



INDIRECT SPECTROPHOTOMETRIC DETERMINATION OF NEOMYCIN BASED ON THE REACTION WITH CERIUM (IV) SULFATE

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abstract: A simple and sensitive spectrophotometric method has been described for the assay of neomycin either in pure form or in pharmaceutical formulations. The proposed method was based on the oxidation of the studied drug by a known excess of cerium (IV) sulfate in acidic medium and estimating the unreacted oxidizing agent by adding a known amount of methyl orange in the same acid medium and measuring the absorbance of the resulted solution at $\lambda_{\text{max}}=498$ nm. Different variables affecting the reaction were studied and optimized. Beer's law is obeyed in the concentration range of $1 \cdot 10^{-6}$ and $1.3 \cdot 10^{-5}$ M and the detection limit of the method was $4.7 \cdot 10^{-7}$ M neomycin. The proposed method was applied successfully to the determination of the examined drug either in a pure or pharmaceutical dosage forms with good precision. No interferences were observed from excipients and the results obtained were in good agreement with those obtained using the official method.

key words: neomycin; spectrophotometry; cerium(IV) sulfate; redox reaction; pharmaceutical formulations.

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1. Introduction

Neomycin [(1R,2R,3S,4R,6S)-4,6-diamino-2- {[3-O-(2,6-diamino-2,6-dideoxy- β -L-idopyranosyl)- β -D-ribofuranosyl]oxy}-3-hydroxycyclohexyl 2,6-diamino-2,6-dideoxy- α -D-glucopyranoside] (Fig. 1) (NOM), is a broad spectrum aminoglycoside antibiotic widely used in clinical therapy of serious infections. It inhibits the growth of both gram-positive and gram-negative bacteria.

Many analytical techniques have been employed for the detection and determination of neomycin and other aminoglycoside antibiotics including potentiometry [1] voltammetry, [2÷6], amperometry [7], fluorometry [8÷12], electrophoresis [13÷16], spectrophotometry [17÷20], chromatography [21÷ 27], and immunoanalysis [28].

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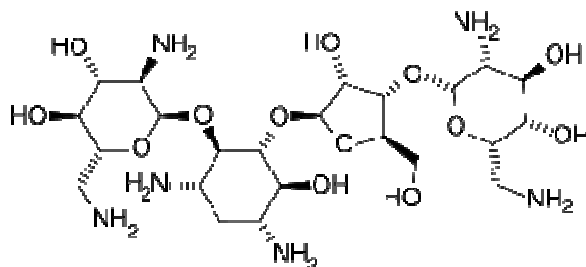


Fig. 1 Molecular formula of neomycin (NOM).

To the best of our knowledge, there is no work in the literature reporting about the application of Ce(IV) for the determination of neomycin. Having in mind the presence of amino and hydroxyl groups in the neomycin molecule and the recent work of El-Didamony et al. [19] describing neomycin indirect assay by oxidation with permanganate as well as the previous use of Ce(IV) as oxidant for indirect determination of other antibiotics [29] we have investigated the possibility of neomycin indirect analysis by oxidation with Ce (IV) sulfate. Thus, the present paper describes a UV-VIS spectrophotometric method for the determination of the cited drug in bulk form and in commercial pharmaceutical formulations. The method is an indirect procedure, involving the addition of an excess of Ce(IV) in acidic media and the determination of the unreacted oxidant by measuring the absorbance decrease of the dye methyl orange (MO).

2. Experimental

2.1. Chemicals

All chemicals used were of analytical reagent grade and double distilled water was used to prepare all solutions.

Stock solution of cerium (IV) sulfate (Sigma-Aldrich) 4×10^{-2} M in 1 M H_2SO_4 (Sigma-Aldrich) was used to prepare the working solution of 2×10^{-3} M $\text{Ce}(\text{SO}_4)_2$ in H_2SO_4 1 M by proper dilution with H_2SO_4 1 M.

The 500 ppm methyl orange (ACS reagent Sigma-Aldrich 85%) (MO) solution was prepared in double distilled water.

A 5 M sulfuric acid stock solution was prepared in a 1 L volumetric flask by adding the appropriate volume of concentrated sulfuric acid (95-98%, density 1.840 g/mL, Sigma – Aldrich) to double distilled water and diluting to the mark. The 1 M sulfuric acid solution was obtained by proper dilution with double distilled water of this stock solution.

