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MASS SPECTROMETRY STUDY OF ASCORBYL PALMITATE AS AN AGENT FOR NANOSOMES FORMATION

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Background: Study of properties and intermolecular interactions of biologically active compounds which can be used for the purposes of transmembrane drug delivery is a topical task of modern molecular biophysics. Ascorbyl palmitate (AP) as a fat-soluble form of vitamin C has recently attracted attention as a promising agent for the formation of nanosomes for the "fat insoluble" drug molecules transfer through membranes. However, AP is not sufficiently characterized by up-to-date soft ionization mass spectrometric techniques.

Objectives: The aim of the present work is to characterize AP and its intermolecular interactions by a number of mass spectrometric techniques: Electrospray Ionization (ESI), Laser Desorption/Ionization (LDI) and Matrix-Assisted Laser Desorption/Ionization (MALDI). The comparison of these techniques applicability to the study of AP intermolecular interactions as a drug delivery assisting agent is scheduled.

Methods: ESI mass spectra are obtained with triple quadrupole Micromass Quattro mass spectrometer. LDI and MALDI experiments are performed by Autoflex II mass spectrometer.

Results: In the ESI experiments in the positive ion mode abundant peaks of protonated and cationized AP molecules as well as the peaks of AP clusters $nAP{\bullet}H^+$ and $nAP{\bullet}Na^+$ ($n=2\div4$) are revealed in the mass spectra. This result testifies to the formation of stable noncovalent complexes of the AP molecules in the polar media and confirms the AP ability of formation nanosomes for drug delivery. Analysis of LDI and MALDI mass spectra of AP in positive and negative ion modes shows that in the presence of molecular ions of AP, the peaks of AP dimers or larger AP clusters are not recorded. The ESI probing of the model system containing AP and dipalmitoylphosphatidylcholine (DPPC) reveals stable $AP{\bullet}DPPC{\bullet}H^+$ complex which models the AP intermolecular interactions with the phospholipid components of biomembranes and/or liposomes under AP functioning as a drug delivery assisting agent.

Conclusions: The current study demonstrates the applicability of all tested mass spectrometric techniques for AP identification in solutions and solid phase, while for the purpose of examining of the AP noncovalent complexes formation and study of AP interactions with biomolecules the ESI is defined as the most effective technique.

KEYWORDS: ascorbyl palmitate; nanosomes for drug delivery; dipalmitoylphosphatidylcholine; noncovalent complexes; mass spectrometry; electrospray ionization; laser desorption/ionization; matrix-assisted laser desorption/ionization.

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Ascorbyl palmitate (AP) is a biologically active compound known as “fat-soluble vitamin C” which demonstrates a number of therapeutic activities [1]. Attachment of a moiety of palmitic fatty acid to ascorbic acid aimed at creating its fat-soluble form [2] resulted in the synthesis of AP whose biological activity appeared to be more extended than the expected antioxidant effect inherent to vitamin C [3]. The pathways of AP biological action involve, along with biochemical reactions, noncovalent intermolecular interactions, the latter being of interest to molecular biophysics. Due to these biologically significant intermolecular interactions AP is considered and studied as a promising agent in drug delivery area. Amphiphilic nature and presence of the hydrophobic tail in the structure of AP as a surfactant [4] favour its incorporation into phospholipid membranes [5]. This property of AP molecules is exploited in tailoring of liposomes for drug delivery [6-8], which can be used as vehicles for the “fat insoluble” drug molecules transfer through membrane structures [9, 10]. A group of AP-based and AP-containing vesicles and liposomes termed as “aspasomes” [11, 12] was designed as nanocarriers for dermal and transdermal drugs transfer [13-15]. While an important application of liposomes is delivery of anticancer drugs [16, 17], it was shown that AP itself can exhibit antitumour activity [18, 19]. At the same time, it was reported that AP can protect DNA from X-ray induced damage [20]. Thus, in accordance with the PubChem database [1] AP is classified as an antioxidant, antineoplastic, and antimutagenic agent. A noticeable share of AP applications is associated with cosmetics and dermatology [21], basing on its antioxidant, anti-aging and whitening effects. Promising developments are related to inclusion of AP into various medicinal forms, such as hydrogels [22] or electrospun nanofibers [23]. Taking into account the described above wide spectrum of AP biological activity and biomedical applications, the relevance of AP intermolecular interactions examining is beyond doubt.

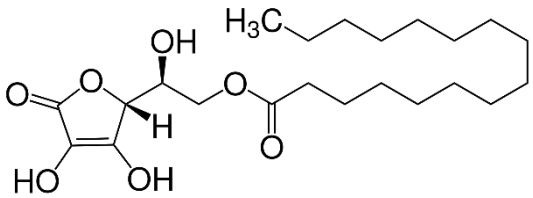
A method of choice for biophysical studies of noncovalent interactions at molecular and supramolecular levels is soft ionization mass spectrometry [24–27]. It permits evaluation not only of pair interactions, but modeling of supramolecular assemblies, such as aggregates of surfactants, micelles [28–32], and fragments of phospholipid membranes [33]. Browsing of the relevant literature has shown that the mass spectrometric data on AP are limited and relate mainly to its identification under synthesis [34]; the data reported for standard electron ionization technique [35] are not informative for biophysics.

