Ref. Ares(2019)7789033 - 18/12/2019

FORMILK

Innovative technology for the detection of enzyme activity in milk

D3.1

Miniature potentiostat, software and sensor

Project acronym	FORMILK
Project start date	1/1/2016
Duration:	48 months
Deliverable type:	Demonstrator
Dissemination level:	Public
Activity and Work package contributing to the deliverable:	WP3 - Transfer of technology [Months: 13-48] SONAS
Due date:	M48
Date:	12.12.2019
Responsible lead organisation:	Powertec ltd. (PTEC)
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Contents

1. Introduction

Modern society depends on quality and reliable food production such as milk production. Ability to measure milk composition is essential for determining milk quality. There are various reports on milk composition and quality based on storage [1], dry period [2] or dietary supplements [3]. Activity of plasmin in milk is one of the quality factors [2]. Several methods of measuring this plasmin activity exist [2, 4, 5], but all of that are using rather large, expensive and not a user-friendly equipment. The objective however is to provide these methods to ordinary day at farms. Milk components can be easily identified by electrochemical methods such as cyclic voltammetry. Cyclic voltammetry is simple method which can be implemented into hand held device. There are commercially available potentiostats, most of them are bulky bench top laboratory devices. Our goal was to develop low cost handheld potentiostat optimized for measuring plasmin activity in milk.

2. First iteration of device

During the first year has PTEC based on the knowledge achieved during completed secondments focused on preparation of preliminary specification of proposed potentiostat. Main part of the potentiostat will covered by LMP91000 analog front end chip from TI for low power chemical sensing applications. This component have integrated transimpedance amplifier with programable gain, and thanks to this PTEC will achieve down to 5 uA full-scale measuring range. There are components that are still in specification phase – the ADC and MCU. These components are being under discussions due to necessary information from other partners of the project consortium that are being collected during undergoing secondments. Within the realized PTEC secondments the electrochemical measurements with nanostructured electrodes modified by ferrocene-peptide by various methods were investigated. Impact of nanostructured film fabrication techniques was observed. The polishing of the rigid gold electrodes had significant effect on maximum response during cyclic voltammetry.

During the first year of the project were further the preliminary measurement of electrical properties of prepared sensing layer, such as charge transport through the layer consisting of caseine and gold nanoparticles. The gold nanoparticles encapsulated by alkylthiols should enhance the limit of detection of developed plasmin sensor. Based on these measurements the charge transport properties have been evaluated and band or hopping transport (1D or 3D hopping) have been found.

These results are crucial for better understand the enzyme sensor and proper sensor design. Besides this a possible scenarios of cooperation on development of specific nano-biosensors used in potentiometric method of measuring plasmin level in milk were composed. It was observed that optimal size of nano-structuralization on biosensor surface has major influence on kinetics of electron transfer which has positive effect to measurement and sensitivity of system, hence it is possible to reach even lower limit of detection.

Design of potentiostat

The core of this frontend is integrated circuit LMP91000. This circuit has integrated transimpedance amplifier is with potentiostat circuit and variable bias. Gain of transimpedance amplifier is programmable to seven values between 2750 V/A and 350 000 V/A or user settable to any value via external resistor. This allows us to reach full scale from ±5 µA to ±700 µA.

Potentiostat is designed to work with two or three electrode systems. Two differential analog to digital converters simultaneously samples voltage between reference electrode and working electrode, and voltage output of transimpedance amplifier representing current flow from counter electrode to working electrode. Internal temperature sensor of LMP91000 is connected to microcontroller’s analog to digital converter, these temperature readings are used for calibration of device. Variable bias is used to generate desired output waveforms, it is controlled via I^{2}C interface by microcontroller.

Preliminary testing of analog front end

Analog frontend was built for purpose of preliminary testing as shown in Fig 1. Circuit was powered from 3.7 V power supply. Digital oscilloscope *RIGOL DS1052D* was used perform analog measurements and conversion into digital domain. Measured waveforms were processed using Matlab. Voltage applied between CE and WE is settable with 73 mV steps with full scale ±0.88/ V. During testing we used triangle wave with 1 second period.