Stock solution of 2.5×10^{-3} M neomycin (NOM) was prepared by dissolving neomycin sulfate (Sigma-Aldrich) in double distilled water and the working solution of 10^{-4} M neomycin was prepared by suitable dilution of this stock solution, with double distilled water.

2.2. Apparatus

All absorbance measurements were carried out with a JASCO (Japan) spectrophotometer model V-530 equipped with matched 1-cm quartz cells.

2.3. Procedure

Different aliquotes (0.25-5 mL) of 10^{-4} M neomycin solution have been pipetted in 25 mL volumetric flasks and diluted with double distilled water to a volume of 10 mL. Then, 2.5 mL H_2SO_4 5 M and 1.5 mL of Ce(IV) sulfate 2×10^{-3} M have been added to each volumetric flask. The flasks were shaken for the solutions homogenisation and then they were let at darkness a well determined time (15 minutes) to assure a complete oxidation of the drug. Subsequently, 1.5 mL of 500 ppm methyl orange solution was added to each volumetric flask and diluted to the mark with double distilled water. The UV-VIS spectrum of each solution was recorded in the range 600-350 nm against a double distilled water blank. The absorbance of each solution was then measured at the wavelength corresponding to the absorption maximum, i.e. $\lambda_{\text{max}}=498$ nm.

Twenty tablets were weighed and ground into a powder. An accurate weight equivalent to the preparation of 100 mL of 2.5×10^{-3} M neomycin stock solution was dissolved in 50 mL double distilled water and then filtered. The filtrate was diluted with double distilled water in 100 mL calibrated flask. This solution was further diluted to a 10^{-4} M neomycin solution with double distilled water and analyzed by the recommended procedure.

An appropriate volume of the pharmaceutical drop preparation was diluted with double distilled water to obtain 100 mL solution of 10^{-4} M neomycin and then analyzed by the developed method.

3. Results and discussion

The proposed method is an indirect spectrophotometric one based on the ability of cerium (IV) sulfate to oxidize neomycin and to bleach the colour of the indicator methyl orange. Neomycin is let to react with a known excess of cerium (IV) sulfate in acidic media. The unreacted Ce(IV) found in excess over neomycin is quantified by adding methyl orange and monitoring the absorbance of the solution at $\lambda_{\text{max}}=498$ nm. In the presence of the oxidant the absorbance of the indicator solution decreases. Assuring that the oxidation reaction is complete, the unreacted cerium (IV) amount is proportional to the drug amount in the sample. According to scheme 1, with increased drug concentration in the sample, the amount of unreacted Ce(IV) bleaching the dye is less and thus the solution absorbance measured at $\lambda_{\text{max}}=498$ nm increases linearly with neomycin concentration (Fig. 2).



measured spectrophotometrically at $\lambda_{\text{max}}=498$ nm

Scheme 1 Reaction scheme of the indirect determination of neomycin by oxidation with Ce(IV) sulfate.

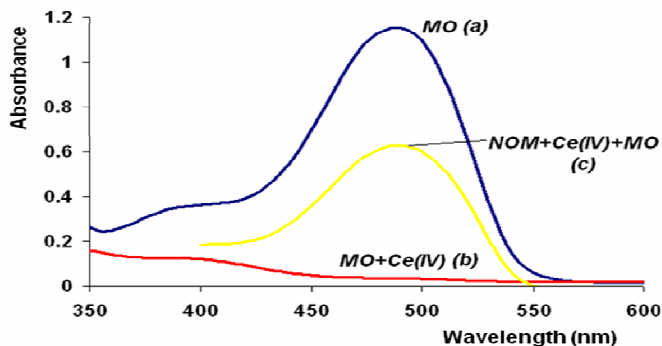


Fig. 2 Absorption spectra of solutions containing 0.5 M H_2SO_4 and

(a) 30 ppm methyl orange (MO); (b) (a) + $1.2 \cdot 10^{-5}$ M Ce(IV) sulfate and (c) (b) + $7 \cdot 10^{-6}$ M neomycin (NOM).

