MOLECULAR COMPLEXES OF GLYCYRRHIZIC ACID MONOAMMONIUM SALT WITH CEPHALOSPORIN ANTIBIOTICS

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Abstract: This article presents the preparation and spectral analysis of complexes formed between the monoammonium salt of glycyrrhizic acid (MASGA) and antibiotics of the cephalosporin class (cefazolin, cefuroxime, ceftriaxone, cefepime). The complexes were obtained at molar ratios of 2:1 and 4:1, and their physicochemical properties were studied using ultraviolet (UV) and infrared (IR) spectroscopy, along with melting point measurements. Spectral analyses confirmed the presence of intermolecular interactions between functional groups (–OH, –COO^-, and –NH_4^+ of MASGA and –C=O, –NH of the antibiotics). The melting point data indicated that higher values compared with the initial substances reflect increased stability of the complexes, while lower values suggest the formation of a new phase. This study may serve as a basis for the development of new antibiotic combinations.

Keywords: glycyrrhizic acid, cephalosporins, supramolecular complex, IR spectrum, UV spectrum, liquidus temperature.

Annotatsiya: Ushbu maqolada glitsirrizin kislotasi monoammoniyli tuzining (GKMAT) sefalosporin sinfi antibiotiklari (sefazolin, sefuroksim, seftriakson, sefepim) bilan komplekslarining olinishi va spektral tahlili taqdim etilgan. Komplekslar 2:1 va 4:1 molyar nisbatlarida olindi va ularning fizik-kimyoviy xususiyatlari ultrabinafsha (UB) va infraqizil (IQ) spektroskopiyasi usullsri yordamida o‘rganildi hamda suyuqlanish haroratlari o’lchandi. Komplekslarning spektral tahlillari funksional guruhlararo **(GKMAT dagi –OH, $–COO^-$ va $–NH_4^+$ hamda antibiotiklardagi –C=O, –NH)** o‘zaro ta’sir mavjudligini tasdiqladi. Suyuqlanish harorati natijalari esa komplekslarning dastlabki moddalar bilan taqqoslaganda yuqoriroq bo‘lishi ularning barqarorligini, pastroq bo’lishi esa yangi faza hosil bo’lishini ko‘rsatdi. Ushbu tadqiqot antibiotiklarning yangi kombinatsiyalarini ishlab chiqish uchun asos bo‘lishi mumkin.

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Kalit so‘zlar: *glitsirrizin kislotasi, sefalosporinlar, supramolekulyar kompleks, IQ-spektri, UB-spektri, suyuqlanish haroratiю*

Аннотация: В данной статье представлены результаты получения и спектрального анализа комплексов, образованных моноаммонийной солью глицирризиновой кислоты (МАГК) с антибиотиками из класса цефалоспоринов (цефазолин, цефуроксим, цефтриаксон, цефепим). Комплексы были получены в мольных соотношениях 2:1 и 4:1, а их физико-химические свойства исследованы с использованием ультрафиолетовой (УФ) и инфракрасной (ИК) спектроскопии, а также методом определения температуры плавления. Спектральный анализ подтвердил наличие межмолекулярных взаимодействий между функциональными группами (–OH, $–COO^-$ и $–NH_4^+$ МАГК и –C=O, –NH антибиотиков). Данные о температуре плавления показали, что её повышение по сравнению с исходными веществами указывает на возрастание стабильности комплексов, тогда как понижение свидетельствует о формировании новой фазы. Настоящее исследование может служить основой для разработки новых комбинаций антибиотиков.

Ключевые слова: глицирризиновая кислота, цефалоспорины, супрамолекулярный комплекс, ИК-спектр, УФ-спектр, температура плавления.

