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Original Research Article

Adsorptive Removal and Solid Phase Microextraction of Neomycin Antibiotic by Graphene Oxide

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ABSTRACT

This study explored the use of graphene oxide (GO) as an efficient adsorbent for the removal and preconcentration of the antibiotic neomycin. Key parameters such as solution pH, adsorbent dosage, contact time, initial concentration, eluent solvent type, and eluent volume were optimized using a one-factor-at-a-time approach. Under optimal conditions, the method achieved a removal efficiency of 95%, and the calibration curve exhibited a linear response within a neomycin concentration range of 0.1-1 ppm. The limits of detection (LOD) and quantification (LOQ) were determined to be 0.033 ppm and 0.099 ppm, respectively. The method demonstrated high selectivity, with no significant interference observed from other substances. Finally, the developed approach was successfully applied to analyze neomycin in three different simulated industrial effluents, highlighting its practical applicability and effectiveness in real samples.

Keywords: **Graphene oxide, Neomycin, Adsorption, Removal, Preconentration**

Introduction

The presence of pharmaceutical contaminants in aquatic environments has emerged as one of the most pressing environmental challenges of the 21st century [1]. Among these contaminants, antibiotics have garnered significant attention due to their widespread use, persistence in the

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environment, and potential to contribute to the development of antibiotic-resistant bacteria [2]. Neomycin, a broad-spectrum aminoglycoside antibiotic, is commonly employed in both human and veterinary medicine for its efficacy in treating bacterial infections. However, its extensive application has led to its frequent detection in surface water, groundwater, and wastewater treatment plant effluents [3]. The environmental persistence of neomycin, combined with its potential ecotoxicological effects, underscores the urgent need for effective and sustainable removal strategies [4]. Conventional wastewater treatment processes often fall short in adequately removing pharmaceutical contaminants, including neomycin. Advanced oxidation processes, membrane filtration, and biological treatments have been explored to address this issue, but these methods are often associated with high operational costs, energy consumption, and secondary pollution. Adsorption-based techniques have emerged as a promising alternative due to their simplicity, cost-effectiveness, and ability to achieve high removal efficiencies. Among the various adsorbents investigated, graphene oxide (GO) has gained significant attention as a versatile material for environmental remediation [5].

Graphene oxide, a two-dimensional derivative of graphene, is characterized by its unique physicochemical properties, including a large specific surface area, high mechanical strength, and an abundance of oxygen-containing functional groups such as hydroxyl, epoxy, and carboxyl groups [6]. These functional groups not only enhance the hydrophilicity of GO but also provide active sites for the adsorption of a wide range of contaminants, including heavy metals, dyes, and pharmaceuticals. Moreover, the tunability of GO's surface chemistry allows for the design of tailored adsorbents with enhanced selectivity and adsorption capacity for specific target compounds [7]. In the context of neomycin removal, GO holds great promise as an efficient adsorbent due to its ability to interact with the antibiotic through hydrogen bonding, electrostatic interactions, and π-π stacking [8]. In addition to its potential as an adsorbent for contaminant removal, graphene oxide has also been explored as a sorbent material for solid-phase microextraction (SPME). SPME is a powerful sample preparation technique that combines extraction and preconcentration into a single step, making it particularly advantageous for the analysis of trace contaminants in complex matrices [9]. The integration of GO into SPME devices has been shown to enhance extraction efficiency and selectivity due to its high adsorption capacity and favorable interaction mechanisms with target analytes [10]. This dual

functionality of GO—as both an adsorbent for removal processes and a sorbent for analytical applications—positions it as a highly versatile material for addressing the challenges associated with neomycin contamination [11]. Despite the growing body of research on graphene oxide-based materials for environmental applications, there remains a need for comprehensive studies that evaluate their performance in real-world scenarios. Factors such as pH, ionic strength, competing ions, and natural organic matter can significantly influence the adsorption behavior of GO and must be systematically investigated to ensure its practical applicability. Furthermore, while the use of GO in SPME offers significant advantages for analytical purposes, its integration into field-deployable devices and its compatibility with various analytical techniques require further exploration [12-15]. This paper aims to address these gaps by investigating the adsorptive removal and solid-phase microextraction of neomycin antibiotic using graphene oxide. The study focuses on evaluating the adsorption capacity and kinetics of GO under varying environmental conditions to elucidate the mechanisms governing neomycin removal. Additionally, the potential of GO as a sorbent material for SPME is assessed through method development and optimization for the trace-level detection of neomycin in aqueous samples [15-19]. By integrating these two complementary approaches, this work seeks to provide a holistic understanding of the role of graphene oxide in mitigating neomycin contamination while advancing its application in environmental monitoring [20].

