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RESEARCH ARTICLE

Indirect Spectrophotometric Determination of Methyldopa using AuNPs-Cr(VI)

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ABSTRACT

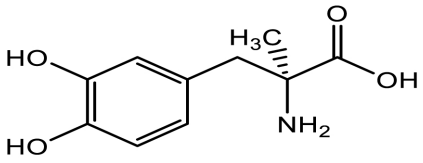
A simple, sensitive, and rapid spectrophotometric method was suggested for the indirect determination of methyldopa. The method depends on the chemical reaction between citrate-stabilized gold nanoparticles and chromium (III) ions, which form during methyldopa oxidation through potassium dichromate. The process results in nanoparticle aggregation, while the solution transitions from red to blue-grey, and the plasmon band moves from 528 nm to 640 nm. The absorption rate increased linearly within the concentration range of 60-140 ng/ml, while the detection limit reached 27 ng/mL. The proposed method showed excellent accuracy and precision because the mean recovery value reached 100.16%, and the relative standard deviation stayed under 1%. The method produced results that matched the labeled content of methyldopa tablet formulations, which demonstrated its effectiveness for pharmaceutical testing.

Keywords: Aggregation, Chromium (III), Methyldopa, Nanoparticles, Spectrophotometric determination

Introduction

Methyldopa (MD), shown in Fig. 1, is chemically (2S)-2-Amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid.[1] It is widely used to treat hypertension. The suggested pharmacological mechanism is based on its metabolic conversion into α-methyl norepinephrine within central adrenergic neurons, which is then released and stimulates central adrenoceptors, particularly alpha-2 adrenoceptor.[2] Methyldopa remains popular with obstetricians for the hypertension of pregnancy because of its apparent safety for the fetus.[3] Recent studies have shown that excessive doses of it cause liver damage due to increased blood urea and creatinine.[4]

Accurately determining methyldopa in pharmaceutical formulations is of undeniable importance due to its therapeutic relevance. Widely used methods for analyzing methyldopa include spectrophotometry,[5–8] spectrofluorimetry,[9] electrochemical[10–12] and chromatography.[13,14] While effective, several of these



Methyldopa ($C_{10}H_{13}NO_4$)

Fig. 1. Chemical structure of methyldopa.

methods often require complex sample preparation or advanced instrumentation, which can limit their accessibility in routine quality control. In contrast, the procedure described in this study offers simplicity, rapidity, high sensitivity and cost-efficitiveness.[15] Recent advances in nanomaterials have expanded the scope of spectrophotometric assays. The localized surface plasmon resonance (LSPR) characteristics exhibited by gold and silver nanoparticles render these noble metal nanostructures effective chromogenic platforms. The nanoparticles show major changes

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in their optical and colorimetric properties when they form aggregates, which makes them suitable for analytical detection methods.[16,17] The majority of sensing systems achieve nanoparticle stability through surface capping agents, which include citrate ions, polymers and thiol-containing compounds that use electrostatic forces and metal–ligand coordination mechanisms to detect Cr(III) species. The physical interaction between nanoparticles causes them to merge into larger clusters, which produces detectable changes that impact both absorption spectrum readings and solution pigmentation.[18–20] The current literature lacks any indirect spectrophotometric method that uses AuNPs to detect methyldopa while providing the same high sensitivity and easy operation as the present research. The proposed method provides a basic and highly sensitive testing method which does not need sophisticated equipment or complicated sample preparation procedures. The novelty of this study lies in the oxidation of methyldopa using potassium dichromate, releasing Cr (III), which interacts with citrate-capped AuNPs through a cooperative metal-ligand reaction. This interaction induces rapid aggregation of the AuNPs and noticeable color shifts from red to purple or blue, depending on the concentration of Cr (III). Thus, the proposed method provides a unique combination of accessibility, rapidity, cost-effectiveness, and demonstrates high sensitivity for methyldopa determination in both pure samples and pharmaceutical formulations.

Materials and methods

Apparatus and chemicals

Citrate-stabilized AuNPs were analyzed using a Shimadzu UV–Visible 1650 PC double-beam spectrophotometer, scanning electron microscope FESEM, TESCAN, MIRA3, Czechia, and Energy-Dispersive X-ray Spectroscopy EDX, TESCAN, MIRA3, Czechia.

