Towards drug delivery systems triggered by ion- selective interactions

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Supporting Information

Experimental

Reagents

Valinomycin, sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB), bis(2-ethylhexyl) sebacate (DOS), poly(vinyl chloride) (PVC), tetrahydrofuran (THF), N,N-dimethylformamide (DMF), multi-walled carbon nanotubes (MWCNTs), sodium carboxymethyl cellulose (NaCMC) and solvatochromic probe of methylene blue (technical grade) were from Sigma Aldrich (Germany). Analytical grade salts were from POCh (Poland). Indium tin oxide coated PET was from Sigma Aldrich (Germany). Film pouches for laminators with 100 μm thickness were from Office Supplies International. Doubly distilled and freshly deionised water (resistivity 18.2 MOhm cm, Milli-Qplus, Millipore, Austria) was used throughout this work.

Methylene blue tetrakis[3,5-bis(trifluoromethyl)phenyl]borate salt (Mb-TFPB) synthesis

150 mg of methylene blue chloride was dissolved in 10 mL of methanol. To this solution solid 416 mg (1eq) of NaTFPB was added and the reaction mixture was stirred 1 h at room temperature. The solvent was evaporated under reduced pressure and the residue was dissolved in ca. 50 mL of dichloromethane. This solution was washed with deionised water (2 x 20 mL) and filtered through a pad of silica gel eluted with dichloromethane. The solvent was evaporated under reduced pressure to afford methylene blue TFPB salt as a purple-blue solid (440 mg, 81%). The NMR spectrum of obtained salt is shown in Fig. S1.

Films and nanofibers mats

Potassium-selective membranes contained (by weight): 1.4% sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB), 2.8% valinomycin, 65.5% bis(2-ethylhexyl sebacate) (DOS) and 30.3% poly(vinyl chloride) (PVC). A total of 100 mg was dissolved in 1 mL of tetrahydrofuran (THF).

Mb-TFPB potassium-selective membranes contained (by weight): 0.9% (Mb-TFPB), 0.7% sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB), 2.8% valinomycin, 65.4% bis(2-ethylhexyl sebacate) (DOS), 30.2% poly(vinyl chloride) (PVC). A total of 100 mg was dissolved in 1 mL of tetrahydrofuran (THF).

Mb-TFPB potassium-selective mats were prepared using the electrospinning technique. Initially, 480.0 mg of PVC and 1039.0 mg of DOS were dissolved in 3 mL of a 1:1 (v/v) mixture of THF and DMF using a magnetic stirrer at 60 °C for 24 hours. Then 44.7 mg of valinomycin, 11.2 mg of NaTFPB, and 14.4 mg of Mb-TFPB were separately dissolved in 1 mL of a THF/DMF solution (1:1 v/v). Both solutions were then combined, stirred magnetically for 30 minutes, and loaded into a syringe for electrospinning.

The resulting mat had an approximate area of 320 cm^2. After electrospinning, the Mb-TFPB potassium-selective mats were left to dry in the laboratory atmosphere overnight and subsequently stored in a refrigerator at 4 °C.

The average mass of the membrane portion used (6 mm diameter) was equal to 1.39 ± 0.10 mg, this portion contained $1.2 \cdot 10^{-8}$ mol of methylene blue ions. The average mass of the portion of nanofiber mat used (6 mm diameter) for experiment was equal to 0.38 ± 0.05 mg, i.e. it was 3.6 times smaller compared to membrane portion used. The absolute contents of the methylene blue ions in the nanofibers mat portion used for experiment was equal to $3 \cdot 10^{-9}$ mol.

Carbon nanotube solutions were prepared by adding 3 mg/mL of MWCNTst to a 1 % sodium carboxymethylcellulose (CMC) aqueous solution.

Sensors intended for selectivity coefficient determination were obtained on ITO covered with layer of MWCNTs obtained from CMC stabilized dispersion, whereas sensors used for optical studies were obtained by placing a membrane directly on ITO support covered with contact

layer of MWCNTs. To obtain MWCNTs layer 5 µL of MWCNTs in CMC was applied directly on ITO and left to dry at room temperature.

A membrane was prepared by applying 0.3 mL of potassium selective cocktail on a microscope slide (2.5 cm ×2.5 cm), after solvent evaporation a film of thickness equal to 30 ±10 µm was obtained. Portions of this film or nanofiber mat, a square 0.8 cm×0.8 cm, were used to prepare sensors. It was carefully placed on ITO (or ITO covered with MWCNTs) and the whole system was sealed by lamination as described earlier.[1]

For all of the spectroscopic measurements the sensors were inserted into 3 mL continuously stirred solutions in fluorometric cuvettes. Fluorescence spectra of the membranes were collected with the membrane fixed to the back wall of the cuvette. Florescence spectra of the solutions were collected with the membrane removed from the excitation path by rotating the cuvette. Measurements were performed without any pretreatment of the membranes.

