*Int. J. Electrochem. Sci.*, 16 **(2021)** Article ID: 210760, doi: 10.20964/2021.07.59

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

# The Determination of Lipase Activity by Measuring pH Using ion-Sensitive Field-effect Transistor

*Tomáš Válek*[1,2], *Miroslav Pohanka*[1]

[1] Faculty of Military Health Sciences, University of Defence, Trebesska 1575, CZ-500 01 Hradec Kralove, Czech Republic
[2] Faculty Of Pharmacy, Charles University, Akademika Heyrovského 1203, 500 05 Hradec Králové, Czech Republic
*E-mail: miroslav.pohanka@gmail.com

*Received:* 22 March 2021 / *Accepted:* 4 May 2021 / *Published:* 31 May 2021

Lipases play an important role in the human metabolism and their abnormal function appear to be associated with many pathological conditions including obesity, Alzheimer disease, atherosclerosis, dyslipideamia associated with diabetes, some infections etc. Measurement of lipase activity therefore becomes important for instrumental diagnosis of the mentioned pathologies. In this work, lipase activity was measured using ion-sensitive field-effect transistor (ISFET) enzyme biosensor based on Tween 20 as a substrate. Developed biosensor is based on measurement of pH change induced by reaction of lipase with Tween 20 when potentiometric ISFET was used for determination of pH changed by the analyte. Tween 20 in concentration range from 0 to 4 % was measured for determination of optimal Tween 20 concentration. Limit of detection was set to be 0.02 %and result curve showed Michaelis–Menten dependency with the Michaelis–Menten constant equal to 0.11 %. Concentration curve of lipase in concentration range from 0 – 4 mg/ml was measured with 2 % Tween 20. Limit of detection was calculated to be 0.02 mg/ml with correlation coefficient 0.998 and Michaelis–Menten constant was equal to 0.36 mg/ml. Effect of interferences and matrix was tested with different concentration. Novel ion-sensitive field-effect transistor method is undemanding for laboratory equipment, chemicals and easy repeatable and shows good analytical results comparable with other analytical methods.

**Keywords:** ISFET, lipase activity, pH, Tween 20, fatty acid

## 1. INTRODUCTION

Lipases are enzymes occurring from microorganisms through fungi, plants and animals to mammals and humans and play irreplaceable role in the elemental metabolic pathways [1]. Lipases are hydrolytic enzymes belonging to the lipase-esterase superfamily (E.C. 3.1.1.3). Their function is to catalyze the hydrolysis or synthesis of a wide range of substrates containing ester bonds in triglycerides, mostly fatty acid and glycerol are created [1-3]. The lipase catalytic center contains active amino acids

residua serine, histidine, glutamate or aspartate, therefore they are also known as serine hydrolases [2,3]. The specific properties of lipases vary with temperature, stability in organic solvents, substrate specificity and pH, so they are suitable for a wide range of applications [4,5]. The use of lipases is important in industries, medicine, pharmacy, analytical chemistry, biotechnology, environmental protection, and the worldwide trade in lipases is growing every year [6].

A wide range of technological processes using lipases is an essential part of biotechnology production. The production of basic compounds used in the cosmetics industry like geranyl isoamyl butyrate acetate, benzyl propionate, is also based on the application of lipases [7]. The production of vegetable oils or biofuels is based on catalytic activity of lipases during fabrication processes [8,9]. As a suitable biorecognition element, lipases are used in a field of analytical chemistry for determination of toxic compounds containing ester bonds like pesticides or organophosphates [10,11]. Biosensors based on determination of lipase activity are used in medicine to diagnose many diseases such as obesity, Alzheimer disease, atherosclerosis, dyslipideamia associated with diabetes, infections etc. [12].

The ion-sensitive field-effect transistor (ISFET) is an electrochemical pH sensor measuring concentration of H+ or OH- ions in solution, it is also able detect pH change induced by different compounds with electric potential [13]. Currently, a wide variety of analytes, such as cells, DNA, proteins, enzymes or enzyme activity, are suitable for the ISFET assay [14,15]. The possibility of miniaturization, fast and easy handling and good sensitivity create the potential for the use of ISFET biosensors [16,17].

Environmental monitoring, industry, food quality control, biotechnology, agriculture and the military are also sectors with a growing interest in ISFET applications [18,19].

For example, an ISFET-based biosensor has been developed to determine hypo or hyperglycemia in patients with diabetes mellitus [18]. The ISFET method for determination of urea based on catalytic reaction of urea and the urease enzyme was published by Chang and co-authors [20]. ISFET pH-sensor with immobilized lipase from porcine pancreas for determination of triglycerides was used in work of Pijanowska and co-authors [21]. The ISFET method using $MnO_2$ nanoparticles for lactate determination was published on work of Xu and co-authors [22].

