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**Screen-printed pH Sensors based on
Ruthenium(IV) Oxide for Measurement
in Food Samples**

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Declaration:

Hereby I declare that this doctoral thesis, my original investigation, and achievement, submitted for the doctoral degree at Tallinn University of Technology has not been submitted for a doctoral or equivalent academic degree. The work reported in this thesis was carried out in the Center of Food and Fermentation Technologies (a.k.a. TFTAK) and supported by European Commission through Horizon-2020 MSCA-ITN project AQUASENSE (grant agreement H2020-MSCA-ITN-2018-813680) and partially supported by Graduate School in Biomedicine and Biotechnology receiving funding from the European Regional Development Fund under program ASTRA 2014-2020.4.01.16-0032 in Estonia.

Maryna Lazouskaya

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**Ruteenium(IV) oksiidil põhinevad
siiditrükiga pH-andurid toiduproovide
mõõtmiseks**

MARYNA LAZOUSKAYA



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List of Publications

The list of author's publications, based on which the thesis has been prepared:

Journal Articles (code 1.1)

- I K. Uppuluri, M. Lazouskaya, D. Szwagierczak, K. Zaraska, and M. Tamm, "Fabrication, potentiometric characterization, and application of screen-printed RuO₂ pH electrodes for water quality testing," [Sensors](#), vol. 21, no. 5399, 2021.
- II M. Lazouskaya, O. Scheler, V. Mikli, K. Uppuluri, K. Zaraska, and M. Tamm, "Nafion protective membrane enables using ruthenium oxide electrodes for pH measurement in milk," [Journal of the Electrochemical Society](#), vol. 168, no. 107511, 2021.
- III Maryna Lazouskaya, Iuliia Vetik, Martti Tamm, Kiranmai Uppuluri, and Ott Scheler "Binary RuO₂-CuO electrodes outperform RuO₂ electrodes when measuring pH in food samples," [ACS Omega](#), vol. 8, pp. 13275-13284, 2023.

Conference articles (code 3.1)

- IV M. Lazouskaya, M. Tamm, O. Scheler, K. Uppuluri, and K. Zaraska, "Nafion as a protective membrane for screen-printed pH-sensitive ruthenium oxide electrodes," [Proceedings of IEEE Biennial Baltic Electronics Conference \(BEC\)](#), vol. 2020-October, pp. 18–21, 2020, Tallinn, Estonia.
- V K. Uppuluri, M. Lazouskaya, D. Szwagierczak, and K. Zaraska, "Influence of temperature on the performance of Nafion coated RuO₂ based pH electrodes," in [IEEE International Conference on Flexible and Printable Sensors and Systems \(FLEPS\)](#), 2021, Manchester, United Kingdom.
- VI M. Lazouskaya, O. Scheler, K. Uppuluri, K. Zaraska, and M. Tamm, "Reusability of RuO₂-Nafion electrodes, suitable for potentiometric pH measurement," in [IEEE International Conference on Flexible and Printable Sensors and Systems \(FLEPS\)](#), 2022, Vienna, Austria.
- VII M. Lazouskaya, I. Vetik, O. Scheler, K. Uppuluri, N. Razmi, K. Zaraska, and M. Tamm, "Cleaning procedure for the screen printed RuO₂-based pH electrodes," in [IEEE Sensors Conference](#), 2022, Dallas, Texas, USA.

Author's Contribution to the Publications

Contribution to the papers in this thesis are (according to CREDiT taxonomy):

- I Methodology, formal analysis, writing – original draft preparation, writing – review and editing, visualization.
- II Conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing – original draft preparation, visualization.
- III Conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing – original draft preparation, writing – review and editing, visualization.
- IV Conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing – original draft preparation, visualization.
- V Conceptualization, methodology, data curation, writing – original draft preparation, visualization.
- VI Conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing – original draft preparation, visualization.
- VII Conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing – original draft preparation, visualization.

Introduction

The assessment of the quality of food products has always been and always will be one of the biggest responsibilities of food researchers. The quality of any food product is evaluated based on various parameters, including adulteration, physical properties, chemical composition, sensory attributes, etc. (Azad & Ahmed, 2016; Biswas & Mandal, 2020; Cheng et al., 2015; Toldrá, 2017). One of the key parameters in food manufacture and food quality analysis is pH. pH is known to be related to food quality and freshness (Abbas et al., 2008; Hopkins et al., 2014; Loudon et al., 2019; Matarneh et al., 2017), can be used to monitor microbial growth in dairy products (Poghossian et al., 2019) and is suitable to reveal mastitis and inflammatory infection in cattle (Kandeel et al., 2019).

At present, to determine the pH of food products, a standard potentiometric method is used (e.g. [ISO 11289:1993](#), [ISO 7238:2004](#)). Potentiometric determination of pH is based on the measurement of the electrochemical potential change between a pH-sensitive electrode and a reference electrode immersed into the test solution. The glass electrode used in conventional pH meters is a combination of pH-sensitive and reference electrodes incorporated in one glass body. However, due to its high cost, fragility of the glass body of the electrode, and possible contamination of samples and the reference junction, novel materials and methods are thoroughly investigated.

Several metal oxides were investigated over the years for application in pH measurement (Manjakkal et al., 2020). Researchers focus their attention on the hydrogen-sensitive metal oxides (e.g., ZnO, SnO₂, IrO₂, TiO₂, Ta₂O₅) (Kurzweil, 2009; Manjakkal et al., 2020). The majority of the oxides have a wide pH range; however, they suffer from noticeable hysteresis that affects the precision of consequent measurements (Kurzweil, 2009). Among the investigated metal oxides, RuO₂ shows good accuracy, low hysteresis, and excellent performance even in the presence of bacteria on the electrode surface (Zhuiykov, 2009).

However, not much attention has been paid so far to the application of the RuO₂ electrodes in real-life samples. Moreover, there is a gap in knowledge regarding the application of novel pH samples to real-life measurements. Most of the research articles concentrate on the fabrication of the electrodes and the characterization of their sensitivity and accuracy in aqueous media. This thesis presents the investigation into the application of potentiometric RuO₂ sensors for pH measurement in food samples. Food samples, especially dairy products, are among the most challenging samples to work with due to their complex compositions. Fats in food samples are known to interfere with pH measurement by blocking the surface of the electrodes and hindering the charge transfer (Upreti et al., 2004). The first part of this thesis is dedicated to the optimization of the electrochemical characteristics of RuO₂ electrodes. Here discussion focuses on the influence of parameters of fabrication of the electrodes (such as sintering temperature and ink composition) on the sensitivity, linearity, hysteresis and drift. Following, the second chapter directs its attention to the key component of making RuO₂ electrodes work in food samples – the Nafion™ (Nafion) membrane. The discussion incorporates the investigation of the properties of the membrane, as well as the electrochemical performance of the Nafion-covered

electrodes. Finally, the third chapter describes the application of the RuO₂ electrodes for pH measurement in real-life samples. Here the results of pH measurement in dairy products are presented and a cleaning approach for the maintenance of the electrodes is proposed.

1 Theory and Literature Overview

1.1 pH measurement in food samples

The standard procedure of pH measurement is regulated by the International Organization for Standardization (ISO) by standards such as [ISO 11289:1993](#) and [ISO 7238:2004](#). The ISO recommends the standard potentiometric method for pH measurement. The theory of potentiometric pH measurement will be discussed in detail in section 1.2.

At present, different methods are scrutinized to find the one allowing fabricating the pH-electrode that would not suffer from asymmetry potential, fragility, or inability to measure pH in some matrices (Bühlmann et al., 2001; Upreti et al., 2004). The most promising results in pH measurement applications are observed for optical sensors, electrochemical sensors, and sensors based on field-effect transistors. Nevertheless, the methods themselves present less interest than miniature and portable sensors that could be used not only in laboratory analysis but also for the modernization of active manufacturing lines for on-line monitoring. Potentiometric sensors attract the most attention as they do not require expensive equipment and are easy to operate.

1.1.1 Potentiometric sensors

The potentiometric method (or Potentiometry) is a cheap and simple method that allows determining the pH in a matter of seconds. Potentiometric detection relies on the selective identification of ions, present in the investigated solution (Karastogianni et al., 2016).

