

Application of Fiber Optic Biosensor in Detection of Sports Analeptic

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Abstract: To improve the efficiency and accuracy of analeptic detection in competitive sports, a new kind of optical fiber biosensor is proposed. The competition law and the ligand binding theory are applied, and according to the combination proportion of competitive molecular of analeptic molecules and its fluorescence labeled in the receptor, the information of analeptic molecular content is transformed into fluorescence signal intensity. The results showed that the lowest concentration of the estrogen and androgen that can be detected by the fiber optic biosensor is 10^{-9} mol/L; physical response time is less than 0.1 seconds; the system error for repeating the experiment is less than 0.25%; agarose SAMs can be saved more than 30 d at -4 °C light avoidance condition. It is concluded that the sensor has good reliability, accuracy, stability and repeatability as well as high sensitivity so that it is especially suitable for the screening detection of analeptic.

Keywords: Fiber optical biosensor, Analeptic, Fluorescence labeling.

Introduction

The rapid development of modern science and technology has led to the transformation of human society into the information age. Human beings will rely on the development, transformation, transmission and processing of information resources for production, life and scientific research. Sensors are the main means of sensing, acquiring, detecting, monitoring and transforming information. Optical fiber sensor is based on optical technology, and the sensitive state is taken out in the form of signal. The optical signal can not only be directly perceived, but also can be converted to electro-optical and electro-optical, which lays the foundation for the development of optical fiber sensor [5, 8]. Fiber optic biosensors use optical fiber sensors to detect biological samples, obtain biological signals, and analyze biological signals. The optical fiber biosensor will be applied to the detection of biological samples. The optical fiber biosensor will improve the detection speed and accuracy of biological samples in the fields of clinical medicine, modern sports, food hygiene and environmental monitoring [3, 6].

Analeptic detection is becoming more and more important in competitive sports. However, the detection methods of analeptic, such as chromatography, mass spectrometry and electrophoresis, often cost a lot of time and money to detect a sample [10]. At present, the domestic and foreign concerned about using a method of biochip to detect analeptic. But due to the small molecular weight of analeptic, preparation of specific antibody has low success rate and high cost so that it will encounter great difficulties in practical application, so the current research progress in this area is not great [1]. With the rapid development of competitive sports, people's demand for analeptic testing, especially for analeptic detection methods applied to large-scale field surveys, is increasing. There is an urgent need for a reagent and method that can be used to test a large number of different kinds of analeptic in a

simple, fast and low cost test. In order to overcome the shortcomings of conventional methods, we will develop an optical fiber biosensor system hardware platform, and develop an optical fiber biosensor for competitive sports to detect hormone analeptic based on the needs of modern sports.

Fiber optic biosensor

Composition summary

The major composition of optical fiber biosensor in this article is shown in Fig. 1.

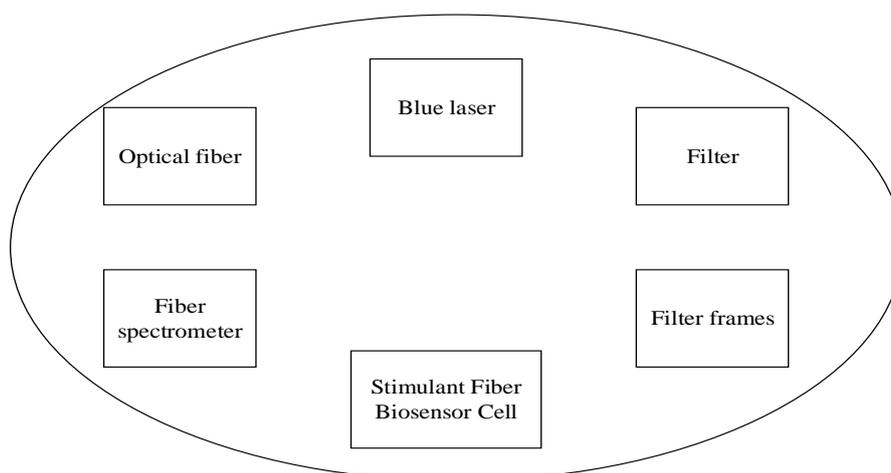


Fig. 1 Composition of optical fiber biosensors

Fiber is the abbreviation of optical fiber, which is made up of fine glass with a diameter of about 0.1 mm. It is transparent and thin, and though it is thinner than the hair, it has a guide wave structure that seals the light in it and propagates along the axis. The single fiber and Y type fiber used in this experiment are both purchased from the Ocean Optics Inc., which is a supporting product of the optical fiber spectrometer of the Ocean Optics Inc.

Blue laser used in the experiment is MBL-II-A type laser produced by Changchun New Industry Optoelectronics Technology Limited Company. The center wavelength is 473 nm, with high brightness, low power consumption, high reliability and stability, and it can improve the flexibility of optical design and mechanical design.