Methyldopa obtained from SDI/Samarra/Iraq was provided as a reference material, with its composition stated to be 99.86 percent w/w methyldopa. Pharmaceutical formulations containing methyldopa were sourced from various suppliers. Additionally, chemical compounds including potassium dichromate, chloroauric acid trihydrate, and trisodium citrate were procured from Fluka.

Solutions

A stock solution of methyldopa was prepared by dissolving 0.01g in 50 mL of deionized distilled water in a standard bottle. The working solution (1 μg/mL) was obtained by diluting the standard stock solution with deionized distilled water to the desired concentration.

A solution of Cr (VI) at a concentration of 300 μg mL^{-1} was prepared by dissolving 0.0424 g of Potassium dichromate ($K_2Cr_2O_7$) in 50 mL of ultrapure distilled water using a standard flask.

Assay of pharmaceutical tablets

Five formulated tablets were crushed after being weighed accurately. From this powder, a quantity equivalent to 0.01g of methyldopa (for both Spain and UK formulations, containing 250 mg of methyldopa per tablet) was carefully weighed separately. The weighed portion was diluted in a 100 mL volume of deionized distilled water using a volumetric bottle. The solution underwent sonication for 5 minutes before researchers performed 4000 rpm centrifugation for 5 minutes to achieve complete separation of insoluble substances. The clear supernatant was subsequently passed through a 0.45 M nylon syringe filter. The solution required appropriate dilution with deionized distilled water to create methyldopa solutions which fell within the needed concentration range.

Preparation of AuNPs

AuNPs were synthesized in aqueous solution using the citrate-mediated reduction of HAuCl4. The researchers performed the synthesis by heating 50 mL of 1 mM $HAuCl_4 \cdot 3H_2O$ solution to the boiling point while they kept the mixture under constant stirring. The solution reached a boiling point; 5 mL of 3.88 $\times$ 10^{-2} M trisodium citrate solution was rapidly added to the mixture, which then underwent 20 minutes of heating. The solution exhibited a color transition from pale yellow to ruby red, which confirmed that the nanoparticles had successfully formed. The AuNP suspension required storage at 4 °C to prevent particle aggregation while maintaining its colloidal stability. The citrate-capped AuNPs underwent characterization through UV–Vis absorption spectrum measurement, which spanned from 250 to 850 nm to show their surface plasmon resonance peak at 528 nm Fig. 2. Additionally, the synthesized gold nanoparticles were characterized using scanning electron microscopy (SEM), and the images are presented in Fig. 3. The spherical shape of citrate-capped AuNPs became evident through 3 which showed that these nanoparticles measured 25.08 nm in diameter on average. Then, energy-dispersive X-ray spectroscopy (EDX) was used to determine the chemical makeup of the samples through elemental analysis present in the gold nanoparticle solution, thereby verifying its

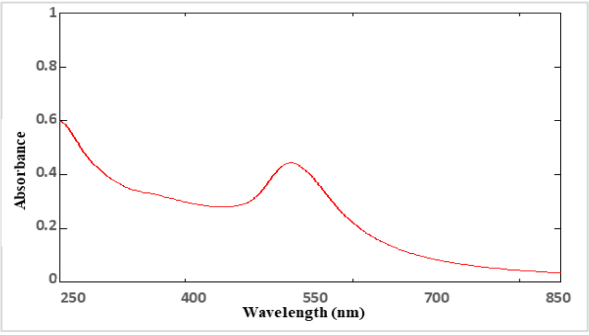

Fig. 2. Absorbance spectrum of AuNPs solution.

purity and confirming the absence of any extraneous contaminants Fig. 4.

General procedure

A series of 10 mL volumetric flasks was prepared by adding 1.0 mL aliquots of the methyldopa standard solution, which ranged in concentration from 0.06 to 0.14 μg/mL. To each flask, 1.0 mL of a potassium dichromate solution (300 μg/mL) was added and allowed to react for 15 minutes at room temperature for reduction. Following this, 1.25 mL of an AuNPs solution was introduced into each flask, and the resulting solution was then diluted to the required final volume using deionized distilled water. The absorption spectra of the resulting solutions were obtained between 400 nm and 800 nm.