Even though a big diversity of pH-sensitive electrodes is known and widely used for various purposes¹, the conventional glass electrode (CGE) is the only type of electrode that is used for pH measurement in food research. The CGE is used in a common pH meter as a combination of a working electrode (WE) and a reference electrode (RE) in one glass body that is called a probe. For the safety and convenience of the measurement, the probe can be covered with stainless steel or inert polymeric material (e.g., polyvinylidene fluoride or polyethyleneimine). One such solution is commercialized by Frontmatec (Denmark). The manufacturer offers a [pH probe \(Figure 1\)](#), covered with a protective steel case with a telescopic sleeve. However, since the CGE has such drawbacks as high cost, brittleness, and therefore the possibility to contaminate food samples with dangerous shattered glass, novel materials that could replace the CGE in standard potentiometric pH measurement are investigated.

In 1986, Korkeala et al. (Korkeala et al., 1986) compared several types of glass electrodes when determining the pH of meat samples. The investigated electrodes showed a significant statistical difference in the calculated pH value.

In 2003, Eftekhari (Eftekhari, 2003) fabricated a pH-sensitive electrode by dip-coating an aluminium rod with lead oxide (PbO₂). The electrode showed a sensitivity of 57.8 mV/pH against the saturated calomel electrode. The author mentions that the electrode was studied in soft drinks and fruit juices with satisfactory results, however, no data is presented in the study. Furthermore, the author indicated that the fabricated electrode is not suitable for pH measurement in complex samples.

¹ such as Normal Hydrogen Electrode, quinhydrone electrode, dropping mercury electrode, antimony electrode, etc.



Figure 1. pH*K21 pH-probe manufactured by Frontmtec, Denmark (the image was taken from [Frontmtec website](#)).

In 2008, Liao and Chou (Liao & Chou, 2008) reported a sputtered ruthenium(IV) oxide (RuO_2) film on a silicon wafer as a solid-state pH-electrode. The electrode showed a sensitivity of 55.6 mV/pH and a difference of 0.14 and 0.50 pH units from the CGE in coke and milk respectively.

In 2015, Manjakkal et al. (Manjakkal et al., 2015) fabricated a binary oxide electrode, based on the screen-printed mixture of RuO_2 and tin(IV) oxide (SnO_2) on top of alumina (Al_2O_3) substrate. Authors demonstrated that their RuO_2 - SnO_2 pH-sensitive electrodes have a sensitivity of 56.5 mV/pH and a pH difference of 0.21 pH units from CGE in lemon juice.

In 2018, Lonsdale et al. (Lonsdale, Shylendra, et al., 2018) presented a solid-state pH sensor that incorporated magnetron-sputtered RuO_2 as the pH-sensitive material. The sensor showed a linear pH response with a sensitivity of 56.6 mV/pH. The electrode was tested in coke, beer, wine, and juice samples and demonstrated a deviation of less than 0.05 pH units from the CGE.

1.1.2 Potentiometric sensors based on polymers

While metals and metal oxides are the most intensively investigated materials for the fabrication of pH-sensors, metal-based sensors based still have the disadvantage of being toxic and not flexible, therefore, limiting the application of such sensors. At present, polymer-based pH sensors are investigated for point-of-care applications (Yoon et al., 2020). Two types of polymer-based pH sensors can be distinguished: (i) metal oxide pH-electrode covered with a protective polymeric membrane, e.g., NafionTM (Lonsdale, Shylendra, et al., 2018; Lonsdale, Wajrak, et al., 2018; K. Xu et al., 2018) and (ii) metal or graphite electrodes covered with a pH-sensitive polymer. Polymer-based sensors are biocompatible, cheap, and flexible, and can be realized on different substrates, including carbon fibre (Yoon et al., 2020), paper (Kawahara et al., 2018), and textiles (Jose et al., 2021; Parilla et al., 2016). Polyaniline (Jose et al., 2021; Lakard et al., 2005, 2007; Yoon et al., 2020) and polypyrrole (Lakard et al., 2005; Mo et al., 2003; Shiu et al., 1999) are the most studied pH-sensitive polymers.

However, only a few papers address the application of the polymer-based pH sensor for measurement in real-life samples. Such an article was written by Upreti et al. (Upreti et al., 2004). Four combinations of polymers were used in that study: tridodecylamine (R_3N) or octadecyl isonicotinate was mixed with plasticisers bis(2-ethylhexyl) sebacate (DOS) or o-nitrophenyl octyl ether (oNPOE). Among the studied polymer mixtures, only those based on R_3N were suitable for measurement in real-life samples. The fabricated electrodes were tested in milk, 20% cheese emulsion, and 70% cheese emulsion.

The difference in pH measured with R₃N/DOS electrode from the CGE was equal to 0.05, 0.25, and 0.01 pH units for milk, 20%, and 70% cheese emulsions respectively. For the R₃N/oNPOE electrode, the pH difference was equal to 0.01, 0.01, and 0.15 pH units for milk, 20% and 70% cheese emulsions respectively.

Another article was published in 2011 by Li et al. (Q. Li et al., 2011). The authors presented an indium tin oxide (ITO)-based electrode modified with electropolymerized bisphenol A. The electrode exhibited a sensitivity of 58.6 mV/pH and was tested in diluted milk and grape juice solutions. The deviation of the pH measured with the fabricated electrode from the CGE did not exceed 0.12 and 0.23 pH units for diluted milk and grape juice respectively.

In 2019, Park et al. (Park et al., 2019) reported a flexible potentiometric pH sensor that incorporated polyaniline as pH-sensitive material. Polyaniline was deposited on a carbon electrode screen-printed on a polyethylene terephthalate (PET) substrate via dilute chemical polymerization. Prior to polyaniline deposition, the carbon and silver inks were screen printed on the PET substrate to create WE and conductive channels respectively. The fabricated electrodes exhibited a sensitivity of 60.4 mV/pH and were tested in milk and on an apple to monitor the spoilage of the sample. The pH values measured with a fabricated electrode during 48 hours of monitoring of milk spoilage were similar to those of the CGE. For the monitoring of apple spoilage, no reference to CGE was performed.

In 2020, Yoon et al. (Yoon et al., 2020) used a polyaniline-coated electrode as a WE. The substrate was fabricated by coating carbon fibre with a self-healing polymer. The same materials were also used for the fabrication of the RE, with the only difference being that the RE was covered with Ag|AgCl instead of polyaniline. The electrode exhibited a pH sensitivity of 58.1 mV/pH and was tested on real-life human fluids and fruits. Unfortunately, the authors did not present the numerical values of measured pH.

1.1.3 Potentiometric biosensors

Electrochemical biosensors can be described as devices, in which a biolayer is used to identify the specific ions in the sample solution. The biolayer can be represented by various materials, including amino acids, enzymes, bacteria, yeast, DNA, antigens, etc. (Vargas-Bernal et al., 2012) (Figure 2). The biolayer responds directly to the presence of a detectable component and generates a signal, depending on the concentration of that component. The main drawbacks of the utilization of biosensors are related to their short stability and shelf-life (Panjan et al., 2017; Shaver & Arroyo-Currás, 2022).

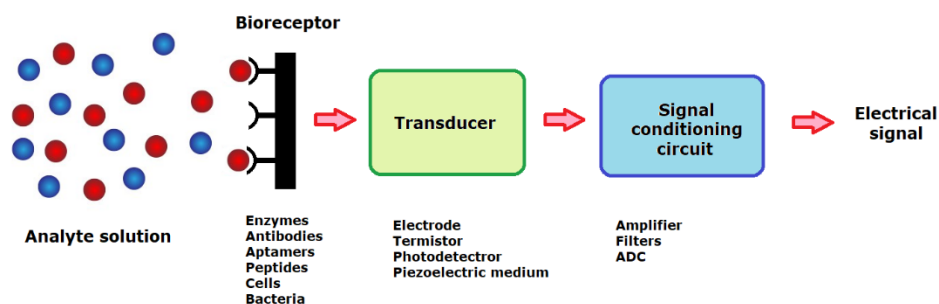


Figure 2. Schematic representation of a biosensor. Interaction of the biolayer with the analyte solution initiate a signal that is later converted to an electrical signal.

In 2018 Zuaznabar-Gardona and Fragoso (Zuaznabar-Gardona & Fragoso, 2018) presented a potentiometric pH sensor based on a polydopamine pH-sensitive layer. Dopamine film was electro- and self-polymerized on a glassy carbon electrode that contained a carbon nano-onion layer. The fabricated electrodes exhibited sensitivities of 58.3 and 60.1 mV/pH for electro- and self-polymerized dopamine respectively. In real-life samples, the deviation of the pH measured with the fabricated electrodes and the CGE was 0.08, 0.22, and 0.16 pH units for vinegar, pineapple juice, and milk respectively.

