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Biosensitivity of Molybdenum Disulfide for Monitoring Breast Cancer Marker CA15-3 Using Quartz Crystal Microbalance

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This paper verified the biosensors of molybdenum disulfide (MoS_2) and the colloidal gold probe to amplify signals studied by using quartz crystal microbalance (QCM) sensor. The chemical reaction was resulted in the formation of colloidal gold probes by immune colloidal gold technique and then verified by double antibody sandwich techniques (ELISA). Nanometer MoS_2 as the sensitive material of sensor was produced by hydrothermal method. The scanning electron microscope (SEM) and transmission electron microscope (TEM) electron microscopy showed that the gold nanoparticles were successfully modified on anti-CA15-3 antibodies and the prepared MoS_2 was typical two-dimensional material with a hexagonal sheet structure. We found that these QCM sensors had high sensitivity ($26.303 \pm 1.139 Hz/scale$, $1 U/ml$) and good linearity (0.960 ± 0.013) within the concentration range of CA15-3 between $0.5 U/ml$ and $100 U/ml$. The results show that the response time of the system was less than $20 s$, had reproducibility and selectivity. It indicated that nanometer MoS_2 is a good biosensor material.

Keyword: molybdenum disulfide; bio-sensitivity; quartz crystal microbalance sensor; immune colloidal gold technique; double antibody sandwich techniques; CA15-3 concentration detection

1. INTRODUCTION

Since the 20th century, cancer has been recognized as one of the major hazards to human health. The incidence of cancer is relatively high in both developed and developing countries. According to the data of the world health organization's international center for research on cancer, cancer caused 7.6 million deaths in 2008, accounting for about 13% of the total deaths. On the basis of the data, the agency estimated that the number of cancer deaths could exceed 13.1 million in 2030 [1]. Breast cancer is one of the major malignant tumors. The incidence and mortality of breast cancer in Chinese women have been on the rise in the past 10 years. According to incomplete statistics, breast cancer patients are

diagnosed in stages as follows: stage I accounts for about 35%, stage II accounts for about 50%, and III and IV account for 15%. Therefore, the rate of early detection of breast cancer is important and needs to be further improved [2].

Breast cancer is one of the common cancers in women. In recent years, the incidence of breast cancer has been increasing year by year. Early detection of malignant tumor markers is particularly important. At present, the common cancer markers detection mainly includes: biosensor[3], immunological methods[4], the proteomics technology[5], liquid biopsy[6] and chemiluminescence immunoassay[7,8], etc. However, the specificity and sensitivity of most serum tumor markers are not ideal. CA15-3 as a relatively sensitive marker has been used with clinical medicine for many years. Detection the serum concentrations of CA15-3 is of great significance to predict cancer. Among them, biosensors are mainly formed by combining specific immune response and biosensors. It converts biological signals into electrical signals for detection, which has the advantages of high sensitivity, low cost and convenient operation. With the development of technology, biosensors are expected to be a new detection method for cancer markers. Immune sensor is a biosensor developed by using photoelectric parameter changes caused by specific response and specific binding of antibodies to antigens[9,10]. Therefore, it has the characteristics of high specificity, high sensitivity and high selectivity.

QCM is a kind of quality testing platform based on the piezoelectric effect of quartz crystal, which is very sensitive to the changes in quality, humidity, density and conductivity, etc.[11]. It's worth mentioning that QCM has the nanogram level of sensitivity. It is a kind of effective research means of micro change and online access to information, which can detect the micro change process and get a lot of online data. Therefore, QCM has gained extensive attention and rapid development in materials, biology, chemistry and other fields in recent years[12]. In this study, a QCM biosensors modified by MoS_2 [13,13] curing anti-CA15-3 antibodies were manufactured for the detection of CA15-3 antigen[15]. By taking the advantage of specific immune response between antigens and antibodies and the high sensitivity to ng-class mass of QCM, the low concentration of antibodies could be detected.

In 1972, Alan Shons and Frank Dorman’s team improved the piezoelectric quartz crystal microbalance to provide a fast qualitative and quantitative method for detecting antibody concentration in solution[16]. Therefore, the application of QCM in biosensors is becoming more and more extensive. Shan Wenqian’s team developed an adaptation-based QCM biosensor for selective sensitivity detection of leukemia cells. Aminophenylboronic acid-modified gold nanoparticles were used for the labeling of cells, which could bind to cell membrane. And then enhanced with silver to achieve significant signal amplification[17].

In July 1990, the first international nanometer science and technology conference was held in Baltimore, USA, which marked that nanometer material science officially became a new branch of material science. Nanotechnology was born. Nanomaterials are usually materials composed of nanoscale particles with extremely small size, which have three characteristics that ordinary materials do not have: surface effect, small size effect and macroscopic quantum tunneling effect[18]. Because of these characteristics of nanomaterials, they have strong adsorption and biological compatibility[19]. The rapid development of nanotechnology promotes the development of biosensors. Biosensors meet a rare opportunity due to these unique properties. Nanomaterials have the following applications in biosensors:

as markers to assist in enhancing the signal and as carrier of biological sensor, such as quantum dots, nanometer electron conduction materials and nanometer colloidal gold body, etc.[20].

