Utilization of Localized Surface Plasmon Resonance of Silver Nanoparticles for the Spectrofluorimetric Estimation of Oxymetazoline in Dosage Forms: Application to Aqueous Humor

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Abstract

A simple, novel, cost-effective and highly sensitive spectrofluorimetric method was developed for estimation of the nasal decongestant oxymetazoline (OMZ) whether *per se* or in its pharmaceutical preparations using colloidal silver nanoparticles (AgNPs). The method is based on the high catalytic potential activity of AgNPs on the fluorescence intensity of OMZ leading to 12-fold increase in its fluorescence intensity. The response was linear over the range of **20.0 to 700.0** ng/mL with lower detection limit of 5.0 ng/mL and limit of quantification of 14.0 ng/mL. The proposed method was applied to the assay of commercial nasal drops, nasal spray and aqueous humor. Interference likely to be encountered from co-administered drugs was studied. The developed method was optimized and validated as per International Council of Harmonization (ICH). An explanation for the drug-AgNPs interaction was proposed.

1. Introduction:

Nanoscience includes the study of the materials within nanoscale (1-100 nm). Among the metal nanoparticles, AgNPs are the most widely used owing to their remarkable chemical and physical properties, including surface enhanced Raman scattering, optical action, electrical conductivity, high thermal, chemical stability and catalytic activity[1]. AgNPs can be used in drug delivery and in the determination of drugs in pharmaceuticals. Although AgNPs have no fluorescence, they were frequently used in the spectrofluorometric analysis of some drugs in biological fluids and dosage forms. They can cause either quenching or enhancement of the fluorescence of drug fluorophores and many drugs were determined based on this fact [2].

Localized surface plasmon resonance (LSPR) is a kind of surface plasmon excitations. It occurs when light hits a nanoparticle having smaller size than the wavelength of the incident light. The incident photon may merge with the metal electrons and start to oscillate coherently[3]. Recent investigations have confirmed that LSPR is an influential technique in biomedical applications due to its sensitivity to material type, size and dielectric constant[4, 5], in addition to its impact on enhancing sensitivity of different spectroscopic techniques including fluorescence[6].

Oxymetazoline hydrochloride (OMZ) Fig. 1 [6-tert-butyl-3-(4,5-dihydro-1H-imidazol-2-ylmethyl)-2,4-dimethylphenol hydrochloride] [7] is a direct acting synthetic adrenergic agonist that stimulates both α_1 and α_2 adrenergic receptors. Oxymetazoline is found in several nasal spray decongestants and ophthalmic drops. It directly stimulates α receptors on blood vessels supplying the nasal mucosa and conjunctiva, thereby producing vasoconstriction and decreasing congestion[8]. It is absorbed from systemic circulation regardless of the route of administration [9]. OMZ is official in each of the United States Pharmacopoeia(USP)[10], the British Pharmacopoeia (BP)[11], and the European Pharmacopoeia [12]. Reviewing the literature revealed few reports for OMZ determination, viz, spectrophotometry[13–18], spectrofluorimetry, [19, 20] gas chromatography[21, 22], HPLC [10][23–28], flow injection-chemiluminescence [29, 30], capillary electrophoresis[31], and electrochemistry[32, 33].

The reported spectrofluorimetric methods for OMZ are less sensitive, tedious and need chemical derivatization with either NBD-Cl reagent with linearity range (1–12 $\mu\text{g mL}^{-1}$) [19] or mucin with concentration range from (2.69 to $26.9 \times 10^{-5} \text{ mol L}^{-1}$) [20], while the proposed study which is based on its interaction with AgNPs leads to 12-fold increase of its fluorescence and hence, a highly more sensitive method for its determination.

2. Experimental

2.1. Materials and reagents:

All chemicals used were of Analytical Reagent Grade.

- Authentic sample of oxymetazoline hydrochloride with a purity of 99.9 % was kindly provided by Pharaonia Pharmaceutical Co., Pharo-Pharma, Alexandria, Egypt.
- Oxymet[®] drops (Batch No. 555 0033) containing 0.5 mg OMZ/ 1 mL, product of the Pharaonia Pharmaceuticals Co., Pharo-Pharma, Alexandria, Egypt.
- Oxymetazoline[®] nasal spray (Batch No.184986) containing 0.5 mg OMZ/1 mL, product of the Medical Union Pharmaceuticals Co., Cairo, Egypt.
- Both preparations were obtained from the local market.
- Methanol, acetonitrile, ethanol, boric acid, sodium hydroxide, sodium acetate trihydrate and acetic acid 96% were obtained from Sigma-Aldrich, Steinheim, Germany.
- Sodium borohydride, silver nitrate and polyvinyl povidone were purchased from Sigma-Aldrich (USA).