Thus, the aim of the present work was to establish mass spectrometric characteristics of AP under Electrospray Ionization (ESI), Laser Desorption/Ionization (LDI) and Matrix-Assisted Laser Desorption/Ionization (MALDI) [36] conditions and to investigate intermolecular interactions of AP related to its functioning as a biologically active compound for the drug delivery applications. Combination of several mass spectrometric techniques provides evaluation of different aspects of AP properties, since ESI technique involves spaying of liquid solutions of biomolecules, while LDI and MALDI ones are based on sputtering of the analytes from the solid state. It was expected that the current study provides information about the different mass spectrometry techniques’ applicability to the following study of AP intermolecular interactions as a drug delivery assisting agent, including interactions of AP molecules with phospholipid components of biological membranes.

MATERIALS AND METHODS

Materials

In our experiments we used ascorbyl palmitate (AP), other names 6-O-palmitoylascorbic acid, ascorbic acid-6-O-palmitate, vitamin C-palmitate [1], whose chemical structure is presented in Scheme 1. Molecular formula of AP is $C_{22}H_{38}O_7$, molecular mass M_r =414.5 g/mol, monoisotopic mass is 414.3 Da.



Scheme 1. Structural formula of ascorbyl palmitate ($C_{22}H_{38}O_7$).

AP was purchased from "Soap4Life" company (Ukraine). Dipalmitoylphosphatidylcholine and solvent methanol were provided by "Sigma-Aldrich" international company. Chemicals for MALDI matrices preparation — α-cyano-4-hydroxycinnamic acid (HCCA) (M_r = 189.9) and 2',4',6'-trihydroxyacetophenone (THAP) (M_r = 168.2) - were purchased from Fluka and Sigma companies, respectively.

Electrospray ionization mass spectrometry

ESI mass spectra of the model systems under study were obtained in the positive and negative ion modes using a triple quadrupole (QqQ) Micromass Quattro Micro mass spectrometer (Waters, Manchester, UK) equipped with the electrospray ion source. This source was operated in the standard ESI mode. The ESI source temperature was set to 120°C and the desolvation temperature was 200°C. The spraying capillary was operated at 3.5 kV. The cone voltage value of 10 V was applied. The analyzed solutions were injected into the mass spectrometer at a constant flow rate of 20 μL•min^{-1}. The spectra were recorded in the mass range of 100–2000 Da. Data acquisition and processing were performed using MassLynx 4.1 software (Waters, Manchester, UK).

AP samples and a model binary system containing AP and DPPC (with 1:1 molar ratio) were prepared as 5mM stock solutions in polar solvent methanol. For spraying procedure in the ESI experiments the primary AP and AP:DPPC solutions to be examined were diluted to the final concentrations of 250 μM.

Note that methanol as the most appropriate solvent is recommended for standard ESI probing of biomolecules and biologically active compounds, according to a number of authoritative studies [24, 25, 29, 36, 37].

As to noncovalent intermolecular complexes of bioactive molecules, the correspondence of the composition of the complexes transferred to the gas phase under ESI conditions with those formed in liquid solutions was addressed in the targeted investigations [29, 37].

LDI and MALDI mass spectrometry

LDI/MALDI mass spectra were recorded in positive- and negative-ion extraction modes, using an Autoflex II mass spectrometer (Bruker Daltonics, Germany) equipped with a nitrogen laser (337 nm).

The samples were ionized in the pulse mode: pulse length 3 ns, frequency 20 Hz. Spectra were recorded in the linear mode using a delayed extraction of 10 ns and an accelerating voltage of 20 keV. The resulting mass spectra were the sum of 20 individual spectra obtained as a result of irradiation with 25 laser pulses in each separate point on the target with the deposited sample. The mass spectra acquired were analyzed using the FlexAnalysis 2.2 software (Bruker Daltonics, Germany).

The sample preparation was as follows. For the matrix-free LDI mode 1 μL of the AP solution in methanol was pipetted on the steel target and dried. In the MALDI mode, this step was preceded by pipetting 1 μL of the matrix solution. For matrix preparation, saturated

solution of either HCCA in acetonitrile-water-trifluoroacetic acid in ratio (70:30:0.1) or THAP in acetone was used.

Photographic images of the samples inside the analyzing system of the mass spectrometer were obtained using the inbuilt video CCD camera and the FlexControl 2.2 software (Bruker Daltonics, Germany).

RESULTS AND DISCUSSION

Electrospray mass spectrometry probing of ascorbyl palmitate solution

Since ESI mass spectra of AP were not properly described in the literature by now, at the initial stage of our experimental study the solution of AP in methanol was investigated by ESI. The positive ions mode of ESI experiments was applied first.

The ESI mass spectrum of AP solution in methanol probed just after preparation is presented in Fig. 1. In the spectrum the characteristic molecular and cluster ions of AP in the protonated and cationized forms can be noted: AP•H^+(m/z 415.3, relative abundance (RA) 100%), AP•Na^+ (m/z 437.3, RA 50%), 2AP•H^+(m/z 829.5, RA 53%), 2AP•Na^+ (m/z 851.5, RA 23%), 3AP•H^+(m/z 1243.8, RA 6%), 3AP•Na^+ (m/z 1265.8, RA %10), 4AP•H^+(m/z 1658.1, RA 3%), 4AP•Na^+ (m/z 1680.1, RA 7%). Other peaks in the mass spectrum (such as peaks with m/z 316.3 and m/z 1067), can be considered as peaks of the AP preparation side products. The most abundant peaks in the spectrum are signals of protonated AP molecule (RA=100%) and protonated AP dimer (RA=53%). The peaks related to Na^+ cationized molecule of AP and its cationized dimer are about two times less intensive in comparison with the corresponding protonated peaks (see Fig. 1). It should be noted that cationization as a way of ion formation is characteristic of the electrospray method of ionization [36] and sodium cations for such ions formation could originate from the trace sodium chloride admixture of solvent or can be solubilized from glassware used. Revealed high intensity of the protonation and cationization processes of AP molecules in the polar solvent is obviously conditioned on the peculiarities of AP molecule structure (see Scheme 1). In particular, the presence of proton-accepting oxygen atoms of carbonyl or hydroxyl groups in the molecular structure provides the centers of protons and sodium cations attachment to the AP molecule.

