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Sensitive Spectrophotometric Detection of Dopamine, Levodopa and Adrenaline Using Surface Plasmon Resonance Band of Silver Nanoparticles

M.R. Hormozi Nezhad[a,b,*], J. Tashkhourian[c] and J. Khodaveisi[c]

[a]*Department of Chemistry, Sharif University of Technology, Tehran, 11155-9516, Iran*

[b] *Institute for Nanoscience and Nanotechnology, Sharif University of Technology, Tehran, P.O. Box 11365-9161, Iran*

[c] *Department of Chemistry, Faculty of Sciences, Persian Gulf University, Bushehr 75169, Iran*

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A simple and effective procedure is proposed for spectrophotometric determination of catecholamines; Dopamine (1), L-Dopa (2) and Adrenaline (3). It was found that the reduction of Ag^+ to silver nanoparticles (Ag-NPs) by these catecholamines in the presence of polyvinylpyrrolidone (PVP) as a stabilizing agent produced very intense surface plasmon resonance peak of Ag-NPs. The plasmon absorbance of the Ag-NPs allows the quantitative spectrophotometric detection of the catecholamines. The calibration curves derived from the changes in absorbance at λ = 440 nm were linear with concentration of Dopamine, Levodopa and Adrenaline in the range of 3.2×10^{-6}- 2.0×10^{-5} M, 1.6×10^{-7} - 1.0×10^{-5} M, 1.5×10^{-6}- 4.0×10^{-5} M, respectively. The detection limits (3σ) were 1.2×10^{-6} M, 8.6×10^{-8} M, 9.7×10^{-7} M for the Dopamine, L-Dopa and Adrenaline, respectively. The method was applied successfully to the determination of catecholamines in Ringer's injection serum.

Keywords: Silver nanoparticles, Catecholamines, Surface plasmon band, Spectrophotometric determination

INTRODUCTION

Catecholamines (include compounds with a dihydroxyphenyl group and an amine group) represent a group of biogenic amines, among which epinephrine, norepinephrine , dopamine and L-dopa act as neurotransmitters in the function of brain and nerve signal transduction or hormones are widely used in the treatment of Parkinson's disease , bronchial asthma, hypertension and in cardiac surgery [1].

The measurement of the level of catecholamines like dopamine and adrenaline in biological fluids has an essential role in the diagnostics of diseases and controlling medicine because many diseases are related to the change of cathecolamines concentration. In particular, various tumours of the sympatho-adrenal system, such as pheochromatocytoma or neuroblastoma are diagnosed by determination of the catecholamines and/or their metabolites in urine and blood [2,3]. On the other hand, pharmaceutical preparations containing catecholamines as pure substances and in dosage forms have been available for many years for the treatment of certain neural disorders; for example, L-dopa that is a dopamine physiological precursor, extensively used for Parkinson's disease. Hence, it is of great importance to develop a method that can effectively detect the neurotransmitters. In recent years, many methods have been reported for the determination of neurotransmitters in pharmaceutical preparations and biological samples, such as electrochemistry [4,5], chemiluminescence [6], spectrofluorimetry [7], high performance liquid chromatography (HPLC) with different detectors [8,9] and

*Corresponding author. E-mail: hormozi@sharif.edu

mass spectrometry [10]. Many current techniques for neurotransmitter detection, such as capillary electrophoresis, liquid chromatography, mass spectrometry and electrochemistry in spite of good sensitivity, require expensive and sophisticated instrumentation or complicated sample preparation and time consuming processes. Thus, the development of new sensitive, fast and practical method for neurotransmitter detection still remains a great challenge.

Nanoparticles made of silver and gold have been the focus of research for many decades as a result of their intriguing optical properties [11–13]. When dispersed in liquid media, these nanoparticles exhibit a strong UV–vis extinction band that is not present in the spectrum of the bulk metal. This extinction band results when the incident photon frequency is resonant with the collective excitation of the conduction electrons and is known as the surface plasmon resonance (SPR) [13,14]. Surface plasmon resonance excitation results in wavelength- selective absorption with extremely large molar extinction coefficients (~$3\times10^{11}M^{-1}$ cm^{-1}) [15] which allowing higher sensitivity in optical detection methods than conventional reagents.

