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A new spectrophotometric method for the determination of promethazine hydrochloride in its pharmaceutical formulation and in biological fluids using CuNPs

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Abstract: A new, simple, and sensitive spectroscopic method has been developed for the determination of PMH in its pure form, in its pharmaceutical formulation, and in biological fluids (blood and urine) spectrophotometrically using copper nanoparticles (CuNPs) synthesized by the green method as a colorimetric detector without the use of chemical reagents. This method was characterized by being easy, sensitive, inexpensive, and environmentally friendly. A colored product (PMH-CuNPs) was formed. The absorbance was measured at the wavelength of 641.4 nm, and the linear range of (20–280) µg/ml, and it was found that the value of the correlation coefficient was (0.9983), the detection limit was (0.39) µg/ml, the quantitative limit was (1.18) µg/ml, and the molar absorption coefficient was (1315.69) L/mol. cm and Sandell significance were (0.2439) µg/cm2, the average value of the percent recovery was 100.4%. The method was successfully applied to estimate Promethazine. HCL (PMH) in its pharmaceutical formulation and in biological fluids, and the characteristics of the colored product (PMH-CuNPs) were diagnosed. By measuring with several techniques, the average diameter was 73.54 nm in AFM technique, and the average crystal size through the XRD technique was 58 nm. SEM was measured, and the complex particles appeared in a spherical or semi-spherical shape, with an average size of 71.89, 82.04, and 79.25. FTIR was measured, and the functional groups were determined.

Keywords: CuNPs, iological fluids Promethazine hydrochloride, spectrophotometric method. Pharmaceutical,

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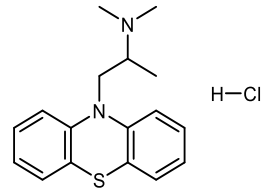
1. Introduction

Promethazine hydrochloride, also known as 2-((dimethyl-amino)-propyl)-phenothiazine monochloride, is a substance that is often used in pharmaceutical formulations. Its structure is shown in Figure (1) below It is frequently used as a sedative, antihistamine, antiemetic, and anesthetic agent and is generally referred to as a "neuroleptic tranquilizer (1–3)."



promethazine hydro chloride

Figure 1. Promethazine hydrochloride Structure

Promethazine HCl in bodily fluids is often analyzed using spectrophotometric and chromatographic techniques, while electro-analytical methods may also be utilized, there are currently several approaches accessible in the literature. Titrimetric measurements are one of the techniques used to quantify promethazine hydrochloride, both in bulk and in pharmaceutical formulations and biological fluids (4,5). It is prescribed for the treatment of symptoms of allergic conditions of the upper respiratory tract and catarrh, which include allergic rhinitis, and as a sedative, Before and after surgeries and childbirth. And as adjunctive therapy with analgesics to control postoperative pain. As well as prevention and control of nausea and vomiting sociated with travel, and contains antiemetic properties associated with anesthesia, Surgery, treatment of vomiting after surgeries, prevention and treatment of motion sickness, and used as a sedative and treatment for insomnia in Sedative; used to treat itchy skin lesions (eczema, pruritus) in adults and children (6). And the best way To use the medicine, take it 33 minutes before travel to prevent dizziness, and the tablets or drink should be taken with food to prevent dizziness, Other symptoms, and it can be used in the form of suppositories (7). The measurement of PM is done using a variety of techniques, such as spectrophotometric approaches(8). Flow injection analysis (9). high-performance liquid chromatographic(10). ion-selective electrode(11,12)

Since more than 10,000 years ago, people have utilized copper, a trace metal that is essential for life, and engaged in many different activities. Due to its supposed low toxicity and antibacterial capabilities, copper has recently attracted the attention of scientists, for people, For use as antibacterial, antifungal, and antiviral medicines, combined pharmacological compounds based on copper are more efficient as antibacterial, antifungal, and antiviral agents (13,14) . A bacteriostatic or bactericidal effect is produced by copper's mechanism, and its concentration has a direct correlation with this effect (15) . Metallic nanoparticles are a new class of materials with applications in medicine, pharmaceuticals, and agriculture. Using biological, chemical, and physical approaches, nanoparticles with amazing properties are obtained. Copper is one of the most abundant elements and plays an important part in the normal functioning of organisms(16). Copper nanoparticles have attracted the interest of scientists and researchers(17) due to their superior selectivity, catalytic efficiency(18), and improved Raman spectroscopy(19). Metal oxide nanoparticles (NPs) are thought to have significant commercial applications in more recent times. Yet another major scientific issue has been the potential toxicity of these nanomaterials. The green production of these particles is a crucial method for assuring lower toxicity levels and so permitting their unfettered deployment in consumer goods for humans(20,21).

2. Materials and Methods

2.1 DEVICES:

1. SHIMADZU UV-Visible-1800 – Japan 2. Sartorius Germany 3.PH meter Jen way England 4. Hot plate 5. Oven 6. Thermometer. 7. Center Fugue –Korea. 8. Electric grinder.

2.2 Reagents and solutions:

1. Preparations solution of CuSo 5H O. 0.1M.Wt. =4.9922 gm was taken from CuSo 5H O and dissolved in 200 ml distilled water.

