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Research Article

Colorimetric Sensor for Determination of Pregabalin Based on Localized Surface Plasmon Resonance of Silver Nanoparticles

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ABSTRACT

The purpose of this study is to develop a novel method for colorimetric sensing and selective determination of the non-chromophoric drug, pregabalin using unmodified silver nanoparticles (AgNPs). Silver nanoparticles are prepared by reducing 0.64 mM silver nitrate with 0.1% w/v sodium borohydride and stabilizing the nanoparticles with 10 mM sodium citrate under vigorous stirring. The citrate-capped silver nanoparticles dispersed in water exhibit a bright yellow color owing to the electrostatic repulsion between the particles due to the presence of a negatively charged surface and have a localized surface plasmon resonance band at 390 nm. Addition of positively charged pregabalin prepared in acetate buffer pH 3.8 induced rapid aggregation of silver nanoparticles through electrostatic interactions with the negative charge on the particle surface. This caused a decrease in the absorbance at 390 nm and the color of the colloidal solution turned from yellow to green upon aggregation. The decrease in absorbance at 390 nm is directly proportional to the concentration of pregabalin with a linearity range of 100 – 500 µg mL^{-1}, a limit of detection of 19 µg mL^{-1}, and a limit of quantitation of 57 µg mL^{-1}. The method was applied for the determination of pregabalin in Lyrica® capsules with a mean %recovery ± standard deviation of 97.809 ± 1.396. The method is simple, rapid, and cost-effective and can be used for the determination of pregabalin in bulk and in its pharmaceutical preparations.

Keywords: Silver nanoparticles; localized surface plasmon resonance; pregabalin; nanoparticle aggregation; colorimetric sensor

1. INTRODUCTION

Pregabalin is a derivative of the neurotransmitter gamma-aminobutyric acid (GABA) and is indicated for the treatment of peripheral and central neuropathic pain and the treatment of generalized anxiety disorder in adults. Also, it is used as adjunctive therapy in adults with partial seizures with or without secondary generalization [1].

Developing analytical methods for the determination of pregabalin is problematic because it is a non-chromophoric compound. Reviewing the literature revealed that most currently available methods are based on sophisticated and expensive techniques such as High-performance liquid chromatography (HPLC) [2–4], liquid chromatography–tandem mass spectrometry (LC–MS/MS) [5–7], gas chromatography (GC) with flame ionization detector (FID) 8, ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) [9], and gas chromatography–mass spectrophotometry (GC–MS) [10,11]. On the other hand,

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the reported methods based on simpler spectrophotometric [12,13] and spectrofluorometric [13,14] techniques require tedious derivatization and sample preparation steps [15].

Recently, nanomaterials have been used in a variety of analytical measurements to enhance the detection of chemical and biochemical analytes [16]. Colorimetric methods based on localized surface plasmon resonance of metal nanoparticles have received considerable attention not only because of their excellent analytical performance exhibited in terms of selectivity and sensitivity but also because of their extreme simplicity and low cost since this kind of assay does not require any expensive or complex instrumentation. Gold, silver, copper, and platinum nanoparticles have been employed in colorimetric sensing of various drugs in pharmaceutical and biological samples [17]. Silver nanoparticles are more commonly used as colorimetric probes due to their high extinction coefficient and low cost [18].

In this work, a simple and fast method is developed for both naked-eye colorimetric detection and spectrophotometric determination of pregabalin by measuring its effect on the absorption spectra of citrate-capped silver nanoparticles. To the best of our knowledge, this is the first visual sensor based on the aggregation of silver nanoparticles for pregabalin detection.

2. MATERIALS AND METHODS

2.1. Chemicals

Pregabalin was provided by Pfizer Egypt with a purity of 100.16%. Silver nitrate, trisodium citrate dihydrate, and potassium dihydrogen phosphate were of analytical reagent grade and purchased from Fischer Scientific (Loughborough, UK). Sodium borohydride was purchased from Loba Chemie (Mumbai, India). Hydrochloric acid and glacial acetic acid were of analytical grade and obtained from Advent Chembio (Mumbai, India). Phosphoric acid (85%, reagent grade) was purchased from Pharmco Products Inc. (Brookfield, United States). Citric acid and anhydrous sodium acetate were of laboratory reagent grade and purchased from ISO-Chem (Pithiviers, France).

