

## SYNTHESIS AND BIOLOGICAL INVESTIGATION OF SALICYLALDEHYDE-BASED AZOMETHINE

Fargana Alizadeh<sup>d</sup>, Aygun Israyilova<sup>a,b</sup>, Alakbar Huseynzada<sup>a,c,\*</sup>, Ulviyya Hasanova<sup>a</sup>, Ulviyya Yolchuyeva<sup>d</sup>

<sup>a</sup>ICESCO Biomedical Materials Department, Baku State University, Baku, Azerbaijan

<sup>b</sup>Laboratory of Microbiology and Virology, Baku State University, Baku, Azerbaijan

<sup>c</sup>ICRL, Baku State University, Baku, Azerbaijan

<sup>d</sup>Institute of Petrochemical Processes, Baku, Azerbaijan

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### Abstract

In the present study, a novel Schiff base of salicylaldehyde was synthesized. Salicylaldehyde-based azomethine was synthesized by the condensation reaction of heteroatom-containing polyamine and aldehydes in acetonitrile in a non-catalyst medium and characterised by <sup>1</sup>H-NMR, <sup>13</sup>C-NMR analysis. The major advantage of the reaction is that it eliminates the requirement for a particular purification process, such column chromatography, for the obtained chemicals. Graphene oxide nanolayers, the second component, have been produced using a modified Hummer technique. The oxidising agent concentration (H<sub>2</sub>SO<sub>4</sub>+KMnO<sub>4</sub>) was increased, allowing for the receipt of purer nanolayers, which is what led to the method's change. Through the use of SEM and XRD techniques to examine the structure and morphology of nanolayers, it was shown that the thickness of these layers is 1 nm. Azomethine was then used to modify graphene oxide. Subsequently, graphene oxide was modified with azomethine. The compound and the ensemble were screened for antibacterial (*S. aureus*, *E. coli*, *A. baumannii*, *P. aeruginosa*, *K. pneumonia*) activities. The minimum inhibitory concentrations of the compounds were also ascertained by the two-fold micro-dilution method. A study revealed that azomethine's antibacterial activity might be enhanced through the addition of graphene oxide nanolayers into the molecule itself.

**Keywords:** salicylaldehyde, azomethine, *A. baumannii*, antibacterial activity, graphene oxide.

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### 1. Introduction

Schiff bases are the condensation products of primary amines and carbonyl compounds, and they originally were outlined by Schiff [1] in 1864. These compounds share the azomethine group, which has the generic formula RHC=N-R1, where R and R1 are alkyl, aryl, cycloalkyl, or heterocyclic groups that can be substituted in a variety of ways. These chemicals are sometimes referred to as anil, imine, or azomethine. The main reasons for interest in these compounds are their structural similarities to naturally existing biological molecules, their relatively easy synthesis processes, and their synthetic flexibility, which allows for the creation of appropriate structural features. They are well-known carriers for arylacetamide, formazone, thiazolidinone, azetidinone, metal complexes, and several other derivatives [16, 17]. It should be noted promptly that Schiff bases have biological or therapeutic implications as potential drug candidates, diagnostic probes, and analytical

instruments. Schiff bases have become well-known in the medical and pharmaceutical fields for their diverse biological activities, which include anticancer, antibacterial, antiviral, antifungal, analgesic, antipyretic, anti-inflammatory, anticonvulsant, antitubercular, antioxidant, anthelmintic, and so on. These compounds can also be used as pesticides, cytotoxins, corrosion inhibitors, and enzyme inhibitors, as well as to prevent DNA damage [2-9].

From another perspective, given that the annual growth of microbial infections causes enormous economic damage and potential risks to human health, and that currently available antimicrobial drugs have significant drawbacks (toxicity, limited efficacy, and environmental problems), the search for safe. The drug discovery process benefits from the distinct advantages that these products have over regular synthetic molecules, making the development of treatments based on them critical. Salicylaldehyde, as one of these molecules, is a useful drug research framework [18- 20]. On the other side, salicylaldehyde (2-hydroxybenzaldehyde) bearing two different active functional groups, namely, a hydroxy group and an aldehyde group, finds wide application as a key chemical in a variety of industrial processes, especially in the large-scale production of pharmaceuticals. Salicylaldehyde and most of its derivatives are commercially available or readily accessible, and hence are ideal starting materials for multicomponent reactions [10- 12].