2. Ginger roots from the local market.

3. Promethazine standard solution with a concentration of (1000 µg/ml) weight (0.1 g) of the active substance of the drug promethazine produced by the State Company for Drug Industry (SDI) in Samarra, Iraq, and dissolved in an appropriate amount of distilled water, and then completed the volume to the extent of the mark with the same solvent in a volumetric vial (100 ml)

4. Twenty tablets of the pharmaceutical drug Dolamine, which contains promethazine, were taken in the form of tablets, produced by a company, Casablanca, Morocco, with a dose of 5 mg, and were grinded well and mixed for the purpose of homogenization. Its weight was 12.4091 grams, enough for 0.1 grams of the active substance of the pharmaceutical preparation, and it was dissolved. With an appropriate amount of distilled water, then filter the solution and complete the volume to the mark with the same solvent in a 100 ml volumetric vial. Other concentrations were prepared with the appropriate dilution.

5. Approximated sodium hydroxide (0.1M) Prepare by dissolving 0.4 g of sodium hydroxide in an appropriate volume of distilled water, then complete Volume up to the mark in a 100 ml volumetric vial.

6. Approximated Hydrochloric acid Solution (0.1M) was prepared by withdrawing 0.83 ml of concentrated hydrochloric acid with a concentration of 12.06 M and added to an appropriate volume of distilled water and then complete the volume up to the mark in a 100 mL volumetric vial.

2.*3.* Procedure:

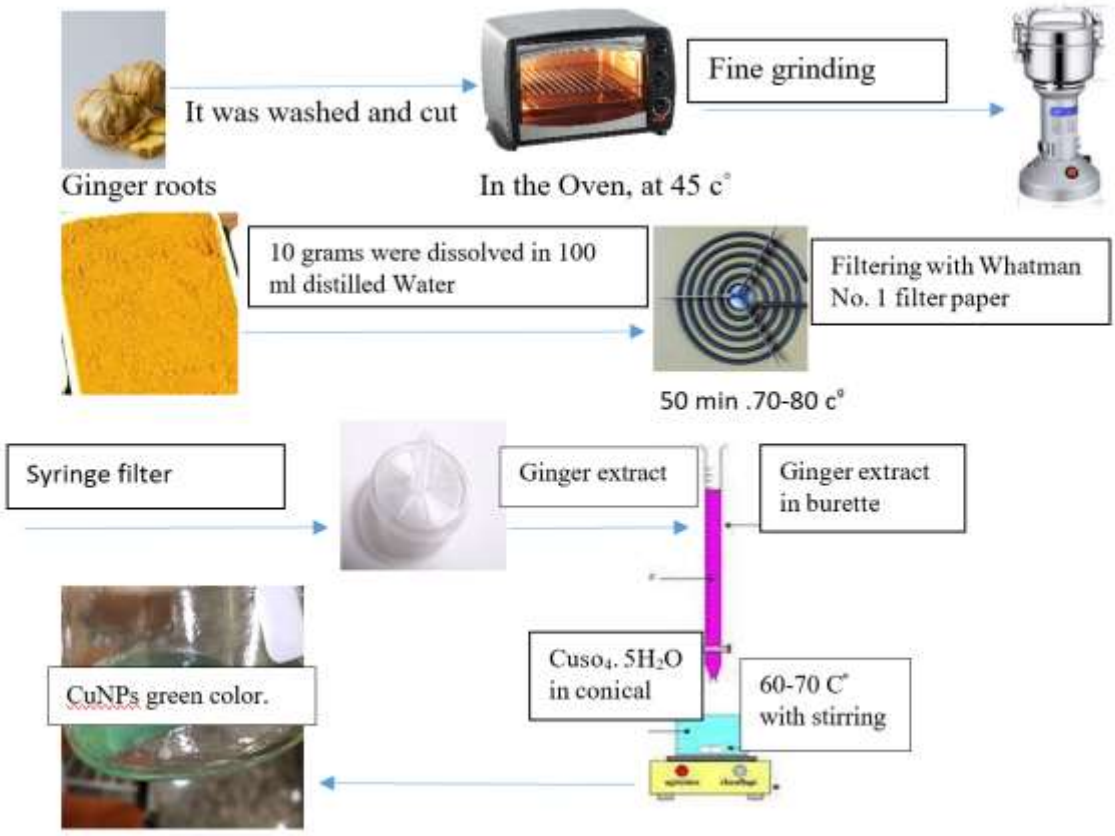


Figure 2. Graphical procedure for Green synthesizes of CuNPs

2.4. Preparations promethazine (CuNPs) complex.

(0.5 ml) of promethazine with a concentration of (1000 µg / ml) was added to (0.5 ml) of sodium hydroxide solution with an approximate concentration of (0.1 M) and (0.5 ml) of the reagent . A greenish blue color appeared and a clear peak in UV – Vis. At **ג *Max=641.*4 nm** as shown in the figure 3.