In this experiment, two filters are used, one is a high pass filter of 490 nm and the other is a high pass filter of 600 nm, both produced by Beijing Daheng Optics Technology Co. Ltd. The optical interference filter (Fig. 2) is a precise optical filter established on the basis of the interference principle of the optical thin film. By designing and changing the structure of membrane system and optical parameters of film, various spectral characteristics can be obtained, which can be used to control, adjust and change the transmission, reflection, absorption, polarization or phase state of light waves. The high pass filter is to filter light with short wavelengths less than the wavelength of the filter, and the long wavelength longer than that of the filter can pass.

The function of the filter frame is to fix the filter and to fix the fiber on both sides of the filter so that the attenuation of light through a filter is minimized. The core diameter of the fiber we use is about 50 μm , so we must be able to aim at the optical fiber on both sides of the filter, so that most of the light passing through the filter enters the optical fiber connected to the rear.

It needs better alignment and coaxiality of the frame. Therefore, in this experiment, we have a very high requirement for the technique of the filter frame, and it is also the key for the smooth completion of the experiment. In addition, the degree of light divergence is proportional to the square of the distance of the optical path. That is to say, the longer the distance is, the greater the degree of light divergence is.



Fig. 2 Optical interference filter

In the analeptic optical fiber biosensor, sample pool is used to place the modified glass slides and fixed on the protein receptor. When the excitation in the sample cell is irradiated by slides, the combined and FITC labeled analeptic molecules on receptor can emit fluorescence under stimulated luminescence, and fluorescence is transmitted to the detection terminal through the optical fiber at the upper end of the sample pool.

USB2000 micro optical fiber spectrometer is a plug and play small palm spectrometer based on the original S2000 micro optical fiber spectrometer. The USB2000 spectrometer can be directly inserted into the USB 2.0 communication serial port of desktop computers or notebook computers, so that the trouble of separating A/D converters from the interface cables can be eliminated. The user can directly insert the spectrometer into a desktop computer without the need to open a computer to search for interruption requests and input and output ports.

Basic principle

The structure of optical fiber biosensor is shown in Fig. 3. It is composed of three parts: the excitation part of biosensor, the detection part of biological sample and the signal processing part [11].

First of all, blue laser (central wavelength of 473 nm) emitted blue light, which is introduced into the sample pool of the biological sample detection part by the input end of the Y type fiber. Under the irradiation of the wavelength stimulated luminescence, the biomolecules in the sample pool can produce a specific wavelength of emission light (the analeptic fiber biosensor is 520 nm). The transmitting light is transmitted from the output end of the Y optical fiber to a fiber optic spectrometer by a high pass color filter (the analeptic fiber optic biosensor is 600 nm) at a specific wavelength. Fiber optic spectrometer is used to collect signals. The signal is transmitted to the computer by analog to digital converter and the fluorescence intensity is analyzed by professional analysis software.

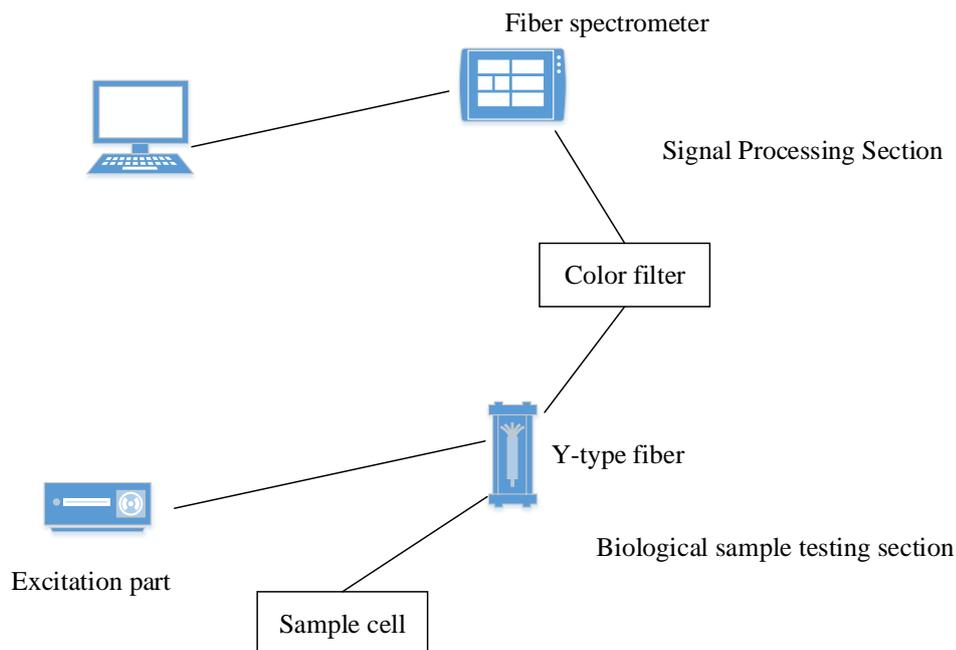


Fig. 3 Optical fiber biosensor

Surface modification method of solid phase carrier

The most important step in detecting analeptic molecules is to immobilize the receptor protein of the analeptic molecules on the surface of the solid phase carrier. The methods of immobilization of protein molecules include two major categories: physical adsorption and chemical coupling. No matter what kind of method is taken, the surface of the solid phase carrier must be modified. The modified method depends largely on the properties of the material selected as the base of the solid phase [4, 9]. Therefore, the selection of the substrate is the first step in our experiment. At present, our detection signals are mainly fluorescent signals, which require good chemical stability and ideal optical properties of substrate, and should be stored for a long time. As a result, we choose glass and quartz as solid carrier for the research.