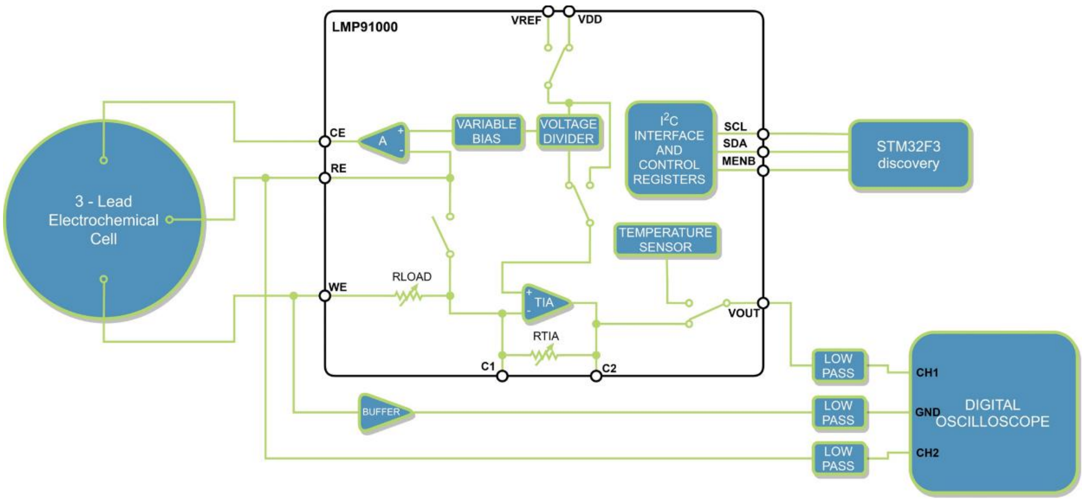
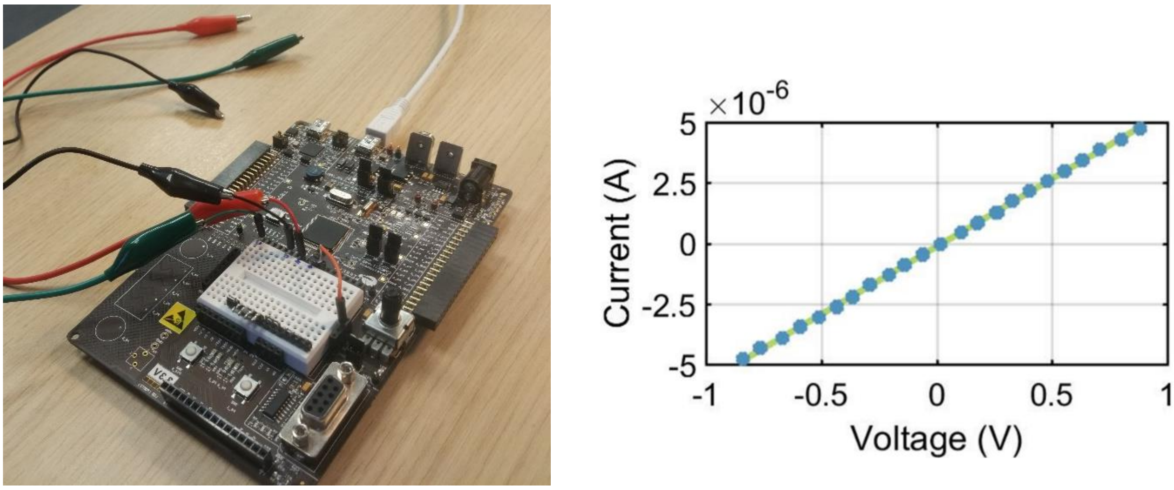


Fig 1. Block diagram of analog frontend.

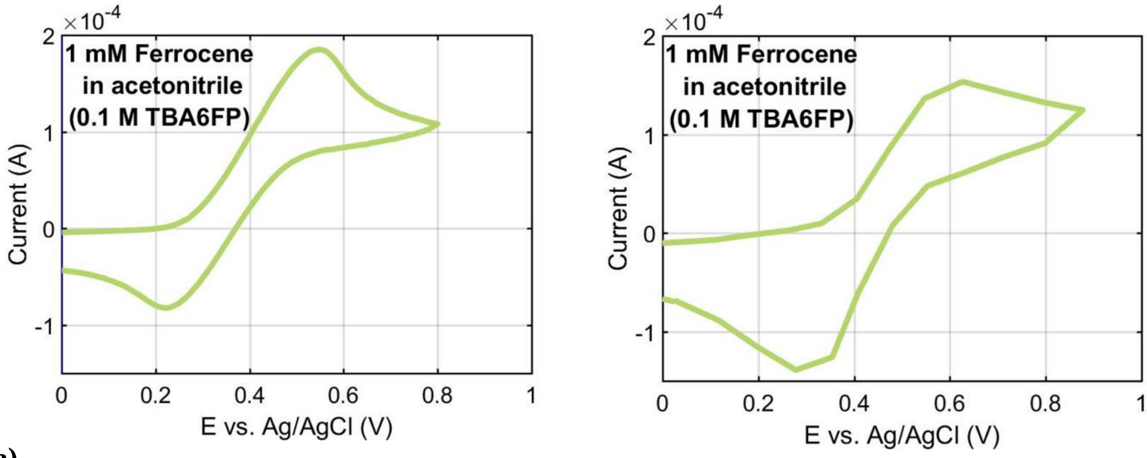
The first tests were done on electronic parts such as resistor for evaluation of accuracy. Fig. 2b. shows measurement results of resistor with nominal value 180 kΩ, calculated resistance using least square linear fit was 178.6 kΩ. Measured value of resistor with multimeter UNIT UT58D was 179.8 kΩ (±1.5 kΩ). These results are within the tolerances.



