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Spectrophotometric determination of phenobarbital in pharmaceutical preparation using gold nanoparticles

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A simple, rapid and accurate spectrophotometric method is proposed to determine Phenobarbital (PHB) based on the coupling of 2,6-Dichloroindophenol sodium salt hydrate (DSH) with PHB to give a new ligand which reacts with gold nanoparticles in alkaline medium at (30-40oC) to give an intense blue-green colored chelate complex having maximum absorption at 600nm. The optimization of the experimental conditions is described. Beer's Law is obeyed at concentration range up to 5-45µg/ml with molar absorptivity 2368.797 L/mol.cm, Sandell's index was 0.09803 (µg/cm^2) The correlation coefficient, limit of detection and limit of quantification were 0.9984, 1.3002µg/ml and 4.3342µg/ml, respectively. The method has been successfully used to determine the Phenobarbital concentration in pharmaceutical preparation.

KEYWORDS

Phenobarbital; 2,6-dichloroindophenol sodium salt hydrate; gold nanoparticles; spectrophotometric method.

Introduction

Neonatal seizures belong among the most common serious neurological disorders worldwide [1]. Although there are several anti-seizure drugs available, phenobarbital still remains the first-line agent for the treatment of neonatal seizures. The drug has several favorable features including the undisputed efficacy against a broad spectrum of seizure types, low risk of serious acute adverse drug reactions, multiple pathways involved in the drug elimination as well as the availability of parenteral drug formulations and their low cost [2]. Phenobarbital could have synergistic neuroprotective effects when applied with therapeutic hypothermia [3]. There are concerns that phenobarbital may negatively impact psychomotor development and neurological outcomes [4]. A few relatively small studies have indicated the possibility that phenobarbital may affect long-term neurodevelopmental outcomes if the drug was administered either in early childhood for the treatment of febrile seizures or prenatally in gestational medication of their mothers [5–7]. Phenobarbital (5-ethyl-5-phenylpyrimidine-2,4,6 (1H,3H,5H)-trione) [8] is used to therapy anxiety and drug withdrawal and to help with surgery , as well as to be used in the treatment of all types of seizures except absence seizures [9]. Molecular weight is 232.235g/mol. Several analytical methods have been proposed for determining phenobarbital such as spectrophotometric [10], chromatographic [11-15] and voltametric [16-18] methods. It is white or almost white, crystalline powder or

colorless crystals, very slightly soluble in water, freely soluble in alcohol. It forms water-soluble compounds with alkali hydroxides, carbonates and with ammonia. Figure 1 shows the chemical structure of the drug [8].

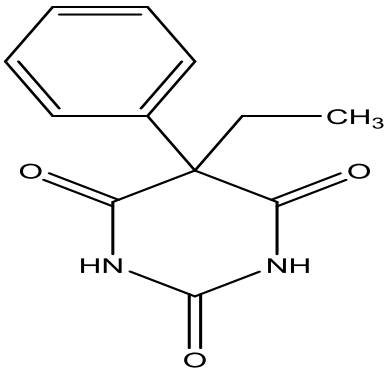


FIGURE 1 Chemical structure of phenobarbital

Nanotechnology is a vast and fast emerging area of scientific research studies resolving several complications related to conservative medication therapies, including underprivileged water solubility, lack of capability to target the problematic cancerous cells in individual bodies, common spreading, universal poisonousness as well as weak therapeutic capabilities. Nano-liposomes are nanometer-scale liposomes that are one of the most useful drug delivery systems in the field of drug release and retention. Important reasons for the use of nano-liposomes in the pharmaceutical industry are their similarity to cell membranes and trapping of hydrophobic and hydrophilic substances, drug delivery to the target tissue, control of drug flow in the bloodstream and good biocompatibility. Another important feature of nano-liposomes is the coating of water-soluble drugs in the central aqueous portion and fat-soluble drugs within its bilayer membrane. Nano-liposomes can be effective in reducing drug toxicity and increasing drug efficacy [19-22]. Gold nanoparticles (AuNPs) have several biomedical applications in diagnosis and disease treatment such as targeted chemotherapy and in pharmaceutical drug delivery due to their multifunctionality and unique characteristics [23].

The aim of the present study was developed a simple and economy method for determining phenobarbital, its raw pure state and in pharmaceutical preparation. The proposed method was based on the chelating complex formation.

Materials and methods

Instrumentals

A Shimadzu UV-Visible-1650–Japan double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements. pH meter, Jenway-3310-England. Sensitive balance with four digit, Sartorius- Germany, the Ultrasonic water bath, LabTech – Korea.

Chemicals

All the utilized chemicals were used by analytical grade, SDI Samarra-Iraq Donated by a PHB and BARABITAL, DSH from BDH, $AuCl_3$ from Sigma Aldrich, while KOH from GCC.

Standard Solutions

The standard solution of phenobarbital (PHB) (100 µg/ml)

In volumetric flask, 0.1 g of phenobarbital in a 100 ml was added and dissolve it in 100 ml of deionized water so that the solution concentration is 1000 µg/mL as a stock

solution, of which the working solution was prepared at a concentration of 100 µg/mL by withdrawing 10 mL of stock solution and diluting it with deionized water in a 100 mL volumetric flask up to the mark with the same solvent.

Preparation of reagent solution 2,6-dichloroindophenol sodium salt hydrate (DSH) (100 µg/ml)

0.01 g of reagent was dissolved in a 100 mL volumetric flask by deionized water and completed the volume up to the mark with the deionized water.

Preparation of gold trichloride ($AuCl_3$) solution (0.1318M)

0.1 g of gold trichloride was weighed in a 25 mL volumetric flask and dissolved in the deionized water and the volume was completed to the mark.