Recently the colorimetric nanoprobes have been developed for sensitive and selective detection of proteins [16], heavy metal ions [17], lectine [18], neurotransmitters [19,20], phenolic compounds [21] thiol-containing amino acids [22] DNA [23] enzymatic activity sensing [19,24] based on optical properties of gold nanoparticles.

In comparison with gold nanoparticles there are limited reports on the optical detection biological active molecules based on silver nanoparticles surface plasmon peak. Hung and coworkers reported a plasmon resonance light scattering (PRLS) detection method for ferulic acid detection based on the formation of silver nanoparticles [25]. Li et al. recently reported a novel and highly selective colorimetric sensor to histidine in water based on para-sulfonatocalix[4]arene modified silver nanoparticles [26]. The surface plasmon peak of silver nanoparticles that synthesized in the presence of humic acids (HA) which acts as capping agents, are sensitive to increasing concentrations of sulfurazon-ethyl herbicide in solution [27]. Also, the colorimetric methods have been developed for assay Concanavalin A (Con A) [28], DNA [29] and ammonia [30] .

In this article we report a very simple and sensitive colorimetric method for detection of a series of neurotransmitters (Dopamine, L-Dopa and Adrenaline) as active reducing agents for the generation of Ag-NPs. The surface plasmon band of the generated Ag-NPs enabled the quantitative analysis of the Dopamine, L-Dopa and Adrenaline.

EXPERIMENTAL

Reagents

All chemicals used were of analytical reagent grade and the solutions were prepared with deionized water. 3-hydroxytyraminium chloride(Dopamine), L-3-(3,4-dihydroxyphenyl)alanine(L-Dopa), (-)-Adrenaline, silver nitrate and polyvinylpyrrolidone (PVP) by average mol wt 10,000 were from Merck and Fluka. All other common laboratory chemicals were of the best grade available and were used without further purification. All solutions were used within 1 h after preparation, and the experiments were performed at ambient temperature (25±2 °C).

Stock solutions of $AgNO_3$ (0.01 M) was prepared by dissolving 0.085 g $AgNO_3$ in deionized water and diluting to 50 ml.

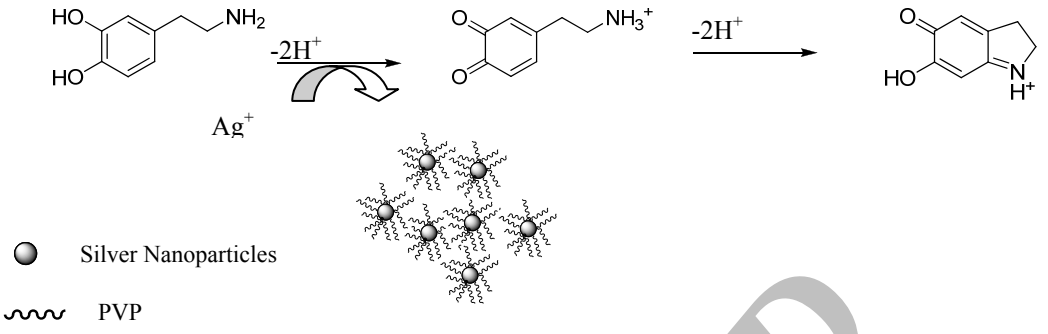
A stock solution of polyvinylpyrrolidone (PVP) (0.4 g/L) was prepared daily by dissolving 0.01 g of PVP (Merck) in water and diluting to 25 ml. Fresh 0.05M solution of 3-hydroxytyraminium chloride(Dopamine),L-3-(3,4-dihydroxyphenyl)alanine(L-Dopa) and (-)-Adrenaline were prepared daily by dissolving the reagent in deionized water. The final pH of the solution was adjusted by adding NaOH.