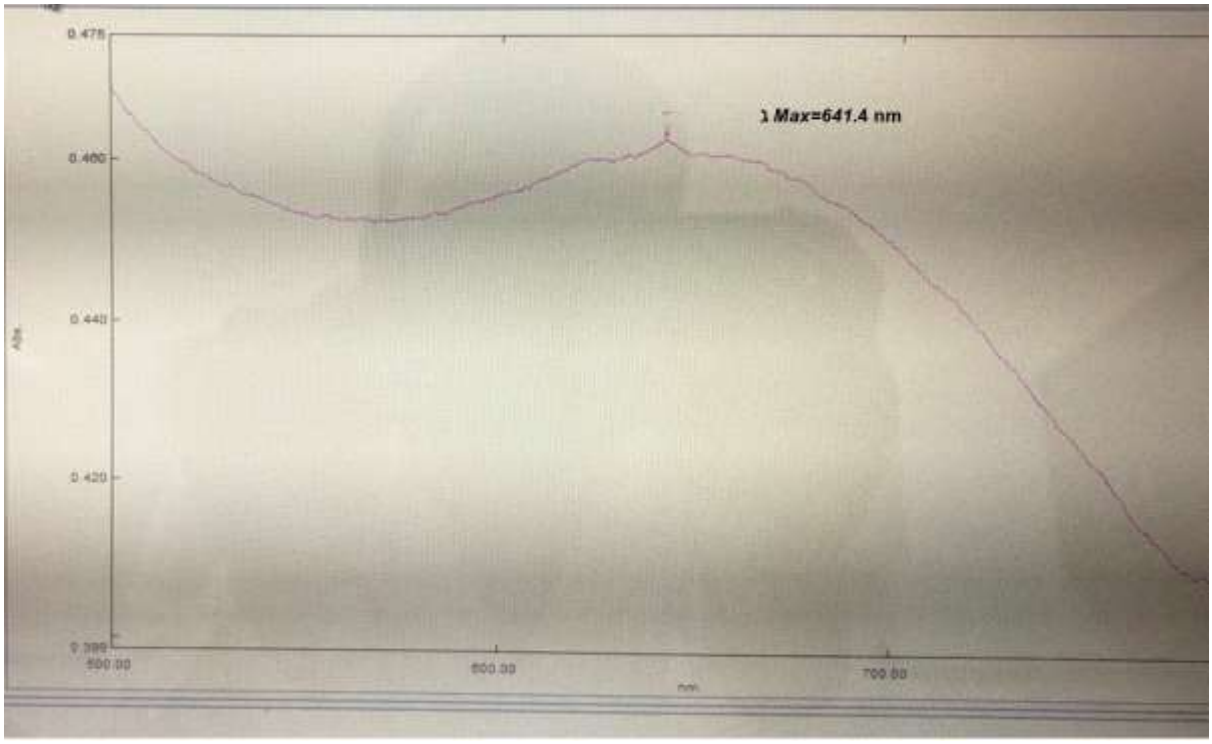


Figure 3. PMH complex with CuNPs

2.5. AFM of CuNPs

Atomic force microscopy (AFM) is a powerful technique that enables the imaging of almost any type of surface, including polymers, ceramics, composites, glass, and biological samples. AFM is used to measure and localize many different forces, including the surface morphology and average particle size. The CUNP AFM images shown in this article are 3D AFM images of smooth, equidistant micro-sphere structures with an average height of 20 nm and a diameter AFM analysis gives the surface roughness Ra = 8.86 nm by AFM microscopy (2D and 3D-dimensional) for CuNPs, through which the shape of the terrain of the surface and the overall height of the surface that was evaluated through AFM are determined, as is the average diameter (55.21 nm) as shown in Figures 4 A, B and C.