Materials and Methods

Chemicals and Reagents

Graphene oxide powder, hydrochloric acid (HCl, 37%), and neomycin sulfate (antibiotic standard) were purchased from Sigma-Aldrich. Sodium hydroxide (NaOH), ethanol (C_2H_5OH), and acetone were obtained from Merck. All chemicals used in this study were of analytical grade and were used without further purification. Deionized water (18.2 MΩ·cm resistivity) was used for all solution preparations.

Instrumentation

Adsorption experiments were conducted using a rotary shaker (IKA KS 4000i). Quantification of neomycin was performed using a UV-Visible spectrophotometer (UNICO 2800 series) at 305 nm.

Preparation of Neomycin Solutions

Stock solutions of neomycin were prepared by dissolving neomycin sulfate in deionized water to achieve a concentration of 1000 mg/L. Working solutions with concentrations ranging from 10 to 100 mg/L were prepared by serial dilution of the stock solution. All solutions were stored at 4°C in amber bottles to prevent degradation.

Batch Adsorption Studies

Adsorption experiments were carried out in batch mode to evaluate the removal efficiency of neomycin by GO. In each experiment, 50 mL of neomycin solution with a known concentration (10–100 mg/L) was placed in a 100 mL Erlenmeyer flask. A predetermined amount of GO (10–50 mg) was added to each flask, and the mixture was agitated on a rotary shaker at 150 rpm at room temperature (25 ± 2°C). The pH of the solution was adjusted using 0.1 M NaOH or HCl before the addition of GO. Samples were withdrawn at regular intervals (10, 20, 30, 60, and 120 minutes), filtered through a 0.22 µm membrane filter, and analyzed for residual neomycin concentration using spectrophotometer.

Real Sample Analysis

The concentration of neomycin in real samples was quantified using HPLC. Chromatographic separation was achieved on a C18 column (250 mm × 4.6 mm, particle size: 5 µm) with a mobile phase consisting of methanol. The flow rate was set at 1 mL/min, and detection was performed at a wavelength of 305 nm. Calibration curves were constructed using standard solutions of neomycin over the concentration range of interest.

Results and Discussion

Optimizations

In this study, paying attention to the precise pH adjustment of solutions as one of the key parameters in optimizing the neomycin removal process is of particular importance. The results (Fig. 1) show that pH changes can significantly affect the removal efficiency of this compound. Specifically, when the pH of the solution is in the more acidic range than 7.0, it is observed that the removal efficiency of neomycin decreases. This reduction could be due to the protonation of carboxylic acid groups and OH groups at the edges of graphene oxide nanosheets. In acidic environment, these groups become protons and lose the ability to absorb neomycin surfaces. On the other hand, at pHs above 8.0, the removal efficiency is also reduced. In these alkaline conditions, the carboxylic and OH groups become negatively charged and become ionized like the carboxylate group. This ionizing state causes the effective interaction with neomycin to be reduced and the removal efficiency is reduced.