Our work describes a new method for determining lipase activity based on measuring the pH change, using an ISFET electrode and Tween 20 as a substrate. Tween 20 is composed of fatty acid esters of polyoxyethylene sorbitan monolaurate [23]. The ester bonds are cleaved by lipase so fatty acids concentration in solution increases. The increasing concentration of fatty acids in solution was measured in time and pH change was observed (Figure 1).

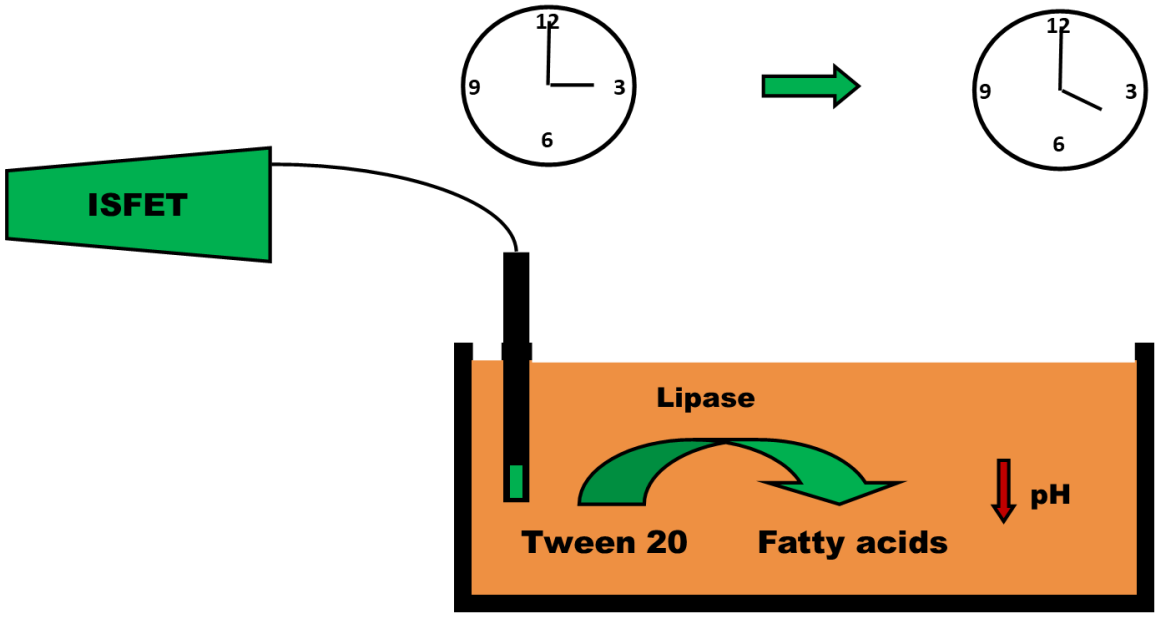


**Figure 1**. Principle of the ISFET assay for determination of lipase activity. Tween 20 is cleaved by lipase and fatty acids in solution are increasing during one hour, so, the pH measurable by the ISFET is decreasing.

## 2. MATERIALS AND METHODS

### *2.1. Chemicals*

All used chemicals apart from plasma samples were obtained from commercials sources without further adjustment. Full fat milk, low fat milk, pure apple juice and beer with 4 % v/v ethanol were obtained from commercial store. The remaining chemicals were obtained in analytical grade. Lipase from *Candida rugosa*, lyophilized powder, ≥40,000 units/mg protein, Tween 20 (polysorbate 20), isopropylalcohol, dimethyl sulfoxide > 99 % (DMSO), calcium chloride, Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCL) were bought from Sigma-Aldrich (St. Louis, MO, USA). Sodium hydroxide, ethyl alcohol 96 %, pure methyl alcohol was purchased from Penta (Prague, Czech Republic). Lipase buffer containing 1 % Tween 20, calcium chloride 80 mmol/l and 20 mmol/l Tris-HCL at pH 7.0 was prepared for standard spectrophotometric assay. Human blood samples were received in the Faculty of Military Health Sciences, University of Defence (Hradec Kralove, Czech Republic). Samples were collected to tubes with heparin and plasma was obtained by centrifugation at 3000 rpm for 15 min.

### *2.2. Apparatus*

Values of pH were determined by pH meter H160 with the ISFET pH electrode from Hach (Loveland, Colorado, USA). Absorbance of measured solution was detected by 96-channel The Epoch

spectrophotometer from BioTech Instruments (Winooski, Vermont, USA). For sonication was used Ultrasonic cleaner 230HT from Zokop (Fuzhou, Fujian, China). Demineralized water was prepared by reverse osmosis process using device Aqua Osmotic 2 from Aqua Osmotic (Tisnov, Czech Republic).