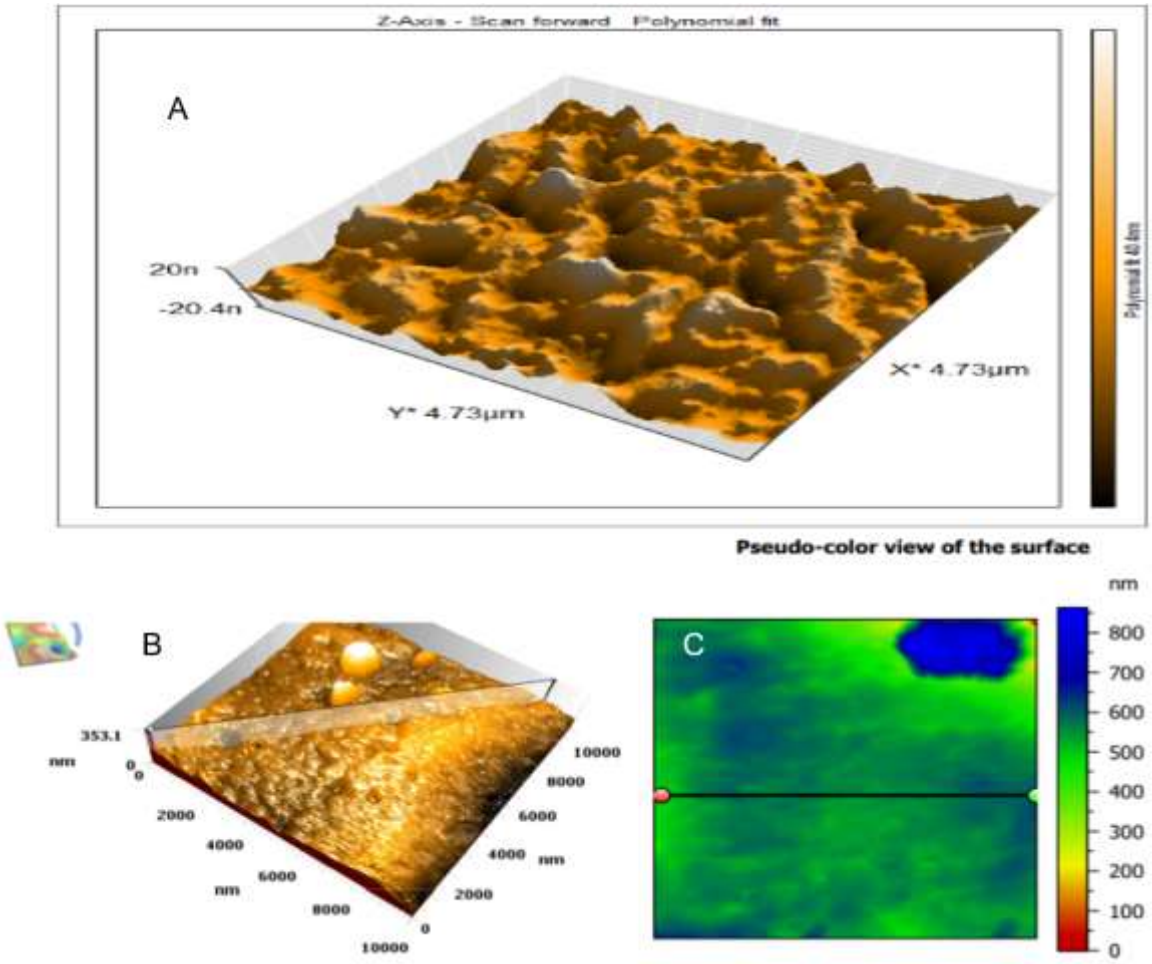


Figure 4. AFM of CuNPs, Roughness average (Ra=8.86 nm

2.6. Atomic force microscopy (AFM) measurements of PMH-CuNPs complex.

The atomic force microscopy images of the complex (PM-CuNPs), through which the shape of the surface topography is clear, and the total surface height that was recorded through (AFM) is (483nm) with an average diameter of (73.54 nm) as shown in Figure 5 A and B.

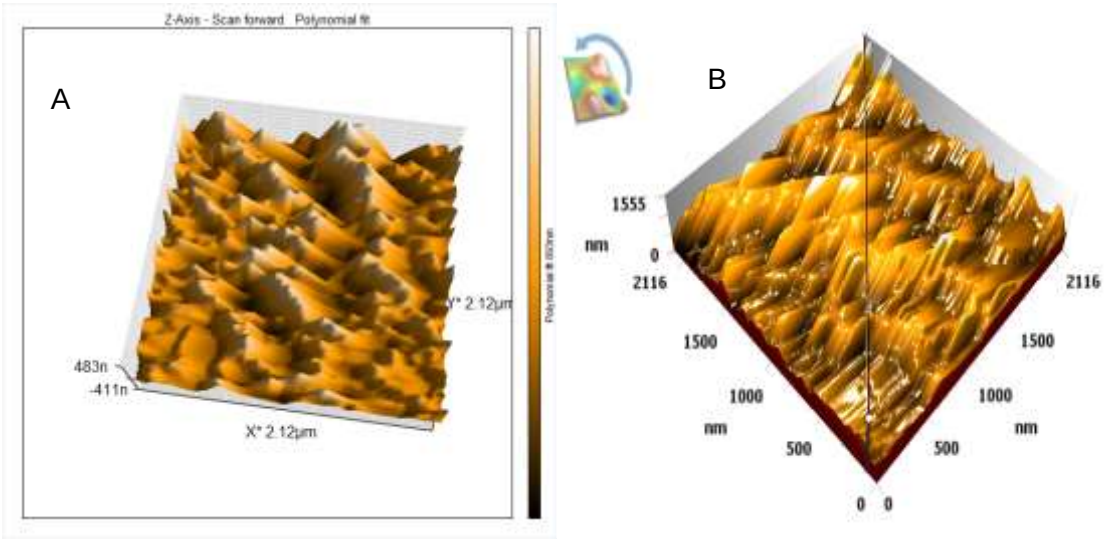


Figure 5. AFM PMH-CuNPs complex

2.7. X-ray diffraction (XRD) measurements of copper nanoparticles

X-ray diffraction of copper nanoparticles, the average size of the crystals was (50.768 nm) nanometers according to the (Scherrer equation)(22) to calculate the size of the crystals (Figure 6) .