2.2. Dosage form

Lyrica® hard gelatin capsules (batch No. 17109), containing 50 mg of pregabalin per capsule, manufactured by Pfizer (Cairo, Egypt) were obtained from the local drug store.

2.3. Instrumentation

UV-Visible absorption spectra were recorded on a Shimadzu UV-1800 double-beam spectrophotometer (Kyoto, Japan) equipped with two matched 1 cm quartz cells. Other instruments include a hot plate magnetic stirrer (DAIHAN, Malaysia), digital balance (Sartorius, Germany), pH-meter equipped with a double junction glass membrane electrode (HANNA, USA), and vortex mixer (Grant Instruments, UK). All glassware was thoroughly washed with nitric acid and then with distilled water before use.

2.4. Synthesis of silver nanoparticles

Silver nitrate was reduced by sodium borohydride and sodium citrate was used as an electrostatic stabilizer [18]. Briefly, 1.0 mL of 10 mM sodium citrate dihydrate is added to 39.0 mL of 0.64 mM AgNO3 under vigorous stirring for 20 minutes. Then, 10 mL of 0.1% w/v NaBH4 is added to the solution and stirred for an hour. The silver nanoparticles solution (0.5 mM) is allowed to stand for 2 hours before use. The silver nanoparticles solution is stored under dark conditions at 4.0 ± 2.0 °C to maintain its stability for several weeks. For use in the colorimetric assay, the silver nanoparticles solution is diluted to 0.2 mM prior to use.

2.5. Pregabalin standard solutions

2.5.1. Pregabalin stock standard solution

A 2000 μg mL^{-1} stock solution of pregabalin was prepared by dissolving 200 mg of pregabalin powder in distilled water and the volume was completed to 100 mL with the same solvent.

2.5.2. Pregabalin working standard solutions

Working standard solutions were prepared in the concentration range of 100 – 500 μg mL^{-1} by pipetting appropriate aliquots of the 2000 μg mL^{-1} pregabalin stock standard solution into 25 mL-volumetric flasks. A volume of 3.0 mL of acetate buffer (pH 3.8) was added to the individual flasks. Finally, distilled water was added to fill the flasks to the 25 mL mark. The blank solution was prepared following the same procedure, but with adding distilled water instead of pregabalin.

2.6. Determination of pregabalin

5.0 mL of pregabalin working standard solution is mixed with 5.0 mL of the 0.2 mM silver nanoparticles solution using a vortex mixer for 20 seconds. The UV-visible spectrum of the mixture is measured after 6 minutes using the UV-Visible spectrophotometer in the wavelength range of 200 – 800 nm against the blank solution. The calibration curve is constructed by triplicate preparation of five concentrations of pregabalin in the range of 100 – 500 μg mL−1 and plotting the average change in absorbance (ΔA) at 390 nm versus pregabalin concentration where ΔA represents the difference in absorbance of silver nanoparticles at 390 nm in the absence and the presence of pregabalin.

2.7. Analysis of pregabalin capsules

The content of ten Lyrica® capsules was accurately weighed. An accurately weighed amount of the capsule powder equivalent to 200 mg of pregabalin was transferred into a 100 mL volumetric flask and dissolved in distilled water. The resulting solution was made up of volume with the same

solvent and filtered to yield a stock solution (2000 µg mL^{-1}). A working solution of 300 µg mL^{-1} was prepared by pipetting an appropriate aliquot of the 2000 µg mL^{-1} stock solution into a 25 mL volumetric flask, adding 3.0 mL of acetate buffer (pH 3.8) then using distilled water to complete the volume. The final solution was analyzed in the same way as that of pregabalin working standard solution. The concentration of pregabalin in the capsules was determined from the calibration curve.