2.2. Instruments:

- Measurements of fluorescence intensity were done using Cary Eclipse Fluorescence Spectrophotometer equipped with Xenon flash lamp. The method was conducted at smoothing factor of 20, high voltage mode (800 V), and slit width of 5 nm.
- Spectrophotometer: UV/Vis. JENWAY Model 6850.
- pH-meter: Consort, P-901, Belgium.
- Magnetic stirrer: product of Daihan Scientific Co, limited, S. korea.
- Vortex Mixer: (IVM-300p, Taiwan)
- Transmission electron microscope (TEM), JEOL, JSM -2100 (Tokyo, Japan). Sample was loaded on 200 mesh carbon coated Cu-grid and examined at 200 KV.

2.3. Preparation of standard solutions:

To prepare standard solution of OMZ (100.0 mg/mL), 10.0 mg of OMZ HCl was dissolved in 100 mL of ethanol in a volumetric flask. The stock solution was diluted using the same solvent to obtain working solution (10.0 mg/mL) which was further diluted as appropriate.

2.4. Preparation of silver nanoparticles:

Colloidal solution of silver nanoparticles was prepared adopting a previously reported method [34] by adding 10 ml of a precursor AgNO_3 (1×10^{-2} M) dropwise to 30 ml of freshly prepared NaBH_4 solution (2×10^{-2} M) with vigorous stirring in an ice bath until light yellow solution was obtained. The obtained solution was further stabilized by addition of 5 ml of PVP (0.3%w/w).

2.5. Procedures:

2.5.1. Construction of Calibration Graph:

The working solutions were prepared by transferring aliquots from the standard solution covering the concentration range (**Table 1**) to a set of 10 mL volumetric flasks followed by 0.1 ml of AgNPs (6×10^{-5} M) then, the volume was completed to the mark using ethanol. After mixing, measurements of fluorescence intensity were done at 306 nm (excitation at 281 nm) against a reagent blank. The corresponding regression equation was derived and a calibration graph was obtained through a plot between RFI and OMZ concentrations (ng/mL).

2.5.2. Analysis of Pharmaceutical formulations:

The contents of each of five bottles of the nasal drops and nasal spray were mixed well. Accurately measured volumes from both Oxymet[®] nasal drops and Oxymetazoline[®] nasal spray were transferred into 100 mL volumetric flasks and completed to the volume with ethanol. Serial dilutions were made as appropriate to obtain working solutions within the working range of proposed method (**Table 1**). The procedure described under "**2.5.1**" was then followed. The contents of each dosage form were calculated using the corresponding regression equation.

2.5.3. Procedure for Content Uniformity Testing:

Assay of 10 Oxymet[®] nasal drops individually was conducted using the proposed method. An accurately measured volume was transferred from each separate preparation into 10 mL volumetric flasks then completed to the volume with ethanol. Serial dilutions were made to prepare 10 volumetric flasks with a final concentration of 500 ng/mL. The procedure described under "**2.5.1**" was then applied. The recovery of each preparation was determined using the regression equation and the content uniformity was calculated applying the official USP [35] guidelines.

2.5.4. Application to aqueous humor:

An aliquot (1mL) of aqueous humor was transferred into a series of 10 mL volumetric flasks. Aliquots of the working solution of OMZ within the concentration range and 0.1mL of AgNPs were quantitatively added, then vortex mixing for 10 second. Complete to the mark with ethanol, then filter. Proceed as described under “2.5.1”.

3. Results And Discussion:

AgNPs were prepared using silver nitrate as a precursor, sodium borohydride as a reducing agent and PVP as a stabilizing agent. AgNPs stabilization achieves through electrostatic and steric stabilization. Electrostatic stabilization (e.g., sodium citrate) is caused by the repulsion between particles, caused by the electrical double layer formed by ions adsorbed at the surface of particles and the corresponding counter ions. Steric stabilization (e.g., PVP) is achieved due to the coordination of sterically demanding organic molecules and polymers that act as protective shields on the metallic surface. In this study PVP was chosen as a stabilizer to prevent agglomeration of silver nanoparticles. In addition to, the sensitivity of method was better during using PVP in compare to sodium citrate[36].