In 2020, Hu et al. (Hu et al., 2020) reported a potentiometric pH sensor based on a graphite electrode modified with tryptophan residues by cyclic voltammetry. Tryptophan was used as pH-sensitive material due to dependency on pH of the tryptophan oxidation. Tryptophan is one of the most commonly oxidized residues of aromatic amino acids in proteins. The sensor exhibited a sensitivity of 52 mV/pH and low deviation from CGE when used in milk and coke (below 0.15 pH units).

1.1.4 Systems consisting of novel working and reference electrodes

The inability of the conventional glass electrode to work in complex media (Eftekhari, 2003; Upreti et al., 2004) cannot be overcome by simply replacing the pH-sensitive working electrode; attention also should be paid to the reference electrode. The reference electrode is known to suffer from the potential drift and inconsistency of reading when used in viscous samples (Amemiya et al., 2007; Mugica et al., 2022). Therefore, alternatives to the conventional glass working electrode are investigated together with the alternatives to the glass Ag|AgCl reference electrode.

In 2017, Li et al. (Q. Li et al., 2017) described their RE electrode based on Ti/Au/Ag/AgCl ion-selective field-effect transistor (ISFET). The electrode was modified with a porous poly(vinyl butyral) membrane and was used together with a poly(ethylene terephthalate)-covered indium tin oxide as a WE. The potentiometric system showed a pH difference exceeding 0.50 pH units in coke, orange juice, beer, milk, and other studied samples.

In 2018, Xu et al. (K. Xu et al., 2018) incorporated an antimony film on top of a copper substrate as a WE together with a Ag|AgCl RE onto a printed circuit board. The WE was additionally modified with a Nafion membrane and the RE was modified with a graphene-chitosan membrane. This potentiometric system showed a pH sensitivity of 54.5 mV/pH and a pH difference from CGE of 0.19 and 0.11 pH units for coke and vinegar respectively.

Also in 2018, Lonsdale et al. (Lonsdale, Wajrak, et al., 2018) incorporated a modification of their previously reported RuO₂-based pH-sensitive electrode and a pH-sensor. The working electrode was fabricated by covering the magnetron sputtered RuO₂ pH-sensitive electrode with protective layers of magnetron-sputtered Ta₂O₅ and drop-casted Nafion. The RE was fabricated by modifying the WE with an acrylic well filled with PVB-SiO₂ junction material. The fabricated WE exhibited a sensitivity of 55.3 mV/pH and the pH-sensor was tested in real-life beverages: coke, sports drink, vinegar, several beer samples, and 2% fat milk. The sensor showed excellent performance with the measured pH value deviation of less than 0.08 pH units from the CGE.

In 2022, Mu et al. (Mu et al., 2022) reported a hydrogel-based flexible pH system. The sensor consisted of ITO as WE directly laser-scribed on a PET substrate, and an Ag|AgCl RE screen-printed on an ITO/PET substrate. The WE and RE electrodes were

covered with a hydrogel consisting of sodium carboxymethylcellulose and calcium alginate. The reported sensor showed a sensitivity of 49 mV/pH and was used to detect total volatile nitrogen (TVB-N) that is highly correlated to fish spoilage (Kyra et al., 1997; Kyra & Lougovois, 2002). Since the components of volatile organics ionize the hydrogel, OH^- is released and a change in pH is observed. The sensors were successfully used to identify the end of the shelf life of tilapia fish.

1.1.5 Conclusion

At present, potentiometric sensors for pH measurement remain the most investigated alternative to the CGE not only from the point of general application but from the point of application in food samples that are complex matrices, in which new alternative sensors cease to work. The development of new potentiometric sensors has a big advantage over other sensors: their work is based on the same principles as the CGE and they allow to use the same electronic devices with the new electrodes as used with the CGE, therefore making the transition to the use of the newly developed pH electrodes less expensive and more probable.

Over the years, potentiometric pH sensors were shown to have excellent pH sensitivity, fast response and accurate pH measurement in aqueous samples. However, most of the research in the field of pH sensors focuses on the materials, fabrication and application of the sensors in aqueous samples and does not address applicability to real-life samples. Much more research is needed to be conducted to make sure that the proposed alternative pH electrodes will prove themselves useful in real-life applications, including food products that are one of the most difficult to work with.

1.2 Principles of potentiometric pH measurement

The potentiometric pH measurement requires the construction of an electrochemical cell that primarily consists of (i) a measuring device – a potentiostat, a voltmeter, a galvanometer, etc., (ii) a working electrode (WE) – an electrode where the analytical reaction is taking place and (iii) a reference electrode (RE) that provides a stable and well-known potential (Figure 3).

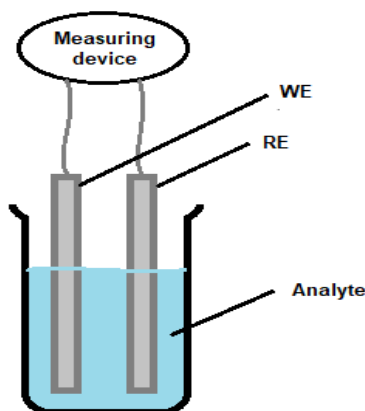


Figure 3. Schematic representation of an electrochemical cell.

The electrical characteristic of an electrochemical cell is electrochemical potential² (potential). The potential of an electrochemical cell (E) is determined as the difference in potentials of the two half-reactions happening on the WE and RE:

$$E = E_{WE} - E_{RE} \quad (1)$$

where E_{WE} is the electrochemical potential of the reaction, taking place on the WE, V; E_{RE} is the electrochemical potential of the reaction, taking place on the RE, V.

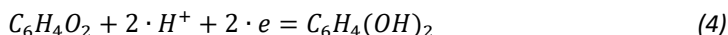
If $E > 0$, the reaction proceeds spontaneously, and if $E < 0$, the reaction proceeds only with the supply of energy from an external current source. Furthermore, if the RE is grounded, its potential is equal to zero, and the potential of the electrochemical cell is equal to the potential of the WE. This allows determining the activities of the ions participating in the electrochemical reaction on the working electrode according to the Nernst equation (Amemiya et al., 2007):

$$x \cdot Ox + n \cdot e \rightleftharpoons y \cdot Red \quad (2)$$

$$E_{Ox/Red} = E_{Ox/Red}^0 + \frac{R \cdot T}{n \cdot F} \ln \frac{[Red]^y}{[Ox]^x} \quad (3)$$

where **Ox** is the oxidized form of the electrode's material, **Red** is the reduced form of the electrode's material; **n** is the number of electrons transferred in the balanced Red/Ox equation; x and y are reaction coefficients; $E_{Ox/Red}$ is the electrochemical potential of the reaction (2), V; $E_{Ox/Red}^0$ is the standard electrochemical potential³, V; **R** is the universal gas constant, 8.314 J/mol·K; **T** is the temperature, K; **F** is the Faraday constant, 96485 J; **[Ox]** and **[Red]** are the activities of oxidized and reduced forms respectively.

For example, the reaction taking place on the quinhydrone WE is described by the following equations:



$$E_{C_6H_4O_2/C_6H_4(OH)_2} = E_{C_6H_4O_2/C_6H_4(OH)_2}^0 + \frac{R \cdot T}{2 \cdot F} \ln \frac{[C_6H_4O_2] \cdot [H^+]^2}{[C_6H_4(OH)_2]} \quad (5)$$

where $C_6H_4O_2$ and $C_6H_4(OH)_2$ are quinone and quinhydrone respectively.

When substituting the constraints and switching from natural logarithm to decimal logarithm, equation (5) takes the form of (6):

$$E_{C_6H_4O_2/C_6H_4(OH)_2} = E_{C_6H_4O_2/C_6H_4(OH)_2}^0 + 0.059 \cdot \ln [H^+] \quad (6)$$

When substituting $-\ln[H^+]$ with pH, equation (6) takes the form of (7)

$$E_{C_6H_4O_2/C_6H_4(OH)_2} = E_{C_6H_4O_2/C_6H_4(OH)_2}^0 - 0.059 \cdot \text{pH} \quad (7)$$

Or in general, for any pH-sensitive electrode

$$E_{Ox/Red} = E_{Ox/Red}^0 - 0.059 \cdot \text{pH} \quad (8)$$

Equation (8) allows determining the pH value of the sample by determining the electrochemical potential of the electrode in the sample solution. The value by which pH is multiplied (0.059 V for equation (8)) is called the sensitivity of the electrode. The value of 0.059 V (or, more commonly, 59 mV) is called Nernstian sensitivity and is considered the theoretical sensitivity of an electrode.