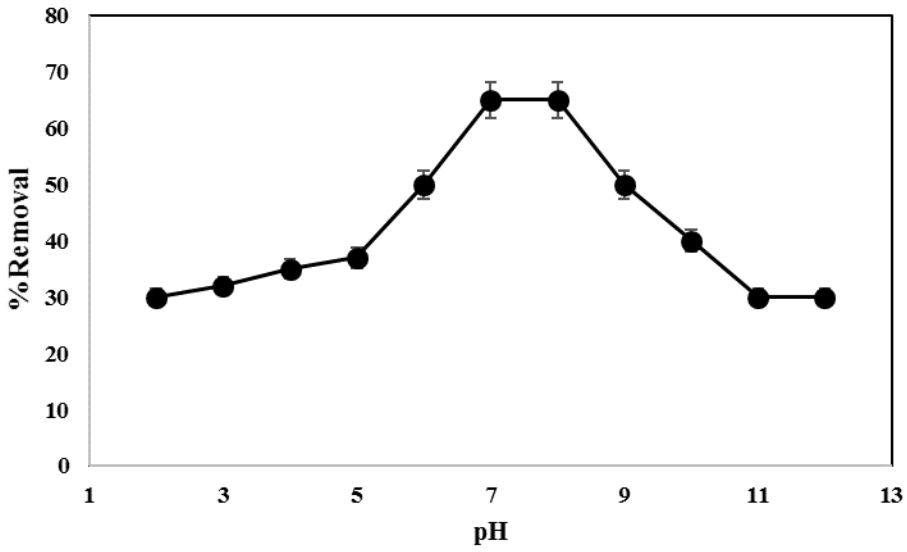

Fig. 1. Optimization of pH

The next parameter that was optimized was the adsorbent value and the results of this study are presented in Figure 2. As the results clearly show, by increasing the adsorbent amount up to 30 mg, the removal efficiency also increases. This increase in removal efficiency is due to the increased adsorbent specific surface area and the availability of analyte for adsorption. In fact, by increasing the adsorbent amount up to 30 mg, more adsorption sites are provided for the analyte and this improves the removal efficiency. However, after this point, i.e. with a further increase in the adsorbent value, the removal efficiency remains constant at 87%. The reason for this

phenomenon can be explained by the fact that after reaching the 30 mg level, there is no longer any analysis in the solution that can be absorbed on the surface of the excess adsorbent. Therefore, a further increase in the adsorbent value has no effect on the removal efficiency, and the efficiency remains at the same level. According to these results, 30 mg was selected as the optimal adsorbent for further experiments. This selection is based on achieving maximum removal efficiency with minimal absorbent consumption, which is considered the best option in terms of both economic and operational efficiency. Also, the use of the optimal adsorbent value can reduce the costs associated with the adsorption process and increase the overall efficiency of the system. Therefore, in future trials, this value will be used as a standard to obtain optimal and reliable results. In addition, further studies can investigate the effects of changes in other parameters such as temperature, contact time, and pH on removal efficiency. More advanced statistical analyses can also help to determine the optimal conditions more accurately. Also, investigating the possibility of reusing the adsorbent after the adsorption process can further strengthen the economic and environmental aspects. Finally, the results of this research can be the basis for improving similar industrial processes and provide solutions for more effective use of available resources.

