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Stabilized chitosan-based biosensor with a tunable linear range for lactate determination in blood and undiluted sweat

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Materials and instrumentations

Experiments were carried out in distilled water. The following compounds were used: hydrogen peroxide H_2O_2 (30% solution), iron (III) hexacyanoferrate $K_3[Fe(CN)_6]$, potassium chloride KCl, iron (III) chloride $FeCl_3$, nickel chloride $NiCl_2$, medium-weight chitosan (Sigma Aldrich, USA), perfluorosulfonated ionomer (PFSI) MF4 SK (Plastpolymer, Russia), lactate oxidase (LOx, EC1.1.3.2) from Pediococcus species (109 U/mg, Sorachim, Switzerland). All compounds were of analytical grade or chemically pure and used without additional purification. Blood serums with normal and pathologic values were purchased from Spinreact (Spain). Planar screen-printed electrode structures containing carbon working electrode (Ø = 1.8 mm), carbon auxiliary and silver reference electrodes were fabricated by Rusens (Russia). Electrochemical measurements were carried out using a Palmsens potentiostats (PalmSens BV, The Netherlands) with the PSTrace software. The flow-injection setup consisted of a Perfusor Compact syringe pump (Braun, Germany), homemade flow-through wall-jet cell with a 0.5 mm nozzle, and 6-port injector (IDEX Health & Science LLC). The flow rate used was of 0.67 $mL \cdot min^{-1}$. The measurements were performed at room temperature (23±2 °C).

(Bio)sensors preparation

Hydrogen peroxide sensors were manufactured using interfacial synthesis of Prussian blue, which included casting a droplet mixture of $K_3[Fe(CN)_6]$ and $FeCl_3$ in 0.1 M KCl, 0.1 M HCl with further reduction with H_2O_2. The PB layer was activated via cyclic voltammetry and the sensors were annealed at 100 °C. Ni-HCF-stabilized sensors were prepared using interfacial synthesis of nickel hexacyanoferrate from mixture of $K_3[Fe(CN)_6]$ and $NiCl_2$ in 0.1 M KCl, 0.1 M HCl. Lactate biosensors were made with drop casting (2µl) lactate oxidase aqueous solution (1.5 mg/mL) in an aqueous solution of chitosan on Prussian Blue modified electrode. Chitosan solutions with final concentrations ranging from 0.01 to 0.1% were prepared by dilution 1% chitosan in 1% acetic acid with water. For PFSI-stabilized lactate biosensors preparation isopropanol droplet (2µl) containing perfluorosulfonated ionomer was applied to chitosan-based biosensor. To prepare test strips, biosensors with 4% PFSI coating were covered with plastic except for a 1 cm wide strip, thus enabling capillary effect.

Electrochemical measurements

Measurements were conducted in flow injection mode at flow rate 40.2 mL/h, E_{dc} = 0.0 V vs. Ag|AgCl in a 0.05 M phosphate buffer with 0.1 M KCl at pH 6.0. The test strip measurements are based on the addition and removal of analyte on the biosensor surface via capillary effect, with the signal recorded in chronoamperometric mode 10 s after the start of the measurement. The (bio)sensor stability, the retention time of 90% of the signal in a 1 mM lactate solution, was measured at room temperature using chronoamperometry in batch mode under constant stirring. All measurements were conducted at E_{dc} = 0.0 V vs. Ag|AgCl in a 0.05 M phosphate buffer with 0.1 M KCl at pH 6.0.

