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Dimethyl sulfoxide as a functional agent for antimicrobial drug's transport facilitating: mechanistic study by mass spectrometry

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Electrospray ionization mass spectrometry (ESI MS) study has been performed to examine biologically significant intermolecular interactions between the molecules of dimethyl sulfoxide (DMSO, known as a functional agent for transdermal and transmembrane drug's transfer facilitation) and some antimicrobial drugs. Formation of stable noncovalent complexes of DMSO with the molecules of antibiotics levofloxacin (LEF) and cycloserine (CYS) in the polar solvent methanol has been revealed by ESI MS probing of model binary systems of DMSO with the mentioned drugs. At the same time ESI MS investigation of the similar model systems containing DMSO and antimicrobial chemotherapeutic preparation decamethoxinum (DEC) has shown that any peaks of the noncovalent complexes between DMSO and DEC are not recorded in the mass spectra, that points to the dependence of DMSO-drug complexation peculiarities on the drug's structure. The data of ESI MS examining of DMSO+dipalmitoylphosphatidylcholine model system reveal that the DMSO molecules also do not form stable noncovalent clusters with the membrane phospholipid molecules in the polar surrounding. The obtained results as to formation of stable noncovalent complexes of DMSO with LEF and CYS in the polar solvent are proposed to be considered as one of the possible molecular mechanisms of action of DMSO as transdermal and transmembrane penetration enhancer in drug delivery. The current study confirms the ESI MS method applicability to examining the DMSO complexation with the drugs molecules and biomolecules with the purpose to predict the DMSO usage efficiency as an agent for the drug's transdermal and transmembrane transport facilitating.

Keywords: dimethyl sulfoxide, antibiotics, electrospray ionization mass spectrometry, noncovalent complexes, transmembrane transfer facilitation.

Диметилсульфоксид як функціональний агент, що сприяє доставці молекул антимікробних ліків: дослідження механізмів методом мас-спектрометрії. *В.А.Пашинська, М.В.Косевич, А.Гомор, Л.Драхош*

Метод мас-спектрометрії (МС) з іонізацією електророзпиленням (ІЕР) застосовано для вивчення біологічно значущих міжмолекулярних взаємодій між молекулами диметилсульфоксиду (ДМСО, відомий як функціональний агент для сприяння трансдермальному та трансмембранному переносу ліків) та молекулами ряду антимікробних препаратів. В ході ІЕР МС досліджень модельних бінарних систем, що містили ДМСО та антибіотики левофлоксацин (ЛЕФ) або циклосерин (ЦИС), встановлено формування стабільних нековалентних комплексів молекул ДМСО з молекулами ЛЕФ та ЦИС в полярному розчиннику метанолі. В той же час, вимірювання методом ІЕР МС подібних модельних систем, що містили ДМСО та антимікробний хіміотерапевтичний препарат декаметоксин (ДЕК), показали, що отримані мас-спектри не містять будь-яких піків нековалентних комплексів ДМСО з ДЕК. Такі результати вказують на залежність

спроможності формування стабільних нековалетних комплексів ДМСО-лікарська молекула від структури молекул ліків. Дані, що отримано в ході ІЕР МС дослідження модельної системи ДМСО+дипальмітоїлфосфатидилхолін, показують, що стабільні нековалентні кластери молекул ДМСО та цього мембранного фосфоліпіду також не формуються в полярному середовищі. Результати щодо формування стабільних нековалентних комплексів молекул ДМСО та антибіотиків ЛЕФ та ЦИС в полярних розчинниках дозволяють запропонувати таке комплексоутворення в якості одного з можливих молекулярних механізмів дії ДМСО як агенту, що сприяє трансдермальному та трансмембранному переносу лікарів. Дане дослідження підтверджує ефективність застосування методу ІЕР МС для вивчення формування нековалентних комплексів між молекулами ДМСО та лікарськими або біологічними молекулами з метою прогнозування потенціальної можливості використання ДМСО у якості агенту, що полегшує трансдермальну та трансмембранну доставку ліків.

