Mass spectrometry and Raman spectroscopy characterization of the bactericidal nanofiber mats with incorporated antibiotics

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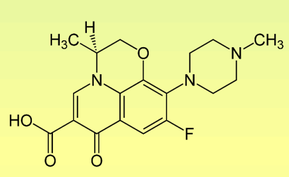
Introduction

Polymeric films with incorporated bactericidal agents are promising biomedical materials which are used in the current medical practice for wounds and burns dressing, tissue engineering etc. Among such films, electrospun polymeric nanofiber mats with incorporated antibiotics or other antimicrobial agents are in the focus of nanoscience related research as healing coating and drug delivery platforms. Our recent study of the nanofiber mats prepared from the polyvinylpyrrolidone (PVP) and polymethylmethacrylate (PMMA) mixture with the addition of biologically active compounds confirmed the mats electrospinning production technology effectiveness and possibility to extract the bactericidal agents from the mats into saline (0.9% aqueous NaCl solution that models physiological conditions) [1]. The current study is devoted to laser desorption/ionization mass spectrometry (LDI MS) characterization of the extracts from the nanofiber mats prepared from PMMA or from a mixture of PVP+PMMA with incorporated antibiotic levofloxacin (LF), as well as to Raman spectroscopy examining of PVP+LF aqueous solutions.

Objects and methods of investigations



Scheme 1. Chemical structure of antibiotic levofloxacin.

For LDI MS measurements, film samples (small pieces of films made of PMMA or PVP+PMMA, which were copolymerized with the antibiotic levofloxacin (LF, MW=361.368) were placed in test tubes and 1 ml of physiological solution was added for extract obtaining.

After extraction, 1 μl samples were taken from each tube and a drop was placed on a metal substrate (target) for measurement in the LDI mode. The drops on the metal substrate were dried before placing the target in the mass spectrometer.

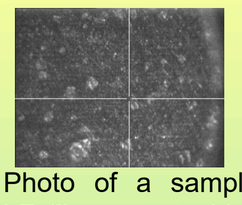


Fig 1. Photo of a sample of the PMMA+LF film extract dried on the target-substrate of mass spectrometer.

For Raman spectroscopic measurements the aqueous solutions of PVP and LF were prepared (LF:PVP - 1:3 and 1:6 molar ratios for mixtures). Laser with a wavelength of 532 nm was used in Raman spectroscopy experiments.

Raman spectroscopy results

The data of the Raman spectroscopy examining of the PVP+LF aqueous solutions testify to the noncovalent interactions of LF with the PVP molecules (Fig. 2). In particular, in the spectral range of 1200-1800 cm^{-1} the small shifts of some bands in the spectra of the solutions of PVP+LF are observed comparing with the spectra of pure LF and pure PVP (Fig.2 b)) that indicates the noncovalent complex appearance. The deposition of the aqueous solutions on the substrate for the measurements does not lead to the destruction of the PVP+LF complexes formed in aqueous solutions.

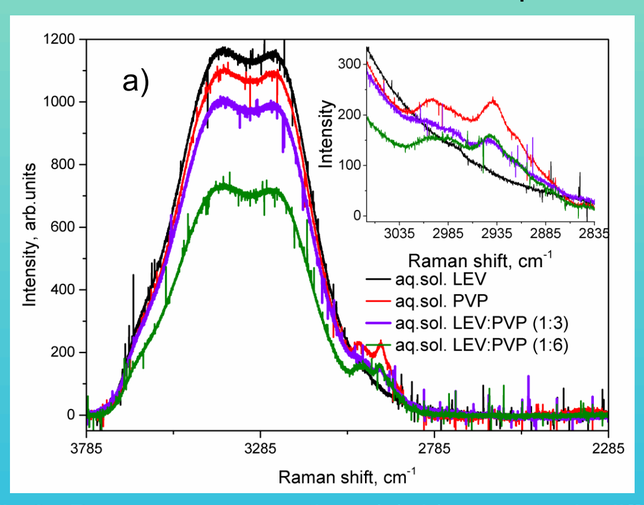


Fig.2 . Raman spectra sections (a), b) – spectra in different spectral ranges) of the aqueous solutions under study: solution of pure levofloxacin (LEV), solution of PVP and LEV:PVP mixtures 1:3 and 1:6. Insert in Fig.2 a) - enlarged spectrum section.