Apparatus

The UV–vis absorbance spectra were recorded on a PerkinElmer (Lambda25) spectrophotometer with 1.0 cm glass cell was used. Measurements of pH were made with a Denver Instrument Model 270 pH meter equipped with a Metrohm glass electrode.

Procedure

In 5ml volumetric flasks, 1 ml of $AgNO_3$ 0.01 M, 0.7 ml of PVP 0.4 (g/L), different concentrations of the catecholamines



Scheme 1. Reduction Process of the Silver Salt by the Dopamine

and 1 ml of NaOH 0.001 M added to obtain the reasonable solution. Then it was mixed slowly and a portion of that was transferred within 7 min into a 1 cm spectrophotometric cell to record the absorbance. It should be noted that the order of the addition of the reagents is very important. The absorbances were measured at 440 nm that is λ_{max} of silver nanoparticles surface plasmon resonance peak at this condition, against a reagent blank.

RESULTS AND DISCUSSION

The systems in this study consist of an aqueous $AgNO_3$ solution that includes polyvinylpyrrolidone (PVP), as stabilizer, at an alkaline medium. Different catecholamines such as Dopamine (1) , L-Dopa (2) and Adrenaline (3) act as effective reducing agents for reduction of silver metal salt (Ag^+) to the Ag NPs without added any seeds (Scheme 1). In the absence of reducing agents, there is no absorption peak in visible region (380-700 nm). Upon addition of catecholamines which act as reducing agent silver ions reduced to silver nanoparticles and then the absorbance characteristic to the plasmon of the Ag-NPs is observed.

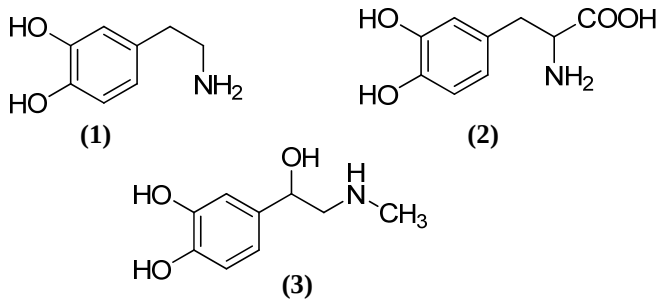


Fig. 1 shows the absorption spectra of the Ag nanoparticles plasmon that produced by the L-Dopa analytes against reagent blank. Based on this peak appearance a method was proposed for the detection of catecholamine compounds. A series of experiments were conducted to establish the optimum analytical conditions for the detection of Dopamine, L-Dopa and Adrenaline. The effects of NaOH concentration, time for color development, type and concentration of stabilizer were investigated to find optimum conditions.

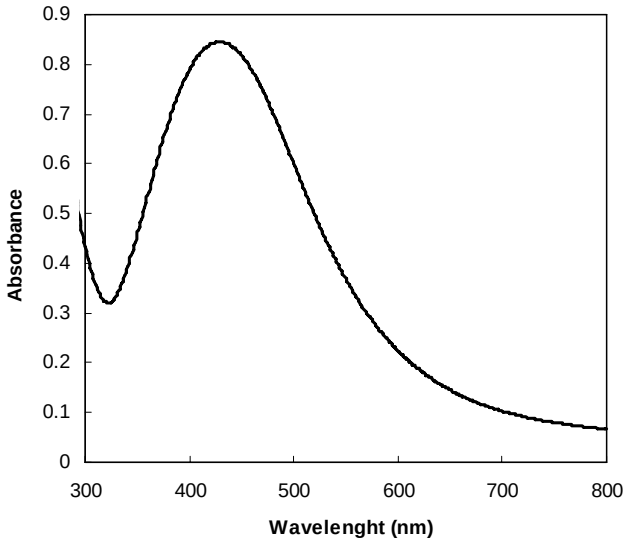


Fig. 1 Absorbance spectra of Ag-NPs formed by 9.6×10^{-6} M of L-Dopa under the optimum conditions