Molecular self-assembly technology is one of the hotspots in the field of micro molecules in recent 20 years[21]. It is a process of molecular aggregation with stable structure formed by spontaneous connection of non-covalent bonds between molecules under certain conditions, such as hydrogen bond[22], van der Waals force[23], $\pi - \pi$ accumulation and hydrophobic force[24,25], etc. Molecules on metal or metal oxide surfaces tend to self-assemble through spontaneous adsorption [26]. The surface of gold nanoparticles is easy to form a highly ordered and tightly enriched molecular layer structure with mercaptan substances, resulting in the strong interaction between metal and sulfur. In this paper, gold nanoparticles and anti-CA15-3 antigens solution will be self-assembled to form a molecular layer. Nano molybdenum disulfide will be used as a sensitive material to enhance the sensitivity to measure the concentration of CA15-3 antigens by taking advantage of the strong interaction between gold and sulfur [27].

Sensors are divided into physical sensors, chemical sensors and biosensors. Chemical sensors have the advantages of high selectivity, high sensitivity, low detection limit and dynamic response of chemical reaction. For these reasons, we chose chemical sensors. In this study, we firstly used immune colloidal gold technique to form immune gold probe, and then verified that gold nanoparticles were successfully assembled on protein molecules through ELISA. Finally, gold-labeled CA15-3 antigens were made. CA15-3 antigens were detected by QCM biosensors modified with MoS_2 curing anti-CA15-3 antibodies. Firstly, a uniform layer of nanometer MoS_2 solution was applied to the QCM gold electrode by coating method. Then, anti-CA15-3 antibodies were fixed on the surface of MoS_2. Finally, these QCM sensors were used to measure different concentrations of CA15-3 antigens. Experiments showed that the sensor had good sensitivity, linearity, reproducibility and fast response time. The results showed that nanometer MoS_2 can be used as one of the sensitive materials for biosensor.

2. EXPERIMENT

2.1. Materials and reagents

CA15-3 antigens were modified by $10nm$, $30nm$ and $60nm$ gold nanoparticles respectively, phosphate buffer saline (PBS，$pH = 7.4$), nanometer molybdenum disulfide(MoS_2), beaker, measuring cylinder, suction filter bottle, semiconductor, quartz crystal microbalances(QCM), magnetic suspension agitator, the centrifuge, vacuum drying cabinet, supersonic jet mill, ultrasonic cleaner, electronic analytical balance, pipette.

2.2. Preparation of colloidal gold probe

The principle of immune colloidal gold technique to label antibody protein is: the surface of colloid gold nanoparticles presents a negative charge state in alkaline solution. Meanwhile, the surface of protein (antibody) appears a positive charge state. Thus, they generate electrostatic attraction and bind

together. The process of gold nanoparticles labeling protein (antibody) does not significantly affect the biological activity of the protein. The solution's pH value, the size of gold nanoparticles and the protein's concentration all affect the labeling adsorption process. The gold nanoparticles, the anti-CA15-3 antibodies and CA15-3 antigen for this experiment were purchased from chongqing vokawei biotechnology co., LTD.

Firstly, the pH value of colloidal gold nanoparticles solution was 7.4 adjusted by potassium carbonate solution. Secondly, colloidal gold nanoparticles solutions with diameters of $10nm$, $30nm$ and $60nm$ were added to anti-CA15-3 antibodies solution respectively, then incubated at room temperature for 30min. Thirdly, appropriate amount of sealing solution was added and incubated at room temperature for 30min. The closed colloidal gold solution was centrifuged at a speed of $8000rpm$ for $20min$ at the temperature of 4℃. Finally, after centrifugation, the supernatant (unbound antibodies) was carefully absorbed and the precipitate was back to the original volume by the $1/20$ concentration of the original colloidal gold nanoparticles solution. Then, the solution was centrifuged again after recovery to the original volume. The unbound antibodies were washed for 4 times to remove unbound antibodies completely. The colloidal gold probes were stored at the temperature of 4℃. TEM and ultraviolet-visible spectrophotometer (UV-VIS) were used to characterize the structure, as shown in Fig. 1.