According to the results reported by our laboratory and years of experience, considering the existing conditions, we choose γ -aminopropyl triethoxy silane (APTES), glutaraldehyde (APTES-GA), polyacrylamide, and agarose these four compounds as the main modified compound, and compare their efficiency of immobilized proteins.

Properties and characterization of APTES and glutaraldehyde

Infrared spectroscopy can be used to identify the structure of functional groups or related compounds, which is widely used in the field of organic chemistry. For the sample compound to be measured, the infrared spectrum is like the "fingerprint zone" [2] of the compound. For the test of compounds, we only need to compare the spectra of the tested samples with those of several standard substances. Once the spectrum and peak positions of a known substance coincide with the sample, the qualitative testing can be completed. In the range of $5000-1300\text{ cm}^{-1}$ spectrum, it is the group region of the infrared spectrum. The important groups of organic compounds have strong absorption in this spectral range, so the spectra recorded in this region are the characteristics of the main reactive groups. The absorption peak of the infrared spectrum is related to the characteristic group contained in the molecule, so the

absorption peak is often able to provide information about the molecular structure of the compounds.

As shown in Fig. 4 (0-9 shows 4500-0 cm^{-1} , interval of 500, the same below), two peaks appear at 3440.4 cm^{-1} and 3445.5 cm^{-1} , respectively, which correspond to N-H bond vibration in IR spectra. From the modified compound APTES, the symmetric extension and asymmetric extension of N-H should correspond to the two peaks of different locations on the infrared atlas. The diagram is consistent with the theoretical analysis. It can be proved that we have successfully modified the APTES to the quartz substrate.

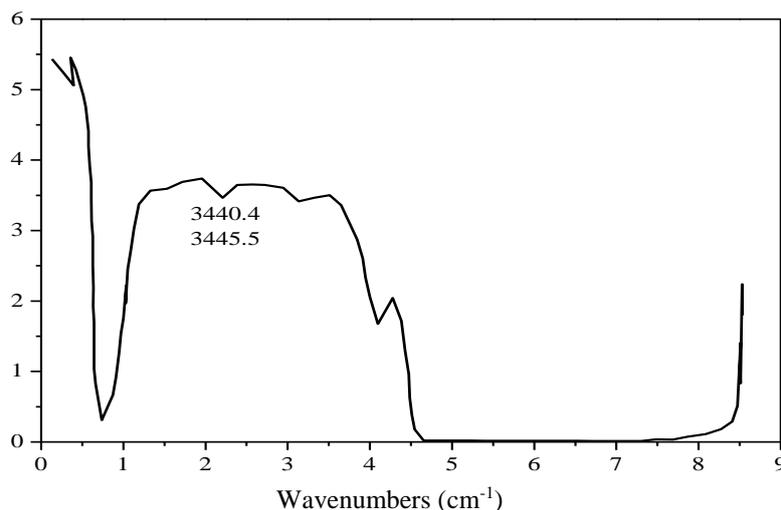


Fig. 4 Infrared transmission spectrum analysis of APTES modified quartz film

Fig. 5 shows a peak at the wavenumber of 2848.2 cm^{-1} , which corresponds to the stretching vibration the C-H bond in the aldehyde group in IR analysis. It corresponds to alkyl carbon atoms in glutaraldehyde molecules, which has modified a strong evidence of glutaraldehyde molecules for the quartz surface.

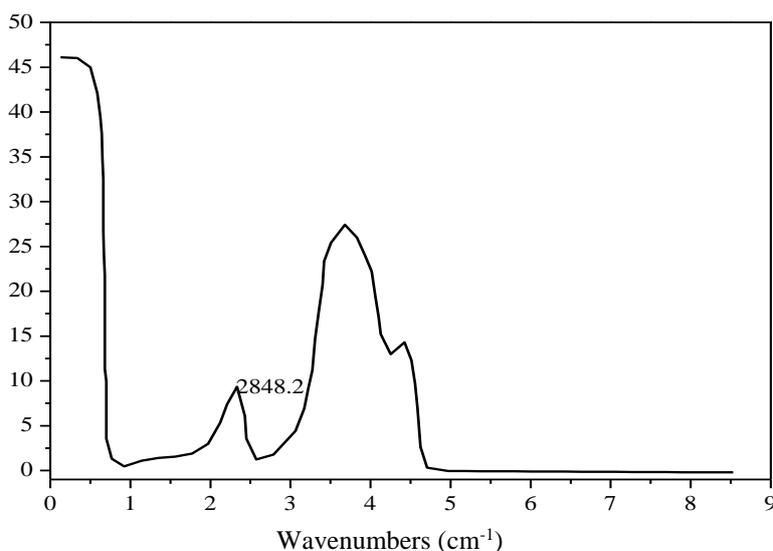


Fig. 5 Analysis of APTES-glutaraldehyde modified surfaces by infrared transmittance spectroscopy

