Antibiotic-loaded PMMA nanofibers as antibacterial material: comparative analysis of levofloxacin and chloramphenicol

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In this work, we fabricated polymer mats formed by PMMA nanofibers loaded with antibiotic levofloxacin (LV) and chloramphenicol (CAM) and characterized them using a microscopic method, UV-visible absorption spectroscopy, and mass spectrometry, analyzed the kinetics of the release of drugs from the mats, and studied the antibacterial properties. The diameter of the obtained nanofibers determined is ranging from 1 to 4μm. The release of antibiotics from the nanofiber mat when soaked in an aqueous solution was studied using UV-Vis spectroscopy and mass spectrometry. These observations showed that ~50% of LV was released within the first 10 minutes, and the remaining 20% within the next 3 hours. In contrast, the release time of CAM was significantly longer: only 10% of the CAM was released from the mat within the first 5 hours, and the next 5% required 45 hours. The overall release fraction was only 35%, which took about 650 hours. It is supposed that LV exhibits a faster and more intense release due to its hydrophilicity and weaker binding to PMMA, while CAM demonstrates a slower and more incomplete release due to its lower water solubility and stronger affinity for the hydrophobic PMMA matrix. The antibacterial properties of the antibiotic-loaded nanofiber matrix were studied against Gram-positive and Gram-negative bacteria. The study showed that both matrices demonstrated high efficacy against these bacteria, with the exception of CAM, which showed lower activity against *P. aeruginosa 9027*.

Sample fabrication

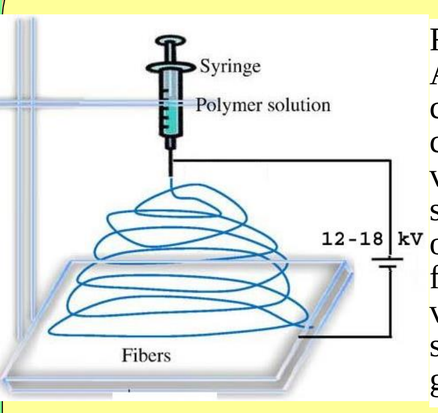


Figure 1. Schema of the conventional electrospinning setup. An electrospinning setup consists of a metal needle as a die, a collector, and of a high voltage power supply. A syringe pump carries the solution from the syringe to the needle. A high voltage is applied to the polymer solution loaded into a syringe. At a certain electric field the electrostatic force overcomes the force of surface tension of the solution which forms Taylor cone on a drop at the tip of a needle. When the voltage becomes above this point, solid fiber is formed. It is supposed that the solvent is evaporated before it reaches the grounded collector.

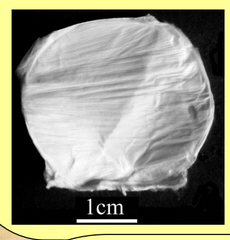
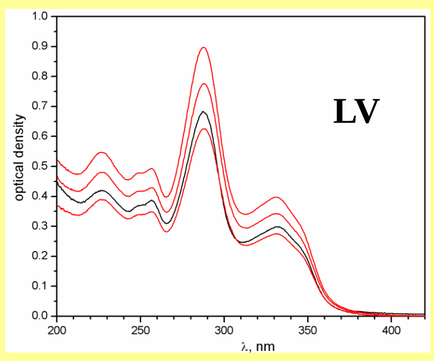


Fig. 2. Photo of a sample of polymer fiber mats with bioactive components added..

Fig. 3. Microphotographs of a sample of thin mat made of PMMA fibers with bioactive components

Analysis of LV and CAM release from PMMA nanofibers



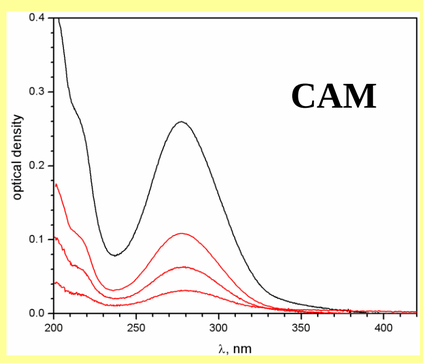
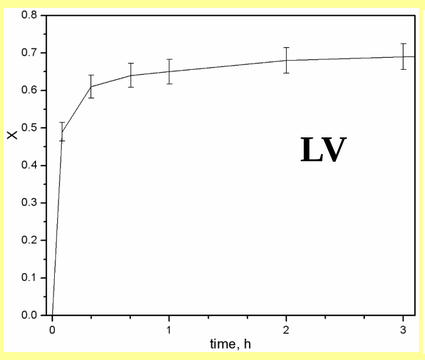


Fig.4. UV absorption spectra of LV and CAM. Black - starting samples in aqueous solution. Red - solutions obtained after soaking mat in water: after 5, 20 min and 50 hours for LV and after 5, 77 and 800 hours for CAM (the intensity increases with time).