Introduction

Antibiotics remain among the most effective agents in combating bacterial infections. Nevertheless, the rapid emergence of antibiotic resistance in recent years has become a major global public health concern, underscoring the urgent need for new strategies to enhance antibiotic efficacy. The monoammonium salt of glycyrrhizic acid (MASGA) has attracted considerable attention as a biologically active compound with the potential to improve drug performance. Previous studies have shown that its complexation with poorly water-soluble drugs can increase their solubility, stability, and therapeutic effectiveness. Complex formation between MASGA and antibiotics may substantially alter their chemical and physical properties, primarily through coordination and hydrogen bonding interactions, thereby conferring new functional characteristics. Such complexes could represent a promising approach for improving the biological activity and antibacterial effectiveness of conventional antibiotics.

ANALYSIS OF THE RELEVANT LITERATURE

Glycyrrhizinic acid (GA) and its monoammonium salt (MASGA) have been widely studied as biologically active triterpene saponins. Their various pharmacological properties, including antibacterial [1], antiviral [2], anti-

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inflammatory [3], anti-cancer [4], and immunomodulatory [5] effects, have been reported in many sources [6]. Chen et al. reported that GA exhibits broad-spectrum antibacterial properties by inhibiting bacterial enzymes, altering cell membrane permeability, and blocking antibiotic efflux channels, thereby promoting antibiotic accumulation and enhancing infection control [7]. According to their data, GA is effective against pathogens such as *Klebsiella pneumoniae*, *Helicobacter pylori*, *Streptococcus mutans*, and *Pseudomonas aeruginosa*, and may have significant activity against antibiotic-resistant strains [7]. At present, the combined application of glycyrrhizic acid (GA) with antibiotics is also being explored as a promising strategy. Singh et al. demonstrated that a synergistic mixture of GA and norfloxacin exhibited potent bactericidal activity against *Staphylococcus aureus* strain that had developed high levels of resistance to multiple antibiotics, primarily through the enhanced generation of reactive oxygen species [8]. Their study further showed that GA significantly reduced the minimum inhibitory concentration of the antibiotic while inducing damage to bacterial DNA. These findings highlight the role of GA as a "bioenhancer" and confirm its potential to improve the efficacy of conventional antibiotics.

Cephalosporins are β-lactam compounds that are widely used in clinical practice. Representatives of the first through fifth generations exhibit broad-spectrum biological activity, being effective against both Gram-positive and Gram-negative bacteria [9]. Recent studies suggest that the combined use of cephalosporins with biologically active natural compounds offers new opportunities to combat antibiotic-resistant strains [10]. In this context, investigations into the supramolecular complexes of the monoammonium salt of glycyrrhizic acid (MASGA) with cephalosporin antibiotics (cefazolin, ceftriaxone, cefepime, and cefuroxime) are of considerable relevance in the fields of pharmaceutical chemistry and drug modification. Furthermore, UV and IR spectroscopy are widely employed to evaluate the stability of supramolecular complexes and to elucidate the complexation process. Literature reports indicate that an increase in the $\Delta\nu$ value (the difference between asymmetric and symmetric stretching vibrations of carboxylate groups), together with shifts in the stretching vibrations of carbonyl groups in IR spectra, as well as bathochromic or hypsochromic effects observed in UV spectra, serve as key indicators of complex formation [11]. Such results provide strong evidence for the formation of true molecular complexes, distinguishing them from simple mechanical mixtures.

The above scientific sources indicate that the formation of stable complexes of MASGA and antibiotics in different molar ratios can increase not only the chemical structure, but also the antibacterial activity. Therefore, the formation of

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supramolecular complexes of MASGA with β-lactam antibiotics, especially representatives of the cephalosporin class, may be a promising direction in the development of new combinatorial approaches to combat antibiotic-resistant microorganisms.

