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Tapered Optical Fiber Bio-Sensor for Testosterone Detection

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Abstract

A large penetration depth of an evanescent wave is the key to success for developing fiber-optic evanescent field sensors. A tapered optical fiber was fabricated through chemical etching method by HF acid. The penetration depth of an optical fiber, stripped off its cladding, is related to the wavelength of incident light, the refractive index of the surrounding medium and the incident angle. Molecular imprinted polymer is used to synthesis the Testosterone receptor which acts as a sorbent material to detect the derivatives of TES. The increase in TES absorption leads to change in refractive index, considerably varying the output intensity of the optical fiber.

Key words: — Tapered Fiber Sensor, Testosterone, Chemical Etching, Evanescent Field Sensors.

1. Introduction

Anabolic and androgenic steroids (AAS) are synthetic substances relating to the male sex hormone, testosterone (androgen). AAS promote the growth of skeletal muscle (anabolic effects) and the development of male sexual characteristics (androgenic effects)[1][3]. AAS are abused by bodybuilders, weightlifters and other athletes in human sports and horse racing as well, and are prohibited by the International Olympic Committee. Their use in horse racing has also been prohibited by the Association of Racing Commissioners' International (ARCI), and they are, therefore, included in the list of prohibited substances in the horse by ARCI[2]. GC-MS is the technique commonly used by forensic laboratories for screening and confirmation of AAS but limited by insensitivity, and tedious procedure [5].

Fiber-optic biosensors (FOBS) have been widely investigated because of their potential sensitivity, detection speed, and adaptability to a wide variety of assay conditions. FOBs measure absorbance, scattering, or fluorescence due to the analyte of interest using a reflection or transmission scheme, such as interferometric immunosensors [10] using long period grating, biconical tapered fiber sensors[9], have been used for both antigen and pathogen (bacteria, etc.)[4] [6] detection. These transmission techniques involve immobilizing antigen. In the present work we have developed a Fiber Optic biosensor to determine the presence of testosterone and derivatives of testosterone using molecular imprinting polymer technique (MIP)[16].

2. Experimental

A. Chemicals Required

Sustanon 250 mg, its pharmaceutical name is Testosterone. It has considerable anabolic and androgenic properties, designed to maximize the synergistic effect of using four testosterones. Total plasma testosterone levels peak approximately 24 hours to 48 hours after administration. Plasma testosterone level returns to the lower limit of the normal range in males in approximately 21 days. Sustanon is a unique blend of 4 different esters of testosterone, Testosterone propionate, Testosterone phenylpropionate, Testosterone isocaproate and Testosterone decanoate. The purpose of attaching an ester to a steroid is to make it more lipophillic, so that when injected intra-muscularly it can remain in the adipose tissue longer and is released in the blood stream over a period of time.

B. Synthesis of Testosterone Receptor

In terms of affinity for the various polymers, testosterone is the most strongly retained compound on the imprinted polymers but â-estradiol is also quite well retained. â-Estradiol has a phenolic A-ring and lacks the C18 methyl group, making the A and B rings fairly planar. Testosterone has a chair, chair structure with a rather sterically demanding C18 methyl group.

More importantly, the phenol of â-estradiol and the R, â-unsaturated ketone of testosterone point in different directions. In spite of these differences in structure and the orientation of their H-bonding functionalities they bind very strongly to the nonimprinted polymer. These are the only steroids possessing a hydroxyl group at the 17th - position and it therefore appears that this single factor is responsible for the affinity of these compounds for both the imprinted and nonimprinted polymers.

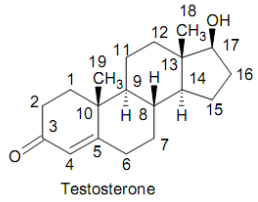

Fig 1. Structure of Steroid

C. Molecular Imprint Technique

The general procedure for creating these MIPs involves

(i) The assembly of polymerizable "functional monomers" around a "template" molecule in a solution containing a high ratio of cross-linker,

(ii) Polymerization of the mixture, and

(iii) Removal of the template to afford the imprinted polymer.

Testosterone (TES) is insoluble in water, but it will dissolve in hot ethanol. For 1M in 10ml, 2.885 g of TES is dissolved in 10ml of ethanol. 1ml of TES contains 250 mg of four different esters, therefore for a 2μL of TES has 0.5003 mg of ester compound.

The covalent imprinting method involves the copolymerization of functional monomers and a template molecule conjugated to a polymerizable group. After polymerization the template is "split off" from the polymer to expose the binding sites. The main disadvantage of covalent imprinting is that, at least for esters and amides, only a small percentage of the templates can be removed from the microcavities. Furthermore, the EGDM cross-linker is also hydrolyzed under the splitting conditions and this result in a more swellable and less selective polymer.

