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Application of silver nanoparticles for the spectrophotometric determination of three benzimidazole anthelmintic drugs in their pharmaceutical preparations

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ABSTRACT

This article describes the application of silver nanoparticles (AgNPs) as a colorimetric probe for the simple and selective determination of three anthelmintic benzimidazole drugs namely; albendazole, flubendazole and mebendazole. The method was based on the AgNPs aggregation induced by their interaction with the cited drugs. This interaction produced a color change from yellow to blue and accompanied by the appearance of a new surface plasmon resonance peak of AgNPs measured in the range of 615 – 630 nm. The linear concentration ranges of the method were 2.0–9.0, 2.0–10 and 2.2–15 μ g mL⁻¹ (r² = 0.9939, 0.9950 and 0.9938) for the studied drugs respectively. The validation of the analytical performance of the method was carefully investigated, and the results were satisfactory. This method was applied successfully to determine the three benzimidazoles in their pharmaceutical samples (tablets and suspensions) with high accuracy and precision. The suggested procedure is characterized by its simplicity since it does not involve solvent extraction or any critical reaction conditions.

INTRODUCTION

Albendazole, mebendazole and flubendazole (Fig. 1) are anthelmintic drugs belonging to benzimidazole family which are widely used in mammal with low toxicity and high effectively against a wide range of helminth species including pin worm, common hookworm, round worm, and whipworm (Köhler 2001). Benzimidazoles specifically bind to tubulin, the microtubule subunit protein, which results in disruption of the microtubule structure and function and in interference with the microtubule-mediated transport of secretory vesicles in absorptive tissues of helminthes (Köhler and Bachmann, 1981; Lacey, 1988; Jasmer *et al.*, 2000).

Many analytical methods were reported for the analysis of the cited benzimidazole anthilmentics in pharmaceutical and biological samples. The techniques have included titrimetry (Mohammed et al., 1986; Basavaiah and Prameela 2003; Basavaiah and Prameela 2003; Basavaiah and Nagegowda 2004; Basavaiah et al., 2004; Basavaiah and Prameela 2004; Basavaiah et al., 2006; Basavaiah et al., 2006), spectrophotometry (Kar 1985; Sastry et al., 1997; Basavaiah and Prameela 2003; Basavaiah and Prameela 2003; Basavaiah and Nagegowda 2004; Basavaiah et al. 2004; Basavaiah and Prameela 2004; Basavaiah et al. 2006; Basavaiah et al. 2006; Kamel et al., 2008; Basavaiah et al., 2009; Tella et al., 2010; Refat et al., 2011; Swamy and Basavaiah 2014), spectrofluorimetry (Baeyens et al., 1985; Küçükkolbaşı et al., 2008; Zhao et al., 2008; Attia et al., 2016), liquid chromatography (Nakos et al., 1994; Fletouris et al., 1997; Balizs 1999; Garcia et al., 1999; Gomes and Nagaraju 2001; Kitzman et al., 2002; Danaher et al., 2007; Varghese et al., 2011) and voltammetry (de Oliveira and Stradiotto 2001).

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Fig 1: Chemical structures of the investigated Benzimidazoles.

Most of the reported spectrophotometric method (Basavaiah and Prameela, 2003; Basavaiah and Prameela, 2003; Basavaiah and Nagegowda, 2004; Basavaiah et al. 2004; Basavaiah and Prameela, 2004; Basavaiah et al. 2006; Basavaiah et al. 2006; Basavaiah et al. 2009) were based on indirect determinations of the cited drugs through their oxidation and subsequent determination of the residual oxidant spectrophotometrically by reaction with another chromogenic reagent. Consequently these methods were time consuming, tedious and involved multi reagents and multi steps reactions. Although the considerable sensitivity of the spectrophotometric methods based on ion-pair complex formation (Sastry et al. 1997; Kamel et al. 2008; Swamy and Basavaiah 2014); they still suffer from the tedious procedure, time-consuming extraction step, and the use hazardous organic solvents. Those reported methods based on CT formation reaction (Sastry et al. 1997; Küçükkolbaşı et al. 2008; Refat et al. 2011) were less sensitive and consume large volumes of organic solvents.