Apparatus

In the potentiometric experiments, a Lawson Labs. Inc. instrument (3217 Phoenixville Pike, Malvern, PA 19355, USA) was used, and stable potential readings (potential change <0.5 mV min−1) recorded were used to construct calibration graphs. The recorded potential values were corrected for the liquid junction potential calculated according to the Henderson approximation. The pump systems 700 Dosino and 711 Liquino (Metrohm, Herisau, Switzerland) were used to obtain sequential dilutions of calibration solutions.

In the electrospinning process, a high voltage (controlled by DC power source (ELSR30P300, Technix) was applied to a stainless-steel needle (27 G), which was mounted on a movable holder connected to a syringe containing the polymeric suspension via a PTFE tube. The flow rate of the polymeric suspension was set to 0.65 mL/h and was controlled by a syringe pump (KDS100, KD Scientific). Fiber mats were collected on an electrically grounded aluminum foil target, placed on a rotating drum collector operating at a speed of 100 rpm. The voltage applied during the electrospinning of nanofiber mats was set to 18 kV, with a distance of 14 cm between the needle and the collector. The electrospinning process was conducted at 21°C with a relative

humidity of 34%, which was regulated using a dehumidifier and air conditioning. The entire process lasted for 2.5 hours.

Fluorimetric measurements were performed using a spectrofluorimeter (Cary Eclipse) (Agilent Technologies). After excitation at wavelength of 665 nm, emission intensity was recorded within the range of 675–800 nm. Excitation and emission slits were both set to 5 and PMT voltage had to be adjusted depending on particular measurement conditions.

Fluorescence images of electrospun mat or membrane cross-sections were collected using Nikon Eclipse LV 100 optical microscope operating in fluorescence detection mode, excitation 510-560 nm, recorded wavelength > 590 nm.

Fluorescence visualization of methylene blue in the volume of electrospun mats was performed using Nikon A1R MP confocal optical microscope. Excitation wavelength was set to 488 nm for the green channel: 500-550 nm and to 641 nm for the red channel 663-738 nm.

Scanning electron microscope (SEM) images were captured using a Carl Zeiss LEO 435 VP model microscope equipped with an SE2 detector. Electrospun fiber samples were coated with Au/Pd using an Emitech sputter coater for 15 seconds and then examined in secondary electron mode at an accelerating voltage of 3 kV.