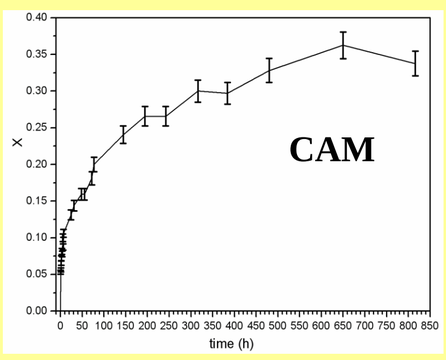
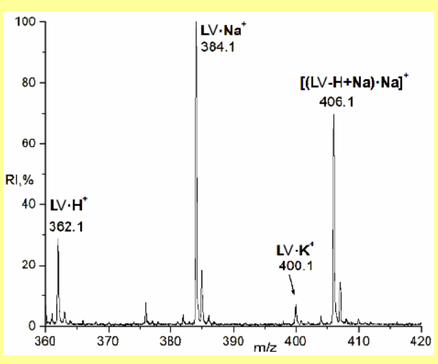


Fig.5. . Release profiles of LV and CAM from PMMA nanofibers obtained from temporal dependence of the absorbance of LV or CAM released. X- the partial release of LV (CAM) from the nanofiber mat.



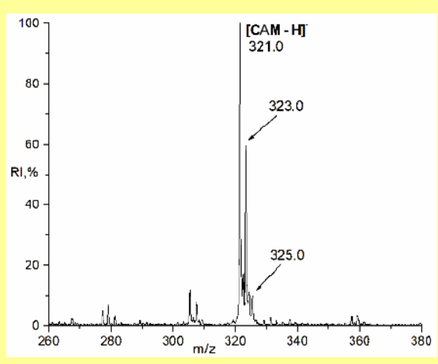


Fig.6. LDI mass spectra of the extracts in saline of the PMMA:LV (left, positive ion mode) and PMMA-CAM (right, negative ion mode) mats.

The LDI data of the probing of the extract from PMMA:LV and PMMA-CAM mats are similarly and confirm the effective loading the PMMA nanofiber mats with LV or CAM during the mats production. The obtained results also testify to active release of LV (CAM) during the extraction into physiological saline that models biomedical application of such mats with loaded antibiotics.

Indicate the significant difference in the release kinetics of LV and CAM from the polymer mat into an aqueous solution. This rate is determined by many parameters, such as the number and size of pores in the nanofibers, the binding energies of the drug molecules with the polymer and solvent, the change in entropy during dissolution, the surface tension at the polymer-solvent interface, temperature, and others. The experimental results suggest that one of the key parameters determining the rate of drug release is its solubility in the liquid. To test this hypothesis, we measured the release of the drugs from the same mats in ethanol. The solubility of CAM in ethanol is approximately 30 times higher than in water (approx. 80 mg/mL and 2.5-3 mg/mL, respectively), and the time to maximum drug release into solution decreases from 300-600 hours (in water) to 10-15 minutes (in ethanol). In the case of LV, the situation is the opposite: its solubility in ethanol (4.5-5 mg/ml) is less than in water (approx. 16 mg/ml), and the time to maximum release of the drug into solution increases from 15-20 minutes to 1.5-2 hours.

Experimental details

Electrospinning was carried out with a needle-to-collector distance of approximately 8 cm and a feed rate of 0.7 mL/h. A metal collector was used to obtain thick mats, while glass substrates (supported on a metal base) were employed to prepare thin samples for high-resolution microscopy.

In our experiments, LV and CAM was extracted from commercial tablets by dissolving them in acetone, followed by purification through filtration and recrystallization. A solution of LV or CAM in acetone was then mixed with a chloroform solution containing a dissolved PMMA. The resulting solution had component concentrations of approximately 82:18 mg/mL for PMMA and LV or CAM, respectively. It is assumed that this ratio is retained in the resulting nanofiber mat, yielding a mass fraction of LV or CAM of about 15–18%.

A dual-channel spectrophotometer (Hitachi M 356, Japan) was used to measure UV–visible absorption spectra. Quartz cuvettes with a 1 mm path length were employed.

To evaluate the partial release of LV (CAM) from the nanofiber mat, 7.4 mg of the mat was incubated in 10 mL of distilled water for a certain period. LV (CAM) release was monitored using UV absorption spectroscopy. The total quantity of LV (CAM) released was determined by comparing the recorded spectra with that of a standard LV (CAM) solution of known concentration.

LDI mass spectra were recorded in the positive and negative ion 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. A samples of the extracts from the mats loaded with antibiotics was deposited on the MALDI target plate.

The in vitro antibacterial efficacy of PMMA discs loaded with antibiotics was assessed against opportunistic strains of *Staphylococcus aureus* 209, *Pseudomonas aeruginosa* 9027, and *Escherichia coli* B using the disk diffusion assay.

