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# Development of spectrofluorimetric method for determination of certain aminoglycoside drugs in dosage forms and human plasma through condensation with ninhydrin and phenyl acetaldehyde

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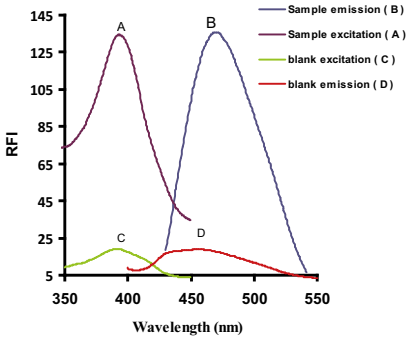
## HIGHLIGHTS

- Development of new spectrofluorimetric method for determination of studied drugs.
- The method is based on reaction of the drugs with ninhydrin and phenylacetaldehyde.
- Validation of the proposed method.
- Application of the proposed method on the analysis of different dosage forms.
- Application of the proposed method on the analysis of amikacin in human plasma.

## GRAPHICAL ABSTRACT

Excitation ($\lambda_{max}$ = 395 nm) and emission ($\lambda_{max}$ = 470 nm) spectra of reaction product of tobramycin (2 μg ml$^{-1}$) with ninhydrin and phenylacetaldehyde.

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## ABSTRACT

A simple and sensitive spectrofluorimetric method has been developed and validated for determination of amikacin sulfate, neomycin sulfate and tobramycin in pure forms, pharmaceutical formulations and human plasma. The method was based on condensation reaction of cited drugs with ninhydrin and phenylacetaldehyde in buffered medium (pH 6) resulting in formation of fluorescent products which exhibit excitation and emission maxima at 395 and 470 nm, respectively. The different experimental parameters affecting the development and stability of the reaction products were carefully studied and optimized. The calibration plots were constructed with good correlation coefficients (0.9993 for tobramycin and 0.9996 for both neomycin and amikacin). The proposed method was successfully applied for the analysis of cited drugs in dosage forms with high accuracy (98.33–101.7) ± (0.80–1.26)%. The results show an excellent agreement with the reference method, indicating no significant difference in accuracy and precision. Due to its high sensitivity, the proposed method was applied successfully for determination of amikacin in real human plasma.



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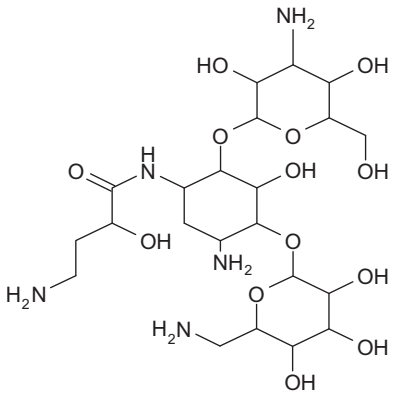
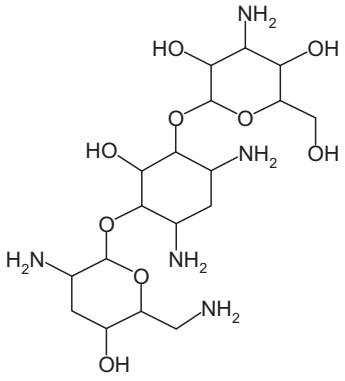
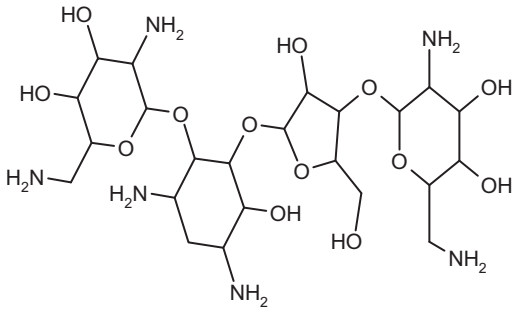
## Introduction

Amikacin, tobramycin and neomycin are aminoglycoside antibiotics that are widely used against both gram-negative and gram-positive bacterial infections in human and veterinary medicine. They have narrow therapeutic ranges and monitoring of these drugs is required to reduce serious side effects such as nephro and ototoxicity [1–3]. Dosage alterations based on the results of drug monitoring have been found to prevent toxicity and ensure efficacy [4].