RESEARCH METHODOLOGY

This study was aimed at investigating the process of complex formation between the monoammonium salt of glycyrrhizic acid (MASGA) and selected antibiotics. Molecular complexes of MASGA with the antibiotics were obtained at two molar ratios (2:1 and 4:1), and several of their physicochemical parameters were determined. The melting points of the resulting complexes were measured using a PTPTU 25-11-1144 instrument. UV spectra were recorded in the range of 200–400 nm with Shimadzu UV-1900i and 1280 spectrophotometers, using quartz cuvettes with a path length of 1 cm. The observed spectral shifts (bathochromic or hypsochromic) provided insight into the formation of the complexes and the nature of their intermolecular interactions. Infrared (IR) spectra were obtained with a Shimadzu IRTracer-100 Fourier transform infrared spectrophotometer (Japan) in the range of 4000–400 cm^{-1}. IR spectroscopy enabled the identification of characteristic vibrations of major functional groups, as well as the detection of hydrogen bonding and intermolecular interactions within the complexes.

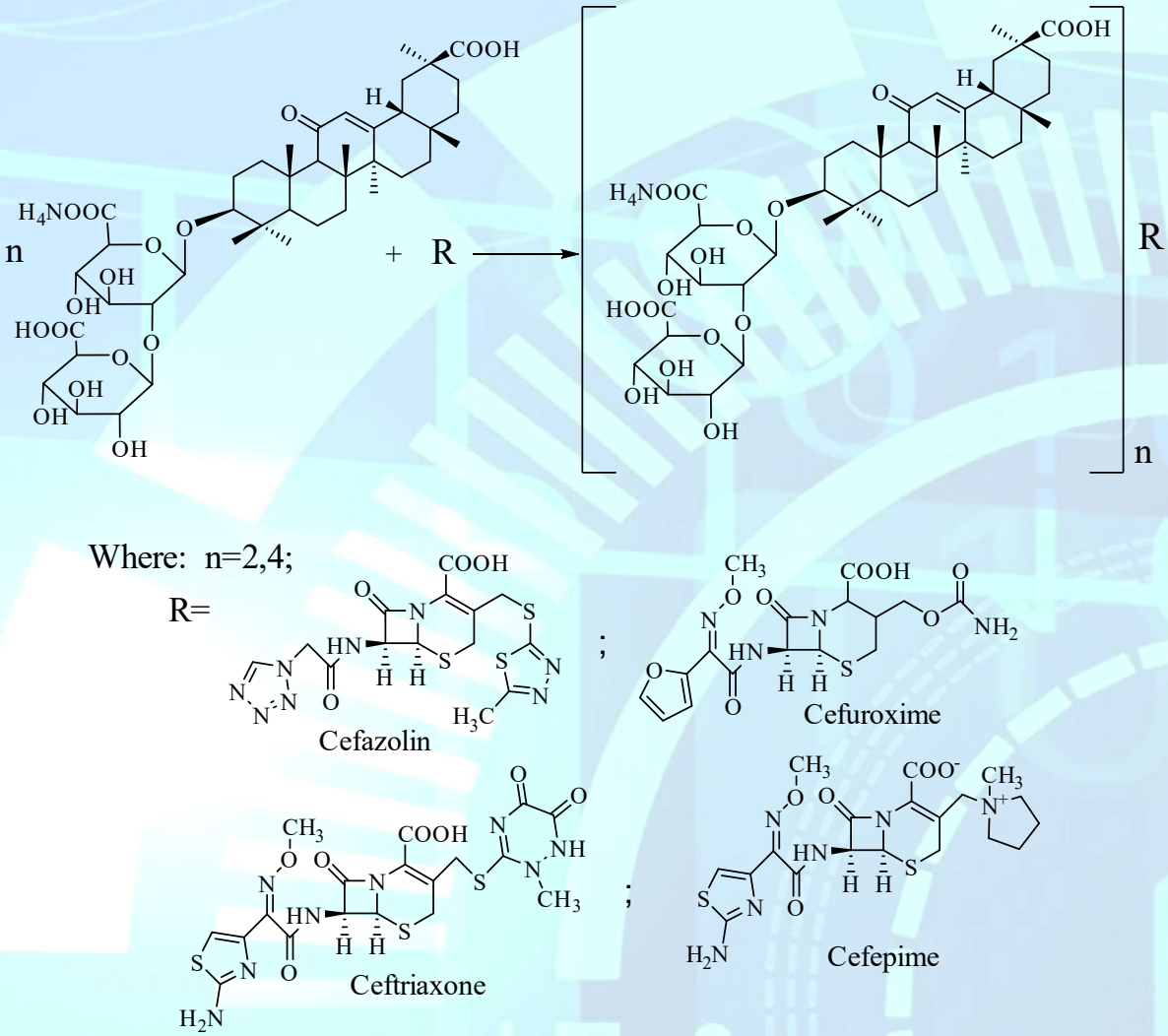
Preparation of MASGA: Cefazolin 2:1 complex.