The X ray photoelectron spectroscopy (XPS) instrument uses conventional X-ray (Mg $k\alpha$ and Al $k\alpha$) as the excitation source to determine the surface element, composition and chemical state of the sample. In this experiment, the XPS analysis uses the Al target, the $k\alpha$ ray energy is 1486.5 eV, and the photoelectron energy spectrum is calibrated with the peak value 285.1 eV of the C-C bond energy spectrum in C1s.

The data in Table 1 are the percentages of each element in the samples. According to the data from the table, the ratio of C and N atoms on the surface of the APTES and GA modified quartz plates is 9.5 and 10.7, respectively, which are slightly higher than the theoretical values. We analyze that it is due to the high concentration of reagents and long reaction time in the modification reaction, so that what formed on the quartz monolayer is not a homogeneous single molecule, but multiple molecular aggregation phenomena in some locations. The information detected by XPS is derived from molecules gathered outside. Some of these molecules may not react with the substrate, but they are aggregated with each other, resulting in a slightly higher ratio.

Table 1. APTES, GA Modified XPS data on quartz wafer surface and XPS data on quartz background

Sample	C1s	O1s	Si2p	N1s
Quartz	0.19	72.57	34.88	1.03
APTES-quartz	36.70	43.15	15.25	3.83
GA-APTES-quartz	53.77	28.18	12.45	5.03

Properties and characterization of polyacrylamide gel membranes

Polyacrylamide gel membrane is a common electrophoretic medium and material applied in the field of molecular biology and medicine. The following atlases are characterized by using quartz as the surface of the solid phase carrier. Fig. 6 (0-8 represent 4000-0 cm^{-1} respectively, interval of 500) shows that, in the infrared spectrum of polyacrylamide, we can observe that the wave number has two absorption peaks in the vicinity of 2496.3-3205.5 cm^{-1} , which corresponds to the characteristics absorption peak of the combination group vibration in the infrared spectrum table. It can be proved that we have successfully modified the polyacrylamide onto the quartz.

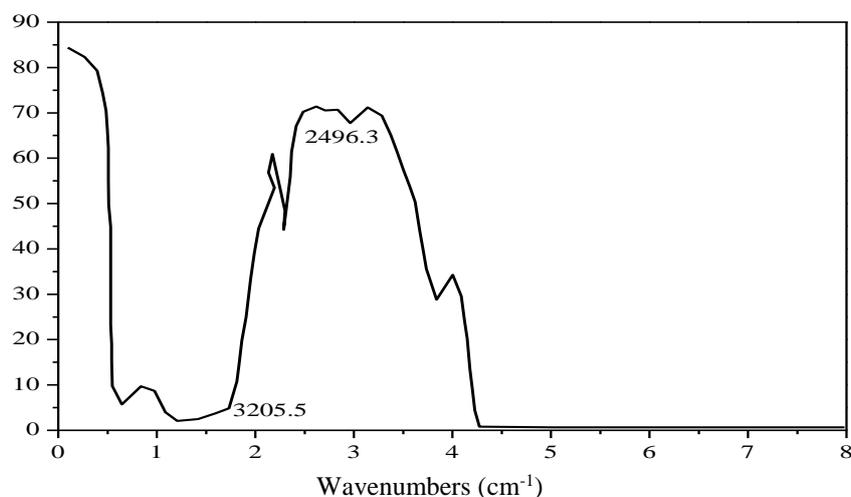


Fig. 6 Polyacrylamide infrared transmission spectroscopy

The XPS data sheet for polyacrylamide is presented in Table 2. The data in the table are the percentages of each element in the samples. From the data in the table, it is seen that the proportion of C and N on the surface of quartz modified by polyacrylamide is approximately 2.2:1. It is almost the same with the proportion of C and N elements in the molecular structure of polyacrylamide, which also confirms that we have successfully modified the polyacrylamide gel film.

Table 2. XPS data of polyacrylamide modified quartz plate surface and quartz background XPS data

Sample	C1s	O1s	Si2p	N1s
Quartz	0.19	72.59	34.88	1.03
Polyacrylamide-quartz	36.70	15.25	3.83	16.40

Properties and characterization of agarose gel membrane

Agarose gel membrane is one of the most commonly used electrophoretic carriers and mediators in modern molecular biology and medicine. Agarose is a chain polysaccharide prepared by agar separation. From the infrared atlas in Fig. 7 (0-8 indicate 4000-2600 cm^{-1} , interval of 200, the same below), we can see clearly that wave number has a very wide hydroxyl peak from 3200-3600 cm^{-1} , which is a typical peak of carbohydrate. As a result, we can conclude that the agarose has been modified on the surface of the quartz.