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LDI MS characterization of extracts of nanofiber mats with levofloxacin

In the positive ion mass spectrum of the extract from PMMA+LF dry mat (Fig. 3) the most intensive signals are the peaks of LF molecular ions: the cationized peak LF•Na^+ with m/z 384.4, relative intensity RI=100%, the protonated antibiotic molecule LF•H^+ with m/z 362.4, RI=29%, and LF•K^+ peak with m/z 400.4, RI=8%. The presence of abundant molecular peaks of the drug in the LDI spectrum indicates intensive extraction of LF from the mat into saline and confirms the efficiency of the production technology of the nanofiber films with the antibiotic loading. It is important to note the stability of the composition of the nanofiber mats over time, since before measurements the dry mats were stored for several months at room temperature. The comparison of the spectra of extracts of PMMA+LF (Fig. 3) and PMMA+PVP+LF films (Fig. 4) shows the general similarity of the spectra, both of which have abundant molecular peaks of the antibiotic, which indicates the efficiency of incorporation of LF in both type mats: prepared from PMMA or from the mixture of polymers PMMA+PVP. At the same time, some differences in the mass spectra of the samples from different films are observed. Namely, in the spectrum of the extract from PMMA+LF mat, the RI of the antibiotic peaks is higher (they dominate in the spectrum) compared to the RI of the LF signals in the spectrum of the PMMA+PVP+LF film extract (RI - below 60). Moreover, in the spectrum of the PMMA+LF system we have a rather intense peak of the protonated LF molecule - LF•H+ with m/z 362.4, while in the spectrum of the PMMA+PVP+LF system this peak is at the noise level. Such changes in the relative intensity of the antibiotic signal may indicate a higher concentration of LF in the films PMMA+LF compared to the films of PMMA+PVP+LF, or that the extraction process from the films based on PMMA monopolymer is more active than from the film with a mixture of PMMA+PVP polymers.

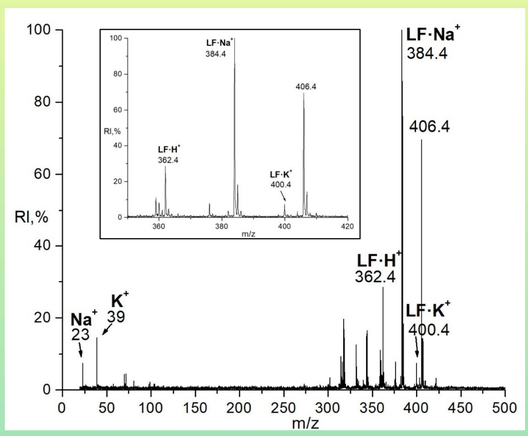

Fig.3 . LDI mass spectrum of the extract of film of PMMA+LF obtained in the positive ion mode. Insert – enlarged section of the spectrum near LF molecular ions area.

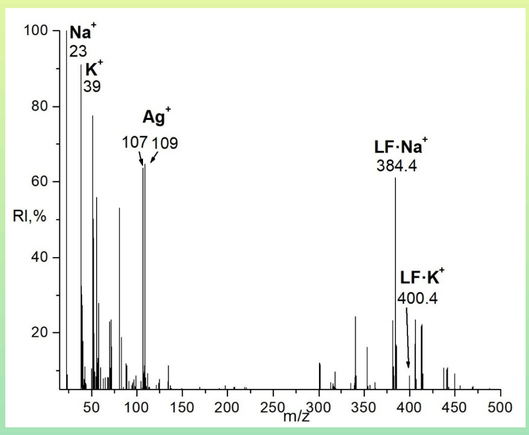

Fig. 4. LDI mass spectrum of the extract of film of PMMA+PVP+LF obtained in the positive ion mode.

Conclusions

The obtained results of the LDI mass spectrometry probing of extracts to saline from the nanofiber mats prepared from PMMA or from a mixture of PVP+PMMA with incorporated antibiotic levofloxacin (LF) demonstrate that significant amount of LF molecules retain their molecular structure within the mat. It is obvious that the components of the nanofiber are not covalently bounded. The noncovalent nature of interactions of LF and PVP is confirmed by the Raman spectroscopy data. The study results verify the effectiveness of the developed technology of production of the nanofiber mats with LF incorporation by electrospinning method. Our findings testify to preservation of the LF molecular structure withing the mat that is very important to provide the antibiotic biological activity after its extraction into the physiological system from the mat.

References

1. A. M. Plokhotnichenko, V. A. Karachevtsev, V. A. Pashynska, P. O. Kuzema, Low Temp. Phys. 50, N3, 215-221 (2024). https://doi.org/10.1063/10.0024959