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# Innovative Surface Plasmon Resonance Aptasensor for Detecting Cocaine in Human Urine

**Gül Keklik Şah and Aybüke A. Isbir Turan ***

Turkish National Police Academy, Institute of Forensic Sciences, Necatibey Cad. No 108, 06570 Ankara, Türkiye; gkeklik9@gmail.com

* Correspondence: aybuke.turan@gmail.com

**Abstract:** This study describes the development of an optical-based surface plasmon resonance (SPR) aptasensor for the detection of cocaine. The aptasensor was prepared by first attaching gold nanoparticles to a clean SPR chip surface, followed by the addition of an aptamer to create a modified surface. This surface was characterized using contact angle and atomic force microscopy, revealing surface roughness values of 0.28 nm and 28.12 nm for the blank and modified surfaces, respectively. The detection of cocaine was carried out in the concentration range of 1 ng/mL to 1000 ng/mL, with a detection time of approximately 8 min and a cocaine limit of detection (LOD) of 0.43 ng/mL. Repeatability studies were conducted, and the stability of the signal response was examined at a concentration of 200 ng/mL. Adsorption isotherm models, including Scatchard, Langmuir, and Freundlich models, were calculated to assess the surface homogeneity of the SPR aptasensor chip, with the results indicating compatibility with the Langmuir isotherm model.

**Keywords:** synthetic urine; aptamer; gold nanoparticle; surface characterization



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## 1. Introduction

Cocaine, a naturally occurring alkaloid with a tropane ring structure, is classified as a local anesthetic. It is well-known for its potent effects and is considered a controlled substance due to its high potential for abuse and addiction. The average lethal dose for a 70 kg individual is 500 mg, although this can increase to 1–2 g in individuals with addiction. Cocaine's excretion from the body varies depending on the route of administration, with nasal excretion taking approximately 75 min, oral excretion about 48 min, and intravenous excretion around 54 min [1]. In toxicology laboratories, a range of methods is used for the analysis of cocaine. These methods include; immunoassay, radioimmunoassay, enzyme-enhanced immunoassay, fluorescent polarization, ion selective electrodes, gas chromatography, gas chromatography/mass spectrophotometry, thin layer chromatography, ultraviolet-visible region spectrophotometry, atomic absorption spectrophotometry, and surface plasmon resonance (SPR). Each of these techniques offers different advantages and can be selected based on the specific requirements of the analysis. Among these, urine is the preferred biological sample due to its ease of collection and the presence of high concentrations of the substance and its metabolites [2–4].

Surface plasmon resonance (SPR) sensors, first described by Wood and later elaborated upon by Fano and Otto, are widely used in fields such as biology, food, electrochemistry, medicine, and environmental monitoring. In medical diagnostics, they are particularly useful for the development of biosensors with rapid response times and label-free methods [4].

Aptasensors, which utilize aptamers as molecular recognition elements, are an emerging alternative to traditional biosensors. Aptamers offer advantages such as high selectivity, easy synthesis, reusability, and a smaller molecular weight compared to antibodies, making them attractive for various applications, including environmental monitoring, toxin detection, food safety, law enforcement, illicit drug detection, and medical diagnostics [5–8].

The development of rapid instruments in fields such as life engineering and genomics has led to new applications for the treatment of many diseases. Consequently, studies in the field of finding markers that will enable the diagnosis and early treatment of diseases and developing analysis methods for these have gained momentum. Today, different methods are used in private laboratories and hospitals for the determination of biomarkers in body fluids. Traditional tests such as the enzyme-linked immunosorbent test (ELISA), the radioimmune test (RIA), the fluorescence immunoassay test (FIA), and the immunoagglutination test (IAA) are commonly employed. These tests have advantages such as high affinity and a wide detection range but often require antibodies and labeling steps, which can be complex and costly [9–11].

Aptamers, molecules with oligonucleotide or polypeptide structures that can bind to target sites with high selectivity and specificity, offer a simpler, more cost-effective, and faster alternative for sensitive and accurate detection of small molecule targets [11–15]. Aptasensors have emerged as an alternative for simple and portable detection applications, from on-site drug testing and personal glucose meters to point-of-care disease biomarker screens. The widespread proliferation of aptasensors is due to their ability to combine high sensitivity and specificity with ease of use, cost-effectiveness, and fast turnaround times [11–16]. Current analytical techniques for cocaine detection include GC, capillary electrophoresis, MS, and HPLC. These methods are sensitive for cocaine detection but can be expensive, complex, and time-consuming, limiting their wide application [17]. Therefore, there is a need for sensitive, fast, and cost-effective analytical methods for the determination of cocaine, especially in pharmacy and forensic sciences. Aptamer-based optical sensors are of great interest due to their simplicity and convenience, with results that can be observed with the naked eye [18].

This research has successfully developed an SPR aptasensor for cocaine detection, demonstrating its potential for rapid, sensitive, and specific analysis. The findings pave the way for future research to explore the application of this biodetection system in real-world scenarios, including clinical diagnostics, forensic analysis, and drug monitoring. The ease of use, cost-effectiveness, and high accuracy of the aptasensor make it a promising candidate for various analytical applications in the field of cocaine detection.