The molecular structure of agarose was almost unchanged after being activated by sodium periodate. Only adjacent hydroxyl groups were oxidized or at least partially oxidized to aldehydes under mild conditions. Because the aldehyde group is less than 1% in the molecular structure, there is hardly any difference in the infrared atlas in Fig. 8, so it cannot be detected. But we can still see that in this picture, the wave number has a very wide hydroxyl peak from 3200-3600 cm^{-1} , which further confirms that we have modified agarose on quartz. On the other hand, the stability of agarose can be confirmed, and it has not been denatured after oxidative activation.

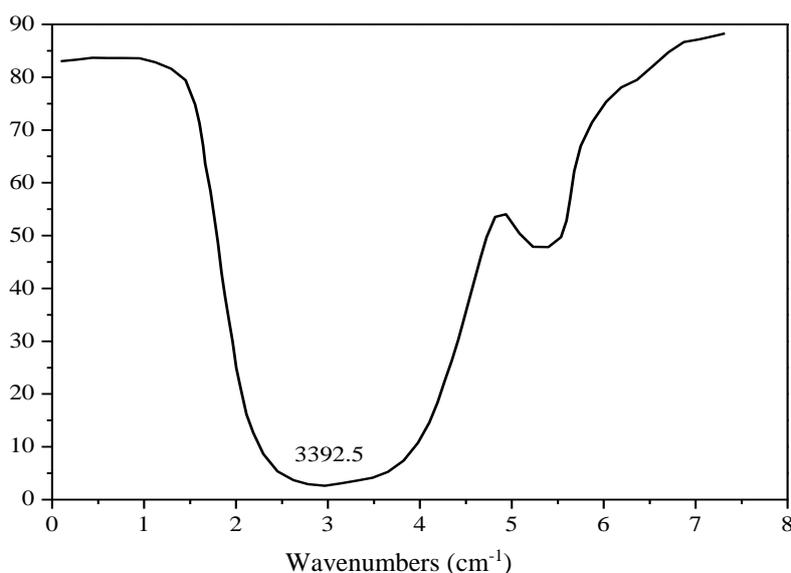


Fig. 7 Agarose infrared transmission spectrum analysis

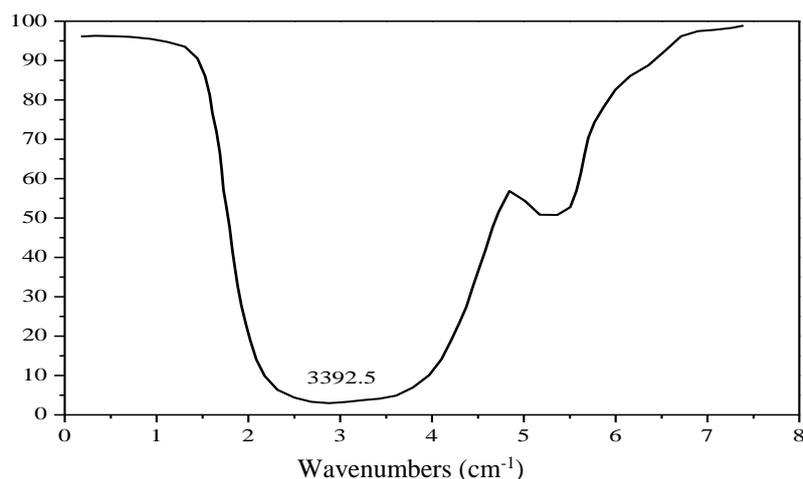


Fig. 8 Analysis of agarose infrared transmission spectrum after activated sodium periodate

Agarose is a typical sugar substance, which contains only three elements, C, H and O, and does not contain N element. Therefore, the kinds of elements are different from those of previous methods. The XPS characterization atlas also contains only three elements of C, O and Si, without N element. This further proves that we have successfully modified the agarose. The following is the XPS data sheet for agarose:

The data in Table 3 are the percentages of each element in the samples. The data in the table shows that, the proportion of C and O on the surface of agarose modified silica is 93.05%, which is consistent with the C and O elements in the molecular structure of agarose. This is also one of the evidences that we have successfully modified the agarose gel film.