Summary table

Biosensor composition	Sensitivity, $mA \cdot M^{-1} \cdot cm^{-2}$	LOD, M	Linear range, M	Stability 90%, min
0.01% chitosan	231 ± 12	$2 \cdot 10^{-7}$	$1 \cdot 10^{-6} – 1 \cdot 10^{-4}$	121± 3
0.05% chitosan	235 ± 28	$3 \cdot 10^{-7}$	$1 \cdot 10^{-6} – 1 \cdot 10^{-4}$	40 ± 6
0.1% chitosan	212 ± 32	$3 \cdot 10^{-7}$	$1 \cdot 10^{-6} – 1 \cdot 10^{-4}$	36 ± 8
0.2% chitosan	162 ± 20	$5 \cdot 10^{-7}$	$1 \cdot 10^{-6} – 1 \cdot 10^{-4}$	82 ± 21
0.3% chitosan	150 ± 40	$5 \cdot 10^{-7}$	$1 \cdot 10^{-6} – 1 \cdot 10^{-4}$	117 ± 11
0.05% chitosan + 0.2% PFSI	97 ± 22	$2 \cdot 10^{-6}$	$5 \cdot 10^{-6} – 5 \cdot 10^{-4}$	94 ± 25
0.05% chitosan + 1% PFSI	7.4 ± 0.3	$8 \cdot 10^{-6}$	$1 \cdot 10^{-5} – 1 \cdot 10^{-3}$	129 ± 23
0.05% chitosan + 2.5% PFSI	1.59 ± 0.02	$2 \cdot 10^{-5}$	$5 \cdot 10^{-5} – 1 \cdot 10^{-2}$	98 ± 25
0.05% chitosan + 3% PFSI	0.563 ± 0.007	$8 \cdot 10^{-5}$	$1 \cdot 10^{-4} – 5 \cdot 10^{-2}$	355 ± 38
0.05% chitosan + 4% PFSI	0.169 ± 0.002	$1 \cdot 10^{-4}$	$5 \cdot 10^{-4} – 1 \cdot 10^{-1}$	428 ± 48
0.05% chitosan + 5% PFSI	0.157 ± 0.006	$3 \cdot 10^{-4}$	$1 \cdot 10^{-3} – 1 \cdot 10^{-1}$	558 ± 75
0.05% chitosan + Ni-HCF	85 ± 7	$6 \cdot 10^{-7}$	$1 \cdot 10^{-6} – 1 \cdot 10^{-4}$	260 ± 28

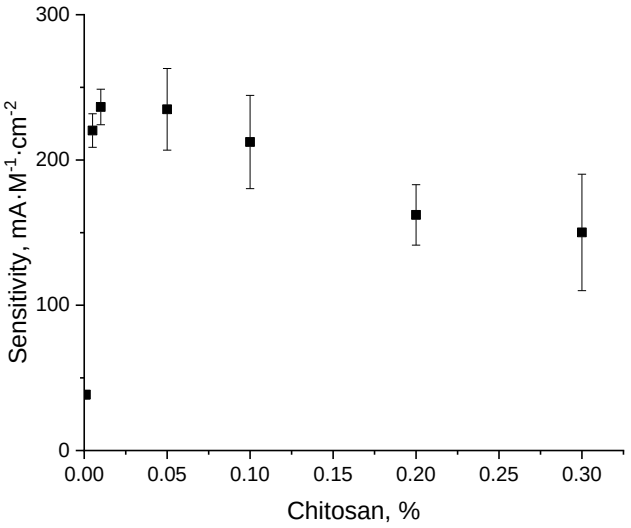


Fig. S1. Dependence of the sensitivity of a lactate Prussian blue based biosensor on chitosan proportion in membrane-forming aqueous mixture, flow injection mode, 0.05 M PBS, 0.1 M KCl, pH 6.0, 0.0 V Ag/AgCl.