3. RESULTS AND DISCUSSION

3.1. Principle of Pregabalin Sensing

The colloidal solution of the synthesized silver nanoparticles appeared bright yellow with its maximum absorption at 390 nm due to the localized surface plasmon resonance of monodispersed silver nanoparticles [19]. The surface of the silver nanoparticles is negatively charged by the citrate caps preventing aggregation by the electrostatic repulsion. Previous literature reported that a positively charged molecule can cause aggregation of the citrate-capped silver nanoparticles [20–22]. Accordingly, pregabalin with its protonated amino group can trigger particle aggregation. The interaction of pregabalin, prepared in acetate buffer (pH 3.8), with citrate-capped silver nanoparticles, results in a color change from yellow to green which is manifested by a decrease in the surface plasmon resonance band of silver nanoparticles at 390 nm and the appearance of a new shoulder at approximately 600 – 700 nm **(Figure 1).**

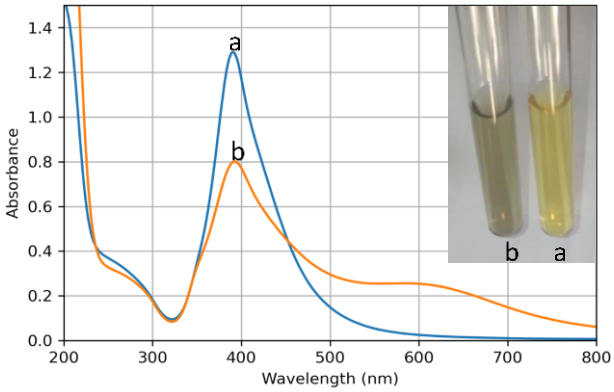

Figure 1: Surface plasmon resonance spectra and color of silver nanoparticles in (a) the absence and (b) the presence of 300 µg mL^{-1} pregabalin.

When pregabalin was prepared in distilled water instead of acetate buffer (pH 3.8), the surface plasmon resonance spectrum and the color of the silver nanoparticles colloidal solution did not change. This result confirms that the mechanism of silver nanoparticle aggregation and color change depends on electrostatic interaction between negatively charged citrate-capped silver nanoparticles and the positive charges of pregabalin. This mechanism is illustrated in **Figure 2.**