Graphene oxide nanolayers are an additional material of the research. Through modification and chemical engineering, new materials with great promise in many scientific fields, particularly the pharmaceutical industry, may be produced. With oxygen-containing groups including hydroxyl, carbonyl, carboxyl, and epoxide interwoven throughout, graphene oxide is an oxidised version of graphene that takes part in modification processes [13- 15]. Because graphene oxide has oxygen functions, it is easily soluble in water and other organic solvents as well as in other matrixes. This is one of its benefits. The characteristics of graphene oxide can be significantly altered by functionalization. Chemically modified graphenes that were produced were used in polymer composites, biodevices, drug delivery systems, and other applications. Furthermore, using nanoparticles shows features of materials that change as a result of the particles' approaching nanosize, and the ratio of a material's surface to volume becomes important. It is an additional advantage of using graphene oxide nanolayers [20- 22].

The benefits of azomethine and graphene oxide nanolayers were taken into consideration while developing the new ensembles based on the aforementioned "blocks of material," which were then synthesised and their antibacterial activity examined. A condensation process in a non-catalyst media was used to synthesise new azomethines from salicylaldehyde and a heteroatom-contained aliphatic polyamine- 2,2-(ethylenedioxy)bis(ethylamine). The structure of synthesised compound was studied by  $^1\text{H}$  and  $^{13}\text{C}$  NMR analysis. The synthesised chemical and ensemble were then tested by measuring minimum inhibitory concentration (MIC) against a variety of bacterial strains, including *S. aureus*, *E. coli*, *A. baumannii*, *P. aeruginosa*, *K. pneumonia*. The results were then compared with ampicillin, a well-known antibiotic.

## 2. Materials and Methods

### General Information

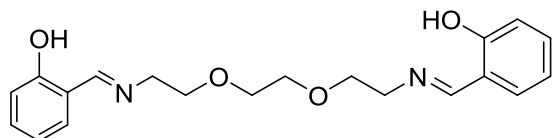
All of the chemicals and solvents used in the experiment were analytical grade products that were purchased from a commercial provider and used without further purification. The purity of the compounds generated and the progress of the reactions were observed using thin layer chromatography (TLC) on Merck silica gel plates (60 F254 aluminium sheets) (visualisation was done under UV light). Melting points were measured using open capillary tubes and an uncorrected Buchi B-540 equipment.

