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Bacteria trapping effectivity on nanofibre membrane in liquids is exponentially dependent on the surface density

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Abstract

Protection against water- and air-borne bacteria as well as their detection at very low levels is a big challenge for the health care profession. The study's main goal was to prepare bacterial filters with a tunable trapping effectivity. We revealed that the trapping efficiency of *Escherichia coli* estimated from the optical density of bacteria passed through the filter was exponentially dependent on the surface density of the polyacrylonitrile nanofibre membranes. This log/linear regression profile was proven for bacterial trapping efficiency higher than 99.9% which opens a door for easy and tunable constructions of ultrasensitive filters and/or nanosensors as well as for the standardization and quality control of nanofibre membranes.

Polyacrylonitrile nanofibres, microparticles, bacterial detection, antibacterial protection, Escherichia coli

Nanomaterials play an essential role in biomedical engineering, as their structure and unique properties enable the construction of new materials for regenerative medicine, theragnostic as well as for ultrasensitive sensors for early-stage disease diagnosis (Eltzov et al. 2011; Ranjbar and Shahrokhian 2018; Ghasemi et al. 2022; Rajamanickam and Yoon Lee 2022). While nanoparticles smaller than 50 nm face major certification issues, the pseudo-two-dimensional nanofibre membrane structures as a material for modern personalized medicine appear to be the most common nanomaterials for this purpose (Pashchenko et al. 2022).

Nanofibre membranes are characterized by an exceptionally high surface-to-volume ratio with an extremely high number of pores. These properties favour nanofibre membranes for broad medical applications and for the production of medical devices, as recently demonstrated, e.g., for wound dressing, drug delivery, and biological sensing (Kenry and Lim 2017; Haider et al. 2018; Bocková et al. 2022; Coradduzza et al. 2022; Pashchenko et al. 2022). The high density of the tiny pores and the ability to adjust their diameter also favour nanofibres for their application as highly effective liquid filters (Qin and Wang 2006; Sahto 2021; Zhou et al. 2022). Whether for water monitoring (Markosian and Mirzoyan 2019; Mao et al. 2020; Mao et al. 2021; Khodayari et al. 2021)

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or purification, pathogen detection, or the production of protective aids (such as masks and respirators), nanofibres are an inexpensive, easy-to-use, and highly effective alternative to the lengthy and expensive laboratory tests (Haider et al. 2018; Bellu et al. 2022).

The filtration effectiveness of electrospun nanofibres depends on their structure. The desired structural configuration can be achieved by suitable manufacturing and polymer parameters. In addition to the type (synthetic or natural) and viscosity of the polymer, manufacturing variables such as the voltage applied, the flow rate of the solution, the needle size, and the distance between the needle and collector affect the size of the pores, the homogeneity of the structure, and in particular, the area density (Krifa and Yuan 2015; Haider et al. 2018; Conte et al. 2020; Anusiya and Jaiganesh 2022; Jafari et al. 2022; Kralovic et al. 2022; Vu et al. 2022).

Nanofibres with different surface densities are suitable for capturing various different-sized particles. Particles smaller than 2.5 µm (PM2.5) are dangerous for humans because their size allows them to diffuse into the bronchi and lungs (Eltzov et al. 2011; Bellu et al. 2022; East et al. 2022). In addition to PM2.5 pollutants (Eltzov et al. 2011; Kim et al. 2018; Matulevicius et al. 2016; Shuvo et al. 2020; Deng et al. 2021), pathogenic microorganisms and viruses also bring a significant human health risk. Moreover, water- and air-borne bacteria (*Legionella* spp., *Escherichia coli*, *Staphylococcus aureus*, etc.) contaminate the environment and without its monitoring, they can cause an epidemiological problem (Bellu et al. 2022).

Due to their flexibility and durability in water, PAN nanofibres are known as suitable for ultrasensitive water and humid air filters. The main goal of the study was the preparation of bacterial filters from PAN nanofibres with tunable trapping effectivity, and developing a methodology for rapid elimination and/or monitoring of bacterial contamination in liquids.

Materials and Methods

Nanofibre preparation and nanofibre quality tests

Nanofibres were prepared from water-insoluble polymer since the nanofibre filter application was intended for liquids and humid air. The polyacrylonitrile nanofibre (PAN) polymer was selected after several preliminary experiments because PAN nanofibres showed very good flexibility, water insolubility, and production reproducibility. Polyacrylonitrile nanofibres of different area densities were obtained from Nanoprogress, s.r.o (Pardubice, Czech Republic).