Preparation of KOH (0.1M)

0.5610 g of KOH was dissolved in a 100 mL volumetric flask with distal water up to the mark with the same solvent.

Preparation of the pharmaceutical preparation BARABITAL (150µg/mL)

10 tablets of the pharmaceutical preparation (supplied from SDI Samarra-Iraq) were weighed and crushed well, taking the equivalent of 0.0681 g weight of one tablet, which contains 15 mg of phenobarbital. The powder was dissolved in 10 mL ethanol in a 100 mL volumetric flask and filled the volume up to mark with deionized water to yield a solution of phenobarbital concentration as 150 µg/mL.

Preparation of gold nanoparticles

20 gm of parsley was weighed after being washed with deionized water and dried, then it was placed in a beaker and immersed in 120 ml of deionized water and boiled for 20 minutes. The extract (brown in color) was filtered and stored in a conical flask. Figure 2 displays the spectrum of parsley extract.

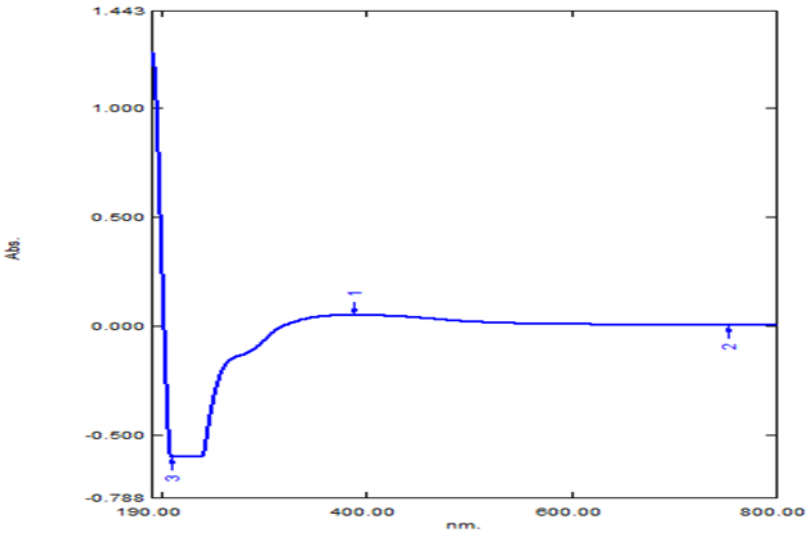


FIGURE 2 The spectrum of parsley extract

In a 100 mL beaker, 10 mL of $AuCl_3$ gold trichloride solution (0.1318M)_as well as 30 ml of parsley extract were added, then the obtained solution placed on a hot plate stirrer at 100 °C, and the magnetic stirrer was set at 400 rpm.

After 10-15 minutes, the color changed from brown to purple, which is evidence for

the formation of gold nanoparticles and their reduction by the vital-content of parsley [24].

The solution was placed in a centrifuge at 3500 rpm for 20 minutes, where the precipitate was collected at the bottom of the test tube and washed several times with deionized water.

The precipitate was placed on a watch glass and dried using a drying oven at 40 °C for 12 hours. The precipitate was stored in a dark place.

0.01 gm of the obtained precipitate was weighed and dissolved with deionized water in a volumetric flask of 100 mL capacity, and completed the volume to the mark so that it was for sonicated for 10 minutes to obtain 100µg/ml from gold nanoparticles solution.

A scan was carried out for wavelengths between 800-200 nm and the absorption was recorded at the maximum wavelength λ_{max} at 556 nm as indicated in Figure 3.

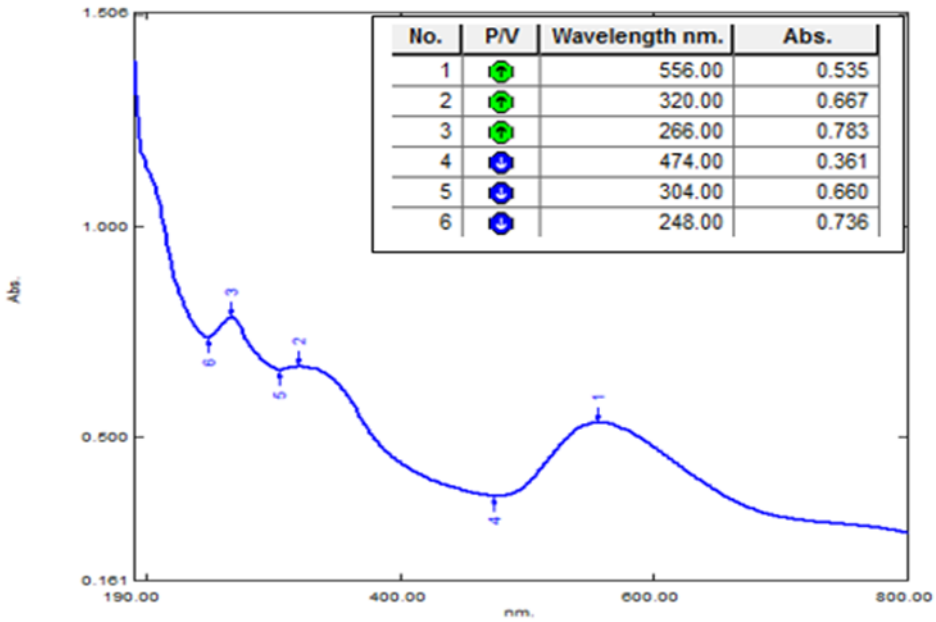


FIGURE 3 The maximum wavelength of gold nanoparticles

Results and discussion

Diagnostics of prepared gold nanoparticles

TEM technique was used to examine, diagnose and determine the shape and size of gold nanoparticles, as the results show that the particles were spherical in shape, of different sizes and at different power's magnifications (50,100 nm) they appeared in the form of dark dots and were within the nano-size, as depicted in Figure 4.