The assay began by pouring 20 mL of molten MPA into Petri dishes, which were then allowed to cool to room temperature and dry for 30 minutes. Subsequently, each plate was inoculated with 0.5 mL of the prepared bacterial suspension. Centrally on each plate, a 7-mm diameter disk of the nanofiber mat, loaded with either levofloxacin or chloramphenicol (approximately 50 μg of antibiotic per disk), was positioned. As a negative control, unloaded nanofiber mat discs were used. The plates then underwent a 24-hour incubation at 37°C. After this period, the diameter of the resulting zone of inhibition was measured.

Evaluation of the Antibacterial Efficacy

The antibacterial performance of LV- and CAM-loaded PMMA nanofiber mats was evaluated by measuring the zones of inhibition against *S. aureus 209, P. aeruginosa 9027,* and *E. coli B*. Both systems produced clear inhibition zones, confirming the release of active drug; however, the PMMA-LV mats consistently generated significantly larger zones than PMMA-CAM for all tested strains. This difference was most pronounced for *P. aeruginosa 9027*, where the inhibition zone diameters were 30.0 mm for PMMA-LV compared with only 8.5 mm for PMMA-CAM. The poor bactericidal activity of PMMA-CAM against this strain can be attributed not only to the intrinsically lower susceptibility of *P. aeruginosa 9027* to chloramphenicol, but also to insufficient drug accumulation in the agar medium, which is likely due to the slow release kinetics and generally low release fraction of CAM from the hydrophobic PMMA matrix. Another most notable result, besides the lowest inhibition values for PMMA-CAM against *P. aeruginosaaeruginosa 9027*, is the observation of the highest inhibition values for PMMA-LV against *E. coli.*

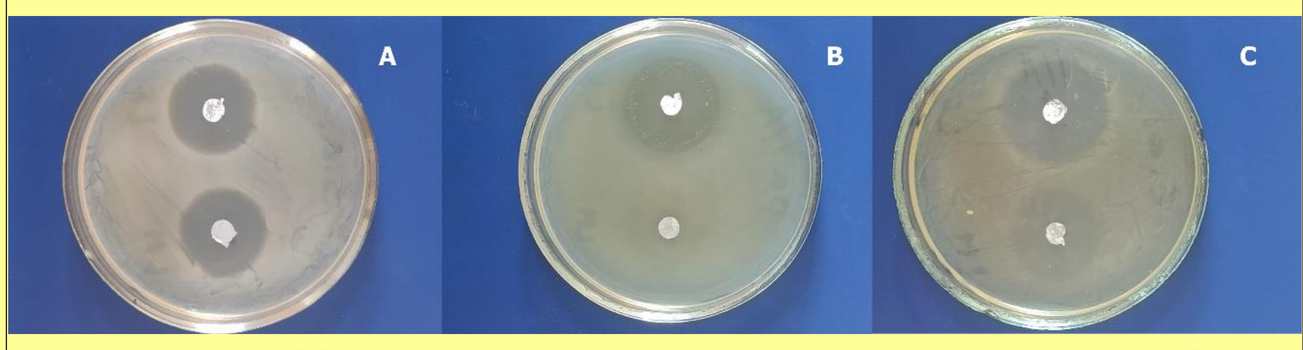


Fig.7. Sensitivity of three strains (A - *S. aureus* 209, B - *P. aeruginosa* 9027, C - *E. coli B)* to LV (upper) and CAM (lower) released from disks prepared from nanofiber mat. The growth-inhibiting capacity of the LV- and CAM-loaded nanofiber mats was quantified by measuring the zones of inhibition against *S. aureus 209, P. aeruginosa 9027, and E. coli B*. The average diameters of the growth inhibition zones for PMMA-LV discs were 28.1 mm for *S. aureus 209*, 32.9 mm for *E. coli B*, and 30.0 mm for *P. aeruginosa 9027*. In contrast, for the PMMA-CAM discs, the zones were 24.9 mm for *S. aureus 209* and 27.1 mm for *E. coli B*, while showing a negligible inhibitory effect on *P. aeruginosa 9027* (8.5±0.71 mm), a zone only marginally larger than the 7-mm disc diameter.

Conclusions

Electrospun PMMA nanofiber mats incorporating the antibiotics levofloxacin (LV) and chloramphenicol (CAM) were fabricated at high loading levels (up to 18 wt%). Release studies revealed distinct kinetics: LV showed rapid release, with ~50% diffusing within 10 minutes and an additional 20% over the next 3 hours, attributed to its hydrophilicity and weaker interaction with PMMA. In contrast, CAM exhibited markedly slower and incomplete release, with only ~35% released over 650 hours, consistent with its poor solubility and stronger affinity for the hydrophobic matrix.

Both antibiotic-loaded mats produced clear inhibition zones against susceptible bacterial strains, confirming that therapeutically relevant drug concentrations were released and maintained during the 24-hour assay. Importantly, the release was sustained rather than uncontrolled, highlighting the functional role of PMMA as a rate-controlling reservoir. These findings underscore the potential of PMMA-based nanofiber mats as localized, long-lasting antimicrobial platforms for wound dressings and implant coatings.