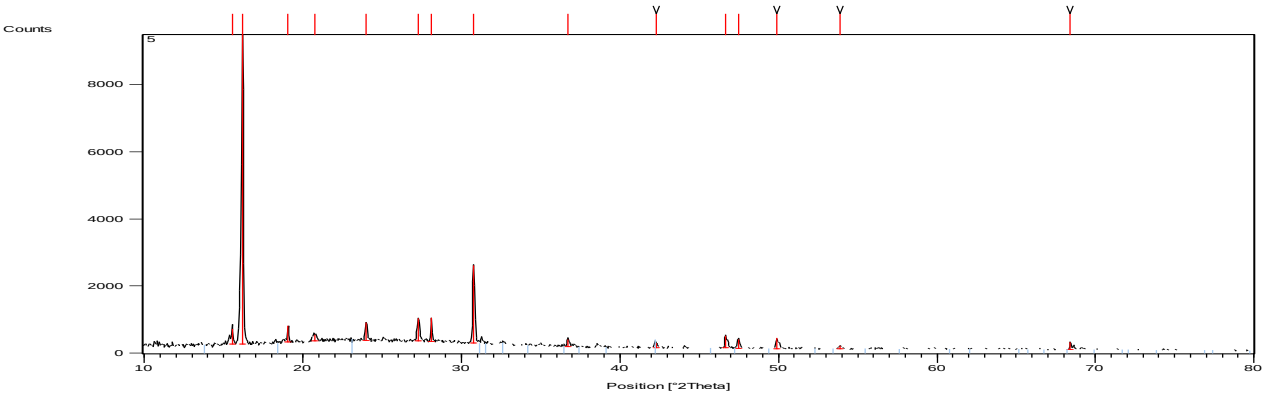


Figure 6. XRD diagram of copper nanoparticles CuNPs.

2.8. X-ray diffraction (XRD) measurements of PMH-CuNPs Nano complex.

X-ray diffraction of the Nano complex (PM-CuNPs), CuNPs), the average particle size was (58) nm according to Scherrer equation (22)to calculate particle size (Figure 7)

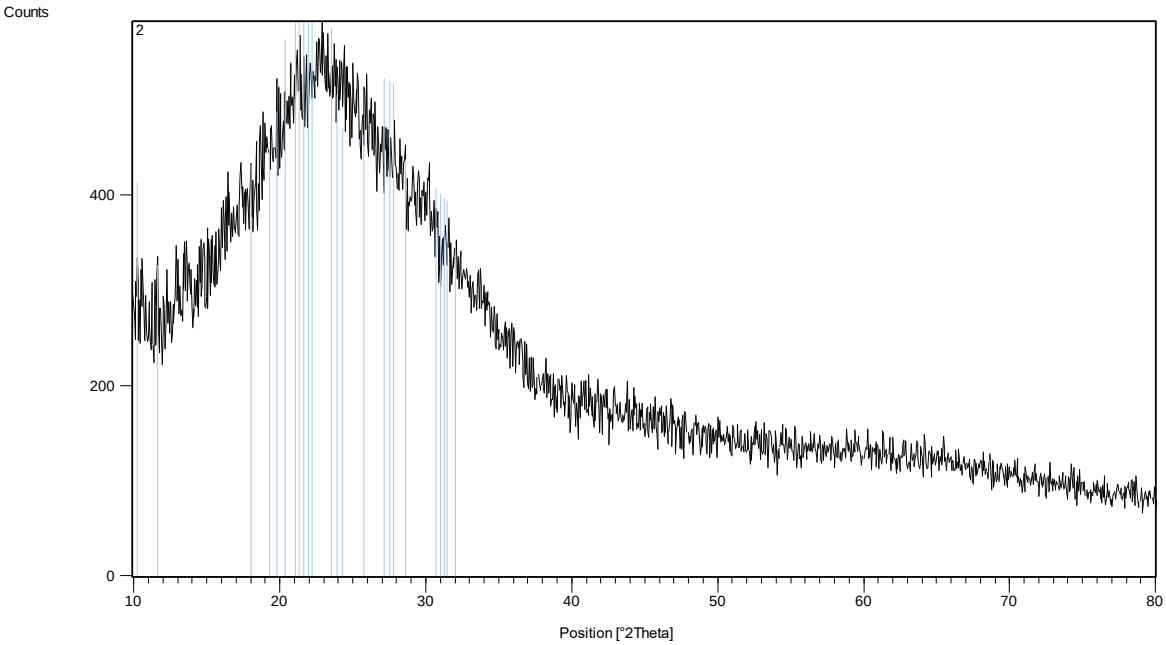


Figure 7. XRD diagram of PMH-CuNPs Nano complex

2.9. Scanning Electron Microscopy (SEM) Measurements of CuNPs

The copper nanoparticles appear under the scanning electron microscope (SEM) with a force of approximation (1µm) in the form of spherical clusters of different sizes from (44.66 nm) to (100.5nm) as shown in the figure 8.