Scanning electron microscopy (SEM) Vega3 SB (TESCAN a.s. Brno, Czech Republic) was employed to visualize the structure of the PAN nanofibre membrane used for filtration experiments. Before sample visualization, samples were sputtered (Sputter Coater Q150R, Quorum Technologies Ltd, Lewes, UK) by a conductive 10 ± 2 nm layer of golden nanoparticles to prevent the samples' charging during SEM visualization. Images were analysed by ImageJ software (NIH, Bethesda, USA) from five different areas of each sample.

Nanofibre diameter distribution was automatically generated by the software OriginPro based on manual measurement performed on SEM images with the use of ImageJ software. Measurements of the nanofibre diameter were performed on five different SEM images from different areas of the PAN nanofibre sample. Analysed SEM images were created at a working distance of 8 mm and a view field of 92.3 µm.

The nanofibre membrane was further characterized for its surface properties. Clearly, the development of a net surface charge affects the distribution of ions in the surrounding interfacial region, resulting in an increased concentration of counter ions (ions of opposite charge to that of the particle) close to the surface. The liquid layer surrounding the particle exists in two parts; an inner region called the Stern layer, where the ions are firmly bound, and an outer, diffuse area where they are less firmly attached. A notional boundary within the diffuse layer is formed in which the ions and particles form a stable entity. When a particle moves (e.g. due to an electric field or gravity), ions within the boundary move with it, but any ions beyond the boundary do not travel with the particle. The respective potential is called the zeta potential and indicates the amount of charged particles to be adhered to the nanofibre surface.

Surface zeta potential was measured on PAN nanofibre samples by Zetasizer nano ZS (Malvern Panalytical Ltd, Malvern, UK) with the use of Universal Dip cell (ZEN1002) accessories to measure the surface zeta potential at five different distances from the PAN nanofibre sample surface. The final surface zeta potential was evaluated automatically by Malvern software using linear regression to calculate the final surface zeta potential at a distance of 625 µm from the PAN nanofibre sample surface.

Model particles (polystyrene latex particles) were used for the determination of the affinity to the PAN nanofibre sample.

Cultivation of *Escherichia coli*

Gram-negative bacteria *Escherichia coli* O26:B6 (*E. coli* DBM 3125 – collection CCM 3988) was used as a model organism for the detection. The reference bacterial strain was provided provided from the bacterial collection of the University of Chemistry and Technology, Prague. The cultivation of bacterial colonies was performed on agar medium. Chemicals used to prepare the agar medium (NaCl, peptone, agar and yeast extract) were obtained from Sigma Aldrich (St. Louis, USA).

Measurement of optical density and data evaluation

Suspensions of *E. coli* (1.12×10^9 cells/ml) were filtered through PAN nanofibre filters with varying surface densities (1.34–6.82 g/m^2). Each trial was replicated three times. Bacterial counts in solutions were quantified by optical density (OD) measurements at 600 nm wavelengths optimal for *E. coli* (Mandelstam et al. 1982). The log-linear decrease was analysed using log-linear regression implemented in the open-source statistical software R (R Core Team, Vienna, Austria). The equation used was $\log(\mathrm{OD}) = -5.93 \times (\text{surface density}) - 5.90$. From these results, we calculated the bacterial trapping efficiency.

Results

The samples of nanofibres examined exhibited randomly oriented fibres with submicrometer diameters, and the nanofibre membranes displayed no significant structural defects (Plate XII, Fig. 1). Naturally, variations in surface density were found to influence the pore size.

The quantification data on diameter distribution of the nanofibres indicated that the diameter of more than 95% of PAN fibres was smaller than 500 nm. Since the number of fibres with a respective diameter range was obtained by simply counting these fibres in the SEM picture, we omitted error bars similarly as in our previous publications. Notably, around 65% of fibres fell within the 100 nm to 400 nm range (Fig. 2).