### *2.3. Data processing*

All samples were measured in pentaplicate and under standard ambient temperature and pressure condition. Values of pH measured by the ISFET was recorded and evaluated. The measured values were processed using Origin 9.1 software (OriginLab, Northampton, Massachusetts, USA). A signal-to-noise equal to three was used to calculate the limit of detection (LOD). Michaelis-Menten constant ($K_m$), correlation coefficient (R) and maximal velocity of the reaction ($V_{max}$) was calculated. From every measured sample was deducted blank value.

### *2.4. Reference method*

Spectrofotometric reference method was inspired by work of Plou and coauthors with a few adjustments [24]. Lipase was dissolved in demineralized water and sonicated for 15 minutes in 37 °C in concentration of 4 mg/ml and measured in concentration range from 0 mg/ml to 4 mg/ml (0, 0.125, 0.25, 0.5, 1, 2 and 4 mg/ml). Tween 20 at a concentration of 1 % in lipase buffer was used as a substrate for detection of lipolytic activity. Lipase buffer with Tween 20 was added into vial in volume of 200 µl and 10 µl of lipase was added into vial just before measurement. Absorbance of solution was detected by spectrophotometer at wavelength 450 nm measured every 10 minutes for 1 hour and results were put into graph.

### *2.5. The ISFET method*

First, the optimal concentration of Tween 20 for measuring of lipase activity was determined as follows: Tween 20 was dissolved in demineralized water in concentration range from 0 to 4 %. Sample containing 1 ml of Tween 20 was put into vial and 100 µl of lipase dissolved in demineralized water in concentration 1 ml/mg was added just before measurement. The pH of every sample was measured by the ISFET every 10 minutes for 1 hour. Final results were recorded and evaluated and put into graph.

To determine lipase activity, lipase was dissolved in demineralized water using sonication for 15 minutes at 37 °C in concentration range from 0 to 4 mg/ml (0, 0.125, 0.25, 0.5, 1, 2, 4 mg/ml). Tween 20 was dissolved in demineralized water at concentration of 2 %. 1 ml of 2 % tween 20 was mixed with 100 µ of lipase and measured every 10 minutes for 1 hour . Results was put into graph.

### *2.6. Effect of solvents*

Influence of solvents used in laboratory was tested by the ISFET method. Common solvents namely methanol, ethanol, isopropanol and DMSO were measured. Two different ratios (1:9 and 1:1) of

solvents were mixed with Tween 20 to final volume 1 ml and tested. Tween 20 concentration in solution was set to be 2 %. 100 µl of lipase in concentration of 1 mg/ml was added to the solution and measured. Demineralized water was used as negative control and 2% Tween 20 was measured as positive control All results were compared with negative and positive control, evaluated and put into graph.

### *2.7. Matrix effect*

Effect of different matrices were measured by the ISFET method. Common foodstuff like beer, full-fat milk, low-fat milk and apple juice and human plasma were tested. All used matrices were measured in concentration of 100 % and 50 % mixed with 4 % tween 20 so final concentration of Tween 20 was 2 %. Demineralized water was measured as negative control and 2% Tween 20 was used as positive control. The results of measured matrices were compared with negative and positive control and put into graph.