### NMR experiments

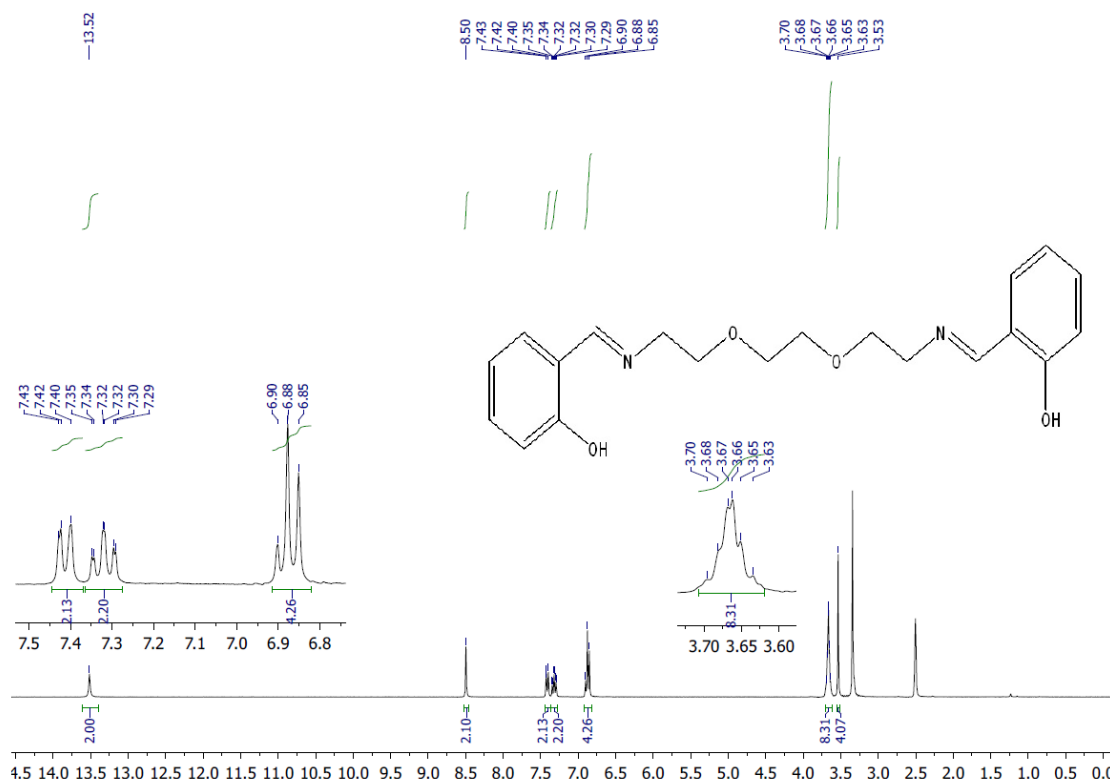
Using Bruker Standard software (TopSpin 3.1), the NMR studies were conducted in 5 mm sample tubes using a BRUKER FT NMR spectrometer AVANCE 300 (Bruker, Karlsruhe, Germany) with a BVT 3200 variable temperature unit (300 MHz for  $^1\text{H}$  and 75 MHz for  $^{13}\text{C}$ ). Chemical shifts were reported as ppm ( $\delta$ ) with internal tetramethylsilane (TMS) as a reference. The following are the stated multiplicities: m (multiplet), q (quadruplet), t (triplet), d (doublet), and s (singlet). J, the coupling constant, is given in Hz. The following are the experimental parameters for  $^1\text{H}$ :  $90^\circ$  pulse-length=10 ms, PL1=3 dB, ns=3, ds=1, d1=1 s, digital resolution=0.23 Hz, SWH=7530 Hz, TD=32 K, SI=16 K, and for  $^{13}\text{C}$  as follows:  $90^\circ$  pulse length=9 ms, PL1=1.5 dB, ns=300, ds=2, d1=3 s, digital resolution=0.27 Hz, SWH=17985 Hz, TD=64 K, SI=32 K. The synthesised compounds were made using DMSO- $d_6$  (99.7%, containing 0.3%  $\text{H}_2\text{O}$ ) and  $\text{CDCl}_3$ , NMR-grades.

## Experimental procedures

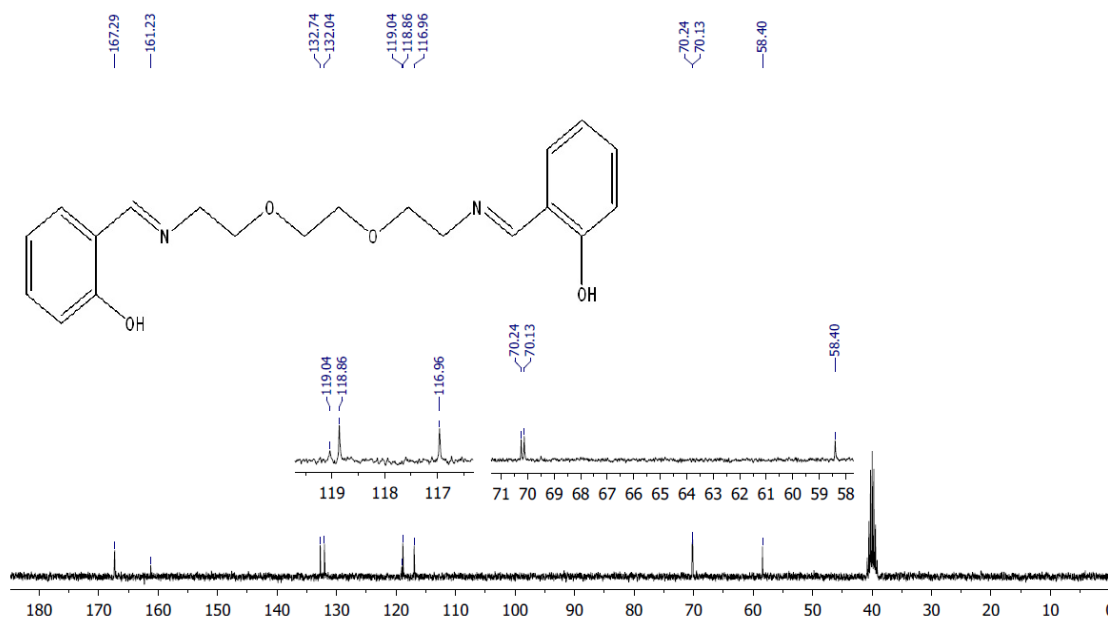
The abovementioned condensation reaction method (Scheme 1) was used to synthesize the imine molecule.



