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Development of Electrochemical and Colorimetric Biosensors for Detection of Dopamine

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Abstract: Neurotransmitters are essential chemical messengers required for proper brain function, and any changes in their concentrations can lead to neuronal diseases. Therefore, sensitive and selective detection is crucial. This study presents a fast and simple colorimetric method for dopamine detection using three reagent solutions: AgNP and MPA, Ag/Au nanocomposite, and mercaptophenylacetic acid. TEM images showed a narrow distribution of Ag and Au nanoparticles with average sizes of 20 nm and 13 nm, respectively, with gold nanoparticles bound to the edges of silver nanoparticles. A paper-based biosensor was created using manual wax printing for the colorimetric detection of dopamine. Visual detection onsite showed color changes with both the silver nanoparticles and mercaptophenylacetic acid mixture and the silver–gold nanoparticle composite. Electrochemical detection using a glassy carbon electrode modified with 8 mM mercaptophenylacetic acid demonstrated high selectivity and sensitivity towards dopamine, with a peak in the range of 0.7–0.9 V. Interferences were minimized, ensuring high sensitivity and selective detection of dopamine.

Keywords: colorimetric sensing; electrochemical sensing; dopamine; paper-based sensor

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1. Introduction

Dopamine (DA) is a biogenic catecholamine that functions as a neurotransmitter in the brain's central nervous system (CNS), facilitating signal transmission between nerve cells. It plays a crucial role in motor control, motivation, arousal, and cognition [1,2]. DA influences the brain's regulation of learning, eating, and certain addictive behaviors. Consequently, abnormal levels of DA in the human body can lead to various diseases such as Parkinson's disease, Alzheimer's disease, psychosis, nausea, schizophrenia, etc. [3–6]. Dopamine is essential for numerous functions within both the central and peripheral nervous systems, acting as a messenger for nerve impulses from the brain. Irregular DA levels in the brain can result in several medical conditions. Symptoms of low DA levels include stress, mental exhaustion, fatigue, and reduced motivation [7]. While dopamine is often regarded as the primary chemical associated with pleasure, current pharmacological perspectives suggest that it mediates incentive salience, indicating the value of a reward to an organism and motivating the actions required to attain it [8].

The determination of dopamine is crucial from a clinical standpoint. Constructing a sensor for the precise and selective estimation of dopamine amid interfering biomolecules can significantly enhance disease detection capabilities [9].

Paper-based sensors have garnered considerable attention from researchers due to their flexibility, convenience, stability, ease of use, and rapid results. The cellulose content of paper can be modified with materials that detect and quantify analytes, providing measurable signals. These materials may include polymers, optically active nanoparticles, or biological components [10]. Such materials might be polymer, optically dynamic nanoparticles [11], or natural components. Such materials may include polymers, optically active nanoparticles, or biological components. Paper can serve as a platform for colorimetric analysis in various fields, including environmental, clinical, medical, geochemical testing, and the food industry. Paper-based tests provide a simple yes/no detection of analytes using known antibodies [12]. Paper-based tests give yes/no detection of an analyte utilizing known antibodies [13]. The development of these tools has brought about a significant advancement, enabling the detection of both simple analytes and complex biomolecules. These tools are referred to as paper-based sensors or point-of-care devices [14]. In this straightforward detection method, paper is utilized as a fundamental material to create microfluidic channels by applying hydrophobic materials to hydrophilic paper [15]. To test natural substances such as blood, sweat, saliva, tears, urine, or environmental reagents like hydrogen sulfide gas and heavy metal particles, the sample is applied to the sensor and travels to the detection area through capillary action without the need for an external pump [16].

Paper, as a sensing material, offers numerous advantages due to its excellent adsorption properties, capillary action, high surface-to-volume ratio, ease of sterilization, and compatibility with biological samples [17,18]. Despite the promising applications of paper-based sensors, there are some limitations regarding precision, sensitivity, and the simultaneous detection of multiple analytes [19]. Paper sensors have been developed by creating hydrophilic channels and hydrophobic barriers [20]. Patterning is employed during fabrication to overcome these limitations. Various detection techniques, such as fluorescence, chemiluminescence, electrochemiluminescence, and electrochemistry, have been utilized for analyte detection [21].

These sensors give an investigative stage to diverse analyte estimations, for example, amperometric estimation [22], colorimetric [23,24], chemiluminescent [25], FRET estimations [26], aptasensor [27], and SERS procedures [28]. A few sensors give colorimetric and electrochemical estimations with respect to metal recognition in indoor and open-air tests [29] and natural toxins [30]. In recent times, much research may incorporate colorimetric detections utilizing paper-based sensors as color estimations [31], which have been finished by utilizing picture scanners, PDA cameras [32], color scanners, and other applications that make it increasingly adaptable and advantageous [33].

To identify specific analytes from a sample, various methods such as electrochemical, colorimetric, electrochemiluminescence, chemiluminescence, and fluorescence are used in paper-based sensors [34]. Modern research uses colorimetric and electrochemical methods of detection more frequently because they are more practical, manageable, and produce fast findings. Colorimetric measurements include methods for identifying a specific analyte by evaluating the color arrangement or shift using mobile phones, direct imaging with a single lens reflex (SLR) camera, desktop scanners, and measurement programming with programs like MATLAB or traditional spectrophotometers by measuring the sample absorbance at specific wavelengths [35]. Since electrochemical methods do not rely on chromophores like spectrophotometric methods do, they are typically employed to ensure non-ultraviolet-absorbing analytes. Electrochemical detections are especially attractive because of their excellent results, straightforward process, and low cost. When the working electrode and counter electrode are associated with the electrolyte solution to provide current to the sample, the analyte is recognized [36,37].

In recent years, tremendous advancements have been made in the field of sensors, especially with the creation of colorimetric and electrochemical sensors that are intended to identify neurotransmitters like dopamine and its precursors. These sensors are essential in many different industries because of their capacity to deliver prompt, sensitive, and focused

detections [38]. Noble metal nanoparticles, such as gold and silver, have become essential components in sensors due to their unique characteristics, which include high surface-area-to-volume ratios, strong catalytic performance, and tunable optical and electrical properties [39]. Marceptophenylacetic acid is used in dopamine detection because it binds to dopamine on its own. MPA exhibits a significant affinity for dopamine in comparison to other neurotransmitters and substances present in biological material.

The synthesis of Au–Ag core-shell nanoparticles, which can exhibit a clear color change from yellow to greenish gray, has been described in notable research studies. These nanoparticles can be used as colorimetric sensing probes for the highly selective sensing of the DA neurotransmitter based on DA-mediated aggregation of the Au–Ag NPs. Raising the pH from 6 to 10 significantly reduced the colorimetric transition response time by a factor of 10, and the estimated DA detection limit was 0.08 μM. Furthermore, Au–Ag NP sensing probes with a pH 10 tuning demonstrated exceptional selectivity for DA against a variety of interfering chemicals [40].