1. Introduction

Development and study of functional materials and substances for drugs delivery and drugs transport facilitation attract significant interest of biomedicine related researchers in recent years. A number of biomaterials providing the effective drugs delivery were proposed [1], including substances employed for transdermal and transmembrane drugs transfer facilitation and more rapid and efficient pass of the medicinal molecules within an organism and to targeting organs and cells. Such substances assisting, in particular, the antimicrobial drugs transportation into the cell are examined to enhance the drug's usage efficiency and safety, to decrease the dose and time of treatment by the applied medications, which is important for antibiotics.

One of the possible ways to facilitate a drug molecule admission into the target biological object is when penetration enhancing agent molecules can form supramolecular complexes with the drug molecules to be delivered [2]. Under this way of admission, the facilitating agent works as a carrier or vehicle [1]. Understanding the role of clusters and noncovalent biocomplexes formation in molecular mechanisms of facilitating the transdermal and transmembrane drugs transfer can stimulate the progress in the modern molecular biophysics, functional materials science and medicine areas related to the drug delivery problem.

Basing on our experience in the soft ionization mass spectrometric techniques application to studies of intermolecular interactions of some drugs with target biomolecules [3, 4] and modulating drugs activity agents [5, 6], we propose an idea that model mass spectrometric experiments can enlighten molecular mechanisms of action of the functional agents facilitating transmembrane drug delivery. As one of the potential mechanisms we consider the formation of stable noncovalent complexes (formally regarded as clusters) between the molecules of the facilitating agent and the drug molecules to be transported through the cell membrane. In the framework of this idea we decided to perform the electrospray ionization mass spectrometry (ESI MS) study devoted to examining the clusters formation between dimethyl sulfoxide (DMSO) which is known as a transdermal and transmembrane penetration enhancer in drug transportation [7] and some antimicrobial drugs with different structures: antibiotic levofloxacin (LEF), anti-tuberculosis agent cycloserine (CYS) and antimicrobial chemotherapeutic preparation decamethoxinum (DEC).

Several assumptions concerning the mechanisms of DMSO biological action and in particular, its interaction with cell membrane structures, supported by molecular dynamics simulations can be found in the scientific literature [8–17]. It was established that the effect of DMSO on phospholipid membranes depends on its concentration. At relatively low concentrations DMSO replaces water molecules near the hydrophilic polar heads of phospholipids causing their dehydration [13, 14], which results in destabilization of the membrane native structure. However, DMSO molecules do not form stable complexes with the phospholipids headgroups at the membrane-water interface [8, 13]. DMSO penetrates into the phospholipid bilayer and diffuses freely within the hydrophobic interior. The most pronounced effect significant for membrane permeability is observed at higher DMSO concentrations and consists in creation of pores within the bilayer. Amphiphilic DMSO molecules in such pores are turned by their hydrophobic side to hydrophobic hydrocarbon tails of phospholipids, while hydrophilic parts of DMSO provide hydrophilic channel accessible to water molecules [11]. Further increase of DMSO content in the system may cause destruction

and disintegration of the lipid bilayer. DMSO-induced change in permeability of phospholipid model membranes was investigated by experimental methods as well, in particular, by means of differential scanning calorimetry [18].

While the DMSO-induced poration of lipid bilayers is actively studied, modeling of the transport of any compounds facilitating by DMSO, to the best of our knowledge, is limited to monoatomic ions [19] and a possible role of DMSO as a vehicle for drug molecules was not paid noticeable attention.

In relation to our subject of biologically-significant intermolecular interactions study under the conditions of ESI MS, two tasks of the current study can be formulated basing on the above information. The first task is to examine the formation of stable noncovalent complexes of DMSO with a number of antimicrobial drug molecules, that may serve as a proof of suggested molecular mechanism of the facilitating role of DMSO in the drug's transmembrane and transdermal transport. And the second task is to study intermolecular interactions of DMSO molecules with the molecules of phospholipid component of cell membranes, dipalmitoylphosphatidylcholine (DPPC), using ESI MS.