In a 50 ml flat-bottomed flask, 1 mmol MASGA (0.840 g) and 25 ml of 50% ethanol were mixed using a magnetic stirrer. After the MASGA was completely dissolved, 0.5 mmol cefazolin (0.238 g) was added to it and stirred at room temperature for 5-6 hours with a magnetic stirrer. Then, the ethanol in the mixture was removed in a rotary evaporator, and the remaining aqueous portion was dried by lyophilization. Yield 0.96 g (89.1%). T.liquid.= 180-182°C.

RESULTS AND DISCUSSION

Complexes of MASGA with cephalosporin class antibiotics (cefazolin, cefuroxime, ceftriaxone, cefepime) were obtained according to the general scheme presented below.

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Scheme 1. General scheme for obtaining complexes of MASGA with antibiotics

Table-1

Some physicochemical parameters and spectral (UV, IR) data of complexes of MASGA with antibiotics

№	Complexes	T.liquid. °C	UV spectrum (ethanol: water), λ_{max}, nm (lgε)	Fundamental vibrational frequencies in the IR spectrum, cm^{-1}
1.	Cefazolin	170±2	271 (4,91)	ν(NH, OH)=3291, ν(CH, CH_2, CH_3)=2956, ν(C=O, β-lactam)=1754, ν(C=O, amide)=1678, ν(COO^-, asim.)=1599, δ(NH, CH)=1542, δ(CH_2, CH_3)=1483, ν(COO^-, sim.)=1380, δ(CH)=1357, 1319, 1280, ν(C–N, C–

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				O)=1240, ν(C–OH)=1098, 1062, δ(=CH)=979, ν(C–S)=653
2.	MASGA: Cefazolin 2:1	180±2	259,5 (4,75)	ν(NH, OH)=3231, ν(CH, CH_2, CH_3)=2925, ν(C=O, β-lactam)=1753, ν(C=O,)=1693, ν(amide I)=1648, ν(COO^-, asim.)=1595, δ(CH_2, CH_3)=1499, δ(NH_4^+,amide II)=1443, ν(COO^-, sim.)=1398,1387, δ(CH)=1365, 1263, ν(C–N, C–O)=1210,1171, ν(C–OH)=1032, δ(=CH)=979, ν(C–S)=649
3.	MASGA: Cefazolin 4:1	180±2	259 (4,72)	ν(NH, OH)=3192, ν(CH, CH_2, CH_3)=2925, ν(C=O, β-lactam)=1711, ν(C=O,)=1701, ν(amide I)=1654, ν(COO^-, asim.)=1592, δ(CH_2, CH_3)=1499, δ(NH_4^+,amide II)=1440, 1413, ν(COO^-, sim.)=1385, δ(CH)=1362, 1265, ν(C–N, C–O)=1212,1170, ν(C–OH)=1038, δ(=CH)=979, ν(C–S)=651
4.	Ceftriaxone	200±2	242 (5,17)	ν(NH, OH)=3433, 3252, ν(CH, CH_2, CH_3)=2935, ν(C=O, β-lactam)=1734, ν(C=O, amide)=1644, ν(COO^-, asim.)=1595, δ(NH, CH)=1530, δ(CH_2, CH_3)=1499, ν(COO^-, sim.)=1394, δ(CH)=1361, 1286, ν(C–N, C–O)=1236, 1184, ν(C–OH)=1102, 1029, δ(=CH)=969, 886 ν(C–S)=728
5.	MASGA: Ceftriaxone 2:1	150±2	258,5 (4,74)	ν(NH, OH)=3201, ν(CH, CH_2, CH_3)=2933, ν(C=O, β-lactam)=1752, ν(C=O, amide I)=1632, ν(COO^-, asim.)=1594, δ(CH_2, CH_3)=1507, δ(NH_4^+,amide II)=1451, ν(COO^-, sim.)=1394, δ(CH)=1359, 1282, ν(C–N, C–O)=1210,1168, ν(C–OH)=1032, δ(=CH)=982, ν(C–S)=660
6.	MASGA: Ceftriaxone 4:1	180±2	258,5 (4,69)	ν(NH, OH)=3209, ν(CH, CH_2, CH_3)=2933, ν(C=O, β-lactam)=1690, ν(C=O, amide I)=1622, ν(COO^-,