² Electrochemical potential of an electrochemical cell is also sometimes referred to as electromotive force (Emf).

³ Standard potential is a measure of the individual potential of a reversible electrode (in equilibrium) in a standard state (concentration 1 mol l⁻¹, pressure 1 atm and temperature 25 °C).

1.3 Metal oxides for pH sensing

The idea of utilizing metal oxides for pH sensing was first suggested in 1947 by Perley and Godshalk (Perley & Godshalk, 1947) and has been investigated since 1982 (Katsube et al., 1982).

According to Fog and Buck, there are several possible mechanisms of the pH sensitivity of metal oxides (Fog, A.; Buck, 1984):

- 1 Ion exchange in the surface layer;
- 2 Equilibrium between the two oxidizing forms of the metal;
- 3 Hydrogen intercalation;
- 4 Oxygen intercalation;
- 5 Steady-speed corrosion of the material.

The authors also indicate that the most probable mechanism for pH sensing is oxygen intercalation: due to non-stoichiometric oxygen content in the oxides, the activity of oxygen in the solid phase should also be considered when calculating the electrode potential.

However, in 1998 Mihell and Atkinson (Mihell & Atkinson, 1998) studied the pH-response of planar RuO₂ electrodes and came to a conclusion that pH response of the electrode is due to the ionic exchange in the surface groups. When metal oxide contacts with an aqueous solution, the dissociative adsorption of water on the surface of the metal oxide leads to the formation of hydroxide groups. Authors attributed the pH-response of the RuO₂ electrode to the interaction between protons and hydroxide groups on the surface of metal oxide (Mihell & Atkinson, 1998).

According to Gláb et al. (Gláb et al., 1989), a metal oxide should satisfy the following criteria:

- ✓ Should be stable;
- ✓ Should not react with interfering ions in all the solutions over a wide pH range;
- ✓ Metal oxide should be reproducible;
- ✓ Should be able to participate in electrode reaction.

Other requirements to the pH electrodes come from the application of the electrodes: in some applications, such as environmental monitoring in food processing, the toxicity of the materials plays key role. For biomedical application, the flexibility and bendability of the sensors can be important. In general, it is desired to have (i) excellent pH-sensitivity and selectivity, (ii) a cost-effective fabrication road and (iii) a straightforward way of integration of sensors into existing applications (Manjakkal et al., 2020).

Among the metal oxides, RuO₂ demonstrates the most favourable characteristics: Nernstian sensitivity, fast response and low drift rate (Figure 4). Comparison of the pH electrodes based on RuO₂ to pH electrodes based on other metal oxides reveals that RuO₂ electrodes have excellent performance, however, the biggest disadvantage is their cost (Manjakkal et al., 2020).

1.4 Ruthenium(IV) oxide

Ruthenium oxide (IV) is the most stable oxygen-containing compound of ruthenium (Drozdzov et al., 2007). Ruthenium oxide (IV) is a blue-black powder with a rutile structure ($a=4.51$; $c=3.11$ Å) (Figure 5) and a melting point of around 1200 °C (Remy, 1956).

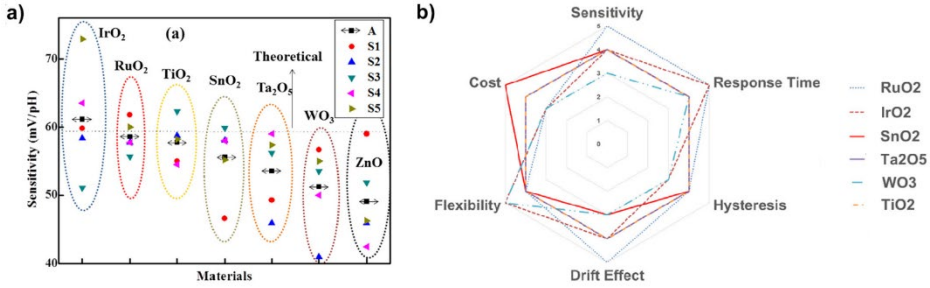


Figure 4. The sensitivity of the pH sensor depends on the material it is made from and the method utilized to fabricate the sensors. Among the investigated metal oxides (a), RuO₂ showed the sensitivity closest to the theoretical Nernstian response (horizontal grey dotted line). Sensors from different batches are indicated as S1, S2, S3, S4, and S5, while A is the average sensitivity. The overall performance of RuO₂ (b) is also exceeding other investigated oxides. Reproduced from Manjakkal et al., 2020.

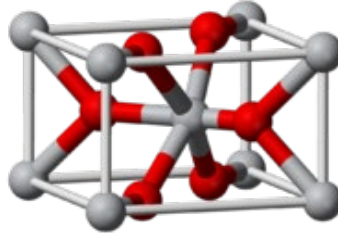
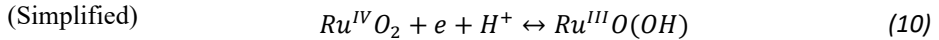
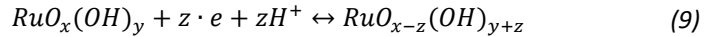


Figure 5. Structure of ruthenium(IV) oxide. Reproduced from Wikipedia.

The electrochemical response of the RuO₂ to the pH change can be described by the equations (9) and (10) (Lonsdale, 2018):



The Nernst equation for this process takes the form of (11)

$$E = E^0 + \frac{R \cdot T}{z \cdot F} \cdot \ln \frac{a_{Ru^{III}}}{a_{Ru^{IV}} \cdot a_{H^+}} \quad (11)$$

Considering that the values of metals activities approximate 1 in solid state and substituting the constants, (11) takes the form of (8).

The fabrication method is known to influence the properties of the RuO₂ electrodes (Table 1). Among the various fabrication methods proposed for electrodes based on metal oxides, the most convenient is screen printing (Manjakkal et al., 2020). From Figure 6a, it can be seen that screen printing allowed to get more reproducible sensitivity of the fabricated electrodes with the mean sensitivity being closer to the theoretical value of 59 mV/pH. The screen-printing technique allows producing electrodes of different sizes and shapes, fast and at a low cost (Zhang et al., 2021). Furthermore, utilizing screen printing approach it is possible to fabricate electrodes with small hysteresis and drift without sacrificing sensitivity and life time of the electrodes (Figure 6b). The printing is commonly described as follows: a roller moving across the screen stencil forces ink or other printable materials past the threads of the woven mesh that supports an ink-blocking stencil to achieve a specifically patterned layer of ink on a suitable substrate. Different woven meshes are prepared to print different parts of the electrode.

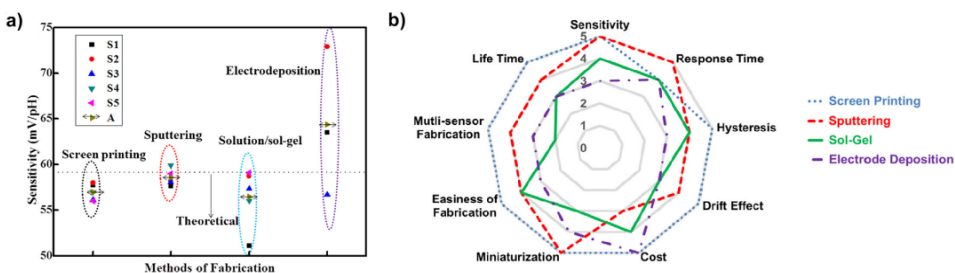


Figure 6. A comparison of the fabrication approaches has demonstrated that sputtering allows reproducibly fabricate electrodes with the sensitivity closest to the theoretical Nernstian response (a). However, even though screen printing is the second best, it has several advantages over the sputtering approach: lower cost and compatibility with mass-production, longer lifetime and compatibility with the fabrication of multi-sensors (b). Sensors from different batches (a) are indicated as S1, S2, S3, S4, and S5, while A is average sensitivity. Reproduced from Manjakkal et al., 2020.

The printing proceeds as a layer-by-layer deposition, where each layer undergoes solidification by thermal treatment. After printing of all the required layers, the surface is covered with an insulative coating (M. Li et al., 2012; Metters et al., 2011).