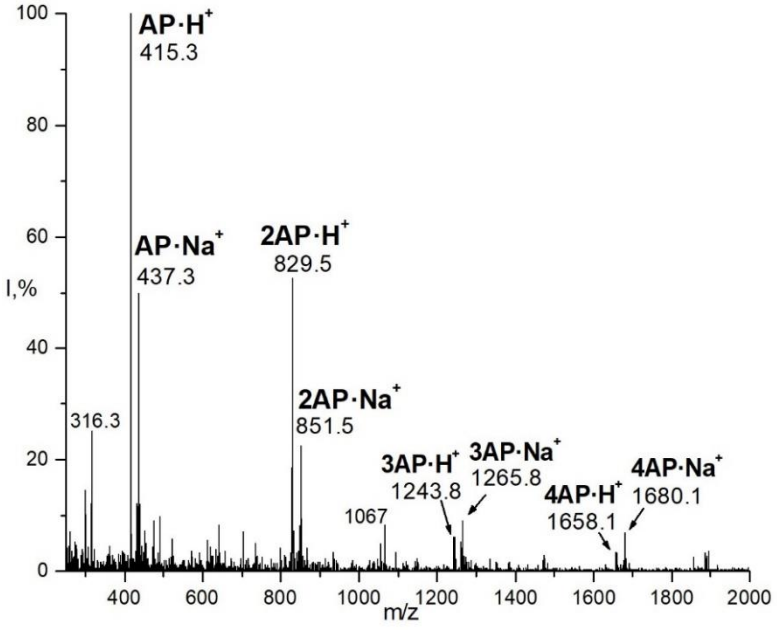


Fig. 1. Positive ion ESI mass spectrum of freshly prepared AP solution in methanol. The m/z value of the first monoisotopic peak is indicated for each group of peaks.

One of the most important results, from the biophysical point of view, is recording the abundant AP clusters of nAP•H^+ and nAP•Na^+ type (n=2–4) in the ESI mass spectrum. This result testifies to the formation of stable noncovalent complexes of the AP molecules in the polar media and confirms the AP prospective in regard to formation of liposomes for different biomedical applications including drugs delivery. From the spectrum analysis it can be also seen that the RA of cationized peaks of AP trimer and tetramer are higher than RA of the appropriate protonated peaks. This fact can point to the higher stability of the big AP clusters stabilized by the sodium cation in comparison with the similar protonated clusters nAP•H^+ (n =3, 4).

Following the purpose of selecting the optimal experimental conditions for the AP intermolecular interactions study by mass spectrometry we also probed the AP solution in methanol after 10 minutes (and more time) keeping at room temperature. Such time delay can be a necessary step in the forthcoming studies of intermolecular interactions of AP with other drug molecules or in the technological processes of formation of AP liposomes carrying the drug molecules for drug delivery purposes. The ESI mass spectrum of AP solution recorded 10 minutes after its preparation is shown in Fig. 2. As we can see in Fig. 2, for the aged solution we have a clearer spectrum (better signal to noise ratio) with less intensive peaks related to the AP preparation admixtures. The following characteristic ions of AP are recorded in the spectrum: AP•Na^+ (m/z 437.3, RA 100%), 2AP•Na^+ (m/z 851.5, RA 52%), 3AP•Na^+ (m/z 1265.8, RA %14), 4AP•Na^+ (m/z 1680.1, RA 6%). It is interesting that the protonated species of AP are not registered after the solution time keeping, that can be explained by the ion-molecular processes going in time, which results in more active replacement of protons by Na^+ ions near the partially negatively charged atoms of AP molecule in the solution studied. The spectrum analysis also demonstrates that the AP solution keeping in time can be even useful for the AP clusters registering in the ESI measurements, since the RA for peaks of AP trimers and tetramers (Fig. 2) is even a little bit higher in comparison with that for the spectrum of freshly prepared AP solution (Fig. 1). The longer time keeping did not effect significantly on the ESI mass spectrum characteristics.

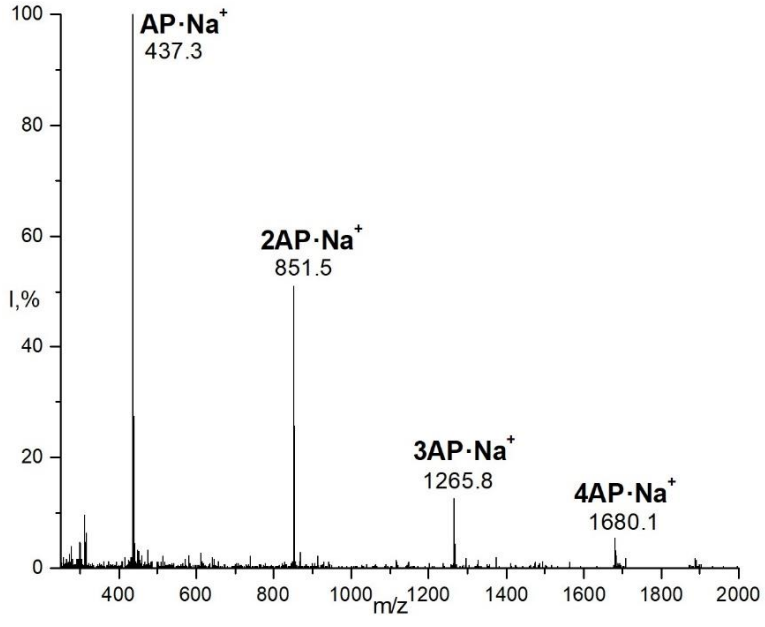


Fig. 2. Positive ion ESI mass spectrum of AP in methanol, recorded after 10 minutes of the sample keeping at room temperature. The m/z value of the first monoisotopic peak is indicated for each peaks group.