**2,2'-((1,11)-5,8-dioxa-2,11-diazadodeca-1,11-diene-1,12-diyl)diphenol:** A round-bottomed flask (10 mL) was charged with salicylaldehyde (1 mmol) and acetonitrile (5 mL) followed by the addition of a 2,2'-(ethylenedioxy)bis(ethylamine) (1 mmol). The reaction mixture was allowed to stir for 24 h at  $80^\circ\text{C}$ . At the end of the reaction time, the precipitate was formed, filtered, washed with distilled water, and dried. Yield 75%, m.p.=  $120\text{-}125^\circ\text{C}$ .  $^1\text{H}$  NMR spectrum: (DMSO- $d_6$ ,  $\delta$ , ppm), 3.53 s (4H,  $2\text{CH}_2\text{N}$ ), 3.63-3.70 m (8H,  $4\text{OCH}_2$ ), 6.85-6.90 t (4H,  $4\text{C}_{\text{Ar}}\text{H}$ ,  $J=15\text{Hz}$ ), 7.29-7.35 m (2H,  $2\text{C}_{\text{Ar}}\text{H}$ ), 7.40-7.43 t (2H,  $2\text{C}_{\text{Ar}}\text{H}$ ,  $J=9\text{Hz}$ ), 8.50 s (2H,  $2\text{CH}=\text{N}$ ), 13.52 s (2H,  $2\text{OH}$ ).  $^{13}\text{C}$  NMR spectrum: (DMSO- $d_6$ ,  $\delta$ , ppm), 58.40 ( $2\text{NCH}_2$ ), 70.13 ( $2\text{OCH}_2$ ), 70.24 ( $2\text{OCH}_2$ ), 116.96 ( $2\text{CH}$ , Ar), 118.86 ( $2\text{CH}$ , Ar), 119.04 ( $2\text{CH}$ , Ar), 132.04 ( $2\text{CH}$ , Ar), 132.74 ( $2\text{C}$ , Ar), 161.23 ( $2\text{C}$ , Ar), 167.29 ( $2\text{CH}=\text{N}$ ).



**Fig. 1.**  $^1\text{H}$  NMR spectrum of the compound in DMSO- $\text{d}_6$  solution



**Fig. 2.**  $^{13}\text{C}$  NMR spectrum of the compound in DMSO- $\text{d}_6$  solution

### X-Ray Powder Diffraction Analysis (PXRD)

To investigate the crystalline structure of the obtained graphene oxide nanolayers, an XRD examination was performed at room temperature using a Rigaku Mini Flex 600 XRD diffractometer with CuK radiation. The materials were scanned between 10 and 80 degrees Bragg at 15 milliamperes. The Williamson-Hall approach was used to determine the crystallite size.

### Scanning Electron Microscopy (SEM) study

The graphene oxide nanolayers were analysed using a SEM JEOL-1400 (Japan) running at 80-120 kV. The ethanol-based ultrasonicated graphene oxide solution was distributed on a carbon-coated grid and allowed to dry at ambient temperature. Morphometric analysis of the images (electronograms) was carried out using Olympus Soft Imaging Solutions GmbH's SEM Imaging Platform programme.

### Synthesis of graphene oxide nanolayers

The Hummer process described in the literature was modified in order to synthesize graphene oxide nanolayers. The procedure was modified by gradually adding more oxidising chemicals and increasing their quantity. There are two steps in the synthesis of graphene oxide:

#### 1. Synthesis of graphite oxide

A three-necked flask with a magnetic stirrer is filled with 10 g of finely powdered graphite, 6 g of sodium nitrate, and 300 ml of concentrated sulfuric acid. The reaction mass is placed into an ice bath, the mixture is stirred briskly, and the temperature is lowered to 0°C following the addition of sulfuric acid. 35 g of potassium permanganate is added to the reaction media within an hour of the temperature reaching the necessary level.