A sensitive and selective electrochemical dopamine (DA) sensor has been created using gold nanoparticles adorned with marimo-like graphene as a modifier of the glassy carbon electrode. Marimo-like graphene was produced by partially exfoliating the meso-carbon microbeads (MCMB) via molten KOH intercalation. The electrode exhibited strong electrochemical activity when it came to DA oxidation. The peak current of oxidation rose linearly with respect to the DA content in a range of 0.02 to 10 μM, with a detection limit of 0.016 μM. The selectivity of the approach was evaluated on real goat serum samples containing 20 μM uric acid. This work demonstrated a workable method for employing MCMB derivatives as electrochemical modifiers to create DA sensors [41].

Motivated by these advancements, we will concentrate on developing a novel dopamine sensor platform with marceptophenylacetic acid and Ag and Au nanoparticles. In this work, colorimetric and electrochemical detections were used to detect dopamine sensitively and selectively. Ag/Au nanoparticle composites, silver nanoparticles, and MPA solution were used in the paper-based colorimetric detections. GCE was treated with a solution of mercaptophenylacetic acid, which was used for electrochemical detection. To develop DA sensors, the effects of various interferences on dopamine detection were also investigated.

2. Materials and Methods

2.1. Chemicals

Ethylene Glycol, PVP, Silver Nitrate, Acetone, Chloroauric acid ($HAuCl_4.3H_2O$), Sodium citrate, Disodium hydrogen phosphate, Sodium dihydrogen phosphate, 4-Mercapto phenylacetic acid, dopamine, ascorbic acid, and uric acid were purchased. All the chemicals were analytical grade and used as received from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Instruments

The UV-Vis absorption spectra were recorded on a UV-visible spectrophotometer (BMS biotechnology medical services K Canada INC UV-1602), Transmission electron microscope (TEM) images for the morphology were recorded, and the electrochemical sensing was carried out on a CHI 660C potentiostat (Origalys, Lyon, France) at room temperature.

2.3. Preparation of Gold Nanoparticles (AuNPs)

Gold nanoparticles were made using a technique documented in the literature [42]. To create a 1 mM gold solution, $HAuCl_4.3H_2O$ was weighed up to 0.0196 g and dissolved in 50 mL of deionized water. The gold solution was added to a hot plate and vigorously stirred until it boiled. Once boiling had begun, 1 milliliter of 5% sodium citrate (0.1 g per milliliter) was gradually added. The solution color changed from yellow to wine red in three to five minutes. The produced AuNPs were kept in a refrigerator at 4 °C.

2.4. Preparation of Silver Nanoparticles (AgNPs)

A slightly modified version of a process described in the literature [43] was used to manufacture silver nanoparticles. PVP was weighed up to 1.2 g, dissolved in 10 mL of ethylene glycol, and heated in an oil bath at 80 degrees Celsius while being constantly stirred until the solution took on a light brown color. Another solution was made by continuously swirling 10 milliliters of ethylene glycol while 0.17 g of silver nitrate was dissolved. Then, the aforementioned solution was gradually supplemented by this solution. After an hour of heating, the mixture was allowed to cool to room temperature without changing color. To halt the process, 10 milliliters of acetone were added. After centrifuging the mixture for five to seven minutes, the precipitates were finally collected, cleaned, and dried at 40 °C. At room temperature, the silver nanoparticles that were produced were kept.

2.5. Preparation of Paper-Based Biosensor

A paper-based biosensor for the detection of dopamine was fabricated using the following method. First, Whatman No. 1 filter paper was immersed in acetone for five minutes, dried, and then baked for 15 min at 80 °C. Using the chart as a guide, a stencil of the necessary shape was made and secured with hair pins on filter paper that had been cleaned with acetone. On the chart, a spoon-like shape was drawn and then cut into the desired shape. The spoon-shaped stencil measurements were specified as 1 cm for width and 2 cm for length for each channel. At 200 °C, wax was melted on a hot plate. Using a paintbrush, the molten wax was evenly applied on the pre-cut filter paper, and the stencil was subsequently removed. On a paper-based biosensor, the hydrophilic channels and hydrophobic barriers were designed. The bare surface functioned as hydrophilic channels, while the wax-coated surface revealed the hydrophobic barriers. The paper-based biosensor was completed and prepared for use.

2.6. Colorimetric Detection Using AgNP and MPA as Reagent Solution

The regent solution was made by constantly swirling a 2 mL silver nanoparticle solution for 1.5 h and then adding 0.5 mL of an 8 mM MPA solution to it. This mixture was agitated once again for 30 minutes and employed as a reagent solution for dopamine detection using a paper-based biosensor. Wax-coated paper-based biosensor hydrophilic channels were filled with 0.05 mL of reagent solution, which was then allowed to dry. Next, 0.05 mL of 0.002 M dopamine solutions with pH values of 7.0, 7.4, 8.0, and 9.0 were applied to each hydrophilic channel one at a time to determine the ideal pH for dopamine colorimetric detection on paper-based biosensors. Therefore, dopamine detection may yield the best colorimetric findings in a 0.002 M dopamine solution at pH 7.4. An additional dopamine solution concentration range was applied to reagent-loaded hydrophilic channels at pH 7.4. Dopamine may easily react to reagent solution in these channels, changing color from yellow to brown, indicating colorimetric dopamine detection on a paper-based biosensor. The biosensor image was taken with a regular phone camera.

2.7. Colorimetric Detection Using Nanocomposite of AgNP+AuNP

A nanocomposite of silver and gold nanoparticle solution was used to detect dopamine in the solution. As a reagent solution for the detection of dopamine in solutions, a mixture of 1 mL of AuNP solution and 1.5 mL of AgNP solution was agitated for 1 h. The reagent solution was separated into four equal parts (0.625 mL), and each part was then filled with 0.5 mL of a 0.002 M buffered dopamine solution with pH values of 7.0, 7.4, 8.0, and 9.0, respectively. The color of the solution instantly changes from purple to orange as the dopamine solution reacts with the reagent solution, indicating colorimetric detection of dopamine.

2.8. Electrochemical Detection

The reagent solution of 4-Mercapto phenylacetic acid (8 mM) was used for the electrochemical detection of dopamine. The electrochemical sensing was carried out on a CHI 660C

potentiostat (CH Instruments, Austin, TX, USA, Ori-gaflex-OGF-Multichannels-OrigaLys) at room temperature. Voltametric measurements were done using the three-electrode system with a silver/silver chloride (Ag/AgCl) as the reference, platinum (Pt) wire as the counter, and glassy carbon electrode (GCE) as the working electrode. A total of 50 µL of reagent solution was applied on the surface of the glassy carbon electrode by drop casting method, dried, and the electrode was then dipped into a 10 mL sample solution of dopamine (2 mM) pH 7.4. The cyclic voltammograms were recorded by cycling the potential between scanning from −0.3 to +1.2 V at a scan rate of 100 mVs^{-1}.