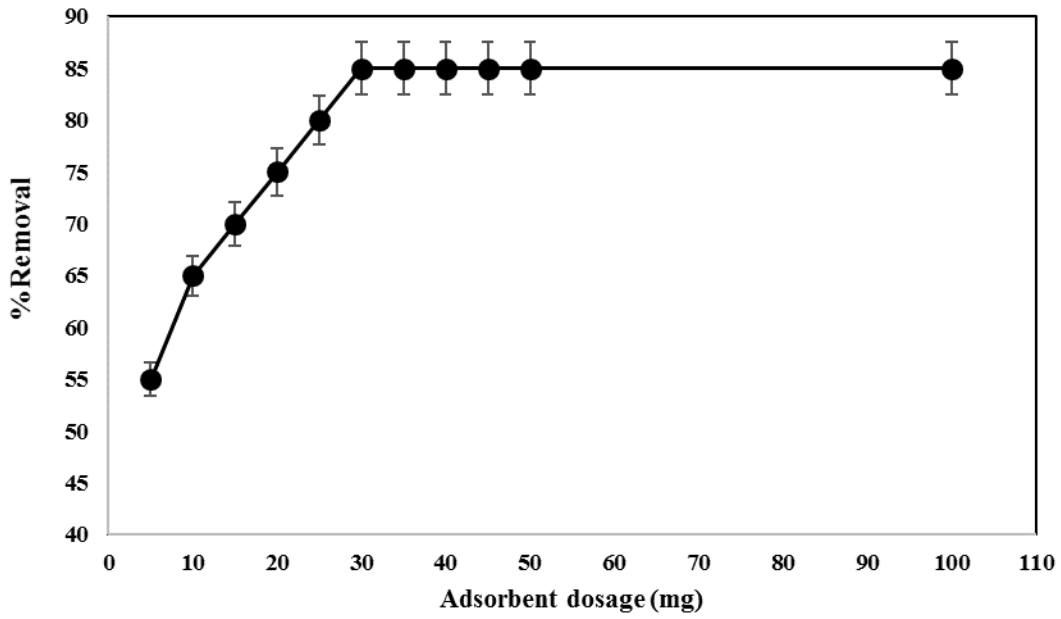

Fig. 2. Optimization of Adsorbent dosage

The next parameter that was optimized was the contact time of the adsorbent with the solution. As the results presented in Figure 3 clearly show, as the contact time increases up to 15 min, the removal efficiency increases and remains constant thereafter. This indicates that the interaction process between neomycin and graphene oxide has fast kinetics. In a period of 15 minutes, the adsorbed species is absorbed by the adsorbent at a high speed, and more time is not required for this process. It seems that in this period of time, all the active sites of the adsorbent are saturated with neomycin molecules, and therefore, increasing the contact time beyond 15 minutes does not affect the removal efficiency.

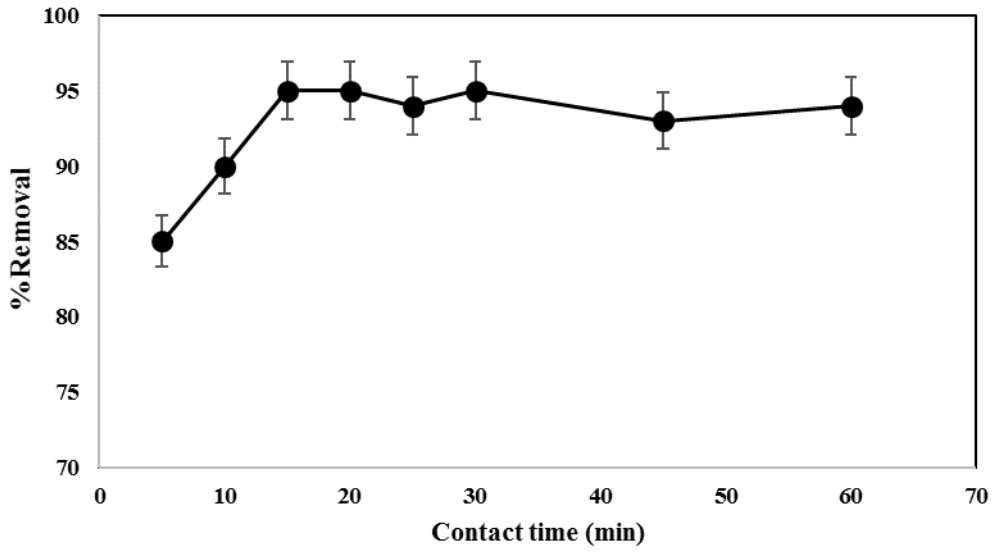

Fig. 3. Optimization of contact time

Under the previously optimized conditions, the concentration of neomycin solution was increased in the concentration range of 5 to 150 mg/L, and the removal efficiency was calculated for all solutions, and the obtained results are plotted in Figure 4. As the presented data clearly show, the adsorbent has maintained its optimal performance by increasing the adsorbent concentration up to 100 mg/L and removed neomycin with high efficiency. This indicates the high ability of graphene oxide nanosheets in adsorption. and removing neomycin from aqueous solutions. Further investigations show that this absorbent material is able to work effectively even at lower concentrations due to its high specific surface area and unique structure. These characteristics make the graphene oxide nanosheet to be considered as an efficient adsorbent for the treatment of water contaminated with antibiotics.

