An Ultrasensitive Electrochemical DNA Biosensor Based on Carboxylated Multi-walled Carbon Nanotube/Molybdenum Disulfide Composites for KRAS Gene Detection

Xiaojing WANG,* Mei YANG,*† Qingyan LIU,* Siyi YANG,* Xintong GENG,* Yixia YANG,*
Huanbao FA,** Yongzhong WANG,* and Changjun HOU*

*Key Laboratory of Biorheological Science and Technology, Ministry of Education, College of Bioengineering, Chongqing University, Chongqing 400044, P. R. China

**College of Chemistry and Chemical Engineering, Chongqing University, Chongqing 400044, P. R. China

In this paper, an ultrasensitive electrochemical biosensor based on carboxylated multi-walled carbon nanotube/molybdenum disulfide composites (MWCNTs-COOH/MoS₂) for the detection of KRAS gene is described. An easy, low-cost method, named one-step hydrothermal, was used for the synthesis of MWCNTs-COOH/MoS₂ nanocomposites, and scanning electronic microscopy (SEM), high resolution transmission electron microscopy (HRTEM), Fourier transform infrared spectroscopy (FT-IR), X-ray photoelectron spectroscopy (XPS) and X-ray diffraction (XRD) were used for characterizing the prepared composites. Furthermore, cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were employed for an electrochemical performance study of this biosensor. Under optimal conditions, the detection limit of target DNA achieved down to 3 fM ($S/N = 3$) with high sensitivity; the linear range with the logarithm of the concentrations of target DNA varied from 1.0×10^{-14} to 1.0×10^{-7} M. Finally, the practicality of our proposed sensor was verified by a determination of the KRAS gene in human serum samples with good accuracy and high precision due to the excellent conductivity and large active surface area of the MWCNTs-COOH/MoS₂ nanocomposites. This proposed biosensor thus provides a practical method for the rapid and sensitive analysis of gene detection.

Keywords KRAS gene, carboxylated multi-walled carbon nanotube/molybdenum disulfide composites, electrochemical DNA biosensor

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Introduction

Methods of cancer screening, where the detection of specific oligonucleotide sequences plays a great important role, has drawn attention of researchers from various fields. Different analytical technologies have been developed, such as PCR,¹⁻³ next generation sequencing (NGS),⁴⁻⁶ HPLC⁷ and so on. However, these technologies rely on professional skills and high cost. In order to find a more convenient method for oligonucleotide sequence detection, researchers are combining the advantages of complementary base pairing of gene with other fields, such as electrochemiluminescence,⁸ electrochemistry,^{9,10} chemiluminescence,¹¹ fluorescence¹²⁻¹⁵ and nanotechnology.¹⁶ Herein, electrochemical DNA biosensors have received much interest concerning the property that they can provide a simpler and more cost-effective platform that is highly sensitive for DNA detection.

To enhance the detection performance of an electrochemical DNA or RNA biosensor, different kinds of signal amplification strategies have been introduced to greatly improve the sensitivity and stability of novel biosensors; for example, nanomaterials

have been designed to provide a promising sensing platform, such as carbon nanotubes (CNTs), 3D graphene, metal nanoparticles, metal oxide nanoparticles.¹⁷⁻²⁰ Moreover, two-dimensional (2D) layered materials,²¹ which we are already familiar with, such as tungsten disulfide (WS₂) and molybdenum disulfide (MoS₂), are popular due to the advantage of photocatalysis, electrocatalysis, electronic and electrochemical properties. MoS₂ is one kind of transition metal sulfides and is now becoming an attractively hot material owing to its remarkable optical properties and excellent electrical performance, which are derived from the unique stacking S-Mo-S structure through weak van der Waals interactions.^{22,23} To improve the poor conductivity of MoS₂,²⁴⁻²⁶ the combination of MoS₂ and other materials, like graphene, MWCNTs, AuNPs, ZnO, polyaniline (PANI), quantum dots (QDs), effectively broadens the properties of MoS₂.²⁷⁻³³ By using Au nanoparticles and hollow MoS₂ microcubes, a binding biosensor for miRNA-21 superior detection was fabricated, along with another signal amplification strategy, the detection limit reached to 0.086 fM ($S/N = 3$).³⁴ To investigate the DNA hybridization process, an ultrasensitive surface plasmon resonance (SPR) biosensor was investigated.³⁵ The novel application that combined MoS₂ with Au nanoparticles and graphene greatly increased the sensitivity and accuracy of the SPR biosensor. Therefore, we can see the great potential of the MoS₂