So, based on the measurements described above, we can summarize that ESI mass spectrometry in positive ion mode is effective for the revealing of the AP noncovalent complexes formation processes in polar solutions. Moreover, the solution time keeping does not affect the ability to register the ions of big AP clusters in the spectrum, that testifies to the high AP clusters' stability during the solution aging.

The next step of the current ESI mass spectrometry study was related to the AP solution probing in ESI negative ion mode. The obtained spectrum is presented in Fig. 3. The spectrum analysis shows that it can be characterized by the presence of abundant molecular and quasimolecular ions of AP, that is similar to the spectrum recorded in the positive ion mode, but these ions have different composition: $[AP\text{-}H]^-$ (m/z 413.3, RA 78%), $AP{\bullet}Cl^-$ (m/z 449.3, RA 46%), $[2AP\text{-}H]^-$ (m/z 827.5, RA 100%). The spectrum in negative ion mode is more clear and free from the peaks of admixtures. Interestingly, the peak of AP dimer is prevailed in the negative ion mass spectrum, but the peaks of bigger AP clusters such as trimers and tetramers are not recorded. At the same time, the peak of complex of the AP molecule with Cl^- anion can be noted in the spectrum that proves the possibility of AP complexation not just with positive but also with negative ions from solvate surrounding.

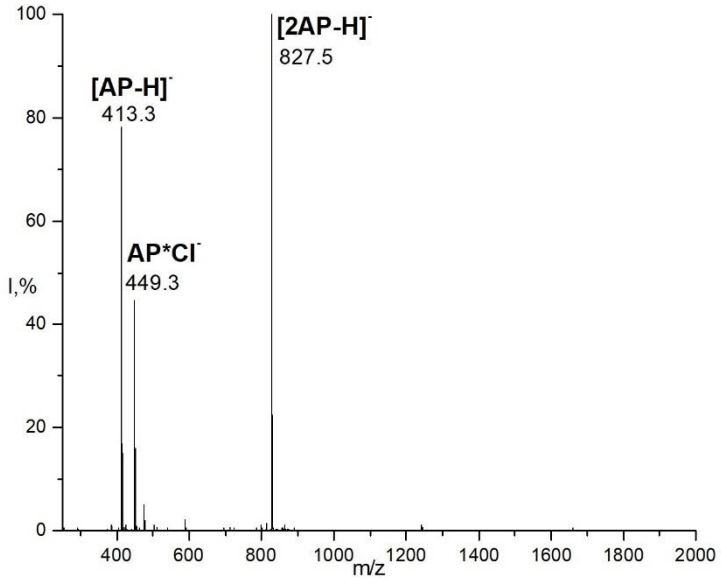


Fig. 3. Negative ion ESI mass spectrum of AP in methanol. The m/z value of the first monoisotopic peak is indicated for each peaks group.

Analysis of the ESI spectrum of AP solution in negative ion mode (Fig. 3) confirms the usefulness of this mode for recording the AP molecules ions and their dimers, while the ESI positive ion mode technique demonstrates the higher effectiveness in registering the bigger AP molecular clusters (such as trimers and tetramers), see Fig. 1 and Fig. 2. So, ESI positive ion mode can be more effective in the study of AP as an agent for liposomes formation for drug delivery purposes.

LDI mass spectrometric study of ascorbyl palmitate

The next stage of our systematic mass spectrometry characterization of AP and its intermolecular interactions was devoted to the examination of AP by LDI mass spectrometry method. As described in the Materials and Methods section of the paper, a drop of AP solution in methanol was deposited on the steel target substrate and dried on air. The photo of the crystallized sample on the steel substrate is presented in Fig. 4. The target was placed in the mass spectrometer and the sample spotted was subjected to the LDI in positive and negative

ion registration modes. It is noticeable that AP was producing abundant peaks in the mass spectra under matrix-free LDI conditions.

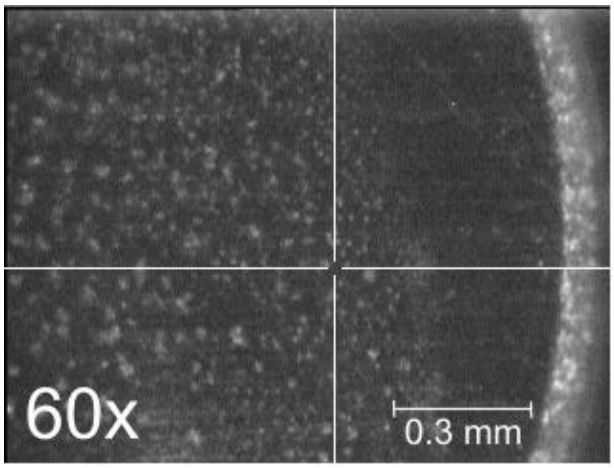


Fig. 4. The photo of AP crystallized from methanol solution deposited onto the steel target for LDI studies.

