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Colorimetric Determination of Thioamide Drugs Based on the Surface Plasmon Resonance Band of Colloidal Silver Nanoparticles

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Abstract

In this article, we report a new method for the determination of two thioamide drugs, propylthiouracil (PTU) and methimazole (MMZ), with unmodified silver nanoparticles (AgNPs). The method principle involves reducing overall surface charges of the AgNPs and subsequent aggregation of AgNPs by thioamide drugs leading to decreasing of the surface plasmon resonance (SPR) peak of AgNPs at 400 nm. The potential effects of relevant experimental conditions including pH, concentration of AgNPs and incubation time on intensity of SPR peak were investigated and optimized. Under the optimum conditions, the calibration graphs were linear in the range of 0.05-1.2 μgmL^{-1} for methimazole and 0.02-0.8 μgmL^{-1} for propylthiouracil, with corresponding limits of detection of 0.01 and 0.007μgmL^{-1} respectively. This method was successfully applied for the determination of PTU and MMZ in pharmaceutical products.

Keywords: Silver Nanoparticles, Thioamide Drugs, Propylthiouracil, Methimazole.

1. Introduction

Antithyroid drugs, which interfere with the synthesis of thyroid hormones, are the treatment of choice for human hyperthyroidism, an illness caused by overactivity of thyroid gland. The thyroid gland with the assistance of peroxidase enzyme is responsible for the production of two thyroid hormones, thyroxine (T4) and triiodothyronine (T3). The formation of these hormones is based on iodine combining with a protein called thyroglobulin. The antithyroid drugs, Propylthiouracil (2-mercapto-6-propylpyrimidin-4-ol) and methimazole (1-methylimidazole-2-thiol) (Figure 1), belong to the family of thioamides.

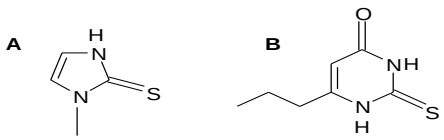

Figure 1. Chemical structures of (A) methimazole and (B) propylthiouracil.

The thioamides, compete with thyroglobulin for oxidized iodide. By competing for oxidized iodide, a selective decrease in the organification and coupling of thyroid hormone precursors occurs and thereby inhibits thyroid hormone production[1,2]. Antithyroid drugs are associated with a variety of side effects such as agranulocytosis, hepatotoxicity, vasculitis, polyarthritis, fever and sore throat, irritation of the skin and so on[3], Thus, the development of methods for the determination of thioamide drugs are obviously of significance.

Up to now, some methods based on various analytical procedures have been described for the determination of MMZ and PTU, such as chromatography [2,4-7], potentiometric and voltammetric [8-11], spectrophotometry[12-14], chemiluminescence method [15], fluorimetry [16], etc. However, these methods have their own limits, like expensive instruments, high cost and robust sample handling. It is necessary to develop simple, accurate and sensitive methods for the determination of methimazole and propylthiouracil.

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Noble metal nanoparticles made of silver and gold have been the focus of research for many decades as a result of their interesting optical properties [17-19]. Gold and silver nanoparticles exhibit surface plasmon resonance (SPR), a phenomenon caused by the collective oscillations of surface electrons induced by visible light, which is manifested by an extinction band in the visible region of their optical spectrum. The plasmon resonance band of noble metal nanoparticles can be modulated by the size, shape, and composition of the nanoparticles, the distance between nanoparticles, and the refractive index of the surrounding medium[20-22]. To date, in many studies gold nanoparticles have been employed as colorimetric probes for sensing biomolecules and metal ions [23-28] but much less attention has been paid to the application of Ag nanomaterials in colorimetric sensing [29-31].

In this paper, we report the use of AgNPs for the sensitive, rapid and simple determination of propylthiouracil and methimazole based upon aggregation phenomenon. Under optimum conditions, the decreasing of SPR band intensity at 400 nm is proportional to concentration of thioamide drugs. PTU and MMZ can combine with the AgNPs through Ag-S covalent bond. This phenomenon leads to the neutralization of nanoparticles surface charges and the loss of surface charges induced aggregation of AgNPs, which could be observed by color change from yellow to deep orange.