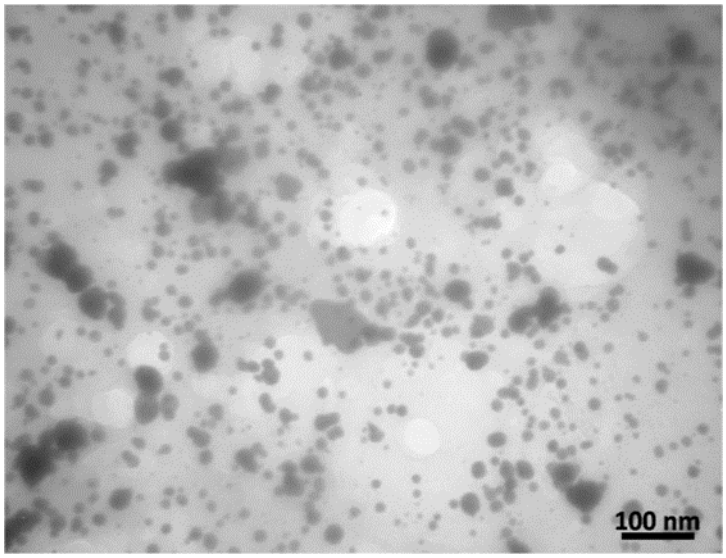


FIGURE 4 Examination of gold nanoparticles under a transmission electron microscope

The prepared gold nanoparticles was identified using the Energy-dispersive X-ray spectroscopy (EDX) technique to know the contents of the sample from the elements and through the peaks of the ray energy, as shown in Figure 5. The presence of quantities of carbon and oxygen indicates the formation of the oceanic network of gold particles and also of hydrocarbon compounds, and the presence of chlorine is due to the formation of golden chloride.

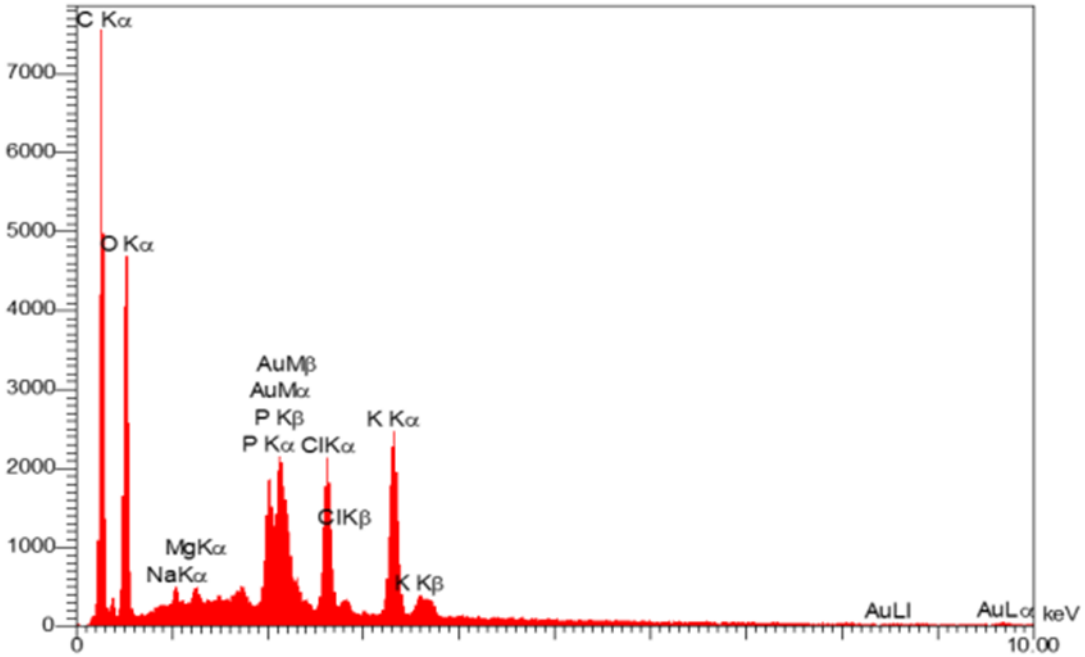


FIGURE 5 shows the results of the examination of gold nanoparticles using X-ray energy scattering

XRD analysis of (AuNps) Crystalline nanoparticles represented by five our peaks corresponding to standard Bragg reflections 111, 200 ,220, 311 and 222. The intense peak at 38.3 (111). Figures 6 and 7 display XRD diffraction and IR spectrum analysis of (AuNps) Crystalline nanoparticles, respectively.

