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ORIGINAL ARTICLE

Synthesis of Silica Nanoparticles Coated by Poly Dopamine Imprinted Polymer for the Determination of Testosterone in Capsules

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KEYWORDS

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ABSTRACT: This study outlines the synthesis and initial evaluation of poly dopamine molecularly imprinted polymers (MIPs) designed for the selective extraction of testosterone. The resulting polymer was utilized as a solid-phase extraction (SPE) sorbent material. The molecularly imprinted polymer was created using a reaction mixture that included silica, the template (testosterone), a functional monomer (dopamine), and water as the solvent. Core-shell structure MIP beads were formed through precipitation and oxidative polymerization. During the polymerization process, a complex was formed between the template and the functional monomer, resulting in a three-dimensional polymer network that encapsulated the template molecules upon completion of polymerization. Subsequently, the template molecules were removed through washing. A control polymer, which was a non-imprinted polymer, was synthesized under similar conditions without the template to facilitate a comparative analysis of performance. Characterization of the synthesized polymer was conducted using Fourier Transform Infrared Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and Thermogravimetric Analysis (TGA). Optimization of parameters such as pH, extraction time, and adsorption capacity was performed. Ultimately, the concentration of testosterone in urine samples was quantified using UV-Vis spectrophotometry following preconcentration with the synthesized MIP. The results obtained demonstrate the effectiveness of the synthesized polymer in extracting testosterone from real samples.

INTRODUCTION

Testosterone is a hormone that is mainly made by your gonads (testicles or ovaries). Testosterone amounts are usually much greater in individual's assigned male at birth compared to individuals' assigned female at birth. If testosterone levels are either excessively high or excessively low, it may lead to specific symptoms [1].

The predominant techniques for quantifying testosterone are primarily those that utilize enzyme-linked immunosorbent assays (ELISA) [2-5]and radio immuno assay (RIA)[6, 7]. These techniques are implemented through the use of commercial kits. Nevertheless, immobilized antibodies must be disposed of immediately

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following their initial application in these assays. High-Performance Liquid Chromatography (HPLC) is an additional method employed for the quantification of testosterone [8, 9] The fluorescence reaction for testosterone has also been employed to determine testosterone levels in samples [10, 11].

The primary drawback of the method was the extensive duration necessary for these analyses. The preparation of target compounds from environmental, biological, pharmaceutical, and food matrices represents one of the most labor-intensive phases in analytical processes. This preparation can be executed through various techniques, contingent upon the type of sample; however, the method most frequently utilized today is solid-phase extraction (SPE). It is widely recognized that SPE employs a diverse range of sorbents, including C18 [12-14], copolymer styrene-divinylbenzene functionalized with different ligands [15-18] and etc. could be used.

Their selection depends on a series of factors that include the kind of sample, the selectivity, and sensitivity required, and cost. For the analysis of testosterone in various types of samples, by simple spectrophotometric determination, the selectivity of the sorbent is very important. Most of commercial sorbents do not have selectivity, and most adsorb polar or nonpolar compounds, and after extraction of the sample, a next separation step with the HPLC or GC method is required. Molecular imprinting is an appealing method for creating very selective polymer receptors that have artificially made recognition sites, enabling them to specifically reattach to a target molecule rather than other similar substances [4, 19-23].

These substances are created by linking together functional and cross-linking monomers around a template molecule, resulting in a very interconnected three-dimensional polymer structure [4, 24]. After the polymerization process, the template molecules are removed, creating binding sites in the polymer network that have shapes, sizes, and functions that match the target analyte. The resulting molecularly imprinted polymers exhibit stability, durability, and resistance to a wide range of pH levels, solvents, and temperatures. MIPs can act as selective sorbent materials for the purification and concentration of modified nucleosides from complex mixtures, like urine, before the separation methods usually used for their analysis. This study introduces, for the first time, the creation of a highly selective MIP for testosterone, which is used as a solid-phase extraction sorbent material, with the MIPs serving as the recognition component.