3.1. Method optimisation

a) Selection of methyl orange concentration. The absorbance variation at $\lambda_{\max}=498$ nm with methyl orange concentration was investigated in the range 10-90 ppm MO. The linear range was 10-50 ppm MO. In order to minimise the photometric error for further investigations a concentration of 30 ppm MO was selected which presented an absorbance of 1.154.

b) Optimisation of Ce(IV) sulfate concentration. The absorbances of solutions containing a fixed concentration of MO (30 ppm) and different concentrations of Ce(IV) sulfate comprised in the range $8 \cdot 10^{-6} \div 1.6 \cdot 10^{-4}$ M Ce(IV) in 0.5 M H_2SO_4 have been measured and it was observed that a concentration of Ce(IV) higher than $1.2 \cdot 10^{-4}$ M $Ce(SO_4)_2$ bleaches completely the solution containing 30 ppm MO (Fig. 3).

c) Influence of the order of reagents addition. It was observed that regardless of neomycin amount added, methyl orange is almost totally bleached if the reagents addition order was dye+ drug+ oxidant or dye+oxidant+drug. The reason of this observation is that the Ce(IV) sulfate didn't have enough time to oxidise the drug because it rapidly bleaches methyl orange. In conclusion, the drug and oxidant solutions respectively must be added first, their addition order doesn't influence the reaction and methyl orange have to be added after a given period of time during which the drug is totally oxidized by $Ce(SO_4)_2$.

d) The influence of time on the oxidation reaction of neomycin by $Ce(SO_4)_2$. It was observed that if methyl orange is added immediatly to the solution containing neomycin and Ce(IV) sulfate in acidic medium the resulted solution is bleached rapidly and the absorbance is very low. This can be explained by the fact that the drug oxidation by cerium(IV) sulfate is a time developing reaction and thus the influence of the reaction time was studied. In this respect, solutions containing 10^{-5} M neomycin and $1.6 \cdot 10^{-4}$ M Ce(IV) sulfate have been let to react at darkness different times before adding the indicator and measuring the absorbance. It was observed that the absorbance of this solutions increases with the time up to 15 minutes remaining then constant (Fig. 4). Thus, for further measurements a reaction time of 15 minutes was selected.

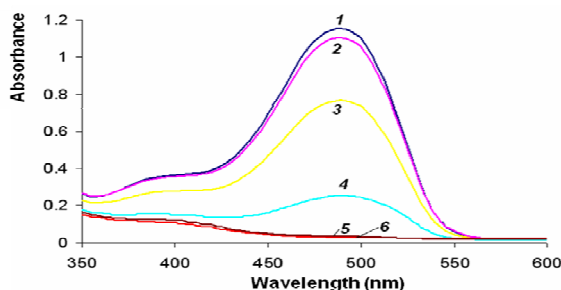


Fig. 3 The influence of the Ce(IV) sulfate concentration on the absorbance of a solution containing 30 ppm MO. Concentration of $\text{Ce(SO}_4)_2$ solution: 1 - 0; 2 - $0.8 \cdot 10^{-5}$ M; 3 - $4.0 \cdot 10^{-5}$ M; 4 - $8.0 \cdot 10^{-5}$ M; 5 - $1.2 \cdot 10^{-4}$ M; 6 - $1.6 \cdot 10^{-4}$ M.

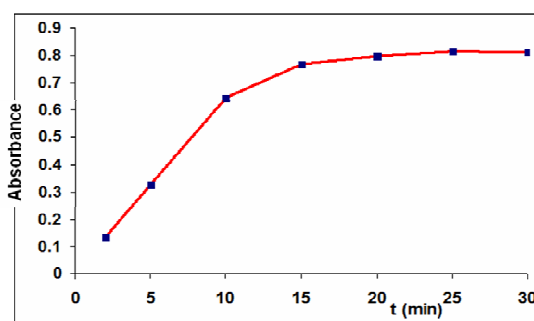


Fig. 4 Influence of time on the reaction of neomycin oxidation by $\text{Ce(SO}_4)_2$ in $0.5 \text{ M H}_2\text{SO}_4$.

e) Influence of neomycin concentration on the absorbance. Molecular absorption spectra have been recorded for solutions prepared as described previously and containing different concentrations of neomycin in the range $1 \cdot 10^{-6} \div 2 \cdot 10^{-5}$ M (Fig. 5).