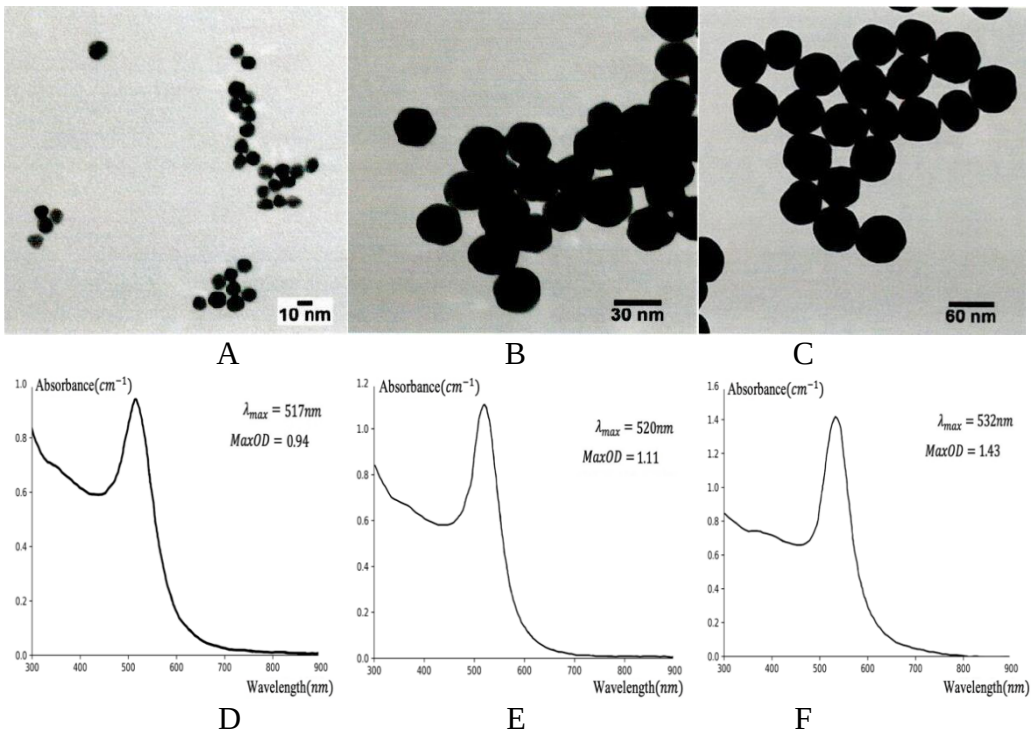


Figure 1. Characterization results of colloidal gold probes; A. TEM characterization of colloidal gold probes; B. Characterization results of colloidal gold probes; C. SEM characterization of nanometer molybdenum disulfide; D. UV-VIS characterization of colloidal gold probes; E. UV-VIS characterization of colloidal gold probes; F. UV-VIS characterization of colloidal gold probes

Fig. 1. showed that the CA15-3 antigens modified by gold nanoparticles had good dispersion and uniform size under TEM. Haiss, D.G.Fernig's team worked on the relationship between gold nanoparticles size and UV-VIS absorption spectrum 28. The λ_{max} in three curves were 517nm, 520nm and 532nm respectively. We could see that the biggest absorption peak was basically around 530nm, according with the standard UV-VIS absorption spectrum of gold nanoparticles. The characterization results of TEM and UV-VIS showed that the gold nanoparticles had been successfully self-assembled and modified on the antigens.

2.3. Double antibody sandwich method (ELISA)

Double antibody sandwich method is shown in Fig. 2. Colloidal gold probes are placed at G (specific antibody 1), antibodies are placed at T (specific antibody 2), anti- colloidal gold probes are placed at C, and absorbent paper is placed at B. Firstly, the material under test (antigen) drops into place A mat (sample pad).

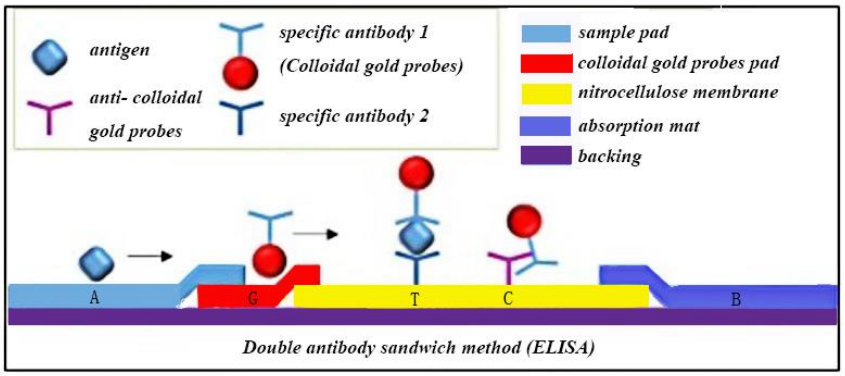


Figure 2. Double antibody sandwich method

Figure 3. Detection result of double antibody sandwich method

Secondly, because of chromatography, material under test moves from A to B. Then, antigens and colloidal gold probes occur specificity immune response through G, generating the compound of colloidal gold probes-antigens. Thirdly, the compound arrives at place T, forming the compound of

colloidal gold probes-antigens-antibodies. As a result of the action of nitrocellulose membrane, colloidal gold probes are fixed in the line T, making the place T appears red lines or red spots. Fourthly, the excess colloidal gold probes move to area C and are captured by anti-colloidal gold probes. Colloidal gold probes are fixed in line C in the same way, making area C appears red lines or red spots. Test result of ELISA test paper was showed in Fig. 3., which was a positive reaction.

2.4 Preparation of nanometer molybdenum disulfide

Firstly, 0.3g of molybdenum trioxide (MoO_3) and 0.1g of thioacetamide (CH_3CSNH_2) were added to a beaker with 80ml of deionized water. Secondly, the solid was stirred by magnetic suspension agitator for 30min till it was completely dissolved in deionized water. Thirdly, put the prepared solution into a vacuum drying cabinet and reacted at 240℃ for 48h. After reaction, took it out and cooled it for 6h. Fourthly, the product was filtered by suction filter bottle and washed with anhydrous ethanol and deionized water for 3 times successively.