D. Polymerization

The procedure for the synthesis of the standard polymer by MIP technique is as follows. According to the work of Mosbach and Sellergren[18], each template requires 3 or 4 equiv of functional monomer in order to produce a sufficiently selective polymer. The template was prepared by 1mml of TES was dissolved in $CHCl_3$ (7.50 mL) in a 50 mL glass flask, and benzonitrile (100 mg), ethylene glycol dimethacrylate [EGDM] (5.00 mL), and the functional monomer acrylic acid [A] (8 mmol) were added.

Benzonitrile acts as an initializer and EGDM act as a cross linker. The samples were then purged with nitrogen. The flasks were then sealed and the mixtures heated at 40 °C for at least 16 h. The bulk polymers were then ground and washed with boiling ethanol until the template could no longer be detected (*λ*=238 nm) in the supernatant.

E. Fabrication of Tapered fiber

One of the simplest ways to create tapers is to use HF as the etching agent. As the fiber is etched, the conditions governing the propagation of light inside the fiber change with the decrease in fiber diameter[8]. To monitor the progress of etching, it is desirable that the fabrication method incorporates a means to measure the light coming in at the output end of the fiber. This will allow the precise control of the etching process and consequently, the management of the diameter of the tapered region. Studies by Sayah et al. have shown a strong correlation of HF etching rates with concentration at room temperature. The dependence in low concentration rage (2/24%) has been claimed to almost quadratic. Etching rate increases with higher concentration of HF. In light of their study and the convenient commercial availability of 49.5 wt % HF is been used.

During the etching process, the diameter of the fiber declines in the region exposed to HF. HF reacts with silica according to the following reactions, with the second reaction dominating at high HF concentration.

$$SiO_2+4HF \rightarrow SiF_4+2H_2O \quad (1)$$

$$SiO_2+6HF \rightarrow H_2SiF_6+2H_2O \quad (2)$$

In the experiments conducted the exposed region is 7 mm. The fiber is surrounded by 49.5% (w/w) HF uniformly. Total volume of HF solution added to the chamber is 15 ml. The total amount of fiber material that can potentially react and dissolve in HF is 0.054 μmol and the amount of HF present in the etching chamber is 375 μmol. Total HF consumed by the dissolution reaction (2), is less than 0.5%

HF followed by the entire reaction chamber being washed twice with NaOH as rapidly as

possible. Fiber was rinsed with NaOH to remove any remnant HF left on the fiber surface or in the Si-O- Si structure inside the fiber would continue to etch the fiber, further reducing the fiber diameter, and often caused the taper to dissolve completely.

Etching was stopped at different times by the procedure described above, and the resulting fiber diameter was measured using an optical microscope (model IMT-2, Olympus, Japan) equipped with a video camera linked to a computer. SCION IMAGE software (Scion Corp.) was used to acquire the microscope images from the camera. With large experimental trials, it became clear that the length of the etching time needed at room temperature was in the order of about 12 min from 125μm to 61μm for a multimode fiber (125 μm/62.5 μm).

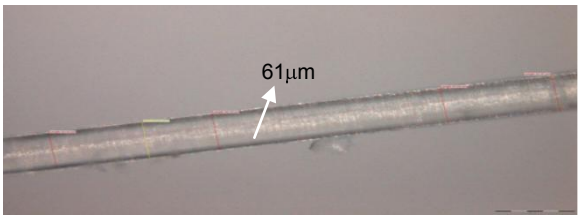

Fig. 2. Image of Tapered MMF with exposed core region.

F. Device Setup and Sensing

The TES receptor synthesized by the MIP technique is coated over the tapered region of the optical fiber. When a beam of light propagates along an optical fiber, the electromagnetic field does not abruptly fall to zero at the core/cladding interface. Instead, the overlap of the incoming beam and the internally reflected beam leads to a field that penetrates into the medium next to the core. This electromagnetic field, which tails but does not propagate into the second medium, is called the evanescent wave, its intensity I(z) decays exponentially with the distance z perpendicular to the interface as follows:

$$I(z) = I_0 \left(\frac{z}{d_p}\right) \qquad (3)$$

where I_0 is the intensity of the incident radiation. The depth of penetration d_p of the evanescent wave is related to the angle of incidence θ at the interface, refractive indexes of core n_1 and cladding n_2, and the wavelength of the radiation λ as follows [4]

$$d_p = \frac{\lambda}{2\pi\sqrt{n_1^2 sin^2\theta - n_2^2}} \qquad (4)$$

The evanescent wave is able to interact with TES receptor within the coating. This mechanism has been employed in rather ingenious way to develop fiber-optic evanescent wave sensors. In this kind of sensors, the parameter measured is the light intensity carried by the fiber that is modulated by the change in the absorption in cladding region [11].