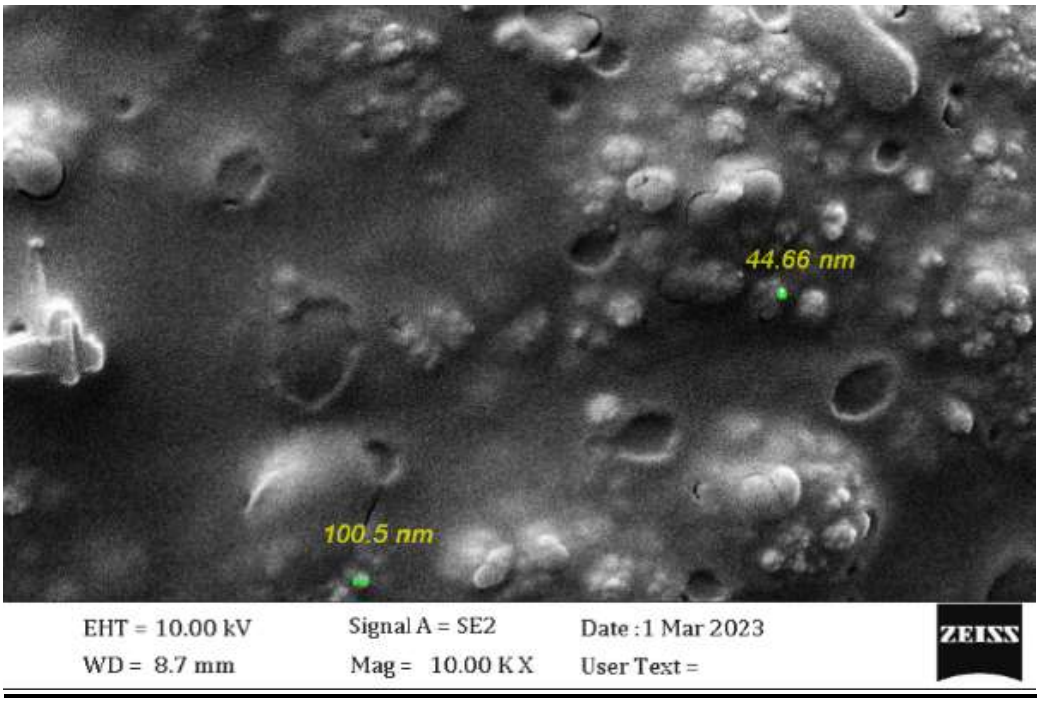


Figure 8. SEM Measurements of CuNPs

2.10 Scanning Electron Microscopy (SEM) Measurements of (PMH-CuNps) .

The SEM of the (PMH-CuNPs) nano complex was measured. In this diagnostic technique, the size and shape of the nanoparticles appear as shown in figure (9). The (PMH-CuNPs) complex appears spherical and its average size was (77.7nm)**.**

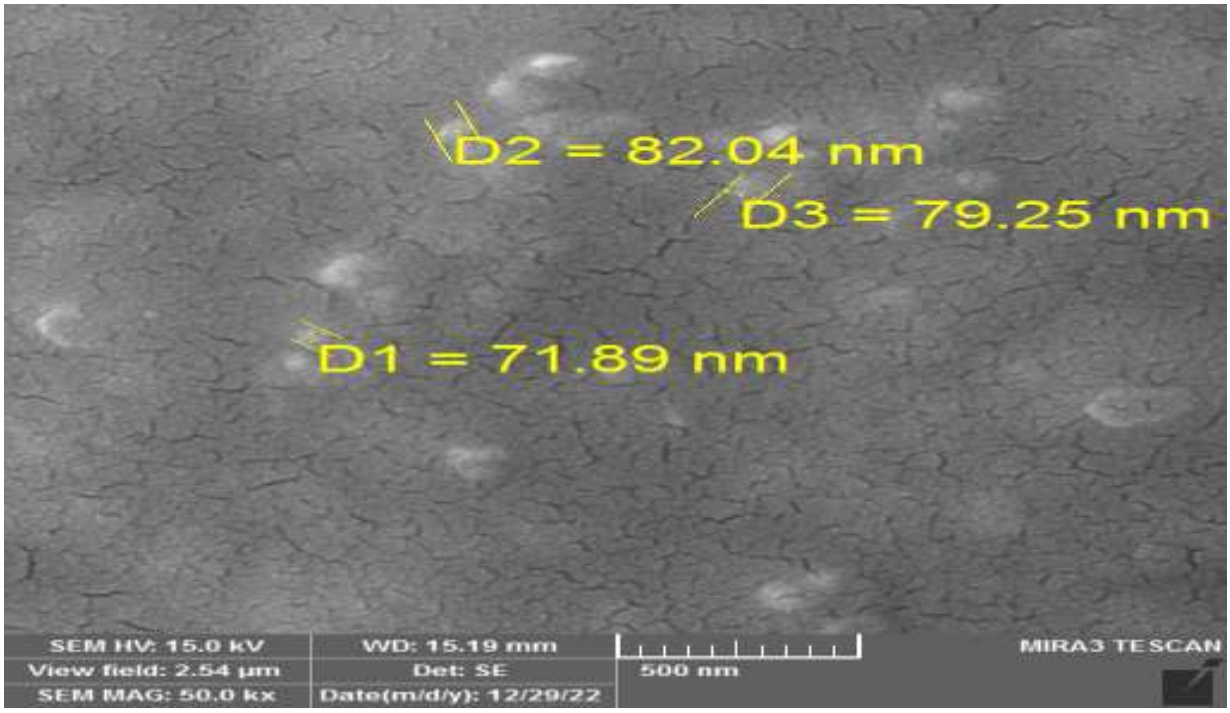


Figure 9. SEM of the PMH-CuNPs nano complex

3. Results and discussion

3.1 Study the optimal conditions for the formed complex

To provide the ideal circumstances for the interaction between the medication and the reagent, the elements influencing the complex's adsorption intensity were investigated (CuNPs).

3.1.1. The effect of nanoparticle size (CuNPs).

Increasing volumes of nano-copper **(CuNPs) (0.2–1.4 ml)** were added to 0.5 ml of the medication and 0.5 ml of NaoH, and the volume was finished in a volumetric vial with a capacity of 5 ml. To evaluate the ideal quantity of nano-copper particle size that delivers the maximum absorption of the resultant complex, it was measured against the blank solution at 642.4 nm. The findings are displayed in the table (1).

Table 1. The effect of nanoparticle size CuNPs.