1.5 Nafion membrane as a protective layer in pH-sensitive electrodes

Nafion is a perfluorinated sulfonic acid (PFSA) ionomer that consists of a fluorocarbon backbone (hydrophobic) and a randomly tethered side chain with a terminal sulphur oxoacid group (Figure 7). Nafion was developed by Walter Grot for Dupont de Nemours (Wilmington, Delaware, USA) in the late 1960s and was the first commercial PFSA ionomer (Grot, 1982). Nowadays, Nafion is the most widely used PFSA ionomer. Nafion is used in the manufacture of fuel cells as a proton exchange membrane (Peighambardoust et al., 2010; Selim et al., 2022) and fabrication of electrochemical sensors as a protective membrane (Lonsdale, Wajrak, et al., 2017; Senthil Kumar et al., 2022) or for detection of ketones (Lucero et al., 2022).

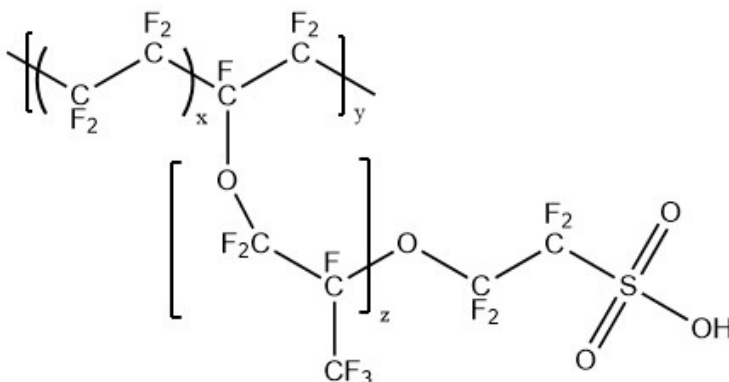


Figure 7. Chemical structure of Nafion.

Similarly to other PFSA ionomers, Nafion has a unique property of enhanced ion and solvent permittivity: the difference in hydrophilic nature of the fluorocarbon backbone and terminal sulphur oxoacid group allows phase separation that increases with the amount of solvent volume.

The permittivity towards H^+ ions is explained by the Grotguss mechanism (Agmon, 1995): diffusion of protons inside the water network via the exchange of bonded H^+ between neighbouring water molecules. During the hydration process, the water molecules ionize the sulfonic acid groups to form $SO_3^- \cdot (H_3O)^+$ ions (Kusoglu & Weber, 2017). However, a further increase in the number of water molecules within the polymer network ($\lambda^4 \approx 1-2$) leads to the dissociation of H^+ from the SO_3^- groups and the formation of complex ions with water (e.g., $H_9O_4^+$, $H_5O_2^+$). Hydrophilic domains enhance phase separation formed in the polymer network. When $\lambda > 2$, domains form an interconnected network, allowing the transport of ions and water molecules through the polymer network. Absorption of water continues till $\lambda \approx 5-6$ when a solvation shell of water molecules is formed around each $SO_3^- H^+$ (Kusoglu & Weber, 2017). This solvation shells of water together with the interconnected water network allow for excellent proton conductivity of Nafion.

Proton conductivity of the Nafion membrane is of key importance in electrochemical electrodes sensitive to cations: since the Nafion membrane consists of negatively charged groups, only small positive cations can travel through the polymer network (Kusoglu & Weber, 2017). This property of the Nafion membrane is of great importance for pH measurement in real-life food samples. The majority of food samples contain proteins and fats that interfere with pH measurement due to the contamination of the sensor. Nevertheless, if an electrode is covered with a Nafion membrane, the proteins in food samples that carry negative charges of $\approx -15 \dots -30$ mV (Tan et al., 2014) cannot pass through the Nafion membrane due to the electrostatic repulsion (Figure 8).

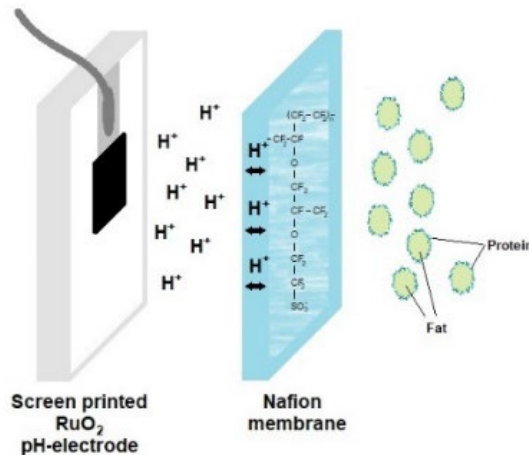


Figure 8. Schematic representation of the application of the Nafion membrane. Reproduced from **Publication II**.

⁴ λ is the water content that is defined as the number of water molecules per mole of sulfonic-acid groups.

Table 1. Fabrication method and material and properties of previously reported RuO₂ pH-electrodes. Reproduced from **Publication I**.

Deposition method	Electrode sensitive material	Substrate	Sensitivity, mV/pH	E ⁰ , mV	R ²	pH range	Response time, s	T, °C	Hysteresis, mV	Drift, mV/h	Reference
Electro-chemical deposition	RuO ₂	Au wire	59.3	521	N/A	2.0 - 12.0	N/A	N/A	N/A	N/A	(Pásztor et al., 1993)
		Au disk	60.5	N/A	0.999	2.0 – 11.0	7	N/A	N/A	N/A	(Shim et al., 2012)
		Pt	56.2	596	0.999	4.0 - 10.0	N/A	37.5	N/A	N/A	(Mingels et al., 2019)
		Pt-Ti	59.3	609	0.999	4.0 - 10.0	N/A	37.5	N/A	N/A	(Mingels et al., 2019)
Screen-printing	RuO ₂	Polyester foil	51.2	606	N/A	2.0 – 10.0	N/A	N/A	N/A	N/A	(Koncki & Mascini, 1997)
		Al ₂ O ₃	56	N/A	N/A	2.0 – 12.0	120	N/A	N/A	N/A	(Manjakkal, Zaraska, et al., 2016)
	RuO ₂ -Ta ₂ O ₅	Al ₂ O ₃	68.2	N/A	0.987	2.0 – 12.0	15	N/A	10	N/A	(Manjakkal, Zaraska, et al., 2016)
	RuO ₂ -Ta ₂ O ₅	Al ₂ O ₃	56	N/A	N/A	2.0 – 12.0	15	N/A	N/A	N/A	(Manjakkal, Cvejic, et al., 2016)
	RuO ₂ -SnO ₂	Al ₂ O ₃	56.5	631	0.998	2.0 – 12.0	9	N/A	7	N/A	(Manjakkal et al., 2015)
	RuO ₂ -Cu ₂ O	Al ₂ O ₃	47.4	N/A	N/A	2.0 – 13.0	N/A	21	N/A	N/A	(Zhuiykov, Kats, et al., 2011)
	RuO ₂ -La ₂ O ₃	Al ₂ O ₃	49.3	N/A	N/A	2.0 – 12.0	N/A	19	N/A	N/A	(Zhuiykov, Marney, et al., 2011)
	RuO ₂ -TiO ₂	Al ₂ O ₃	56.6	630	0.999	2.0 – 11.0	15	N/A	5	N/A	(Manjakkal et al., 2014)
RuO ₂ -Pt	Al ₂ O ₃ -Pt	58	640	N/A	2.0 – 13.0	2-Jan	23	~ 0	1.5	(Zhuiykov, 2009)	
Sol-gel Pechini	RuO ₂ -CNT	Au, Co, steel	63.1	647	1	2.0 – 12.0	50	N/A	N/A	N/A	(Kahram et al., 2014)
	RuO ₂ -TiO ₂	Ti	56	N/A	0.998	2.0 – 12.0	N/A	25	N/A	N/A	(Pocrička et al., 2006)
Radio-Frequency Magnetron Sputtering	RuO ₂	Pt wire	60	913	N/A	2.0 – 12.0	90	25	30	3	(McMurray et al., 1995)
		Ordered m/p carbon	57.8	598	0.999	2.0 – 12.0	180	22	3.14	19	(Lonsdale, Wajrak, et al., 2017)
			58.4	670	0.999	4.0 – 10.0	30	22	1.13	5	(Lonsdale, Maurya, et al., 2017)
		Carbon	59.2	800	1	4.0 – 10.0	25	22	5.44	20.5	(Lonsdale, Maurya, et al., 2017)
		Pt	58.6	925	0.999	4.0 – 10.0	20	22	6.45	23.4	(Lonsdale, Maurya, et al., 2017)
		Si	55.6	N/A	N/A	1.0 – 13.0	<1	N/A	4.36	0.38	(Liao & Chou, 2008)
		Si	51.7	N/A	0.978	2.0 – 10.0	N/A	N/A	N/A	N/A	(Yao et al., 2020)
		Si	55.8	N/A	0.998	2.0 – 10.0	N/A	N/A	N/A	N/A	(Yao et al., 2020)
	Si	56	N/A	N/A	1.0 - 12.0	N/A	N/A	N/A	N/A	(Chou et al., 2005)	
	RuO ₂	Al ₂ O ₃	73.8	N/A	0.998	4.0 - 10.0	3	22	~ 5	N/A	(Sardarinejad et al., 2015)
		Al ₂ O ₃	58.8	N/A	0.999	2.0 – 12.0	30	22	1.3	2.9	(Lonsdale, Wajrak, et al., 2018)
	RuO ₂ -Ta ₂ O ₅ -Nafion	Al ₂ O ₃	55.3	288	1	2.0 – 12.0	136	22	0.7	7.2	(Lonsdale, Wajrak, et al., 2018)
RuO ₂ -CNT	Ta	55.5	643	1	2.0 – 12.0	40	25	10.2	~ 3	(B. Xu & Zhang, 2010)	

2 Aims of the Thesis

The main objective of the thesis was to fabricate a potentiometric pH electrode based on ruthenium(IV) oxide for pH measurement in food samples.