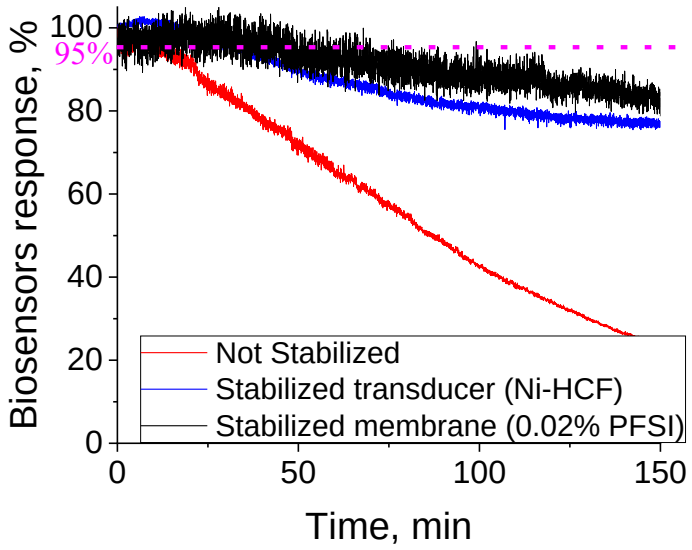


Fig. S2. Chronoamperograms of lactate biosensors based on Prussian blue and lactate oxidase immobilized in 0.05% chitosan aqueous solution and the same stabilized with Ni-HCF layer or 0.02% PFSI, batch mode upon stirring, 1 mM lactate solution in 0.05 M PBS, 0.1 M KCl, pH 6.0, 0.0 V Ag/AgCl.

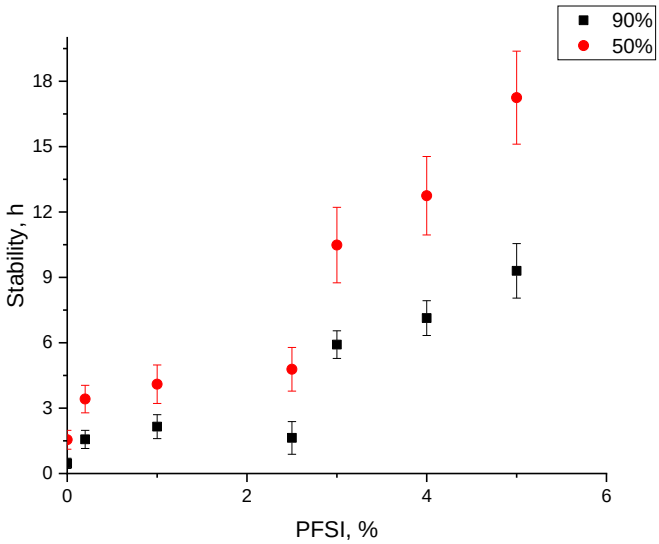


Fig. S3. Dependence of chronoamperometric signal stability period duration in 1 mM lactate solution of lactate biosensors based on Prussian blue, lactate oxidase immobilized in 0.05% chitosan aqueous solution and perfluorosulfonated ionomer on proportion of PFSI in isopropanol solution, batch mode upon stirring, 0.05 M PBS, 0.1 M KCl, pH 6.0, 0.0 V Ag/AgCl

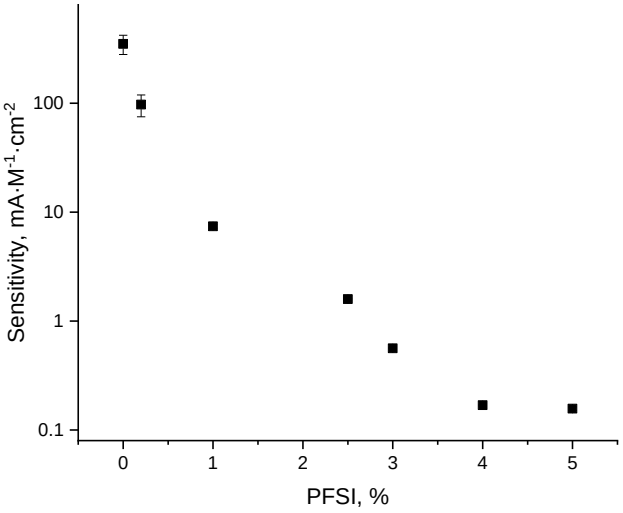


Fig. S4. Dependence of sensitivity of lactate biosensors based on Prussian blue, lactate oxidase immobilized in 0.05% chitosan aqueous solution and perfluorosulfonated ionomer on proportion of PFSI in isopropanol solution, flow injection mode, 0.05 M PBS, 0.1 M KCl, pH 6.0, 0.0 V Ag/AgCl.