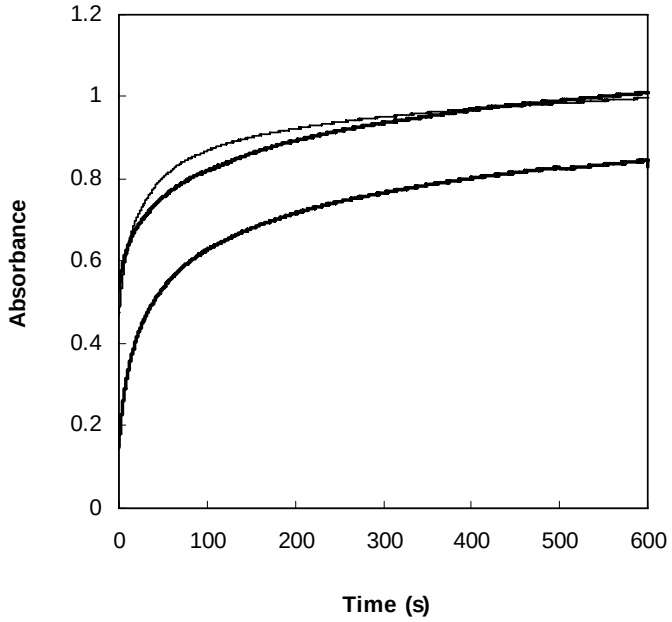


Fig. 2 Kinetic curves corresponding to the absorbance changes (at $\lambda = 440$ nm) of the catecholamines stimulated growth of Ag-NPs. Conditions; $AgNO_3$ (2×10^{-3} M), PVP (0.06 g/L), NaOH (0.2 mM) , Dopamine (2×10^{-5} M) , L-Dopa (9.6×10^{-6} M) and Adrenaline (3.2×10^{-5} M).

Effect of Time for Color Development

Fig. 2 shows the changes of the absorbance at 440 nm with time during the first few minutes from initiation of the reaction for different analytes. As it is obvious, the absorbance reaches a maximum after about 7 minutes and remains constant afterwards. Therefore, all the absorbance measurements were performed after 7 minutes from initiation of the reaction.

Effect of Stabilizer Type and Concentration

Protective agents or stabilizers play a crucial role in stabilizing the nanoparticle colloidal metals from agglomeration according to the two basic meodes: *electrostatic* and *steric* stabilization [31]. Electrostatic stabilization is caused by the Coulombic repulsion between particles, caused by the electrical double layer formed by ions adsorbed at the particle surface (e.g., sodium citrate) and the corresponding counter ions. Steric stabilization is achieved because of the coordination of sterically demanding organic molecules and polymers that act as protective shields on the metallic surface (e.g. PVP) [32]. In this study we selected PVP and sodium citrate as stabilizer for preventing of silver nanoparticles agglomeration. Further study shows that sensitivity and linear range of catecholamines were better when we used PVP in compare to sodium citrate. Because H^+ was produced from the reaction between silver ions and catecholamines, consumption of H^+ can promote silver ion reduction. When PVP was introduced, it would coordinate with Ag^+ and H^+ which can produce complex compounds, $Ag(PVP)^+$ and $H(PVP)^+$ then Ag^+ and H^+ were stabilized [33]. The stabilization of Ag^+ restrained the silver nanoparticles formation reaction, but the stabilization of H^+ facilitated it. The latter affection may be stronger than the former one, and the reaction was accelerated. The variation of absorbance at 440 nm that is λ_{max} surface plasmon peak of the Ag-NPs as a function of the concentration of PVP is shown in Fig. 3. The results show that the maximum intensity was obtained at 0.06 g/L PVP for different analytes.