## 2. Materials and Methods

### *2.1. Chemicals and Equipment*

The following chemicals and reagents were used in the experiments: 6-Mercapto-1-hexanol, gold (III) chloride hydrate, 3-Aminopropyl trimethoxysilane, HEPES, phosphate buffer, Tween 20 (Sigma-Aldrich), dimethylaminopropyl (EDC-HCl) (ROTH), and an aptamer (100 µM) with the sequence 5′-Thiol-C6–AAG GAT AAA TCC TTC AAT GAA GTG GGT CTC CC-3′ (Helix Biotechnology, Knoxville, TN, USA) [19]. Standard cocaine solution and synthetic urine (Sigma-Aldrich, Istanbul, Turkey) were also used, with the pH adjusted to 7.4 with PBS. Glassware and consumables were treated with 4 M $HNO_3$ for 24 h to ensure sterilization.

The SPR imager II (GWC Technologies, Madison, WI, USA) device was used for SPR sensor measurements, along with an SPRchipTM gold SPR sensor chip (SPRimager®II GWC Technologies). The dimensions of the gold chip were 1 mm × 18 mm × 18 mm, with a surface thickness of the gold part of approximately 50 nm. The size analysis of the synthesized gold nanoparticles was determined using a Nano Zetasizer (NanoS, Malvern Instruments, London, UK).

### *2.2. Synthesis of Gold Nanoparticles*

Gold nanoparticles (AuNPs) were synthesized using the Turkevich method, where gold (III) chloride trihydrate ($HAuCl_4.3H_2O$) salt is reduced to Au using sodium citrate [20]. A solution of 12 mL of preheated sodium citrate solution (1%, *w*/*w*) and 8.5 mg of $HAuCl_4$ salt was added to 100 mL of boiling water. The solution was strongly stirred while heating

until it turned into a deep red color for 20 min. After cooling, the AuNP solution was diluted to a final volume of 100 mL.

### *2.3. Surface Plasmon Resonance Chip Modification*

For the functionalization of the cocaine aptamer with AuNPs, 20 µL of AuNPs colloid was added to 20 µL of 3′-thiol-terminated DNA aptamer, 10 µL of NaCl (0.1 M), and 10 µL of PBS (0.1 M, pH 7.4). The mixture was mixed at room temperature and stirred for 24 h. The AuNPs were thiolated and attached to the aptamer via covalent S-Au bonds, similar to the method described by Akgönüllü et al. [8]. After 24 h of incubation, 10 µL of the solution was taken and dropped onto the chip surface. It was kept under room conditions for 24 h.

The hydrophilicity of the modified chip surface was analyzed using contact angle measurements. These measurements were made on a KRÜSS DSA100 (Hamburg, Germany), which includes a high-resolution camera, microscope optics, a syringe, a lens, and focusing and control elements in a closed housing. A stainless steel needle (0.5 mm) with a PP Luer-lock connector was used with a disposable syringe (1 mL). The liquid–liquid interfacial tension and surface tension were examined using the sessile drop method. As a result, the approximate contact angle value of the SPR chip was determined.

Contact angle analysis was performed to demonstrate the hydrophilicity of the surface-modified SPR chip. The contact angle between a liquid substance and a solid surface (for example, the SPR chip surface) was measured with the KRÜSS DSA100 device. An angle, that is, a tangent, is formed between the solid surface and the liquid. The possibility of finding tensions on solid surfaces due to contact was concluded by Young in 1805 [21]. The contact angle of a liquid substance on a solid surface is defined by mechanical equilibrium under the influence of solid–liquid, solid–vapor, and liquid–vapor interfacial tensions. A contact angle below 90° indicates that the solid surfaces are susceptible to moisture and that there is a liquid drop spread over the solid surface in an expanding area. A contact angle greater than 90° generally indicates that the surface is not suitable for moistening and creates a liquid drop on the solid surface, reducing the contact.

By dropping 10 µL of distilled water onto the gold surface of the SPR chip, the contact angle was determined via the adhered drop method [22]. Two different frames were photographed by dropping water on different areas of the chip gold surface, and the contact angle was calculated separately for each examination. The values of the calculated contact angle were determined as the contact angle taken from the left side of the droplet with the surface and the contact angle taken from the right side. In addition, the average of the values of these two points was calculated. The same processes were applied to the modified chip surface.

### *2.4. Characterization Studies of SPR Aptasensor*

Contact angle analysis of both the blank chip surface and the Au-modified SPR aptasensor chip surface was performed using a KRÜSS DSA 100 (Hamburg, Germany) instrument. The sessile drop method was applied for the measurements, and average contact angle values were calculated for the SPR aptasensors. The SPR gold surfaces were characterized by AFM (Park Systems, XE-100E, Suwon, Republic of Korea). Visualization studies were conducted in non-contact mode. The sample area was visualized with a resolution of 256 × 256 pixels and a scanning rate of 2 µm $s^{-1}$. The SPR aptasensor gold surface was modified using the self-assembled monolayers (SAMs) technique. After preparing the SPR aptasensor chip with the SAM technique, it was placed in the device, and a pH 7.4 equilibrium PBS buffer was passed through the system to equilibrate the chip surface. To examine the relationship between cocaine concentration and the SPR aptasensor signal change, cocaine solutions at different concentrations were prepared. These cocaine samples, prepared with PBS 7.4 buffer solution, were then sent to selected areas on the SPR aptasensor chip surface.