Results and discussion

Citrate-capped gold nanoparticles exhibit unique optical properties, such as color changes and scattering effects.[21] The analytical detection of metal ions, especially chromium species, becomes possible because of these features, which enable their broad application. Research findings demonstrate that Cr(III) ions can be detected through AuNP-based methods, which show no significant response to Cr(VI) ions.[19,22] This is achieved through the strong interaction of Cr(III) ions with AuNP surfaces. The reaction leads to fast nanoparticle aggregation, which produces a visible color transformation from red to blue. The researchers investigated the feasibility of applying this principle for methyldopa detection; the absorption spectrum of the AuNPs was examined in the presence of methyldopa, Cr (VI) ions, and mixed solutions of both. The results indicate that neither methyldopa nor Cr (VI) alone caused aggregation of AuNPs. However, when methyldopa was combined with Cr (VI), distinct aggregation occurred, as evidenced by the appearance of a new absorption band in the 600 to 700 region and a simultaneous decrease in the plasmon peak at 528 nm, Fig. 5. This phenomenon can be explained by the in-situ formation of C r(III) during the oxidation of methyldopa by Cr (VI), which subsequently interacts with citrate-stabilized AuNPs. This can be explained by the different behaviors of Cr (VI) and Cr (III) when interacting with citrate-capped AuNPs. Chromium in its hexavalent state, Cr (VI), exists as the (Cr_2O_7) ion, which induces strong electrostatic repulsion when interacting with citrate anions ($C_6H_5O_7$–), thereby preventing AuNPs aggregation. In contrast, trivalent Cr (III) behaves as a hard Lewis acid with high affinity for negatively charged oxygen groups, allowing it to form stable six-coordination complexes with ligands containing oxygen, nitrogen, or sulfur atoms.[23] When Cr (III) binds to citrate-capped AuNPs, cooperative metal–ligand interactions result in the formation of Cr (III)–citrate complexes. facilitating nanoparticle aggregation and producing the characteristic optical shift from red to blue.[24] Thus, the oxidation of methyldopa by Cr (VI) generates Cr (III), which triggers AuNP aggregation via this mechanism, as illustrated in Fig. 6.

The optimal conditions

Effect of buffer solutions

The research studied the analytical properties through experimental tests, which studied its

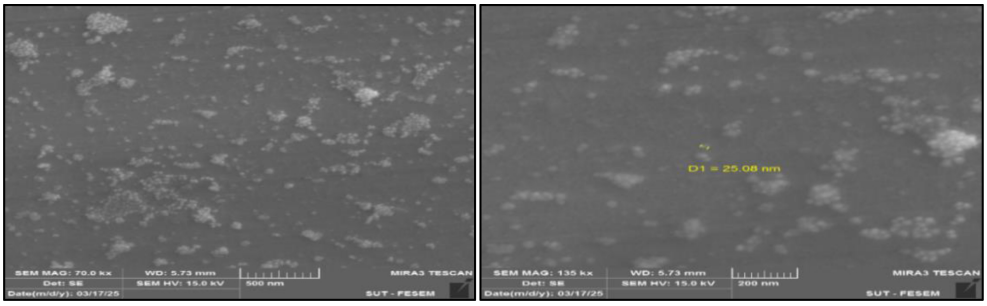

Fig. 3. The SEM images.

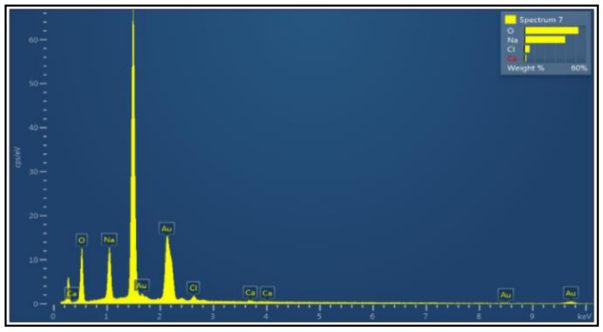

Fig. 4. The energy-dispersive X-ray spectroscopy (EDX) for AuNPs.

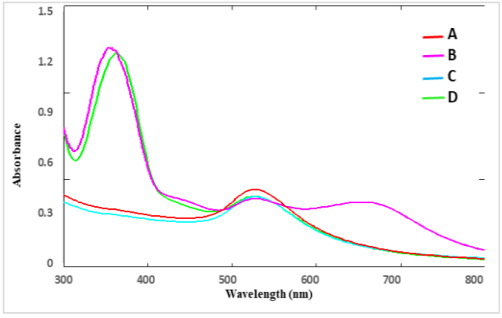

Fig. 5. Absorption spectra of AuNPs (A), Methyldopa, Cr (VI) and AuNPs (B), Methyldopa and AuNPs (C), Cr (VI) and AuNPs (D).