† To whom correspondence should be addressed.
E-mail: yangmei@cqu.edu.cn

Scheme 1 Schematic illustration of the strategy for KRAS gene detection with proposed biosensor.

nanocomposite in electrochemical detection.

It has been reported that anti-EGFR agents could not obtain the desired antitumor effects in chemotherapy for some part of patients suffering from non-small cell lung cancer (NSCLC). The reason is mutation of the KRAS gene, an important downstream molecule in the EGFR signal transduction pathway. Thus KRAS screening is greatly critical to determine if a patient's tumors will or not react to monoclonal antibody inhibitors of EGFR, and help the doctor to timely change treatment programs.³⁶ Sanger sequencing, the NGS-based method, PCR and high-resolution melting analysis (HRM) have been used for KRAS gene detection, but it is limited due to the high cost and being time-consuming.^{37–42} Through the application of the toehold-mediated strand displacement reaction (TSDR) and isothermal DNA amplification, a fluorescent biosensor of the KRAS gene was developed, and reached a relatively low detection limit of 1.8 pM among other fluorescent biosensors, but is not sensitive enough for gene detection or comparable to electrochemical biosensor.¹² Electrochemical biosensors using enzyme capped gold nanoparticles for signal amplification were fabricated for KRAS gene detection,⁴³ and achieved a very high sensitivity of 0.035 fM. However, the activity of the enzyme is susceptible to environmental influences, which might limit the development of field-deployed detection.

In the present work, we designed an ultrasensitive electrochemical biosensor that is based on carboxylated multi-walled carbon nanotube/molybdenum disulfide composites (MWCNTs-COOH/MoS₂) for detecting the KRAS gene. The procedure for target detection is presented in Scheme 1. Firstly, a MWCNTs-COOH/MoS₂ nanocomposite was prepared by the one-pot hydrothermal method. Secondly, a MWCNTs-COOH/MoS₂ nanocomposite was added on the prepared GCE electrode to immobilize the amino-modified capture probe through the amide reaction. In this step, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) played an indispensable role, EDC formed amide bonds between carboxyl of MWCNTs-COOH and the amino groups of capture probes, while NHS was used to improve the stability of EDC. With the amide reaction, the capture probe was tightly covalent to the surface of the electrode, and then the DNA/MWCNTs-COOH/MoS₂ decorated electrode was incubated into Tris-HCl buffer containing various concentrations of target DNA, after hybridization between the capture probe and the target probe.

Table 1 Synthetic oligonucleotide sequences used in this study

Nucleotide name	Sequence (5' → 3')
Capture probe	NH ₂ -(CH ₂) ₆ -TCAACCTCGACC-ACCGCATCCGTT
Target DNA	AACGGATGCGGTGGTCGAG-GTTGA
MT-1 (Single-base mismatch)	AACGGATGCGGTGTTTCGAG-GTTGA
MT-3 (Three-base mismatch)	AACGGAAGCGGTGTTTCGAG-CTTGA
NCS (Non-complementary sequence)	CCTAATCTGACTTAGCGGGT-TCCC

Methylene blue (MB) was used as an electrochemical detection indicator that can bind with DNA strands through the electrostatic interaction. Finally, we tested the practical ability of this biosensor in human serum, and obtained a desirable result. Altogether, the designed biosensor showed wonderful sensitivity and good selectivity, suggesting that this MWCNTs-COOH/MoS₂ nanocomposite enhanced biosensor has a promising application in molecular detection.

