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Label-free gold nanoparticles for the determination of neomycin

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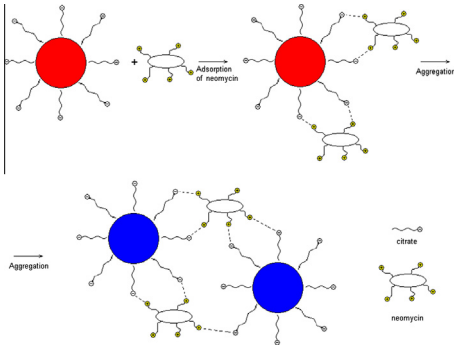
HIGHLIGHTS

- Determination of neomycin using label-free gold nanoparticles.
- Erythromycin, ampicillin, oxytetracycline and sulfamethazine did not affect.
- The bigger concentration of EDTA, the lower amounts of neomycin cause aggregation.
- Limit of detection 28 ng mL^{-1}.
- The method was applied to the determination of neomycin in eye- and ear-drops.

GRAPHICAL ABSTRACT



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ABSTRACT

A new spectrophotometric method for the determination of neomycin has been developed. The method is based on aggregation of label-free gold nanoparticles leading to change in absorption spectra and color of the solution. Influence of different factors (the concentration of ethylenediaminetetraacetate (EDTA), pH, the concentrations of neomycin and the nanoparticles) on the aggregation and analytical performance of the method was investigated. EDTA plays an important role not only as a masking agent to eliminate interferences of metal cations but strongly affects the sensitivity of the nanoparticles relative to neomycin. The method allows to determine neomycin with detection limit of 28 ng mL^{-1}. It was applied to analysis of eye- and ear-drops. The sample pretreatment is simply done by diluting the formulation with water.

Introduction

Neomycin belongs to a chemical class of aminoglycoside antibiotics produced from actinomycete *Streptomyces fradiae*. It is often used in a variety of antibiotic pharmaceutical products. This antibiotic poseses good antibacterial activity with respect to gram-negative and gram-positive bacteria. However, aminoglycosides (and especially neomycin) cause some toxic effect such as oto- and nephrotoxicity. Uncontrolled using aminoglycoside antibiotics in medicine and veterinary for infective diseases treatment results in their accumulation in different objects and may be dangerous for people's health. Therefore, it is important to develop sensitive, selective, simple and cost-effective methods for the determination of neomycin [1].

Different analytical methods for the determination of neomycin have been described such as HPLC [2–10], capillary electrophoresis [11,12], electrochemical methods [13], immuno assays [14,15]. Neomycin has no absorption ability in UV–vis range, and most determinations have to use different kinds of derivatization or indirect approach [11]. These procedures often are either complex and expansive or time-consuming. Only one work found deals with direct UV-detection of neomycin at 200 nm in capillary electrophoresis [12]. The same reason restrains a development of sensitive

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and simple spectrophotometric methods for the determination of neomycin. Only one spectrophotometric procedure of neomycin determination was found in the literature of the last years [16]. This method is based on ion-association of neomycin with chromotropic acid azo-derivatives.

Gold nanoparticles have been suggested recently as peculiar chromogenic reagents [17–19]. Their optical properties are conditioned by surface plasmon resonance (SPR) that appears as an intense absorbance band in the visible range [20]. Its position strongly depends on aggregative state of the nanoparticles. The change in color during aggregation process is contrasting and can be used for detection of an analyte. This optical phenomenon has been already used for the determination of different metal ions [21], anions [22–26] and organic compounds [27–33].

The aim of this work was to develop a simple and cost effective spectrophotometric method for the determination of neomycin using label-free gold nanoparticles.

Material and methods

Materials

Chemically pure hydrogen tetrachloroaurate, sodium citrate and analytical grade neomycin sulfate, erythromycin, ampicillin, oxytetracycline, sulfamethazine, ethylenediaminetetraacetate disodium salt (EDTA), sodium hydroxide and hydrochloric acid were used. The substances stock solutions were prepared by dissolving their weighted portions in deionized water; stock 0.25 mol L^{-1} solution of EDTA was prepared by dissolving the weighted portion of EDTA in 0.25 mol L^{-1} NaOH.

Instrumentation

Absorption spectra of solutions were recorded by SF-103 spectrophotometer (Akvilon, Russia), pH was measured by Ekspert 001 ion meter (Ekoniks, Russia). TEM-images of the samples were recorded using transmission electron microscope LEO912 AB OMEGA (Carl Zeiss, Germany). Chromatographic determination of neomycin in eye- and ear-drops was performed using the PerkinElmer Series 200 system with a refractometric detector on Thermo Scientific Hypersil Gold column (50 × 4.6); the sample volume was 5 μL; eluent–water solution containing 260 mmol L^{-1} trifluoroacetic acid and 75 mmol L^{-1} sodium hydroxide; flow rate was equal to 0.7 mL min^{-1}.