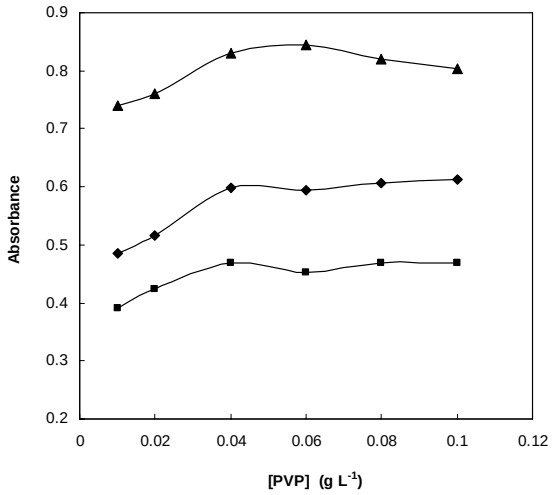


Fig. 3 Effect of PVP concentration on the absorbances plasmon of the Ag - NPs. Conditions; $AgNO_3$ (2×10^{-3} M), NaOH (0.2 mM), Dopamine (2×10^{-5} M), L – Dopa (9.6×10^{-6} M) and Adrenaline (3.2×10^{-5} M).

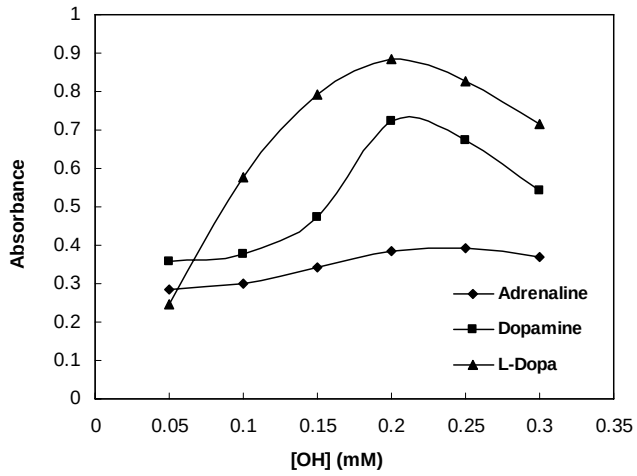


Fig. 4 Dependence of the surface plasmon band intensity on the concentrations of NaOH. Conditions; $AgNO_3$ (2×10^{-3} M), PVP(0.06 g/L) , Dopamine (2×10^{-5} M), L-Dopa (9.6×10^{-6} M) and Adrenaline (3.2×10^{-5} M).

Effect of NaOH Concentration

The influence of pH on Ag^+ reduction by catecholamines is expected since catecholamines has a dihydroxyphenyl group which can lose H^+ during oxidation and o-quinone formation process (Scheme 1). Because buffered condition failed to obtain silver nanoparticles we added NaOH for provide enough alkalinity. Fig 4. shows the effect of NaOH concentration on silver nanoparticles surface plasmon peak intensity. As it is seen, the signal increases up to a known concentration of NaOH then decreases which might be due to the Ag_2O formation. Thus, a concentration of 0.2 mM NaOH was selected as the optimum NaOH concentration.

Analytical Figures of Merit

Calibration Curves

Fig. 5 (a–c) is depicted the spectra of the Ag-NPs formed upon treatment with different concentrations of the analytes for a fixed time interval corresponding to 7 min. As the concentration of the analytes increases, the absorbance correspond to the plasmon of the Ag-NPs are intensified. Under the optimum experimental conditions, the calibration curves for Dopamine, L-Dopa and Adrenaline were linear from 3.2×10^{-6}- 2.0×10^{-5} M, 1.6×10^{-7} - 1.0×10^{-5} M, 1.5×10^{-6}-4.0×10^{-5} M, respectively.