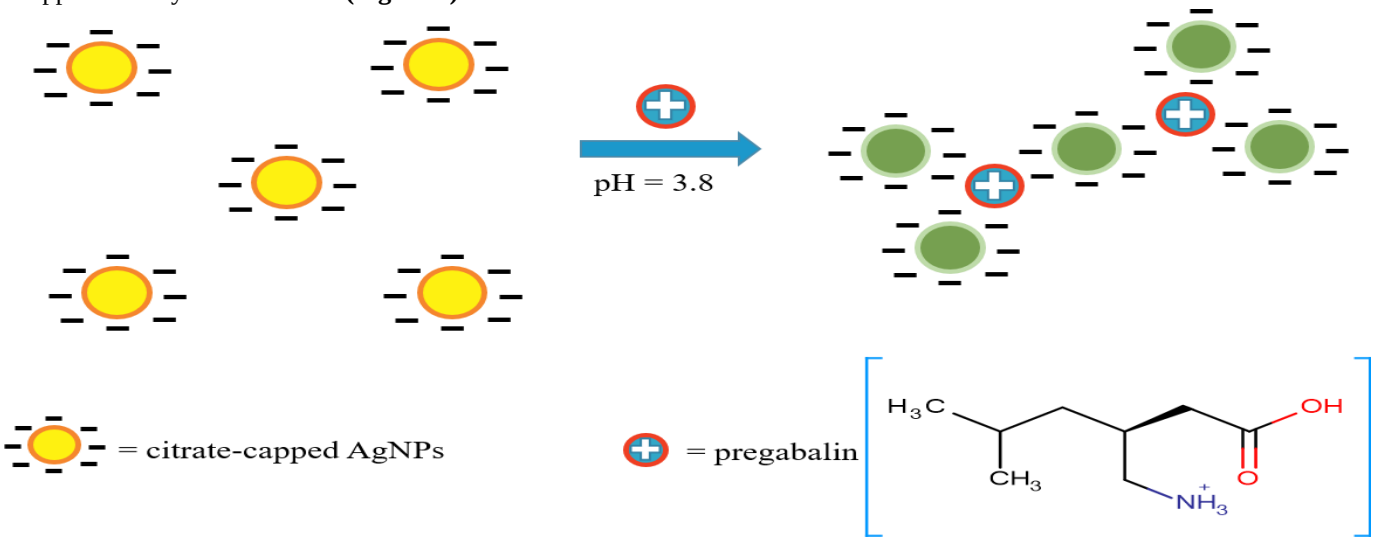

Figure 2: Schematic illustration of the colorimetric detection of pregabalin based on the aggregation of citrate-capped silver nanoparticles.

3.2. Optimization of the experimental conditions

Different factors affecting the colorimetric assay of pregabalin were thoroughly studied and optimized. The aim of the optimization process was to get the green color of aggregated silver nanoparticles with higher ΔA values where ΔA represents the difference in absorbance of silver nanoparticles at 390 nm in the absence and the presence of pregabalin.

3.2.1. Effect of citrate concentration

Different concentrations of trisodium citrate dihydrate in the range of 1 – 25 mM were studied. The optimum concentration of citrate was found to be 10 mM concerning ΔA as shown in **Figure 3**. Citrate concentration is a critical factor in the colorimetric assay of pregabalin because lower citrate concentrations (<10 mM) can induce self-aggregation of silver

nanoparticles in the absence of pregabalin which will decrease ΔA while higher citrate concentrations (>10 mM) can produce highly stabilized silver nanoparticles which will be insensitive to pregabalin, and this will also decrease ΔA.

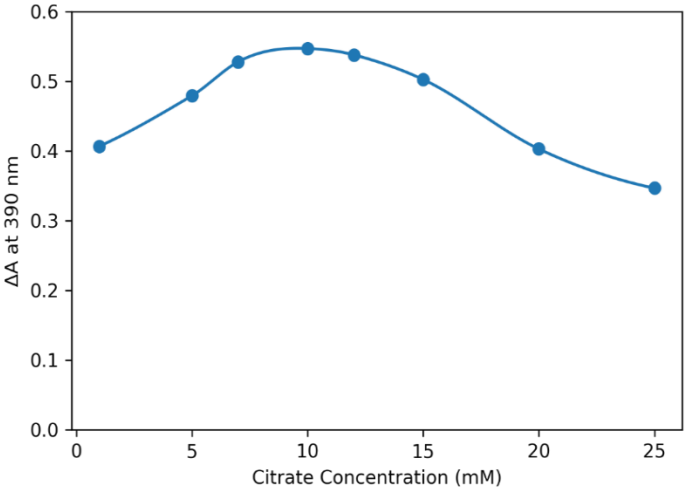

Figure 3: Effect of citrate concentration on ΔA at 390 nm. Pregabalin concentration is 300 µg mL^{-1} prepared in acetate buffer pH 3.8.

3.2.2. Effect of buffer type

The medium surrounding the nanoparticles has a great effect on the interaction between silver nanoparticles and pregabalin. Four different acidic media were investigated namely, acetate buffer, citrate buffer, phosphate buffer, and hydrochloric acid. The pH was adjusted to 3.8 for all these media. As indicated in **Figure 4**, pregabalin in hydrochloric acid was not able to cause nanoparticle aggregation and the color of the colloidal solution remained yellow. Phosphate buffer induced nanoparticle aggregation and color change without the addition of pregabalin. This effect may be due to the adsorption of phosphate anions on the surface of silver nanoparticles, reducing the surface charge and decreasing the stability of silver nanoparticles [23]. Citrate molecules in citrate buffer were added to the citrate caps on the surface of silver nanoparticles and resulted in more stabilized nanoparticles which were more resistant to aggregation caused by pregabalin. Acetate buffer was the optimum medium with respect to ΔA at 390 nm as shown in **Figure 4.**

3.2.3. Effect of pH

The pH of the medium has a dual effect on the interaction of pregabalin with citrate-capped silver nanoparticles. PH influences not only the degree of ionization and charge of pregabalin but also the degree of ionization and charge of citrate ions on the surface of silver nanoparticles. Citrate is a weak tricarboxylic acid (pKa = 3.1, 4.7, and 6.4), so it tends to be ionized and more negatively charged at higher pH values. This renders the silver nanoparticles extensively stabilized. On the other hand, the positive charge of pregabalin decreases at higher pH values and this positive charge would be insufficient to overcome the higher repulsion forces among nanoparticles.