It should be between 17 and 20°C in this instance. The ice bath is taken out and the temperature is allowed to increase to 35±3°C after all of the potassium permanganate has been added. For thirty minutes, the solution is stirred once the temperature reaches the necessary level. Major gas evolution takes place at this point. The mixture's "hiss" progressively disappears as it thickens over time. A minor quantity of gas is released after 20 minutes, causing the mixture to turn pasty. The paste that is produced is grayish-brown in color. Thirty minutes later, the temperature goes up to 98°C when 460 ml of distilled water is added very slowly (to avoid strong boiling and splashing) while vigorously stirring the reaction mass. If the reaction is set up with a small amount of graphite, higher heating may be needed.

The reaction mass turns brown and is vigorously stirred for 15 minutes at the required temperature following the addition of the prescribed amount of water. In addition, 300 ml of 3% hydrogen peroxide and 1.4 l of warm water (between 40 and 50°C) are added to the mixture and vigorously stirred for five minutes. The solution takes on a yellowish colour upon the addition of hydrogen peroxide. After filtering the precipitate further, a paste that is brownish-yellow is produced. Filtration needs to be done promptly while the solution is still warm because, when the solution cools, a reaction byproduct known as the sparingly soluble salt of mellitic acid precipitates. The precipitate is washed with around 42 litres of heated (60–70°C) distilled water after filtration. Barium nitrate is added to the solution after the final wash to determine whether sulfuric acid is present. The yellowish-brownish substance is dried in a desiccator for ten hours if no precipitate forms. If not, further washing of the resulting graphite oxide is required.

## 2. Graphene oxide synthesis

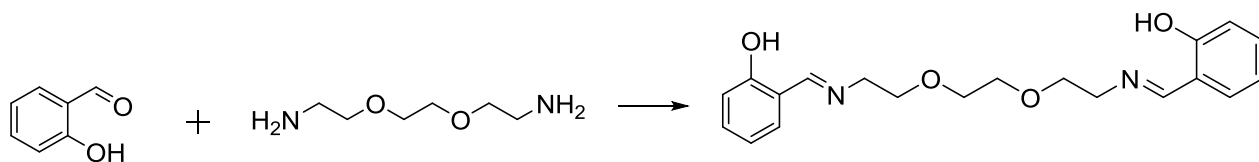
10 mg of the produced graphite oxide and 15 ml of DMSO are added to a 25 ml beaker. Graphite oxide nanolayers are then formed by sonifying the solution for five minutes. During the day, the resultant solution is totally stable. After a month, graphene oxide completely precipitates. The resultant solution can be utilised straight away or centrifuged at 9000 rpm to separate the solid graphene oxide.

## Biological activity

The antibacterial activity of supramolecular ensemble comprising synthesised graphene oxide nanolayers and azomethine compound was determined according to CLSI guidelines. The activity of the samples was evaluated by 2 fold microdilution assay [23, 24]. *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were used as test strains during the experiment. The bacterial strains used in the study were from Baku State University's culture collection at the microbiology department in Azerbaijan. Muller Hinton medium ("Liofilchem") was used to inoculate the bacterial strains. The concentration of the compounds was ranged from 1024 to 8 µg/mL. The concentration or density of the test cultures was adjusted to 0.5 McFarland by using a digital densitometer. After inoculation of bacterial strains in each well, the plates were incubated for overnight at 37°C. After incubation, 30 µL of resazurin dye (0.01%) (Sigma Aldrich) was added to each well and microplates were again placed in an incubator for 3-4 hours. The colour change from blue to pink is considered as evidence of bacterial growth. This way the minimum inhibitory concentration (MIC) is considered as the concentration after which the tested compounds do not inhibit this change in colour. MIC of the studied compound was compared with the MIC of ampicillin.