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				asim.)=1596, δ(CH_2, CH_3)=1513, δ(NH_4^+,amide II)=1431, ν(COO^-, sim.)=1390, δ(CH)=1359, 1259, ν(C–N, C–O)=1212, 1167, ν(C–OH)=1029, δ(=CH)=977, ν(C–S)=687
7.	Cefepime	180±2	237 (4,85)	ν(NH, OH)=3186, 3051, ν(CH, CH_2, CH_3)=2936, ν(C=O, β-lactam)=1767, ν(C=O, amide I)=1651, 1632, ν(COO^-, asim.)=1566, δ(CH_2, CH_3)=1548, δ(amide II)=1418, ν(COO^-, sim.)=1388, δ(CH)=1321, ν(C–N, C–O)=1266, 1176, ν(C–OH)=1038, δ(=CH)=904, ν(C–S)=693
8.	MASGA: Cefepime 2:1	200±2	257,5 (4,64)	ν(NH, OH)=3282, ν(CH, CH_2, CH_3)=2958, ν(C=O, β-lactam)=1757, ν(C=O, amide I)=1678, ν(COO^-, asim.)=1596, δ(CH_2, CH_3)=1540, δ(NH_4^+,amide II)=1490, ν(COO^-, sim.)=1374, δ(CH)=1355, ν(C–N, C–O)=1237,1180, ν(C–OH)=1098, 1061, δ(=CH)=1008, ν(C–S)=781
9.	MASGA: Cefepime 4:1	165±2	258 (4,84)	ν(NH, OH)=3209, ν(CH, CH_2, CH_3)=2933, ν(C=O, β-lactam)=1724, ν(C=O, amide I)=1617, ν(COO^-, asim.)=1588, δ(CH_2, CH_3)=1540, δ(NH_4^+,amide II)=1454, ν(COO^-, sim.)=1391, δ(CH)=1348, ν(C–N, C–O)=1213,1166, ν(C–OH)=1028, δ(=CH)=982, ν(C–S)=656
10.	Cefuroxime	180±2	257 (5,21)	ν(NH, OH)=3527, 3364, 3245, ν(CH, CH_2, CH_3)=2932, ν(C=O, β-lactam)=1754, ν(C=O, amide I)=1696, 1665, ν(COO^-, asim.)=1625, δ(CH_2, CH_3)=1543, δ(amide II)=1482, ν(COO^-, sim.)=1397, δ(CH)=1328, ν(C–N, C–O)=1243, 1153, ν(C–OH)=1046, δ(=CH)=980, ν(C–S)=749