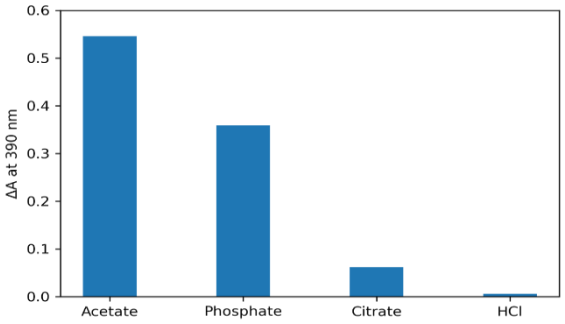

Figure 4: Effect of buffer type on ΔA at 390 nm. Pregabalin concentration is 300 µg mL^{-1} prepared in the corresponding buffer

Therefore, aggregation of silver nanoparticles and color change does not occur. At lower pH values, carboxylates of citrate are more protonated, decreasing the negative charges on particle surfaces and the amino group of pregabalin is more protonated increasing its positive charge and resulting in enhancement of aggregation and color change of silver nanoparticles upon addition of pregabalin. The pH of acetate buffer was varied in the range of 3.8 – 5.8 to study the effect of pH on the interaction of pregabalin with citrate-capped silver nanoparticles. As expected, the lower the pH of the acetate buffer, the more significant the effect of pregabalin on the surface plasmon resonance spectrum of silver nanoparticles. Accordingly, 3.8 was the optimum pH as shown in **Figure 5.**

The pH of acetate buffer (pKa of acetic acid = 4.76) cannot be lowered further below 3.8 because this leads to a loss of buffer capacity.

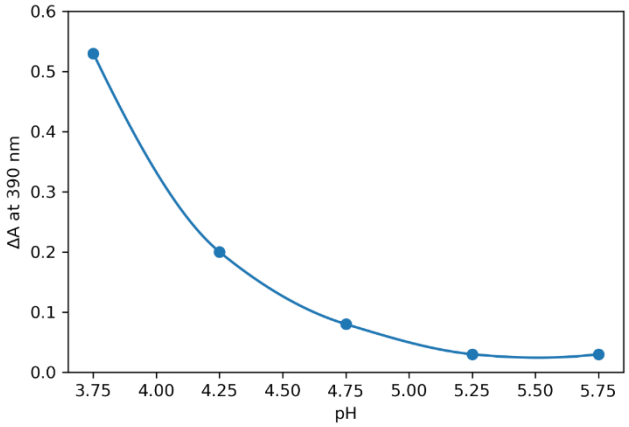

Figure 5: Effect of pH of acetate buffer on ΔA at 390 nm. Pregabalin concentration is 300 µg mL^{-1} prepared in acetate buffer.

3.2.4. Effect of ionic strength

Various concentrations of acetate buffer in the range of 0.05 – 1.2 M were investigated. It was found that 1.0 M was the optimum concentration with regard to ΔA at 390 nm as demonstrated in **Figure 6**. Typically, environments with high ionic strength can minimize the charge repulsion of negatively charged nanoparticles due to the electrostatic shielding effect [18]. This facilitates the action of pregabalin as an aggregation inducer.

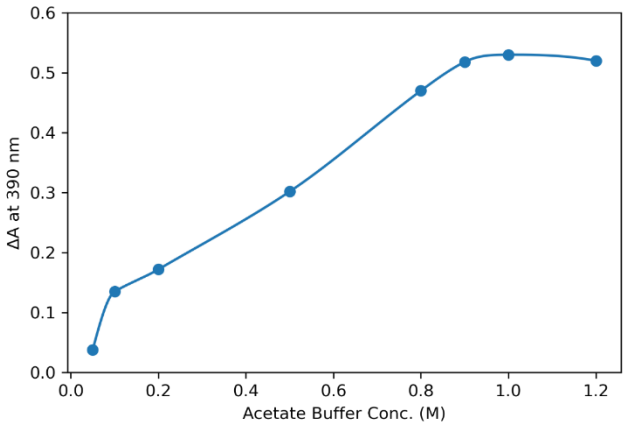

Figure 6: Effect of ionic strength of acetate buffer on ΔA at 390 nm. Pregabalin concentration is 300 µg mL^{-1} prepared in acetate buffer.