The optical fiber was a standard multimode optical fiber with core and cladding diameters of 62.5 and 125 μm, respectively. The unpolarized laser light from He–Ne laser source (<5mW) was passed from one end of the fiber and the output intensity was measured at the other end of the optical fiber.

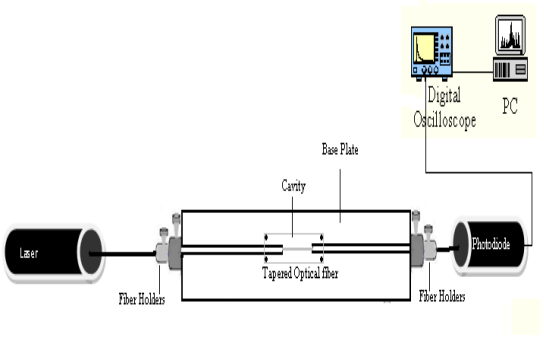

Fig. 3. Schematic illustration MIP receptor coated sensing element in a device assembly.

During our experimental observations setup, the analyte was prepared by above said method and made to interact with the polymer receptor in order to act as a sorbent substance. A closed cavity was formed where the analyte sample solution and the TES receptor coated tapered fiber is enclosed. To increase the concentration of TES, 1μL to 10μL of TES is added with 10 ml of ethanol. A thermometer with least count of 0.1 ◦C was also positioned together for monitoring the temperature inside the chamber. The complete experimental setup is shown in Fig.3. All experiments were conducted at room temperature (25◦C).

3. Characterization & Results

The produced template has been characterized using Thin Layer Chromatography, UV-Visible Spectroscopy, IR Spectroscopy techniques and shown. The tapered optical fiber was employed with TES receptor as clad with a desired thickness as homogenous cast as possible.

A. Thin Layer Chromatography

Synthesized polymer can be Verified by Thin Layer Chromatography(TLC). The below figure clearly shows that polymer is formed. Testosterone, benzonitrile, EGDM, Acrylic acid and testosterone receptors are placed in silica gel plate through micropipette. The plate is

placed inside the beaker where solvent is kept. The solvent absorb and move up. All components will travel at different speed and have different spot. This shows and conform that polymer is synthesized.

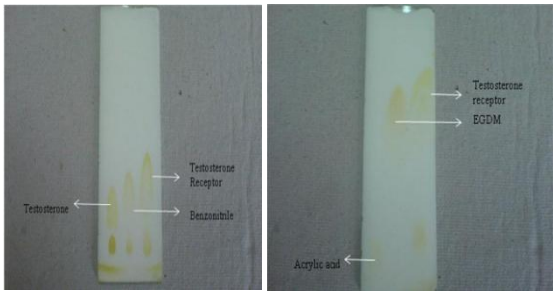

Fig. 4. TLC method identifying polymer receptor

B. UV Absorption Spectra

Wavelength absorption of TES is 238nm respectively (gathered from literature). The absorption spectra taken by spectrophotometer(Hitachi u – 2800), shows peak at 290nm. Since, TES used in this work mixed with oil.

At different concentration, variation in absorbance can be seen. Here as the concentration increase absorption also increase. The graph shows the absorption peak at 1.19 for 15µL concentration.

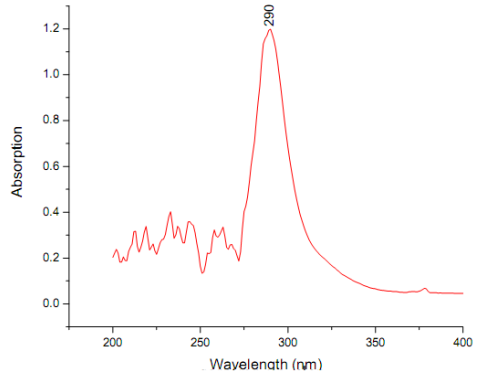

Fig. 5. UV Spectra showing absorption of TES

According to Beer – Lambert Law,

Absorbance, $A = \log_{10} P_0 / P$

Transmittance was calculated to be 6.4% for the above concentration. If absorption increase than transmittance start to decrease and the relation is shown by different concentration. For concentrations of 2, 4, 6, 8, 10 and 15 µl of TES is dissolved in hot ethanol and corresponding absorption peak is taken.