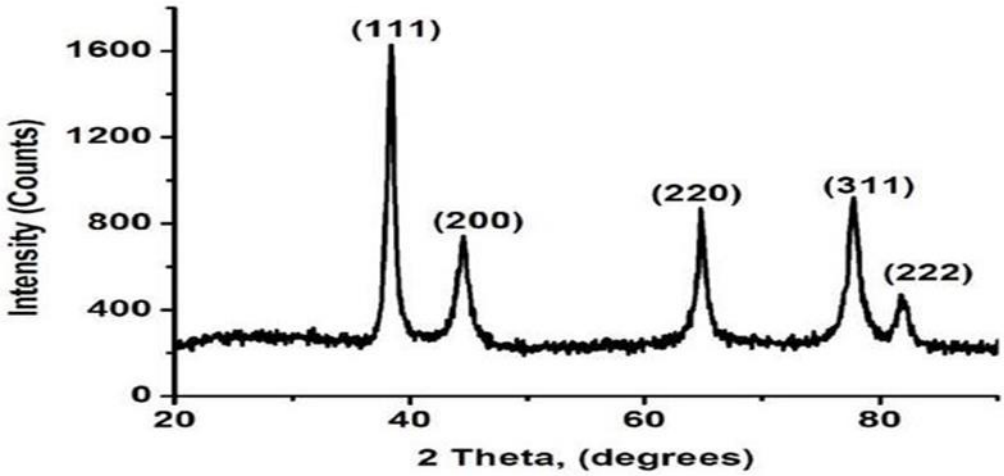


FIGURE 6 XRD analysis of (AuNps) Crystalline nanoparticles

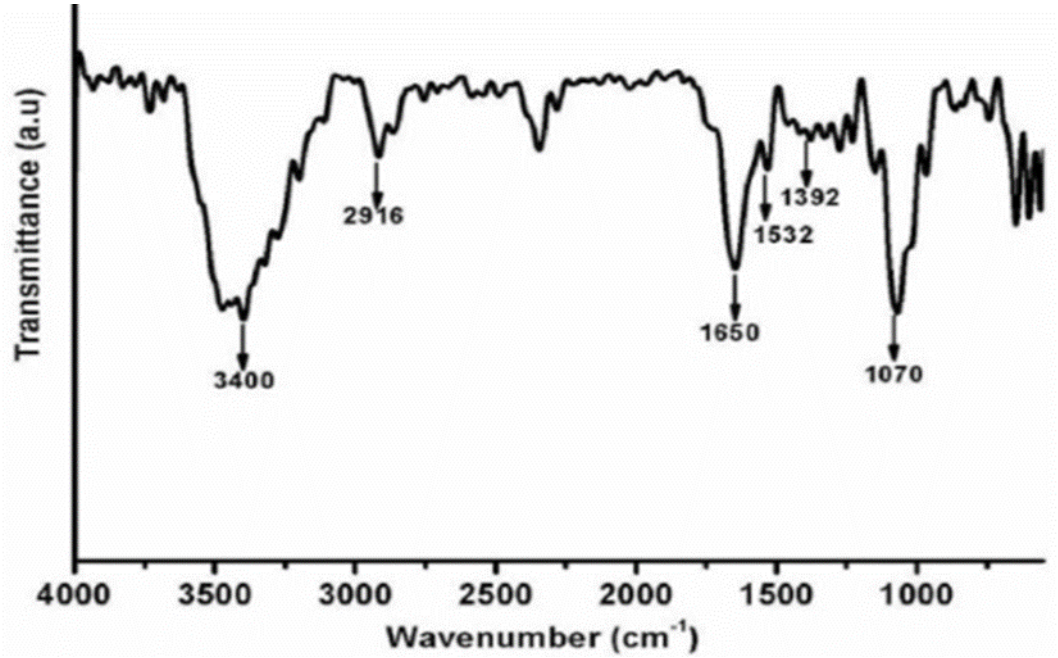


FIGURE 7 IR spectrum of (AuNps)

Preparation of phenobarbital-gold nanoparticles complex (AuNPs)

0.5 mL of KOH (0.1M) was added to 1 mL of PHB (100 µg/mL) and 0.2 mL of DSH reagent (100 µg/mL), and then 1 mL of gold nanoparticle solution (100 µg/mL) was added, as well. The color of the resulting solution was greenish blue and the maximum wavelength of the resulting complex is fixed at 600nm.

Setting optimum conditions

1- Effect of base quantity

Different volumes of KOH solution (0.1M) were added from 0.1-1 mL to 1 mL of PHB (100 µg/mL) and 0.2 mL of the reagent solution and 1 mL of gold nanoparticle solution and the absorbance was measured at 600nm wavelength as shown in Figure 8. It was found that 0.5 mL from KOH exhibited the best absorption.

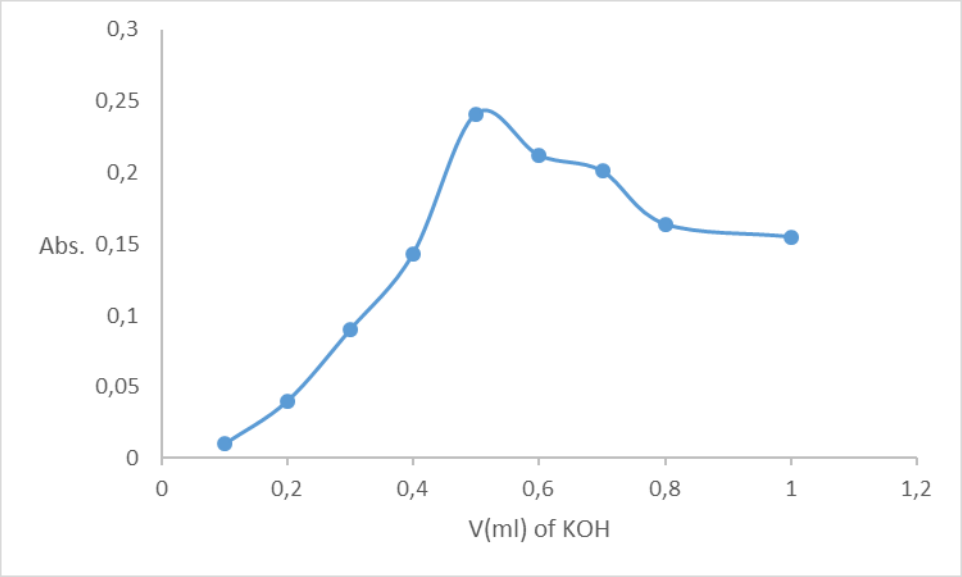


FIGURE 8 The optimum base volume

2- Optimum reagent concentration

A study was conducted to amount of DSH reagent solution giving the greatest absorption of the colored product, as 0.5ml of (0.1M) KOH was added to 1ml of PHB (100 µg/ml) and increasing volumes of 0.1-1 mL of DSH reagent solution (100 µg/mL) and 1ml of gold nanoparticles solution, and as the results indicates, it is evident that 0.5 mL of the reagent is the optimum volume as this volume was used in subsequent experiments, as depicted in Figure 9.