(a) **(b)**

Fig 2. (a) First prototype of devices and (b) measurement of resistor.

Final tests were in solution of 1 mM Ferrocene in acetonitrile with 100 mM tetrabutylamonium hexafluorophosphate. Gold working electrode, Platinum counter electrode and Ag/AgCl reference electrode were used. Reference measurement (Fig 3a.) was done with commercially available potentiostat EmStat3+. Redox potential E vs. Ag/AgCl was E1/2ref = 320 mV and redox peak current was i1/2ref = 108 µA. Using same solution and setup test measurement of designed analog frontend was done. Results of test measurement (Fig 3b.) was redox potential E vs. Ag/AgCl E1/2test = 350 mV and redox peak current was i1/2test = 102 µA. Test measurement is in are in conformity with reference measurement. Voltage steps between CE and WE of 73 mV were too rough and had significant influence on current flow.



(a) **(b)**

Fig 3. Reference measurement (a) and analog frontend testing measurement (b).

3. Second iteration of device

During the second year has PTEC developed first prototype of a miniature potentiostat. The device properties have been carefully designed in accordance to requirements of the experiment conditions defined by project partners. The prototype was designed using programmable system on chip (PSoC) from Cypress semiconductor with ARM processor Cortex M3. Since the potentiostat setup is done through the USB port, the device is during the experiment controlled by single button only what enables make it smaller and handheld. Current on working electrode (WE) is changed on voltage by the transimpedance amplifier and measured by 18 bit Delta-Sigma ADC with 2000 samples per second (SPS). All device properties and components have been under discussions due to necessary information from other partners of the project consortium that are being collected during undergoing secondments. The potentiostat performance have been tested by the cyclic voltammetry study done on 10mM ferrocyanide/ferricyanide redox couple in PBS solution with gold working electrode and Ag/AgCl reference electrode. To demonstrate measurement reliability a comparison with common desktop potentiostat (FRA2 μAUTOLAB type III) have been done, Fig. 5, and identical voltammograms have been recorded (difference lies in the range of common reproducibility of electrochemical measurements). The further improvement of the device is planned in accordance to discussion with project partners.

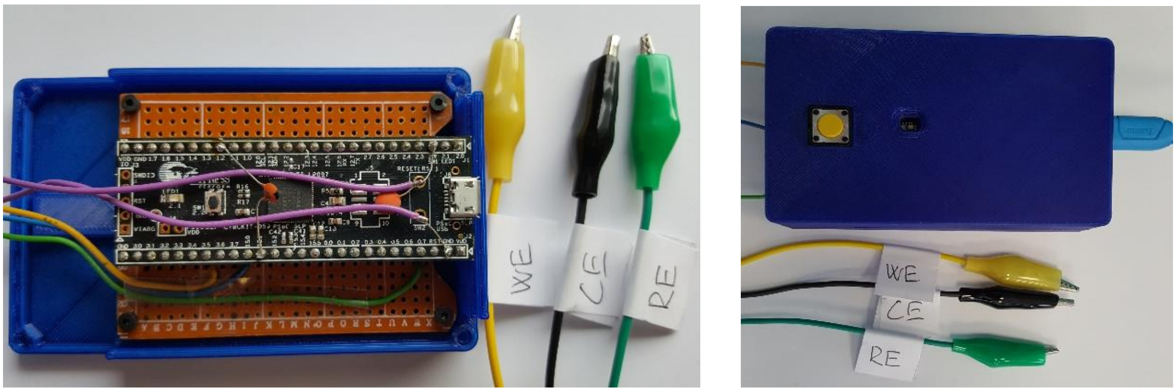


Fig. 4. Second generation miniature potentiostat with crocodile clips for electrodes and 3D printed enclosure.