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11.	MASGA: Cefuroxime 2:1	180±2	262 (4,76)	ν(NH, OH)=3202, ν(CH, CH_2, CH_3)=2921, ν(C=O, β-lactam)=1746, ν(C=O, amide I)=1675, ν(COO^-, asim.)=1655, δ(CH_2, CH_3)=1591, δ(NH_4^+,amide II)=1443, ν(COO^-, sim.)=1388, δ(CH)=1324, ν(C–N, C–O)=1210,1163, ν(C–OH)=1032, δ(=CH)=977, ν(C–S)=751
12.	MASGA: Cefuroxime 4:1	170±2	258,5 (4,93)	ν(NH, OH)=3192, ν(CH, CH_2, CH_3)=2928, ν(C=O, β-lactam)=1746, ν(C=O, amide I)=1690, ν(COO^-, asim.)=1637, δ(CH_2, CH_3)=1588, δ(NH_4^+,amide II)=1437, ν(COO^-, sim.)=1407, δ(CH)=1325, ν(C–N, C–O)=1212,1160, ν(C–OH)=1041, δ(=CH)=982, ν(C–S)=745
13.	MASGA	190±2	258,5 (4,7)	ν(NH, OH)=3209, ν(CH, CH_2, CH_3)=2923, 2865, ν(C=O,)=1700, ν(COO^-, asim.)=1645, 1586, δ(CH_2, CH_3)=1447, δ(NH_4^+)=1411, ν(COO^-, sim.)=1384, δ(CH)=1351, 1258, ν(C–O)=1216,1170, ν(C–OH)=1038, δ(=CH)=983.

Based on the changes in the fundamental vibrational frequencies of the functional groups in the IR spectra of the starting materials, it is possible to determine what types of interactions occur in the formation of MASGA, cefazolin, and their complex in a 2:1 molar ratio.

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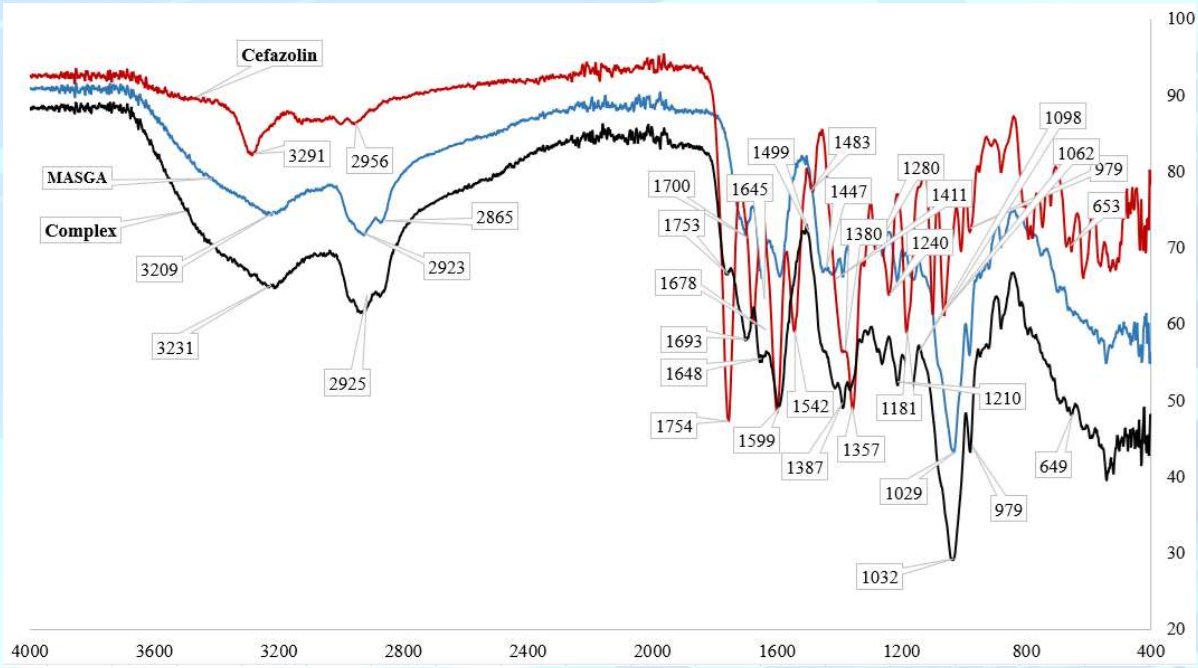


Figure 1. IR spectra of MASGA, Cefazolin, and MASGA: Cefazolin 2:1 complex

In the IR spectrum at 3231 cm^{-1}, a broad shoulder-shaped vibrational frequency was observed, indicating the formation of hydrogen bonds (-O–H….N–H) between the hydroxyl and amide groups in the MASGA and cefazolin molecules. In addition, a slight decrease in the Δν value between the asymmetric and symmetric vibrations in the carboxylate groups indicates the presence of ion-pair or partial hydrogen bonding. At the same time, the vibrational frequency corresponding to the carbonyl group (C=O) in the β-lactam ring (1753 cm^{-1}) remained almost unchanged, indicating that the pharmacologically active center of the antibiotic was preserved.