MATERIALS AND METHIDS

Material

All chemicals were obtained from Sigma-Aldrich, located in St. Louis, MO, USA, unless stated differently. Hydrochloric acid, sodium hydroxide, methanol, and TRIS buffer were bought from Merck. Ultrapure water was produced using a Milli-Q water purification system based in Bedford, MA, USA. Stock solutions of testosterone at strength of 1.0 mg per mL were made by mixing the correct amount in a methanol and water blend. These solutions stayed stable for 48 hours at room temperature. Working solutions of testosterone at a concentration of 2 mg L^{-1} were created by diluting the stock solutions with the methanol-water mixture. The pharmaceutical preparation, Utregestan® soft capsule, was produced by Besins Pharmaceutical Industries, France. The commercially obtained testosterone capsules were reported to contain 100 and 200 mg of testosterone per capsule, along with TiO_2 as an excipient.

Apparatus

A UV-VIS spectrophotometer (Varian-Cary100, Australia) was employed to quantify testosterone in standard solutions following interaction with the polymer. The soxhlet extraction apparatus facilitated the removal of the target molecule from the polymer network. pH adjustments were conducted using a model 3510 Jenway pH meter. The Fourier transform infrared (FTIR) spectra for the template molecule (p), non-imprinted polymers, and molecularly imprinted polymers were recorded using a 6700 Thermo Nicolet FTIR spectrometer, with infrared spectra captured over the range of 400-4000 cm^{-1}. The surface morphologies and particle sizes of the molecularly imprinted polymers coated on silica nanoparticles were analyzed using scanning electron microscopy (SEM; model VEGA, TESCAN, Brno, Czech Republic) equipped with a field

emission gun, as well as transmission electron microscopy (TEM; model Tecnai 20, FEI). TEM provides imaging capabilities at a significantly higher resolution compared to light microscopes, due to the reduced de Broglie wavelength of electrons.

Preparation of surface molecularly imprinted polymer for Testosterone

Silica nanoparticles (0.4 g) were first mixed in 20 mL of Tris buffer (10 mM, pH 8.5). After this, 0.2 g of dopamine (monomer) and 0.02 g of testosterone (template) were put in, and the blend was stirred mechanically for 12 hours at room temperature. This led to the self-polymerization of dopamine and the natural creation of a thin, sticking polydopamine (PDA) layer on the silica surface. Next, the testosterone molecules were removed from the PDA layer through washing, which helped form a testosterone-imprinted SiO2@MIPs composite. Soxhlet extraction was done overnight with a 70:30 (V/V) methanol/acetic acid mixture to get rid of the template. The polymer was then rinsed several times with pure methanol to clear away remaining acetic acid and assist in drying. The dried polymer is now ready for tests. The total removal of testosterone was confirmed through spectrophotometric examination of the washing solution. The process for making the non-imprinted polymer (NIP) was very similar to that of the molecularly imprinted polymer (MIP), except that the template (testosterone) was not included during the NIP preparation. The polymerization process is shown in Figure 1.

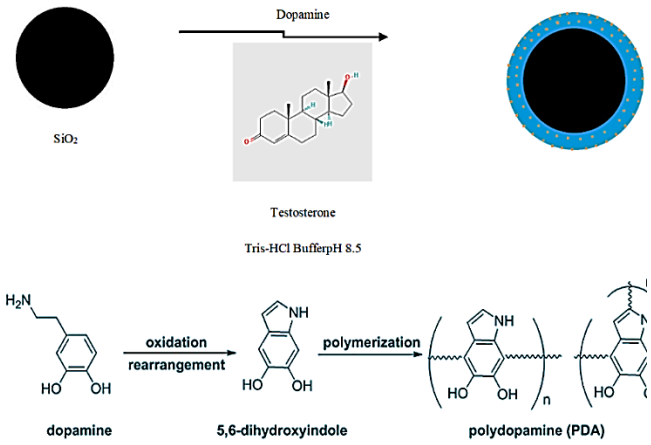

Figure 1. The polymerization mechanism

Batch rebinding experiments

To assess the binding affinity of the imprinted polymer, static adsorption was conducted by suspending 1 mg of molecularly imprinted polymers (MIPs) in varying concentrations of testosterone (ranging from 1 to 50 ppm). Following a stirring period of 15 minutes, the polymer particles were subjected to centrifugation at 11,000 rpm. The quantity of testosterone bound to the MIP was ascertained by calculating the difference between the total testosterone present and the residual amount remaining in the solution, utilizing a UV spectrophotometer. The binding capacity of the SMIP was determined using the following equation:

$$Q=\frac{(Co-Ce)V}{W} \qquad (1)$$

The variable Q represents the binding capacity (mg g^{-1}), while C0 and Ct denote the initial and residual concentrations (mg L^{-1}) of testosterone or its analogs, respectively. Additionally, V indicates the volume of the solution (mL), and M refers to the mass (mg) of the MIP or NIP particles employed in the adsorption experiments.