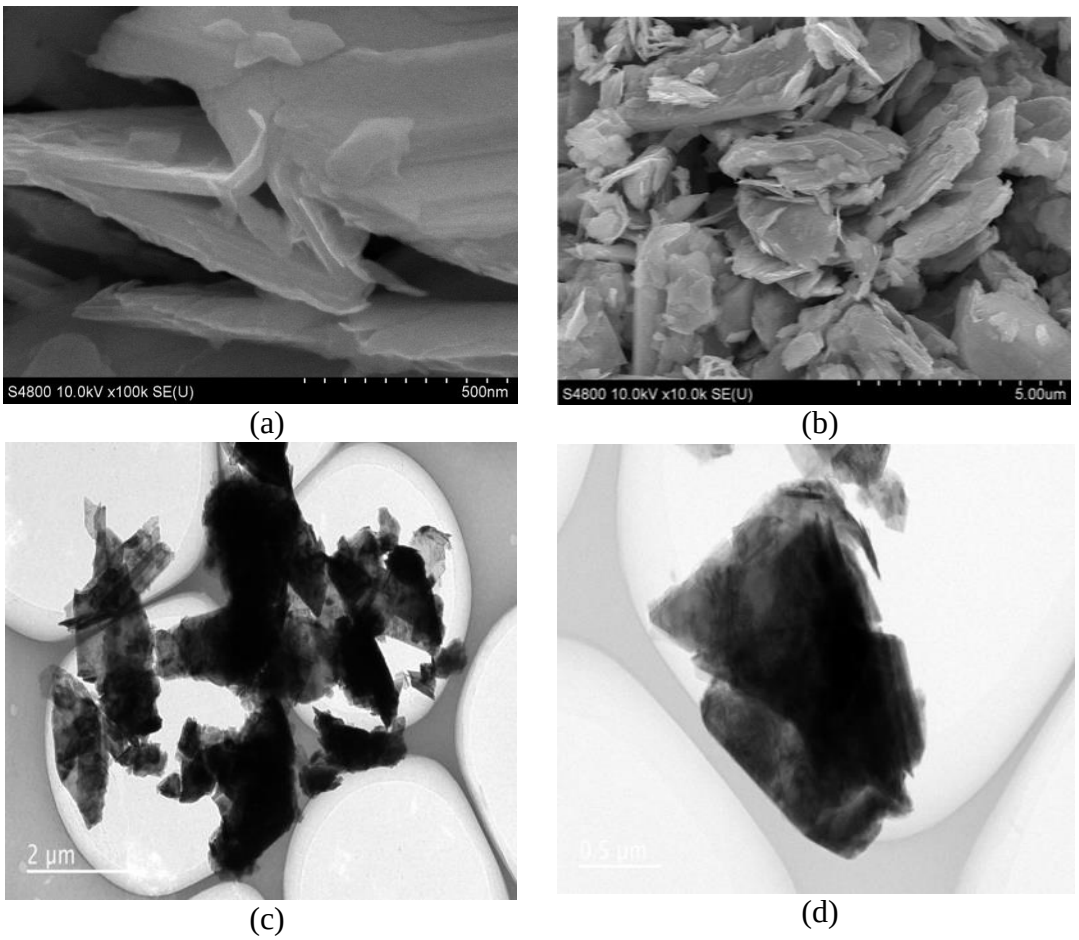


Figure 4. SEM and TEM characterization of nanometer molybdenum disulfide; a**.** SEM characterization of nanometer molybdenum disulfide; **b.** SEM characterization of nanometer molybdenum disulfide; **c.** TEM characterization of nanometer molybdenum disulfide; **d.** TEM characterization of nanometer molybdenum disulfide

The product was placed in a vacuum drying cabinet and dried at 60℃ for 6h to obtain solid products. Finally, MoS_2 sheet was stripped under the condition of air flow with high speed and high pressure by supersonic jet mill, so as to prepare MoS_2. SEM, TEM and X-ray diffraction (XRD) were used for characterization. SEM and TEM characterization results were shown in Fig. 4., and XRD characterization result was shown in Table 1 X-ray diffraction data of nanometer MoS_2 and Fig. 5. The data in Table 1. show the peak points of X-ray diffractometer.

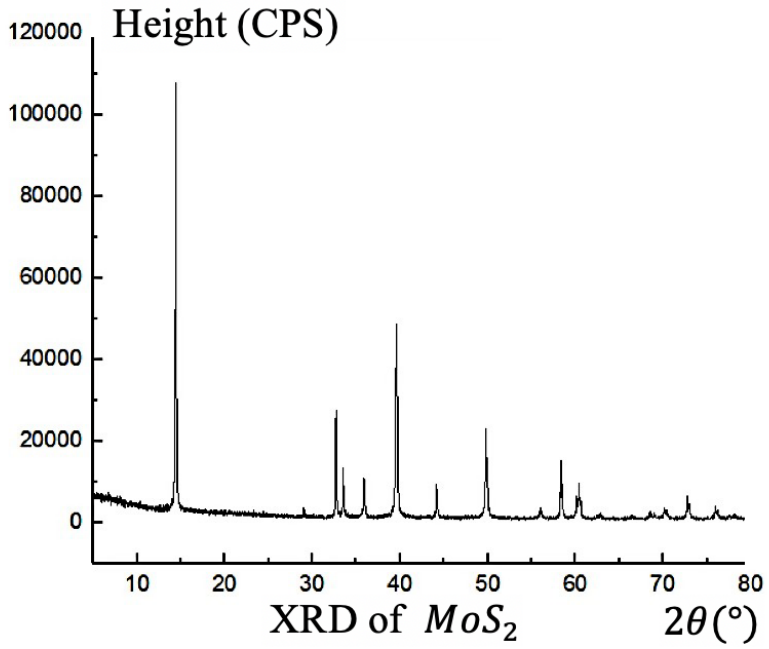


Figure 5. X-ray diffraction instrument result of nanometer MoS_2

It could be seen from Fig 4. that the morphology of nanometer MoS_2 was mainly lamellar structure and a typical two-dimensional material. MoS_2 particles were uniformly dispersed with a small particle size of about 500nm~2um and an average particle size of 1um. The nanocrystalline layers were distributed evenly with less accumulation, and the thickness of the nanocrystalline layers was relatively small.