3.2.5. Effect of silver nanoparticles concentration

The as-synthesized silver nanoparticles have a concentration of 0.5 mM calculated based on the final concentration of silver nitrate. This colloidal solution was diluted to 0.05 – 0.25 mM prior to reaction with pregabalin. 0.2 mM was chosen as the optimum silver nanoparticles concentration for the colorimetric assay as it exhibited higher sensitivity to pregabalin as shown in **Figure 7.**

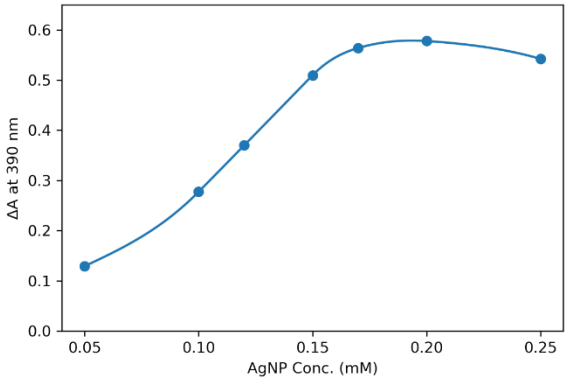

Figure 7: Effect of silver nanoparticles concentration on ΔA at 390 nm. Pregabalin concentration is 300 µg mL^{-1}

3.2.6. Effect of reaction time

The reaction time was investigated by monitoring the decrease in the silver nanoparticles absorbance at 390 nm as a function of time, after the addition of pregabalin. As shown in **Figure 8,** the absorbance gradually decreased and then remained constant after 6 minutes. Therefore, the proposed method is considered rapid as 6 minutes are sufficient for the complete reaction.

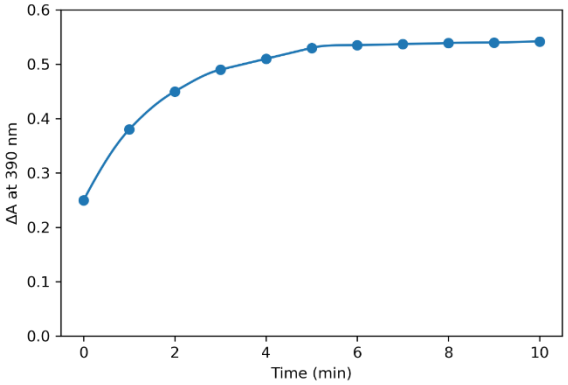

Figure 8: Effect of reaction time on ΔA at 390 nm. Pregabalin concentration is 300 µg $mL^{-1.}$

3.3. Analytical performance and validation of the proposed method

Under optimum experimental conditions discussed before, silver nanoparticles were used as a colorimetric probe for naked-eye detection and quantitative determination of pregabalin. The color of silver nanoparticles solutions changed from yellow to green with increasing concentrations of pregabalin. The color change is associated with a decrease in the absorbance intensity of colloidal solutions at 390 nm and the appearance of a shoulder at 600 – 700 nm as shown in **Figure 9.** The developed method has been validated in compliance with the guidelines of the International Conference on Harmonization (ICH) [24]. The method linearity, range, limit of detection, limit of quantitation, accuracy, precision, and selectivity were investigated.

3.3.1. Linearity and range

A calibration curve was constructed by plotting the change in absorbance (ΔA) at 390 nm versus pregabalin concentration (**Figure 10**). The proposed method was linear over the range of 100 – 500 µg mL^{-1}. The statistical parameters of the regression equation summarized in **Table 1** indicate that the proposed colorimetric method has acceptable linearity over the concentration range of 100 – 500 µg mL^{-1}.