Ethanol solution of oxymetazoline exhibits an emission peak at 306 nm upon excitation at 281 nm, as demonstrated in (Fig.2). The addition of AgNPs was found to enhance the emission band of the drug by 12-folds (Fig.3). It was previously reported that, the free space absorption conditions of the fluorophores is modified upon adding metal-nanoparticles, and this intensely changes the spectra obtained in the absence of metallic nano surfaces[37]. Figure 4 shows the spectra of increasing concentrations of OMZ upon addition of fixed volume (0.1 mL) of AgNPs (6×10^{-5} M) at 306 nm.

3.1. Characterization of PVP capped AgNPs:

In order to characterize the prepared colloids, UV-Visible spectrum was recorded for the prepared AgNPs showing intense sharp absorption peak at 385 nm (Fig.5). In addition, TEM images were used for further confirmation for the prepared AgNPs indicating spherical AgNPs with size range of 142 nm (Fig.6).

3.2. Synergistic impact based on LSPR of OMZ:

Metal induced fluorescence enhancement phenomenon is proposed to be due to interactions of the excited fluorophores with surface plasmon resonances in metals. In this study, the interactions are suggested to be between AgNPs and nitrogen atoms of the imidazoline ring of OMZ [5,6] leading to remarkable enhancement of OMZ fluorescence intensity, as illustrated in Schem.1.

3.3. Optimization of experimental parameters:

The ability of AgNPs to enhance the fluorescence intensity of OMZ was affected by different experimental parameters. These parameters include AgNPs volume, diluting solvents, pH, terbium ion

effect, incubation time. All these factors were carefully studied in order to optimize the performance of the proposed method.

3.3.1. Effect of AgNPs volume:

The effect of AgNPs (6×10^{-5} M) solution volume was studied, and the maximum fluorescence intensity was obtained using 0.1 mL, higher volumes led to decreasing the fluorescence intensity (**Fig.7**).

3.3.2. Effect of diluting solvents:

Different diluting solvents including; distilled water, methanol, ethanol and acetonitrile were attempted. The impact of diluting solvents was studied using 0.1 mL of AgNPs added to OMZ (500 ng/mL). Both acetonitrile and ethanol gave the maximum fluorescence intensity (**Fig.8**) but ethanol was used as optimum diluting solvent in further experiments because it is more green and eco-friendly [38].

3.3.3. Effect of pH:

During this study, two buffer solutions were used including 0.2M acetate buffer with pH range 3.0-5.0; and 0.2M phosphate buffer with pH range 6.5–9.5. The pH was found to have no considerable impact on the enhancement of the fluorescence intensity.

3.3.4. Effect of terbium (Tb):

Terbium was added to the reaction mixture, hopefully, it would enhance the fluorescence intensity of OMZ. The method, known as lanthanide-sensitized luminescence, and is applicable to many aromatic ketones and aldehydes and a variety of biologically important compounds[39]. It was found that, Tb has no effect, and hence it was excluded in this study.

3.3.5. Effect of incubation time:

The incubation time was investigated upon addition of 0.1mL of AgNPs to OMZ solution. The enhancement in fluorescence intensity was studied at different time intervals from starting one to 30 min. The interaction between OMZ and AgNPs was found to be completed within 1 min and the fluorescence intensity was stable for more than 30 min.

3.4. Validation of the proposed method:

Evaluation of the validity of the proposed method was done according to ICH Q2R1 recommendations[40], where linearity range, LOQ, LOD, precision, accuracy, sensitivity, robustness, selectivity and specificity, were studied.

3.4.1. Linearity range:

The fluorescence intensity of OMZ upon addition of AgNPs increased linearly by increasing drug concentration. Rectilinear range (**20.0 – 700.0**) ng/ml with perfect correlation coefficient of 0.9999 was obtained. The data of statistical analysis proving the linearity of the proposed method are summarized in **Table 1**

Both LOQ and LOD were determined according to ICH Q2R1 recommendations [40], where the values of LOQ and LOD are 14.0 ng/mL and 5.0 ng/mL, respectively(**Table 1**).

3.4.2. Accuracy and precision:

The accuracy of the proposed method was attained during comparing the outcomes of the analysis of OMZ samples with the results obtained upon using official USP method (HPLC method) [10] for both raw material and commercial dosage forms.

No considerable differences were observed concerning the accuracy and precision between the performance of the proposed and official USP methods using both Student t-test and variance ratio F-test [41] as illustrated in **Table 2**.

The precision of the proposed method is investigated through both intra-day and inter-day precisions.