2. EXPERIMENTAL

All chemicals used were of analytical reagent grade and all solutions were prepared with doubly distilled deionized water (obtained from Ghazi Serum Co. Tabriz, Iran). Stock solutions of $AgNO_3$ (1.0×10^{-3} mol L^{-1}), sodium citrate (0.1 mol L^{-1}) and $NaBH_4$ (3.5×10^{-3} mol L^{-1}) was prepared by dissolving appropriate amount of $AgNO_3$ (Merck, Darmstadt, Germany), sodium citrate (Merck) and $NaBH_4$ (Merck) in deionized water. The stock solution of $AgNO_3$ was stored in a dark place. Methimazole (MMZ, 99%) and propylthiouracil (PTU, 99.5%) were kindly provided by Iran Hormon (Tehran, Iran), respectively. Their stock standard solutions (1000 μgmL^{-1}) were prepared by dissolving the appropriate amount of sample powder in water. These solutions were protected from light and stored at 4°C. Working standard solutions were prepared daily by appropriate dilution of stock solutions. Sodium acetate buffer (0.1 mol L^{-1}) was prepared by dissolving the appropriate amount of sodium acetate in water and adjusting the pH value.

The UV–Vis absorption spectra were measured using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) with 1.0 cm quartz cells. The pH value was measured using a pH meter (HANA 211, Romania). 20 mL of 1.0×10^{-3} M $AgNO_3$ aqueous solution was mixed with 0.5 mL of 0.1 M sodium citrate solution, and then 1 mL of 3.5×10^{-3} M $NaBH_4$ (freshly prepared) was added to the above mixture drop wise under vigorous stirring. After reaction under vigorous stirring for 2 h, the product was stored in a brown bottle at room temperature for 24 h prior to further use[32]. Into a 10 mL volumetric flask, 1.0 mL AgNPs, appropriate amounts of MMZ or PTU, and 0.5 mL acetate buffer solution (pH 6.0) were added in turn. After addition of each reagent, the resulting solution was mixed. The mixture was finally diluted to the mark with water. After shaking, an appropriate portion was transferred into a 1.0 cm cell to record the absorbance. The absorbance values were measured at 400 nm against doubly distilled deionized as blank.

Twenty tablets of methimazole and propylthiouracil (Iran Hormon) were weighed in order to find the average mass of each tablet. The contents were then powdered and mixed. 10 mg of this powder was accurately weighed and dissolved in about 10 mL water, filtered into a 25 mL volumetric flask and diluted to the mark with water. A suitable aliquot of this solution was taken for the determination of MMZ or PTU according to the recommended procedure. The recovery assays were carried out using the same procedure but adding the known amounts of pure drug.

3. RESULTS AND DISCUSSION

In our experiment, the as-synthesized AgNPs were transparent and immediately after synthesis showed a rather weak surface plasmon resonance (SPR) band at 400 nm.

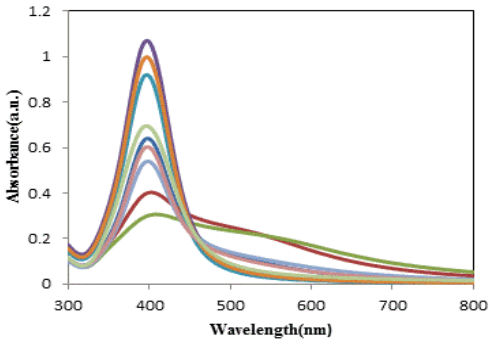

Figure 2. UV‑vis absorption spectra of the (1) 1.0 mL of AgNPs; in the presence of various concentrations of MMZ; (2) 0.05 µg/mL; (3) 0.1 µg/mL; (4) 0.4 µg/mL; (5) 0.5 µg/mL; (6) 0.6µg/mL; (7) 0.8 µg/mL; (8) 1.0 µg/mL; (9) 1.2 µg/mL at pH = 6.0.

After 24 h of storage, the SPR band absorption intensity became more intense. Absorption spectrum of AgNPs exhibited that the maximum absorption wavelength was located at 400 nm. By adding PTU or MMZ SPR intensity decreased which ensured a simple and selective colorimetric assay for them (Figure 2).

The effect of important variables (pH, kind of buffer, concentration of buffer, the amount of AgNPs and incubation time) on the measurement parameter was studied by one-at-a-time method. The initial pH values were adjusted from 2 to 9, using HCl or NaOH solution. The results are shown in Figure 3.a.