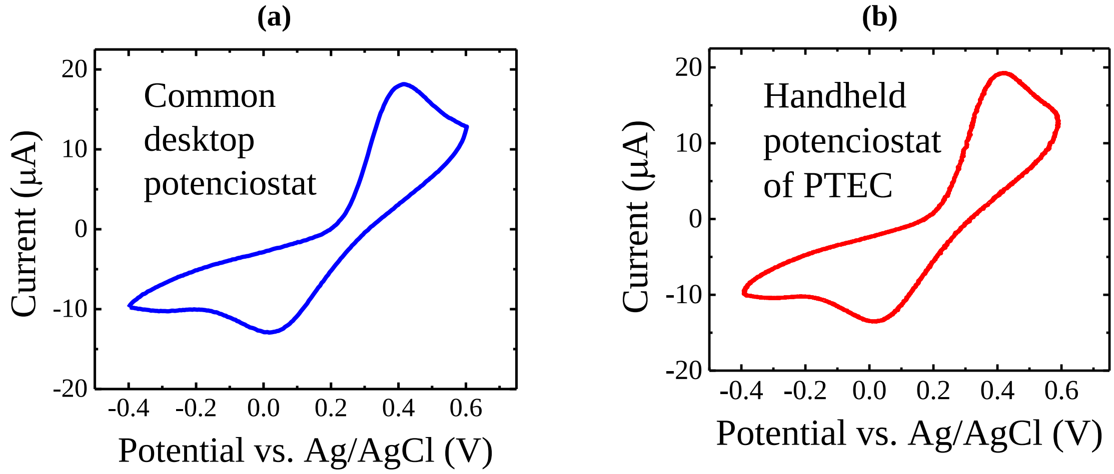


Fig. 5. Cyclic voltammetry study of 10mM ferrocyanide/ferricyanide redox couple in PBS at a scan rate of 100 mV/s recorded by (a) common desktop potentiostat (FRA2 μAUTOLAB type III) and (b) handheld potentiostat designed by PTEC.

The linear increase of the voltage applied to the electrode is an ideal case that never happens. The voltage ramp is digitalised, and staircase-like ramp simulates the linear growth. Since the voltage steps are rather small, the abrupt increase is neglected and the voltage ramp is assumed as a smooth linear one. However, it was found that the measurement conditions are not ideal enough to suppress the capacitive current. In other words, the current charging the capacitance can be expressed as a capacitance multiplied by the derivative of voltage with respect to time. Due to the abrupt change of the voltage, the current spike appears followed by the exponential decrease to the steady-state value. The time required to stabilize the current is denoted as relaxation time and is defined as a product of the capacitance and the resistance. Please note that the capacitance as the equivalent electrical circuit element stands for the Helmholtz double-layer, whereas the resistance represents the reciprocal value of the solution conductivity. As a very first step, the delay time in measurement was introduced. The voltage step is followed by the delay; hence, the current is measured in steady-state conditions. It was found that this trivial solution offers reasonable results even though the voltage ramp digitalization is present. Reduction of the electrode area also helps to reach a higher sweep rate with higher resolution.

4. Third iteration of device

The final iteration of the device has been carefully designed in accordance with the requirements of the experiment conditions defined by project partners.

The final device was designed using a programmable system on chip (PSoC) from Cypress semiconductor (CY8CKIT-059) with ARM processor Cortex M3. The potentiostat communicates with the computer via a USB port using the virtual port.

The potentiostat setup is done through the graphic interface created using LabView. The device is during the experiment controlled by a single button only what enables to make it smaller and handheld. The power supply of the final version potentiostat prototype has been upgraded - the device is powered via a USB port (no external power supply required).

Current on working electrode (WE) is changed on voltage by the transimpedance amplifier and measured by 12bit Delta-Sigma ADC with 2000 samples per second (SPS). Due to 12 bit AD converter potentiostat achieves sufficient accuracy.