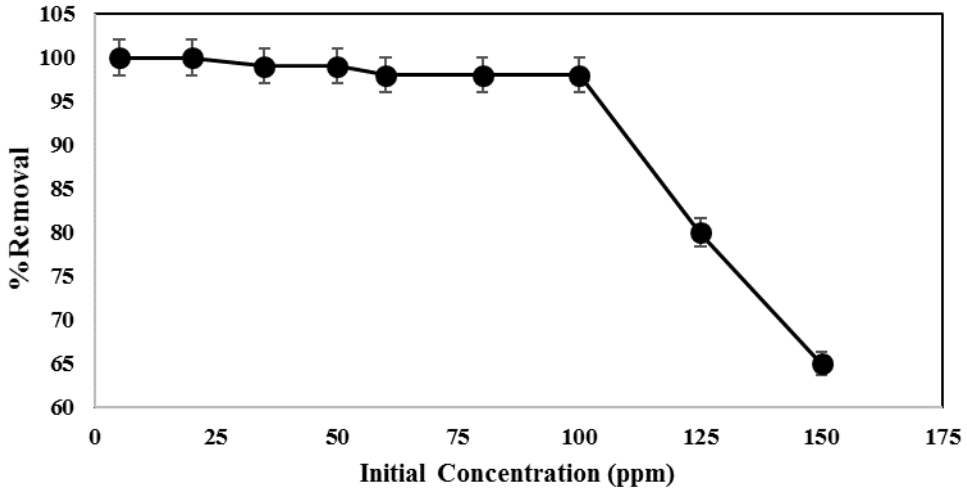

Fig. 4. Optimization of Initial Concentration

After optimizing all the parameters affecting the removal of neomycin, the process of drug absorption was also carefully examined. In this regard, the nature of the detergent or desorbent solvent was evaluated first, and as the recovery percentages presented in Figure 5 clearly show, among the methanol, ethanol, acetone, and propanol solvents, methanol is the solvent with the highest desorption efficiency. has been an analyte. Therefore, methanol was chosen as the optimal solvent. In terms of polarity, methanol is more similar to water than other organic solvents, and it is also less toxic. These characteristics make methanol environmentally preferable and in the context of green chemistry over other investigated solvents. The use of methanol not only increases the absorption efficiency, but also brings less environmental risks. At this stage of the research, the optimization of the volume of the desorbing solvent was investigated and the results of these investigations are presented in Figure 6. The results show that in volumes less than 1 ml, the amount of recovery is very small. But from the volume of 1 cc to 5 cc, the recovery percentage is completely done. Considering the main goals of this research, which includes neomycin preconcentration, the smallest possible volume, i.e. 1 ml, was selected. This choice is due to the fact that the volume of the initial analyte solution is 20 ml and the volume of the desorbing solvent is 1 ml, so the concentration factor of neomycin is 20.