Volume of (CuNPs) ml	Absorbance
0.2	0.160
0.4	0.426
0.6	0.410
0.8	0.395
1	0.378
1.2	0.347
1.4	0.305

The optimal size for CuNPs is 0.4 ml, as can be seen from the table above, where it offers the greatest absorption.

3.1.2. The effect of volume NaoH

After selecting the best size of copper nanoparticles, which was 0.4 ml with 0.5 ml of the drug at a concentration of 1000µg/ml, the effect of the base size of sodium hydroxide was studied on the absorbance value at wavelength 642.4 nm against the blank solution, and the results are shown in the table (2).

Table 2. Effect of NaoH volume

Volume of NaoH in ml	Absorbance
0.2	0.390
0.4	0.405
0.6	0.430
0.8	0.440
1	0.410
1.2	0.403
1.4	0.380

From the table, it is clear that 0.8 ml of base sodium hydroxide is the best volume.

3.1.3. Temperature effect:

The complex had the highest absorbance at temperature (30 C^{o}), as indicated in the table (3), after the absorbance was measured at various temperatures between (25-55 C^{o}).

Table3. Temperature effect

Temperature C^{o}	Absorbance
25	0.435
30	0.453
35	0.437
40	0.426
45	0.415
50	0.390
55	0.350

3. 1.4The effect of pH

After selecting the best volume of (CuNPs) 0.4 mL with 0.5 mL of the drug at a concentration of 1000 µg/mL and 0.8 ml of NaoH, the effect of the pH within the range of (2-11) was studied through sodium hydroxide solutions and hydrochloric acid solutions at a concentration of 0.1 M, and the absorbance was measured of the solution versus the blank solution at the wavelength 641.4 nm as shown in the table (4).

Table 4. The effect of pH

The pH	Absorbance
2	0.008
3	0.0013
4	0.0018
5	0.0020
6	0.316
6.5 pH function at first addition before addition an additional amount of the base, or the addition of HCl acid.	0.453
7	0.243
8	0.126
9	0.110
10	0.09
11	0.08

The optimal pH value is 6.5, as seen in the above table.

3.1.5. Study the effect of using other bases

The effect of changing the used base was studied, where the same volume of the base (0.8 ml) was taken and the concentration of 0.1 M each of sodium carbonate and potassium hydroxide with the complex formed between (CuNPs) and the drug promethazine against the blank solution at a wavelength of 641.4nm as shown in table (5).

Table 5. Effect of using other bases

Bases	Absorbance
NaOH	0.453
KOH	0.138
Na_2CO_3	0.262

It is clear from the table that sodium hydroxide is better than potassium hydroxide and better than sodium carbonate, so it was used as a base medium in all subsequent experiments with promethazine and reagent (CuNPs) to form the colored complex.

3.2 Drawing of Calibration Curve:

After studying and fixing the optimal conditions for the reaction, a series of concentrations of the active substance solution were taken Promethazine under study to make a standard titration curve for it using a series of concentrations starting from (20 µg/ml) to (280 µg/ml) by taking increasing volumes of the drug (0.1 -1.4 ml).At a concentration of (1000 µg /ml) into a series of volumetric vials, capacity (5ml), then add (0.8ml) of sodium hydroxide to each vial, then add (0.4ml of CuNPs reagent) at a concentration of (0.1M), then complete the volume to Limit the mark with distilled water, and record the average absorbance of six readings at a wavelength of 641.4 nm against the blank solution. It was noted that the concentrations that give compatibility with Beer-Lambert's law ranged from (20 µg/ml) to (280 µg/ml) of the promethazine solution in a final volume (5ml) It was found that the value of the correlation coefficient (0.9983), the straight-line equation (y = 0.0041 x + 0.051), the detection limit (0.

39), the quantitative limit (1.18), the molar absorption coefficient (1315.69L/mol.cm), and Sandel's significance (0.24295µg/cm^2)

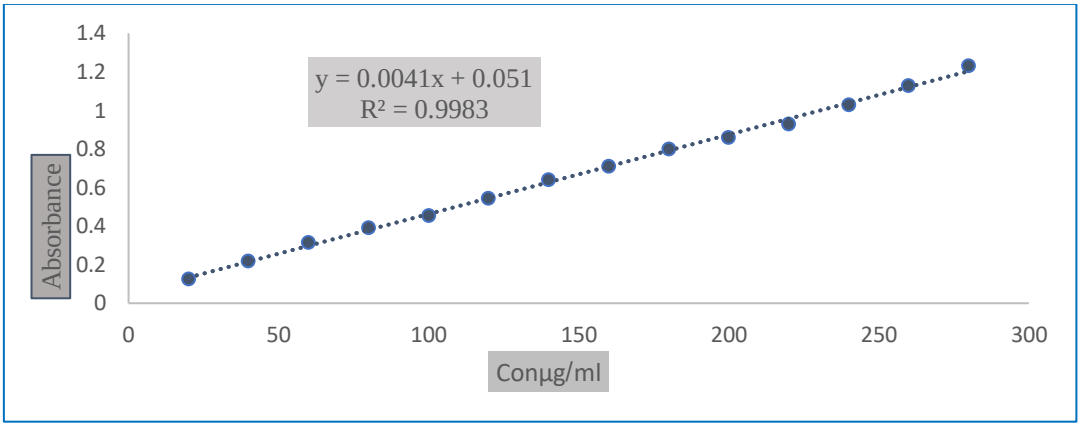


Figure 10. Calibration curve for the determination of promethazine

3.3 Accuracy and Precision

After studying and fixing the best conditions for the proposed reaction to estimate promethazine, to know the accuracy and Precision of the method Based on (ICH)(12), six readings were conducted for each measurement process for each of the concentrations that fall within the calibration curve for promethazine HCL, and the absorbance rate was chosen for the concentrations of the calibration curve, and the percentage recovery value Rec% was used to express the accuracy of the results, and it was found that the method has good accuracy and Precision As the value of the percentage recovery rate was 100.4%, and the relative standard deviation rate was (0.1921%), as shown in Table (6) .