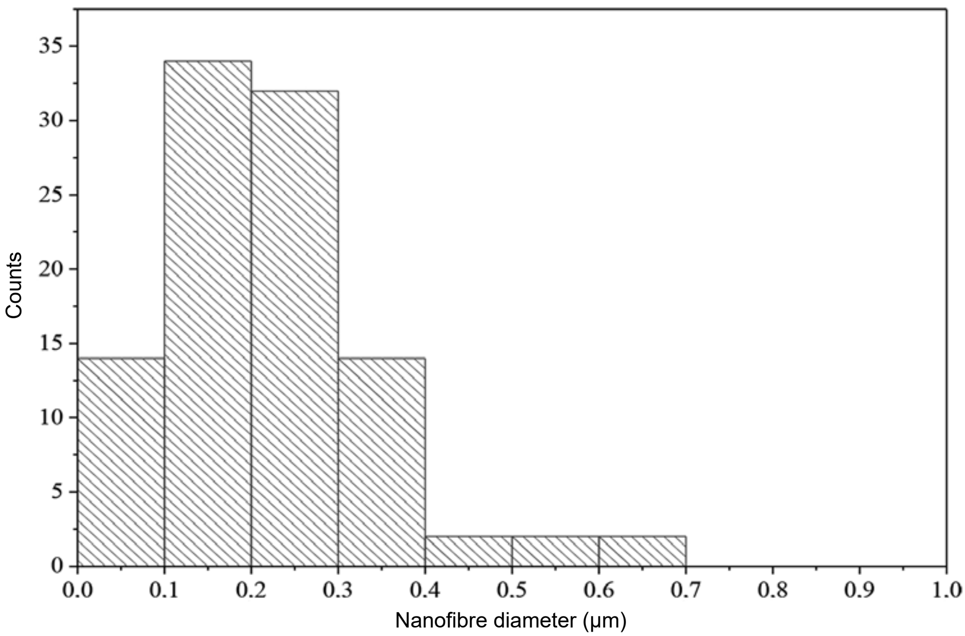

Fig. 2. PAN nanofibre diameter distribution as obtained from the SEM image by computer analysis

Measurement of the zeta potential of PAN nanofibres is shown in Fig. 3 (Plate XII). The surface zeta potential of PAN nanofibre membrane at a distance of 625 μm from the sample surface was measured as −34.7 mV ± 2.8 mV, indicating strong affinity of bacteria surface to the PAN nanofibre membrane and its suitability for a biosensor.

Results of the characterization of the PAN nanofibre-based sensor for *E. coli* detection in liquids are presented in Fig. 4. Standard errors were negligible and, therefore, are not shown.

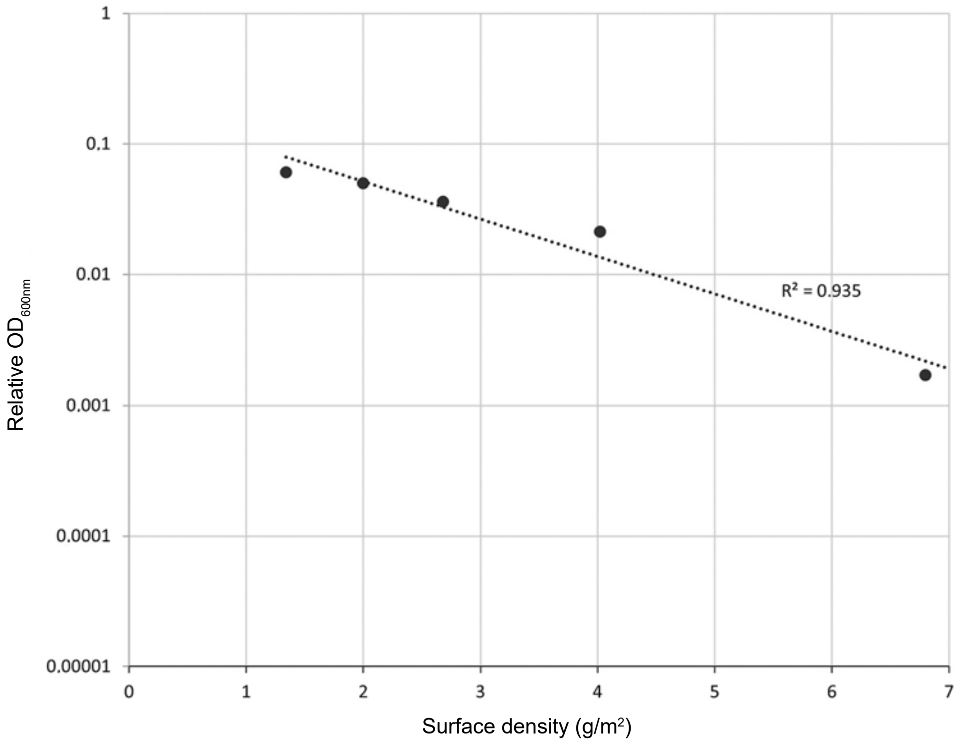

Fig. 4. Dependence of optical density (OD) (600 nm) of *Escherichia coli* at the filtrate on the surface density of the nanofibre filter. The Y axis is logarithmic, and the lines denote the results of log-linear regression. The Pearson regression coefficient was $R^2 = 0.935$.