Table 3. XPS data on agarose modified quartz plate surface and quartz background XPS data

Sample	C1s	O1s	Si2p	N1s
Quartz	0.19	72.59	34.88	1.03
Agarose-quartz	32.85	60.19	3.83	16.40

Comparison of the molecular efficiency of immobilized proteins with different modified substrates

Immobilization and characterization of protein molecules

We fixed the same concentration of FITC- Goat anti-human IgG on different modified glass slides, and observed the efficiency of different methods to modify glass slide protein by fluorescence microscope. Among them, APTES and APTES-GA modified FITC-Goat anti-human IgG, FITC labeled Goat anti-human IgG reached 8 times more than the dilution of fluorescence that was almost undetectable on FITC-modified polyacrylamide; Anti-human IgG of FITC-sheep on polyacrylamide modified glass diluted to more than 16 times, which almost cannot be detected by fluorescence; for agarose modified FITC-Goat anti-human IgG, FITC labeled Goat anti-human IgG diluted to more than 32 times, which almost cannot be detected by fluorescence. From the above analysis, we can see that the agarose immobilization effect is the best and the fluorescence signal intensity obtained is higher. The polyacrylamide is better than APTES-GA and APTES-GA is better than APTES, and APTES protein immobilization has the worst effect.

Comparison of immobilization ability of different modified substrates to protein molecules

The fluorescence spectrophotometer is based on the biochemistry, especially the principle of molecular biology. Matter is irradiated by light, emitting light of longer wavelengths, which are called fluorescence. Using photomultiplier tube to detect the intensity and spectral characteristics of fluorescence, the component of the sample can be analyzed qualitatively or quantitatively [7]. In this paper, FITC labeled Goat anti-rabbit IgG was used, with the excitation wavelength of 495 nm and the emission wavelength of 520 nm. Therefore, the emission intensity of this study was measured at 520 nm wavelength. The results were shown in Fig. 9 (0-5 represent 32, 16, 8, 4, 2, and 1 dilution times, respectively).

From Fig. 9, it is seen that fluorescence intensity curve of agarose modified surface immobilized fluorescent labeled Goat anti-human IgG has always been at the polyacrylamide modified surface; fluorescence intensity curve on the polyacrylamide modified surface of immobilized fluorescence labeled Goat anti-human IgG is always higher than the fluorescence intensity curve of APTES-GA modified surface; the fluorescence intensity curve of APTES is the lowest, and the lowest only detected 4 times diluted concentration fluorescence, while the fluorescence intensity of agarose modified glass slide can detect fluorescence diluted 32 times.

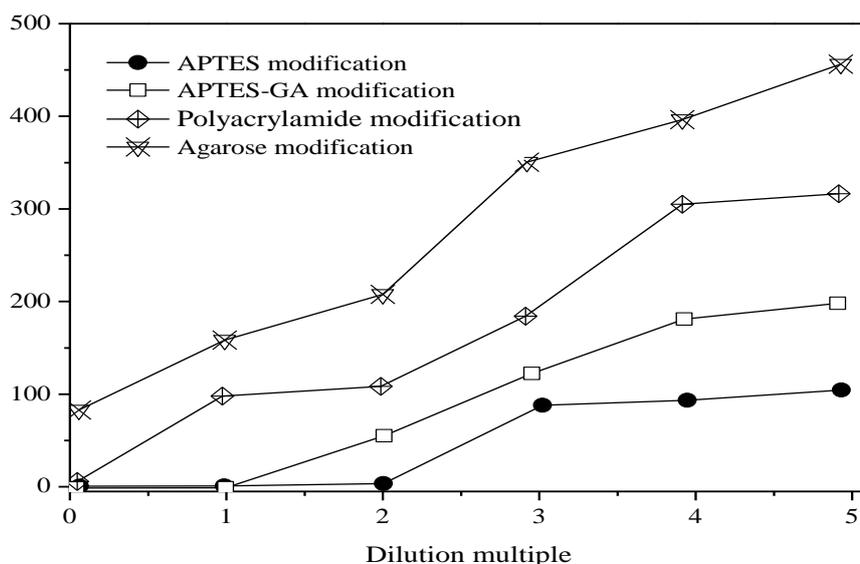


Fig. 9 Fluorescence intensity determination of fixed protein (FITC-goat anti-human IgG) on glass slides

Therefore, we can further explain that under the same conditions, the amount of protein molecules immobilized on the agarose modified glass surface is the highest, followed by polyacrylamide. The immobilization effect of APTES-GA is lower than that of polyacrylamide, and that of APTES modified glass is the least.

Results and analysis

Determination of saturation concentration

With a fixed concentration of receptor protein and different concentrations of fluorescent ligands incubated together, the specific binding and ligand concentration map can draw a rectangular parabola, that is, the saturation curve. Then, the maximum binding capacity of the

receptor B_{\max} and the equilibrium dissociation constant K_d can be obtained by Scatchard analysis (Figs. 10 and 11). The Scatchard equation is as follows:

$$B/F = -B/K_d + B_{\max}/K_d.$$

The straight line in Fig. 10 is $y = -0.17x + 25.72$, $K_d = 5.95 \times 10^{-8}$ mol/L.