Assay of testosterone in capsules

The contents of ten capsules, each containing either 100 mg or 200 mg of testosterone, were ground using a

mortar and pestle. A precise aliquot of this powdered material was weighed and placed into a 100-mL volumetric flask. Ethanol was subsequently added, and the flask was agitated for 30 minutes. The volume was then adjusted to 100 mL, and the solution was filtered through paper. The procedure outlined in the batch procedure section was adhered to.

The concentrations of testosterone obtained through the proposed method are presented in Table 1. The results indicate that the method is effective in quantifying testosterone in complex samples, leading to the conclusion that it is suitable for determining testosterone in pharmaceutical formulations.

Table 1. Determination of testosterone in capsules.

Amount of drug in formulation (mg)	Amount of drug added (mg)	Amount of drug measured (mg)	Recovery%
100	0	98.12	--
	10	107.51	97.7
	15	110.2	95.8
200	0		
	10	201.1	95.7
	15	203.21	94.5

RESULTS AND DISCUSSION

The objective of this study was to assess the practicality of employing molecularly imprinted polymers (MIPs) as a selective solid-phase adsorbent for the extraction of testosterone from bovine milk.

FT-IR spectra

The synthesized molecularly imprinted polymers (MIPs) and the control polymers (NIPs) were analyzed using Fourier-transform infrared spectroscopy (FT-IR). The IR spectra of both polymers are comparable, suggesting a resemblance in their backbone structures (Figure 2).

The prominent peak observed at, 3431 cm^{-1} was associated with the asymmetrical stretching of aromatic O–H bonds. A peak at 1630 cm^{-1} was linked to the stretching vibration of C=C bonds. The peak at, 1519 cm^{-1} was derived from the scissoring vibration mode of NH2 groups. The peaks at 1103 cm^{-1} corresponded to various vibration modes, including CH2 bending, C–O–H bending, C–O symmetry, and C–C stretching. Additionally, the peak at 806 cm-1 was attributed to C-H vibrations. Notably, strong peaks at 471, 807, and 1103 cm^{-1} were associated with the vibrations of SiO2. In contrast, the FTIR spectrum of synthetic PDA@SiO2 nanoparticles displayed a significant and broad peak in the range of 3600–3200 cm^{-1}, indicating the presence of hydroxyl groups and water.

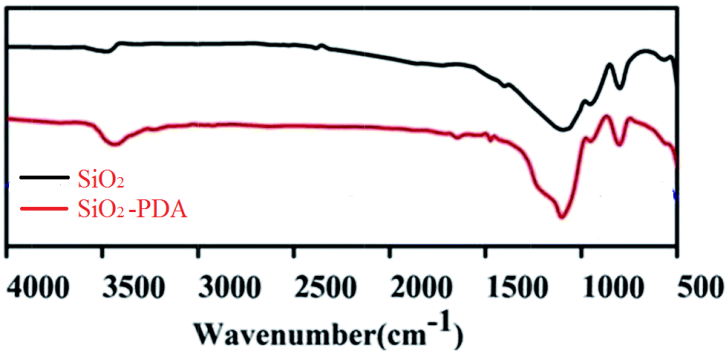

Figure 2. FTIR spectra of SiO_2 nanoparticles and PDA@silica nanoparticles

Morphology study

The morphological characteristics of MIP were analyzed using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). Figures 3 and 4 illustrate that the PDA@SiO_2 nanoparticles exhibit a spherical morphology with an approximate particle size of 30 nm. The core-shell architecture of MIP is distinctly observable in both SEM and TEM images. The silica nanoparticles and the polymer are clearly identifiable. The core, which appears darker, corresponds to the SiO_2 nanoparticles, while the lighter outer layer represents the MIP shell. The SiO_2 nanoparticles are encapsulated within the MIP layer. This core-shell configuration is anticipated to enhance mass transfer between the solution and the MIP surface.