3.3.2. Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) of the proposed method were calculated from the slope and standard deviation of the intercept (SD intercept) of the calibration curve as follows:

$$LOD = \frac{3.3\ SD_{intercept}}{slope}$$

$$LOQ = \frac{10\ SD_{intercept}}{slope}$$

The calculated LOD and LOQ were found to be 19 and 57 µg mL^{-1} respectively indicating that the method has adequate sensitivity for the determination of pregabalin in bulk, in diluted solutions, and in pharmaceutical dosage forms.

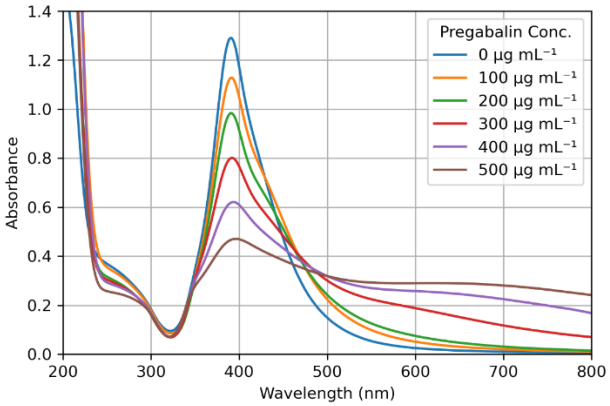

Figure 9: Surface plasmon resonance spectra of silver nanoparticles solutions with various concentrations of pregabalin

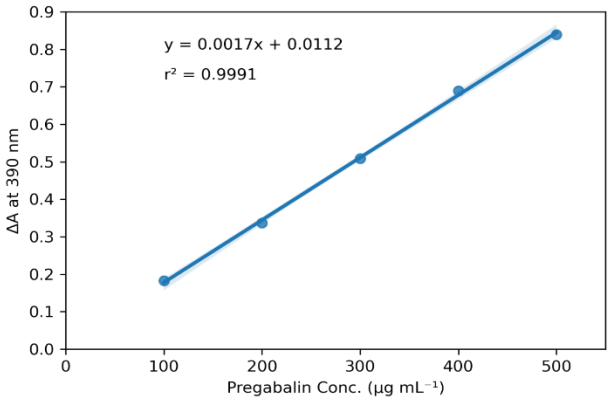

Figure 10: Calibration curve of pregabalin representing the linear relationship between ΔA at 390 nm and pregabalin concentration.

3.3.3. Accuracy and precision

The accuracy of the proposed method was assessed by triplicate measurement of three different concentrations of pregabalin within the linearity range. **Table 2** presents the percent recovery for each concentration and demonstrates the accuracy of the method expressed as mean % recovery ± standard deviation.

Table 1: Results of performance data and statistical parameters of the regression equation for determination of pregabalin using the proposed method.

Linearity range	**100 – 500 µg mL^{-1}**
Correlation coefficient (r)	0.9996
Slope	0.0017
Intercept	0.0112
Standard deviation of slope	2.88×10^{-5}
Standard deviation of intercept	9.55×10^{-3}
Standard deviation of regression	9.11×10^{-3}

The precision of the proposed method was investigated by assessing the repeatability and the intermediate precision. Regarding the repeatability of the method, three different concentrations within the linearity range were determined by triplicate measurement on the same day, while for intermediate precision the three concentrations were assayed on three consecutive days with triplicate determination each day. As shown in **Table 3**, the small values of relative standard deviation (< 2.0%) indicate that the method is precise.

Table 2: Recovery results of pregabalin in pure form by the proposed colorimetric method.

Conc. taken (µg mL^{-1})	**Mean conc. found* (µg mL^{-1})**	**% Recovery**	**Mean % recovery ± Standard deviation**
150	150.157	100.105	100.249 ± 0.737
350	353.667	101.048	
450	448.177	99.595	

*n=3

3.3.4. Selectivity

Acceptable values of the mean percent recovery ± SD and %RSD in recovery studies of pregabalin capsules **(Table 4)** indicate that there is no potential interference of the inactive ingredients. Also, the surface plasmon resonance spectrum obtained from the solution of pregabalin capsules is typical of that obtained from the standard solution of pregabalin at the same concentration.