Table 6. The accuracy and Precision of the method for promethazine.HCL.

Conc. Taken µg/ mL	Abs. Average for six reading	Conc. Found µg/ ml	Rec%	RSD%
20	0.131	19.5	97.5	0.37
40	0.217	40.5	101.3	0.15
60	0.305	62	103.3	0.14
80	0.389	82.4	103.1	0.12
100	0.453	98.	98	0.22
120	0.544	120.2	100.2	0.13
140	0.640	143.7	102.6	0.12
160	0.710	160.7	100.4	0.27
180	0.797	182	101.1	0.26
200	0.857	196.59	98.3	0.23
220	0.929	214.05	97.3	0.14
240	1.028	238.29	99.3	0.09
260	1.128	262.68	101	0.17
280	1.230	287.56	102.7	0.25

3.4 Method application:

The suggested procedure must be used with some medicinal formulations that include in order to determine if it is successful about the medication promethazine. HCL, the direct technique was used with the pharmaceutical preparation DOLAMINE made in Casablanca, Morocco, which comprises a concentration of 5 mg of promethazine.

3.4.1Direct Method (direct application to the calibration curve):

The direct method was applied to the preparation The pharmacist, DOLAMINE in the form of tablets, where I took three concentrations (160,200, 260 µg \ ml) Which is located within the calibration curve, and the same method was used when preparing the calibration curve, and the absorbance values of the mentioned concentrations were measured at a rate of three readings for each concentration at the wavelength of 641.4 nm. The percentage recovery value was used to express the accuracy of the results, REC.%, As for the expression of the precision of the results were expressed using the relative standard deviation (RSD) %.

Table 7. Direct method

Drug	**Taken Abs.**	**Abs.** µg/mL	**Found** µg/mL	**Average of Rec%**	**RSD%**
DOLAMINE	160	0.706	159.7	% 99.79	0.13
	200	0.869	199.5		0.19
	260	1.115	259.5		0.23

3.4.2Application of the method to biological fluids:

The method was applied by taking (1) ml of blood serum for each concentration separately, and the volume (0.8, 1, and 1.3 ml) was added, respectively, from the standard drug solution with a concentration (1000µg\ml) to prepare the appropriate concentration in a volumetric vial of (5) ml, then added (0.8 ml) of NaoH approximately concentration (0.1M) and then (0.4 ml) was added of (CuNPs) solution as a colorimetric reagent, and complete the volume to the mark with distilled water, and the concentrations were prepared (160µg\ml, 200µg\ml, 260µg\ml) and the solutions were treated in the same manner as used in the preparation of the calibration curve, and the results are shown in the table (8) .

Table 8. Results of applying the method to serum

Taken Abs. (µg/mL)	**Abs.**	**Found (µg/mL)**	**Average of Rec%**	**RSD%**
160	0.701	158.5	%99.62	0.11
200	0.867	199		0.27
260	1.120	260.8		0.15

The same method was used with blood serum, with the same volumes, and for three concentrations for each concentration separately, and the results are shown in the table (9)

Table 9. The results of applying the method on urine

Taken Abs. (µg/mL)	**Abs.**	**Found (µg/mL)**	**Average of Rec%**	**RSD%**
160	0.704	159.2	% 99.86	0.09
200	0.875	201		0.17
260	1.113	259		0.21

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1. Conclusions

An easy, fast, and economically inexpensive spectroscopic method was developed, as no reagent was used in this method A commercial chemist, but the reagent that was previously prepared from ginger root extract, was used in a non-nanoscale way Expensive and environmentally friendly, as this method relies on the reaction of the drug that contains rich donor atoms Electrons with the detector that contains copper nanoparticles as a metallic bond (M-Ligand) , component of a colored complex with a blue-green color that appears immediately after addition, in an alkaline medium of sodium hydroxide, which gives the highest absorption at the wavelength of 641.4 nm and follows Beer-Lambert's law in the concentration range 20 - 280 µ/ml, and the resulting complex is stable for more than From 65 minutes, and this time is suitable for conducting the analysis, and the value of the molar absorption coefficient for this method is1315.69 L /mol cm, and Sandel's indication. 0.2439 µg/cm2 the method is considered to have a degree of accuracy and precision, as the recovery value was 100.1% and the standard relative deviation value RDS% was not exceeded 0.37%. This method is distinguished by not needing special conditions or reagents. And extraction processes or special solvents, and this method was successfully applied to the pharmaceutical preparation (DOLAMINE 5mg) in the form of tablets. The method was also applied to biological fluids such as blood and urine.

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Conflicts of Interest: The authors declare no conflict of interest.

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