3. Results

3.1. Characterization

TEM analysis was conducted to confirm the formation and size of NPs. The TEM images of the Ag/Au composite, Au and Ag, are shown in Figure 1. The size of silver nanoparticles was confirmed by transmission electron microscopy, and it was depicted as 20 nm. The size of gold nanoparticles was confirmed by transmission electron microscopy, and the prepared AuNPs have a size of about 13 nm. The TEM image of the composite showed that scattered gold nanoparticles bound at the edges of silver nanoparticles formed composite clusters, and the formation of nanocomposite was confirmed.

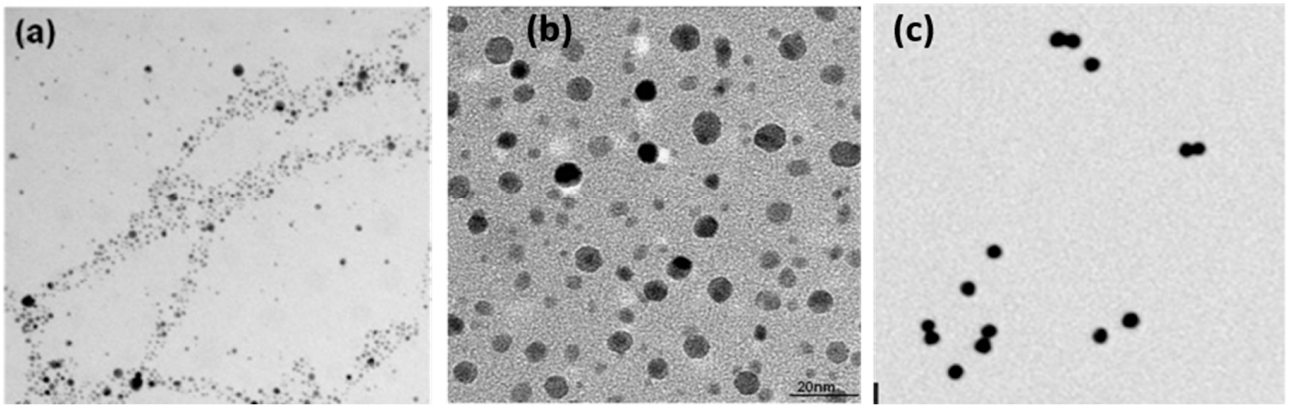

Figure 1. TEM image of (**a**) Composite (AgNP+AuNP), (**b**) AuNP and (**c**) AgNP.

As shown in Figure 2, silver nanoparticles show a UV-visible peak at 425 nm, and this wavelength was matched with the value given in the literature [44]. UV-visible spectra of gold nanoparticles show an absorption peak at 530 nm, and this wavelength was matched with the value given in the literature [45]. UV-visible spectra of Ag/Au nanocomposite show absorption at 428 nm and 510 nm, which was confirmed by comparing with values given in the literature [46]. When dopamine interacts with the R2 Ag/Au nanocomposite, absorption peaks may quench and shift to 437 nm and 520 nm, respectively. These changes in wavelength indicate the successful detection of dopamine by nanocomposite, as shown in Figures 3 and 4.

UV-visible spectra of mercaptophenylacetic acid (MPA) are depicted in Figure 3. The obtained surface plasmon resonance peak of MPA was at a wavelength of 460 nm. Dopamine gave an absorption peak at a wavelength of 465 nm in the UV-visible spectra. The figures acquired are in proximity to the previous reports [47]. The reagent (R1) of the AgNP and MPA solutions gave an absorption peak at a wavelength of 430 nm. Dopamine (2 mM) at pH 7 in the presence of reagent AgNPs and MPA (R1) gives an absorption peak at the 440 nm wavelength, as shown in Figure 4. This shift of the peak to a higher wavelength indicates the detection of dopamine by the reagent.

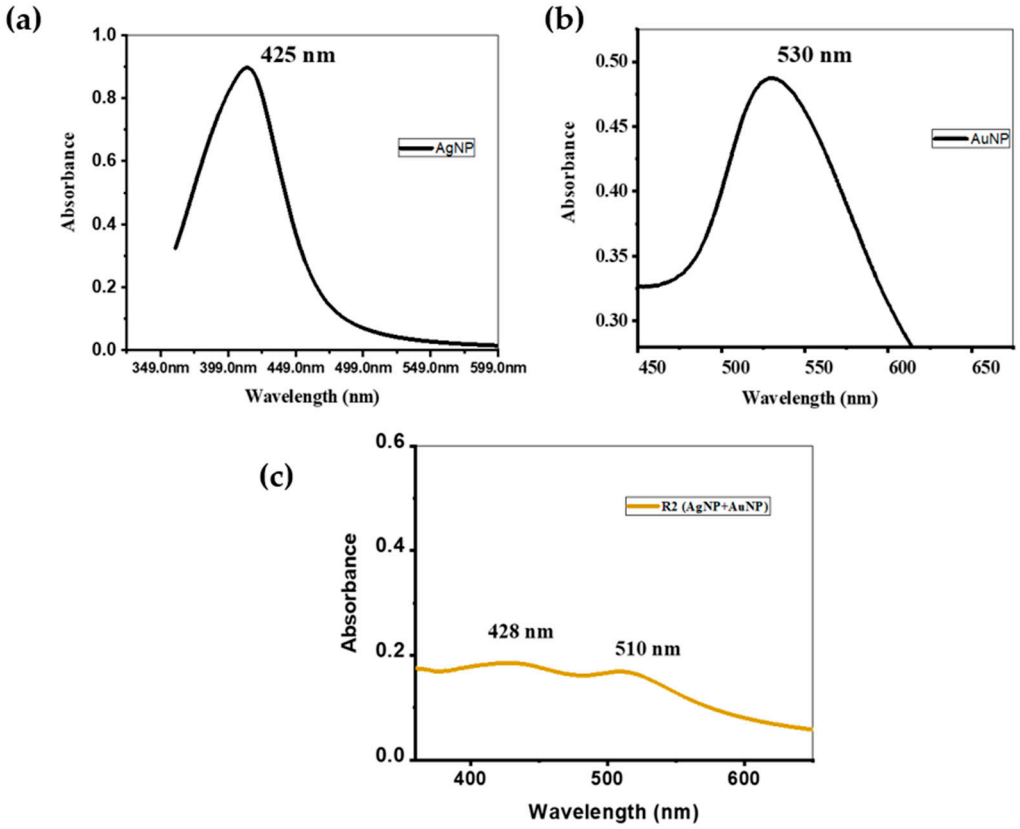

Figure 2. UV-visible spectra of (**a**) AgNP, (**b**) AuNP, and (**c**) Composite (AgNP+AuNP).