Table 1. X-ray diffraction data of nanometer MoS_2

$2-Theta$	$d(A)$	BG	$Height$	1%	$Area$	1%	$FWHW$
14.400	6.1459	100	3132	100.0	47517	100.0	0.129
32.689	2.7372	56	771	24.6	10533	22.2	0.116
39.541	2.2773	55	1408	45.0	34814	73.3	0.210
49.780	1.8302	40	653	20.8	18410	38.7	0.240

1、$2-Theta$ denotes the diffraction Angle

2、$d(A)$ denotes interplanar crystal spacing

3、BG denotes the intensity at the end of back

4、$Height$ denotes the intensity of the diffraction

5、 1% (the first) denotes the relative intensity

6、 $Area$ denotes the diffraction area

7、 1% (the second) denotes the relative area

8、 $FWHW$ denotes half tall wide

It could be seen from the Fig. 5. and Table 1. that the crystal structure was good, and the Height (diffraction peak strength) of 2θ equaled 14.400(002), 32.689(100), 39.541(103) and 49.780(105) was relatively high, which was basically consistent with the standard X-ray powder diffraction pattern of MoS_2 crystal, indicating that the sample prepared was MoS_2 with hexagonal crystal system.

2.5. Apparatus

The QCM measurement system is shown in Fig 6. The working electrode is a gold-plated ($0.2cm^2$) quartz crystal oscillator. The natural resonance frequency is 7.995MHz, and it is AT cutting type. To measure the solution, the crystal electrode was sandwiched between two plexiglass blocks with a neoprene circular seal. One of the crystal electrode's surfaces was exposed to the reaction solution. We called the instrument QCM container for convenience.

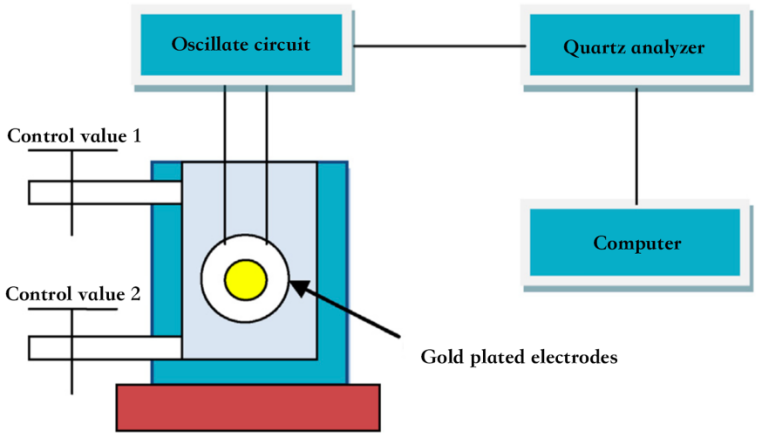


Figure 6. Block diagram of testing system

The antigens solution to be measured and PBS buffer were added to the tank of QCM container. The solution to be tested entered QCM container through the control valve 1. Waste water was discharged through control valve 2. The frequency measurements were delivered to a computer for analysis at room temperature (25℃) with a QCM device.

2.6 Preparation of gold electrode for QCM sensor

Prior to coating the QCM electrode with MoS_2, the working electrode of the quartz crystal was cleaned with concentrated sulfuric acid, rinsed with anhydrous ethanol, and dried for later use. At this

point, the resonant frequency of QCM was recorded as the reference frequency. Fig. 7. showed the reference frequency of optical crystal without coating any sensitive material. From the diagram, we found that frequency of the sensor varied by no more than $16Hz$ during the whole process, which showed that the frequency was not caused by the change of antibodies concentration to be measured when the number changed within $20Hz$.

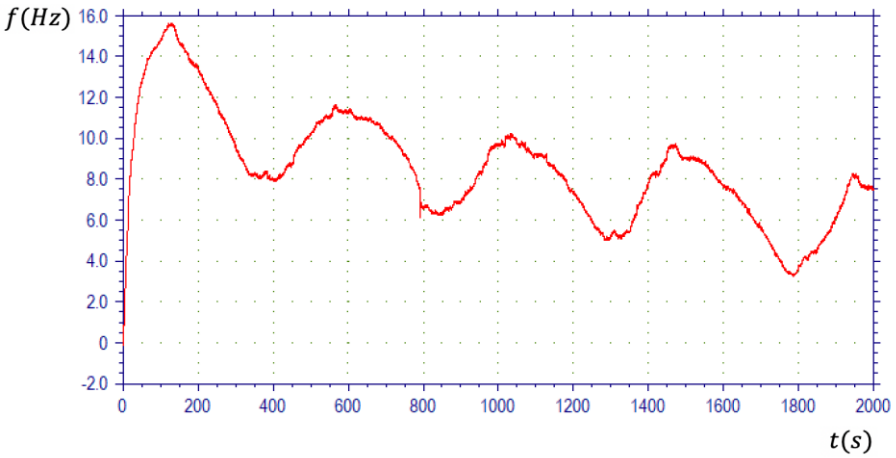


Figure 7. Variation of measurement frequency with time

And the numerical value relative to the reference frequency $7.995MHz$ electrode was negligible which showed that the cleaned gold electrode frequency was relatively stable near the reference frequency.