2. Materials and methods

2.1. Objects of investigations

The objects of study are dimethyl sulfoxide (DMSO, C_2H_5OS, *MW* = 78.13 Da) and a number of antimicrobial drugs with different structure: antibiotic levofloxacin (LEF, $C_{18}H_{20}FN_3O_4$, *MW* = 361.37 Da), anti-tuberculosis drug cycloserine (CYS, $C_3H_6N_2O_2$, *MW* = =102.09 Da), and antimicrobial preparation decamethoxinum (DEC, $C_{38}H_{74}N_2O_4Cl_2$, *MW*(average) = 693.92 Da, monoisotopic mass 692.50 Da) (Scheme 1).

Decamethoxinum was synthesized at the Institute of Organic Chemistry of the National Academy of Sciences of Ukraine (Ukraine). Levofloxacin and cycloserine medications were produced by pharmaceutical company "Lekhim" (Ukraine). L-α-dipalmitoylphosphatidylcholine (DPPC, *Mr* = 734.04) was provided by "Alexis Biochemicals" (Switzerland). Methanol (MeOH) was purchased from the Sigma-Aldrich company (Switzerland).

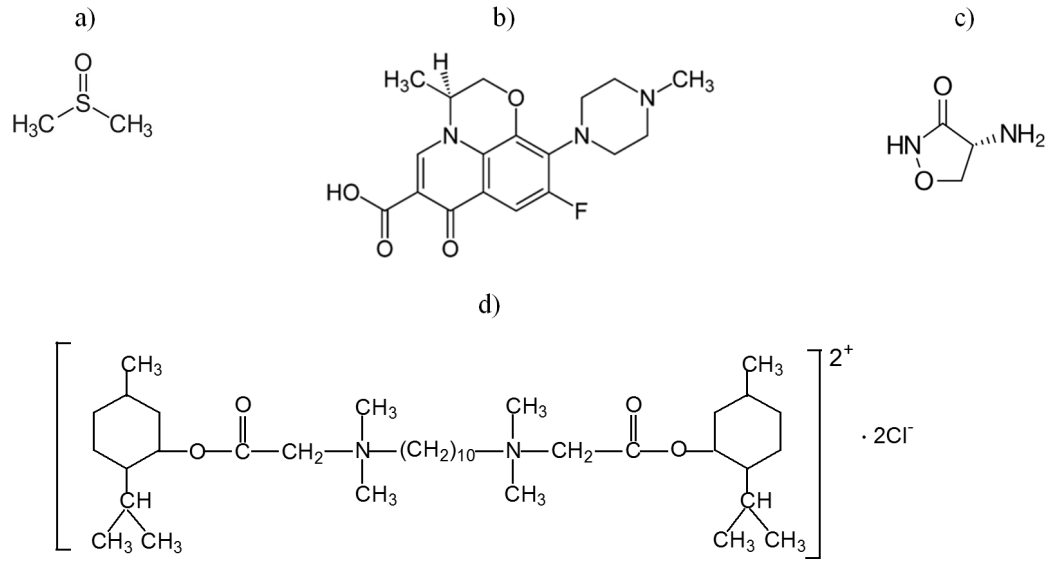
Stock solutions of investigated substances (5 mM) are prepared in methanol (standard for ESI MS polar solvent) and used for preparation of binary model systems. The mixtures are kept at room temperature for at least 10 minutes before the ESI MS analysis. The spraying procedure requires dilution of the solutions to the final 250 mM concentration of the most concentrated component of the model systems in each solution. *2.2. ESI mass spectrometry*

The ESI mass spectra are obtained in the positive ion mode using a triple quadruple (QqQ) Micromass Quattro Micro mass spectrometer (Waters, Manchester, UK) equipped with the electrospray ion source. This source operates in the standard ESI mode. The ESI source temperature is set to 120°C and the desolvation temperature is 200°C. The spraying capillary is operated at 3.5 kV. The cone voltage (CV) value of 10 V is used. The analyzed solution (20 mL) is injected into the mass spectrometer at a constant flow rate of 0.2 mL/min of the methanol solvent. ESI spectra are recorded in the mass/charge range of *m/z* 100-2000. Data acquisition and processing are performed using MassLynx 4.1 software (Waters, Manchester, UK).