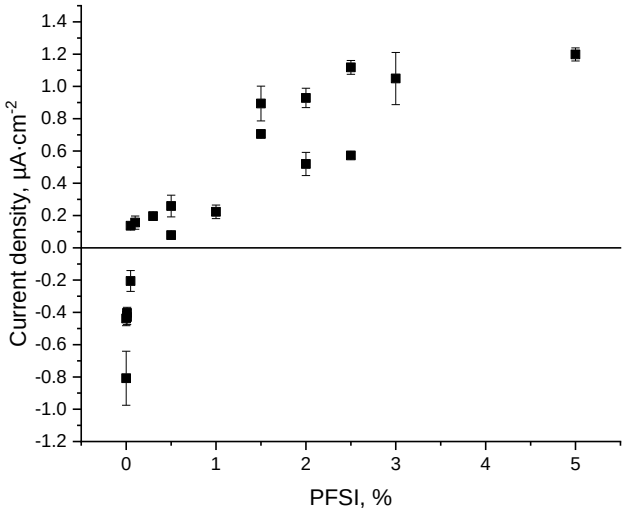


Fig. S5. Dependence of amperometric signal upon injection of 0.2 M KCl solution in flow injection mode of sensors based on 0.05% chitosan aqueous solution and perfluorosulfonated ionomer on proportion of PFSI in isopropanol solution, 0.05 M PBS, pH 6.0, 0.0 V Ag/AgCl.

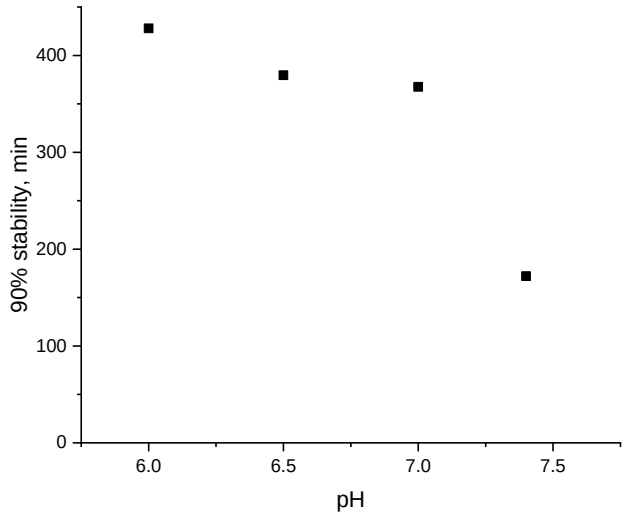


Fig. S6. Dependence of 90% chronoamperometric signal stability period duration in 1 mM lactate solution of lactate biosensor based on Prussian blue, lactate oxidase immobilized in 0.05% chitosan aqueous solution and 4% perfluorosulfonated ionomer isopropanol solution on value of pH, batch mode upon stirring, 0.05 M PBS, 0.1 M KCl, 0.0 V Ag/AgCl.