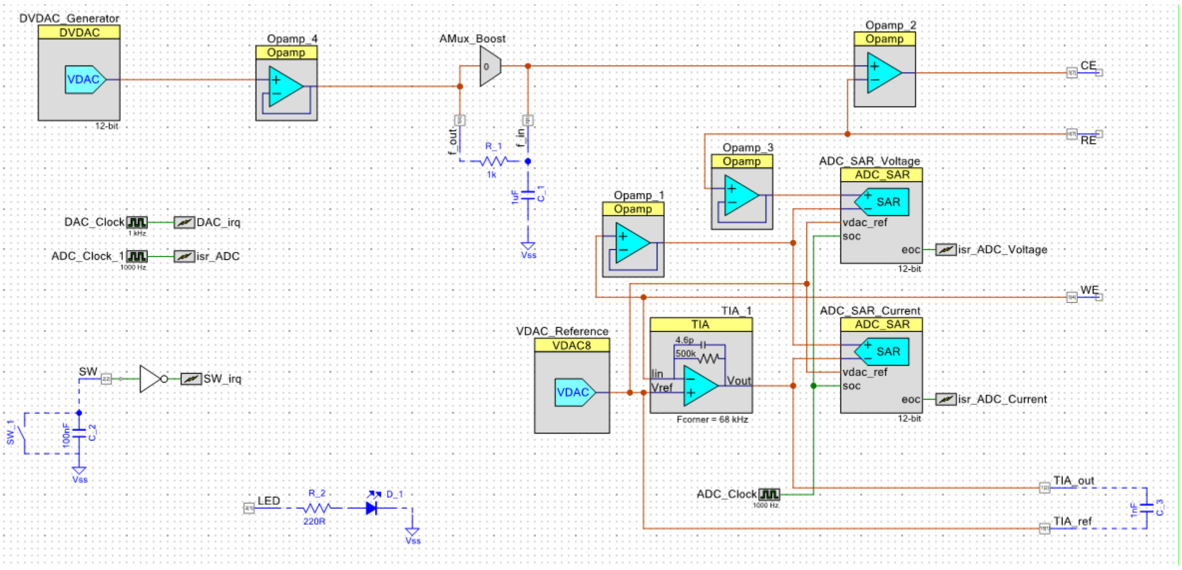
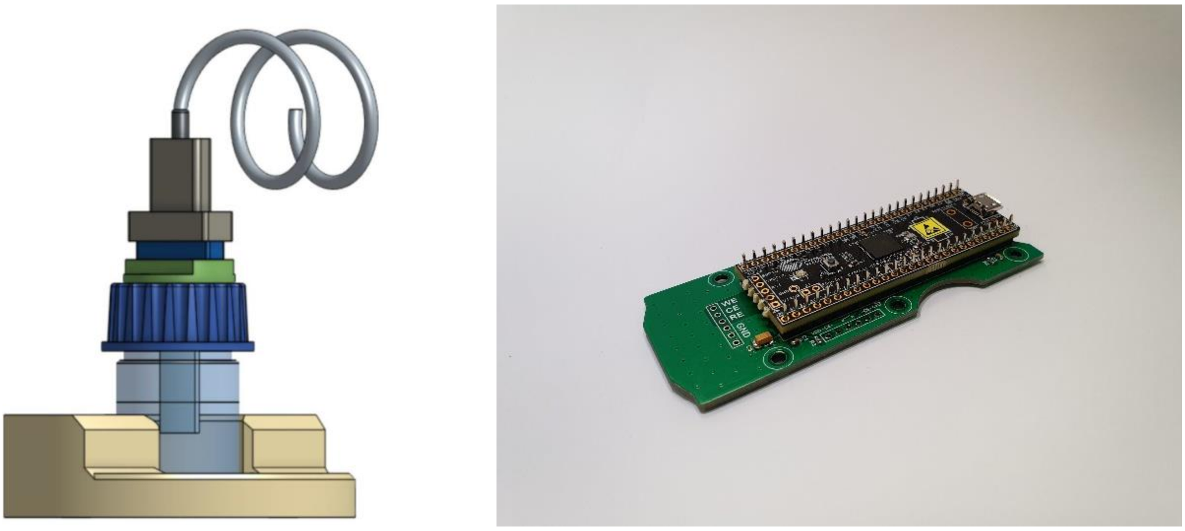


Fig. 6. Final circuit diagram of potentiostat.

Measurement voltage	-2000 - 2000 mV
Scan rate	100 - 1000 mV/s
Quiet time	0 – 10 000 ms
Current range	±2, ±4, ±8, ±20, ±25, ±50, ±66, ± 100µA
AD/DA converters	12 bit

Tab. 1. Operating parameters.



(a) **(b)**

Fig. 7. (a) Test screen printed electrodes in the electrochemical cell and (b) final PCB of potentiostat.

Fig.8. Final PCB potentiostat and electrode.

5. Software

5.1.First iteration of software

First iteration of software used serial port and HTerm interface to communicate. Press the button to turn on the device. Parameters are set via serial port according to help below. The program is simple, while waiting to press the button, we can use help when we send the "HELP" command to the potentiostat.

<u>HELP:</u>
QUIET - Quiet time in ms (0 to 10000)
START - Initial voltage in mV (0 to 2000)
HIGH - Highest voltage in mV (0 to 2000
LOW - Lowest voltage in mV (- 2000 to 0)
RATE - Scan rate in mV/s (100 to 1000)
CURRENT - Maximum current, see below (0 to 7)
COUNT - Number of scans (1 to 100)
POLARITY - Positive: 1, Negative: -1

PULSE AMPLITUDE - Amplitude of pulse for DPV in mV (only for DPV)
PULSE WIDTH - Width of pulse for DPV in ms (only for DPV)
PULSE PERIOD - Period of pulse for DPV in ms (only for DPV)
SAMPLE TIME - Sample time before pulse for DPV in ms (only for DPV)