The preparation process of the gold electrode was divided into two steps: firstly, the dispersed solution of MoS_2 treated by ultrasonic was uniformly coated on the gold electrode, and the solution was dried at 60℃ in a vacuum drying cabinet to ensure the complete volatilization of anhydrous ethanol and formed a nanometer film; secondly, the anti-CA15-3 antibodies were fixed on the surface of MoS_2 by simple physical adsorption method. Then it was left to dry for $30min$ and refrigerated overnight until the surface of the gold electrode was completely dry. In this experiment, we should make 20 sensors label with #1-#20 for detection. These sensors were all made according to the processing above.

3. RESULTS AND DISCUSSION

3.1 Influence of gold nanoparticles with different particle sizes on measurement results

In order to study the influence of anti-CA15-3 antibodies labeled by gold nanoparticles with different particle sizes on the measurement results, frequency shifts of $10nm$ labeled anti-CA15-3, $30nm$ labeled anti-CA15-3 and $60nm$ labeled anti-CA15-3 at concentrations of $13U/ml$, $18U/ml$ and $20U/ml$ were measured. The experimental results were shown in Fig. 8.

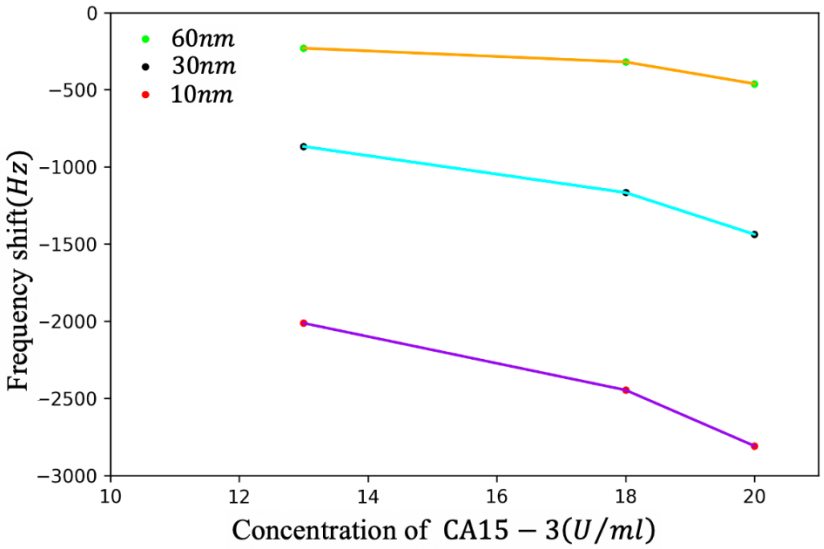


Figure 8. Frequency of anti-CA15-3 antibodies labeled by different gold nanometer

Fig 8. showed that the frequency shift of gold nanoparticles with different particle sizes was different when you measuring the same concentration of anti-CA15-3 antibodies solution. The bigger the particle size of gold nanoparticles modified antibodies, the greater the frequency shift. When the concentration was equal, the quality was greater if the particle size of gold nanoparticles was bigger. The Sauerbrey equation shows that the bigger the mass, the bigger the frequency shift. It conforms to QCM sensing model theory based on mass effect.

3.2 Linearity characteristic of the MoS_2 modified sensors

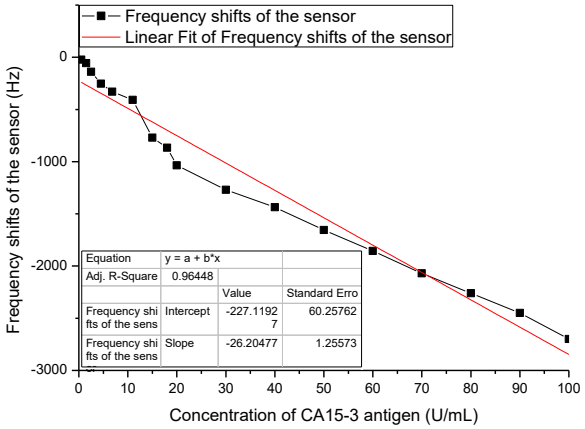


Figure 9. Concentration-frequency shift curve of sensor #1

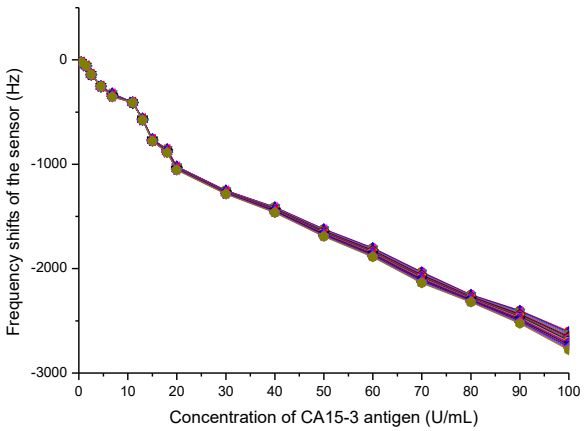
Firstly, we prepared 20 independent QCM sensors which were modified by MoS_2 curing anti-CA15-3 antibody to detect CA15-3 antigens with different concentrations labeled by $30nm$ gold nanoparticles. The sensor was numbered #1 to #20. The experimental process was consistent, that is, the preparation process of the 20 sensors was consistent. In the next section, we will analyze the reproducibility of the sensor.