A logarithmic decrease of OD was observed with increasing surface density of PAN nanofibre membranes. This linearity was observed for all test densities, excluding the control (0 g/m^2 surface density). This control represented the bacterial count prior to filtration.

It was determined that a 99.9% efficiency in bacterial trapping could be achieved at a PAN nanofibre surface density of 6.46 ± 0.14 g/m^2 for OD at 600 nm.

Discussion

Adequate protection against particles and organisms, medical devices, and filters must meet the defined health standards (e.g., the EU standard for medical face masks EN 14683:2019) (Park 2020). The unsatisfactory methodology that was applied for the determination of nanofibre filter protection effectivity during the recent COVID-19 pandemic wave caused an obvious need for structural standardization of nanofibre materials and a more profound knowledge of structural/functional relations of nanofibre membranes. This is necessary for the preparation of proper protective nanofibre membranes against particles and microorganisms characterized by different sizes and shapes, as well as for the determination of their effectivity.

This study (1) proves an exponential dependence of *E. coli* trapping effectivity on surface mass on a large scale and (2) presents a simple methodology for the modulation of filter efficiency.

We have shown that the trapping efficiency of *E. coli* estimated from the optical density of bacteria passed through the filter was exponentially dependent on the surface density of the polyacrylonitrile nanofibre membranes. This log/linear regression was proven for bacteria trapping efficiency higher than 99.9% which opens a door for the easy and tunable construction of ultrasensitive filters and/or nanosensors. In addition, this finding can be effectively applied in standardization processes and for quick quality control of nanofibre membranes.

Clearly, the advantage of nanofibre membranes lies in their very small pore sizes which vary with surface density. Due to their biomimetic features, such as submicron fibre diameter and high porosity, these membranes are predicted to be effective for the ultrafiltration of small particles (Reddy et al. 2021). Filtration may be either specific, where a functional component on the surface selectively binds particles, or non-specific, characterized by the physical retention of all particles large enough to be intercepted by the pores (Tang et al. 2022). Specific filtration involves a functional element on the surface that binds certain particles, although non-specific binding may occur. These non-specifically bound entities can be washed away, leaving behind the specifically bound particles (Horne et al. 2020).

Efficient non-specific bacterial retention on PAN nanofibre filters was demonstrated at surface densities ranging from approximately 1 g/m^2 to 6 g/m^2. Within this range, the logarithm of the filtrate's OD linearly decreases with increasing surface density, indicating a consistent exponential decline in the number of *E. coli*. This relationship facilitated the development of a methodology for assessing the efficiency of such filters. Measurement of the surface zeta potential with the use of model particles suggests the suitability of the PAN nanofibre membrane for the construction of sensors highly sensitive to bacterial contamination. Our structural analysis confirmed that the samples met the established criteria for nanofibres and that the uniformity of PAN nanofibres was sufficient for the subsequent fabrication of sensors. PAN nanofibre-based filters certified on the interception effectivity of particles of different sizes could thus be used for an easy, quick, and one-step determination of bacterial (particle) pollution.

Surface mass appeared, naturally, as a critical quantity influencing the unspecific binding of particles to nanosensors. Such a methodology with a certified filter could also be easily used for the classification of the filter efficiency and for concentration calculation of the suspended particles in the liquid in general, having knowledge of the number of retained particles on the nanofibre filter. Clearly, such information is key for preparing and classifying protective masks or sensors. Notably, the surface density of the nanofibre membrane plays a vital role in filtration efficiency. We have experimentally verified (data not shown) that nanofibre membrane deposited on non-woven spun-bond fabric captured 93.98%, whereas nanofibres with the same surface density deposited on aluminium foil retained 94.03% of the bacteria.

Furthermore, the surface properties of the nanofibre membrane have been characterized. The development of a net surface charge alters the ionic distribution at the interface, remarkably increasing the concentration of counterions near the surface. This phenomenon is critical for the non-specific binding of particles to nanosensors, as indicated by the measured zeta potential.