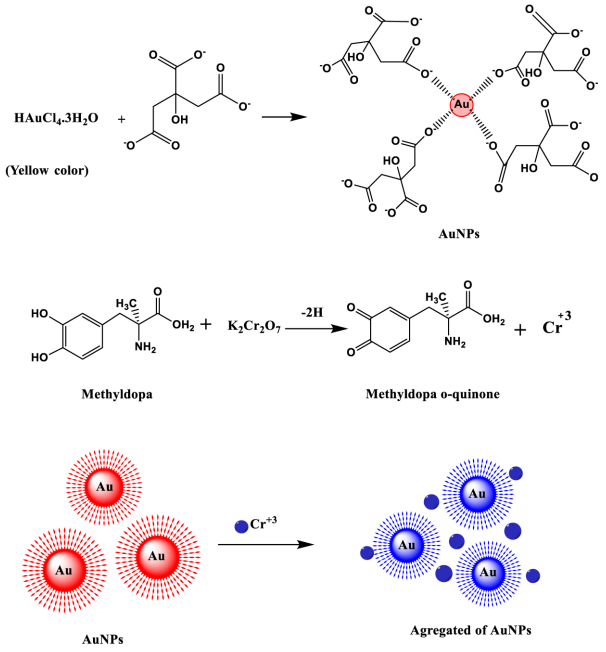

Fig. 6. Suggested mechanism of reaction.

reactions under various pH conditions and buffer solutions, and overall stability. The AuNPs–Cr(III) sensing system achieved its highest stability when the solution pH was maintained at 5 ± 0.4. To investigate the influence of various buffering environments, different buffer media were examined, as illustrated in Fig. 7. The data in Table 1 show that phthalate, acetate, and citrate buffers produced significant decreases in the absorbance ratio (A at 640 nm /A° at 528 nm). The experiments continued without buffer solutions because all measurements needed to take place in an unbuffered solution to achieve the highest possible analytical response.

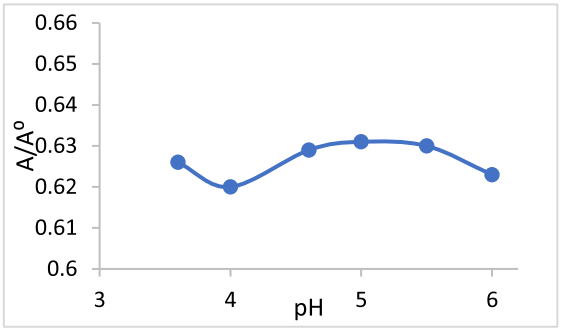

Fig. 7. Effect of pH.

Table 1. Effect of buffer solutions.

Type of medium	A / A°	pH
Phthalate buffer	0.561	5.32
Acetate buffer	0.630	5.40
Citrate buffer	0.600	5.24
without	0.634	5.21

Effect of amount Cr (VI)

The effect of Cr(VI) concentration on the analytical signal obtained for methyldopa was examined over the concentration interval of 100–500 μg/mL. As illustrated in Fig. 8A, the absorption ratios (A/A°) increased progressively with Cr(VI) concentration and reached their maximum at 300 μg/mL, beyond which no further enhancement was observed. This indicates that 300 μg/mL represents the optimal concentration of the oxidizing agent required to achieve complete oxidation and stable complex formation with AuNPs.

Additionally, the effect of oxidizing agent volume was evaluated by adding 0.5 to 1.5 ml of potassium dichromate solution at a fixed concentration of 300 μg/mL. As shown in Fig. 8B, the highest absorbance ratio was obtained when 1.0 mL of Cr(VI) solution was used. Therefore, this volume was selected as the optimum condition for all subsequent analyses to ensure maximum sensitivity and reproducibility of the assay.