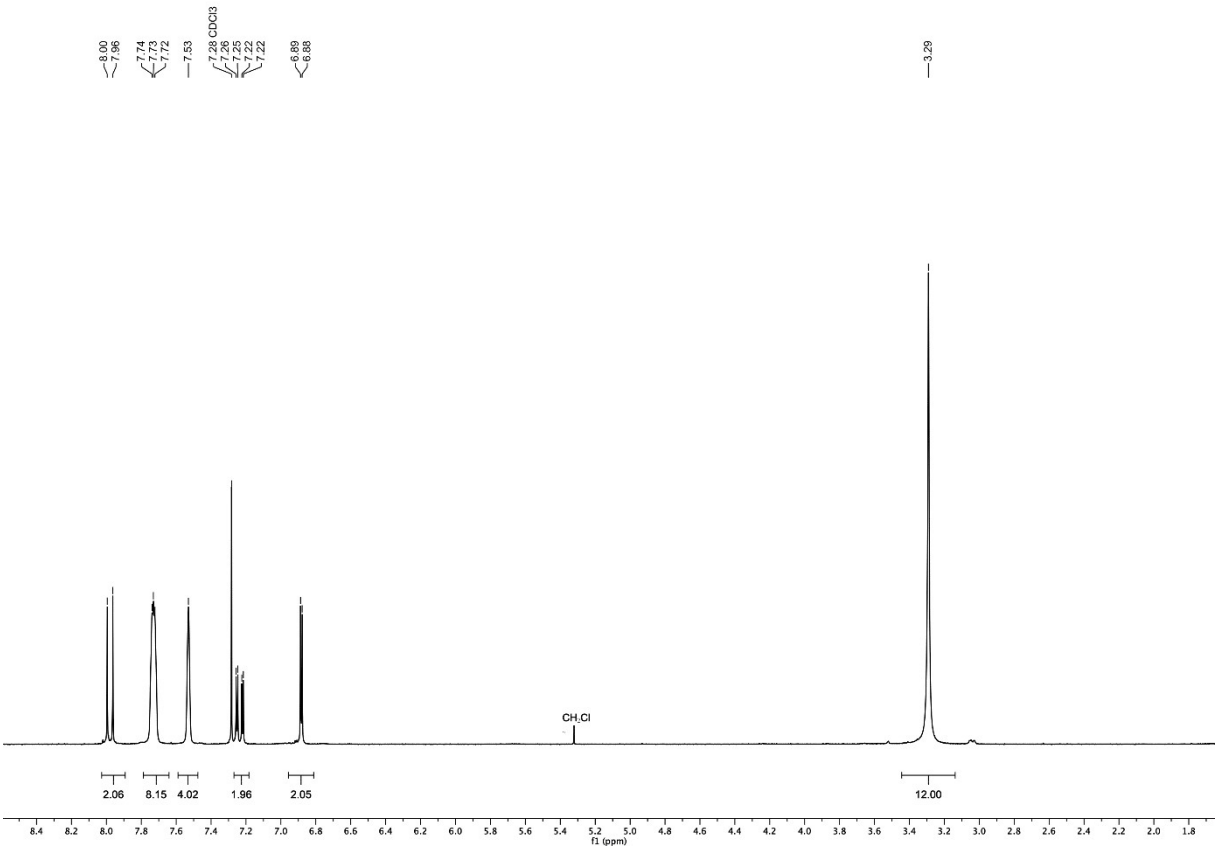
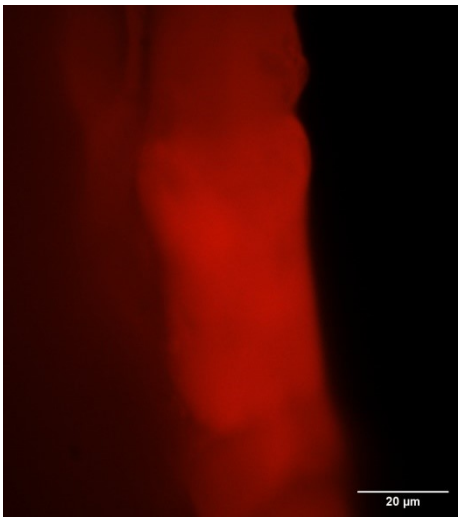
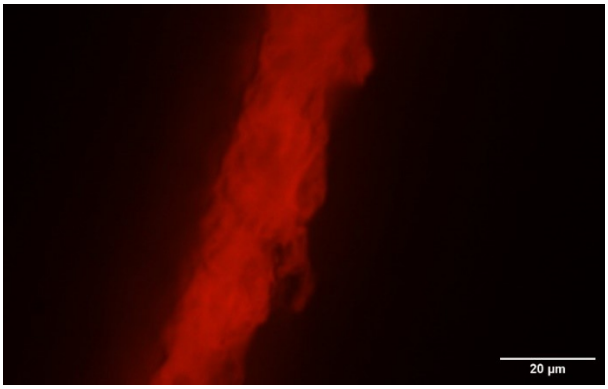


Fig S1. 1HNMR spectrum of obtained Mb-TFPB salt.

A)



B)



C)

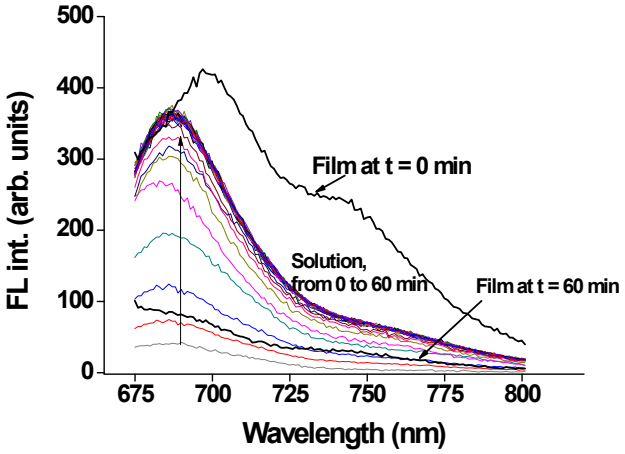


Fig. S2 A) Cross section of as prepared ITS-DDS A) film, B) nanofibers mat. C) Emission spectra of: (black) lines as prepared IST-DDS membrane and membrane post 60 min contact time with aqueous solution of 10^{-4} M KCl, and changes of solution emission – membrane was not in optical path but placed by the wall of the cuvette and parallel to excitation beam.

Fluorescence of methylene blue in lipophilic phase displays a more pronounced shoulder at around 740 nm compared to water solution. This fluorescence (~740 nm) is accentuated due to reabsorption/quenching of fluorescence below 700 nm by Mb^+ as it is present at high concentration and characterized by a small Stokes shift.