It can be seen that the optimum pH ranges are 4.0 – 5.0 for PTU and the optimum pH is 6.0 for MMZ, respectively. At higher or lower pH a marked decrease in the ΔA values is observed. Therefore, pH 5.0 and pH 6.0 were selected for PTU and MMZ determinations, respectively. Among three buffer solutions tested (i.e. acetate, phosphate and BR buffer solutions), acetate buffer gave the best results in terms of sensitivity and repeatability for both drugs. Then, the concentration of acetate buffer by adding different volumes of buffer (0.1 M) was optimized. For both of drugs, by using 0.5 mL buffer maximum ΔA obtained (Figure 3.b).

The effect of concentration of AgNPs solution on ΔA values was also investigated. The results (Figure 3.c) indicated that1 mL of synthesized AgNPs was suitable for PTU and MMZ. At higher or lower concentration, a marked decrease in the ΔA values is observed.

Finally, the influence of incubation time on intensity of SPR at 400 nm was studied. According to the obtained results, for both drugs the intensity is enhanced rapidly following the addition of the drugs and reaches a plateau in about 15 min (Figure 3.d).

According to the above procedure, different concentrations of PTU and MMZ were used to construct the calibration curves. The absorbance was obtained at 400.0 nm for all measurements of thioamide drugs. There were linear relationship between the intensities and the analytes concentrations over the range of 0.05-1.2 μgmL^{-1} for MMZ and 0.02-0.8 μgmL^{-1} PTU, with the corresponding detection limit (3σ) of 0.01 and 0.007 μgmL^{-1} respectively. The relative standard deviation (RSD) for five replicate determinations of 0.5 μgmL^{-1} PTU and MMZ were measured 1.01% and 1.2% respectively.

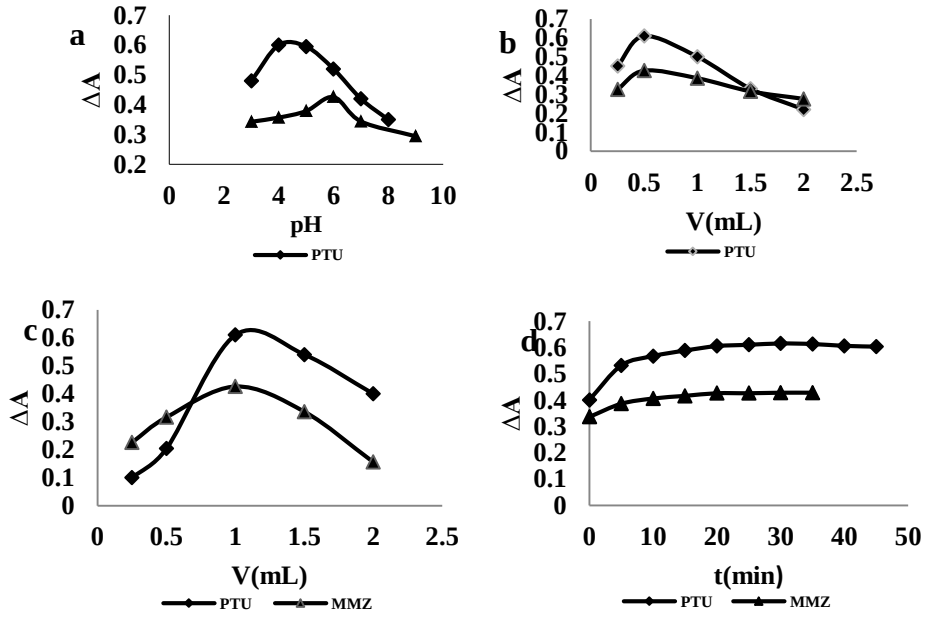

Figure 3. Effects of (a) pH (amount of AgNPs =1.0 mL, $C_{Analyte}$= 0.5 µg/mL), (b) concentration of buffer (amount of AgNPs =1.0 mL, $C_{Analyte}$= 0.5 µg/mL, and pH = 6.0 (for MMZ) and 5.0 (for PTU)), (c) concentration of AgNPs ($C_{Analyte}$= 0.5 µg/mL, pH = 6.0 (for MMZ) and 5.0 (for PTU), amount of buffer=0.5 mL) and (d) incubation time (amount of AgNPs =1.0 mL, $C_{Analyte}$= 0.5 µg/mL, pH = 6.0 (for MMZ) and 5.0 (for PTU), amount of buffer=0.5 mL) on the intensity of absorbance AgNPs at 400 nm.