### *2.5. Preparation of Standard Cocaine Solution*

Cocaine solutions were prepared to examine the relationship between total cocaine concentration and SPR aptasensor signal. Cocaine samples at concentrations of 1, 5, 10, 50, 100, 200, 500, and 1000 ng/mL were prepared using a PBS 7.4 buffer solution and passed through the system simultaneously to selected regions on the SPR chip surface. The determination of cocaine and cocaine in synthetic urine was performed in a highly sensitive and precise manner with a determination time of approximately 8 min in the SPR aptasensor. The limit of determination (LOD) of cocaine was found to be 0.43 ng/mL.

### *2.6. Preparation of Standard Cocaine Solution with Urine*

Cocaine solutions with urine were prepared to examine the relationship between total cocaine concentration and SPR aptasensor signal. Cocaine solutions with urine at concentrations of 1, 5, 10, 50, 100, 200, 500, and 1000 ng/mL were prepared using urine samples and a PBS 7.4 buffer solution and passed through the system simultaneously to selected regions on the SPR chip surface.

## 3. Results

Gold nanoparticles were synthesized using the Turkevich method. The characterization of the synthesized gold nanoparticles was carried out with a zeta-size analysis device. The size of gold nanoparticles was calculated as 20.55 nanometers based on the zeta-size analysis results. Zeta-size analysis data and the PdI value of gold nanoparticles are shown in Figure 1.

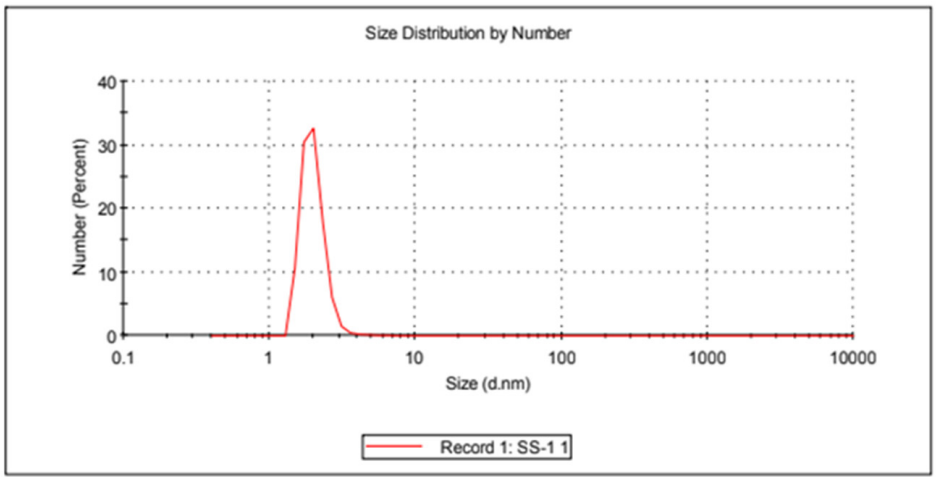
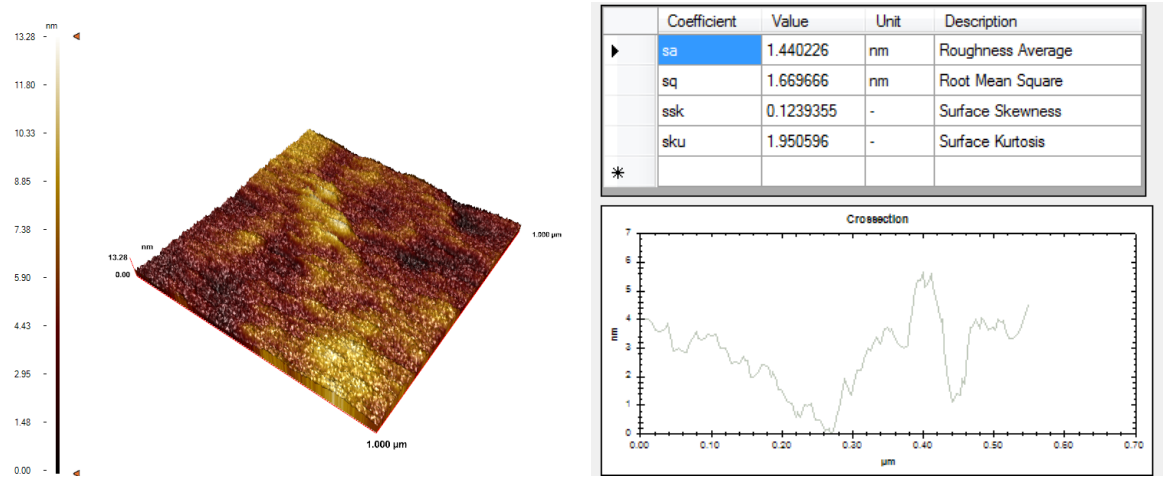



