RESEARCH ARTICLE

Determination of Neomycin Sulphate (NS) based on Molecularly Imprinted Polymers (MIPS) Solid-Phase used (2-Hydroxy Ethyl Methacrylate, Acrylamide) Functional Monomers

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ABSTRACT

Neomycin Sulphate (NS) selective molecularly imprinted polymers (MIPs) were based on ion-pair by prepared (four polymers MIPs using NS as the template a well as 2-Hydroxy ethyl methacrylate (2-HEMAC), Acrylamide (AAM) as a monomer, used Divinyl Benzene (DVB) as cross-linker and used benzoyl peroxide (BPO) as initiator. NIPs were prepared by using the same composition of MIPs except for the template (NS). The MIPs were prepared using the variation ratio of monomer and cross-linker. These MIPs applicant as solid-phase extraction for determination (NS) in pharmaceutical preparation used UV-Spectrophotometer as detector.the results gave a good response, where the reconstruction percentage Rec% took values of (97.857909–100.735053) %, and RSD% took values of (0.041720–0.438022) % of (NS) drug for the Neomycin Sulphate pharmaceutical.

Keywords: Molecularly imprinted polymers, Neomycin Sulphate, Solid-phase extraction.

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INTRODUCTION

Neomycin Sulphate (NS) is an aminoglycoside antibiotic that is produced naturally by the actinomycete bacterium Streptomyces fradiae via the fermentation process.[1] Its found in many topical medications such as creams, ointments, and eye drops.[2] Use of the treatment of gastrointestinal infections. It has bactericidal properties against gram-negative bacteria and partial also against gram-positive bacteria. Neomycin sulfate (Figure 1) is mainly composed of the two isometric components Neomycins B and C. The component B has higher antibiotic activity than component C (3) chemically it is known as (2RS,3S,4S,5R)-5-amino-2-(aminomethyl)-6-((2R,3S,4R,5S)-5-((1R,2R,5R,6R)-3, 5-diamino-2-((2R,3S,4R,5S) -3-amino-6- (aminomethyl) -4,5-dihydroxytetrahydro-2H-pyran-2-yloxy)-6-hydroxy cyclohexyl oxy)-4-hydroxy-2-(hydroxymethyl) tetrahydrofuran-3-yloxy) tetrahydro -2H-pyran-3,4-diol(4).

Numerous techniques by which near-body objects are examined are found in pure pharmaceuticals. These techniques are electrophoresis techniques,[5] visible spectroscopy techniques,[6] UV-spectroscopy techniques,[4,7] chromatography techniques,[8,11] and various analytical techniques.[12,15]

MATERIALS AND METHODS

Instrumentation: Monitoring of the analyses was performed using UV-Visible Spectrophotometer 1800 pc (SHIMADZU-Japan) using the (1 cm) quartz cells and Scanning Electron Microscopy (SEM) (JSM.6390A) (Tokyo-Japan) and IRAffinity-1S (FTIR) - 8000 (SHIMADZU -Japan), hot plate

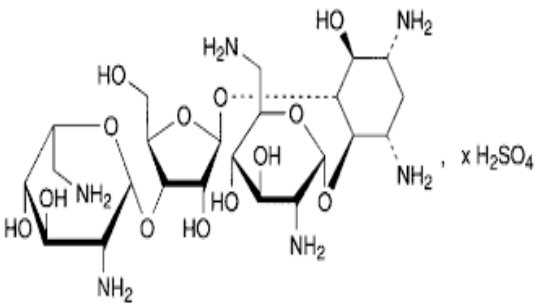

Figure 1: Chemical structure of Neomycin Sulphate.

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with stir (Germany). During the polymerization process, pure Neomycin Sulphate shows an absorption band at (310 nm), this band can be used to ensure that all NS was removed after washing. It was measured by using UV-vis spectrophotometer. An ultrasonic Sensitive Water Bath from (SONEX-GERMANY) was used for stirring the polymer solution.