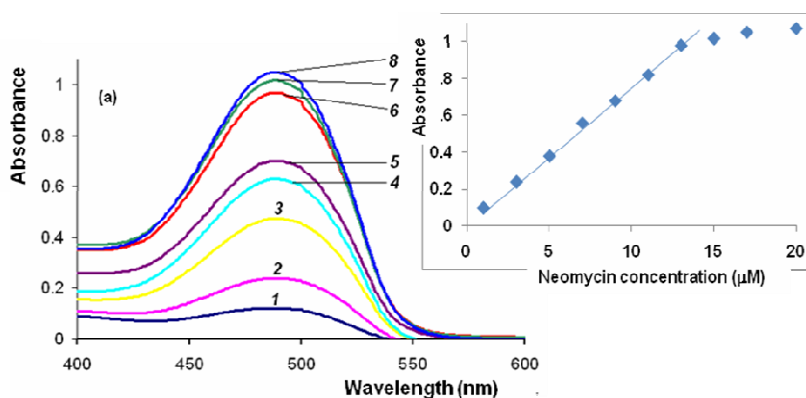


Fig. 5 (a) Spectra of solutions containing 30 ppm methyl orange (MO) added after 15 minutes to $1.2 \cdot 10^{-4}$ M $\text{Ce(SO}_4)_2$ in H_2SO_4 and different concentrations of neomycin: 1 - $1 \cdot 10^{-6}$ M; 2 - $3 \cdot 10^{-6}$ M; 3 - $5 \cdot 10^{-6}$ M; 4 - $7 \cdot 10^{-6}$ M; 5 - $9 \cdot 10^{-6}$ M; 6 - $13 \cdot 10^{-6}$ M; 7 - $15 \cdot 10^{-6}$ M and 8 - $20 \cdot 10^{-6}$ M and (b) the dependence of the absorbance of this solutions on neomycin concentration ($\lambda_{\text{max}} = 498 \text{ nm}$).

A linear dependence of the absorbance on drug concentration was observed in the range $1 \cdot 10^{-6} - 1.3 \cdot 10^{-5}$ M neomycin. The regression equation of the calibration graph, obtained by the least squares method was:

$$A = 0.0732C_{\text{NOM}} + 0.0246, r^2 = 0.9987 \quad (2)$$

where A=absorbance; C_{NOM} =neomycin concentration in μM . The molar absorptivity was determined to be $7.54 \cdot 10^4 \text{ mol L}^{-1} \text{ cm}^{-1}$.

f) Evaluation of methods performance characteristics. The detection limit (DL) calculated according to the 3σ criterion was $4.7 \cdot 10^{-7}$ M.

The RSD (%) was evaluated for five replicate analyses at three concentration levels ($3 \cdot 10^{-6}$, $7 \cdot 10^{-6}$ and $1 \cdot 10^{-5}$ M) of neomycin and the obtained values were less than 2 % indicating a good precision of the proposed method.

g) Analytical applications. The proposed method was applied to neomycin determination in commercial drops and tablets. Neomycin content in Afacort drops with the declared amounts of 0.83 mg dexamethasone (1.09 mg dexamethasone sodium phosphate)/mL and neomycin 3.5 mg (5.8 mg neomycin sulfate)/mL and in Negamicin (neomycinum) tablets with 500 mg neomycin/tablet has been analyzed by the above described indirect spectrophotometric method and the results obtained are listed in table 1. No interference was observed from dexamethasone in the drops and neither from the common tablet excipients in Negamicin. The results obtained with the above described method were in good agreement with those obtained by an official method [30].

Table 1 Assay results of commercial preparations containing neomycin.

Pharmaceutical dosage form	Supplier	Declared content of neomycin	% found $\pm$ SD ^(a)	
			Proposed method	Official method [30]
Afacort drops	Anfarm Hellas (Greece)	3.5 mg/mL	101.21 $\pm$ 1.57	99.84 $\pm$ 0.83
Negamicin (neomycinum)	Antibiotice Iasi (Romania)	50 mg/tablet	99.76 $\pm$ 1.23	99.95 $\pm$ 1.39

^(a) Average of five determinations

4. Conclusions

An indirect spectrophotometric procedure has been developed for the determination of the antibiotic neomycin, relying on the ability of cerium (IV) sulfate to oxidize quantitatively both neomycin and methyl orange in strongly acidic medium. A known, excedentary amount of cerium (IV) sulfate is added first over the sample solution. After completing the neomycin oxidation, a suitable amount of methyl orange is added and the absorbance of the final solution is then measured at 498 nm. Advantageously, the employed analytical signal is directly proportional with the neomycin content in the sample.

The experimental parameters of the method have been optimized, a critical importance presenting the order of reagents addition and the time of neomycin oxidation. Because these parameters are easy to control, the performance characteristics of the method are satisfactory and the method can be applied in the analysis of some pharmaceutical formulations without the interference of the excipients, as we tested on two products.

The described procedure is reliable, very simple and conveniently applicable in most laboratories due to the accessibility of the reagents employed and to the time of analysis reasonably low.

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