Detection Limit and Reproducibility of the Method

The detection limit ($3S_b$) [34] obtained for determination of Dopamine, L-Dopa and Adrenaline were 1.2×10^{-6} M, 8.6×10^{-8} M, 9.7×10^{-7} M, respectively. A study of the precision of the method was made with six independent experiments and solutions of various concentrations of analytes under the optimum conditions. Table 1 features the analytical characteristics of the method.

In Table 2, the linear range and detection limit of the proposed method are compared with the corresponding values for the detection of neurotransmitters based on formation of Au-nanoparticles [19]. From the data given in Table 2 it is immediately obvious that the linear range and detection limit of the proposed method is superior to those reported before [19].

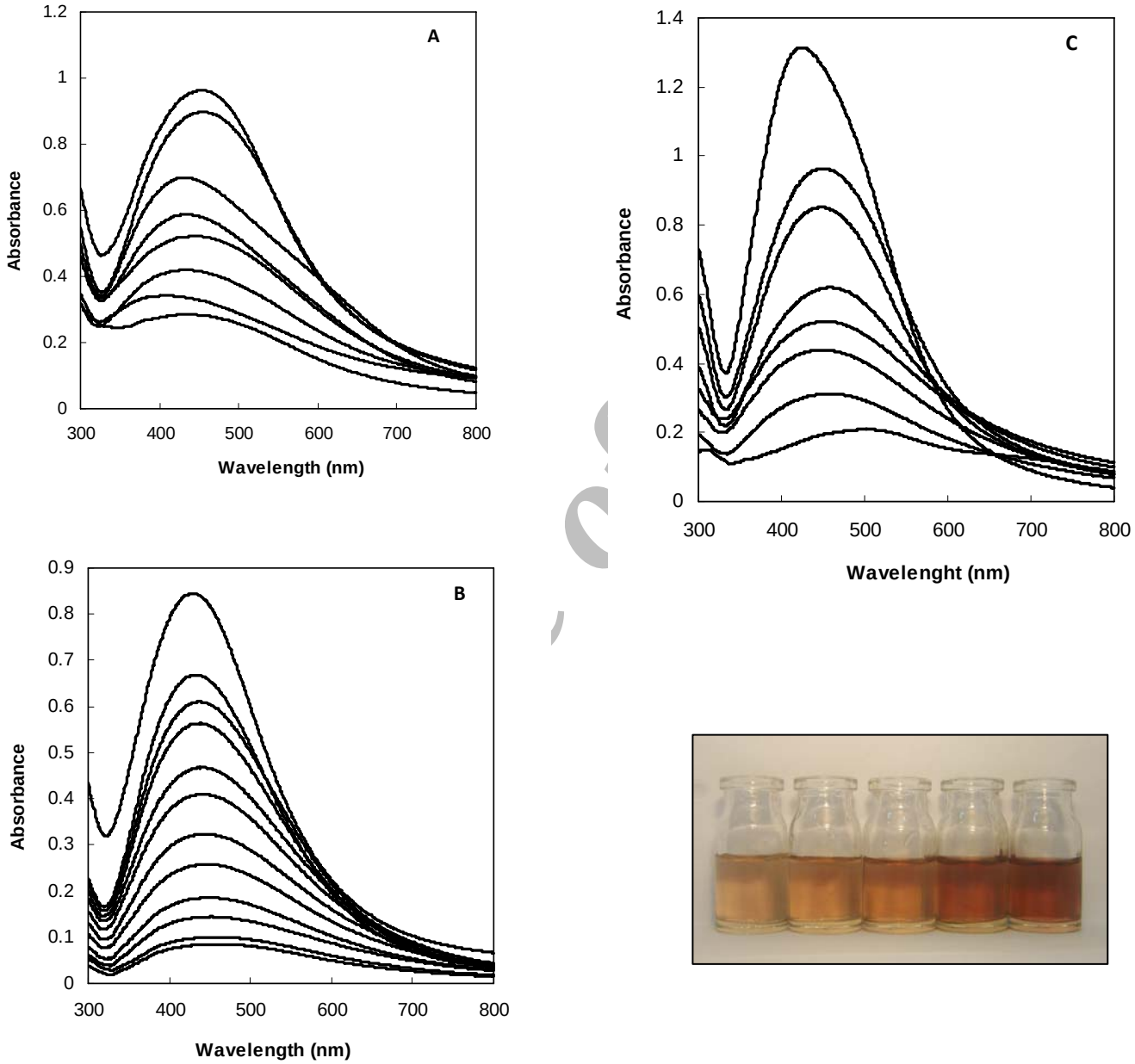


Fig. 5 Absorbance spectra of Ag-NPs in the presence of different concentration (shows in parenthesis) of A) Dopamine (3.2×10^{-6}- 2.0×10^{-5}), B) L-Dopa (1.6×10^{-7} - 1.0×10^{-5}) , C) Adrenaline (1.5×10^{-6}- 4.0×10^{-5}) D) Color images of the glass bottle containing Ag-NPs formed in the presence of different concentrations of L-Dopa.

Table 1 Analytical characteristics of the method

Parameters	Dopamine	L-Dopa	Adrenaline
Linear Range (M)	3.2×10^{-6}- 2.0×10^{-5}	1.6×10^{-7} - 1.0×10^{-5}	1.5×10^{-6}- 4.0×10^{-5}
Calibration Equation	A=0.0483C[a]+0.1192	A=0.0832C+0.0123	A=0.0263C+0.1
	(R^2=0.997)	(R^2=0.994)	(R^2=0.995)
LOD[b] (M)	1.2×10^{-6}	8.6×10^{-8} M	9.7×10^{-7} M
%RSD[c]	3.2[1] - 4.5[2]	2.5[1] - 3.8[2]	2.1[1]- 3.2[2]

[a] Unit of concentration in calibration equations are μM.

[b] Theoretical detection limit (blank plus three times its S.D.) [34]

[c] RSD for six measurements that correspond to 1) 4×10^{-6} M and 2) 1.5×10^{-5} M.