Preparing of Standard Solutions

Preparing of standard solution (0.01 mol/L) Neomycin Sulphate by dissolving (0.615 gm)of standard Neomycin Sulphate in the diluted with the amount of triple-distill water and a (0.4 mL) of 0.1 M NaOH and completed to (100 mL) in the volumetric flask, the other solutions were prepared in100 mL at the ranged from (0.0085–0.004 mol/L) in the same procedure.

Synthesis of the Imprinted Polymers NS- (MIP1-2-HEMAC) & NS- (MIP2- AAM)

Polymeric materials (MIP1 and MIP2) were prepared by taking two test tubes (25 mL) and added to the first 0.3 mmol and to the second washing solution and under 5pa vacuumed pressure.

Preparation of Pharmaceutical Form (Neomycin sulphate)

The pharmaceutical form (tablets) containing NS, which is available in the local market produced by Memphis Pharmaceuticals and Chemical Industries / Egypt, has weighed 10 tablets (500 mg) of NS where its weight was 6.325 g, it was grounded using a ceramic mortar, the mean weight of one tablet was 0.6325 g, then a concentration of 0.01 mol/L was prepared by taking a 0.615 g of the powder, putt in a 100 mL volumetric flask, dissolved with tri-distill water and added 4 mL of 0.1 M NaOH after being placed in ultrasound water bath, the solution is filtrated using Whatman No. 42 filter paper to get rid of any non-dissolved substance and the filtrated solution which containing 0.01 mol/L NS was obtained. This solution was used in the next experiments.

Procedure of NS Standard Solution

1.1 mmol of the template material of NS, which was dissolved in 10 mL of methanol and added 4.6 mmol of polymer 2-HEMAC and 13.3 mmol of the AAM to the first and second tubes respectively. After that was centrifuged using the ultrasonic water bath for 5 minutes, then added 10 mmol and 85.5 mmol DVB as cross-linking to the first and second tubes, respectively. Added 0.2 mmol from benzoyl peroxide as the initiator of polymerization reaction per tube, the bubbles were expelled from the solution by exposing it to the high purity of nitrogen for 30 minutes and immediately the tube was shut completely using a rubber stopper and the tube was put in a water bath at a temperature 60°C without moving for five days. After the completion of the formation of the polymer, the template was removed by repeated washing of polymer using a mixture of 10% (v/v) of acetic acid/methanol by using a soxhlet extractor for 24h and drying it at 40°C for 1-hour Then the material was milled and converted into powder using a mortar of granite and using a porous steel sieve 125 µm. To estimate the extracted product, a 3 mL plastic syringe was filled with polymer without a template. a Standard solution within the calibration curve was prepared, allows it to pass through the plastic syringe (column), and then removed from the column using a Different volumes 4-8.5 mL of 0.01 mol/L NS standard solution were transferred to a series of 10 mL volumetric flasks, diluted with amount of triple-distill water and a 0.4 mL of 0.1 M NaOH. The volume was completed to the volume with same solvent. Then the wavelength scanned between 190-400 nm, the maximum absorbance was at 310 nm.

RESULTS AND DISCUSSION

Absorption Spectra

The neomycin sulfate absorption was measured against the blank solution at 400–190 nm wavelengths. NS showed maximum absorption at wavelength 310 nm, as in Figure 2A. Calibration curve as in Figure 2B showed the linearity of NS was 0.0085-0.004 mol/L and the determination coefficient $R^2 = 0.9993$. The detection limit and the quantification limit were 0.000301 µg.mL^{-1} and 0.001003 µg.mL^{-1} respectively. Rec% was between% 98.381806–101.437787% and RSD% was between 0.102036- 0.994888%.