<u>Use these values for current range setting:</u>
0 - 100 uA
1 - 66 uA
2 - 50 uA
3 - 25 uA
4 - 20 uA
5 - 8 uA
6 - 4 uA
7 - 2 uA\

<u>To set new parameters send string as follows:</u>
SET: QUIET, START, HIGH, LOW, RATE, CURRENT, COUNT, POLARITY, PULSE AMPLITUDE, PULSE WIDTH, PULSE PERIOD, SAMPLE TIME

EXAMPLE 1:

To set up parameters for CV:

QUIET: 500, START: 1000mV, HIGH: 1100mV, LOW: -900mV RATE: 500 mV/s, CURRENT: 100 uA, COUNT: 3 POLARITY: Positive

Send this string:
SET: 500, 1000, 1100, -900, 500, 0, 3, 1\

EXAMPLE 2:

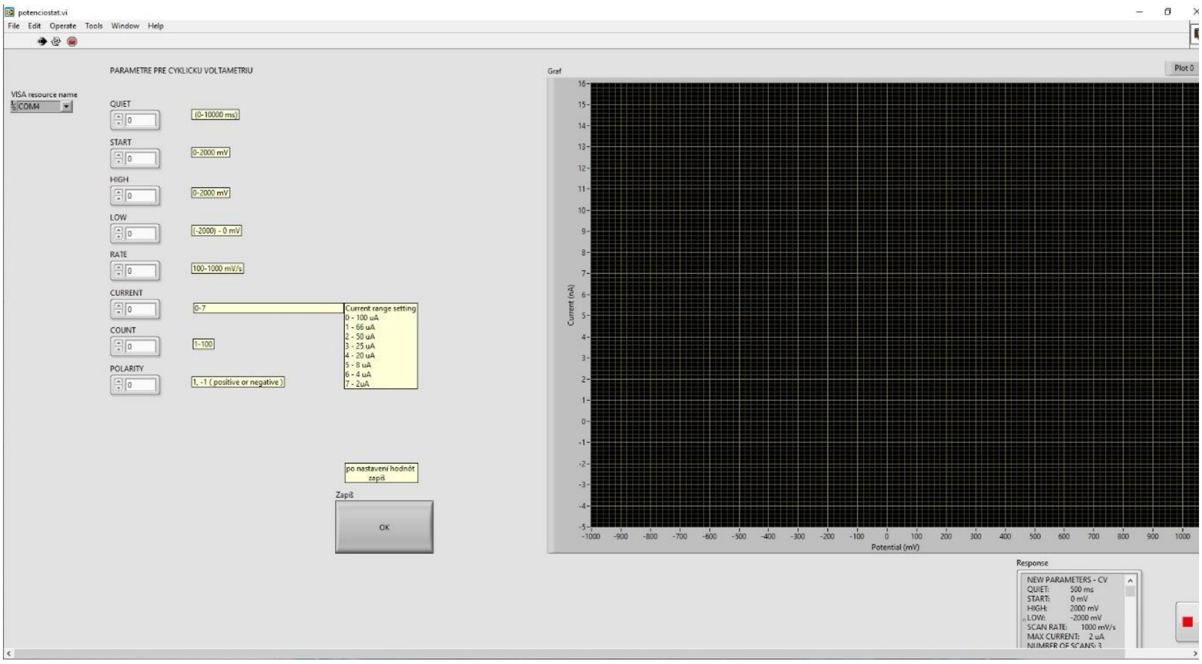
To set up parameters for DPV:

QUIET: 500, START: 1000mV, HIGH: 1100mV, LOW: -900mV, RATE: 500 mV/s, CURRENT: 100 uA, COUNT: 3, POLARITY: Positive, PULSE AMPLITUDE: 500 mV, PULSE WIDTH: 50 ms, PULSE PERIOD: 200 ms, SAMPLE TIME: 17 ms

Send this string:
SET: 500, 1000, 1100, -900, 500,0, 3, 1, 500, 50, 200, 17

5.2.Second iteration of software

In the second iteration of software the potentiostat setup was created through the graphic interface using programming language LabView. The device is possible during the experiment controlled by a single button only what enables to make it small size and handheld.



VISA resource name: COM4

QUIET: 0 (0-10000 ms)
START: 0 0-2000 mV
HIGH: 0 0-2000 mV
LOW: 0 (-2000) - 0 mV
RATE: 0 100-1000 mV/s
CURRENT: 0 0-7
COUNT: 0 1-100
POLARITY: 0 1, -1 (positive or negative)

Current range setting
0 - 100 uA
1 - 66 uA
2 - 50 uA
3 - 25 uA
4 - 20 uA
5 - 8 uA
6 - 4 uA
7 - 2uA

po nastavení hodnôt zapiš

```
NEW PARAMETERS - CV
QUIET:          500 ms
START:          0 mV
HIGH:           2000 mV
LOW:            -2000 mV
SCAN RATE:      1000 mV/s
MAX CURRENT:    2 uA
NUMBER OF SCANS: 3
```

Fig. 9. The graphic interface created using LabView.