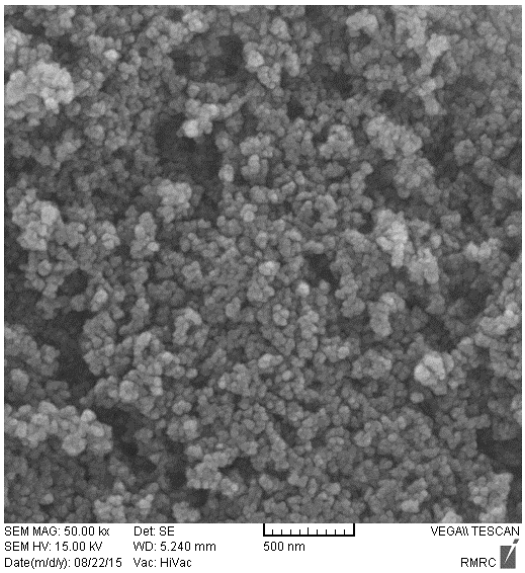

Figure 3.SEM image of PDA@SiO_2 nanoparticles

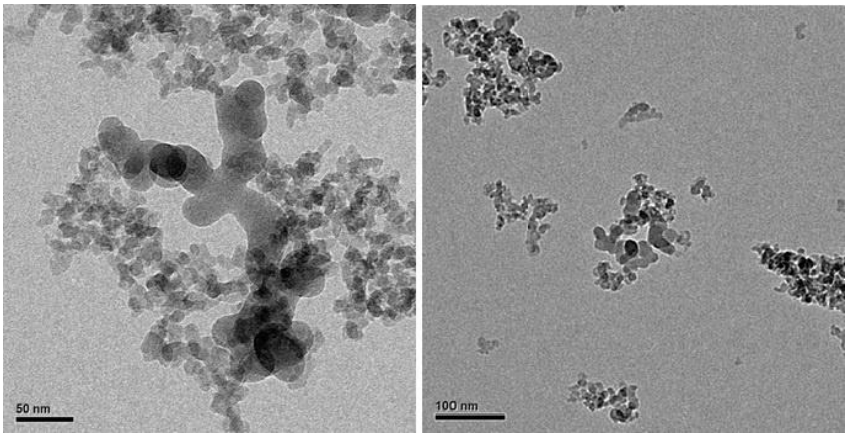

Figure 4.TEM image of PDA@SiO_2 nanoparticles.

Thermal gravimetric analysis

Thermal gravimetric analysis (TGA) serves as an effective method for assessing the thermal stability of PDA@SiO2 nanoparticles and provides valuable compositional insights. The temperature at which weight loss is observed is crucial for evaluating a material's performance under severe conditions. The corresponding mass versus temperature graph (Figure 5) indicates that the evaporation of components, such as water from the composite, occurs between 70 and 110 °C, while polymer decomposition takes place at temperatures ranging from 340 to 380 °C. This graph illustrates that 6% of the composite consists of PDA, which undergoes decomposition as temperature rises.

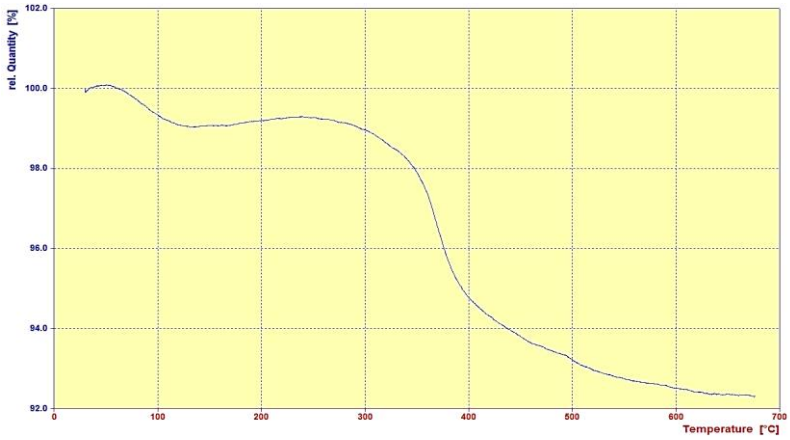

Figure 5. Thermo gravimetric analysis of PDA@silicananoparticles.