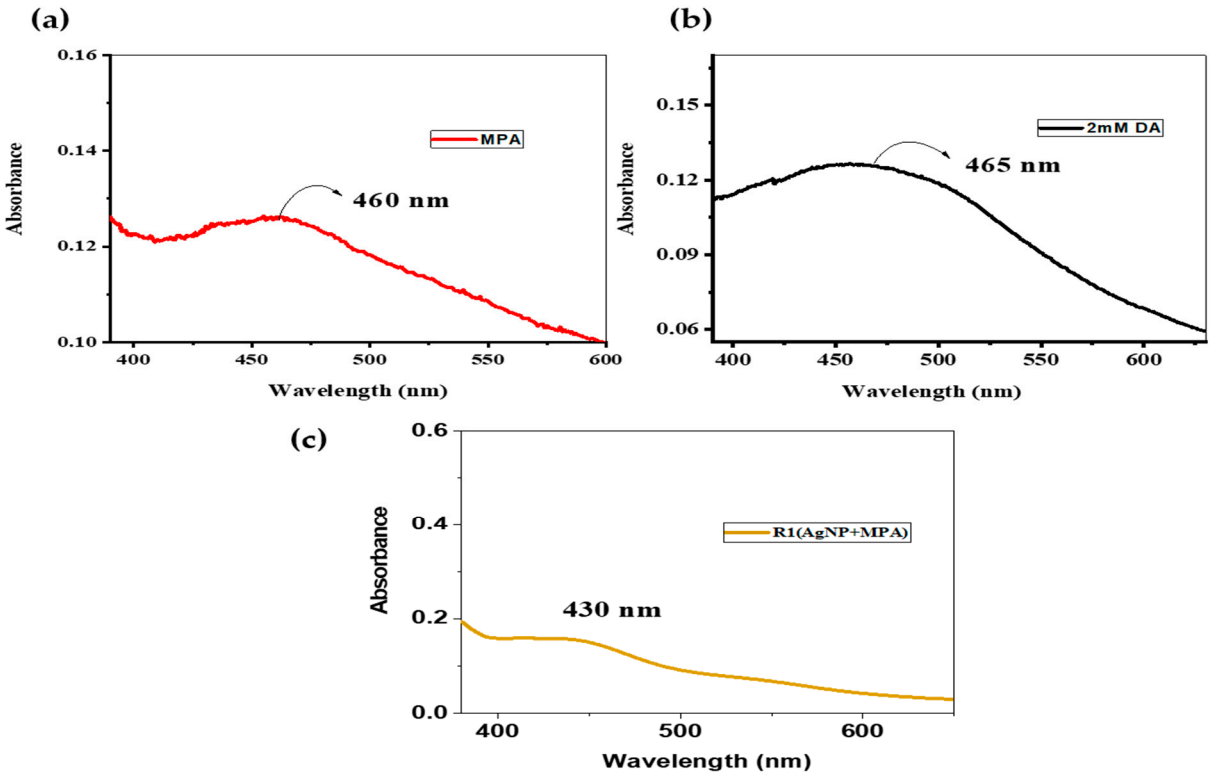

Figure 3. UV-visible spectra of (**a**) MPA, (**b**) DA, (**c**) reagent R1 (AgNP+MPA).

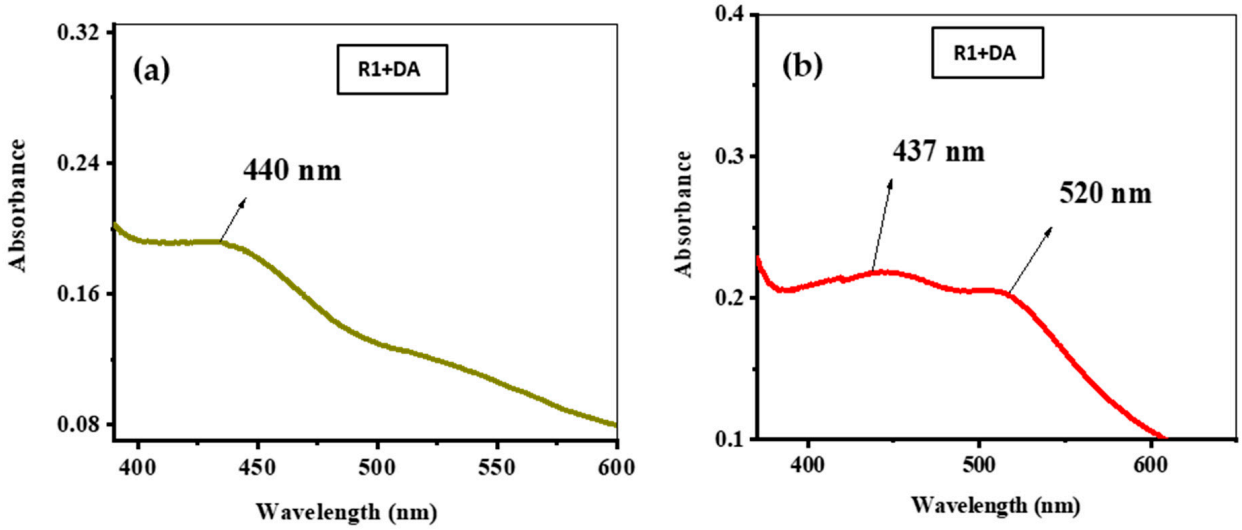

Figure 4. UV-visible spectra of (**a**) dopamine reaction with R1 (AgNP+MPA) and (**b**) dopamine with R2 (Ag/Au composite solution).

3.2. Interaction of AgNP+MPA with Different Concentrations of DA

From the UV-visible spectra of concentrations of 2 mM–10 mM of dopamine solution that interacts with a reagent solution composed of AgNP+MPA, the absorption peaks were quenched and showed absorption at 440 nm, as depicted in Figure 5. Furthermore, a direct relationship between absorbance and dopamine concentrations was observed; as the concentration of dopamine increased, the absorbance increased.

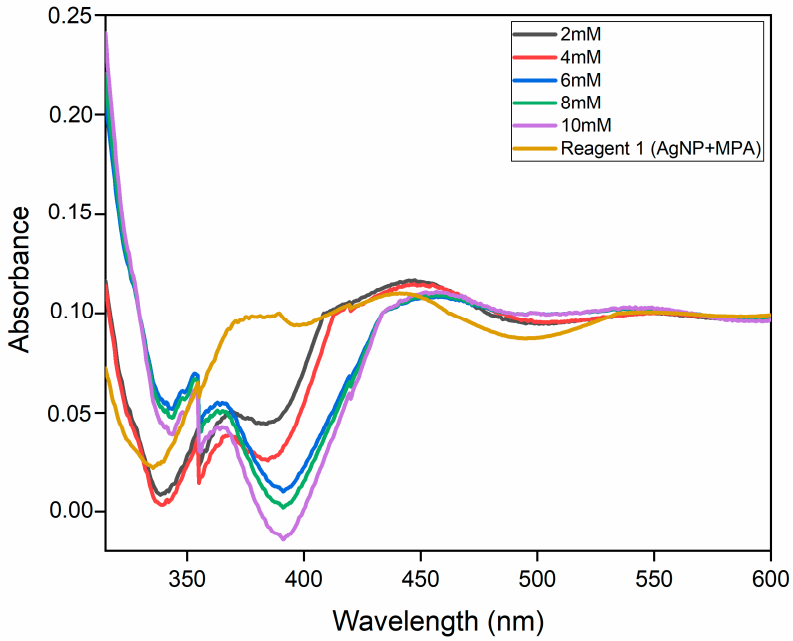

Figure 5. UV-visible spectra of reagent 1(AgNP+MPA) without dopamine and in the presence of dopamine concentrations 2 mM, 4 mM, 6 mM, 8 mM, and 10 mM.