The intra-day precision is tested through measuring three different concentrations of OMZ and every concentration was repeated three times. While measuring the same concentrations of OMZ on three consecutive days represented the inter-day precision. The precision of the method is confirmed through the low values of %RSD which (less than 2%) indicating good repeatability and reproducibility (**Table 3**).

3.4.3. Robustness:

The robustness was evaluated through testing the impact of minor changes influencing the fluorescence intensity during routine work. The impact of AgNPs volume ($0.1 \text{ mL} \pm 0.02$) has been studied with no significant effects on recovery percentages and % RSD values, (**Table 4**).

3.4.4. Selectivity:

Interference study:

The interference likely to be encountered from some commonly co-administrated and/or co-formulated drugs was studied. The tolerance limits of these compounds, namely; Xylometazoline hydrochloride, triamcinolone acetonide and cromolyn sodium, were determined. The high values of the obtained tolerance limits reveal the high selectivity of the proposed method. (**Table 5**).

3.5. Applications:

3.5.1. Analysis of OMZ in its dosage forms:

The proposed method was successfully applied to estimate OMZ in its nasal drops and nasal spray preparations. The high percentage recoveries obtained pointed out that no interference was encountered from the existing excipients. A perfect harmonization is achieved between the outcomes recorded in **(Table 6)** with those found via official USP method [10]. The results of statistical analysis obtained using both Student's t-test and variance ratio F-test [38] indicated no considerable differences between the proposed method and the USP method performance.

3.5.2. Content uniformity testing:

Due to remarkable sensitivity of the proposed method, it was exploited for content uniformity testing. The fluorescence intensity of Oxymet[®] nasal drops was determined according to USP guidelines [35]. [39] Dosage uniformity requirements are met as the acceptance value (AV) of the randomly selected 10 preparations is less than to L1 (L1 =15%), as illustrated in **(Table 7)**.

3.5.3. Analysis of OMZ in aqueous humor:

The high sensitivity of the proposed method allowed the determination of OMZ in aqueous humor. The results revealed that the mean absolute recoveries and the SD of OMZ in aqueous humor is 100.05 ± 1.06 **(Table 8)**.

4. Conclusion:

AgNPs are one of metal nanoparticles, that are chemically synthesized using reducing agent (sodium borohydride), stabilizing agent (PVP) and a precursor (AgNO_3). The PVP coated AgNPs caused significant enhancement of OMZ fluorescence. There is no encountered interference from other drugs and ingredients during determination of OMZ in dosage forms or aqueous humor.

Declarations

Funding

No funding was received for conducting this study.

Conflict of Interest

The authors declare that they have no conflict of interest.

Availability of data and materials

All the data and the materials are available all-over the study.

Code Availability

Not applicable.

CRediT authorship contribution statement

Shymaa M. Abd Elhaleem: Methodology, Formal analysis, Validation, Investigation, Writing - original draft.

Fawzi Elsebaei: Validation, Writing - Review & Editing, Supervision.

Shereen Shalan: Validation, Writing - Review & Editing, Supervision.

Fathalla Belal: Conceptualization, Validation, Writing - Review & Editing, Resources, Supervision.

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Tables

Due to technical limitations, table 1-8 is only available as a download in the Supplemental Files section.

Figures

Fig.1

Figure 1

Chemical structure of oxymetazoline hydrochloride.

Fig.2

Figure 2

Excitation and emission spectra of OMZ (500 ng/mL) in ethanol(a,a').

Fig.3

Figure 3

Emission spectra of OMZ in ethanol. Where: (a): OMZ emission peak at concentration (500 ng/mL). (b): OMZ – AgNPs emission peak when the concentration of OMZ is (500 ng/mL) using 0.1 mL of AgNPs (6×10^{-5} M).

Fig.4

Figure 4

Emission spectra for increasing concentrations of OMZ in ethanol during using 0.1 mL of AgNPs (6×10^{-5} M), where (a-h) are (20.0,30.0, 50.0,70.0, 100.0, 300.0, 500.0, 700.0 ng/mL OMZ).

Fig.5

Figure 5

UV-visible spectrum of the prepared AgNPs (6×10^{-5} M).

Fig.6

Figure 6

TEM images of the prepared AgNPs (6×10^{-5} M).

Fig.7

Figure 7

Effect of volume of AgNPs (6×10^{-5} M) on fluorescence intensity of OMZ (500 ng/mL).

Fig.8

Figure 8

Effect of diluting solvents on the fluorescence intensity of OMZ (500 ng/mL) using 0.1 mL AgNPs (6×10^{-5} M).

Supplementary Files

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