6. Device case

The device consists of few individual parts: A) On/off button, B) Red LED diode - indicates the status of the measurement, C) Green LED diode – indicates the status of the power supply, D) stainless steel screws M2.5, E) metal connector, F) spacers, G) USB connector and H) PCB.

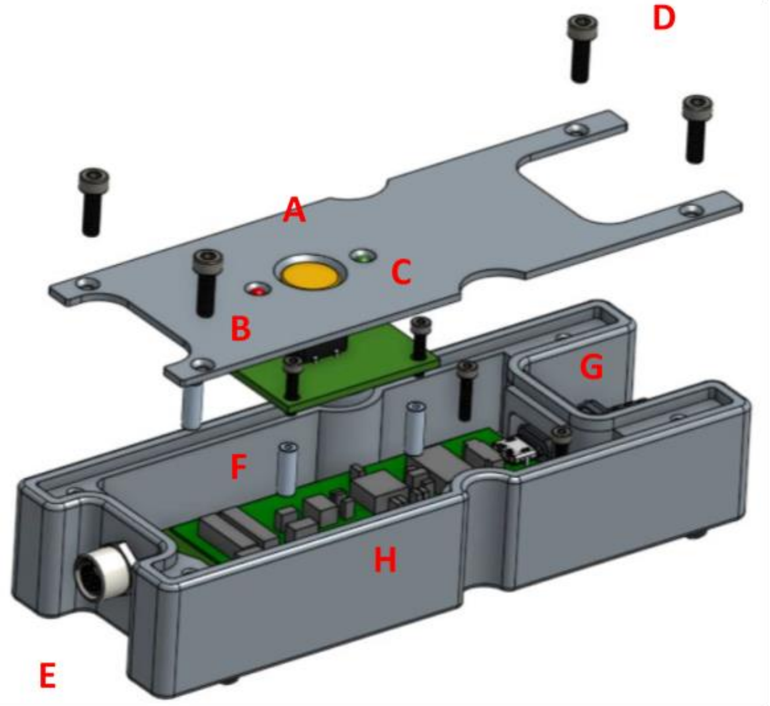
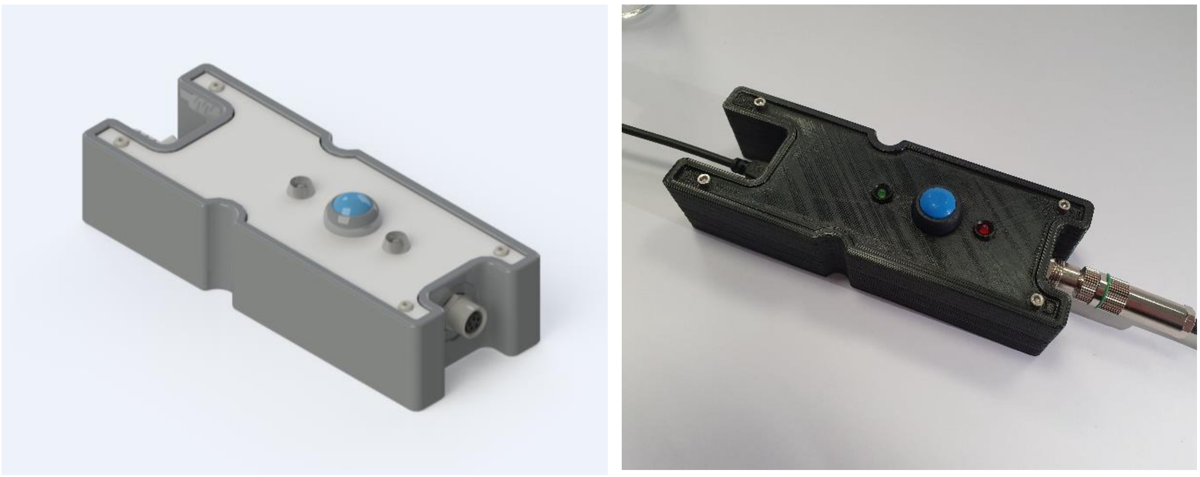


Fig. 10. Individual parts of the device.



(a) **(b)**

Fig. 11. (a) Model of potentiostat case and (b) real device case created by 3D printing technology.