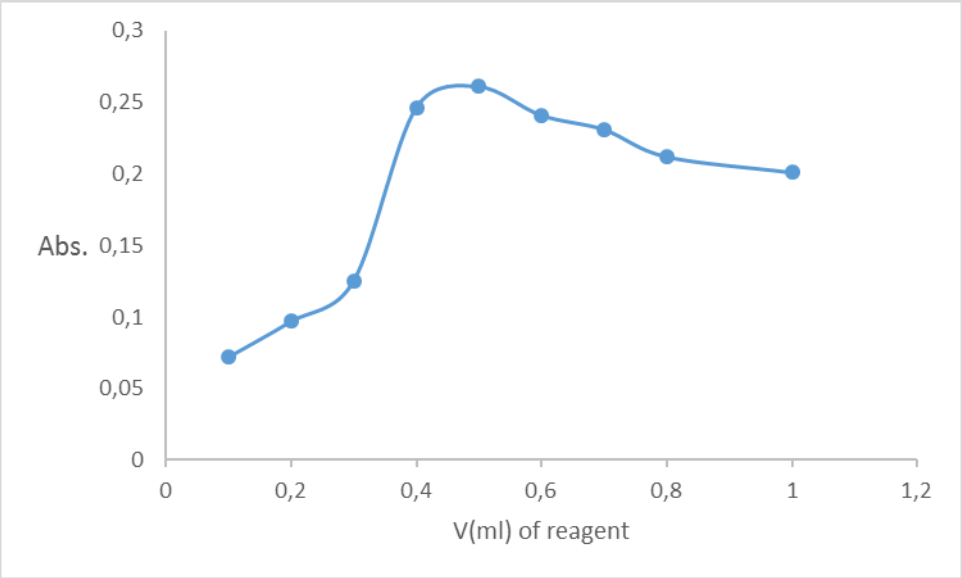


FIGURE 9 Effect of reagent volume (100µg/mL) DSH on the absorption of the colored complex

3- Effect of gold nanoparticles concentration

1 mL of the PHB was put into a series of 10 mL volumetric flask, 0.5 mL of (0.1M) KOH and 0.5 mL of (100 µg/mL) DSH reagent solution and the increasing volumes of gold nanoparticles solution (0.5-5 mL) were added, then the absorbance was measured at 600nm. It was found that the volume of 2.5 mL of gold nanoparticle solution gave the highest absorbance, which was adopted in the subsequent experiments, as displayed in Figure 10.

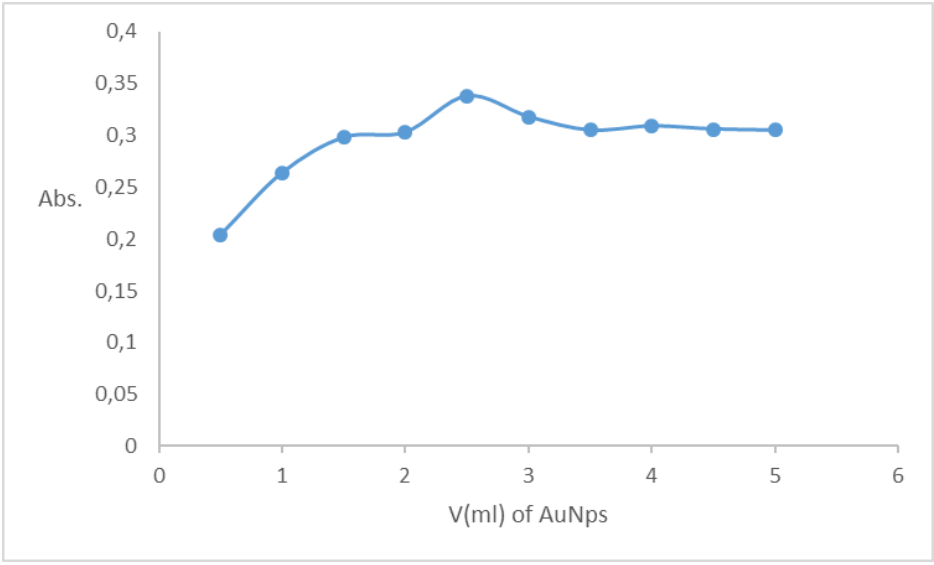


FIGURE 10 Effect of gold nanoparticles concentration

4- The effect of temperature

Using a water bath, the effect of temperatures from 15-60 °C on the formation of the complex was studied. Through the optimal conditions obtained from previous experiments, it is clear in this study and as depicted in Figure 11, that the best absorption is at a temperature of 30-40 °C.