**Figure 1.** Zeta-size analysis data.

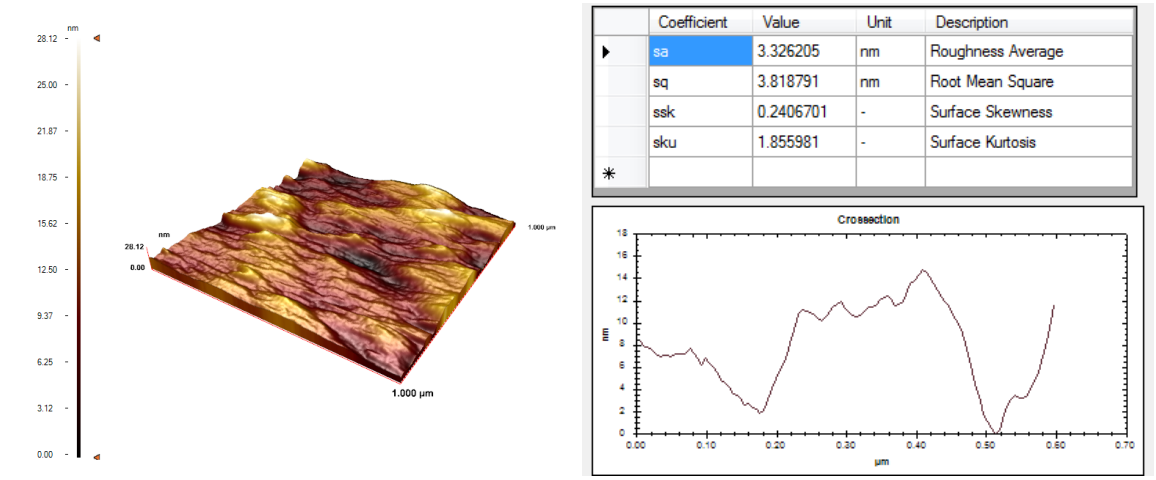
Characterization studies of the SPR aptasensor chip were performed with contact angle and AFM. The contact angle values of the blank and modified SPR aptasensor chip were investigated using the adhered drop method by dropping a drop of water on its surface. Two different contact angle values were recorded, and images were obtained by dripping water on different parts of the SPR aptasensor chip. The contact angle value of the blank gold surface was measured at 69.6°, while that of the aptamer-modified surface was 62°. According to the results, it was obtained that the contact angle decreased after the blank gold chip surface was modified with aptamer. This decrease indicates that hydrophilic groups were attached to the surface.

Surface characterization studies were performed with AFM to examine the surface morphology of the blank SPR chip surface and the Au-modified SPR aptasensor chip. The surface of the blank SPR chip was cleaned with an acidic piranha solution. The surface depth of the unmodified blank SPR chip was measured at 13.28 nanometers. The average thickness of the SPR aptasensor chip modified with gold nanoparticle–aptamer

was determined as 28.12 nm. It is shown from the results that the modification process was performed correctly on the gold surface of the SPR chip. In Figure 2, AFM images of the blank SPR chip and the Au-modified SPR aptasensor chip were given.




(**a**)




(**b**)

**Figure 2.** (**a**) Blank chip; (**b**) Au-modified-SPR-aptasensor chip.

The evaluation of surface roughness is crucial for various fundamental problems, including friction, contact deformation, heat, and electric current conduction, and the tightness of contact joints [23]. The average surface roughness values of the blank SPR chip and the modified SPR chip were calculated as 1.44 nm (a) and 3.32 nm (b), respectively, as shown in Figure 2a. The higher average surface roughness value in Figure 2b compared to Figure 2a indicates that the surface modification was successfully carried out.

### *3.1. Data Evaluation of Cocaine Determination at Different Concentrations*

Figure 3 shows the sensograms obtained from the interaction of synthetic cocaine solutions prepared at different concentrations with the SPR aptasensor. During the measurements, firstly, the equilibrium buffer solution was passed through the SPR system for about three minutes, then cocaine samples were given from the smaller to the bigger concentration value for about five minutes. Towards the end of about eight minutes, the system reached equilibrium again. Then, 0.1 M pH 2.5 glycine-HCl desorption solution

was passed through the system for about three minutes. Between two consecutive measurements, a PBS equilibrium buffer at pH 7.4 was given to the system for fifteen minutes to allow the system to reach equilibrium again [24].

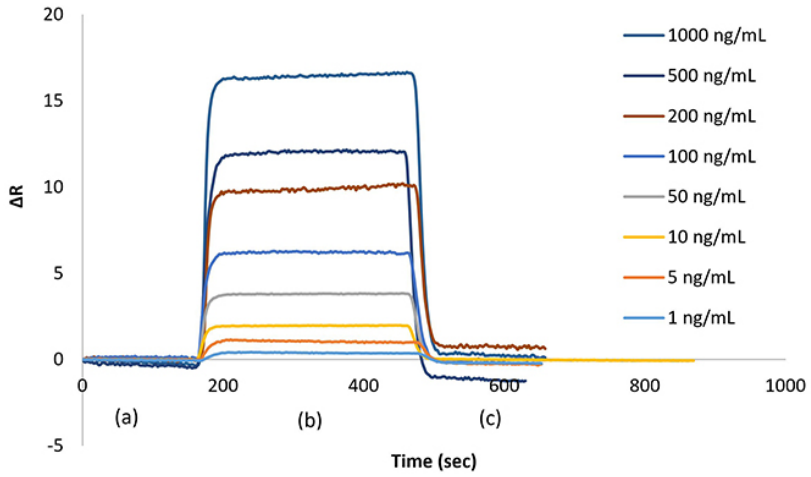



**Figure 3.** Sensogram plot of the signals of interactions between the SPR aptasensor and total synthetic cocaine in the concentration range of 0–1000 ng/mL; (**a**) PBS 7.4 equilibrium solution; (**b**) adsorption step; (**c**) desorption step.