Table 3: Repeatability and intermediate precision of the proposed colorimetric method.

Conc. taken (µg mL^{-1})	Repeatability (n = 9)			Intermediate precision (n = 27)		
	Mean conc. found (µg mL^{-1})	SD	%RSD	Mean conc. found (µg mL^{-1})	SD	%RSD
150	150.157	1.006	0.670	153.515	2.126	1.385
350	353.667	4.275	1.209	354.452	4.785	1.350
450	448.177	4.844	1.081	448.776	7.360	1.640

SD: standard deviation, %RSD: relative standard deviation

To further assess the selectivity of the method, a placebo containing the excipients of the pharmaceutical product (matrix) was prepared, including lactose monohydrate, cornstarch, and talc as shown in the dosage form product information file of Lyrica® capsules. The placebo was analyzed in the same way as that of Lyrica® capsules and the matrix gave the same response as that of the blank solution. Thus, the method was found to be selective and pregabalin can be determined in its capsules without interference from the present inactive ingredients. This may be because the interaction with silver nanoparticles depends on the presence of an amino group in the drug molecule which is not available in the excipients.

3.3.5. Robustness

Robustness was investigated by making small deliberate changes in various experimental conditions such as working wavelength (± 1 nm), pH (± 0.05 units), volume, and concentration of reagents. Such small changes which may occur during the experimental procedure did not have a significant effect on the ΔA values observed for pregabalin and the %RSD values were found to be less than 2.0% indicating that the proposed method is robust.

3.4. Analysis of pregabalin capsules

The proposed colorimetric method was applied for the determination of pregabalin in the commercially available Lyrica® capsules. Recovery studies were performed by measuring ΔA of a certain concentration repeated six times and the mean percent recovery was calculated and listed in Table 4. The assay results were consistent with the labeled amount of the dosage form indicating that excipients and other sample matrices did not interfere with pregabalin. The recovery results of pregabalin capsules obtained by the proposed method were statistically compared to those obtained by a reported method[25].This reported method is based on the derivatization of pregabalin with ninhydrin to form a blue violet-colored product which is then measured spectrophotometrically at 402.6 nm within the linearity range 50 – 1000 µg mL^{-1}. F-test and t-test were carried out at a 95% confidence level and the p-value for both tests are listed in **Table 4.** The results of the F-test indicate that there is no significant difference between the variances of the two methods. Assuming equal variances of the two methods, t-test results demonstrate that there is no significant difference between the mean percent recovery of the two methods. This proves the reliability of the proposed method for the determination of pregabalin in its pharmaceutical preparations.

Table 4: Determination of pregabalin in Lyrica® capsules by the proposed method and the reported spectrophotometric method.

	Conc. taken (µg mL^{-1})	Mean conc. found* (µg mL^{-1})	Mean % recovery ± SD	p-value	
				F-test	t-test
Proposed method	300	293.427	97.809 ± 1.396	0.431	0.140
Reported method[25]	300	298.446	99.482 ± 0.731		

* n = 6, SD: standard deviation

4. CONCLUSION

A novel method for the detection and selective determination of pregabalin was developed using citrate-capped silver nanoparticles as a colorimetric probe. The concept of sense depends on the interaction between positively charged pregabalin and negatively charged silver nanoparticles which induces aggregation of particles leading to a change in the surface plasmon resonance band and color of the colloidal solution. The proposed method can be operated at room temperature without derivatizing agents. Moreover, the interaction between pregabalin and silver nanoparticles proceeds rapidly, requiring only 6 minutes per measurement. The method is cost-effective and more practical for routine use due to its simplicity. Unlike other methods reported in the literature, this colorimetric probe does not involve complicated sample preparation steps and does not require high levels of technical skill. The proposed method is validated in terms of accuracy, precision, linearity, selectivity, and robustness, so it is suitable for the routine analysis of pregabalin in bulk and its pharmaceutical formulations without interference from excipients.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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