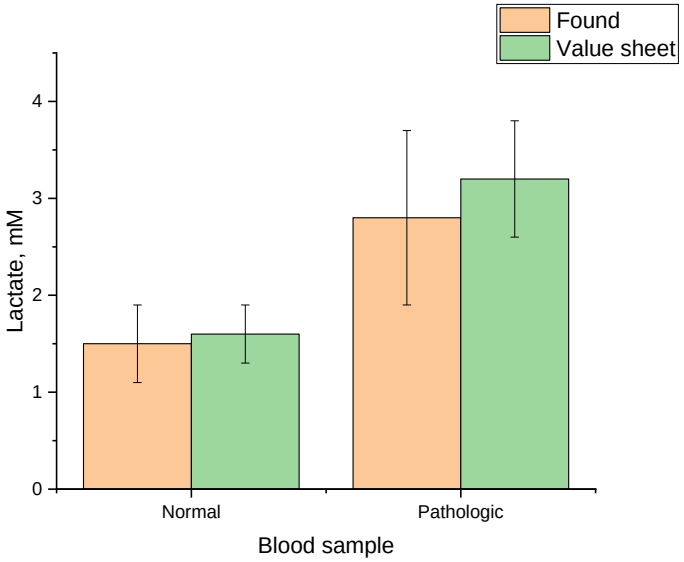


Fig. S7. Results of lactate detection in human blood serum samples in flow injection mode with 100 times dilution using biosensor based on Prussian blue, lactate oxidase immobilized in 0.05% chitosan aqueous solution, 0.0 V Ag/AgCl.

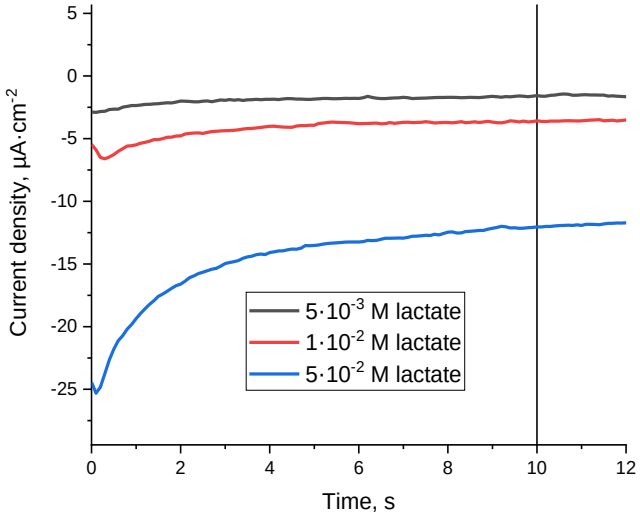


Fig. S8. Chronoamperograms of lactate biosensors based on Prussian blue and lactate oxidase immobilized in 0.05% chitosan aqueous solution and 4% perfluorosulfonated ionomer isopropanol solution, capillary mode, 0.05 M PBS, 0.1 M KCl, pH 6.0, 0.0 V Ag/AgCl.

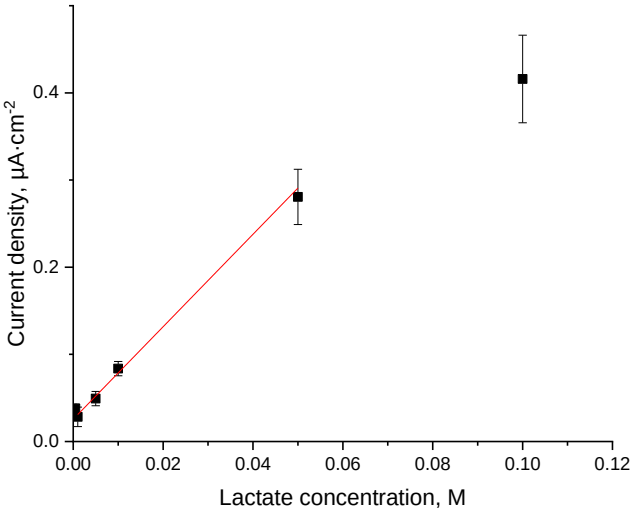


Fig. S9. Calibration curve of lactate biosensor based on Prussian blue and lactate oxidase immobilized in 0.05% chitosan aqueous solution and 4% perfluorosulfonated ionomer isopropanol solution, capillary mode, 0.05 M PBS, 0.1 M KCl, pH 6.0, 0.0 V Ag/AgCl.