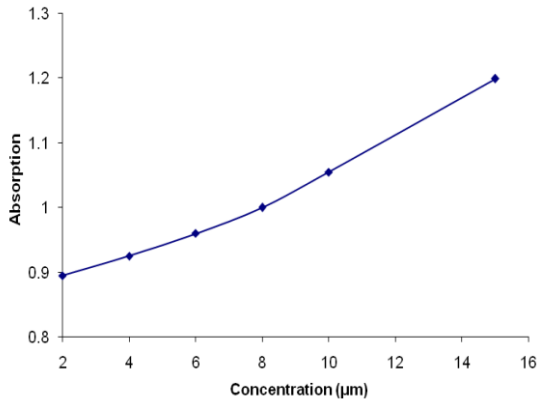

Fig 6. Absorption at different concentration.

C. IR Spectroscopy Method

TES contain O and OH groups. Similarly EGDM, acrylic acid has some functional group. We have to see the TR functional groups through FTIR spectroscopy (Thermo Nicolet – AVATAR 330). IR spectroscopy was taken to all individual compounds and for resultant TR. The result shows peak at 3428 cm $^{-1}$, these conforms that some chloroform is still present in the product. Since chloroform is a solvent we don't take much care on it. Below 1723 cm-^{1}, all peaks are same as Ethylene glycol dimethacrylate peak. Between 3200-3500 cm^{-1} alcohol (OH) groups, H-bonding is present. Similarly between 2850-3000 cm^{-1} alkanes, CH_3, CH_2, CH group, 2240-2260 cm^{-1} Nitrile group, 1705-1720 cm^{-1} Carboxylic acid group, C=O (H bonded) and 2100-2270 cm^{-1} -N=C=O, -N=C=N- group will present.

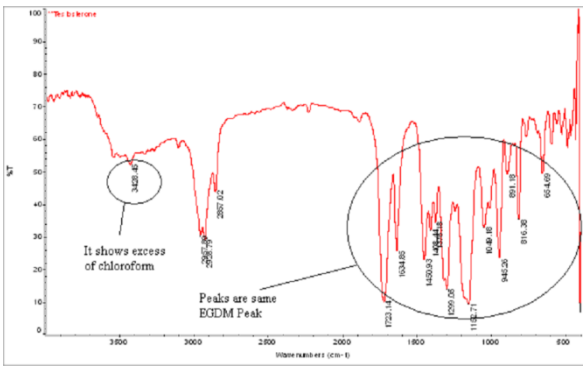

Fig.7. IR Spectral analysis confirming TER Receptors

D. Testosterone sensing behavior

The TES sensing behavior of TES receptor coated tapered optical fiber is evaluated with the change in optical permeability of the sensing probe as a function of concentration of TES binding factor and was recorded in a closed chamber at room temperature. Untapered optical fiber output is -780mv and for tapered optical output is -560 mv. When the tapered fiber is coated with TES receptor and

immersed at different concentrations from 1µl to 10µl of TES in 10 ml of ethanol, the variation in optical intensity is shown

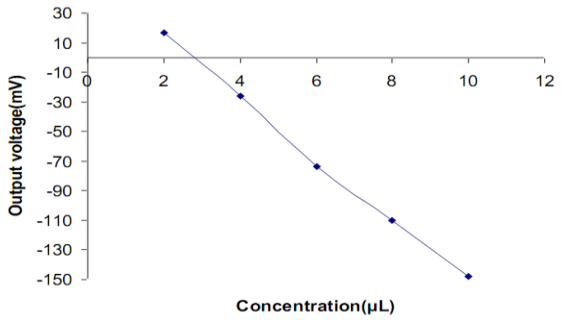

Fig.8. Linear relationship between optical intensity variation and concentration of TES

4. Conclusion

MIP technique is used to increase the selectivity of TES steroid and this act as a sorbent to TES. When the concentration is increased, density also increased and hence refractive index changes. According to the change in the refractive index the output intensity too varies. The resultant TES receptor formed has the refractive index of 1.410 which is verified by Abbe's refractometer.

As concentration of TES increases absorption also increases and transmission start to decrease and hence optical intensity attenuates. Intensity is different for different waist diameter and length and RI. The velocity of light in the medium is reduced when concentration of TES increases and so RI increases. Sensitivity that achieved through this work is 250mg/ L and resolution is 20mv/µL. N.A is high for this optical fiber. This sensor has high sensitivity and selectivity with greater affinity towards all kind of TES species.

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