Effect of the amount of AuNPs solution

The influence of the volume of prepared AuNPs solution that can be used in the estimation of methyldopa was studied by testing various volumes (0.1-2.0

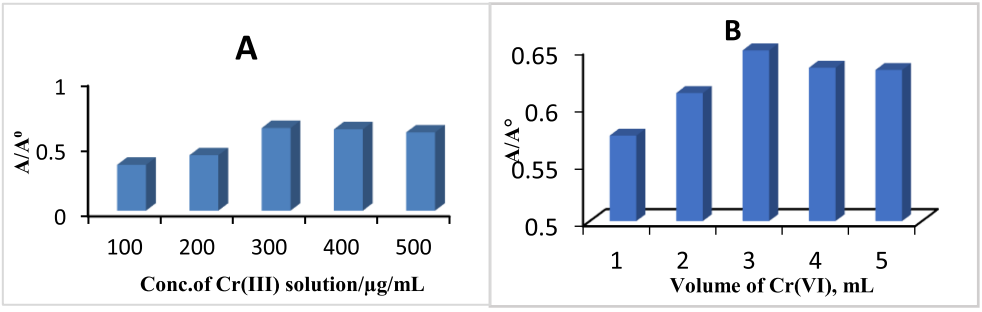

Fig. 8. (A) Effect of the concentration of Cr (VI) solution, (B) Effect of the volume of Cr (VI) solution.

mL). As presented in Fig. 9, the absorbance ratio increased with increasing AuNPs volume, reaching a maximum at 1.25 mL. This volume provided optimal nanoparticle aggregation, indicating efficient interaction between methyldopa and the AuNPs–Cr(VI) system. Therefore, 1.25 mL of the AuNP solution was selected as the optimal volume, and it was adopted in subsequent experiments.

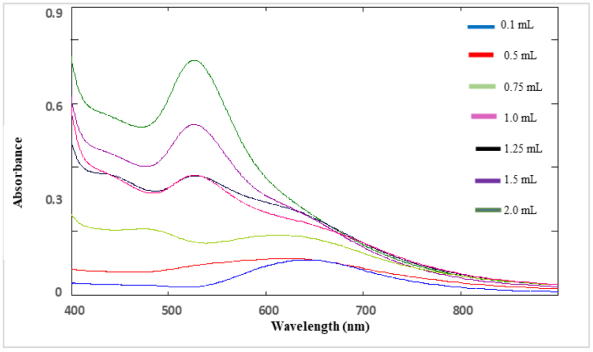

Fig. 9. Effect of changing the amount of AuNPs.

Effect of oxidation period and stability

The study aimed to evaluate the time required for the oxidation of methyldopa and the simultaneous reduction of Cr (VI) to Cr (III). A 1.0 μg/ml of methyldopa was reacted with an optimized amount of potassium dichromate at different time intervals. As shown in Table 2, complete oxidation of methyldopa was achieved within 15 minutes. The subsequent aggregation of AuNPs, induced by their interaction with the generated Cr (III) ions, was complete after 10 minutes and remained stable for at least 50 minutes.

To investigate the influence of temperature on the aggregation behavior of AuNPs, experiments were conducted at different temperatures (0-50°C). The absorbance variations were recorded over time, as illustrated in Fig. 10. The results indicate that gold nanoparticle aggregation occurs rapidly after the release of Cr (III) ions at room temperature (25±2°C). Any deviations from this temperature, whether above or below, resulted in a decrease in the absorbance ratio, suggesting that the optimal reaction temperature for stable and reproducible aggregation is room temperature.

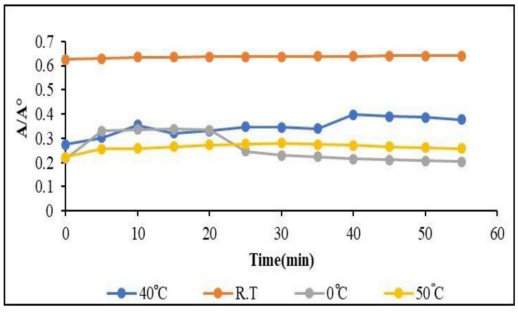

Fig. 10. Effect of temperature on the reaction.

Final absorption spectrum and calibration curve

Aggregation of AuNPs was evidenced by a reduction in a characteristic plasmonic absorption band at 528 nm, together with a corresponding increase in absorbance around 640 nm. The absorbance ratio at 640 nm and 528 nm (A/A°) functions as a quantitative

Table 2. Effect of the oxidation period of methyldopa.