7. Summary

One of the issues identified in the first part of the project is to provide a methodology for the preparation of electrodes for electrochemical measurements. Used electrodes are expensive (gold wire or film) and surface preparation have a huge impact on results. There was designed a surface modifying process that can eliminate this issue by using commercial thin-film electrodes. This modified electrode system allows faster and cheaper monitoring of plasmin activity. It has been found that the sensing layer thickness and/or homogeneity play a key role in the electrical and sensing properties of the layer. As a result, proper deposition techniques should be developed to improve the layer deposition conditions and enhance the sensing layer reproducibility.

We have constructed prototype frontend of designed potentiostat were able to measure it's basic performance. We have verified accuracy by measuring resistance of resistor with known value. Test measurements on Ferrocene solution were consistent with reference measurement. The final prototype of miniature handheld potentiostat has been constructed and validated with the commercially available instrument. Validation of potentiostat with the sensors in real samples and further commercialization was demonstrated on FORMILK 2019 school at the period 2018-2019.

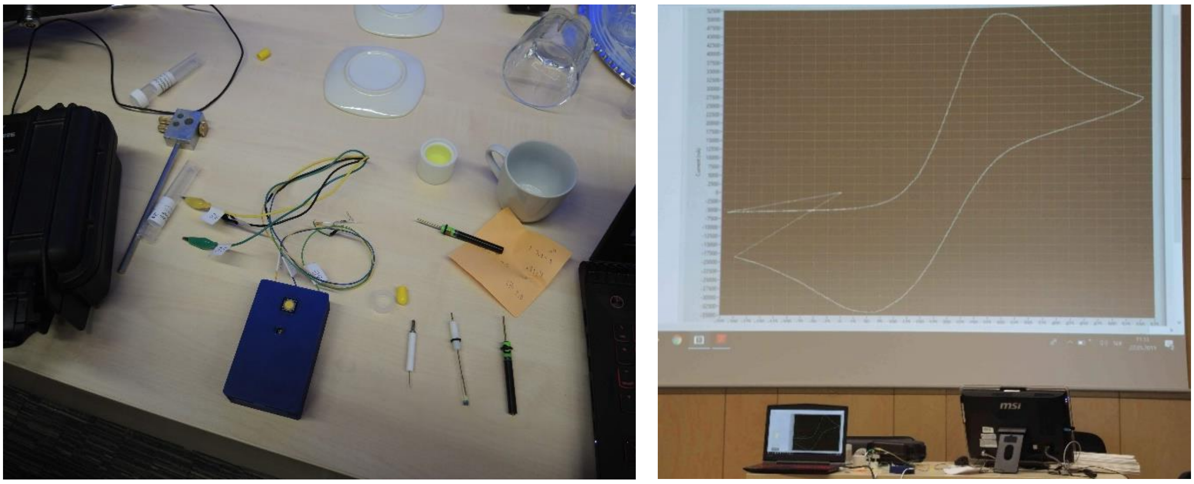


Fig. 12. Validation of potentiostat with the sensors in real samples and further commercialization was demonstrated on FORMILK 2019 school at the period 2018-2019.

8. References

[1] A. O’Connell, A.L. Kelly, J. Tobin, P.L. Ruegg, D. Gleeson, The effect of storage conditions on the composition and functional properties of blended bulk tank milk, Journal of Dairy Science, Volume 100, Issue 2, 2017, Pages 991-1003, ISSN 0022-0302,

[2] R. de Vries, M. Brandt, Å. Lundh, K. Holtenius, K. Hettinga, M. Johansson, Short communication: Influence of shortening the dry period of Swedish dairy cows on plasmin activity in milk, Journal of Dairy Science, Volume 99, Issue 11, November 2016, Pages 9300-9306, ISSN 0022-0302

[3] Hameed, Aneela & Anjum, Faqir & Zahoor, Tahir & Rahman, Zia & Akhtar, Saeed & Hussain, Majid. (2016). Effect of oxytocin on milk proteins and fatty acid profile in Sahiwal cows during lactation periods. Turkish Journal of Veterinary and Animal Sciences. 40. 163-169.10.3906/vet-1506- 29.

[4] Castillo, G., Pribransky, K., Mező, G., Kocsis, L., Csámpai, A., Németh, K., Keresztes, Z. and Hianik, T., Electrochemical and Photometric Detection of Plasmin by Specific Peptide Substrate. Electroanalysis, 27: 789–798., (2015) doi:10.1002/elan.201400622

[5] A. Poturnayova, I. Karpisova, G. Castillo, G. Mező, L. Kocsis, A. Csámpai, Z. Keresztes, T. Hianik, Detection of plasmin based on specific peptide substrate using acoustic transducer, Sensors and Actuators B: Chemical, Volume 223, February 2016, Pages 591-598, ISSN 0925-4005