Table 2. Comparison of present work and previous study that use Au-nanoparticles for analysis of neurotransmitters

Parameter	Analyte	Ref.[19]	This Work
Detection Limit (M)			
	Dopamine	2.5×10^{-6}	1.2×10^{-6}
	L-Dopa	2.5×10^{-6}	8.6×10^{-8}
	Adrenaline	2.5×10^{-5}	9.7×10^{-7}
Linear Range(M)			
	Dopamine	2.5×10^{-6} - 1.5×10^{-5}	3.2×10^{-6}- 2.0×10^{-5}
	L-Dopa	5.0×10^{-6} - 3.0×10^{-5}	1.6×10^{-7} - 1.0×10^{-5}
	Adrenaline	4.0×10^{-5} - 2.0×10^{-4}	1.5×10^{-6}- 4.0×10^{-5}

Table 3 Determination of catecholamines in Ringer's injection solution

Analyte	Added (μM)	Found (μM)	Recovery (%)
Dopamine	1.00	1.02	102.00
	5.00	4.91	98.20
L-Dopa	0.40	0.39	97.50
	2.00	2.05	102.50
Adrenaline	2.00	1.96	98.00
	4.00	3.89	97.25

Application

The proposed method was successfully applied to the determination of Dopamine, L-Dopa and Adrenaline in Ringer's injection solution. Ringer's serum is nonpyrogenic and sterile solution which containing common electrolyte solution with a variety of clinical uses likes fluid and electrolyte replenishment. For the determination of catecholamines in Ringer's serum, a certain amount of each the compounds was spiked to the samples without further treatment and their concentrations were determined by the proposed methods. The results are given in Table 3.

CONCLUSION

The potential application of silver nanoparticles as chromogenic agent has been demonstrated in this work for optical detection of catecholamines based on the seedless production of Ag-NPs. The proposed method is simple, sensitive, and inexpensive for the determination of catecholamines. This analytical protocol may be important for monitoring and optical detection of catecholamines in various biological samples.

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