3.3. Interaction of Ag/Au Composite with Different Concentrations of DA

From UV-visible spectra of the concentration range from 2 mM to 10 mM of dopamine solution that interacted with reagent solution (Ag/Au nanocomposite), it was recorded that there was a direct relationship between absorbance and concentration, as the concentration of dopamine decreased, absorbance also decreased as shown in Figure 6.

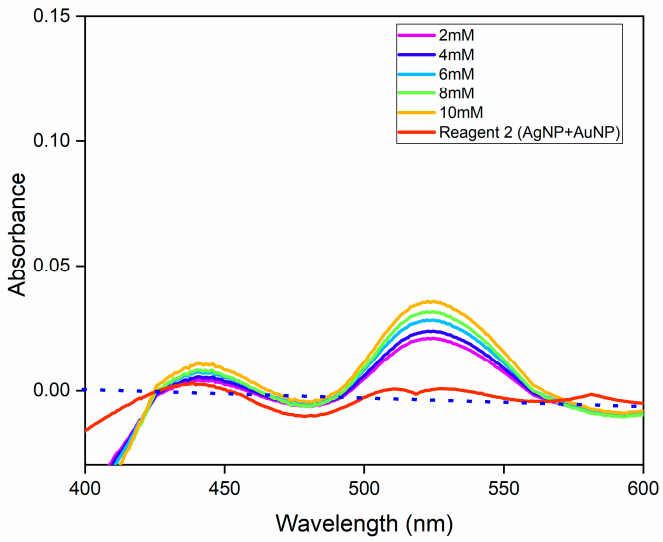

Figure 6. UV-visible spectra of reagent 2 (AgNP+AuNP) without dopamine and in the presence of dopamine concentrations 2 mM, 4 mM, 6 mM, 8 mM, and 10 mM.

3.4. Colorimetric Detection of Dopamine

3.4.1. Reagent Solution (AgNP and MPA)

Dopamine was determined calorimetrically on paper and in solution using reagent solutions of AgNPs and MPA.

The colorless dopamine solutions were treated with a reagent solution (AgNPs+MPA), where a change in color from yellow to blackish brown indicated colorimetric detection of dopamine in the solution, and this color became darker with the increase in pH of the dopamine solution. Figure 7 indicates the visual detection of dopamine.

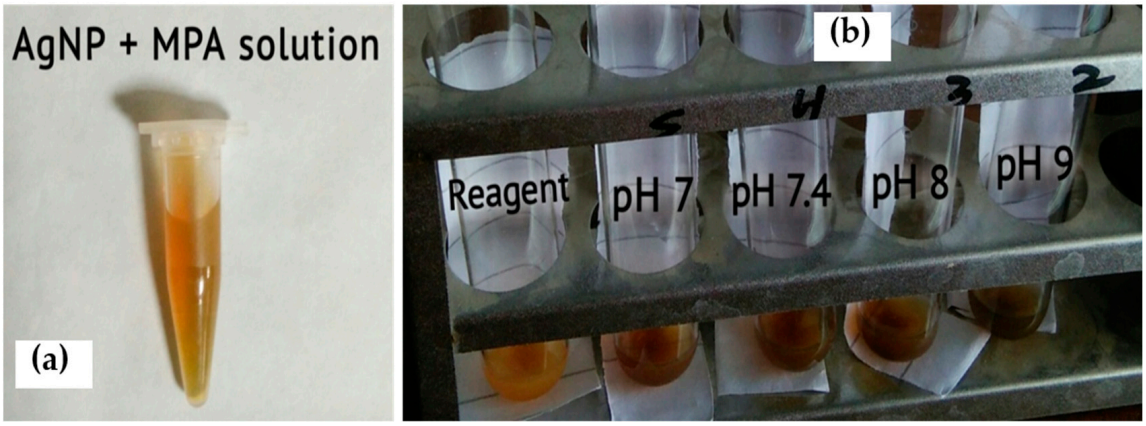

Figure 7. (**a**) Reagent solution of silver nanoparticles (AgNPs) and mercapto-phenyl-acetic acid (MPA) (**b**) visual detection of dopamine (2 mM) in solution using AgNPs and MPA solution at pH 7.0, 7.4, 8.0 and 9.0.

3.4.2. Colorimetric Detection of Dopamine on Paper-Based Biosensor Using AgNP and MPA as Reagent Solution

The colorimetric detection of dopamine on a paper-based biosensor was carried out using AgNP and MPA as reagent solutions. A total of 0.05 mL of reagent solution was applied to a spoon-shaped paper device, and a yellow color appeared on the paper after drying at room temperature. Colorimetric detection of dopamine was carried out by applying 0.05 mL of a 2 mM colorless solution of dopamine to dried paper, and the color changed from yellow to blackish brown on the paper. Later, a photo of this sensor was

taken with a cell phone camera, and the RGB value was estimated using Image J software, as shown in Figure 8. The RGB value for reagent (AgNPs+MPA) was 86 K, and reagent + dopamine was 98 K. The increase in the intensity of colors confirmed the colorimetric detection of dopamine on the paper-based sensor.