## 3. RESULTS

### *3.1. Reference method*

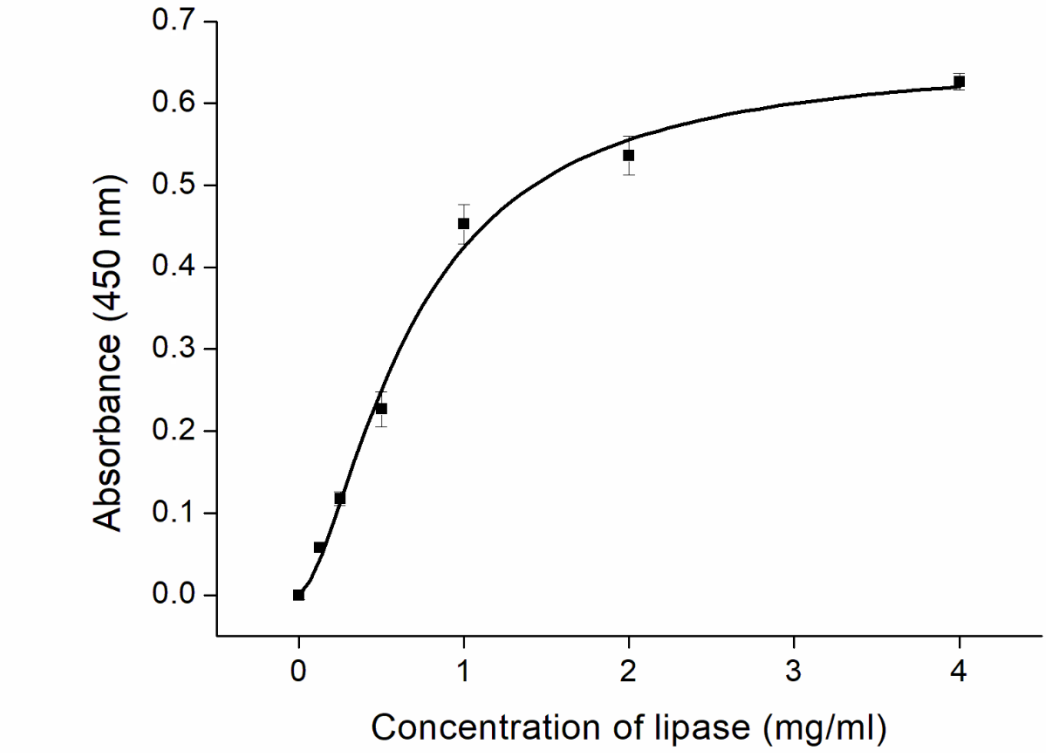


**Figure 2.** Concentration curve of lipase in concentration range from 0 – 4 mg/ml measured by standard spectrophotometric assay for determination of lipase activity, the result curve represents difference between the first and the last measurement. Error bars indicates standard deviation for n = 5.

Standard spectrophotometric method for lipase activity determination was performed and results are shown in Figure 2. Assay was performed in concentration range of lipase from 0 to 4 mg/ml. The LOD was calculated to be 0.059 mg/ml (0.88 µmol/l) with the R equal to 0.996 and $V_{max}$ equal to 0.66. Calibration curve corresponds with Michaelis-Menten dependency with $K_m$ equal to 0.68 mg/ml (10.2 µmol/l). All values are summarized in Table 1.

### *3.2. The ISFET method*

Concentration curve of Tween 20 from 0 to 4 % was measured by the ISFET electrode and lipase at concentration of 1 mg/mg was added. The LOD was equal to 0.02 % (179 µmol/l) with the R equal to 0.981 and $V_{max}$ equal to 0.62. The Michaelis-Menten dependent calibration curve is shown in Figure 3. and $K_m$ was set to be 0.11 % (986 µmol/l).

Concentration curve of lipase activity was measured in the same concentration range of lipase as reference method, for precise comparation of results. Calibration curve is shown in Figure 4 corresponding with Michaelis-Menten dependency with $K_m$ equal to 0.36 mg/ml (5.2 µmol/l). The LOD was set to be 0.022 mg/ml (0.33 µmol/l) and the R was calculated to be 0.998 and $V_{max}$ equal to 0.71. All values are summarized in Table 1.