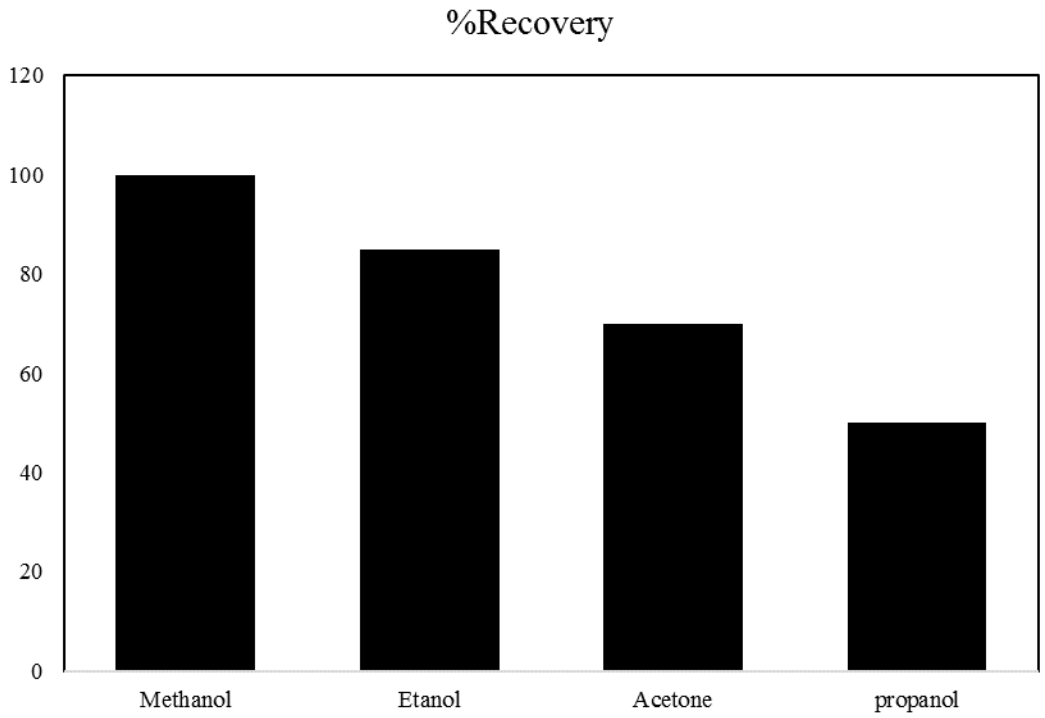

Fig. 5. Optimization of eluent solvent nature

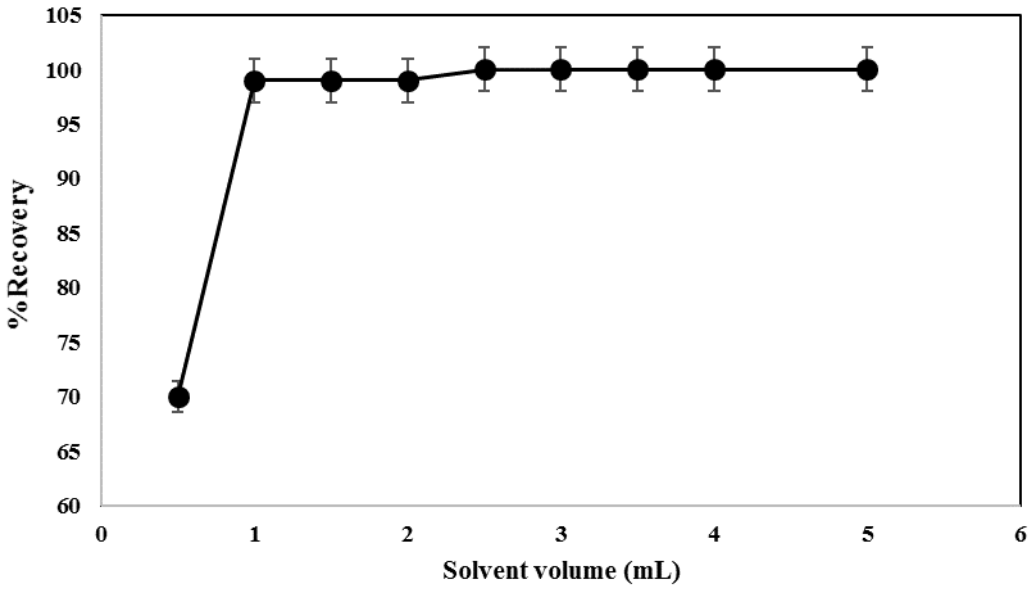

Fig. 6. Optimization of eluent solvent volume

Method Validation

In this research, after optimizing all pre-concentration parameters, a calibration curve for neomycin has been drawn. The obtained data is displayed in Figure 7. In this curve, the absorption of neomycin after pre-concentration in the concentration range of 0.1 to 1 mg/liter is characterized by a linear relationship, the slope of which is 1.6333 and the width from its origin is -0.0512. The limit of detection and the limit of quantification of the proposed method are also calculated based on the guidelines of the International Association of Pure and Applied Chemistry. These values are equal to 0.033 and 0.099 ppm respectively. Tolerance limit values have been calculated using standard methods. The results obtained in Table 1 clearly show that the proposed method has a very high selectivity compared to other species that may be present in the wastewater of pharmaceutical industries. In this study, neomycin has been considered as one of the main pollutants. The results show that the proposed method is able to isolate neomycin from other compounds with high accuracy and sensitivity. This indicates the high efficiency of the method in removing specific pollutants from wastewater. Tolerance values obtained for neomycin have been reported to be in the range of 50 to 300. This range indicates the method's ability to tolerate different concentrations of neomycin without reducing efficiency.