3. Results and discussions

With the purpose to examine the idea stated in the Introduction as to a possibility of stable supramolecular complexes formation between the molecules of the studied antimicrobial drugs and facilitating transmembrane transport agent DMSO, we performed the following ESI MS investigations of the model systems containing the drugs and DMSO.

At the first stage of our study the ESI MS probing of model binary systems of (DMSO+LEF) and (DMSO+CYS) (with 1:3 molar ratio, assuming that the medicinal antibiotic components prevailed over facilitating agent DMSO in the model systems) were carried out. Formation of stable noncovalent clusters of DMSO with the molecules of antibiotics LEF and CYS in the polar solvent methanol has been revealed in the experiments on the basis of recording protonated DMSO·drug·H^+ complexes in the ESI mass spectra obtained (Fig. 1 and Fig. 2). The types of observed clusters depend on the composition of the systems studied. Thus, in the (DMSO + CYS) system preferentially protonated clusters are formed (Fig. 2), including DMSO dimers 2DMSO·H^+ (*m/z* 157), and the clusters of the drug with one and two DMSO molecules CYS·DMSO·H^+ (*m/z* 181) and CYS·2DMSO·H^+ (*m/z* 259) respectively. Presence of residual sodium ions



Scheme 1. Chemical structures of the medications under study: a) dimethyl sulfoxide; b) levofloxacin; c) cycloserine; d) decamethoxinum.

in the (DMSO + LEF) system (Fig. 1) is reflected in domination of the Na^+ cation-containing DMSO clusters $DMSO{\cdot}Na^+$ and $2DMSO{\cdot}Na^+$. LEF clusters with DMSO are present both in protonated $LEF{\cdot}DMSO{\cdot}H^+$ (m/z 440) and cationized $LEF{\cdot}DMSO{\cdot}Na^+$ (m/z 462) forms.

Further, the examining of the model system (DMSO + DEC) with different molar ratio of the mixture components was performed. Taking into account that DEC is a salt and after dilution of the medication in the polar solvent (methanol in our experiments) its intact organic dications are available in the solution and have better ability to form the ions under ESI conditions in comparison with DMSO molecules, firstly we probed the (DMSO + DEC) system with 1:1 molar ratio. In the spectrum obtained (Fig. 3a) the peaks of the individual components of the mixture were only recorded: $DMSO{\cdot}Na^+$, m/z 101, I (relative intensity) = 42 %; $2DMSO{\cdot}Na^+$, m/z 179, I = 100 % for DMSO and the dication $DECdic^{2+}$, m/z 311, I = 5 % for DEC. Any peaks of the complexes between DMSO and DEC are not recorded.

The complexation of DMSO with DEC is not revealed in the model system (DMSO + DEC) with higher 1:3 molar ratio too (Fig. 3b). The increasing of the DEC concentration in the model system logically resulted in the changing of the relative intensity of the peaks of the individual components of the mixture (see Fig. 3b): peak of $DECdic^{2+}$, m/z 311 became the most intensive with I = 100 %, comparing with intensity of the peaks of DMSO — $DMSO{\cdot}Na^+$, m/z 101, and $2DMSO{\cdot}Na^+$, m/z 179, which relative intensity decreased less than 20 %. Moreover with the increasing of the DEC concentration in the model system the cluster ion of the dication with one counterion $DECdic{\cdot}Cl^+$, m/z 657 [20], appears in the spectrum, but DMSO is not attached to this type of associates as well.

It is known that DEC dication forms stable complexes with both inorganic and organic anions at the ESI MS conditions [20, 21]. To explain the results obtained in the current ESI MS study we consider that partial negative charge at the oxygen atom of DMSO (regarded as aprotic compound) is not sufficient for its efficient binding with the positively charged alkylammonium groups of DEC and formation of the stable noncovalent complex between DMSO and DEC dication, especially in the presence of Cl^- counterions of DEC salt in the solution. At the same time, presence of proton-donor groups in the LEF and CYS molecules facilitate their hydrogen bonding with proton-acceptor -S=O group of DMSO, that can provide the formation of stable clusters of DMSO with LEF and CYS molecules in solution reflected in the peaks of these protonated clusters in the mass spectra (Fig. 1 and Fig. 2).