LDI mass spectrum of AP solution in methanol in positive ion mode is shown in Fig. 5a. As we can see in the figure, the mass spectrum can be characterized by good signal to noise ratio and the most abundant peaks in the spectrum are those of cationized AP molecule: AP•Na^+ (m/z 437.3, RA 100%) and AP•K^+ (m/z 453.3, RA 72%). In this mass spectrum along with the AP ion cationized by sodium cation we recorded the AP molecular ion cationized by potassium cation (which we did not note in the ESI mass spectra). It can be explained by the presence of traced amounts of potassium salts on the surface of steel metal substrate. The most significant result obtained is that in the contrast to situation in the ESI mass spectra (see Fig. 1 and Fig. 2) in the LDI mass spectrum of AP there are no peaks of cationized AP dimers (2AP•Na^+, m/z 851.5) and no peaks of other noncovalent AP complexes (3AP•Na^+, m/z 1265.8; 4AP•Na^+, m/z 1680.1). Such a fact indicates that the LDI conditions of ionization are less "soft" in comparison with the ESI conditions. Desorption/ionization of the crystalized AP sample by laser beam irradiation does not provide survival of the noncovalent clusters of AP like those we can observe in the ESI mass spectrometry experiment.

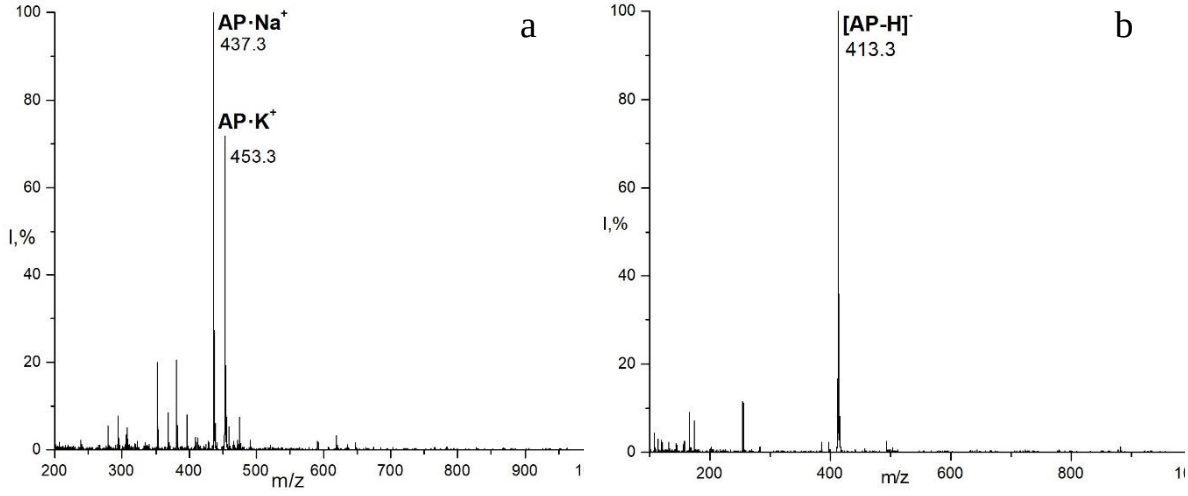


Fig. 5. LDI mass spectra of AP deposited from solution in methanol: a) positive ion mode; b) negative ion mode.

The AP sample was also probed by the LDI method in the negative ion mode; the appropriate mass spectrum is presented in Fig. 5b. Expectedly, in the mass spectrum we can find the peak of deprotonated AP molecule — $[AP - H]^-$ (m/z 413.3, RA 100%) similarly to the ESI mass spectrum in negative ion mode. However, in contrast to the case with ESI mass spectrum, we did not record any signals of AP intermolecular complexes, such as $AP•Cl^-$ or $[2AP - H]^-$.

Based on the LDI mass spectral data obtained and comparison of the results of AP probing by ESI and LDI mass spectrometry we can conclude that ESI conditions (in positive as well as in negative ion modes) are softer and more suitable for the studies on the AP noncovalent complexation in the solution.

MALDI mass spectrometric study of ascorbyl palmitate

Based on the classical mass spectrometry knowledge [36] that in matrix-assisted LDI mass spectrometry experiments (MALDI) the matrix usually provides softer conditions for the samples ionization [38], at the next stage of our systematic study we examined the AP by MALDI mass spectrometry. Firstly, we used HCCA matrix and prepared the samples as described in the methodical section of the paper. Photo of the crystals formed after drying of methanol AP solution and HCCA matrix solution deposited onto the steel target is presented in the insert in Fig. 6. Comparison of the photos in Fig. 4 (dried AP solution in methanol without matrix) and in Fig. 6 (dried AP solution in methanol + HCCA matrix) shows that the matrix addition to the system results in the formation of larger crystals (probably due to AP-HCCA co-crystallization). Moreover, the amount of the crystals increased significantly, that obviously related to the presence of HCCA matrix in the system.