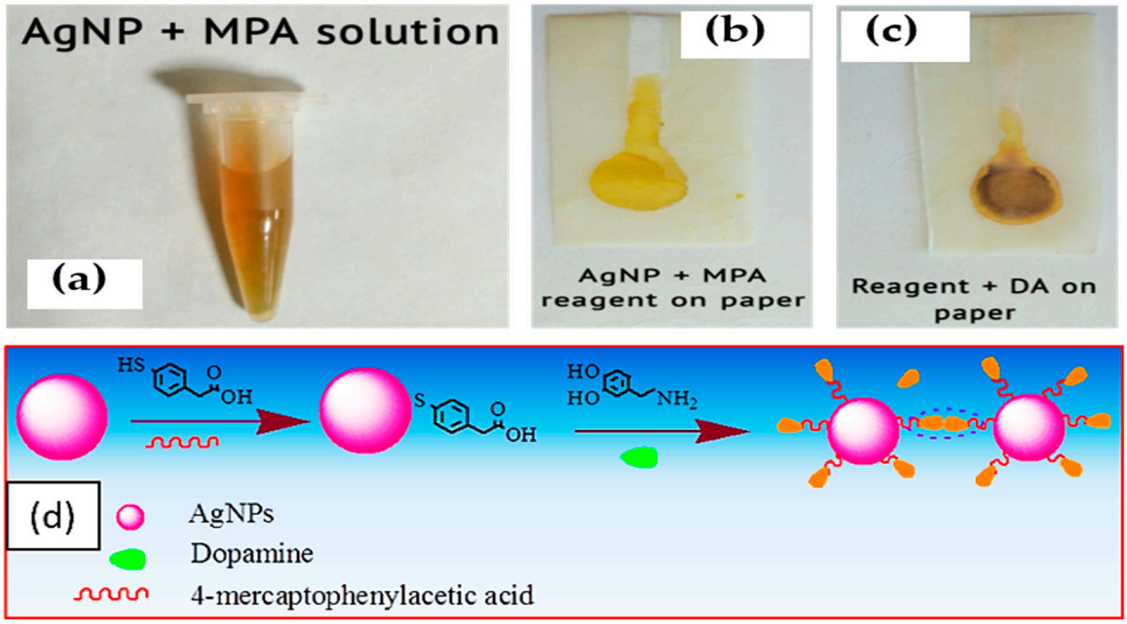

Figure 8. (**a**) AgNPs and MPA solution (**b**) AgNPs and MPA solution on spoon-shaped paper device (**c**) dopamine (2 mM) detection using AgNPs and MPA solution on paper device (**d**) Schematic diagram of reaction of dopamine with AgNP+MPA reagent.

The reagent AgNP+MPA solution has a negatively charged acetate group that interacts with the positively charged amine group of dopamine. Hence, hydrogen bonding originates between dopamine molecules, where the aggregation of nanoparticles indicates a change in color from yellow to blackish brown. This phenomenon confirms the onsite colorimetric detection of dopamine, as shown in Figure 8d.

The mechanism of interaction between marceptophenyl acetic acid and dopamine is hydrogen bonding and electrostatic interaction. The hydroxyl groups of dopamine and the carboxylic acid group of MPA form hydrogen bonds among themselves. The electrostatic interaction occurs between the amine group of dopamine and the acidic carboxyl group of MPA. The thiol groups in MPA also interact with dopamine through coordination bonds.

3.4.3. Ag/Au Nanocomposite Solution

A composite solution of silver and gold nanoparticles was prepared by mixing their solutions at 1:2, respectively. A sample solution of 2 mM dopamine was prepared and divided into four parts. The pH was maintained at pH 7, pH 7.4, pH 8, and pH 9 using a buffer solution. All colorless solutions were treated with a reagent solution (AgNP and AuNP), which produced a color change in the solution from purple to orange. The color change became darker with the increase in the pH of dopamine, as shown in Figure 9.

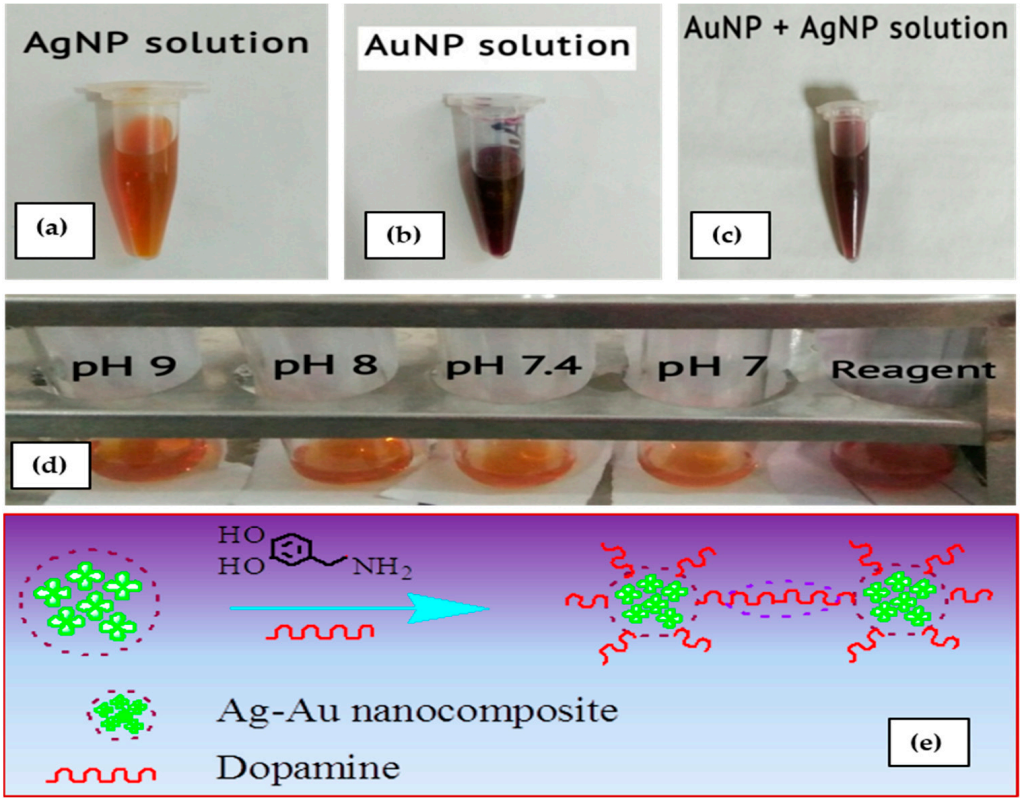

Figure 9. (**a**) silver nanoparticles solution, (**b**) gold nanoparticles solution, (**c**) reagent solution of Ag/Au nanocomposite, (**d**) colored detection of 2 mM solution of dopamine in the reagent solution at pH 9.0, 8.0, 7.4 and 7.0, and (**e**) Schematic diagram of reaction of dopamine with Ag/Au nanocomposite.

Ag and Au nanocomposites are stabilized by negatively charged particles, and these particles are effectively adsorbed on the positive end of the dopamine molecule. Hydrogen bonding originates between adjacent dopamine molecules with the aggregation of composite molecules that indicate a change in color from purple to orange and confirm the colorimetric detection of dopamine in the solution state, as shown in Figure 9e.

The mechanism of interaction between Ag and Au nanocomposite is surface adsorption by catechol-metal interaction and electrostatic interaction. The catechol group in dopamine has a strong affinity for metals, so both Au and Ag form coordination bonds with the hydroxyl groups of catechol, leading to the adsorption of dopamine to the surface of nanoparticles. Second, depending on the surface charge of nanoparticles, electrostatic interactions occur between the positively charged amino group of dopamine and the negatively charged surface of functionalized nanoparticles.

3.5. Electrochemical Detection of Dopamine and Selectivity and Sensitivity for Dopamine Sensing

Electrochemical detection of dopamine was carried out using a glassy carbon electrode modified with 50 µL of 8 mM solution of 4-Mercaptophenylacetic acid by drop casting method.