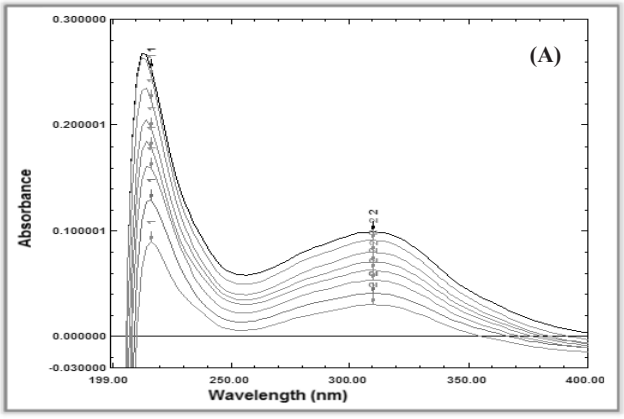

Figure 2: A shows the zero-order spectra of NS at wavelength 310 nm; B shows the calibration curve of NS with concentrations of (0.0085-0.004 mol /L)

Accuracy and Precision

The accuracy and precision of the method were tested by calculating the %Rec and the relative standard deviation %RSD for two concentrations from the calibration curves. Table 1 shows these results, RSD% value was between 0.102036–0.994888%, and %Rec value was between 98.381806–101.437787%.

Synthesis of MIPs for Betamethasone Sodium Phosphate (BMSP)

Two MIPs of neomycin sulfate were prepared by the polymerization process, which NS is a template and the selection of monomers that play an important role in the interaction with the template to the formation of molecularly printed polymers. Two monomers were used: 2-Hydroxy ethyl methacrylate (2-HEMAC) and acrylamide (AAM), the molecularly printed polymers needed a suitable quantity and cross-linking to complete the polymerization process to make the polymer highly selective and more solid. In addition, the type of solvent used limits the polymerization process. High polar organic solvents were used to complete the polymerization process; these solvents were methanol to be the appropriate solvent.

Several attempts were made to prepare molecularly printed polymers and included selecting optimal ratios of the drug: monomer: cross-linker for the preparation of MIPs and NIPs.

Prepared of MIPs and NIPs have the appropriate characteristics to perform their work and are presented in Table 2.

FTIR Analysis

The FTIR spectra of NS, which are between 400–4000 cm^{-1} using KBr when MIPs are formed based on the monomer 2-Hydroxy ethyl methacrylate and acrylamide, show the presence of basic functional groups (before and after removal of the drug) as in Figures 3 and 4 and Table 3.

The FTIR spectra indicated that there were two peaks of OH group and that their wave number became higher because the peaks that appeared have belonged to OH in NS and those in the 2-HEMAC; in addition, we note that the groups of carbonyl

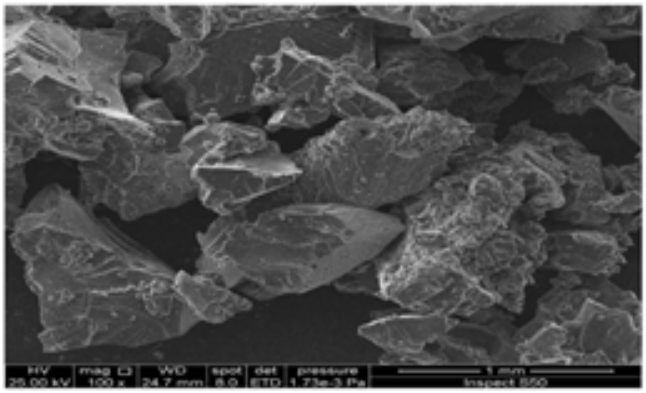

Figure 3: Shows NS-MIP2-AAM SEM (1000 μm) obtained before removing the NS template molecule by the polymerization process.

Table 1: Accuracy and consistency of NS drug

	Drug Conc (mol/L)			
Sample	*Taken*	*Found*	*Rec %*	*RSD %*
NS	0.004	0.004058	101.4378	0.280741
	0.0045	0.004427	98.38181	0.235394

*An average of seven determination.

Table 2: The different ratios of (D: M: C) used in the preparation of MIPs and NIPs for NS.