Experimental

Reagents and materials

All oligonucleotide sequences were synthesized and purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China); the oligonucleotide sequences are listed in Table 1. The methylene blue (MB), Tris-HCl, and ethylene-diaminetetraacetic acid disodium salt (Na₂EDTA), *N*-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from Sigma-Aldrich (St. Louis, MO). Potassium ferricyanide (K₃Fe(CN)₆) and potassium ferrocyanide (K₄Fe(CN)₆) were provided by Sinopharm Group Chemical Reagent Co., Ltd. (Beijing, China), Normal human serum were purchased from Solarbio Tech. Co., Ltd. (Beijing, China), MWCNTs-COOH and L-cysteine were purchased from Aladdin Chemicals Co., Ltd. (Shanghai, China), Na₂MoO₄·2H₂O was purchased from Shanghai Chemical Reagent Corporation (Shanghai, China). Other reagents were purchased from Chongqing Chuan-

dong Chemical Co., Ltd. All chemicals were of analytical grade. Deionized (DI) water was produced using a Milli-Q system.

Instrumentations

All electrochemical measurements were performed on a CHI660D electrochemical workstation (Shanghai CH Instruments Co., China) with a regular three-electrode system composed of a platinum wire as an auxiliary electrode, a saturated calomel electrode (SCE) as a reference electrode and a 3-mm diameter GCE as a working electrode. Cyclic voltammetry (CV) measurements were used to record all electrochemical properties while constructing the biosensor. DPV measurements were used for all detections of the electrochemical performance of the target DNA, and performed between 0.1 and -0.7 V, along with a 0.05 V of pulse amplitude, 0.05 s of the pulse width and 0.5 s of pulse period. The morphologies of the nanocomposites were demonstrated on a LIBRA 200 transmission electron microscope (Zeiss, Germany) and a JSM-7500F scanning electron microscope (JEOL, USA). The Fourier-transform infrared spectrum (FT-IR) was recorded on a Nicolet6700 (Thermo Fisher Scientific, USA). The X-ray photoelectron spectrum (XPS) in this work was measured on an Escalab 250Xi (Thermo Fisher Scientific, USA). The X-ray diffraction (XRD) pattern here was obtained on a Bruker D8 (Bruker, Germany).

Preparation of the MWCNTs-COOH/MoS₂ nanocomposite

MWCNTs-COOH/MoS₂ nanocomposites were prepared by the one-step hydrothermal method, as reported before.⁴⁴ In order to keep the original structure of the final nanocomposites, we used a vacuum freeze-drying method to get the final material while the reported method was dried by a vacuum at 80°C for 20 h. The specific process was as follows: First, 0.017 g MWCNTs-COOH was added in 40 mL deionized water and well dispersed by ultrasonic irradiation for 20 min. Second, 0.3 g Na₂MoO₄·2H₂O was added with ultrasonic irradiation for 30 min. Then pH value was adjusted to 6.5, followed by the addition of 0.8 g L-cysteine. The mixture was hardly stirred for more than 1 h, and 40 mL deionized water was added during the stirring of mixture, then transferred into a 100-mL Teflon-lined stainless-steel autoclave and kept at 180°C for 48 h. After the hydrothermal reaction, the vessel was kept at room temperature and cooled, then opened in a ventilated kitchen, the composite was firstly collected by filtration and repeated washing with water, and then collected by centrifugation and washed with both distilled water and absolute ethanol, and then dried by vacuum freeze-drying for 24 h.

Fabrication of electrochemical DNA biosensor

The fabrication process of this biosensor is shown in Scheme 1. The GCE was polished to an eligible standard with 0.3 and 0.05 μm alumina slurries. It was then washed ultrasonically with ultrapure water and absolute alcohol three times, and dried with nitrogen. The concentration of the MWCNTs-COOH/MoS₂ nanocomposite we used was 2 mg/mL, and then 5 μL of the MWCNTs-COOH/MoS₂ nanocomposite mixture was carefully dropped on the polished GCE, and dried at room temperature. Before immobilization of the capture probe, the MWCNTs-COOH/MoS₂ composite modified electrode was incubated into a PBS buffer (pH 7.4, 0.01 M, NaCl) containing 5 mM EDC and 8 mM NHS for 45 min, and then washed with PBS buffer containing 0.1% SDS (PBS buffer I) and dried with nitrogen; 5 μL of the 1 μM capture probe was dropped on the electrode and kept at room temperature for 2 h with protection of the centrifuge tube. The purpose of this step

was to make sure the amino modified capture probe could be covalently immobilized on the electrode, and then washed with PBS buffer containing 0.1% SDS. Here the electrochemical biosensor was fabricated and stored in a refrigerator at 4°C for the following steps.