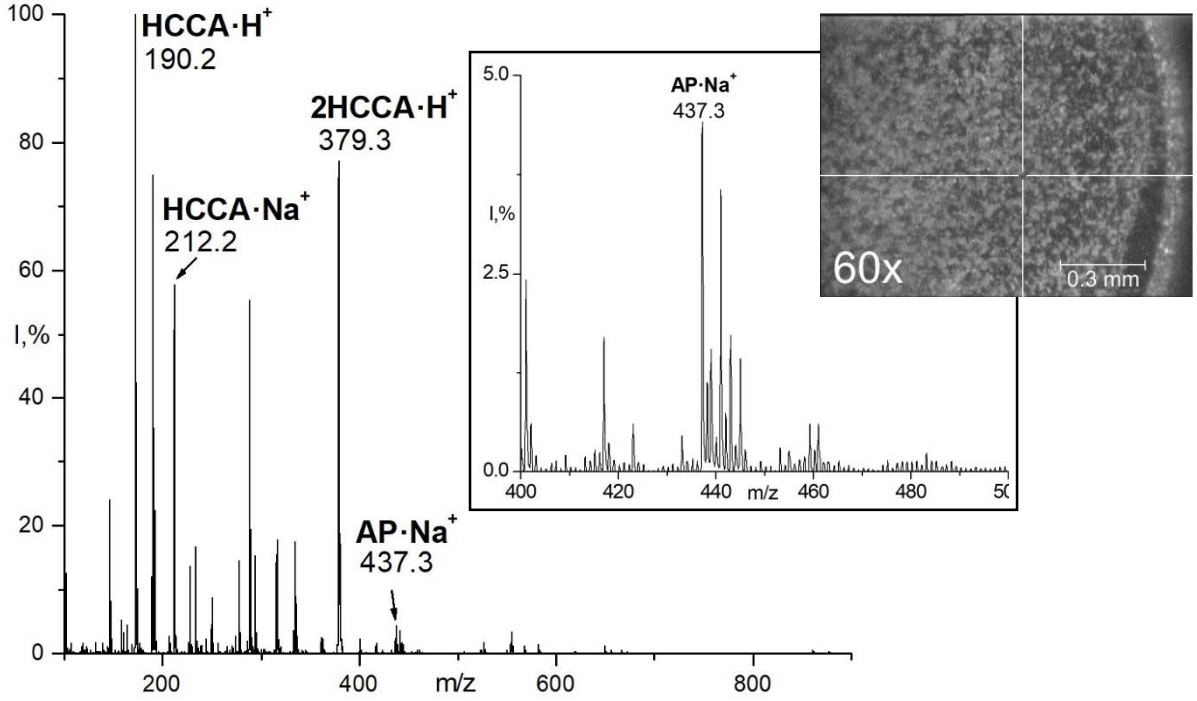


Fig. 6. Positive ion MALDI mass spectrum of AP with HCCA matrix.
Insert: Photo of the dried (crystallized) mixture of AP solution in methanol and HCCA matrix deposited onto the steel target.

In the spectrum of AP solution in HCCA matrix obtained in the positive ion mode (Fig. 6) the domination of the peaks of matrix can be noted. The most abundant peaks in the spectrum

can be attributed to the matrix: HCCA•H^+ (m/z 190.2, RA 100%), HCCA•Na^+ (m/z 212.2, RA 57%), 2HCCA•Na^+ (m/z 379.3, RA 76 %). At the same time, the nonabundant peak of cationized AP molecule AP•Na^+ (m/z 437.3, RA 4%) is also recorded in the spectrum (Fig. 6). The peaks of any intermolecular complexes of AP are not registered.

At the last stage of the MALDI experiment we decided to try another conventional MALDI matrix to probe AP solution. THAP matrix solution in acetone was chosen as less "aggressive" matrix in comparison to highly acidic solution of HCCA in water-acetonitrile-TFA, although higher laser power was necessary to be applied to generate MALDI mass spectra with acceptable analyte signal-to-noise ratio. Photo of dried AP sample in THAP matrix is shown in the insert of Fig. 7. This photo demonstrates relatively large light crystals formed (compare with the samples photos in Fig. 4 and insert of Fig. 6) that is related to the presence of THAP matrix in the sample.