Synthesis of label-free nanoparticles

Label-free nanoparticles (NPs) were prepared by reducing metal salt precursor (hydrogen tetrachloroaurate, $HAuCl_4$) in a liquid phase by citrate according to the Frens method [34] with a slight modification.

Briefly, 1 mL of 1% $HAuCl_4$ was introduced in 250 mL bulb, diluted with 100 mL of deionized water and heated until boiling. 1.4 mL of 1% sodium citrate was added to the hot solution at stirring. The solution was boiled in 5 min till stable ruby color. The mixture was cooled at stirring and kept in the dark for 24 h to complete stabilization and re-crystallization of NPs. The concentration of NPs in the final solution was 70 μg mL^{-1} (0.35 mM in terms of gold).

It has been shown in the previous work [35] that gold NPs obtained as described above have surface plasmon resonance band at 520–530 nm in the absorption spectra of their water solutions. Nanoparticles of 18–26 nm predominate in the solution, the average diameter of NPs being 23 nm.

Determination of neomycin

To construct a calibration curve, 0–25 ng of neomycin were introduced in a test-tube and diluted with deionized water up to 3.9 mL. Then 0.1 mL of 0.25 mol L^{-1} EDTA and 1 mL of NPs solutions were added successively. The final volume was 5 mL. Absorption spectra were recorded after 3 min of the reaction. The calibration curve was constructed as A_{700}/A_{520} ratio versus the concentration of neomycin.

Results and discussion

The label-free gold NPs are negatively charged in the solution due to the dissociation of citrate on their surface. This charge results in electrostatic repulsion of the particles that prevents their aggregation. Therefore, some positively charged species decreasing the charge of NPs would decrease repulsion of the NPs and can cause aggregation. We investigated the influence of antibacterials representatives of different classes on the NPs. There were the representatives of aminoglycosides (neomycin), macrolides (erythromycin), penicillins (ampicillin), tetracyclines (oxytetracycline) and sulfanilamides (sulfamethazine). Addition of different antibiotics showed that only neomycin caused aggregation of NPs, being already at the concentration of 40 ng mL^{-1} (Fig. 1). Erythromycin, ampicillin, oxytetracycline and sulfamethazine did not remarkably affect NPs aggregative state even at concentration higher 140 ng mL^{-1}. Aggregation resulted in appearing the absorbance band of NPs aggregates at 700 nm and decreasing the band of single NPs at 520 nm (Fig. 1). The color of the solution was changed from ruby to blue. TEM-images of the solution after addition of neomycin showed the presence of NPs aggregates of different shape and size. Several aggregates and their parts had prominent chain shape (Fig. S1).

This effect can be ascribed to different charges of the substances owing to their different ionic state under the experimental

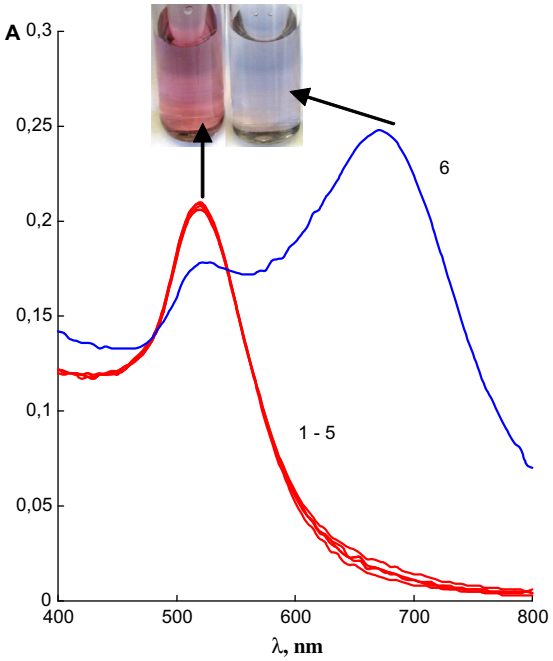

Fig. 1. Absorption spectra of label-free gold NPs water solutions in the absence of antibiotics (1) and in the presence of erythromycin (400 ng mL^{-1}), ampicillin (190 ng mL^{-1}), oxytetracycline (250 ng mL^{-1}), sulfamethazine (140 ng mL^{-1}) (2–5) and neomycin (40 ng mL^{-1}). c_{NPs} = 0.07 mmol L^{-1} Au, c_{EDTA} = 5 mmol L^{-1}, pH 7.5.