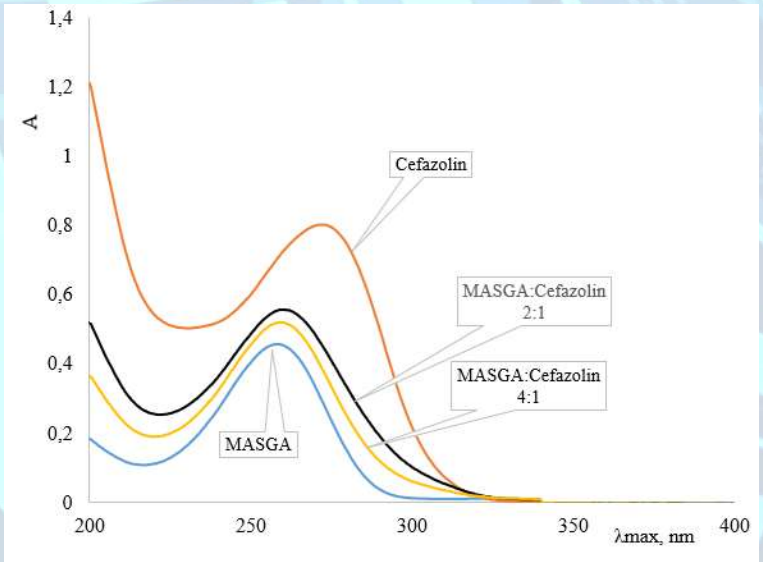


Figure 2. UV spectra of MASGA, Cefazolin, and MASGA: Cefazolin 2:1, 4:1 complexes

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Analysis of the UV spectra of these compounds showed the following results. In the spectrum of pure cefazolin, absorption maxima corresponding to n→π* electronic transitions were recorded in the regions of 202 and 237 nm, and to π→π* electronic transitions around 281 nm. These are electronic transitions characteristic of aromatic and carbonyl groups in the antibiotic molecule. In the spectrum of MASGA, the main absorption maximum appeared around 252–255 nm. This is the result of n→π* electronic transitions associated with the conjugated double bond located in the "C" ring in the aglycone part of MASGA. In the UV spectrum of the MASGA: Cefazolin 2:1 complex, an absorption maximum was observed around ~260 nm, which showed "bathochromic" shifts relative to the absorption maximum of MASGA (258 nm) and "hypsochromic" shifts relative to cefazolin (281 nm). These changes indicate a change in the electronic environment during intermolecular interactions between MASGA and cefazolin molecules.

CONCLUSION AND RECOMMENDATIONS

In conclusion, many molecular complexes of MASGA with cephalosporin class antibiotics (cefazolin, cefuroxime, ceftriaxone, cefepime) in molar ratios of 2:1 and 4:1 were obtained, and some of their physicochemical parameters were determined. In order to determine the nature of the interactions in the process of complex formation, their spectral analysis (UV-, IR-) was carried out. The increase in the Δν value between the asymmetric and symmetric vibration frequencies of carboxylate groups in the IR spectra, the shift of carbonyl vibration frequencies, as well as the observation of "bathochromic" and "hypsochromic" effects in the UV spectra, allowed us to assess the process of complex formation. These data can serve as a scientific basis for analyzing the properties of molecular complexes. These obtained complexes can be one of the promising directions in the development of new methods and strategies to increase the effectiveness of antibiotics in the future.

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