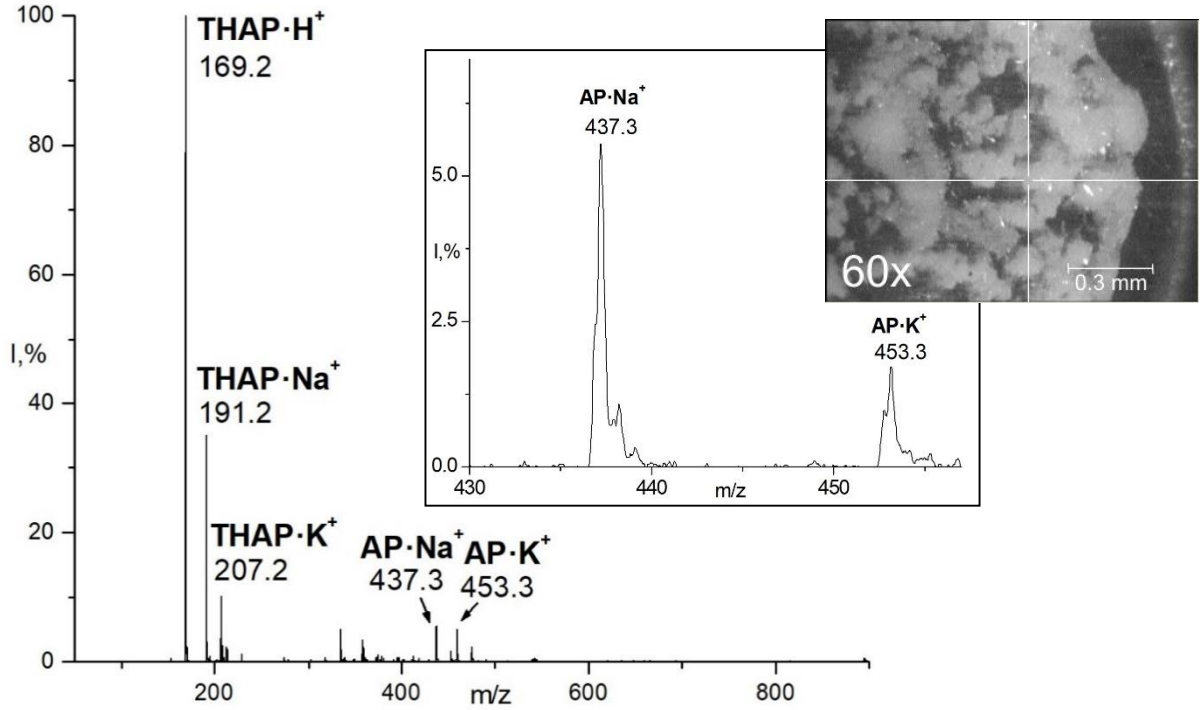


Fig. 7. Positive ion MALDI mass spectrum of AP with THAP matrix.
Insert: Photo of the dried (crystallized) mixture of AP solution in methanol and THAP matrix deposited onto the steel target.

In the MALDI mass spectrum of the studied system in the positive ion mode (Fig. 7) we can see the peaks of THAP matrix domination similar to the situation with the experiment using HCCA matrix. We can note the following peaks in the spectrum: THAP•H^+ (m/z 169.15, RA 100%), THAP•Na^+ (m/z 191.15, RA 36%), THAP•K^+ (m/z 207.15, RA 10%), AP•Na^+ (m/z 437.5, RA 5%), AP•K^+ (m/z 453.5, RA 3%). As we can see, the abundance of the molecular ions of AP is also relatively low in the spectrum with THAP matrix similar to the experimental results with HCCA matrix (Fig. 6), no peaks of AP noncovalent complexes being recorded, as well.

Taking into account the THAP matrix's good applicability to the MALDI measurements in negative ion mode, we also examined the AP with THAP matrix in this mode. The spectrum obtained is presented in Fig. 8. It is characterized by the presence of signals of ions of the matrix as well as AP molecular ions: $[THAP - H]^-$ (m/z 167.15, RA 100%), $[2THAP - H]^-$ (m/z 335.15, RA 24%), $[AP - H]^-$ (m/z 423.15, RA 10%). In contrast to the spectrum of AP with the same matrix in the positive ion mode, in the current spectrum we can note the peak of the dimer

[2THAP - H]$^-$ (m/z 335.15), however it is a dimer of just matrix molecules, and again we did not record any peaks of noncovalent complexes of AP molecules.

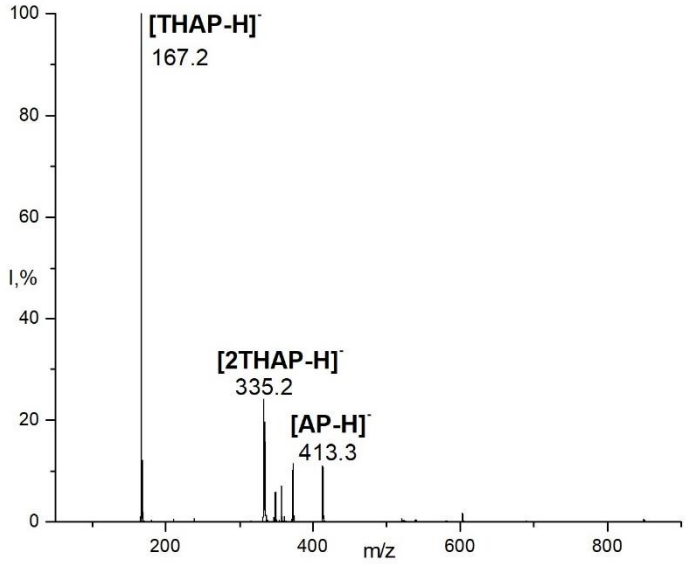


Fig. 8. Negative ion MALDI mass spectrum of AP with THAP matrix.

Electrospray study of ascorbyl palmitate – dipalmitoylphoshatidylcholine system

To check a possibility of interaction of AP with biomolecules including phospholipid components of biomembranes and liposomes, a binary system composed of AP and DPPC (1:1 molar ratio) was tested by ESI technique optimized in AP solutions measurements described above. Mass spectrum of the system presented in Fig. 9 contains sets of peaks characteristic of AP, which were attributed in Fig. 1 and Fig. 2 above: nAP•H^+ (n = 1, 2), nAP•Na^+ (n = 1–4), and the peak of DPPC in the protonated form DPPC•H^+ (m/z 734.6). The main object of search which fits the available mass range is the cluster of AP with DPPC in the protonated form: AP•DPPC•H^+ (m/z 1148.9) (Fig. 9).

