridization based on peptide nucleic acid probes with MB on carbon paste and modified gold electrode were also reported by Erdem group [24,25]. This article reports method for synthesizing NaA zeolite, studies of the electrochemical behavior of Ag(I) incorporated in zeolite-modified carbon paste electrode and the application of the modified electrode for electrochemical DNA biosensor.

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2. Experimental methods

2.1. Regents and material

The NaA zeolite was synthesized in our laboratory. Sodium aluminate was from Fison. Graphite powder, sodium metasilicate pentahydrate, silver nitrate were purchased from Fluka. Sodium hydroxide used in this work was also purchased from Merck. All reagents were of analytical grade and used as received without further purification. In this work a 15-mer oligonucleotide as the probe and its complementary as the target nucleotide were used. Some oligonucleotides were utilized as non-complementary oligonucleotides. All oligonucleotides were synthesized by MW G-BIOTECH Company (Germany). The sequences of these oligonucleotides are as below:

Probe DNA	5′-TGG GGA TGC AGA ACT-3′
Complementary DNA	5′-AGT TCT GCA TCC CCA-3′
Non-complementary DNAs	
NC1:	5′-GTT ACT GTT GTA GAT ACT AC-3′
NC2	5′-GTA GTA TCT ACC ACA GTA AC-3′

Stock solution of the oligonucleotides (100 μM) were prepared with TE buffer solution (10 mM Tris–HCl, 1 mM EDTA, pH 8.00) and kept frozen. More diluted solutions of the oligonucleotides were prepared using 0.50 M acetate buffer solution (pH 4.80) containing 20 mM NaCl. Other chemicals were of analytical reagent grade. Distilled, deionized and sterilized water was used in all solution preparation. Each measurement consisted of immobilization of probe and detection of target DNA (immobilization/detection cycle) carried out on a fresh ZMCPE and Ag/ZMCPE surface. All the experiments were performed at room temperature in an electrochemical cell.

2.2. Instrumentation

The X-ray diffractograms of zeolitic sample was measured using an X-ray diffractometer (XRD, GBC MMA Instrument, $\lambda = 1.5418$ Å and 28 mA). FT-IR spectrum was recorded at room temperature using FT-IR spectrometer (Vector 22-Bruker), in the range of 450–2000 cm^{-1}. Scanning electron microscopy (SEM) was done on selected samples to determine the crystallite size and morphology using a JEOL JXA-840 SEM. Electrochemical experiments were performed using AUTOLAB PGSTAT 30 electrochemical analysis system and GPES 4.9 software package (Eco Chemie, The Netherlands). The utilized three electrode system was composed of a ZMCPE and Ag/ZMCPE (surface area of 0.015 cm^2) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode for DPV and a platinum wire as the auxiliary electrode.

3. Procedure

3.1. Synthesis and characterization of NaA zeolite

Sodium aluminate and sodium metasilicate pentahydrate were used as Al and Si sources, respectively, the sodium source were NaOH pellets. A sodium hydroxide solution was prepared which split into two portions prior to any other experiments. In the first step, a suitable amounts of sodium aluminate ($NaAlO_2$, 61 wt% of Al_2O_3, Merck) was added and stirred until clear. To the second half of the sodium hydroxide solution, suitable amounts of sodium metasilicate pentahydrate (SiO_2 28%, Na_2O 29%, H_2O 43%) was added. The dissolution of the sodium metasilicate was very slow at room temperature; therefore, heating assistance was applied to the sample at 70 °C for 1 h which is then filtered. The two clear solutions were then recombined and the resulting white gel was shaken until homogeneous. The synthesis of zeolite NaA was attempted under only microwave heating [26]. A suitable composition of gels was employed for microwave heating at 30 and 60 min. The resulting white powder products were filtered and rinsed with distilled water before drying at 60 °C for overnight. The XRD pattern is shown in Fig. 1A.

Fig. 1A shows that the crystallization of NaA zeolite was completed in 30 min microwave irradiation and with increasing microwave irradiation time to 60 min; it displayed a phase transformation of zeolite NaA into sodalite.

All the characteristic reference IR bands of the zeolite NaA with 30 min microwave irradiation are observed for the synthesized NaA (Fig. 1B). The reference IR wave numbers were given as 1002, 672, 557 and 464 cm^{-1} for NaA zeolite [27,28]. The broad band at about 3446 cm^{-1} and band at about 1652 cm^{-1} are attributed to zeolitic water, whereas the band at about 1002 cm^{-1} is due to asymmetrical vibrations related between tetrahedral.

SEM micrograph of the synthesized zeolite sample with 30 min microwave irradiation is illustrated in Fig. 1C, which the formation of cubical crystallites with the smaller size of zeolite crystals. This is most likely due to the accelerated rate of heating and gel dissolution, which means the gel, is consumed quickly, forming more nuclei from which crystals grow. As limited reagents are left after the nuclei formation period, they produce many smaller crystals. The fact that the gel is heated for a shorter time also means that the time for crystal formation is not enough. Results showed that this method is a faster, simpler, higher purity and very energy efficient method.

3.2. Preparation of the working electrode

For preparing the ionic silver–zeolite sample containing 33 wt% silver (Ag–NaA-33), 2 g of NaA zeolite was washed by distilled water and then stirred for 6 h with 160 mL $AgNO_3$ (0.05 M) at 35 °C. To have a completely ion-exchanged silver–zeolite, the samples were again mixed with fresh $AgNO_3$ solution at the same conditions.

Finally, the Ag/NaA zeolite particles were filtered and then dried at 50 °C in an oven. All the steps were carried out in the absence of direct light.

SEM micrograph of Ag/NaA zeolite particles is showed in Fig. 1D. The silver content of ion-exchanged samples was determined by EDS. The sodium elements were detected in minor amount by EDS in Ag^+/NaA samples, indicating the complete ion exchange of NaA zeolite (Fig. 1E).

The ZMCPE (zeolite modified carbon paste electrode) and Ag/ZMCPE (Ag/NaA zeolite modified carbon paste electrode) was prepared by mixing NaA-zeolite and Ag/NaA-zeolite and high viscosity paraffin (density = 0.88 g cm^{-3}) from Fluka in a ratio of 5:95%, 15:85%, 30:70% (w/w) of NaA zeolite and Ag/NaA zeolite to graphite powder in a mortar.

Fig. 2 shows SEM and EDS of ZMCPE and Ag/ZMCPE in a ratio of 30:70% (w/w) NaA zeolite and Ag/NaA zeolite to graphite powder. As seen in this figure, zeolite particles were dispersed into the carbon paste very well.

A portion of the resulting paste was then inserted in the bottom of a glass tube. The electrical connection was implemented by a copper wire lead fitted into the glass tube. The surface of the resulting paste electrodes were smoothed on a weighing paper and rinsed carefully with distilled water.

3.3. Electrochemical activation of the Ag/ZMCPE and ZMCPE

The polished electrode was pretreated at optimized potential of 1.80 V vs. SCE for 5 min for electrochemical activation of electrode