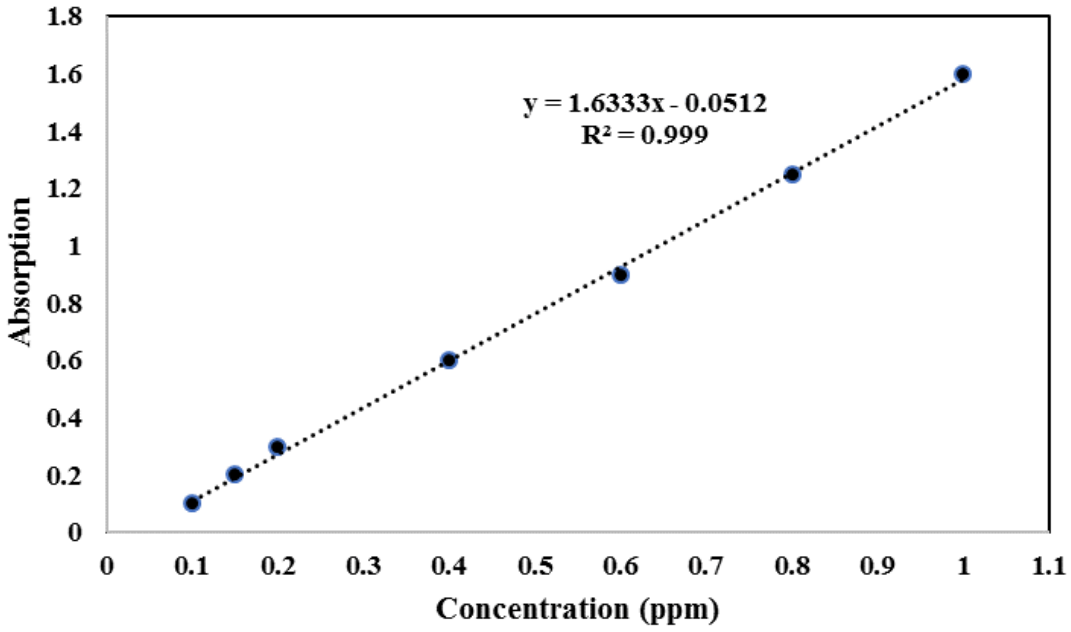

Fig. 7. Calibration Curve

Table. 1. The calculated tolerance limit

Interfering Species	Tolerance limit
Na^+, K^+, Cl^-, NO_3^-	300
Ca^{2+}, Mg^{2+}, F^-	150
Mn^{2+}, Cu^{2+}, ClO_4^-	50

In order to investigate the exact performance of the proposed preconcentration method in measuring low amounts of neomycin, as described in detail in the second chapter, three simulated effluent samples were prepared. In each of these samples, a certain amount of neomycin was added to the wastewater. Then, the analysis processes were performed on all three samples and the absorption rate of these samples after resorption and recovery was measured using an ultraviolet-visible spectrophotometer. The results are presented in Table 2. The obtained data show that the measured values are in good agreement with the added values and the obtained recovery percentages vary between 97.78 and 104.00%. This suggests that the proposed method was able to measure neomycin in these matrices without systematic error. In addition, the relative standard deviation values, which are all reported to be less than 5%, indicate the high accuracy of this method.

Table. 2. Real sample analysis

	Added (ppm)	Found (ppm)	%RSD	%Recovery
Simulated effluent 1	0.100	0.098	4.12	98.00
Simulated effluent 2	0.500	0.520	3.98	104.00
Simulated effluent 3	0.900	0.880	4.45	97.78

Conclusion

Due to the significant increase in sensitivity observed in this study after conjugation of solid-phase microextraction with spectrophotometry, as well as its very high selectivity and good reproducibility, it is suggested that the high-performance liquid chromatography method should be discarded for other pharmaceutical compounds and in future researches, researchers should further develop microextraction methods and increase sensitivity while improving selectivity. These new methods can help reduce the time and costs associated with the analysis of pharmaceutical compounds. In addition, due to recent advances in microextraction technologies, it is possible to further improve the accuracy and accuracy of the results, which can be significantly effective in research and industrial processes. One of the main advantages of using solid-phase microextraction combined with spectrophotometry is the reduction of the need for organic solvents, which is not only economically cost-effective but also environmentally important. Reducing the use of chemical solvents leads to a reduction in environmental pollution and a reduction in the production of chemical waste, which is a positive step towards sustainable development. Also, since these methods do not require complex and expensive equipment, they can also be used in smaller laboratories with limited budgets. This could provide wider access to cutting-edge research in the field of pharmaceuticals and analytical chemistry. Finally, due to all these advantages and significant advances made in this field, it is expected that in the near future, microextraction methods will become a viable alternative to more traditional methods such as high-performance liquid chromatography and will be accepted as a new standard in research and industrial laboratories. Researchers should continue to look for ways to optimize these methods so that they can get the most out of them.

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