Hybridization with target ssDNA and electrochemical measurements

The DNA/MWCNTs-COOH/MoS₂ modified electrode was kept in a 20 mM Tris-HCl buffer (pH 7.4, 20 mM NaCl) containing various concentrations of the target DNA sequences at 30°C for 1 h, and then rinsed with PBS buffer I to remove any non-specifically adsorbed DNA. It was incubated in a 50 μM MB solution for 15 min and then washed smoothly with ultrapure water. Finally, the electrochemical response was operated by DPV in 0.1 M PBS (pH 7.4 0.01 M NaCl) on a CHI660D electrochemical workstation.

Detection in normal human serum

To investigate the practicality of this proposed biosensor, we tested the biosensor with normal human serum. Firstly, the serum was 30-fold diluted by Tris-HCl buffer; secondly, 900 μL diluted serum was added to 100 μL of 1.0×10^{-11} M target probe and 100 μL of 1.0×10^{-12} M target probe separately, and used as a hybridization solution for serum sample detection. The other step was completely the same as 2.5.

Results and Discussion

Characterization of the MWCNTs-COOH/MoS₂ nanocomposites

Here, SEM, HRTEM, FT-IR, XPS, XRD were introduced to verify the characteristic of the nanocomposite we prepared. Figure 1A clearly shows layer-nanoflowers of the MoS₂ which is the same as reported,⁴⁴ revealing the flower-like morphology of MoS₂. An SEM image of MWCNTs-COOH was displayed in Fig. 1B, which illustrates the nanotube of MWCNTs-COOH. The MWCNTs-COOH/MoS₂ composite is exhibited in Fig. 1C, showing the combination of sheets of MoS₂ and nanotube MWCNTs-COOH. Moreover, Figs. 1D – 1I show TEM and HRTEM images of MoS₂, MWCNTs-COOH and MWCNTs-COOH/MoS₂ composites, and further demonstrates the structure of the as-prepared materials. Figures 1D – 1E obviously show the nanosheet shape of MoS₂ and the nanotube structure of MWCNTs-COOH. Figure 1F clearly shows the good admixture and insert of MWCNTs-COOH/MoS₂ composites. As shown in Figs. 1G – 1H, the lattice spacing of MWCNTs-COOH/MoS₂ was 0.68 nm, the lattice spacing of MoS₂, and the lattice spacing of MWCNTs-COOH were 0.48 nm. According to the increase of the lattice spacing between MoS₂ spacers and the decrease of MWCNTs-COOH spacers, we could draw the conclusion that the MWCNTs-COOH was inserted and wrapped around the MoS₂.

The FT-IR spectrum (Fig. 2) was obtained between 4000 and 400 cm^{-1} . The O-H absorption peaks appearing at 3430 and 1630 cm^{-1} were due to the stretching vibration and bending vibration of the adsorbed water between the molecular layers; the bands nearby 1384 cm^{-1} appearing in all of these three samples were attributed to stretching vibrations of the C-O bonds. The absorption peaks appearing at 1120 cm^{-1} were due to the asymmetry and symmetric stretching vibration of the S=O functional group. The peaks of MWCNTs-COOH/MoS₂ which appeared at 600 cm^{-1} were assigned to Mo-S vibration, and the absorption peaks, which belong to MWCNTs-COOH/MoS₂ and MoS₂, appearing at nearby 956 cm^{-1} was derived from the stretching vibration of the Mo-O, suggesting that the

Fig. 1 SEM images of MoS₂ nanosheets (A), MWCNTs-COOH (B) and MWCNTs-COOH/MoS₂ composites (C); HRTEM images of MoS₂ nanosheets (D, G), MWCNTs-COOH (E, H) and MWCNTs-COOH/MoS₂ composites (F, I).