## 3. Results and discussion

### Chemical synthesis.



### Scheme 1. Synthesis scheme of novel salicylaldehyde-based azomethine

Salicylaldehyde and 2,2-(ethylenedioxy)bis(ethylamine), aliphatic polyamine with heteroatoms, performed a simple condensation process to synthesise the molecule. The primary advantage of the reaction is that it may occur in a media without catalyst, and the formed chemicals don't need to be purified using a specific technique like column chromatography.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy was used to determine the structure of the synthesised molecule. A thorough examination of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra showed the absence of any signals associated with amine or aldehyde groups. This demonstrates that the salicylaldehyde aldehyde group condensed with the main amine group of the heteroatom-contained aliphatic polyamine. Furthermore, the azomethine (CH=N) group's signal is seen in the 8–8.5 ppm range in  $^1\text{H}$  NMR spectra and the 160–170 ppm range in  $^{13}\text{C}$  NMR spectra.

The modified Hummer process was then used to synthesise the graphene oxide nanolayers, which were then examined by PXRD (Fig. 3) and SEM (Fig. 4) analyses.

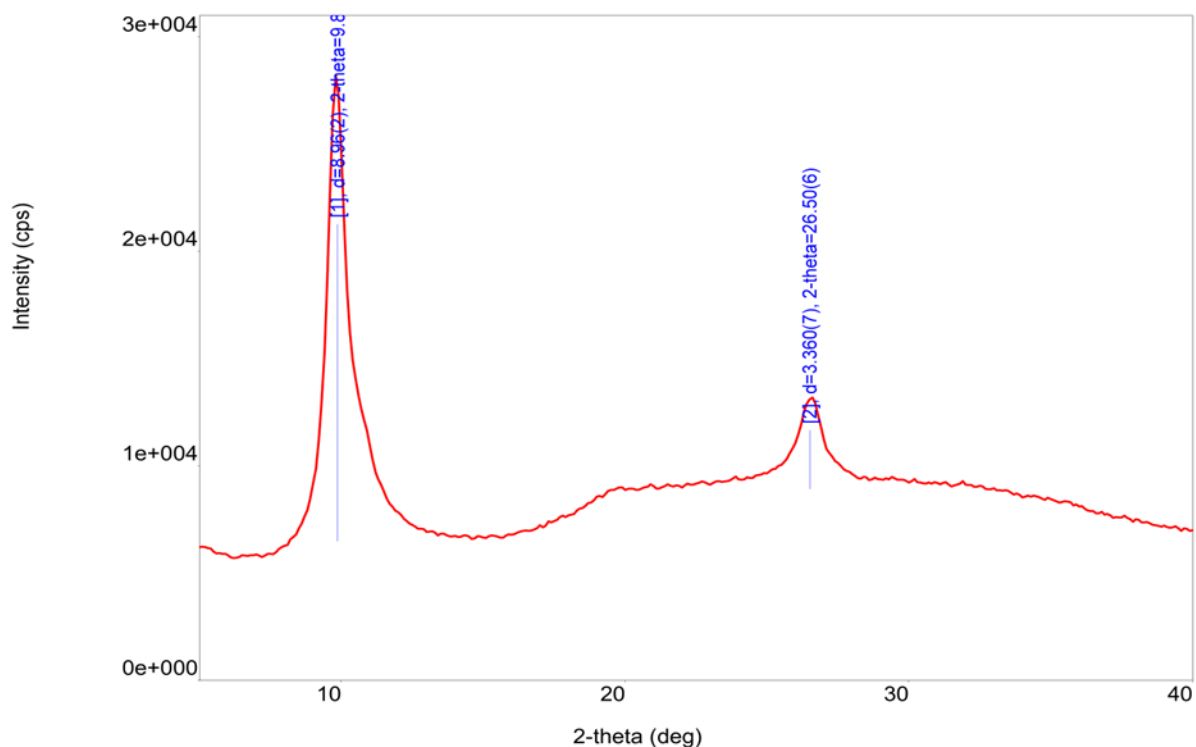


Fig. 3. Powder XRD spectrum of graphene oxide nanolayers.