A bare glassy carbon electrode gave an oxidation peak at a potential in the range of 0.38 to 0.6 volts in a 2 mM solution of dopamine at pH 7. A modified glassy carbon electrode gave an oxidation peak at a potential in the range of 0.7 to 0.9 V in a 2 mM solution of dopamine at pH 7. Although the carboxyl group on the modified electrode surface became deprotonated and interacted with positively charged dopamine molecules to facilitate electron transfer, this comparison of both peaks indicated that electrodes modified with mercaptophenylacetic acid enhanced the oxidation of dopamine and increased peak current.

Also, both peaks were obtained in different potential ranges, as shown in Figure 10. The bar electrode gave a peak in the potential range of 0.38 to 0.6 V, while dopamine gave a peak in the range of 0.7 to 0.9 V; hence, the modified electrode gave a good electrochemical detection of the analyte with a higher potential range.

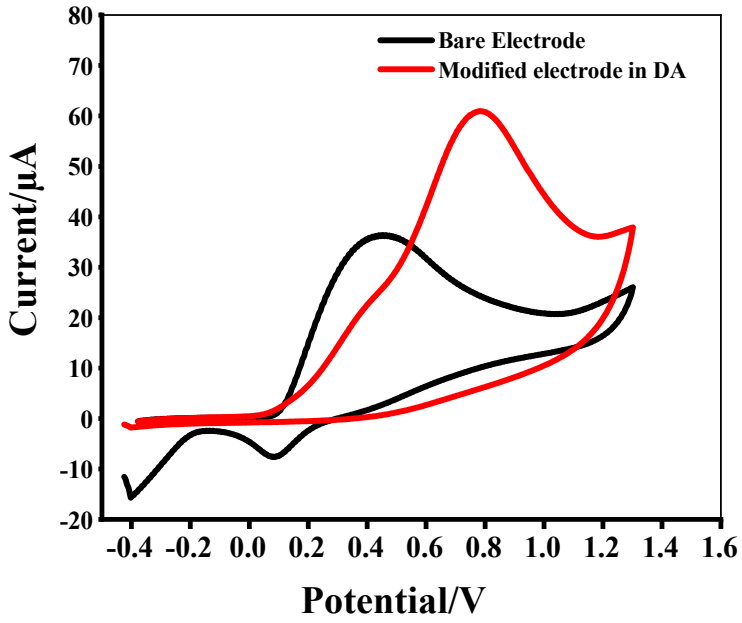

Figure 10. Cyclic Voltammograms of bare electrode and electrode modified with MPA in 2 mM solution of dopamine at pH 7.

3.6. Electrochemical Detection with Different Concentrations of DA

From cyclic voltammograms of concentration ranging from 2 mM to 10 mM of dopamine solution interacted with a modified glassy carbon electrode, it was observed that there was a direct relationship between current and concentration. As the concentration of dopamine increased, the current also increased, as shown in Figure 11.

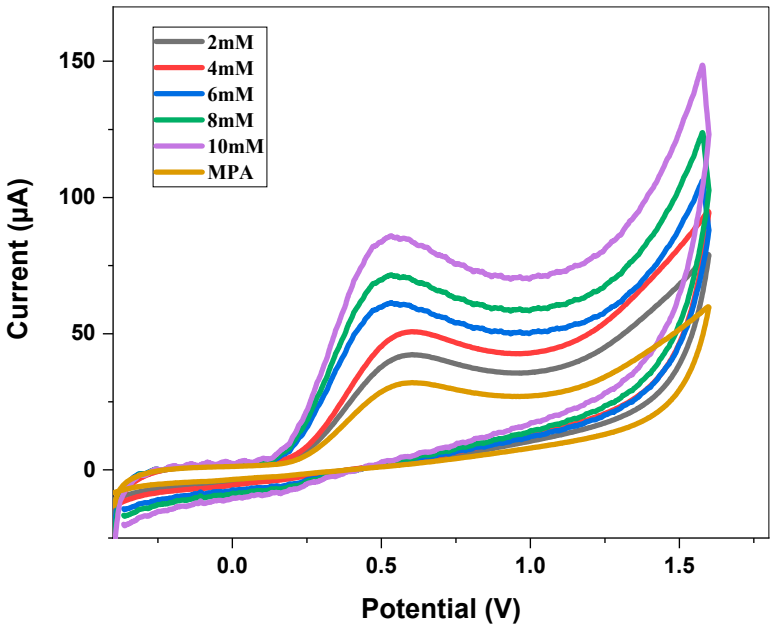

Figure 11. Cyclic Voltammograms of the modified electrode without dopamine and in the presence of various concentrations from 2 mM, 4 mM, 6 mM, 8 mM, and 10 mM solution of dopamine at pH 7.

The cyclic voltammogram was recorded in the interfering species, as shown in Figure 12. A modified glassy carbon electrode gave an oxidation peak of uric acid with a potential of 0.40 to 0.8 V in a solution of uric acid and ascorbic acid at pH 7. The peak current of uric acid and ascorbic acid was lower than that of dopamine, and it was shifted to a lesser potential range. This comparison showed that in the presence of interfering substances, dopamine gave a well-defined peak at a higher current and potential value, while uric acid and ascorbic acid gave a lower peak. The potential ranges for peaks were also different; dopamine had a higher oxidation potential than ascorbic acid and uric acid; therefore, the developed modified electrode gave a selective and sensitive detection of dopamine.

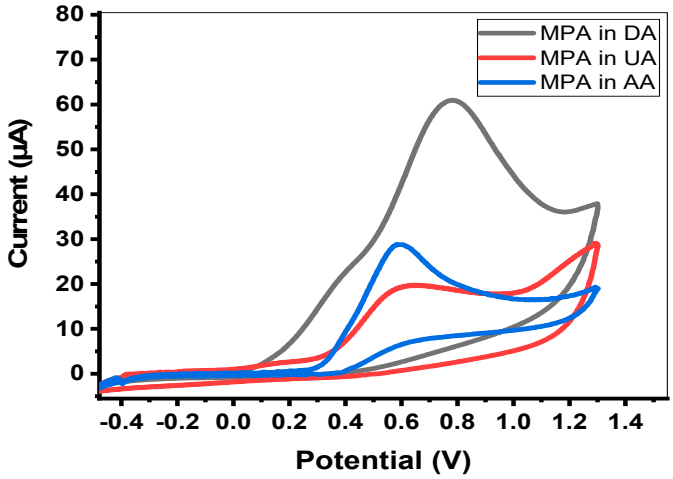

Figure 12. Cyclic Voltammograms of modified electrode in dopamine, ascorbic acid, and uric acid.