Standing time before adding AuNPs, (minute)	(A /A°) /Standing time (minute)											
	5	10	15	20	25	30	35	40	45	50	55	60
5	0.329	0.332	0.335	0.336	0.338	0.341	0.346	0.345	0.349	0.348	0.352	0.355
10	0.472	0.479	0.486	0.492	0.497	0.504	0.512	0.519	0.524	0.534	0.542	0.546
15	0.625	0.630	0.634	0.635	0.636	0.636	0.637	0.638	0.638	0.640	0.640	0.641
20	0.527	0.539	0.546	0.553	0.558	0.560	0.564	0.566	0.568	0.571	0.578	0.580

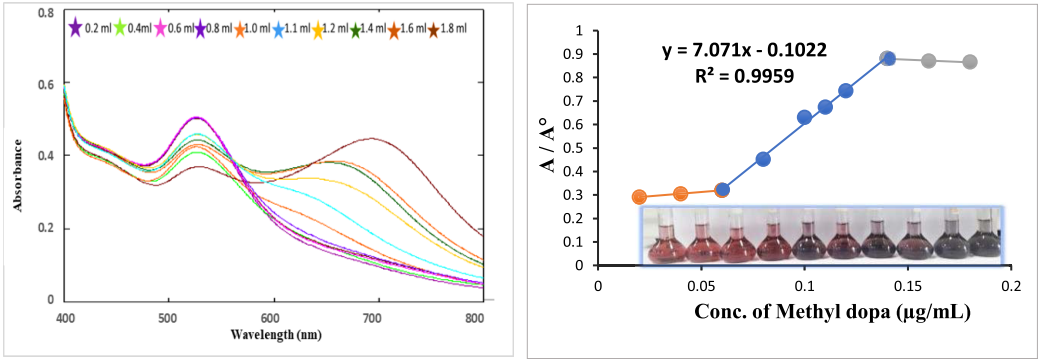

Fig. 11. Final spectrum and calibration curve.

Table 3. Accuracy and precision.

Conc. of methyl dopa (μg/mL)	*Recovery (%)	Average recovery (%)	*RSD (%)
0.08	99.82	100.16	±1.057
0.1	100.19		±0.343
0.12	100.48		±0.723

*Average of five repeated measurements.

Table 4. Determination of methyldopa in tablets.

Drug (250 mg)	Amount present (μg/ml)	Drug content found (mg)	*Recovery (%)	*RSD (%)
Aldomet	0.08	248.33	99.33	±1.190
Aspen	0.1	252.73	101	±1.149
	0.12	249.66	99.86	±1.593
Accord (UK)	0.08	248.89	99.55	±0.609
	0.1	255.85	102.34	±1.053
	0.12	245.63	98.25	±0.912

*Average of three repeated measurements.

parameter that determines the degree of nanoparticle aggregation. The researchers established a calibration plot through their experiments, which linked the absorbance ratio to methyldopa concentrations measured in μg mL^{-1} under their optimized experimental setup. The calibration curve Fig. 11 showed a perfect linear relationship within the range 0.06-0.14 μg/mL concentrations, which proved that the method offers high sensitivity for quantitative methyldopa analysis.

Method accuracy and precision

The method's accuracy and precision were evaluated through determination of the recovery percentage and the relative standard deviation (RSD) using five replicate measurements at three different methyldopa concentrations. The results, which appear in Table 3, show that the developed method provides both precise and accurate analytical results.

The method showed consistent results through recovery tests, which used identical concentration levels because the RSD values remained at low levels. The analytical procedure shows strong stability because the recovery percentages remain consistently high, which proves that the system based on AuNPs–Cr(VI) works effectively for methyldopa quantification in drug products.

Method validation

The proposed method underwent validation through its application for quantifying methyldopa in Aspen and Accord commercial tablet products. The recovery values reported in Table 4 indicate a high level of analytical accuracy and demonstrate

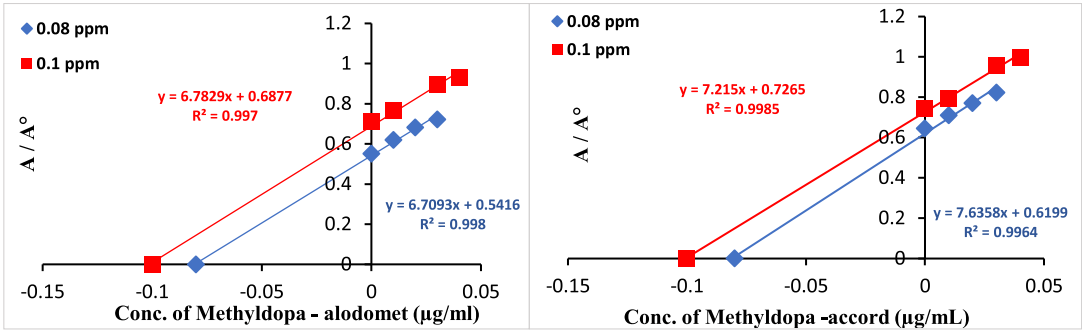

Fig. 12. Curves of standard addition method.