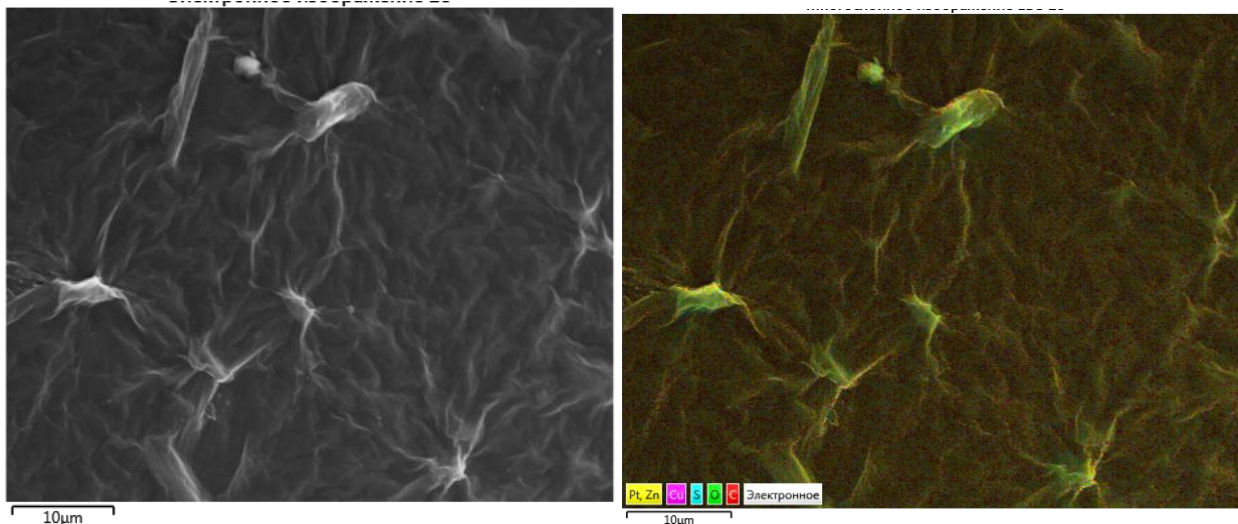


Fig. 4. SEM images of graphene oxide nanolayers.



Fig. 5. Elemental analysis data of graphene oxide nanolayers.

The diameter of the layer has been determined to be 1 nm by SEM examinations.

Furthermore, elemental analysis (Figure 5) verifies that graphene oxide nanolayers are formed throughout this process.

Additionally, azomethine solution (1 mg/mL) was added after graphene oxide solution (1 mg/mL) was sonicated, and this enabled for the receive of the supramolecular ensemble. Given that Schiff bases have

antimicrobial activity, it was determined to investigate the impact of adding graphene oxide (in an ensemble). Antimicrobial analyses of Schiff base and individual ensembles were thus carried out. The data that were obtained were compared to the ampicillin activity results. The results of antibacterial activity were demonstrated in the table. According to results, graphene oxide nano layer enhance activity of azomethine and increase the releasing rate for the test compound. Table shows that when compared to pure azomethine and ampicillin, the ensemble exhibits greater activity in case of *E.coli*, *P.aeruginosa* and *K.pneumoniae*. In case of *S.aureus* Azomethine+GO (32 µg/mL) showed better activity (128 µg/mL) compare with the azomethine.

**Table.** Minimum inhibitory concentration (µg/mL) of test compounds.

Investigated Compounds	Bacterial Strains			
	<i>E.coli</i>	<i>S.aureus</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>
Azomethine	256	128	128	512
Azomethine+GO	32	32	16	256
Ampicillin	64	16	512	512

#### 4. Conclusions

A straightforward condensation procedure was used to develop the new azomethine derivative based on a salicylaldehyde scaffold and heteroatom-contained aliphatic amine. A potential advantage of this procedure is that it doesn't require a catalyst to carry out the reaction or a specific purification method for the substance that is received, such as column chromatography. We obtained new ensembles based on azomethine and graphene oxide, which were produced using a modified Hummer technique. Novel ensembles based on azomethine and graphene oxide, synthesised using the modified Hummer technique, were received. Although azomethine has antibacterial activity, it was determined to investigate how its biological properties will alter in the context of the supramolecular ensemble. Antimicrobial activity studies on *K.pneumoniae*, *P.aeruginosa*, *A.baumannii* and *S.aureus* revealed that supramolecular ensemble (Schiff base+graphene oxide) outperformed pristine Schiff base and Ampicillin.

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