The crucial difference of the results obtained for the model systems of DMSO with LEV and CYS (in which the stable clusters of the DMSO with the drugs were revealed)

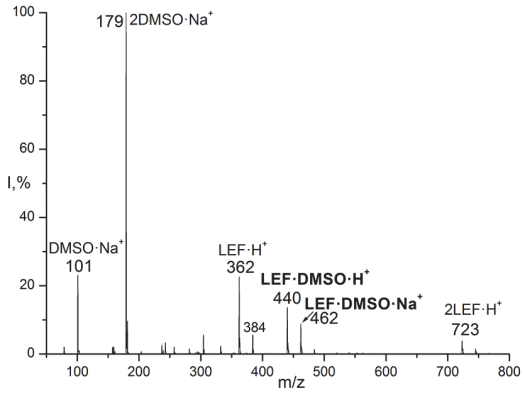


Fig. 1. ESI mass spectrum of the (DMSO + LEF) system (1:3 molar ratio).

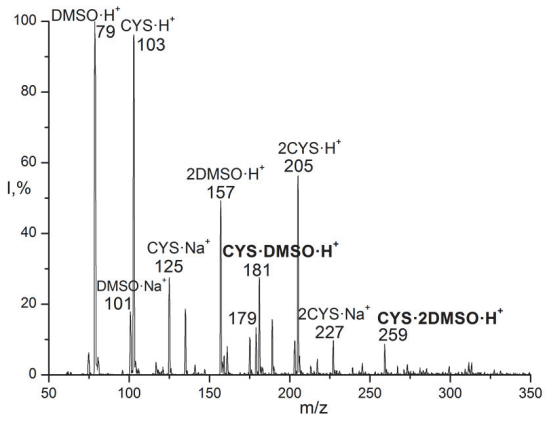


Fig. 2. ESI mass spectrum of the (DMSO + CYS) system (1:3 molar ratio).

from the results obtained for (DMSO + DEC) model system (in which the peaks of the noncovalent complexes between the molecules of the system components were not recorded) points to existence of selectivity in DMSO-drug complexation. Obviously, such selectivity and the noncovalent complexes formation ability and stability are related with the structural features of the molecules of the drugs under study.

To address the second task of our study we also performed the ESI MS examining of DMSO intermolecular interaction with DPPC phospholipid molecules with the purpose of modelling the processes accompanying the DMSO transmembrane and transdermal penetration (see Introduction). The model system of (DMSO + DPPC) (1:3 molar ratio) has been measured in our current ESI MS study. In the obtained spectrum (Fig. 4) the peaks of the individual components of (DMSO + DPPC) mixture are registered: for DMSO — $DMSO{\cdot}Na^+$, m/z 101, I = 54 %, and $2DMSO{\cdot}Na^+$, m/z 179, I = 100 %; for DPPC - $DPPC{\cdot}Na^+$, m/z 756.9, I = 16 % and $2DPPC{\cdot}Na^+$, m/z 1491, I = 3 %. The peaks of noncovalent complexes of DMSO with DPPC are not recorded in the spectrum. Such a result is in a good correlation with the results of other investigations by other methods, pointing to high speed of penetration of DMSO molecules through phospholipid membranes: the penetration of DMSO into the lipid membrane is not hampered by its adsorption at the membrane surface, since DMSO does not form stable complexes with the polar heads of membrane phospholipids [8, 13].

A reason for absence of efficient binding of DMSO with ammonium group of the DPPC polar head may be the same, as discussed above for DMSO-DEC interactions.