Recently several nanometals have been synthesized and utilized for the colorimetric determination of different analytes (Ling et al., 2008; Gao et al., 2014; Patel et al., 2015). Silver nanoparticles (AgNPs) is one of the nanometals which gained much interest in chemical analysis due to its high extinction coefficient and cost effectiveness of the analysis. AgNPs have a characteristic surface plasmon resonance (SPR) peak appears at 390 nm which could be shifted to higher wavelength upon the interaction with the analyte accompanied by a change in the color of the colloidal solution. The reaction between nano-particles and analyte is generally based on electrostatic interactions (Ling et al. 2008; Gao et al. 2014), which causes the interparticle distance to decrease, leading to particle aggregation. The aim of the present work is to utilize citrate-capped Ag NPs as colorimetric probe for development of a simple, sensitive, efficient, and economical spectrophotometric method for the determination of three benzimidazole anthilmentics (albendazole, flubendazole and mebendazole) in bulk and pharmaceutical preparations.

EXPERIMENTAL

Chemicals and materials.

All chemicals were of analytical grade and used without further purification. Silver nitrate, sodium borohydride, sodium

citrate dihydrate were purchased from Sigma–Aldrich. Albendazole powder was kindly supplied by the Egyptian International Pharmaceutical Industries Company (EIPICO, Cairo, Egypt). Flubendazole powder was kindly supplied by Unipharm (Cairo, Egypt). Mebendazole powder was obtained from The Arab Drug Company for pharmaceutical and chemical industries

Toerell & Stenhagen (Pesez and Bartos 1974) buffer solution was prepared by mixing the aqueous solutions of; phosphoric acid (3.5 mL /100 mL, El-Nasr chemical company, Cairo, Egypt), citric acid (7 g/100 mL, United company for Chemical and Medical Preparations, Cairo, Egypt) and sodium hydroxide (4 g /100 mL, Isochem specifications, Cairo, Egypt). The volume was completed to 1000 mL with distilled water to give buffer stock solution. An appropriate volume from the stock solution was adjusted to the required pH with 0.1 M hydrochloric acid (36%, Lab-Chem fine chemicals and reagents, Cairo, Egypt).

Pharmaceutical formulations

All pharmaceutical preparations were purchased from the local market. Alzental tablet (Egyptian International Pharmaceutical Industries Company, EIPICO, Cairo, Egypt) labeled to contain 200 mg of albendazole per tablet. Fluvermal tablet (produced by Minapharm under license of Janssen Pharmaceutica Belgium) labeled to contain 100 mg of flubendazole per tablet. Antiver tablet (Alexandria Co. for Pharmaceuticals & Chemical Industries, Alexandria, Egypt) labeled to contain 100 mg of mebendazole per tablet. Alzental, Fluvermal and Antiver suspensions were labeled to contain 20 mg per mL of albendazole, flubendazole and mebendazole, respectively.

Instrumentation.

Shimadzu UV-1601PC UV-Visible was used for measuring the UV-visible spectra. The Scanning spectrophotometer detector was silicon photodiode. The pH was measured using pH-meter, model AD11P (Adwa, Romania).

Preparation of standard solution.

An accurately weighted amount (10 mg) of the studied drug was dissolved in 100 ml methanol to give the stock standard solution of the drug. The stock solution was diluted with distilled water to obtain working standard solutions having the required drug concentrations $(1.0 - 15.0 \ \mu g \ ml^{-1})$.

Preparation of Silver Nanoparticles

Citrate-capped Ag NPs were prepared according to the previously reported procedure (Guo *et al.*, 2008). Silver nitrate was reduced by sodium borohydride in the presence of sodium citrate as a stabilizer. In brief, 10 mL of 1 mM of silver nitrate solution was added drop wise to 30 mL of a freshly prepared aqueous solution of 2 mM sodium borohydride with vigorous stirring. After 10 min, 3 mL of sodium citrate solution (1 % w/w) was added to stabilize the formed yellow AgNPs. The colloidal solution was then stirred for 20 min and stored at 4 °C for 2 days before use.