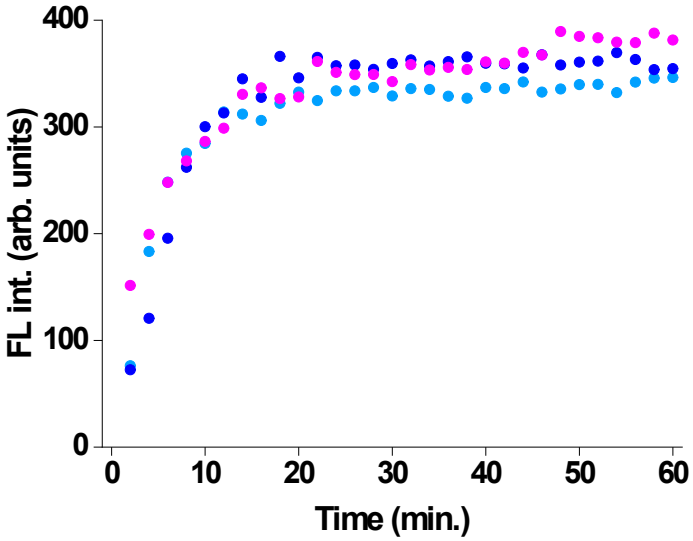


Fig. S3 The effect of anion present in solution on emission changes in response to contact time of IST-DDS film system: (●) 10^{-4} M KCl, (●) 10^{-4} M KNO_3 (●) $5 \cdot 10^{-5}$ M K_2SO_4.

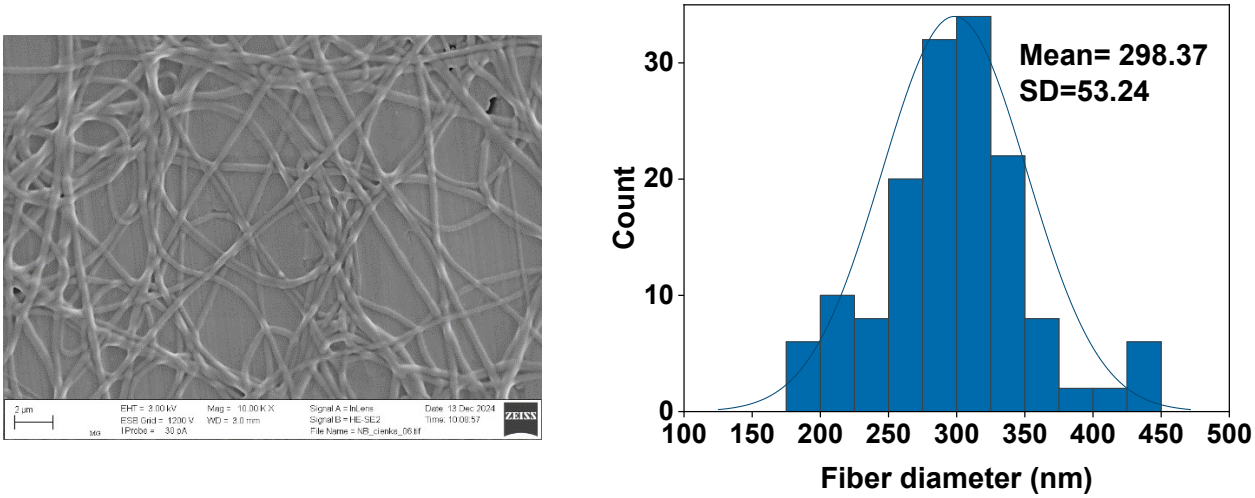


Fig. S4 Nanofibers used as IST-DDS: A) SEM image obtained of significantly thinner mats than used in optical experiments (to allow better visualization of nanofibers). B) Histogram of diameter of obtained nanofibers.

Confocal images of nanofibers in the absence or presence of potassium ions

Fig. 4 shows confocal images of nanofibers in the absence of K^+ ions in as prepared, dry nanofiber mat (left column) and in the presence of 10^{-2} M KCl aqueous solution, recorded after 8 min (middle column) or after ~20 min (right column). In the red channel, at 20 min higher amplification was used (voltage change from 71 to 110 V). 488 nm or 641 nm lasers were used for sample excitation for green and red detection channels, respectively. Green detection channel was set at 500-550 nm and presumably shows mostly scattering by the nanofibers while the red channel shows fluorescence of the methylene blue in the 663-738 range. Green channel scattering allows observation of nanofibers structure, red channel emission of methylene blue.

References

[1] E. Stelmach, J. Kalisz, B. Wagner, K. Maksymiuk, A. Michalska, *Anal. Chem.,* 2024, **96**, 3253-3258.