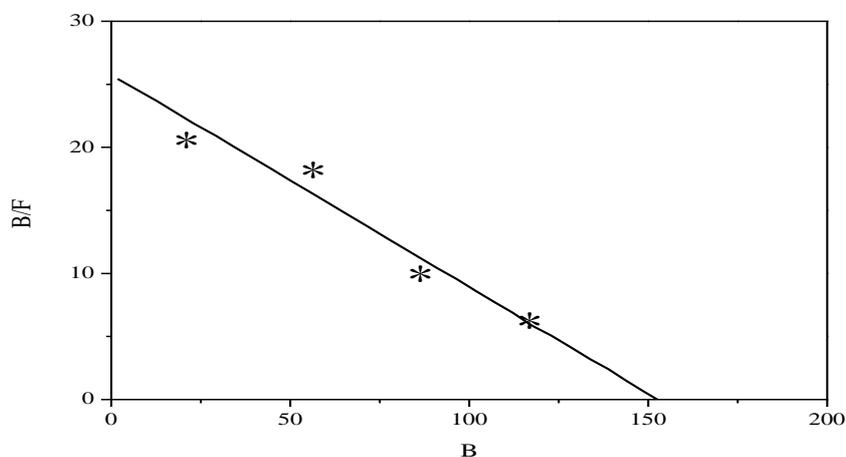


Fig. 10 Determination of saturated estrogen concentration – Scatchard analysis of mapping

The straight line in Fig. 11 is $y = -1.61x + 29.75$, $K_d = 6.30 \times 10^{-9}$ mol/L.

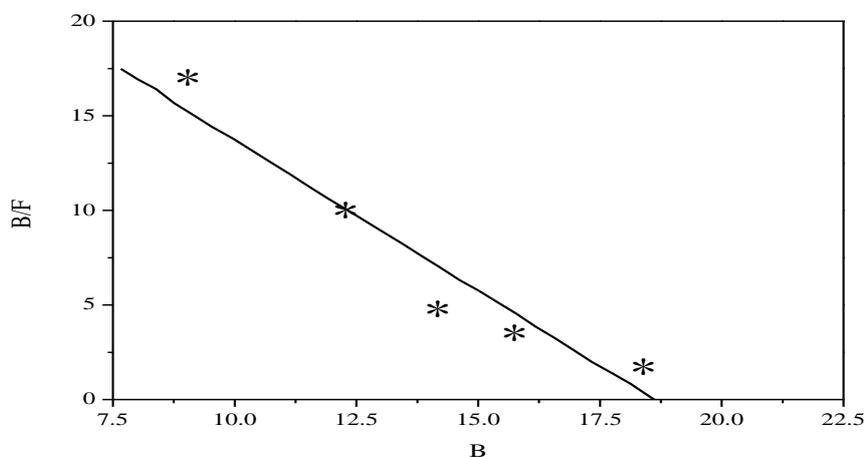


Fig. 11 Determination of androgen saturation concentration – Scatchard analysis of mapping

Determination of the content of stimulant molecules by competition method

The competition binding test is used to determine the ability of non-labeled drugs to compete for the same receptor with fluorescent ligands. Unlabeled drugs are usually used to show the concentration of 50% of the fluorescent ligand binding to the receptor (IC_{50}). If we compare the intensity of various non-labeled drugs, the apparent dissociation constant K_i can be obtained and the formula is as follows. The compound I is a competitive inhibitor, and the binding of L or I to the receptor is reversible. When L and I compete with R at the same time, according to the law of mass action, we can get the following result:

$$K_i = IC_{50} / (1 + L / K_d).$$

The calculation of IC_{50} : first of all, the fluorescent ligand concentration is stipulated, the tested drug 6-7 different concentrations are selected, and then the inhibition percentage of tested drugs combined with fluorescent ligand and receptor ($I\%$) is calculated and the formula is: $I\% = (\text{total combined pipe RFU} - \text{competition binding pipe RFU}) / (\text{total combined pipe RFU} - \text{non-specific binding pipe RFU}) * 100\%$. A dose-effect semi logarithmic drawing method is applied to draw the S curve and the most effective method transforming S curve into straight line is the logit conversion method, $\text{logit} = \text{Ln}(I / (100 - I))$. Using logit-log (I) drawing the figure, IC_{50} is the concentration of drug when $I = 50$ and the logarithm is zero.

The straight line in Fig. 12 is $y = -1.30x - 11.05$, $IC_{50} = 2.70 \text{ mol/L}$ and $K_i = 1.45 \text{ mol/L}$. This shows that the lower limit of estrogen detection is 10^{-9} mol/L , and the linear range is the best in 10^{-7} - 10^{-8} mol/L .