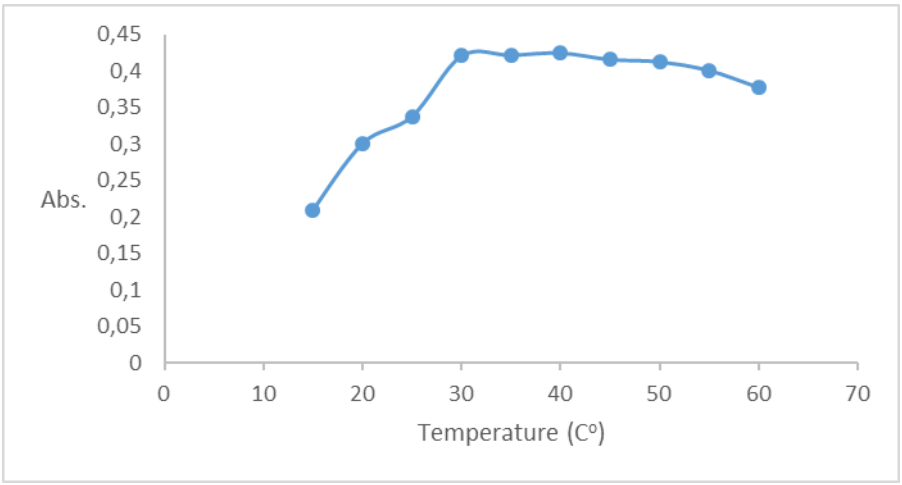


FIGURE 11 effect of temperature

5- Addition sequence effect

It was noted from the obtained results indicated in Table 1 that the following sequence as: Drug + Base + Reagent + Nano-gold solution, respectively gives the highest absorption after heating the complex to a temperature of 40°C. Therefore, this sequence was followed in subsequent experiments.

TABLE 1 Effect of the additions sequence on the absorption of the colored product

The additions sequence	Absorbance
Drug+ Reagent + Nano-gold+ Base	0.322
Drug+ Base + Reagent + Nano-gold	**0.338**
Drug + Nano-gold+ Reagent + Base	0.316
Nano-gold+ Base + Drug + Reagent	0.275
Nano-gold+ Reagent + Drug + Base	0.208

6- Measuring of complex stability

It was experimentally found that the absorption of the complex stabilizes after 5 minutes and remains stable for 45 minutes, as shown in Table 2.

TABLE 2 Study of complex stability time

Time (min.)	Absorbance
0	0.314
5	**0.336**
10	**0.336**
15	**0.336**
20	**0.337**
25	**0.337**
30	**0.338**
35	**0.338**
40	**0.338**
45	**0.338**
50	**0.337**
55	0.305
60	0.295

Final absorption spectrum

After stabilizing the optimal conditions, 0.5 mL of KOH (0.1M), 0.5 mL of DSH reagent (100µg/mL) and 2.5 mL of gold nanoparticle solution, the absorption of the formed colored product was measured against blank solution in the wavelength range between 200-800 nm, and it was found by the results obtained in Figure 12 that the wavelength of the highest absorption is 600 nm.

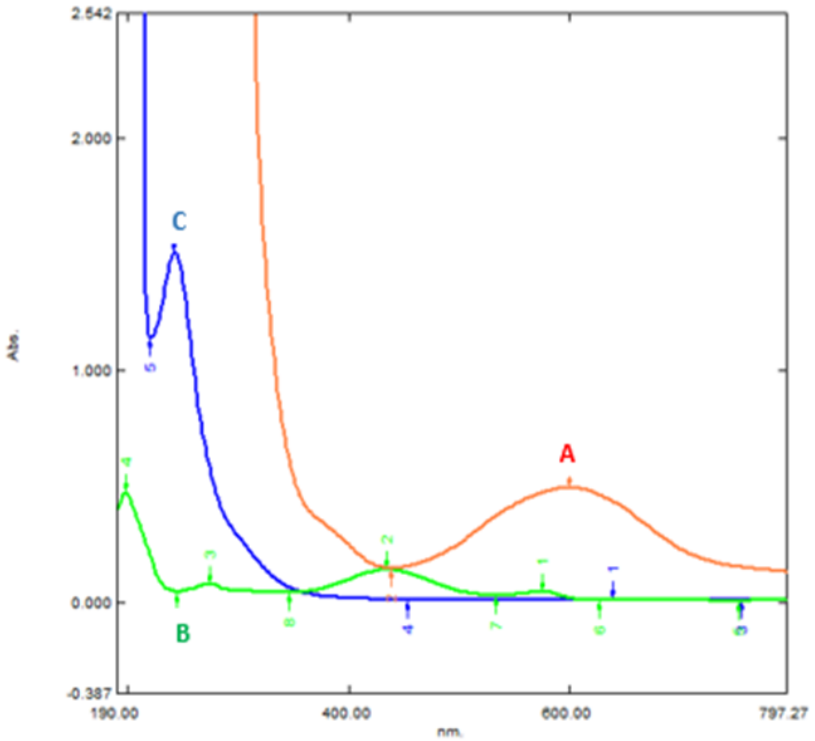


FIGURE 12 Absorption spectrum of complex (A), absorption spectrum of blank (B) and (C) absorption spectrum of drug

The calibration curve

Increasing volumes (0.1 - 6 mL) of PHB (100µg/mL), 0.5 mL of KOH (0.1M), 0.5 mL of DSH reagent solution (100µg/mL) and 2.5 mL of (100µg/mL) of gold nanoparticle solution were added to a series of 10 mL volumetric flask, filled to the mark with deionized water, and after the solution was heated to 40 ˚C, the absorption was measured against the blank at length. The 600 nm wavelength and Figure 13 indicate the linear calibration curve which shows that Beer's law was followed at a range of concentrations (5-45µg/mL) of the drug. The molar absorption of the resulting compound was calculated and found to be 2368.797 L $mol^{-1}.cm^{-1}$. Sandal's significance is 0.09803 µg.cm^{-2}.