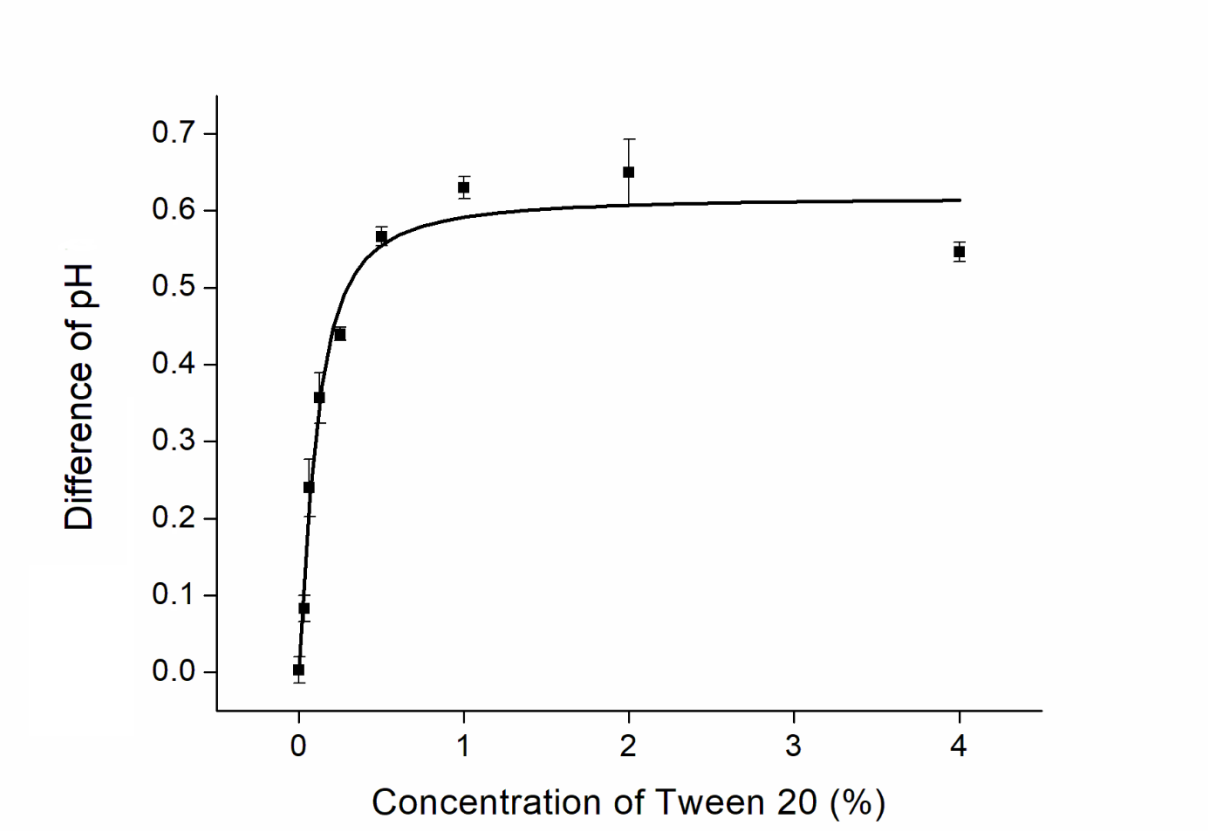


**Figure 3.** Calibration curve of Tween 20 from 0 to 4 % depending on pH change, the result curve represents difference between the first and the last measurement. Error bars indicates standard deviation for n = 5.

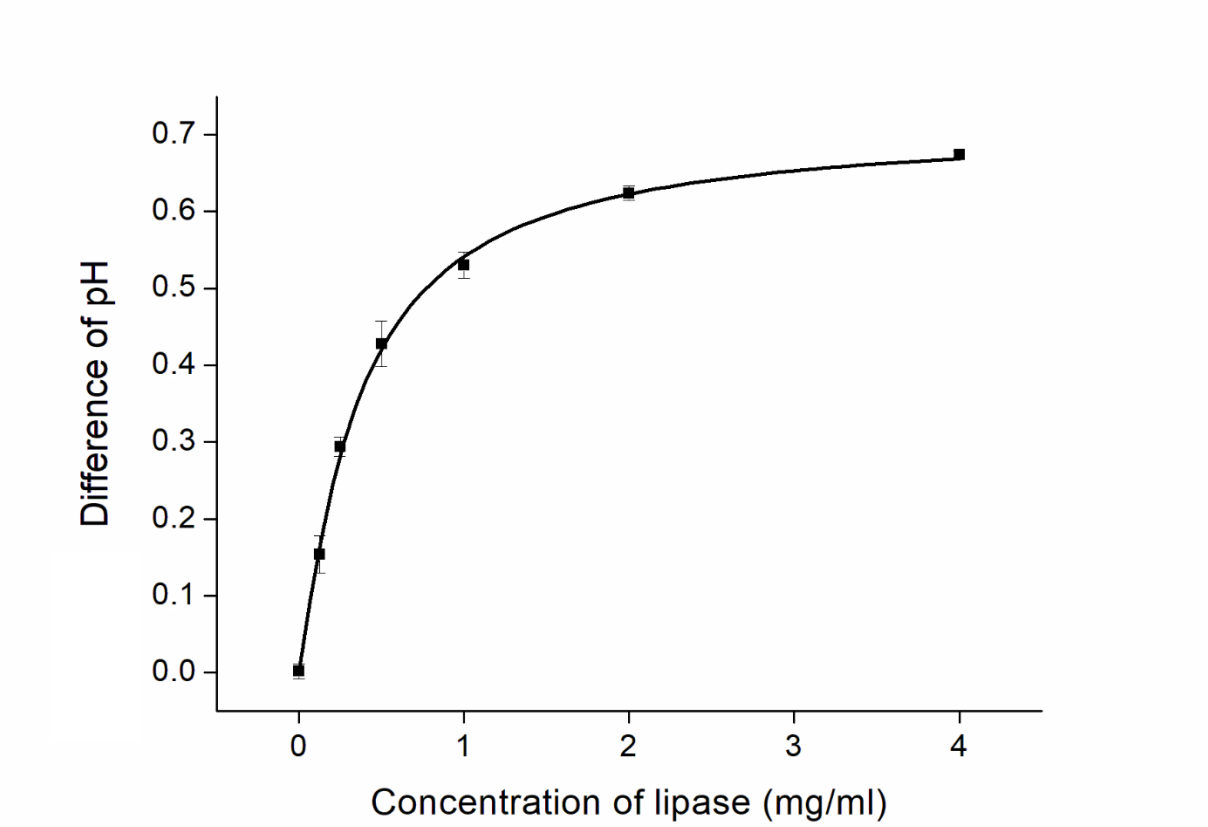


**Figure 4.** Concentration curve of lipase measured by the ISFET assay for determination of lipase activity, the result curve represents difference between the first and the last measurement. Error bars indicates standard deviation for n = 5.