Table 5. Estimation of methyldopa in dosage forms by standard addition method.

Drug (250 mg)	Present amount (μg/ml)	Drug content found (mg)		Recovery (%) of standard addition method
		Current method	Standard addition method	
Aldomet	0.08	248.33	252.26	100.9
Aspen	0.1	252.73	253.46	101.38
Accord	0.08	248.89	253.69	101.47
(UK)	0.1	255.85	251.73	100.69

close agreement with the labeled drug content. The research findings demonstrate that the AuNPs–Cr(VI) method serves as an effective analytical method that can be used for regular quality control tests of methyldopa in pharmaceutical products.

The standard addition method was applied to evaluate method selectivity for the determination of methyldopa in commercial tablet samples without any interference from pharmaceutical additives or co-administered drugs. The results illustrated in Fig. 12 and summarized in Table 5 showed excellent consistency with the calibration curve, confirming that the added analyte concentrations were accurately recovered. These results confirm that the method based on AuNPs–Cr(VI) is highly specific, with minimal interference from common excipients, thus ensuring its suitability for routine pharmaceutical analysis.

Conclusion

The spectrophotometric method for methyldopa detection became simpler because it employed the AuNPs–Cr(VI) system to develop a colorimetric sensing platform. The proposed method showed linear results between 0.06–0.14 μg/mL concentrations, and it detected 0.027 μg/mL as its lowest measurable amount. The research achieved its goal through this method because it provides a specific and dependable analytical method to measure methyldopa concentrations. The method shows both specific binding and consistent results, which make it appropriate for pharmaceutical quality control tests and also suitable for biological sample testing. The present research demonstrates that gold nanoparticles function as effective colorimetric probes, which enable the creation of fast, affordable, and sustainable analytical methods.

Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all Figures and Tables in the manuscript are ours. Furthermore, any Figures and images that are not ours have been included with the necessary permission for republication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at the University of Mosul.

Authors' contributions statement

Z. Z. conceptualized and supervised the project, wrote the manuscript and analyzed the results. A. A. conducted the experiments. Both authors collaborated in discussing the findings and contributed to the final version of the manuscript.

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التقدير الطيفي غير المباشر للمثيل دوبا باستعمال AuNPs-Cr(VI)

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الخلاصة

تم تطوير طريقة طيفية غير مباشرة سريعة ودقيقة لتقدير مستويات المثيل دوبا. تعتمد هذه الطريقة على التفاعل الكيميائي بين جسيمات الذهب النانوية المثبتة بالسترات وأيونات الكروم الثلاثي (Cr^{3+}) الناتجة عن أكسدة المثيل دوبا باستخدام ثنائي كرومات البوتاسيوم. ويؤدي هذا التفاعل إلى تجمع الجسيمات النانوية مصحوبًا بتغير لون المحلول من الأحمر إلى الرمادي المزرق، إضافةً إلى حدوث إزاحة حمراء لحزمة البلازمون السطحي من 528 نانومتر إلى 640 نانومتر. أظهرت قيم الامتصاص زيادة خطية ضمن مدى تراكيز يتراوح بين 60 و140 نانوغرام/مل، مع حد كشف بلغ 27 نانوغرام/مل. كما بيّنت الطريقة المطوَّرة دقة وتوافقًا عاليين، إذ بلغ معدل نسبة الاسترجاع 100.16% وكان الانحراف القياسي النسبي أقل من 1%. وقد أظهرت نتائج تطبيق الطريقة على أقراص المثيل دوبا الدوائية توافقًا جيدًا مع المحتوى المعلن، مما يؤكد كفاءتها في التحليل الصيدلاني.

الكلمات المفتاحية: تجميع، الكروم (III) ، مثيل دوبا، الجسيمات النانوية، التقدير الطيفي.