Fig. 2 IR spectra of MoS₂ nanosheets, MWCNTs-COOH and MWCNTs-COOH/MoS₂ composites.

Fig. 3 XRD patterns of MoS₂ nanosheets, MWCNTs-COOH and MWCNTs-COOH/MoS₂ composites.

MWCNTs-COOH/MoS₂ was well prepared.^{44,45}

All of the above results were proved to be consistent with the findings in Fig. 3, which display the XRD pattern of MoS₂, MWCNTs-COOH and MWCNTs-COOH/MoS₂ composite, respectively. Since we could see in Fig. 3, the MoS₂ accurately displayed the crystalline peaks at $2\theta = 13.65^\circ$, 33.12° , 42.84° and 56.42° corresponding to the (002), (100), (103), and (110) planes of MoS₂ (JCPDS No. 37-1492), respectively.⁴⁶ For MWCNTs-COOH, the typical diffraction peaks that appeared at $2\theta = 25.91^\circ$ and 43.82° were considered to be (002) and (100) reflections of the hexagonal graphite structure, revealing the

high electrical conductivity of MWCNTs-COOH.⁴⁷ For the MWCNTs-COOH/MoS₂ composites, the typical diffraction peaks assigned to the (002) plane of MoS₂ were absent, showing that the MoS₂ of the composites was single-layer. Other diffraction peaks of MoS₂ were rather weak because the MWCNTs-COOH was inserted in the composite and the flower like MoS₂ transformed into less layer structure. The major diffraction peaks of MWCNT were weaker, because MoS₂ nanosheets reduced the stacking of MWCNTs-COOH, while the insert of MWCNTs-COOH between MoS₂ nanosheets induced a larger layer-space of the MoS₂ nanosheets attributed to the

Fig. 4 XPS survey spectra of MWCNTs-COOH/MoS₂: overall survey MWCNTs-COOH/MoS₂ (A); high-resolution Mo 3d XPS spectra of MWCNTs-COOH/MoS₂ (B); high-resolution S 2s XPS spectra of MWCNTs-COOH/MoS₂ (C).

diffraction peaks appearing at $2\theta = 9.82^\circ$.⁴⁸ This demonstrated the successful preparation of MWCNTs-COOH/MoS₂ composites.

To further confirm the MWCNTs-COOH/MoS₂, an XPS study was introduced. Figure 4A shows the overall survey MWCNTs-COOH/MoS₂; the appearance of C, Mo and S revealed a successful preparation of the materials. High-resolution Mo 3d XPS spectra of MWCNTs-COOH/MoS₂ (Fig. 4B) show the three typical main peaks of Mo 3d_{5/2} (229.0 eV), Mo 3d_{3/2} (232.2 eV) and S 2s for MoS₂ (226.4 eV). The high binding energy peak of Mo 3d (235.5 eV) and other two high binding energy peak of Mo 3d_{3/2} (233.2 eV), Mo 3d_{5/2} (230.1 eV) was considered to be the peak MoO₃, due to the preparation of the catalyst.^{30,49} Figure 4C shows the S 2p peak, which contains three doublets. The doublet at S 2p_{3/2} = 161.9 eV and S 2p_{1/2} = 163.1 eV was assigned to the S 2p_{3/2} and S 2p_{1/2} (C-S-C) lines of

Fig. 5 Cyclic voltammograms of different modified electrodes in 1.0 mM Fe(CN)₆^{3-/4-} containing 0.1 M KCl solution of this biosensor. Bare GCE (a), MWCNTs-COOH/MoS₂/GCE (b), ssDNA/ MWCNTs-COOH/MoS₂/GCE (c), dsDNA/MWCNTs-COOH/MoS₂/GCE (d).