**Table 1.** Summarized results of reference method and ISFET method.

| Method | LOD | $K_m$ | $V_{max}$ | R |
|---|---|---|---|---|
| Reference method | 0.059 mg/ml (0.88 µmol/l) | 0.68 mg/ml (10.2 µmol/l) | 0.66 | 0.996 |
| ISFET method Tween 20 | 0.02 % (179 µmol/l) | 0.11 % (986 µmol/l) | 0.62 | 0.981 |
| ISFET method lipase | 0.022 mg/ml (0.33 µmol/l) | 0.36 mg/ml (5.2 µmol/l) | 0.71 | 0.998 |

*3.3. Effect of solvents*

Influence of common solvents used in laboratories was measured by the ISFET electrode. Each sample was compared with negative and positive control and evaluated as shown in Figure 5. DMSO in ratio 1:1 shown no significant change from positive control, other solvents shown significant decrease of pH change.

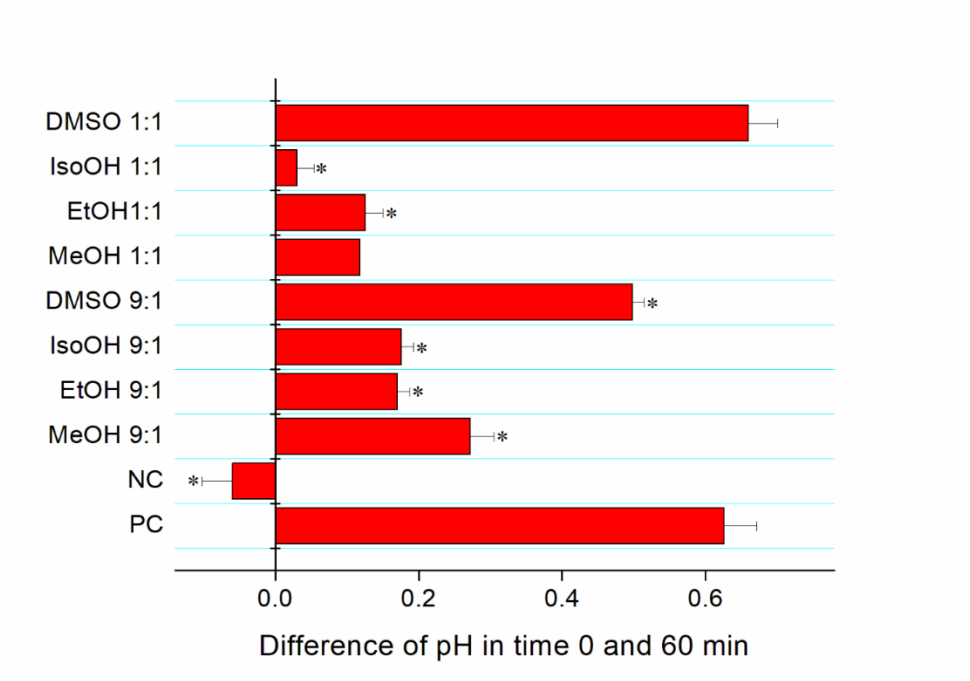


**Figure 5.** Effect of solvents to the ISFET assay in ration 1:1 and 9:1. DMSO – dimethyl sulfoxide, IsoOH – isopropanol, EtOH – ethanol, MeOH – methanol, NC – negative control, PC- positive control. Results significantly different from positive control are marked by symbol * above the column. Error bars indicates standard deviation for n = 5.