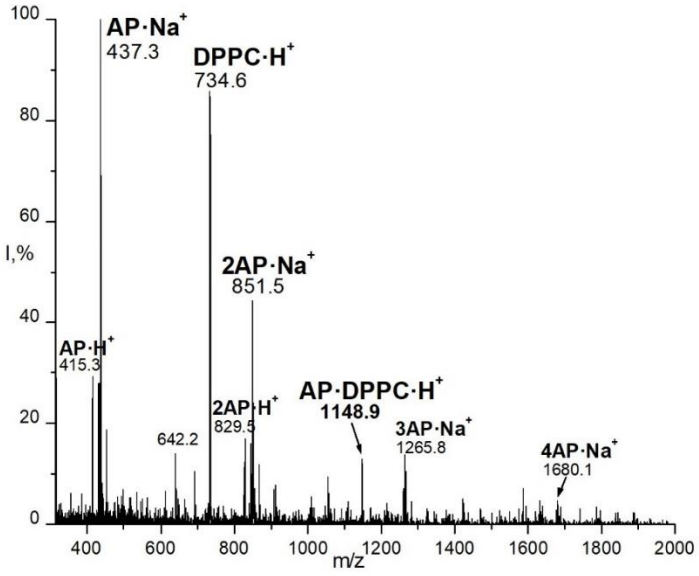


Fig. 9. Positive ion ESI mass spectrum of (AP + DPPC) binary system in methanol.

Registering of this associate proves intermolecular interactions of AP with DPPC which is a component of natural biological membranes and artificial liposomes. Formation of such stable noncovalent complexes of AP with DPPC in the conditions of the polar solvent models similar possible noncovalent interactions of the AP molecules with the phospholipids of biomembranes and liposomes when AP is functioning as a drug delivery assisting agent.

CONCLUSIONS

The current mass spectrometry study of AP solutions demonstrated the applicability of soft ionization mass spectrometry method in the research of AP noncovalent complexes formation as well as in the AP identification in different forms including solutions and crystal phase samples. While the both used soft ionization techniques — ESI and MALDI — were useful in determination of AP presence in the samples studied, the ESI mass spectrometry examining of AP solutions in polar solvent confirmed the ESI technique effectiveness in the revealing of the AP molecules clusterization and noncovalent complexes formation in the polar surrounding. ESI study of model system contained AP and DPPC confirmed the formation of stable noncovalent complexes between AP molecules and molecules of the membrane phospholipid that models the intermolecular interactions of AP as an agent assisting in drug transmembrane delivery. Based on the current study results we can recommend the ESI mass spectrometry technique to be used in AP nanosomes formation examination. Moreover, the ESI mass spectrometry might be useful in the following studies of AP intermolecular interactions with the molecules of medications and biomolecules when the AP is used as an agent enhancing the drug molecules transmembrane transfer.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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МАС-СПЕКТРОМЕТРИЧНЕ ДОСЛІДЖЕННЯ АСКОРБІЛПАЛЬМІТАТУ ЯК АГЕНТУ, ЩО ЗДАТНИЙ ФОРМУВАТИ НАНОСОМИ

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Актуальність. Вивчення властивостей і міжмолекулярних взаємодій біологічно активних речовин, які можуть бути використані для поліпшення трансмембранного транспорту лікарських сполук, є одним із актуальних завдань сучасної молекулярної біофізики. Аскорбілпальмітат (АП), як жиророзчинна форма вітаміну С, нещодавно привернув увагу дослідників як агент, перспективний для створення наносом, що здатні сприяти переміщенню через клітинну мембрану молекул «нерозчинних у жирах» лікарських сполук. Однак, АП та його супрамолекулярні комплекси досі не були охарактеризовані сучасними м'якоіонізаційними методами мас-спектрометрії.

Мета роботи. Мета даної роботи — охарактеризувати АП та його міжмолекулярні взаємодії за допомогою низки мас-спектрометричних методів: з іонізацією електророзпиленням (ІЕР), лазерною десорбцією/іонізацією (ЛДІ) та матрично-активованою лазерною десорбцією/іонізацією (МАЛДІ). Заплановано порівняння застосовності зазначених методик для вивчення міжмолекулярних взаємодій АП як агента, що сприяє доставці ліків.

Матеріали і методи. Спектри ІЕР одержано за допомогою квадрупольного мас-спектрометра Micromass Quattro. Експерименти ЛДІ та МАЛДІ виконувались за допомогою мас-спектрометра Autoflex II.

Результати. ІЕР експерименти в режимі позитивних іонів виявили наявність у мас-спектрах піків протонованих і катіонізованих молекул АП, а також кластерів АП типу nАП•H^+ і nАП•Na^+ (n=2÷4). Цей результат свідчить про утворення стабільних нековалентних комплексів молекул АП у полярних середовищах і підтверджує здатність АП формувати наноструктури для доставки ліків. Аналіз ЛДІ та МАЛДІ мас-спектрів АП, зареєстрованих у режимах позитивних та негативних іонів, показав, що за присутності сигналів від молекулярних іонів АП, піки димерів чи більших кластерів АП відсутні. Вивчення методом ІЕР модельної системи, що містила АП та дипальмітоїлфосфатидилхолін (ДПФХ), виявило утворення в розчині стабільного комплексу АП•ДПФХ•H^+, який моделює міжмолекулярні взаємодії АП з фосфоліпідними компонентами біомембран та/або ліпосом в умовах функціонування АП як агенту, який сприяє доставці ліків.

Висновки. Проведене дослідження демонструє застосовність усіх протестованих методів мас-спектрометрії для ідентифікації АП у розчинах та твердій фазі, тоді як для вивчення утворення міжмолекулярних нековалентних комплексів АП та взаємодій АП з біомолекулами найбільш ефективним методом є мас-спектрометрія з ІЕР.

КЛЮЧОВІ СЛОВА: аскорбілпальмітат; наносоми для доставки ліків; дипальмітоїлфосфатидилхолін; нековалентні комплекси; мас-спектрометрія; іонізація електророзпиленням; лазерна десорбція/іонізація; матрично-активована лазерна десорбція/іонізація.