Optimization of Adsorption Conditions of testosterone on polymer

Effect of sample pH on adsorption of testosterone

The impact of different pH levels on the absorption of testosterone was studied using a batch method. Five samples of standard or solution (6 mL) with testosterone (2 ppm) were placed into 20 mL beakers, and the pH was adjusted between 4 and 8 using 0.01 mol L^{-1} HNO3 or NaOH. Then, precisely 0.1 g of MIP adsorbent was added to each beaker, and the mixtures were shaken strongly for 180 minutes to help the testosterone stick to the imprinted polymer particles. Based on the findings illustrated in Figure 4, it was observed that the amount of absorbed testosterone increases at pH = 5. Therefore, pH 5 was selected for additional tests, since the polymer's adsorption ability decreases at other pH levels (Figure 6). In MIPs, the key element influencing analyte adhesion is the pull resulting from the dimensions, form, and suitability of the analyte molecules and MIP spaces, even though additional forces like hydrogen bonding and ionic connections also play a role in the adhesion of the analyte to MIP fiber [21]. Testosterone has not acid or base properties but poly dopamine in hard basic or acidic properties; thus, adsorption of testosterone to the synthesized imprinted polymer is pH sensitive.

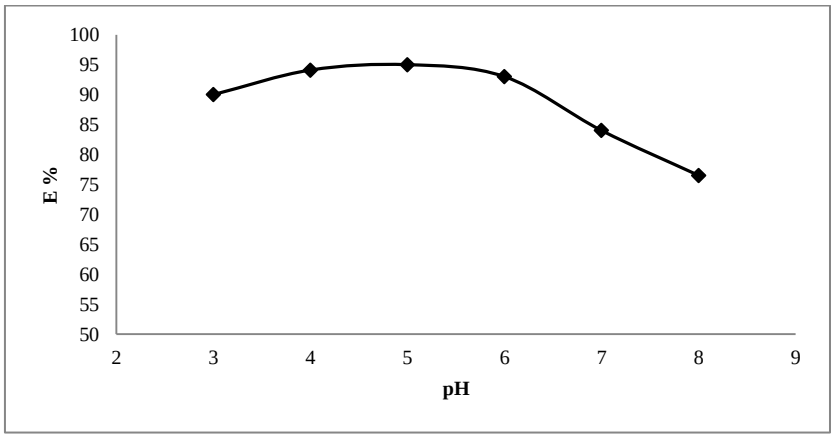

Figure 6. Effect of solution pH on testosterone uptake.

Effect of time on Adsorption of Testosterone

Five portions of standard or sample solutions (3 mL each) that contained testosterone at a level of 2 ppm were placed in 20 mL beakers. Then, 0.2 g of molecularly imprinted polymer (MIP) adsorbent was added to each beaker, and the mixtures were stirred vigorously for times of 5, 10, 15, 30, 60, and 120 minutes to enhance the adsorption of testosterone onto the imprinted polymer particles. After centrifuging the solutions, the amount of unadsorbed testosterone in the liquid was assessed using spectrophotometry. Figure 7 shows that a settling time of about 15 minutes was needed to reach 97% absorption. The amount of testosterone that was attached to the polymer was calculated by deducting the quantity of unadsorbed substance from the starting concentration of the template.

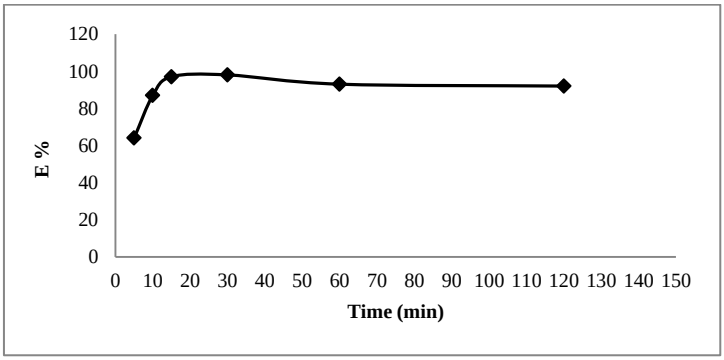

Figure 7. Influence of adsorbing time on the extraction of testosterone.

