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Silver nanoparticle probe for fast colorimetric determination of Tobramycin in pharmaceuticals with greenness assessment.

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KEYWORDS

Factorial design, Silver nanoparticle, Colorimetry, Tobramycin, GAPI.

SHORT SUMMARY

A fast eco-friendly spectrophotometric method is developed for determination of a non-UV absorbing drug, Tobramycin sulphate, in its bulk powder, pharmaceutical dosage form and spiked human plasma using PVP-capped silver nanoparticles as a colorimetric probe. This method is based on measuring the decrease in the absorbance spectrum of silver nanoparticles at 415 nm by increasing the concentration of Tobramycin sulphate. The absorbance quenching is due to the formation of hydrogen bonds between the PVP-capped silver nanoparticle and the drug, resulting in an aggregation which leads to color change and could be measured quantitatively. Different factors affecting the spectrophotometric determination of Tobramycin sulphate as; silver nanoparticle concentration, pH, buffer type and reaction time were studied and optimized using full factorial design, in which the predicted model fits well to the experimental data as proved by ANOVA results. Validation of the proposed method was performed according to ICH guidelines and was found to be within the acceptable ranges. The proposed method was successfully applied for determination of Tobramycin sulphate in its pharmaceutical dosage form; Tobrin ophthalmic Solution®, Tobrin ophthalmic ointment® and spiked human plasma with high accuracy and selectivity.

EXTENDED ABSTRACT

Since COVID-19 pandemic, it was found that the viral pneumonia caused by COVID-19 can cause secondary bacterial infection which can be treated by various antibiotics including aminoglycosides, to which Tobramycin belongs[1]. Moreover, it was found that COVID-19 patients had symptoms of conjunctivitis, which can be treated with Tobramycin [2]. It was found that upon TOBRA administration as ophthalmic eye drops, some systemic absorption occurs, and the mean peak serum level reaches Cmax =5 µg/mL Therefore, it was an urgent need to find a fast and sensitive method for quantification of TOBRA in human plasma. Some Spectroscopic[3,4] and chromatographic methods[5,6] have been reported for the determination of Tobramycin using various derivitizing agents but these methods have disadvantages of being tedious and time-consuming due to derivatization steps. In this study, we employed a simple, quick green spectrophotometric method for the determination of tobramycin using PVP-capped silver nanoparticles (PVP-AgNPs). (PVP-AgNPs) have particle size between(1-100nm.)[7],unique optical, electronic, antibacterial properties and are widely employed in bio-sensing, photonics and in antimicrobial applications. Moreover, they exhibit a yellowish brown color which turns to orange upon drug interaction due to hydrogen bonds formation and aggregation of PVP-AgNPs[8].Recently, many methods using AgNPs have been reported for the determination of drugs[9,10].The principle of our proposed method depended on that the absorbance of the PVP-AgNPs is decreased upon the addition of Tobramycin, due to the aggregation with the drug and hence, color change occurred from yellowish brown to orange which could be detected by the naked eye and is equivalent to the drug concentration. The full factorial

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experimental design was conducted to choose the best experimental conditions to achieve maximum absorbance difference and hence the maximum method sensitivity and accuracy. The studied factors were silver nanoparticle concentration, pH, buffer type , keeping the reaction time constant as shown in **Table 1**. Silver nanoparticles concentration was found to be the most significant factor. The optimized method was validated according to ICH guidelines and was found to be linear in the concentration range of (0.35-4.00 µg/mL) as shown in **Figure 1**, accurate with mean recovery of (99.51%±0.950) and precise with mean %RSD equals 0.871. LOD and LOQ values were found to 0.08 µg/mL and 0.24 µg/mL, respectively. The proposed method was applied for the determination of Tobramycin in different pharmaceutical formulations as eye drop , eye ointment and spiked human plasma showing good recoveries of (100.74%±1.282), (99.17%±1.258) and (101.67%±0.577) respectively. Additionally, the green character of our developed method was evaluated by the recent tool; Green Analytical Procedure Index (GAPI).GAPI tool has the advantage of assessing the green aspect of the whole analytical method, starting from sample collection till reaching the final determination; it is also helpful in comparing procedures[11]. The proposed method shows good green character as the green color appears in many pentagrams as shown in **Figure 2.**

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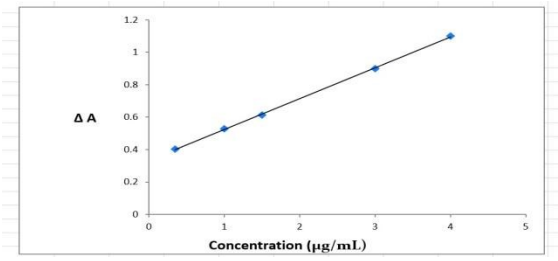

Figure 1 Calibration curve of ΔA versus the concentration of Tobramycin sulphate (0.35- 4.00 µg/mL) using PVP-AgNPs.

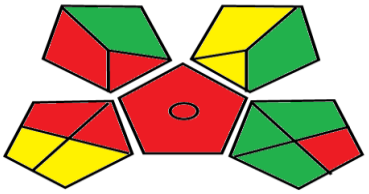

Figure 2 GAPI assessment of the green profile for the proposed spectrophotometric methods.

Table 1 The three variables at two levels that were chosen for optimization of conditions by quality by design.

Variable	Level (-1)	Level (+1)
Factor (A) = pH	7	9
Factor (B) = Buffer Type	Carbonate buffer	Britton-Robinson buffer
Factor (C) =PVP-AgNPs concentration	53.93 µg/mL	215.74 µg/mL

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