		Drug	*Monomer*	*Cross-linker*	*Initiator*		
No. MIP	*Ratio*	*NS*	*2-HEMAC*	*DVB*	*BPO*	*Solvent*	*Result*
MIP1	%	1.32	39.48	59.22	0.3	±10 mL CH3OH	Yellow emulsion
	mmol	0.2	6	9	0.2		
MIP1	%	1.71	34.19	64.1	0.3	±10 mL CH3OH	Yellow emulsion
	mmol	0.4	8	15	0.2		
MIP1	%	2	30.87	67.11	0.3	±10 mL CH3OH	Yellow glass material
	mmol	0.3	4.6	10	0.2		
NIP1	%		30.87	67.11	0.3	±10 mL CH3OH	Yellow glass material
	mmol		4.6	10	0.2		
No.MIP	Ratio	Drug	Monomer	Cross-linker	Initiator	Solvent	Result
		NS	AAM	DVB	BPO		
MIP2	%	1.23	16.46	82.3	0.3	±10 mL CH3OH	Yellow emulsion
	mmol	0.75	10	50	0.2		
MIP2	%	2.39	16.27	81.34	0.3	±10 mL CH3OH	Yellow emulsion
	mmol	2.2	15	75	0.2		
MIP2	%	1.1	13.31	85.59	0.3	±10 mL CH3OH	Yellow glass material
	mmol	1.1	13.3	85.5	0.2		
NIP2	%		13.31	85.59	0.3	±10 mL CH3OH	Yellow glass material
	mmol		13.3	85.5	0.2		

All ratios of MIPs and NIPs were prepared using a water bath at 60-70ºC.

Table 3: Demonstration of the most recognized peaks in FTIR spectra of the molecular printed polymer of NS using 2-HEMAC as a functional monomer.

No.	*Functional Group*	*NS*	*NS-(MIP_1-2-HEMAC) before template removal*	*NS-(MIP_1-2-HEMAC) after template removal*
1	O-H str.	3425	3461, 3375	3492
2	N-H str.	3363	3436	---
3	C-H aromatic.	3078,3043	3085,3043	3083,3049
4	C-H aliphatic.	2993, 2950	2910,2839	2920,2852
5	S=O str.	1631	1627	---
6	C=O str.ester.	--	1600	1600
7	C=O str.α.β.unsaturated	--	1683	1681
8	C-H bending	1458	1442	1440

Table 4: Showing the most peaks identified in the FTIR spectra of the molecularly printed polymer of NS using AAM as a functional monomer.

No.	*Functional Group*	*NS*	*NS- (MIP_2- AAM)before template removal*	*NS- (MIP_2- AAM)after template removal*
1	O-H str.	3425	3427 (drug)	---
2	N-H str.	3363	3537(Monomer)	3425(Monomer)
3	C-H aromatic.	3078,3043	3082,3020	3083,3049
4	C-H aliphatic.	2993, 2950	2898,2833	2918, 2850
5	S=O str.	1631	1627	---
6	C=O str. ester.	---	1600	---
7	C=O str.amid	---	1703	1701
8	C-H bending	1458	1442	1442

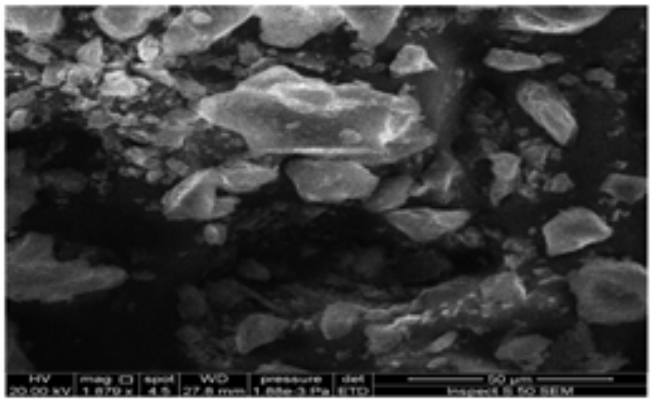

Figure 4: Shows NS-MIP2-AAM SEM (1000μm) obtained after removal of the NS template molecule by the polymerization process.