Adsorption Capacity of testosterone by MIP

The collection of testosterone from the sample solution was studied using batch experiments. This stage aimed to evaluate how sample concentration affects testosterone collection to determine the best concentration for working solutions. Several testosterone levels were made, with the pH set at 5.0. Solutions with testosterone concentrations of 1, 2, 4, 6, 8, 20, 30, 40, 50, and 60 ppm were prepared, adjusting the pH to 5.0 with 0.01 mol L−1 HNO3 or NaOH. Then, 0.01 g of the molecularly imprinted polymer (MIP) adsorbent was introduced to each container, and the mixtures were stirred rapidly for 20 minutes to improve the absorption of testosterone onto the imprinted polymer particles. To reach saturation, the initial testosterone levels were gradually raised until plateau values, indicating the adsorption capacity, were achieved. The findings are shown in Figure 8, revealing an average maximum adsorption capacity of 0.011 for the MIP (as displayed in Figure 8).

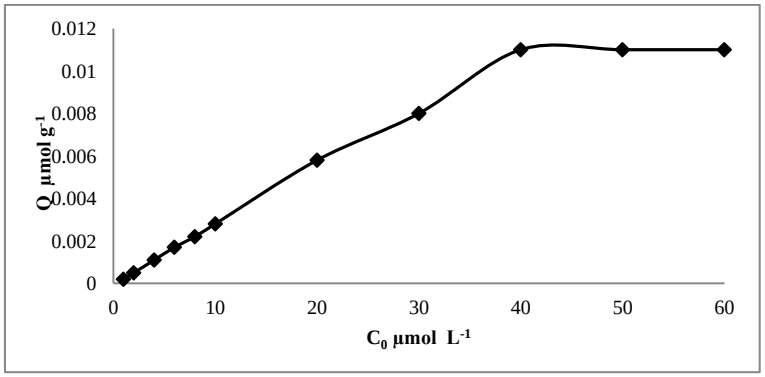

Figure 8. The effect of testosterone initial concentration on the adsorptionquantity of synthesized polymer (MIP).

Calibration Curve and Sensitivity

The calibration curve showed that it followed Beer's law within the concentration range of 0.004 to 0.058 µg of testosterone for each mL in the tested solution. The linear regression equation obtained was $A = 0.0189 \times 10^3 C$ (µg mL^{-1}) + 0.0076, with a correlation coefficient of r = 0.9922. The molar absorptivity was calculated to be 0.25×10^{-6} L mol^{-1} cm^{-1} at a wavelength of 315 nm. At a concentration of 0.5 µg of testosterone per mL the relative standard deviation based on 10 repeated measurements, was determined to be 1.5%.

Interference effects

Following the assessment of the MIP's efficiency, an examination of the polymers' selectivity was conducted. The recovery yields of the MIP were analyzed after the extraction of testosterone in the presence of testosterone, uric acid, and keratin. During the elution phase, testosterone was recovered with yields ranging from 91.4% to 84.2% for the MIP. These findings validated the potential for removing interfering compounds from the MIP.

CONCLUSIONS

This study explained the use of a new type of molecularly imprinted polymer for extracting testosterone from solid materials. The unique adsorbent was developed using the surface imprinting technique, with testosterone serving as the template and modified silica particles acting as the base material. Grafting methods can help create molecular imprints on the surfaces of polymer/silica beads, leading to MIP composites that have more available binding sites and improved mass transfer rates compared to those made through standard bulk polymerization techniques. The suggested method has many advantages, such as being inexpensive, having a high capacity with great recovery rates, and outstanding extraction efficiency. Additionally, this technique provides a selective, simple, and effective way to measure testosterone.

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Conflict of interests

There is no conflict of interest.

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