Experimental verification has shown that the substrate onto which nanofibres are deposited has a negligible effect on filtration efficiency, with the membrane's pore size being the decisive factor. This is pertinent to the antibacterial and antiviral efficacy of membranes used in face masks, especially those conforming to FFP1, FFP2, and FFP3 standards, which denote the level of protection based on European standards.

In conclusion, our data prove that *E. coli* can be effectively trapped on PAN nanofibre membranes within the commonly produced surface densities of about 1 g/m^2 to 5 g/m^2, which are advantageous for handling. The linear dependence of the log(OD) on the surface density provides a simple yet effective method to determine the efficiency of nanofibre filters.

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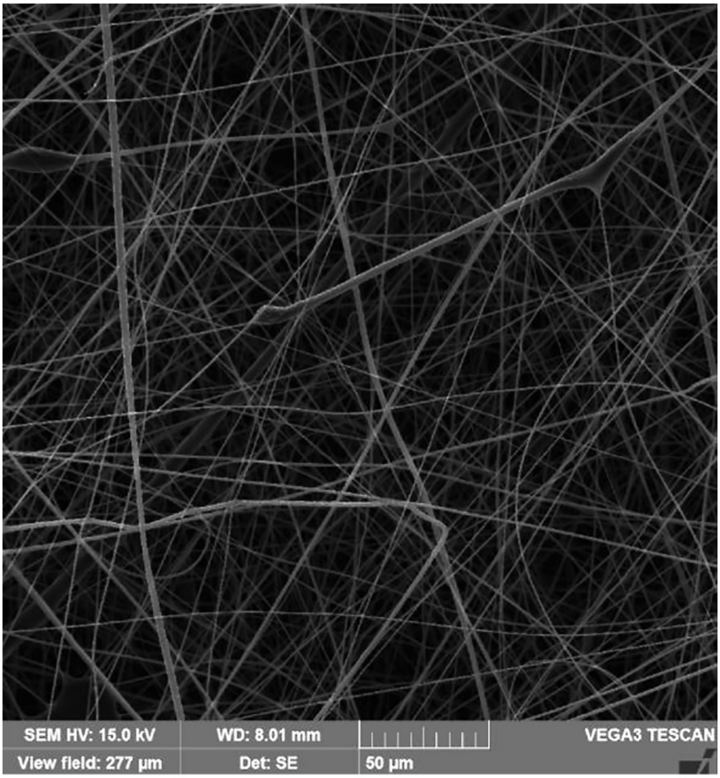

Fig. 1. Scanning electron microscopy image (with a scale bar) of the PAN nanofibre membrane with a density of 2.68 g/m²

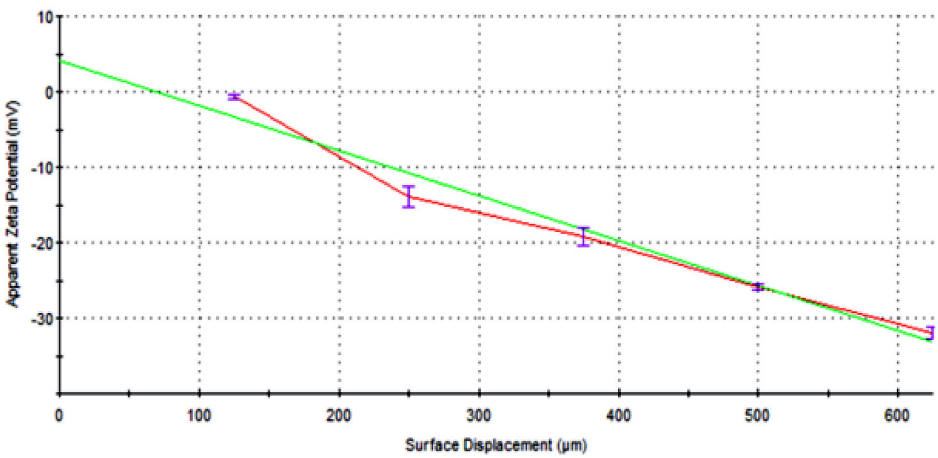

Fig. 3. The mean values and standard deviations obtained from 15 determinations. Surface zeta potential of PAN nanofibres (-34.7 mV ± 2.8 mV)