To reach the aim of the thesis, the workflow was separated into the following steps:

- Aim 1 Find the fabrication parameters for the screen printing of ruthenium(IV) oxide pH electrodes.
- Aim 2 Find fabrication parameters for the deposition of the protective Nafion membrane. Investigate the properties of the ruthenium(IV) oxide pH electrodes covered with the Nafion membrane.
- Aim 3 Investigate the performance of the fabricated electrodes in food samples and during food processing.

3 Materials and Methods

Detailed description of the materials in methods can be found in the corresponding publications. This section is dedicated to briefly introducing utilized experimental procedures.

3.1 Fabrication of the RuO₂ electrodes (Publications I-III)

Ruthenium(IV) oxide electrodes were fabricated by screen printing technique that consisted of 4 main steps (Figure 9a): (i) screen printing of conductive layer (Ag layer), (ii) screen printing of pH-sensitive layer (RuO₂ layer), (iii) attachment of copper wire to later connect an electrode to a measuring device, and (iv) insulating the electrical contact with silicone-based resin. The dimensions of the fabricated RuO₂ electrodes are presented in Figure 9b,c. Furthermore, in *Publication I* the influence of the sintering temperature on the performance of the RuO₂ electrodes was investigated. The properties of the fabricated RuO₂ electrodes are discussed in *Publications I, II, IV* and *V*.

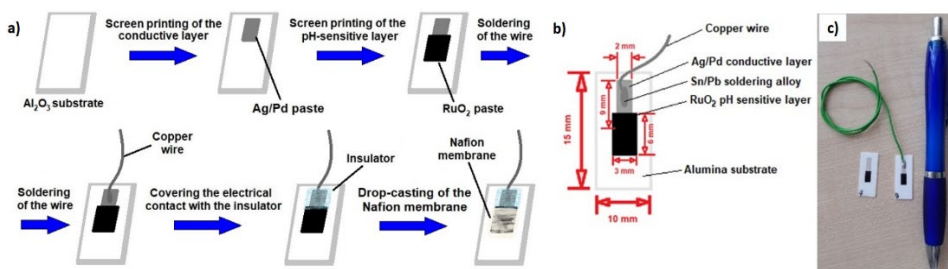


Figure 9. The RuO₂ electrodes were fabricated by screen printing of a conductive layer of Ag/Pd on an Al₂O₃ substrate and consequently printing of the pH-sensitive layer of RuO₂. (a) After the thermal treatment of both printed layers, a copper wire was attached to the Ag/Pd layer via soldering with a Pb/Sn alloy. For the fabrication of the RuO₂-Nf electrode, the RuO₂ part of the RuO₂ electrode was covered with Nafion membrane by drop-casting technique. The fabricated electrodes had small dimensions (b). A photograph of the fabricated electrodes is presented in figure (c) for the visualization of the fabricated electrodes. Adopted from *Publication III*.

3.2 Modification of the fabricated electrodes with Nafion membrane (Publication II) and investigation of the properties (Publications II, IV-VII)

In *Publication II* the conditions and parameters of the deposition of the Nafion membrane were investigated. The following conditions of the deposition were evaluated: (i) concentration of Nafion solution, (ii) number of Nafion layers, (iii) the time between layers deposition and (iv) drying temperature. The performance of the modified electrodes was evaluated on the basis of (i) membrane morphology, (ii) performance in milk, (iii) electrochemical characteristics (sensitivity, hysteresis and drift) and (iv) stability over long period of time. In *Publication VI*, the reusability of the RuO₂ electrodes after Nafion membrane deterioration was investigated and in *Publication VII* the cleaning procedure for the fabricated electrodes is discussed.leaning approach is one of the most important steps in accurate pH measurement. Usage of the electrode that was not properly cleaned can lead not only to false readings, but also the cross-contamination of

the next sample and possible destruction of the electrode in use. Furthermore, in *Publication V* the reversibility of thermal treatment of the Nafion membrane was investigated.

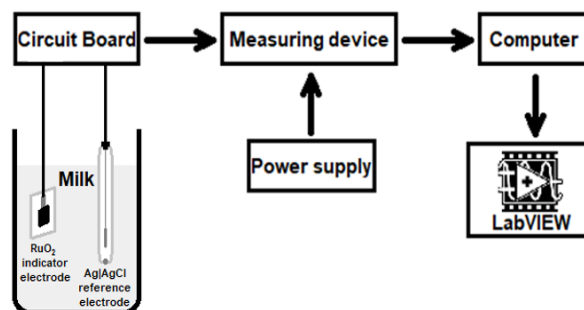
3.3 Electrochemical characterization of the fabricated electrodes

3.3.1 Setup

The setup for the work with the fabricated RuO₂-based electrodes consisted of the units presented in [Table 2](#) and [Figure 10](#). To evaluate the performance of the fabricated electrode, several parameters were evaluated: sensitivity (or the basis of the Nernst equation) and linearity (R²), hysteresis and drift rate.

Table 2. Electrochemical setup used in this work.

Unit	Electrode and equipment used	Function
Working electrode	RuO ₂ , RuO ₂ -Nf, RuO ₂ -CuO, RuO ₂ -CuO-Nf electrodes	pH-sensitive electrode
	Standard glass ion-selective Ag AgCl KCl electrode (HI1053P, Hanna Instruments, USA)	Accurate pH measurement
Reference electrode	Standard glass ion-selective Ag AgCl KCl electrode (HI1053P, Hanna Instruments, USA)	Stable potential readings
Measuring device	Data Acquisition (DAQ) device, USB-6259, National Instruments, USA	Recording of the data
Power supply	High-performance digital power supply (E3631A, Agilent, USA) with an interference-free input voltage of 12 V	Powering up the measuring device
Software	LabVIEW program (National Instruments, USA)	Registering the data



*Figure 10. Schematic representation of the setup used in this work. Reproduced from **Publication III**.*

3.3.2 Electrochemical characteristics

Sensitivity

As mentioned in section 1.2, sensitivity rises from the Nernst equation and is used to evaluate the performance of an electrode and to calculate the pH of the sample from the measured potential value. Sensitivity is determined by checking the response of an electrode to pH change. For that, several buffer solutions of different pH are prepared

and the potential of an electrode in these solutions is recorded. In this work, citric, phosphate and carbonate buffers were prepared in pH ranges 3-6, 7-8 and 9.2-11.8 respectively. The buffers were prepared according to (Dawson, P.; Elliott, D.; Elliott, A.; Johns, 1991). The pH of the buffers was determined with a conventional pH meter (Seven2Go Advanced Single-Channel Portable pH Meter, Mettler Toledo, Switzerland).

The theoretical (expected) response of the pH sensor depends on the temperature and at a temperature of 21 °C should be equal to 58.4 mV/pH (Table 3). However, the sensitivity of printed electrodes most of the time is different from the theoretical response and depends not only on the materials used for the fabrication of the electrode but also on the fabrication methods and conditions (Kurzweil, 2009; Manjakkal et al., 2020; Zhuiykov, 2012).

Table 3. Theoretical sensitivity at common room temperatures.