(C = O ester) and (C = O α.β.unsaturated) have remained after the removal of the template molecule because they belong to the monomer. NH and S = O groups of NS, which disappeared after removing the template molecule, showed that the repeated washing process MIPs using a mixture of 10% (v/v) of acetic acid: methanol and removal it

Table 4 shows NS were measured in pure form and NS-MIP2-AAM (before and after removing the template molecule) by scanning it in a range of 400–4000 cm^{-1} using the KBr method.

The fourier transform infrared spectroscopy (FTIR) tables indicated a broad peak of the NH group and that its wave number becomes higher because this peak was the sum of the NH group of the NS with the NH group of AAM. In addition, the carbonyl groups (C = O of ester and C = O of amid) has remained after the removal of template molecule because it belongs to the monomer, as groups of (NH) and (S = O), due to the NS, which appeared during the MIPs formation and disappeared after template molecule removal process.

Morphological Characterization

Morphological analysis is important to clarify the design of particles and their sizes before and after removing the template molecule of NS from the polymer. Covalent or non-covalent reactions arrange monomers carrying certain functional groups around the template molecule, and therefore, there will be a high degree of polymerization with linking bonds, where the functional group is kept in place by the polymer network. The template molecule is subsequently solved by using solvents, leaving cavities complementing the template molecule in terms of shape, size, composition, and arrangement of functional groups.

From the SEM images, as shown in Figures (3,4), there was a difference in size and structure of the molecules before and after washing, where the polymeric molecules had rectangular shapes and small sizes 0.06566-0.06958 μm for NS-MIP2-AAM before removing the template molecule (NS) and 0.5452-0.4324 μm for MIP2-AAM after removing the template molecule (NS).

Application of Method

The method was applied using solid-phase extraction for two concentrations (0.005 and 0.007 mol/L) of a pharmaceutical form of NS were carried out within the calibration curves by performing three readings for each measurement, and the wavelength between 200-400 nm was scanned. The results showed that the method has good accuracy and precision, where the value of Rec% ranged between (97.857909–

Table 5: Results of applying the method to NS-MIP1-2-HEMAC and NS-MIP2-AAM prepared using solid-phase extraction of two concentrations 0.005 and 0.007 mol/L in pure form.

		Conc (mol/L)			
Sample	*Method*	*Taken*	*Found*	*Rec %*	*RSD %*
Standard solutions (NS)	NS-MIP_1-2-HEMAC	0.005	0.004893	97.857909	0.041720
		0.007	0.0070513	100.7350532	0.190874
Standard solutions (NS)	NS-MIP_2-AAM	0.005	0.004912	98.248065	0.438022
		0.007	0.006981	99.722592	0.113894

Table 6: Results of applying the method to NS-MIP1-2-HEMAC and NS-MIP2-AAM prepared using solid-phase extraction of two concentrations of 0.005 and 0.007 mol/L for the pharmaceutical preparation (Neomycin Sulphate).

		Conc (mol/L)			
Sample	*Method*	*Taken*	*Found*	*Rec %*	*RSD %*
Neomycin sulphate Tablet 500mg	NS-MIP_1-2-HEMAC	0.005	0.0051094	102.188980	0.925215
		0.007	0.0069489	99.27031043	0.291131
Neomycin sulphate Tablet 500mg	NS-MIP_2-AAM	0.005	0.0050892	101.783175	0.862544
		0.007	0.0070195	100.2781795	0.358109

100.735053) and value of %RSD between 0.041720–0.438022% for the standard NS, while the value of Rec% ranged between 97.857909–100.735053% and the value of RSD% is between 0.041720–0.438022% for NS pharmaceutical form as shown in Tables 5 and 6.

CONCLUSION

A new method for the determination of NS using molecularly imprinted polymers based on solid-phase extraction has been developed to be selective in the determination of (NS) regardless of the presence of additives or different prepared polymers.

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