The ΔRmax values increase with increasing cocaine concentration. As the concentration of cocaine increases, the amount of analyte binding to the active selective binding sites also increases, resulting in a higher ΔRmax value.

### *3.2. Data Evaluation of Cocaine Determination with Urine*

The sensograms obtained from the interaction of cocaine-containing synthetic urine solutions with different concentrations with the SPR aptasensor are shown in Figure 4. During the measurements, an equilibrium buffer solution was first passed through the SPR system for approximately 3 min. Then, synthetic urine samples containing cocaine were passed for approximately 5 min. Towards the end of approximately 8 min, the system reached equilibrium again. A 0.1 M pH 2.5 glycine-HCl desorption solution was then passed through the system for about 3 min. For consecutive measurements, pH 7.4 PBS equilibrium buffer was given to the system for 15 min to allow the system to reach equilibrium between both measurements.

In Figure 4, it is easily observed that as the cocaine concentration increases, the ΔRmax value also increases. This is because the increase in cocaine concentration leads to an increase in the number of active binding sites and, consequently, an increase in the concentration of the binding analyte. No significant changes in ΔRmax were observed when maximum binding occurred. The ΔR values signaling in the 0–50 ng/mL concentration range were calculated using the linear equation $y = 0.0673x + 0.5677$, and in the 200–1000 ng/mL range, the linear equation was $y = 0.0104x + 6.5595$. Calibration charts prepared for the determination of total cocaine are shown in Figure 5.

As a result of the calculations, the determination of cocaine was found to be approximately 89% accurate in the 0–50 ng/mL concentration range and approximately 93% accurate in the 200–1000 ng/mL concentration range, as shown in Figure 5.

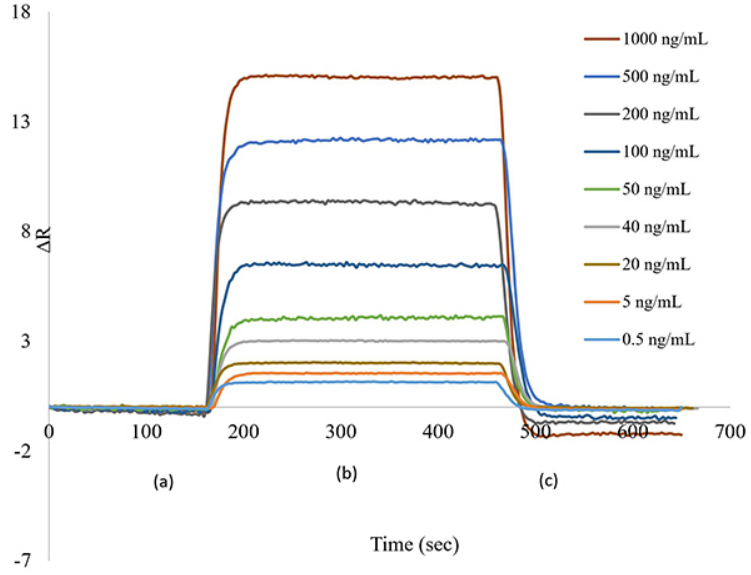



**Figure 4.** Sensogram plot of the signals of interactions between the SPR aptasensor and synthetic urine samples containing total cocaine in the concentration range of 0–1000 ng/mL; (**a**) PBS 7.4 equilibrium solution; (**b**) adsorption step; (**c**) the desorption step.