General analytical procedure:

An appropriate volume of the working drug solution which gave a final concentration of $2-15 \ \mu g \ mL^{-1}$ of the studied drugs was transferred to 10 mL calibrated volumetric flask. A volume of 2 ml of 0.1 mM AgNPs solution and 1 ml of Toerell & Stenhagen buffer solution (pH 8 for albendazole and mebendazole and pH 7 for flubendazole) were added. The solution was mixed thoroughly and allowed to stand for 10 min. The absorbance of mixture was measured at the specified wavelength. Blank experiment was carried out in the same time following the previously mentioned procedure omitting the drug solution.

Preparation of pharmaceutical samples for analysis

Tablets and suspension

Ten tablets of the commercially available in the local market were accurately weighed and pulverized into fine powder. An accurately weighed amount of the powdered tablets (or aliquot of the suspension) equivalent to 10 mg the drug was transferred into a 100 ml volumetric flask and 50 ml of methanol was added. The mixture was shacked well and sonicated for 20 - 30 min. The solution was then completed to the mark with the same solvent and filtered off. Further dilutions of the filtrate were made to obtain sample solutions in the required final concentrations (3, 5 and 7 µg mL⁻¹). Finally the general analytical procedure was followed mentioned above.

RESULTS AND DISCUSSION

Sliver nanoparticles are colloidal solutions of yellow color with SPR absorption at 390 nm. Addition of citrate ions stabilize the AgNPs as the ionized polar citrate carboxyl groups on the surface of the citrate-capped AgNPs rendered the particles negatively charged, and hence dispersed from each other by the electrostatic repulsion. It was reported that positively charged molecules can effectively trigger citrate capped nanoparticles to aggregate (Ling *et al.* 2008; Gao *et al.* 2014). A positively charged compound can act as a 'molecular bridge' which initiates negatively charged nanoparticles causing them to aggregate through electrostatic attraction. It is well known that the position of the SPR band of AgNPs is much affected by the distance between the particles (Evanoff and Chumanov 2004; Kumbhar *et al.*, 2005). Reduction of the distance between the particles during the aggregation of AgNPs shifted the SPR band to higher wavelength. Although the molecule of the studied drug contains exocyclic amino group and nitrogen hybrid rings, it could not associate with the AgNPs surface by the ligand exchange with citrate ions, due to steric hindrance. However, if the pH of the solution was adjusted so that makes drug molecules positively charged, then they can neutralize the electrical repulsion of silver nanoparticles (Ling *et al.* 2008; Gao *et al.* 2014),

It was observed that the addition of the studied drugs changed the color of the AgNPs solution to blue and shifted the SPR band to 615 - 630 nm, depending on the structure of investigated drugs. The dramatic changes of the absorption spectra and solution color of citrate-capped Ag NPs on exposure to three benzimidazole drugs confirmed that the aggregation mechanism of AgNPs was caused by electrostatic interaction between negatively charged citrate stabilized particle surfaces and the positive charges of benzimidazole molecules.

The aggregation-based changes in the absorption band and color change of the AgNPs solution represent the basis of the proposed spectrophotometric method and the optical detection of the cited drugs.

Optimization of Reaction Conditions

AgNPs have their unique SPR band, which strongly depends on their size, shape, interparticle distance and the surrounding medium. In order to enhance the detection sensitivity of this colorimetric probe, factors facilitating the SPR change caused by the investigated drugs were critically studied and optimized.

Effect of pH

The adjustment of the pH of media is of a great importance as it is not only promote the electrical charge of analyte molecules, but also affect the stability of the nano-particles (Patel et al. 2015). Previous study indicated that the agglomeration of metallic NPs surfaces could occur at pH below 4 without addition of analytes due to the neutralization of NPs surface charges resulting in their aggregation and the changes in their SPR band (Basu et al., 2007). Solutions having different pH values (4 - 10) were prepared and investigated to select the best pH for effective colorimetric sensing of benzimidazole drugs with citratecapped Ag NPs. (Fig. 2). It was found that the highest absorbance values were obtained in the pH range of 7 - 9 for albendazole and mebendazole while in the case of flubendazole the pH range was 6 - 8. Higher or lower pH resulted in decreasing the absorbance. Therefore pH values of 8 for albendazole and mebendazole, and pH 7 for flubendazole, were selected as the optimum pH through the current work. The optimum pH for flubendazole was lower than that for both albendazole and mebendazole, may be due to the lower pka value for flubendazole (pka=7.08) compared to albendazole (pka = 9.51) and mebendazole (pka = 8.44).