4. Conclusions

Formation of stable noncovalent complexes of DMSO with the molecules of antibiotics LEF and CYS in the polar solvent

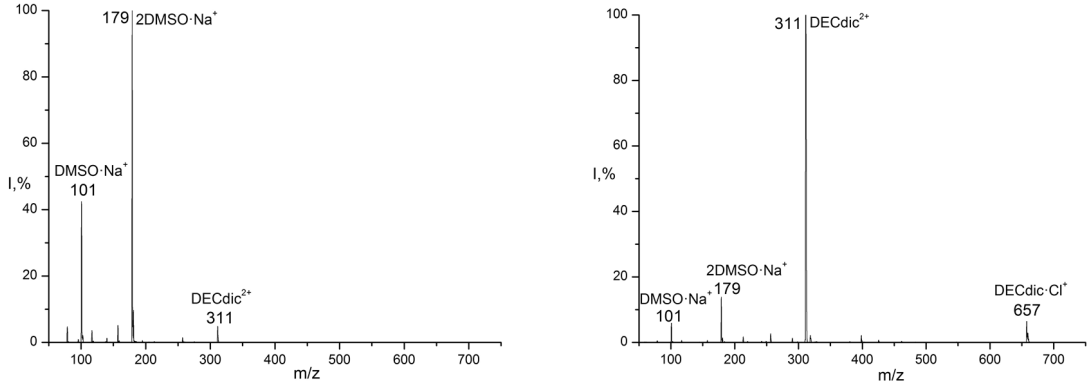


Fig. 3. ESI mass spectra of the (DMSO + DEC) system with different molar ratio of the components: a) 1:1 molar ratio; b) 1:3 molar ratio.

methanol is shown in the current ESI mass spectrometry study. At the same time the ESI MS probing of the similar model systems containing DMSO and antimicrobial drug DEC with different molar ratio demonstrates that peaks of stable noncovalent clusters of DMSO and DEC are not recorded in the mass spectra. Such results point to the dependence of DMSO-drug intermolecular binding peculiarities on the drugs structure. Hydrogen bonding of available proton-donor groups of the LEF and CYS molecules with proton-acceptor group of DMSO can provide the formation of stable clusters of DMSO with LEF and CYS molecules in solution that is confirmed by our ESI MS measurements. At the same time partial negative charge at the oxygen atom of DMSO is not sufficient for its efficient binding with the positively charged alkylammonium groups of DEC dication in the conditions when Cl^- counterions of DEC salt compete with DMSO for the dication binding in the solution. The obtained data as to formation of stable noncovalent complexes of DMSO with LEF and CYS in the polar media are proposed to be considered as one of the possible molecular mechanisms of action of DMSO as transdermal and transmembrane penetration enhancer for the drug delivery.

The results of the ESI MS examining of (DMSO + DPPC) model system testify to the idea that the DMSO molecules do not form stable noncovalent complexes with the DPPC in the polar surrounding. Our experimental data are in a good correlation with the data of other researchers as for fast and easy penetration of DMSO through membrane structures without stable complexation with the membrane molecular components.

The results obtained confirm the ESI MS method applicability to study the DMSO complexation with the drugs molecules and to revealing the molecular mechanisms of DMSO action as a functional agent for the drug?s transdermal and transmembrane transfer facilitation.

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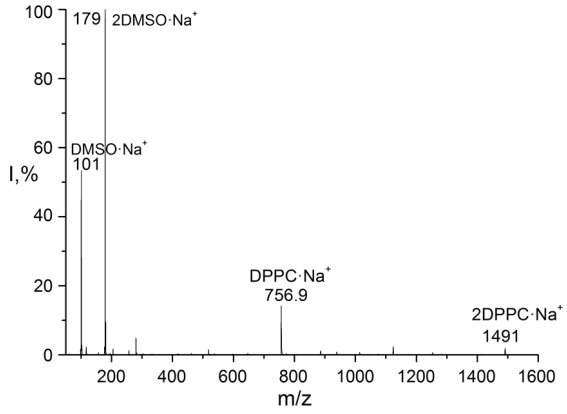

Fig. 4. ESI mass spectrum of the (DMSO + DPPC) system (1:3 molar ratio).

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