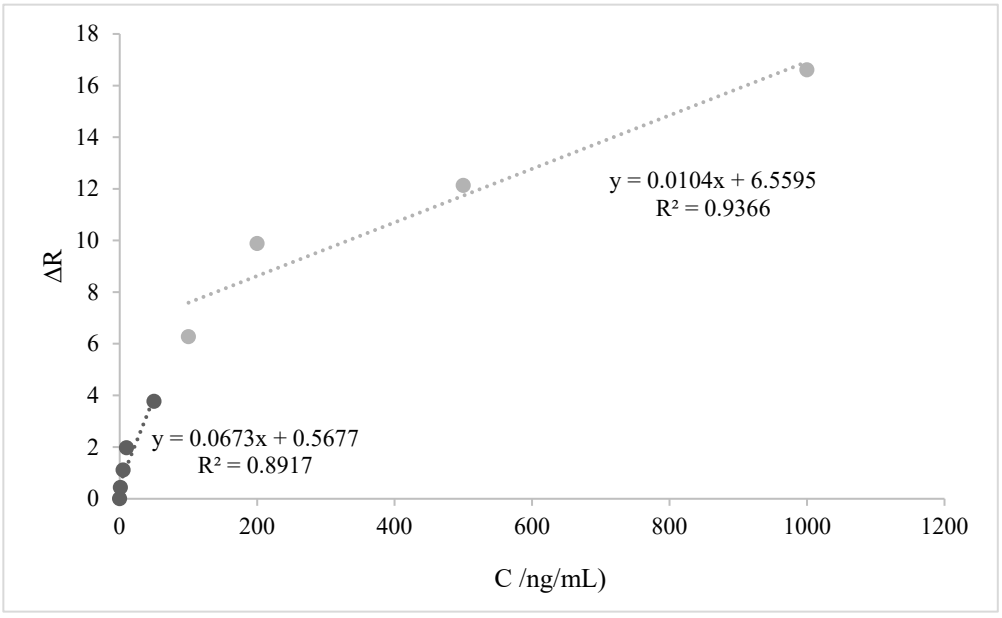



**Figure 5.** Concentration-dependent calibration plots with SPR aptasensor for cocaine concentrations ranging from 0 ng/mL to 50 ng/mL and from 200 ng/mL to 1000 ng/mL, using PBS 7.4 buffer.

### *3.3. Repeatability Results of SPR Experiments*

One of the significant advantages of SPR aptasensors is their repeatability. The ability to perform analyses on SPR-based sensors without any labeling and the minimal variation in analysis results demonstrate this advantage. Therefore, a cocaine solution with a concentration of 200 ng/mL was chosen for the repeatability step. This concentration was selected because it is neither too low nor too high, ensuring reliable results.

A 200 ng/mL concentration of cocaine standard was sent to the SPR system four times in succession to observe the repeatability of the signals. As shown in Figure 6, the detection of cocaine was carried out successively without any decrease in signal change by the cocaine aptasensors.

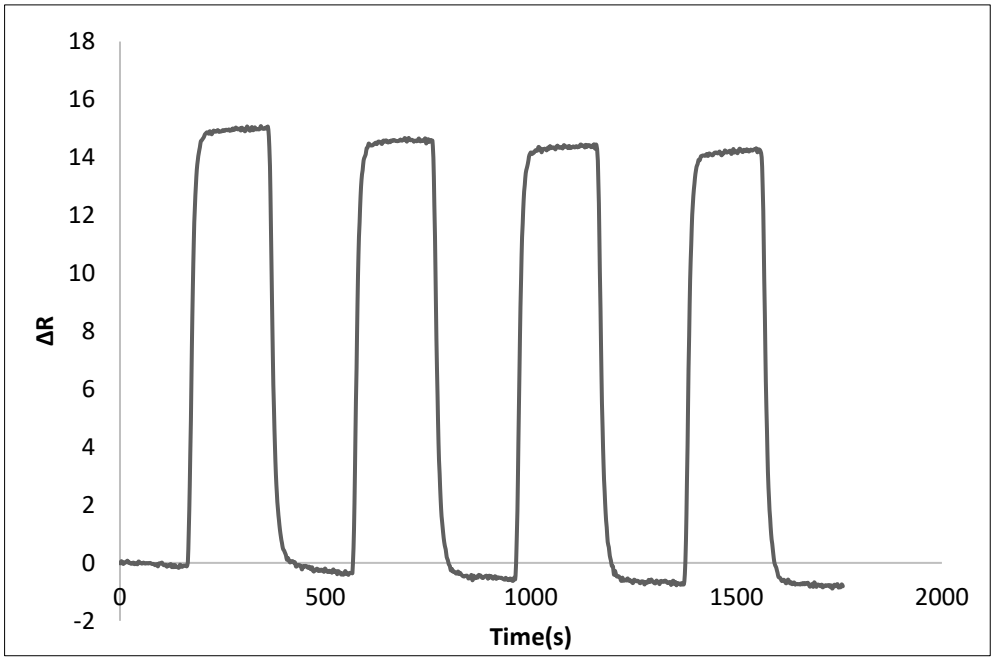



**Figure 6.** Repeatability results of the SPR aptasensor at a concentration of 200 ng/mL.

### *3.4. Kinetic Model of Aptasensor*

Kinetic analysis of SPR aptasensors has been performed to demonstrate the interaction between the target molecule and the SPR chip. The Scathard and attachment kinetic lines, including values such as '$\Delta R_{max}$', '$k_a$', '$k_d$', '$K_A$', and '$K_D$', obtained from the equations, are provided in Table 1.

**Table 1.** Balance and binding kinetic parameter values of SPR chip [25].

| **Scatchard** | | **Binding Kinetic Analysis** | |
|---|---|---|---|
| $\Delta\mathbf{R_{max}}$ | 13.88 | $k_a/(ng/mL)^{-1}\ s^{-1}$ | 0.0005 |
| $\mathbf{K_A}/(ng/mL)^{-1}$ | 0.018 | $k_d/s^{-1}$ | 0.0042 |
| $\mathbf{K_D}/ng/mL$ | 55.55 | $K_D/ng/mL$ | 8.4 |
| $\mathbf{R^2}$ | 0.5932 | $K_A/(ng/mL)^{-1}$ | 0.11 |
| | | $R^2$ | 0.9161 |

Adsorption isotherms were constructed by plotting the concentration of the solute remaining without being adsorbed against the amount adsorbed per unit mass of adsorbent when the solutions reached equilibrium at a constant ambient temperature. The Langmuir, Freundlich, and Langmuir–Freundlich adsorption isotherm models were examined to assess the surface binding homogeneity of the SPR aptasensor chip and to describe the interaction between the SPR aptasensor and cocaine. The results obtained from the Langmuir and Freundlich isotherm models are presented in Table 2.