Fig 2: Effects of pH (4-10) of the Toerell & Stenhagen buffer solution on the SPR absorbance of the reaction products between AgNPs and 7 μ g/ml of albendazole (- \circ -), flubendazole (- \blacksquare -) and mebendazole (- \square -).

Effect type and volume of the buffer

Buffer solutions having different composition with the recommended pH value and their effects on the absorbance of the reaction product were evaluated. The studied buffers were phosphate, Britton-Robinson, borate, and Toerell & Stenhagen buffers (Fig. 3). Toerell & Stenhagen buffer gave the best results and it was chosen for subsequent study.



Fig 3: The influence of the buffer types (pH 8) on the SPR absorbance of the reaction product between AgNPs and albendazole (7 ug/ml).

It was observed that increasing the volume (0.2-2.2 mL) of Toerell & Stenhagen buffer produced a gradual increase in the absorbance up to 0.8 ml and remained unchanged till 1.2 ml. Further increase in the volume of the buffer slightly reduced the

absorbance of the reaction product. As a result, 1.0 mL of the buffer was selected for the recommended general analytical procedure.

Effect of AgNPs concentration

The general analytical procedure was carried out using different concentrations of AgNPs solutions. By using a range of volumes (0.2 - 2.8 ml) of 0.1 mM AgNPs solutions, it was found that the absorbance was gradually increased by increasing the volume of the reagent. Maximum values were obtained The results indicated that 2.0 mL AgNPs (1.0×10^{-4} M) solution was sufficient and satisfactory determination results of studied drugs by AgNPs detection system.

Effect of reaction time and temperature

The progress of the reaction between the studied drugs and AgNPs was monitored at different time intervals by measuring the absorbance at the specified wavelength (615 - 630). The reaction was rapid and completed within 8 min as the absorbance reached its maximum value. The obtained absorbance remained constant upon standing for 30 min. As a result all the absorbance measurements were carried out at 10 min after the mixing reagents.

A significant reduction in the absorbance was observed when the reaction was performed at elevated temperature (higher than 35 °C). The suitable temperature was 25 - 35 °C, so the reaction was carried out at room temperature.

Method Validation

The developed procedure was fully validated according to International Conference on Harmonisation guidelines (Branch 2005) in respect to linearity, range, accuracy, precision.

Linearity and range

A series of the standard drug solutions were analyzed using the general analytical procedure. Calibration curves were constructed by plotting absorbance against the corresponding concentration. The obtained linear concentration ranges were 2.0–9.0, 2.0–10 and 2.2–15 μ g ml⁻¹ with correlation coefficients of 0.9939, 0.9950 and 0.9938 for albendazole, flubendazole and mebendazole respectively. Other statistical parameters were calculated and summarized in Table 1.

Table 1: Analytical parameters for determination of the investigated drugs by the proposed method.

Parameters	Albendazole	Flubendazole	Mebendazole	
Linear range	2.0 - 9.0	2.0 - 10.0	2.2 - 15	
Slope \pm SD	0.0344±0.0011	0.0551±0.0016	0.0610±0.0020	
Intercept \pm SD	0.0360 ± 0.0066	0.0894 ± 0.0109	-0.0006±0.0136	
Correlation coefficient r ²	0.9939	0.9950	0.9938	
Determination coefficient, r	0.9969	0.9975	0.9969	
Limit of detection, LOD	0.63	0.66	0.74	
Limit of quantitation LOQ	1.91	1.99	2.23	

* the concentration is in μ g/ml.