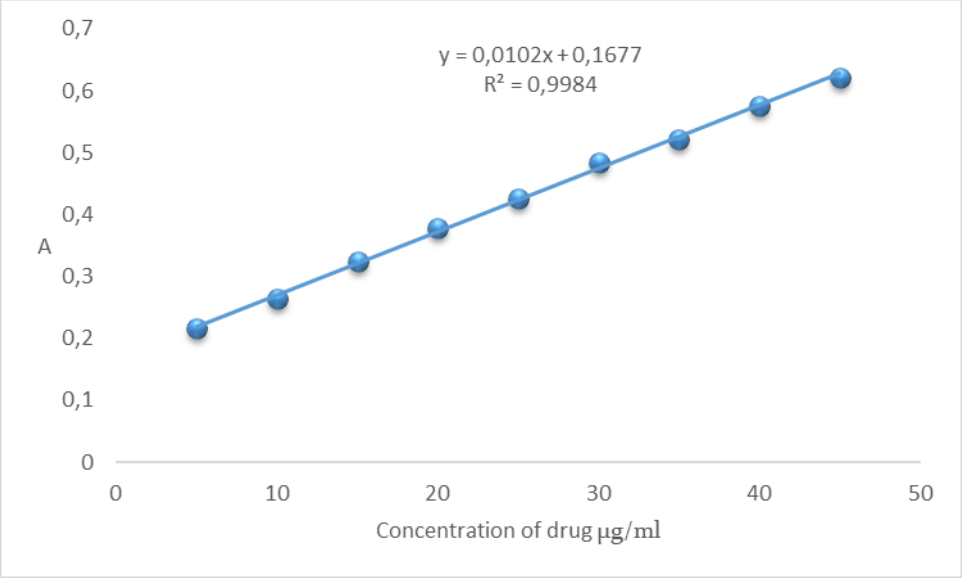


FIGURE 13 The calibration curve

Accuracy and precision

The accuracy and precision of the method were tested, since the recovery percentage (Rec%) and relative standard deviation (RSD%) values were 100.021-98.971% and 0.1276-0.3215%, respectively. These values demonstrated good accuracy and precision, as indicated in Table 3.

TABLE 3 The accuracy and precision results

Conc.of PHB taken µg/ml	A	Conc.of PHB found µg/ml	Rec %	RSD %
20.000	0.376	20.4212	102.1078	0.0729
30.000	0.478	30.4215	101.4052	0.1272
40.000	0.570	39.4411	98.6029	0.2475

The direct method

The direct method was applied to three concentrations of the pharmaceutical preparation BARABITAL (15mg), which are 15, 25, 35 µg/mL and for six replications for each concentration. The results are indicated in the Table 4.

TABLE 4 The direct method results

Conc.of PHB taken µg/ml	Absorption	Conc.of PHB found µg/ml	Rec %	RSD %
15.000	0.314	14.343	95.6209	0.621
25.000	0.424	25.127	100.509	0.0226
35.000	0.518	34.343	98.123	0.0439

Multi standard additions method

The drug has been estimated in pharmaceutical preparation (BARABITAL 15mg) by standard additions method as depicted in Figure 14 and Table 5.

TABLE 5 Multi standard additions method

Conc. Taken (µg/ml)	Conc. Found µg/ml	Rec.%	RSD%
5	5.0709	101.4193	1.736

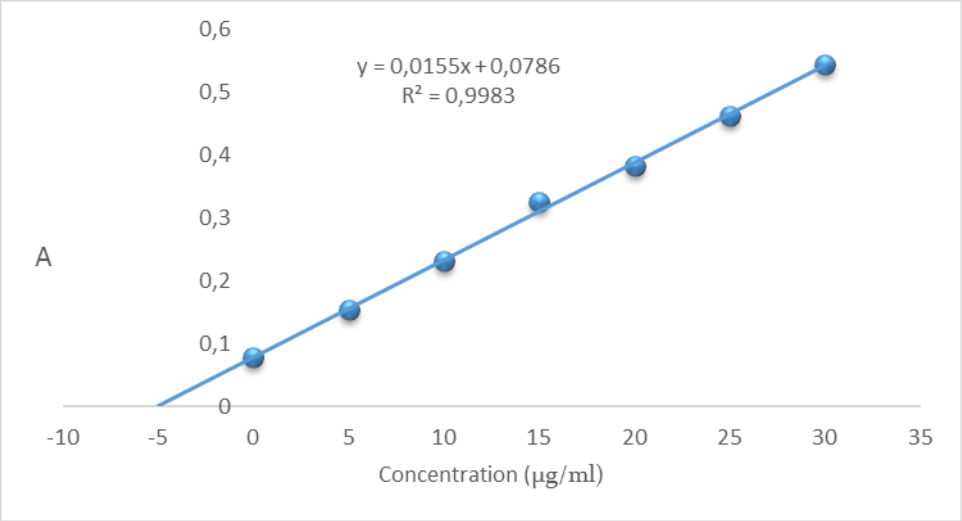


FIGURE 14 Multi standard additions method results

Stoichiometry of the reaction

Under the optimum conditions, the stoichiometry of the reaction between PHB and AuNps were investigated by continuous variations methods. The ratio was found to be 1:1, as displayed in Figure 15.