**Table 2.** Langmuir, Freundlich, and Langmuir–Freundlich isotherm model parameters of the SPR aptasensor [26].

| **Langmuir** | | **Freundlich** | | **Langmuir–Freundlich** | |
|---|---|---|---|---|---|
| $\Delta\mathbf{R_{max}}$ | 10.86 | $\Delta R_{max}$ | 1.69 | $\Delta R_{max}$ | 3.70 |
| $\mathbf{K_D}/ng/mL$ | 66.57 | 1/n | 0.53 | 1/n | 1.88 |
| $\mathbf{K_A}/(ng/mL)^{-1}$ | 0.01 | $R^2$ | 0.9891 | $K_D/ng/mL$ | 7.66 |
| $\mathbf{R^2}$ | 0.9992 | | | $K_A/(ng/mL)^{-1}$ | 0.13 |
| | | | | $R^2$ | 0.8932 |

According to Table 2, the Langmuir isotherm model was determined to be the most suitable adsorption model based on the results of experimental studies in cocaine determination. Additionally, an $R^2$ value of 0.9992 and a $\Delta R_{max}$ value of 10.86 were obtained for cocaine. These results indicate that the binding sites on the designed SPR aptasensor chip surface are homogeneously distributed, suggesting an equi-energy, monolayer formation with minimal lateral interaction.

Furthermore, when the correlation coefficient values (R) of Figure 7a–c were compared, it was observed that the Langmuir isotherm model was the most suitable for the SPR aptasensor and cocaine. The highest signal value ($\Delta R_{max}$) calculated from the Langmuir isotherm was obtained as 0.9992. This result indicates that the binding properties of cocaine on the aptasensor surface are characterized by a monolayer formation, with minimal lateral interaction and homogeneous distribution at the same energy level.

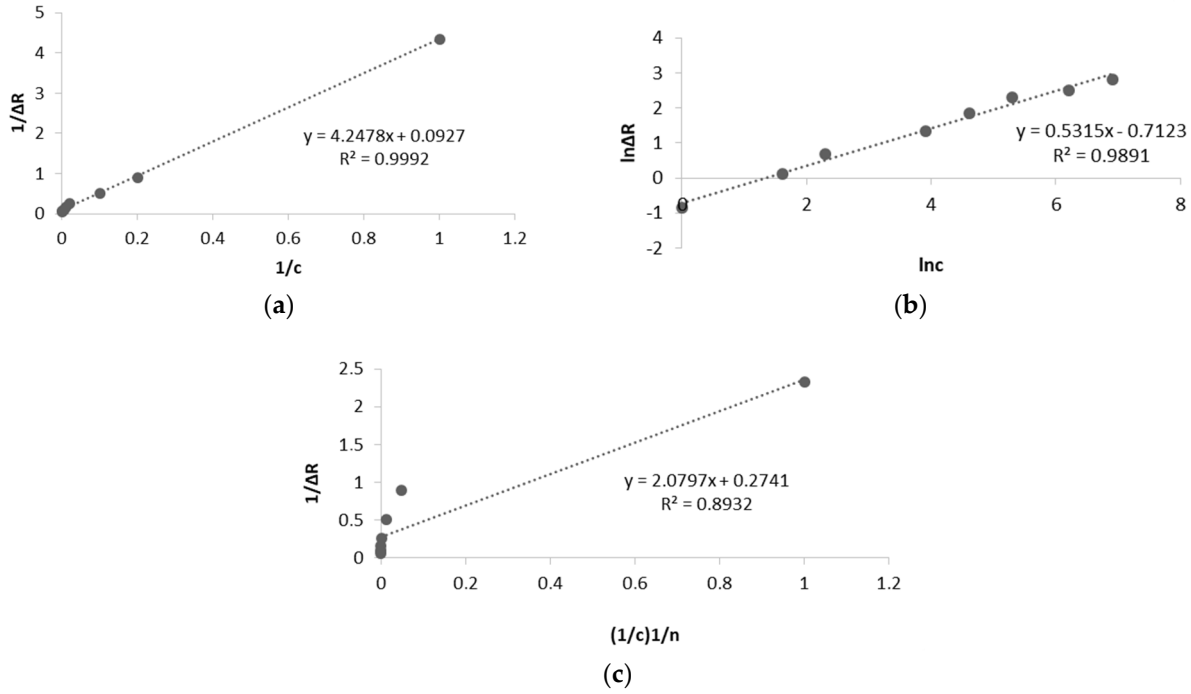



**Figure 7.** (**a**) Plot of the modified SPR aptasensor Langmuir adsorption model, (**b**) modified SPR aptasensor Freundlich model, (**c**) modified SPR aptasensor Langmuir–Freundlich model.