3.7. Interference Effect of Ascorbic Acid and Aric Acid

The interference effect of ascorbic acid and uric acid was studied (Figure 13) in the presence of reagent 1 (AgNP+MPA) and reagent 2 (Ag/Au composite). From UV-visible spectra, it was indicated that in the presence of reagent 1, dopamine was absorbed at 0.18, while ascorbic acid, uric acid, and their mixtures were absorbed at 0.09, 0.076 and 0.17, respectively. From UV-visible spectra, it was indicated that in the presence of reagent 2, dopamine was absorbed at 0.184, while ascorbic acid, uric acid, and their mixtures were absorbed at 0.125, 0.10, and 0.176, respectively. The absorbance value of dopamine confirmed the selective detection of dopamine in the presence of interfering substances.

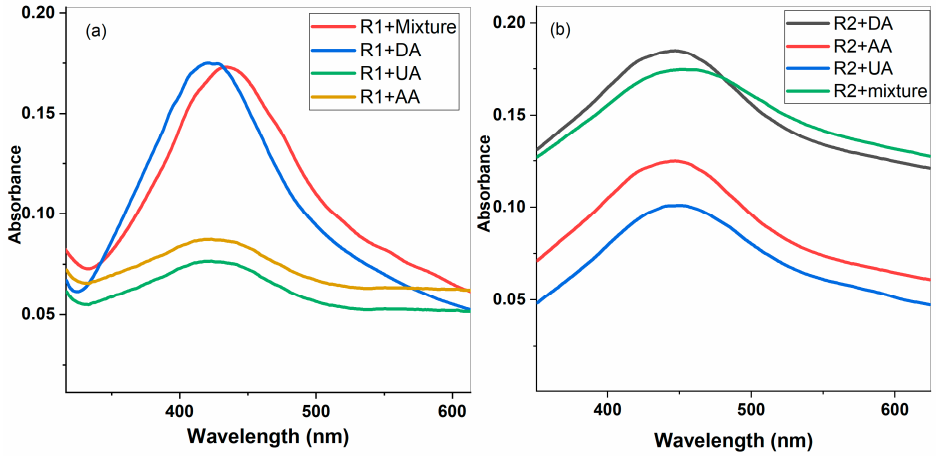

Figure 13. UV-visible spectra of dopamine, ascorbic acid, uric acid, and their mixture in the presence of (**a**) reagent 1 (AgNP+MPA) and (**b**) reagent 2 (Ag/Au composite).

The linearity of dopamine detection using AgNP+MPA represented the R2 value as 0.9949, with the measured value of the limit of detection as 2.51 µM. The linear range for dopamine detection was reported as 1–10 µM, which indicates a linear relationship between absorbance and concentration, as shown in Figure 14. Other colorimetric data were compared with recent work, as shown in Table 1. The reproducibility test was also performed using electrochemical cyclic voltammetry by taking five measurements in a 2 mM dopamine solution. The readings obtained were 46.38, 43.99, 45.20, 46.04, and 46.49. The standard deviation was calculated as SD = 1.71. The obtained results showed that the proposed sensor had excellent fabrication reproducibility.

Table 1. Comparison of developed method of dopamine detection with the latest literature.

Probe Material	Technique	Linear Range	Detection Limit	Reference
Procaterol hydrochloride	Electrochemical	1.0–100 µmol/L	0.3 µmol	[48]
Chiral ZnO nanoparticles	Fluorescent	5–13 µg/mL	0.15 µg/mL	[49]
Graphene modified electrodes	Electrochemical	4–100 µmol/L	2.64 µmol	[50]
Unmodified silver nanoparticles	Colorimetric	0 to 0.6 mM	60 nM	[51]
Aptamer and unmodified citrate-capped gold nanoparticles	Colorimetric	5.4×10^{-7} M to 5.4×10^{-6} M	3.6×10^{-7} M	[52]
Mn_3O_4 and graphene oxide in a nafion film, along with gold nanoparticles	Electrochemical	1.0 µmol/L to 1.45 µmol/L	0.25 µmol/L	[53]
Poly zincon layer	Electrochemical	1.16 to 401 µM	0.38 µM	[54]
Present method	Colorimetric andElectrochemical	1 to 10 µM	2.51 µM	Recent work

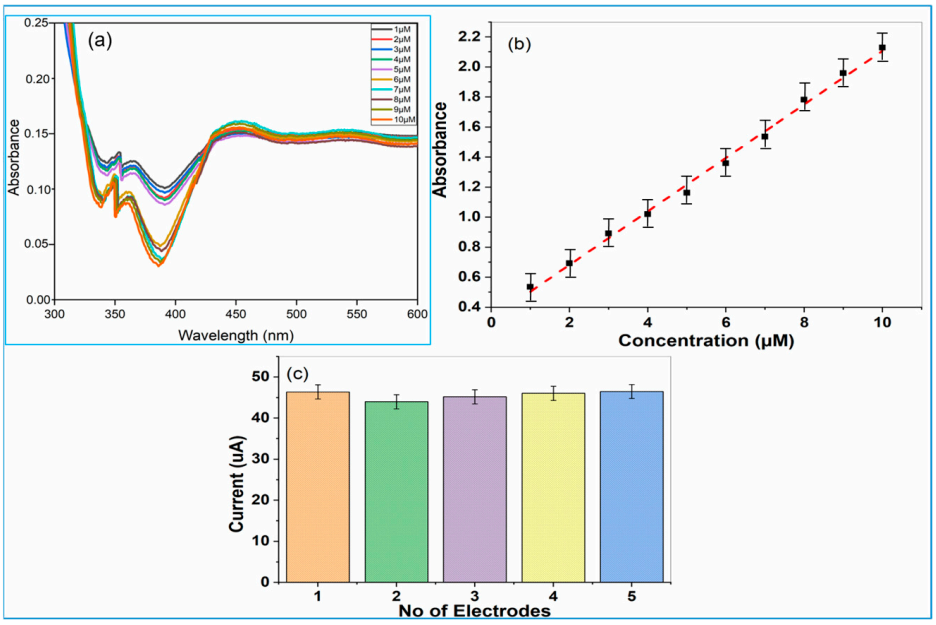

Figure 14. (**a**) UV-visible spectra of dopamine at various concentrations from 1 to 10 micromolar in the presence of reagent 1 (AgNP+MPA), (**b**) Calibration graph for dopamine detection using AgNP+MPA, and (**c**) bar chart of the reproducibility of the developed sensor (n = 5).

4. Conclusions

This study successfully developed a simple and inexpensive paper-based microfluidic biosensor for onsite dopamine detection. Whatman filter paper No. 1 was treated with hot wax to create hydrophilic channels, and reagent solutions were applied and dried at room temperature. When dopamine solution was added, a visual color change was observed using solutions of silver nanoparticles with mercaptophenylacetic acid and a gold-silver nanoparticle nanocomposite. This color change occurred at pH levels 7.0, 7.4, 8.0, and 9.0 and was confirmed by UV-visible spectroscopy. Additionally, an electrochemical sensor was created by modifying a glassy carbon electrode with mercaptophenylacetic acid, which showed excellent performance for dopamine oxidation. This dual-sensing system, notable for its simplicity, fast response time, and high sensitivity, offers a cost-effective and scalable solution for medical diagnostics.

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