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Conc. level	÷ *	% Recovery ^a ± SD	
μg ml ⁻¹	Albendazole	Mebendazole	Flubendazole
		Intra-day assay	
3	100.22 ± 1.26	99.37 ± 1.39	100.23 ± 0.70
5	100.31 ± 1.10	100.87 ± 1.53	100.28 ± 1.31
7	99.97 ± 1.09	100.83 ± 1.00	99.97 ± 1.07
Mean	100.17 ± 0.18	100.356 ± 0.85	100.16 ± 0.17
		Inter-day assay	
3	99.78 ± 1.47	98.15 ± 1.61	101.36 ± 1.35
5	100.07 ± 0.49	100.22 ± 0.67	99.96 ± 0.79
7	99.91 ± 1.07	100.74 ± 0.85	100.57 ± 1.41
Mean	99.92 ± 0.15	99.54 ± 1.30	100.63 ± 0.70

Table 2: Intra- and inter-day analysis for the evaluation of the accuracy and precision using the proposed method.

^a the value is the average of three determinations.

 Table 3: Analysis of the studied benzimidazole in their pharmaceutical formulations using the proposed and reported methods.

Dosage forms	% Recovery ^b ± SD		F-value ^c	t-test ^c
	Proposed method	Reported method		
Alzental 200 mg tablet	101.07 ± 1.06	100.01 ± 1.39	1.72	1.14
Alzental suspension ^a	100.22 ± 0.88	99.60 ± 1.15	1.71	0.80
Fluvermal 100 mg tablet	100.06 ± 1.41	98.99 ± 1.04	1.84	1.23
Fluvermal suspension ^a	100.37 ± 0.83	100.52 ± 1.8	4.70	0.14
Antiver 100 mg Tablet	100.02 ± 1.09	100.63 ± 1.59	2.13	0.59
Antiver suspension ^a	99.85 ± 1.39	99.50 ± 1.72	1.53	0.30

^a the concentration of suspension is 20 mg/mL.

^b the value is the mean of five and three replicate measurement for the proposed and reported methods, respectively.

^c The tabulated values of t- and F-test at 95% confidence limit are 1.943 and 6.95. respectively

Accuracy and precision

In order to determine the accuracy and precision at the intra- and inter-day levels, the standard drugs solutions at three different concentrations within the working range were prepared and analyzed by the proposed method in three replicates. The analysis was carried out within the same day in case of intra-day analysis or on three consecutive days for inter-day analysis (Table 2). The closeness of the percentage recoveries to 100% indicated the satisfactory accuracy of the proposed method. The calculated relative standard deviations did not exceeded 2 % in both intra- and inter-day analysis indicated the excellent precision of the proposed procedure at both level of repeatability and intermediate precision

Selectivity and effect of Interferences

The effect of commonly utilized excipients in drug formulation was studied. The specificity of the method was conducted to prove that the drug is free from interference of commonly used tablet excipients. The investigated benzimidazole drugs were studied in the presence of various excipients e.g. lactoseas glucose, fructose, lactose, maltose, sucrose, and magnesium stearate which were prepared in the proportion corresponding to those which are used in the final dosage form (20 μ g ml⁻¹) and analyzed with the proposed method. It was found that there was no significant interferences effect by the studied excipients on the results of the method.

Application to pharmaceutical dosage forms

The proposed method was applied to the determination of the investigated benzimidazolea in dosage forms (tablet and

suspension). The results obtained were statistically compared with those of the reported methods for albendazole (Tella *et al.* 2010) flubendazole (Baeyens *et al.* 1985) and mebendazole (Baeyens *et al.* 1985) by applying the Student's *t*-test for accuracy and *F*-test for precision.

The calculated t and F-values at 95% confidence level, and results in Table 3 showed that there is no difference between the calculated and theoretical values of both t- and F-values indicating good levels of accuracy and precision of the proposed method, respectively. The sensitivity, simplicity and minimal volume of samples make the method suitable for routine analysis of the studied drugs in quality control laboratories.

CONCLUSION

This article demonstrated the use of silver nanoparticles as chromogenic agent for the determination of three anthelmintic drugs. The reagent involved in the proposed method was inexpensive, readily available, and fairly stable in aqueous solution compared to many reagents used in other methods. The developed procedures did not include any critical reaction conditions such as rigid pH control, tedious or time-consuming extraction or heating steps.

Moreover, the proposed analytical methodology is considered a green procedure which could be applied for monitoring and optical detection of investigated benzimidazole drugs in pure and pharmaceutical dosage forms. Hence, the methods can be used in routine analysis of these drugs in quality control laboratories

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