## 4. Discussion

The cocaine aptasensor, prepared with good sensitivity and repeatability, has demonstrated its potential as a striking optical method for cocaine detection in urine samples. The robustness and ease of design of the SPR chip make it advantageous for creating portable devices, which could be beneficial for on-site testing and point-of-care applications. The binding of aptamers to gold nanoparticles in urine cocaine analysis indicated that the sensitized SPR aptasensor was suitable for this application. These analyses demonstrate the feasibility of the biodetection system for cocaine detection in real biological samples, suggesting potential applications in forensic science, clinical toxicology, and drug monitoring. The results obtained from this study highlight the potential of aptamer-based SPR sensors for the detection of small molecules like cocaine. The high sensitivity, specificity, and repeatability of the aptasensor make it a promising tool for various analytical applications. The successful integration of gold nanoparticles with aptamers on the SPR chip surface enhances the sensor's performance, making it a viable option for the detection of cocaine in complex biological matrices.

Furthermore, the study provides insights into the kinetic and binding properties of the aptasensor, which are crucial for understanding the interaction between the aptamer and the target molecule. The Langmuir isotherm model, which best fits the experimental

data, indicates a monolayer binding with minimal lateral interaction, confirming the homogeneity and specificity of the aptamer binding sites on the SPR chip surface.

## 5. Conclusions

An affinity-based aptasensor was utilized for cocaine detection, demonstrating a rapid and economical method that is highly sensitive to cocaine. The aptasensor chip, with increased conductivity due to gold nanoparticles, was designed for more sensitive and selective determination of analytes with low molecular weight and low concentrations. The sensitivity of the aptasensor was significantly enhanced with the aptamer-containing gold nanoparticles. This aptasensor successfully detected the presence of cocaine in different concentrations and synthetic urine.

Gold nanoparticles were successfully synthesized using the Turkevich method and characterized by zeta-size analysis. The size of the gold nanoparticles was measured as 20.55 nanometers, and the PDI value was 0.513. The SPR chip surface was modified using a gold nanoparticle-treated aptamer. For this, the gold nanoparticle-treated aptamer solution was dropped onto the gold surface and left overnight.

The surface morphology of the modified chip was characterized using an atomic force microscope (AFM) and contact angle measurements. A three-dimensional image was taken with an AFM to visualize the morphology of the modified chip. The depth of the blank SPR chip surface was found to be 13.28 nanometers, while the surface depth of the modified chip was measured as 28.12 nanometers. These results indicate that the gold surface of the SPR chip, with and without aptamer, is homogeneously coated with gold nanoparticles. The contact angle was determined using the adherent drop method by dropping a drop of water onto the modified SPR chip surface. The contact angles of the blank gold surface and the aptamer-modified chip surfaces were measured as 69.6° and 62°, respectively.

According to these results, when the empty gold surface of the chip is compared with the chip whose modification is completed, it is concluded that hydrophilic groups are attached to the surface due to the reduction of the contact angle. Characterization studies of the modified chip revealed a linear correlation between the sensor response system and cocaine concentrations ranging from 1 ng/mL to 1000 ng/mL and the logarithm of increasing concentrations. A linear relationship was also noted between the sensor response system and the logarithm of cocaine concentrations in synthetic urine ranging from 0.5 ng/mL to 1000 ng/mL. For cocaine and cocaine in synthetic urine, the lower and upper limits of detection were calculated as 0.43 ng/mL and 1.27 ng/mL, respectively. The concentration range scale and detection limit of cocaine samples gave results in parallel with previous studies in the literature.

The analysis of cocaine and cocaine in synthetic urine was performed with great sensitivity in the SPR aptasensor, with a detection time of approximately 8 min. To examine the reusability of aptamer-based SPR aptasensors, a cocaine solution with a concentration of 200 ng/mL was sent to the SPR system four times in succession, and the stability of the signal response was observed. The aptamer-based cocaine aptasensor, prepared with good sensitivity and reproducibility, has demonstrated its potential to be a striking optical method for cocaine detection in urine samples. The robustness and ease of design of SPR chips make them an alternative for designing portable devices. Sensitive results were obtained at low concentrations, where the aptamer-based SPR aptasensor system gave consistent results when compared to traditional analysis methods. For example, Helander et al. reported that LC-MS/MS was utilized, necessitating sample pre-preparation. The study focused on the complex form of cocaine and its effects, with the robustness of this complex aiding in the development of the determination method. Borgul et al. reported that the determination of cocaine metabolites was conducted using electrochemical methods. The determination method incorporated the use of capillaries and silver, the latter of which made the process economically expensive. Atta and Vo-Dodhin reported that a SERS device was employed. The determination time with this method was longer than with SPR.

However, the SERS method yielded results at concentrations as low as picograms, making it a valuable approach [27–29].

The binding of aptamers to gold nanoparticles in urine cocaine analysis indicates that the sensitized SPR aptasensor is suitable These analyses demonstrated the feasibility of the designed biodetection system for cocaine detection in real biological samples. The successful integration of gold nanoparticles with aptamers on the SPR chip surface enhances the sensor's performance, making it a viable option for various analytical applications in the field of cocaine detection.

**Author Contributions:** A.A.I.T.; conceptualization, methodology, writing-review and editing and supervision and G.K.Ş.; formal analysis, investigation, resources, writing—original draft preparation, visualization. All authors have read and agreed to the published version of the manuscript.

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