*3.4. Matrix effect*

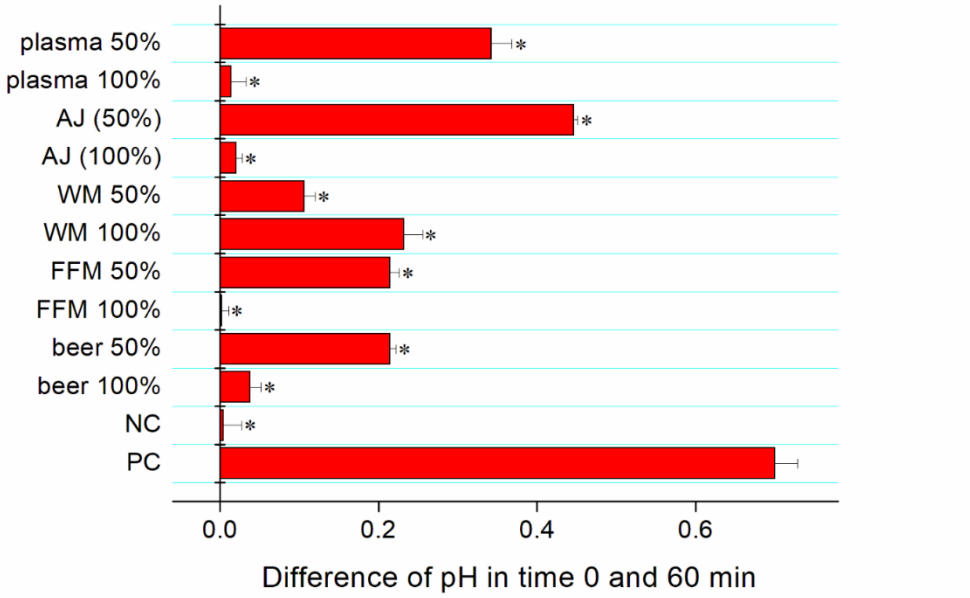


**Figure 6.** Effect of plasma and foodstuff in concentration 50 % and 100 % to the ISFET assay. AJ – apple juice, WM – whole milk, FFM – fat free milk, NC – negative control, PC- positive control. Results significantly different from positive control are marked by symbol * above the column. Error bars indicates standard deviation for n = 5.

Effect of different matrices like foodstuff or plasma was tested by optimized the ISFET method. Samples were compared with negative and positive control and result are shown in Figure 6. Effect of matrix was proved in all samples by significant decrease of pH change. Effect of matrices was reduced by diluting of matrices by demineralized water to 50 % concentration.

## 4. DISCUSSION

Novel ISFET method was developed for lipase assay and it exerts better results than standard method concerning LOD and $K_m$. LOD equal to 0.022 mg/ml for the ISFET method is sufficient for analytical purposes and nearly three times lower than using reference spectrophotometric assay. In the work of Narwal and co-authors amperometric triglyceride biosensor was constructed using combination of three enzymes immobilized on nanoparticles with LOD 0.1 nmol/l [25]. Bioelectrocatalytic sensor for triglycerides in human skin using enzymatic cascade reaction was developed and LOD was set at 15 mg/dL [26]. Indoxyl acetate method for lipase activity determination measured by spectrophotometer shown LOD 2.78 mmol/l [2]. Triglyceride biosensor based on a platinum nano particle and polypyrrole nano composite electrode was developed by Chauhan and co-authors with LOD equal to 25 mg/dl [27].

$K_m$ value is a parameter characterizing catalyst and affinity of catalyst to analyzed substrate, that means the lower $K_m$ value the stronger affinity of catalyst to substrate. $K_m$ value was significantly lower using the ISFET electrode (5.4 µmol/l) than spectrophotometric assay (10.2 µmol/l) for the determination of lipase activity.

An improved method to measure lipase activity in aqueous media was developed by Hernandez-Garcıa and co-authors. The lowest $K_m$ was proved for meristate 0.57 mmol/l and palmitate 0.88 mmol/l using lipase from *Pseudomonas fluorescens* [28]. Pohanka and co-authors (2018) used 20 as substrate for spectrophotometric determination of lipase activity in bacterial homogenate. $K_m$ value was calculated to be 217 µmol/l [29].

Solvents and matrix effect were tested by the ISFET method. Common solvents used in laboratories like ethanol, methanol, isopropanol and DMSO was tested. DMSO shown no influence even in high concentration and other solvents had significant effect on the method. With decreasing concentration of the solvent, effect of solvents decreased as well. Matrix effect was tested with common foodstuff (milk, apple juice, beer and human plasma). Effect of matrix was proved in all samples, influence of matrix decreased with decreasing concentration in measured sample. At high concentrations of strong buffers, such as organic solvents or foodstuff, it is not possible to change the pH by the lipase-tween 20 reaction.

## 5. CONCLUSION

The ISFET method for determining lipase activity using tween 20 as a substrate is a very simple tool, easy repeatable and not demanding on chemicals or laboratory equipment. It is possible to use the

ISFET method in field condition, no electricity is needed, only a pH meter with battery. Analytical time is suitable for medical purposes, laboratories, industry or the food industry and immediate evaluation of results is possible without the use of a computer. Interference and matrix effect were measured at different concentrations, and the effect was unfortunately proved in all samples except DMSO. Influence decreases with decreasing concentration of solvent or matrix in sample, this effect attribute to ability maintain stable pH and lipase-tween 20 reaction cannot change pH by fatty acid origin. The ISFET method for lipase activity determination promises a novel pathway to analytical purposes in wide range utilization as an easy and low-cost tool in the practice.