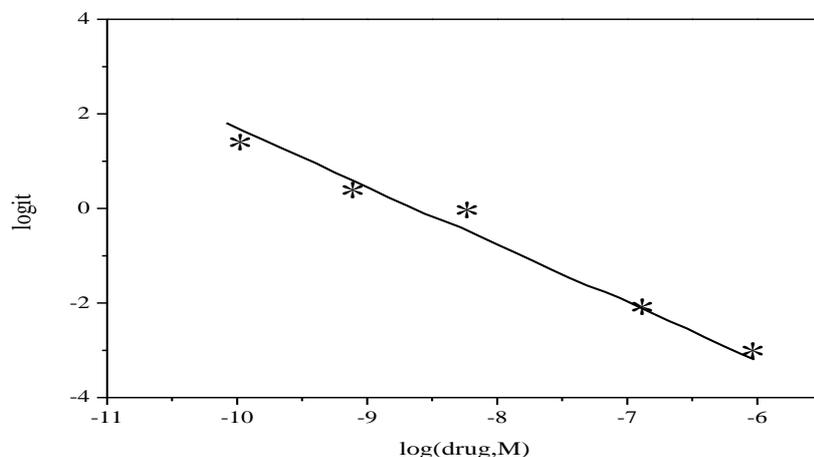


Fig. 12 Competition curve of estrogen samples

The straight line in Fig. 13 is $y = -0.88x - 7.60$, $IC_{50} = 2.20 \text{ mol/L}$ and $K_i = 0.86 \text{ mol/L}$. It can be seen that the lower limit of androgen test is 10^{-9} mol/L , and the linear range is the best in 10^{-8} - 10^{-9} mol/L .

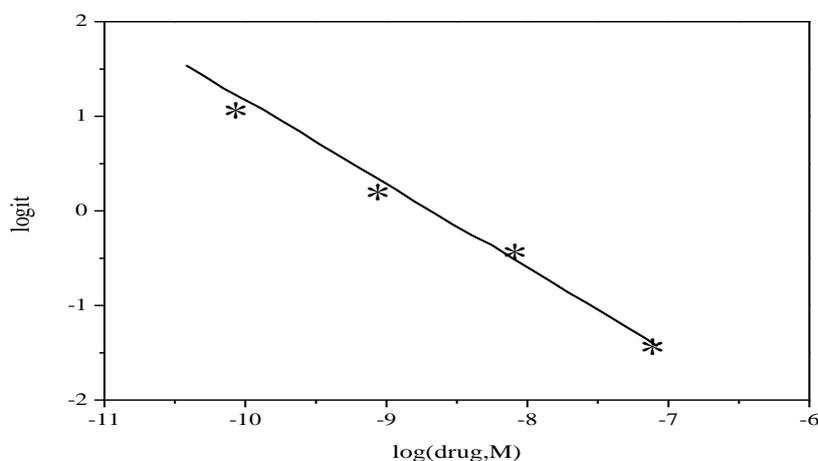


Fig. 13 Competition curve for androgen samples

Performance error of fiber optic biosensor

The performance of the signal acquisition path we have set up is measured and tested to see if it is in accordance with the requirements of the experimental accuracy. A group of ideal working concentrations were used to fix the receptor, and the fluorescence detection was carried out by fiber optic biosensor. The purpose is to verify whether the error of fluorescence intensity detected by optical fiber biosensor is within the allowable error range of normal experiment, that is, to measure the accuracy of fiber biosensor we use. Under the same condition, the sample under working concentration was measured 8 times. The experimental data are shown in Table 4.

Table 4. Error analysis

The number of measurements	1	2	3	4	5	6	7	8
Data result	602.2	598.4	600.5	601.8	599.9	600.1	600.8	603.1

It is known from the calculation that the experimental error is $X/S = 0.25\%$. It is within the range of normal system error allowed by the experiment.

In the experiment, the substrate we used is the glass slides modified by agarose gel membrane. In general, 100 pieces of agarose slides were made in one time and they were stored and activated before being used. The agarose modified glass slides were stored at $-4\text{ }^{\circ}\text{C}$ for light and moisture retention. The fluorescence intensity of FITC was measured every 10 days; that is to say, the experiment is repeated every 10 days to detect the fluorescence intensity at ideal working concentration. The obtained fluorescence intensity data are shown in Table 5.

Table 5. Stability analysis

The number of measurements	1	2	3	4	5	6
Data result	601.1	597.7	590.0	571.9	483.1	321.4

By measuring the fluorescence of the sample for two months, we found that agarose can be preserved for more than a month in the condition of avoiding light and moisturizing and the stability is ideal.

Conclusion

The optical fiber biosensor is used as the hardware platform, and for the needs of modern sports, optical fiber biosensor is developed for the detection of hormone stimulants in competitive sports. In the analeptic optical fiber biosensor, glass and quartz are selected as solid phase carrier for different chemical self-assemblies on it. And in terms of the ligand protein immobilized on the receptor and fluorescein labeled, the competition method is applied to transform the content of target biological samples into the fluorescence intensity of fluorescein. The fiber spectrometer is used for visual feedback of the results. A fast and accurate fiber optic biosensor for detecting analeptics is developed, and the reliability, accuracy, stability and reproducibility of the sensor are verified. The future research work can further improve the surface modification method and optimize the combination of light path, so as to improve the sensitivity, accuracy and anti-interference ability of sample detection.

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