Fig. 9. showed the concentration-frequency shifts curve of a QCM sensors to detect the concentration change of CA15-3. The figure indicated there was a linear relationship between concentration and frequency shifts. In order to see the linear effect, we fitted the line of concentration and frequency based on the least square method.

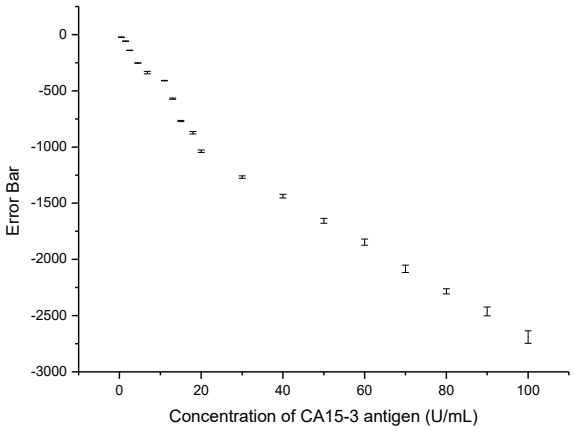
Fig 9. was a scatter diagram of 17 concentrations of sensor #1 within $0.5\sim100U/ml$. The detection limit of the sensor approached 0.5U/mL. Based on the straight line fitted by the least square method, the regression coefficient was 0.9648 and the sensitivity was $26.2Hz/scale(1U/m)$. But when the sensor can detect at the confidence level of 0.05, it indicated that the sensor had good linear relationship and high sensitivity. When the concentration of CA15-3 was above 100 U/mL, the sensor tends to be saturated, mainly because of the limited MoS_2 or antibody coated on the sensor.

3.3 Reproducibility

The reproducibility of sensor is defined as: the response frequency shifts of the sensor were consistent or within an allowable range (relative standard deviation of the measurement results is less than 2%) when detecting different concentration of CA15-3 antigens solution by QCM sensors. In order to verify the reproducibility, we prepared 20 QCM sensors which were modified by MoS_2 again to detect CA15-3 antigens labeled by $30nm$ gold nanoparticles. The detected concentration range was still $0.5U/ml$ to $100U/ml$. The measured data were shown in Fig. 10.



(a)



(b)

Figure 10. Concentration-frequency shifts curves of 20 sensors; **a.** Concentration-frequency shifts curves of 20 sensors; **b**. Error bar of the concentration-frequency shift curves of 20 sensors

Fig. 10. was the scatter diagram of 12 concentrations of 20 sensors within $0.5U/ml$ to $100U/ml$ respectively. From Fig. 10a, we found that the scatter points of these 20 sensors had a strong linear relationship. The regression coefficient and sensitivity of these sensors were similar. The regression coefficient is 0.960 ± 0.013 and the sensitivity is $26.303 \pm 1.139Hz/scale$ $(1U/ml)$. It meant that the frequency shift error of these sensors was small at the same concentration. From Fig. 10b, the error bar for each concentration is small relative to the mean, which is within an acceptable range. What's more, the sensors were stored in a refrigerator for a few days and measured again, the results were within the margin of error. Therefore, it could be considered that the QCM sensors which were modified by MoS_2 curing CA15-3 antigens had reproducibility.

3.4 Response time

In addition to linearity and reproducibility, the sensor response time was also tested in this study. Response time is defined as: when the input parameter changes, the response time will not change to a new state immediately, but will stabilize in the new state after a period of time, which is called the response time. Fig. 11. showed four stable states: the sensor oscillated in PBS buffer, then successively added a certain amount of anti-CA15-3 antibodies solutions labeled by $10nm$, $30nm$ and $60nm$ gold nanoparticles separately.

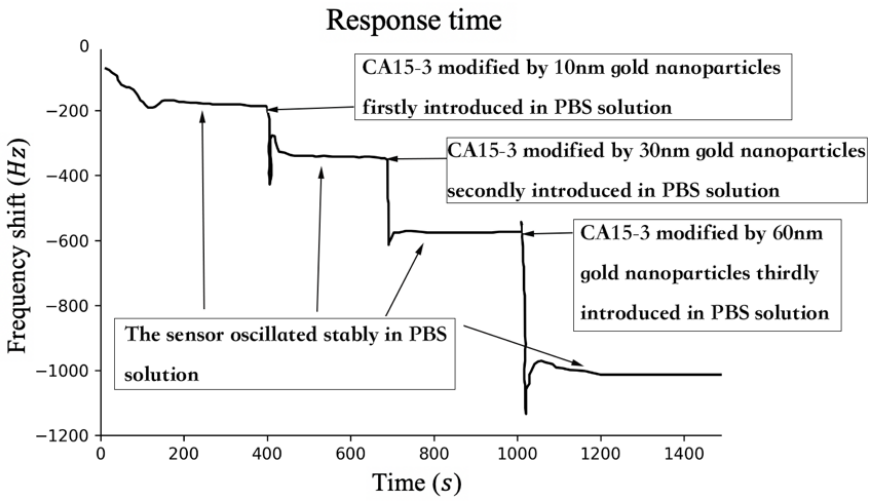


Figure 11. Response time of QCM sensor

As you could see from Fig. 11. , the frequency would return to a stable state after a short period of time (less than 15s) by rapidly dropping CA15-3 antigens solution labeled by $10nm$, $30nm$ and $60nm$ gold nanoparticles separately in the container. The response rate was fast.