MoS₂, and the binding energy at S 2p_{3/2} = 163.1 eV and S 2p_{1/2} = 164.6 eV were matched to bridging S₂²⁻ and/or apical S²⁻ ligands. High-energy components appearing at 168.8 and 170.0 eV could be explained as being high-valence S species C-SO_x-C.^{50,51}

Electrochemical behavior of modified electrodes

CV measurements were used to characterizing the electrochemical processes. The CV curves were recorded in a 1.0 mM [Fe(CN)₆]^{3-/4-} solution containing 0.1 M KCl at a scan rate of 50 mV s⁻¹; the potential scanning range was from -0.2 to 0.6 V. Curves from a to d (Fig. 5) represent different kinds of modified electrodes; the MWCNTs-COOH/MoS₂ enhanced electrode (curve b) shows a higher redox peak current than the polished bare electrode (curve a) because of the good conductivity of the nanocomposites. After incubation of the capture DNA, the redox peak current of the ssDNA/MWCNTs-COOH/MoS₂ modified electrode (curve c) decreased because of the negatively charged DNA. When target DNA (1.0 × 10⁻⁷ M) was added for successful hybridization, the peak current further declined (curve d), because of blocking of the electron transfer on the surface, which was caused by the double-stranded structure of the capture-target DNA.⁵²⁻⁵⁴

Optimization of experimental conditions

To optimize the detection conditions of the target DNA, the operational conditions were investigated, including the temperature and time of double-stranded hybridization reactions, the incubation time of MB, and the concentration of MB used at the last step, respectively. The concentration of the target DNA we used in this part was 1.0 × 10⁻⁷ M. The results could be recorded by DPV curves.

As shown in Fig. 6A, the current grew while the hybridization time was increasing from 20 to 80 min, and reached to the maximum value after 60 min, and then basically remained stable, indicating that hybridization of DNA has completely finished; 60 min is thus the optimal hybridization time. Figure 6B shows that the hybridization temperature was monitored in a range from 27 to 33°C. It can be observed that the signal increased as the temperature increased from 27 to 30°C, but decreased from over 30 to 33°C, indicating that 30°C is the optimum temperature for DNA hybridization reactions. Moreover, the incubation time of MB was carried out from 15 to 20 min; as we can see from Fig. 6C, this did not change a lot,

Fig. 6 Optimization of the experimental conditions: (A) effects of hybridization time, (B) effects of hybridization temperature, (C) effects of incubation time of MB, (D) effects of concentration of MB.

so we chose 15 min as the optimized incubation time of MB. Also, the ultimate concentration of MB was set as 50 μM after being optimized, as shown in Fig. 6D.

Analytical performance of the DNA biosensor

To investigate the analytical performance of the MWCNTs-COOH/MoS₂ based DNA biosensor, different concentrations of target DNA were added and incubated under the optimal conditions. The DPV measurement was the main technology used in this part to record the change in current. It can be clearly observed that the signal intensity of MB was growing along with the increasing concentration of target DNA (Fig. 7A). The calibration plot illustrated that this biosensor holds a nice linear relationship between the electrochemical currents and the logarithm of the concentration of target DNA from 1.0×10^{-14} to 1.0×10^{-7} M, as shown in Fig. 7B, with a correlation coefficient of 0.9978. The linear-regression equation was $I(\text{A}) = 50.95 + 3.39 \log C(\text{M})$, and the actual detection limit was 3 fM. Compared with other reported electrochemical biosensors for oligonucleotide sequences detection (Table 2), the biosensor of this work had reached a relatively lower detection limit or a relatively wider detection range. We found that the flower-like MoS₂ exceed the reaction surface for detection, while the insert of MWCNTs-COOH increased the conductivity of the electrode. Therefore, we can conclude that this MWCNTs-COOH/MoS₂ nanocomposite that enhanced the biosensor had a good electrochemical performance of high sensitivity.

Specificity, stability and reproducibility of biosensor

The specificity of the sensor was studied by comparing three different kinds of interferences: single-base mismatch (MT-1), three-base mismatch (MT-3) and noncomplementary probe

Fig. 7 (A) DPV curves after hybridization with increasing concentrations of target ssDNA (from a to i): 0, 1.0×10^{-14} , 1.0×10^{-13} , 1.0×10^{-12} , 1.0×10^{-11} , 1.0×10^{-10} , 1.0×10^{-9} , 1.0×10^{-8} , and 1.0×10^{-7} M. (B) Logarithmic plot of peak current vs. the concentration of target ssDNA.