T, °C	Theoretical sensitivity, mV/pH	T, °C	Theoretical sensitivity, mV/pH	T, °C	Theoretical sensitivity, mV/pH
16	57.4	21	58.4	26	59.4
17	57.6	22	58.6	27	59.6
18	57.8	23	58.8	28	59.8
19	58.0	24	59.0	29	60.0
20	58.2	25	59.2	30	60.2

The sensitivity [mV/pH], E^0 [mV] and linearity of the response (R^2) of the fabricated electrodes were determined by measuring the electrochemical potential in pH buffers and plotting the measured electrochemical potential as a function of the pH (Figure 11). The value of the potential was taken 90 seconds after immersing the electrode into a solution to let the electrode reach stable potential readings). The values of sensitivity and E^0 were determined from the linear equation calculated from the least squares regression.

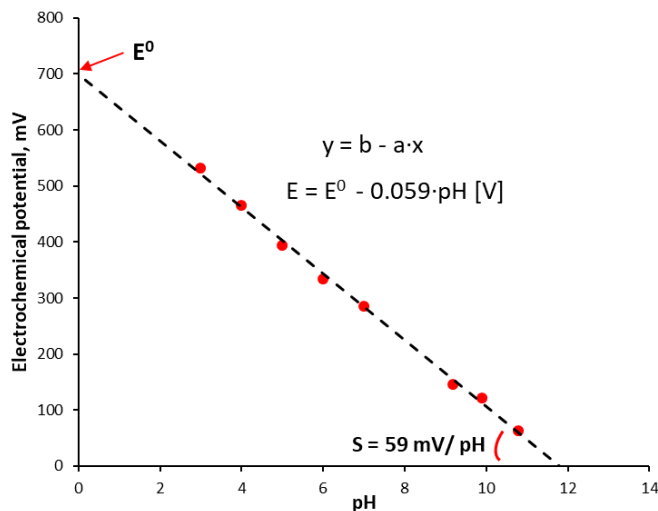


Figure 11. An example of a pH-electrode calibration curve.

Hysteresis

Hysteresis [mV] is the so-called 'memory effect' of an electrode and is used to describe the impact of previous measurements on the current one (Figure 12). Hysteresis is related to the composition of the double layer on the surface of an electrode and the changes happening in the double layer when an electrode is exposed to solutions of different pH. The pH measurement loop is known to impact the hysteresis of pH electrodes fabricated from metal oxides (Manjakkal et al., 2020). Hysteresis of the fabricated electrodes was determined by measuring the pH of the buffers cyclically: the electrodes were exposed to pH buffer solutions from pH 3 to 7 and back to 3 (3–5–7–5–3) and from pH 11 to 7 and back to 11 (11–9–7–9–11) to evaluate acidic and basic hysteresis respectively. After submerging a fabricated electrode into a buffer solution, the electrochemical potential was recorded for 5 minutes and the value of hysteresis was determined by comparing the potential at pH 3. The electrode was rinsed with distilled water in between the measurement in the buffer solutions.

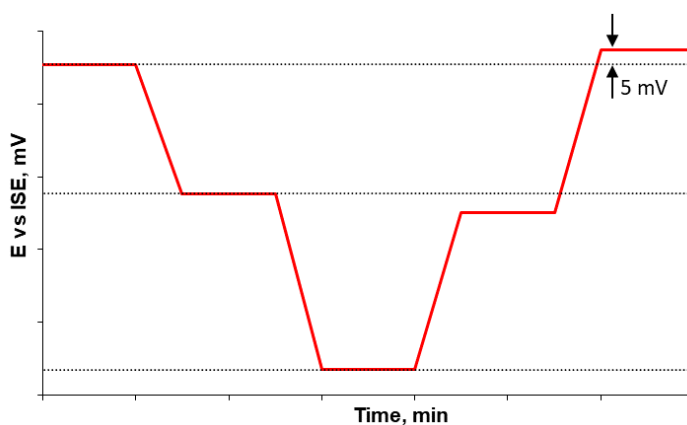


Figure 12. An example of a hysteresis graph.

Drift

Drift [mV/h] of an electrode is a change of the potential of an electrode with time. Drift can be used to determine the reliability of an electrode and its applicability for continuous measurement. The drift was determined by the method of the slope of the line of line-of-best-fit after monitoring the potential of an electrode continuously for 2 hours by calculating the change of the potential in 1 hour period.

Cross-sensitivity

Solid-state electrodes designed to measure pH can sometimes be cross-sensitive to other ions in the sample (Figure 13) (Manjakkal et al., 2015; Manjakkal, Zaraska, et al., 2016). Therefore, the sensitivity of the fabricated RuO₂-Nf electrodes was evaluated in the presence of interfering ions. Change of the sensitivity of the fabricated electrodes was evaluated in the presence of chlorides of common cations – LiCl, KCl, NaCl, and NH₄Cl. For that, the sensitivity of the fabricated electrodes was first measured in buffer solutions as described in section 3.3.2 and then in the same buffer with the addition of the above-mentioned compound (the concentration of the compound was 0.1 M).

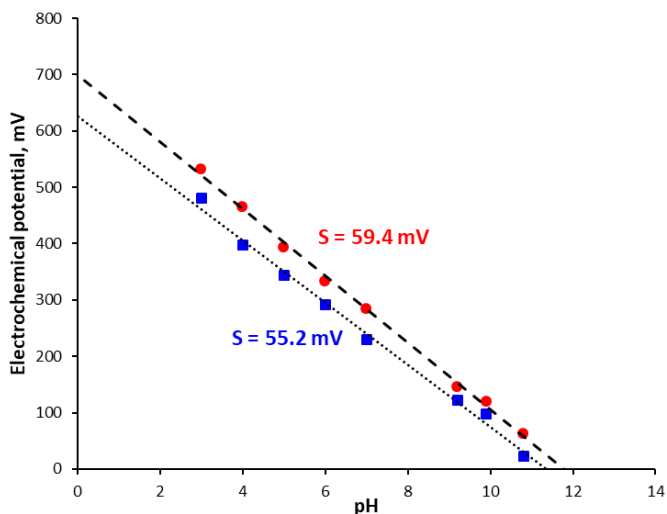


Figure 13. An example of the sensitivity of the RuO_2 electrode in the absence (red) and in the presence of interfering ions (blue).

3.4 Measurement in real-life samples

In *Publication III*, the performance of the $\text{RuO}_2\text{-Nf}$ electrodes in food samples was investigated. Among others, pH measurement in dairy products was conducted. The electrodes were pre-calibrated by two-point calibrating procedure and then used to measure pH of a dairy sample. The performance of the fabricated electrode was evaluated based on formula (12). All the measurements were made in triplicate for 2 electrodes.

$$pH_{\text{difference}} = pH_{\text{fabricated}} - pH_{\text{CGE}} \quad (12)$$

Furthermore, continuous measurement of pH change during milk coagulation was performed. For that, first, continuous measurement in milk sample was performed over a period of 1 hour and then the electrode was cleaned and used for pH measurement during milk coagulation. Coagulation of milk was shown to happen during first 5 minutes of the experiment. The pH change was monitored for 35 minutes. The measurement was conducted for 2 electrodes in parallel.

4 Results and Discussion

4.1 Fabrication parameters for the screen printing of the RuO₂ pH electrodes (Publications I and II)

Sintering temperature is known to impact the properties of the solid-state electrodes fabricated from metal oxides: higher sintering temperatures lead to improved crystallinity, increased density and lower porosity of the fabricated layer, therefore, forming compact microstructures (Javanbakht et al., 2016; Oketola et al., 2022; Syaizwadi et al., 2018). Furthermore, an increase in sintering temperature leads to lower electrical resistance and therefore improved electrical characteristics (Syaizwadi et al., 2018). For the fabricated screen-printed RuO₂ electrodes, the morphology and microstructure of the RuO₂ layers did not change much with the sintering temperature (Figure 14). All three electrode types, RuO₂-800, RuO₂-850 and RuO₂-900, had low porosity and grain size of ~0.5-2.0 μm. However, better adhesion of the RuO₂ ink to the Al₂O₃ substrate was observed for the electrodes sintered at 800 °C.