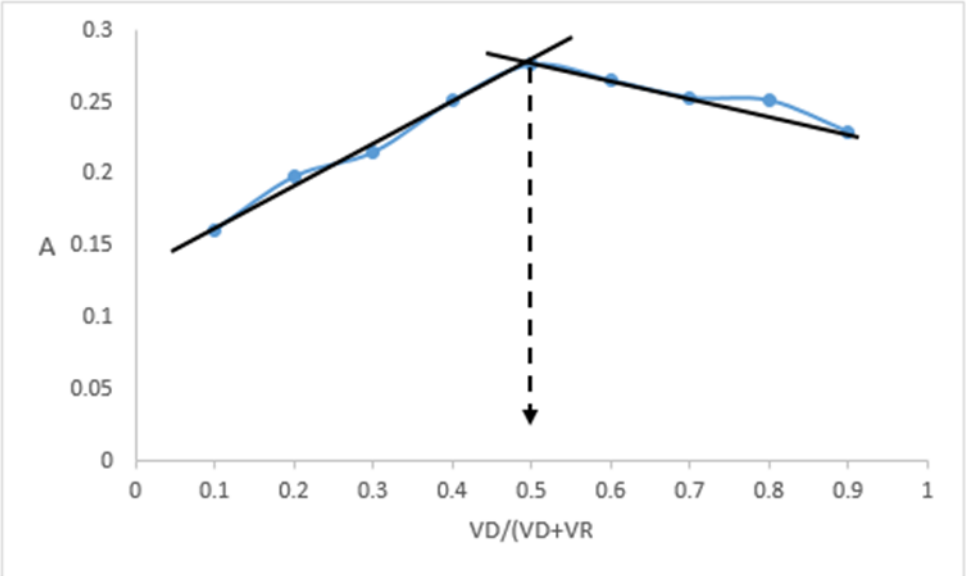
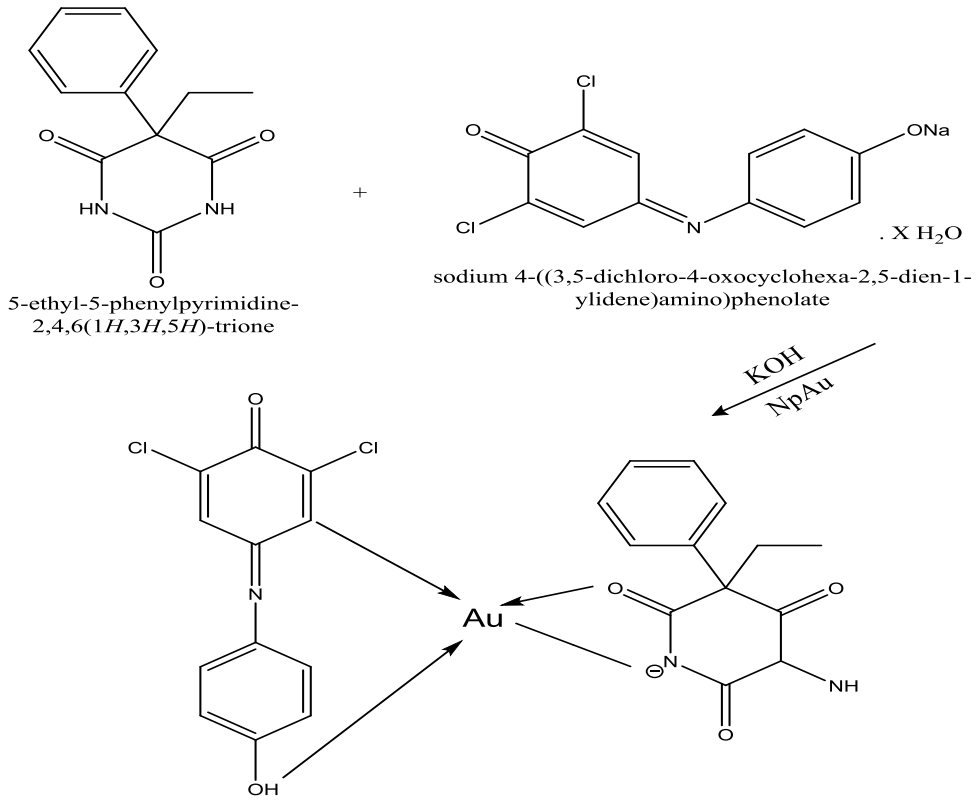


FIGURE 15 Continuous variations method of complex between ligand and metal

Suggested reaction

Suggested reaction can be as in the following equation in Scheme 1.



SCHEME 1 Suggested reaction equation: Preparation of phenobarbital-gold nanoparticles complex (AuNPs)

Method Comparison

The proposed method was compared with another spectroscopic method, as depicted in Table 6.

TABLE 6 Comparing the proposed method to another spectral method

Parameters	Present Method	Other Method[12]
λ_{max} (nm)	600	215
Beer's law range (µg/mL)	5-45	3-9
L.O.D (µg/mL)	1.3002	0.40
L.O.Q (µg/mL)	4.3342	1.22
Correlation coefficient (R^2)	0.9984	0.998
Sandell's index (µg/cm^2)	0.09803	————
ε (L/mol.cm)	2368.797	————
Average Rec%	100.7053	99.9646
RSD%	0.0729 - 0.2475	0.266 - 0.747

Conclusion

The developed method is simple and sensitive which is used to determinate Phenobarbital in its pure raw material form and pharmaceutical product and pharmaceutical form. This method included the reaction of PHB with DSH reagent to form a ligand that reacted with gold nanoparticles in alkaline medium to give blue-green colored chelate complex having maximum absorption at 600nm. The results obtained showed the percentile recovery values, the relative standard deviation, the detection limit, and the quantitative limit that the method is accurate and precision, which indicates the success of the proposed method for determinate PHB.

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