Table 2 Comparison of linear ranges and detection limits of the different electrochemical biosensors

Modified electrode	Detection technique	Linear range/M	Detection limit/fM	Reference
GOD/AuNPs/MoS ₂ /MWCNTs/GCE	CV	1.0×10^{-14} – 1.0×10^{-8}	0.79	44
MoS ₂ /carbon aerogel/GCE	DPV	1.0×10^{-12} – 1.0×10^{-8}	300	46
Thin-layer MoS ₂ /GCE	DPV	1.0×10^{-16} – 1.0×10^{-10}	0.019	55
AuNPs/MoS ₂ /GCE	EIS	1.0×10^{-14} – 1.0×10^{-9}	0.45	56
ZnO/MoS ₂	DPV	1.0×10^{-15} – 1.0×10^{-6}	0.66	57
CdS/MoS ₂	Photocurrent response	1.0×10^{-15} – 1.0×10^{-10}	0.39	58
MWCNTs-COOH/MoS ₂ /GCE	DPV	1.0×10^{-14} – 1.0×10^{-7}	3	This work

Fig. 8 Comparison of DPV responses of the blank and the four different types of DNA sequences (single-base mismatch target, MT-1; three-base mismatch target, MT-2; noncomplementary sequence, NCS). The concentration of different DNA sequences was 1.0×10^{-7} M.

Fig. 9 Stability of this biosensor after being kept in 4°C refrigerator for 15 days.

(NON) with the target DNA. Figure 8 shows different current responses of each type of detector. The discrimination between the mismatch oligonucleotide and the target DNA is apparently obvious. The current of MT-1 was 38% of the complementary target ssDNA, indicating that the base mismatch prevented completion of hybridization. The current of MT-3 was only 23% of complementary target ssDNA, and the current of NON was 13%. The results indicated that this DNA biosensor holds a good selectivity and DNA mismatch recognition ability.

The reproducibility of the DNA biosensor was also analyzed by using five sensors for repeated tests with 1.0×10^{-7} M target sequences within 1 day. The relative standard deviation was 4.4% ($n = 5$), showing acceptable reproducibility for target DNA detection. The stability of this biosensor was also studied. As shown in Fig. 9, after storage in a refrigerator at 4°C for 15 days, the sensor still preserved about 95.2% of peak current

Table 3 Determination result of the KRAS gene in serum samples with the biosensor (each result was the average of three determinations)

Sample No.	DNA added/M	Average value of DNA/M	Recovery, %
1	1.0×10^{-11}	9.56449×10^{-12}	95.64
2	1.0×10^{-12}	9.84226×10^{-13}	98.42

compared with the initial response current value, which showed that the DNA sensor had good stability and could be considered to have a good ability for application studies.

KRAS gene detection in normal human serum

To improve the practicality of the DNA sensor, we investigated recovery experiments by detecting different amounts of target DNA in serum samples from normal people. The interference effect of the complicated biological fluids was measured by DPV. As shown in Table 3, the recoveries obtained were 95.64 and 98.42% with RSD of 2.0 and 2.4%, (which added 10 and 1 pM, to diluted serum respectively), revealing that the fabricated biosensor is reliable for the detection and diagnosis of the KRAS gene in serum samples.

Conclusion

Hence, we have fabricated an ultrasensitive electrochemical biosensor based on MWCNTs-COOH/MoS₂ nanocomposites for detecting the KRAS gene. Introducing the MWCNTs-COOH/MoS₂ nanocomposite can excellently improve the detection capacity. The nanocomposite can exceed the immobilization area for the capture, while MWCNTs-COOH elevates the conductivity of the electrode. The biosensor exhibited a low detection limit of 3 fM, a wide linear range with 1.0×10^{-14} to 1.0×10^{-7} M, and presented outstanding sensitivity. Therefore, we believe that this displayed biosensor can play a critical role in the on-time screening of DNA in molecular diagnosis.

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