ACKNOWLEDGEMENTS
This work was supported by a Ministry of Defence of the Czech Republic - long-term organization development plan Medical Aspects of Weapons of Mass Destruction of the Faculty of Military Health Sciences, University of Defence.

## References

1. M. Pohanka, *Molecules*, 24 (2019) 616.
2. T. Valek, A. Kostelnik, P. Valkova and M. Pohanka, *International Journal of Analytical Chemistry*, 2019 (2019) 8538340.
3. M. Widmann, P. B. Juhl and J. Pleiss, *BMC Genomics*, 11 (2010) 123.
4. J. C. S. dos Santos, C. Garcia-Galan, R. C. Rodrigues, H. B. de Sant' Ana, L. R. B. Gonçalves and R. Fernandez-Lafuente, *Enzyme and Microbial Technology*, 60 (2014) 1.
5. A. A. Mendes, P. C. Oliveira and H. F. de Castro, *Journal of Molecular Catalysis B: Enzymatic*, 78 (2012) 119.
6. A. B. Vermelho, E. F. Noronha, E. X. F. Filho, M. A. Ferrara and E. P. S. Bon, in E. Rosenberg, E.F. DeLong, S. Lory, E. Stackebrandt, F. Thompson (Editors), The Prokaryotes: Applied Bacteriology and Biotechnology, Springer Berlin Heidelberg, Berlin, Heidelberg, 2013, p. 213.
7. N. N. Gandhi, *Journal of the American Oil Chemists' Society*, 74 (1997) 621.
8. F. Hasan, A. Shah and A. Hameed, *Enzyme and Microbial Technology*, 39 (2006) 235.
9. N.-C. Shang, R.-Z. Liu, Y.-H. Chen, C.-Y. Chang and R.-H. Lin, *Journal of the Taiwan Institute of Chemical Engineers*, 43 (2012) 354.
10. F. Kartal, A. KilinÇ and S. Timur, *International Journal of Environmental Analytical Chemistry*, 87 (2007) 715.
11. K. Gangadhara Reddy, G. Madhavi and B. E. Kumara Swamy, *Journal of Molecular Liquids*, 198 (2014) 181.
12. J. R. Mead, S. A. Irvine and D. P. Ramji, *Journal of Molecular Medicine*, 80 (2002) 753.
13. C.-S. Lee, S. K. Kim and M. Kim, *Sensors*, 9 (2009) 7111.
14. S. Caras and J. Janata, *Analytical Chemistry*, 52 (1980) 1935.
15. J. Janata and S. D. Moss, *Biomed Eng*, 11 (1976) 241.
16. D. Pijanowska and W. Torbicz, *Bulletin of the Polish Academy of Sciences: Technical Sciences*, 53 (2005) 251.
17. M. J. Schöning and A. Poghossian, *Electroanalysis*, 18 (2006) 1893.
18. V. K. Khanna, *IETE Journal of Research*, 54 (2008) 193.
19. J. C. Dutta, in 2012 2nd National Conference on Computational Intelligence and Signal Processing (CISP), 2012, p. 185.
20. K.-M. Chang, C.-T. Chang and K.-M. Chan, *Sensors (Basel, Switzerland)*, 10 (2010) 6115.
21. D. G. Pijanowska, A. Baraniecka, R. Wiater, G. Ginalska, J. Łobarzewski and W. Torbicz,

*Sensors and Actuators B: Chemical*, 78 (2001) 263.
22. J.-J. Xu, W. Zhao, X.-L. Luo and H.-Y. Chen, *Chemical Communications*, 14 (2005) 792.
23. W. Zhang, Y. Tang, J. Liu, L. Jiang, W. Huang, F.-W. Huo and D. Tian, *Journal of Agricultural and Food Chemistry*, 63 (2015) 39.
24. F. J. Plou, M. Ferrer, O. M. Nuero, M. V. Calvo, M. Alcalde, F. Reyes and A. Ballesteros, *Biotechnology Techniques*, 12 (1998) 183.
25. V. Narwal and C. S. Pundir, *Enzyme and Microbial Technology*, 100 (2017) 11.
26. C. Y. Jeong, Y. D. Han, J. H. Yoon and H. C. Yoon, *Journal of Biotechnology*, 175 (2014) 7.
27. R. Chauhan, B. Nagar, P. Solanki and T. Basu, *Materials Focus*, 2 (2013) 316.
28. S. Hernández-García, M. I. García-García and F. García-Carmona, *Analytical Biochemistry*, 530 (2017) 104.
29. M. Pohanka, J. Zakova and I. Sedlacek, *Sensors and Actuators B: Chemical*, 273 (2018) 610.