Different analytical methods have been developed for determination of aminoglycosides in pharmaceutical formulations and biological fluids. These methods include chromatographic [5–17], spectrofluorimetric [18–24], spectrophotometric [25–30], polarographic [31], capillary electrophoresis [32] and immunoassay methods [33,34].

The purpose of this study was to develop a simple, rapid, sensitive, applicable and low cost method for determination of these drugs in pure forms, pharmaceutical formulations, spiked and real human plasma through condensation reaction with ninhydrin and phenylacetaldehyde depending on the presence of a primary amine moiety in the chemical structures of studied drugs, (Table 1).

**Table 1**
Chemical Structures of studied aminoglycoside drugs.

| Name | Structure |
|---|---|
| Amikacin |  |
| Tobramycin |  |
| Neomycin |  |

## Experimental

### Apparatus

Spectronic™ Genesys™ 2PC UV/VIS spectrophotometer (Milton Roy Co, USA) with matched 1 cm quartz cell connected to IBM computer loaded with winspec™ application software, A Perkin Elmer LS 45 Luminescence spectrometer (UK) connected to an IBM PC computer loaded with the FL WINLAB™ software, laboratory centrifuge 4000 c/s (Bremsen ECCO, Germany) and MLW Milwakee SM 101 pH meter (Portugal), were used in this work.

### Materials and reagents

All materials used in this work were of analytical grade. Aminoglycosides pure drugs were generously supplied by their respective manufacturers and were used without further purification; **Amikacin sulfate**, **and Tobramycin** were kindly provided by Egyptian International Pharmaceutical Industries Co (E.I.P.I.CO., Egypt). **Neomycin sulfate** was kindly provided by Memphis Co for Pharmaceutical & Chemical Industries, Egypt. **Ninhydrin** (Sigma–Aldrich Chemie GmbH, Germany) was freshly prepared daily as 0.1% (w/v) in distilled water. **Phenylacetaldehyde** (Sigma–Aldrich Chemie GmbH, Germany) was prepared as 0.02% (v/v) in ethanol and the solution was stable at least one week at 4 °C. All other chemicals such as ethanol, methanol, acetonitrile, acetone, dimethylformamide, phosphoric acid, citric acid, sodium hydroxide and hydrochloric acid were obtained from El Nasr chemical Co, Egypt.

### Pharmaceutical formulations

The following available pharmaceutical preparations were analyzed; Amikin® vials (GlaxoSmithKline, Egypt) labeled to contain 100 mg amikacin sulfate in 2 ml aqueous solution, Neomycin® tablets (Memphis Co for Pharmaceutical & Chemical Industries, Egypt), labeled to contain 500 mg Neomycin sulfate per tablet, Tobrin® eye drops and Tobrin® eye ointments (Egyptian International Pharmaceutical Industries Co; E.I.P.I.CO., Egypt), labeled to contain 0.3% w/v and 0.3% w/w tobramycin, respectively. Tobradex® eye drops (Alcon-Couvreur), labeled to contain 0.3% w/v tobramycin and 0.1% w/v dexamethasone.

### Stock standard solutions

A stock solution of each drug (0.5 mg $ml^{-1}$) was prepared using distilled water. Further dilutions were made to obtain working standard solutions in the concentration range of 5–80 μg $ml^{-1}$.

### Buffer solutions

Teorell and Stenhagen buffer solutions [35] of pH range 5.0–8.5 were prepared in distilled water. The buffer is composed of phosphoric acid, citric acid and 1 M sodium hydroxide, and it was adjusted to the required pH with 0.1 M hydrochloric acid.

### General analytical procedure

One milliliter of Torell and Stenhagen buffer solution (pH 6.0) was transferred into test tube and then 0.6 ml of ninhydrin reagent (0.1%, w/v in water), 1.0 ml of the standard solution of drugs in the concentration range of 5–80 μg $ml^{-1}$ and 0.6 ml of phenylacetaldehyde reagent (0.02%, v/v in ethanol) were added. The reaction was allowed to proceed for 10 min in water bath at 80–85 °C, 70–75 °C and 70–80 °C for amikacin, neomycin and tobramycin,