The influence of some potentially interfering substances on the determination of 0.5 mgL^{-1} PTU and MMZ using the developed method was examined. The tolerance limit was taken as the concentration of the added species causing less than 5 % relative error. The obtained results are presented in Table 1. MMZ and PTU were satisfactorily determined in pharmaceutical products commercialized in Iran (Methimazole (MMZ) and Propylthiouracil (PTU)) with nominal contents of 5.0 and 50.0 mg, respectively by the proposed method. The obtained results are summarized in Table 2.

Table 1. The influence of some potentially interfering substances on the determination of 0.5 mgL^{-1} propylthiouracil (PTU) and methimazole (MMZ) drugs using the developed method

Foreign substances	Tolerance limit (Foreign substance to analyte ratio)	
	MMZ	PTU
Mg^{2+} ,Ca^{2+},K^{+},Ni^{2+},Na^{+}, Cl^{-} , SO_4^{2-}	≥1000	≥1000
Co^{2+} , NO_3^{-}, Ba^{2+}	500	500
Cr^{3+} , I^{-}	250	250
Cu^{2+} , Fe^{2+}	1	1

Table 2. Concentrations of propylthiouracil (PTU) and methimazole (MMZ) drugs obtained by the proposed method in real samples

Sample	Nominal Content (mg/sample)	Found[a] (mg/sample)	Recovery (%)
Methimazole Tablet	5	5.1± 0.3	102
Propylthiouracil Tablet	50	49.9 ± 0.5	99.4

[a]Average of three determinations ± SD.

Statistical analysis of the assay results showed satisfactory precision of the proposed method with no significant difference between certified and experimental results. Recovery experiments on pharmaceutical preparations spiked with different amounts of drugs were also carried out. The results are given in Table 3 indicating that the proposed method has sufficient precision and accuracy (satisfactory recoveries between 97 and 100%) for the determination of MMZ and PTU in pharmaceutical products.

Table 3. The results obtained by the proposed method on pharmaceutical products spiked by propylthiouracil (PTU) and methimazole (MMZ) drugs

Sample	Analyte	Amount Added (µg/mL)	Amount Found (µg/mL)[a]	Recovery (%)
MMZ tablets	MMZ	0.2	0.195±0.005	97±3
		0.4	0.39±0.01	98±2
PTU tablets	PTU	0.2	0.198±0.005	99±2
		0.4	0.4±0.0	100±2

4. CONCLUSION

In this work, a low-cost, simple and fast colorimetric method for the detection and quantification of thioamide drugs with unmodified silver nanoparticles was introduced. The interaction between AgNPs and thioamide drugs results in aggregation of silver nanoparticles and decreasing of its SPR peak at 400 nm which was proportional to concentration of thioamide drugs. This method was successfully applied for the determination of two thioamide drugs, PTU and MMZ, in pharmaceutical products. Comparison of analytical features of this method with those of some previously reported methods (Table 4) indicates that LOD of the developed method are better than or comparable with most of the other methods.

Table 4. Analytical feature comparison between previously reported methods and the proposed method for determination of propylthiouracil (PTU) and methimazole (MMZ) drugs

Method	Analyte	Linear range (μg/mL)	Limit of detection	Reference
Fluorimetry	MMZ	0.005-0.570	0.003	[16]
Voltametry	MMZ	0.114-11.4	0.057	[11]
Voltametry	PTU	0.017-1.70	0.008	[8]
Liquid chromatography	MMZ	0.25-50	0.150	[5]
HPLC	PTU	0.068-0.170	0.051	[33]
Spectrophotometry	MMZ	0.02-6.00	0.015	[14]
Spectrophotometry	PTU	0.08–1.00	0.03	[34]
	MMZ	0.04–0.50	0.02	
Chemiluminescence	MMZ	2-100	1.0	[15]
Spectrophotometry-based SPR of AgNPs	MMZ	0.05-1.2	0.01	Proposed method
	PTU	0.02-0.8	0.007	

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