3.5 Selectivity of the sensor

In order to study the selectivity of QCM sensors which were modified by MoS_2 curing anti-CA15-3 antibodies.

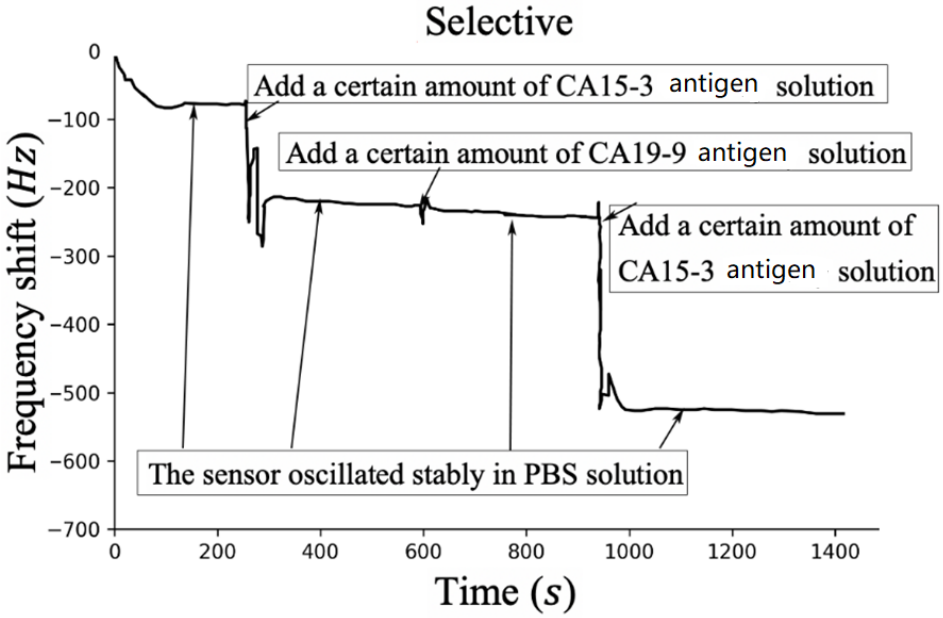


Figure 12. Selectivity characteristic of the QCM sensor

You could see from Fig. 12 that the frequency was significantly decreased when the gold-labeled CA15-3 antigen solution was added for the first time when the sensor was stabilized in PBS buffer. After the frequency reached the stable state again (about $600s$), added CA19-9 antigen solution, it could be

seen that the frequency did not shift. When the gold-labeled CA15-3 antigen solution was added again, the frequency decreased significantly again.

Therefore, it could be concluded that the sensor had a good adsorption effect on CA15-3 antigens solution but could not adsorb CA19-9 antigens solution.

3.6 Performance comparison with other types of sensors

The comparison the sensor with similar sensors for Monitoring Breast Cancer Marker CA15-3 that were described in literature. The results were showed in table 2. From the table, it can be seen that compared with the ZnO nanorod QCM sensor[30], our sensor has a wider detection range and higher sensitivity. On the other hand, compared with electrochemical sensors [32], our sensor also has good wider detection range. Compared with electrochemical sensor, QCM sensor has a good real-time online function, and the output of QCM sensor is digital signal, which is very conducive to the later data processing and analysis.

Table 2. The comparison the sensor with similar sensors

Biosensor	Targer molecular	Linear range of detction	Detection limit	Sensitivity of QCM sensor
ZnO nanorod QCM sensor [29]	CA15-3	0.5U/mL-25U/mL	0.5	25.34 ± 0.67 Hz/scale (1 U/mL))
glucose oxidase (GOD) in Pt NCs/CNTs-OrgSi@chitosan composite matrices immunosensors electrochemical immunoassay [30]	CA15-3	0.1-160U/mL	0.1	
graphene-modified electrode electrochemical immunosensor [31]	CA15-3	0.1-20U/mL	0.1	
our sensor	CA15-3	0.5-100U/mL	0.5	$26.303 \pm 1.139 Hz/scale$

4. CONCLUSION

The CA15-3 biosensor based on QCM modified by nanometer MoS_2 was developed. The sensor had good linearity, sensitivity, reproducibility and fast response time. All the results showed that the proposed biosensor had good selectivity and specificity, which could effectively measure the concentration of CA15-3 antigens. Due to the wide application prospect of sensors in environmental monitoring, food safety, clinical medicine and other fields, it is hoped that this study can broaden the application of quartz crystal in biological sciences. Compared with the method of ultrasonic light scattering imaging to detect CA15-3 concentration, such as the paper of “Diagnostic value of breast ultrasound DOT combined with serum CA15-3 and CEA detection in breast tumors”, this method has the advantages of higher sensitivity and shorter response time

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