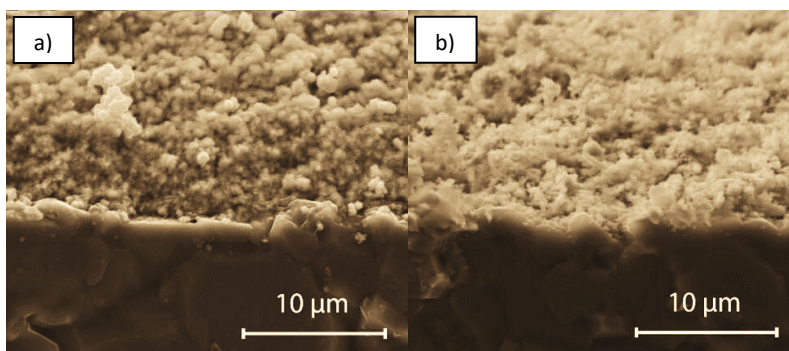


Figure 14. Scanning Electron Microscopy images (cross-section) of the RuO₂ electrodes sintered at (a) 800 °C and (b) 900 °C. Reproduced from **Publication I**.

Next, the electrochemical characteristics of the fabricated RuO₂ electrodes were investigated (Table 4). The fabricated electrodes exhibited sensitivity close to the theoretical Nernstian response and excellent linearity of the response to the pH change ($R^2 > 0.094$) with sensitivity values slightly improving with the increase of the sintering temperature (Table 4). The drift was small for all three electrode types and was below 1 mV/h. The hysteresis was smaller for the acidic loop; furthermore, the hysteresis was the smallest for the RuO₂ electrodes sintered at 850 °C (4.6 mV and 26.3 mV for acidic and basic hysteresis respectively). All the 3 electrode types showed characteristics similar to those previously published by different authors (Table S1 in **Publication I**). Considering all the electrochemical characteristics, RuO₂-850 electrodes were selected for further studies. Therefore, in the upcoming paragraphs phrase ‘RuO₂ electrodes’ will imply ‘RuO₂ electrodes sintered at 850 °C’.

Next, the stability of the readings of the fabricated electrodes was investigated. The results are presented in Figure 15. The sensitivity of the electrodes was changing for the first 4 weeks of conditioning. This change of sensitivity is explained by the slow speed of the formation of the double layer on the surface of the RuO₂ electrodes. It was shown before (Kurzweil, 2009; Pásztor et al., 1993) that solid-state electrodes need some time to reach stable composition of the double layer.

Table 4. Electrochemical characteristics of the RuO₂ electrodes sintered at different temperatures. Adopted from **Publication I**.

	Electrode type		
	RuO ₂ -800	RuO ₂ -850	RuO ₂ -900
S, mV/pH	61.8 ± 1.0	60.5 ± 1.4	56.1 ± 2.1
E ⁰ , mV	681.9 ± 5.0	664.2 ± 11.3	624.0 ± 27.8
R ²	0.996	0.997	0.996
Hysteresis A, mV	25.1	4.6	1.2
Hysteresis B, mV	35.4	26.3	40.4
Drift, mV/h	0-5	0-5	0-5

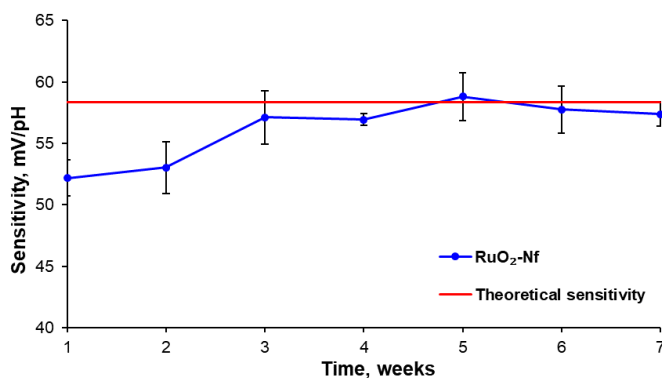


Figure 15. Change of the RuO₂ electrode sensitivity (Y-axis) with time. The sensitivity was changing for the first 4 weeks of storage in water and reached stable sensitivity values on 5th week.

RuO₂ solid-state electrodes were previously shown to work in aqueous samples and even in some more complex samples, such as coke and juices (Liao & Chou, 2008; Manjakkal et al., 2015). However, screen-printed RuO₂ electrodes do not work well in milk (Figure 16). Therefore, it was decided to cover the pH-sensitive part of the RuO₂ screen-printed electrode with a Nafion membrane. From Figure 16, it can be seen that Nafion-covered RuO₂ electrodes exhibit excellent pH dependency, identical to the CGE.

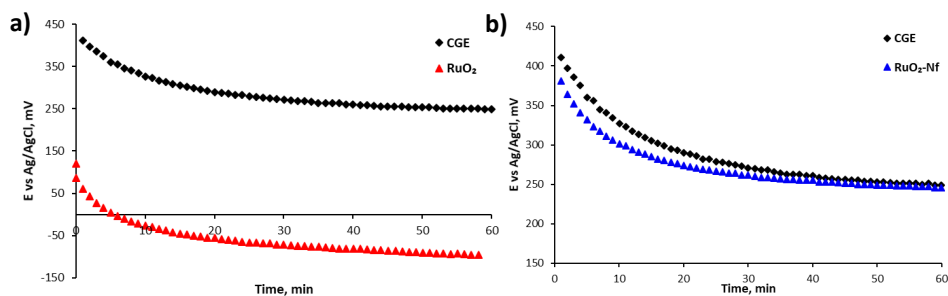


Figure 16. After placing the RuO₂ electrode in milk (**a**, red), its electrochemical potential abruptly dropped to negative values, indicating that electrodes cannot be used to measure the pH of the milk sample. On the other hand, the potential of the RuO₂-Nf electrode (**b**, blue) was similar to the CGE. Modified from **Publication II**.

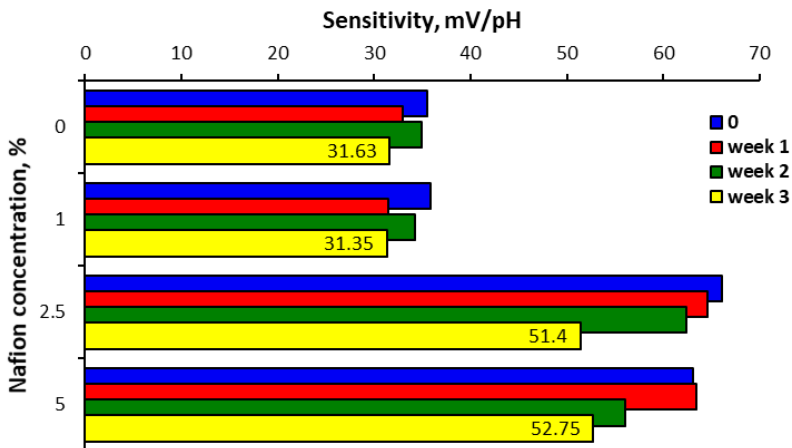
4.2 Fabrication parameters for the deposition of the Nafion membrane and properties of the RuO₂-Nf electrodes (Publications II-VI)

After discovering the improved performance of the RuO₂ electrodes after covering them with the Nafion membrane, it was necessary to devote more attention to the deposition of the Nafion membrane. The parameters of the deposition of the Nafion membrane, such as method of membrane casting and its conditions (e.g., annealing temperature, pre-treatment, humidity), as well as polymer characteristics of Nafion (equivalent weight, length of side chain) are known to impact the proton conductivity of the membrane (Kusoglu & Weber, 2017). Among the deposition techniques reported by different authors (Table 1 in **Publication II**), drop-casting of Nafion solution is the most common one. However, there is a big difference in how Nafion membranes were drop-casted. The previously published papers vary in the thickness of the Nafion membrane and drying temperature.

4.2.1 Concentration of Nafion casting solution (Publication IV)

The influence of the Nafion concentration on the performance of the RuO₂-Nf electrodes was evaluated by utilizing 5 % Nafion solutions diluted with 1:1 mixture of distilled water and ethanol to the following concentrations (v/v %): 1, 2.5 and 5. The sensitivity of the fabricated electrodes was monitored for 3 weeks and the results are presented in [Figure 17](#). It can be seen that, when the 5 % Nafion solution to cover the RuO₂ electrodes, the RuO₂-Nf electrodes had the sensitivity, closest to the theoretical Nernstian response. Therefore, 5 % Nafion solution was selected for further studies.

To be certain that Nafion solution evenly covers the surface of the RuO₂ electrodes, the optical microscopy images of the electrodes with and without Nafion coatings were taken. [Figure 18](#) demonstrates that is evenly distributed over the RuO₂ microparticles, creating a uniform protective layer.



*Figure 17. Change of the sensitivity for the RuO₂-Nf electrodes fabricated from the solutions with Nafion concentration of 1, 2.5 and 5 %. The sensitivity was measured before covering the RuO₂ electrodes with Nafion membrane (blue), 1 (red), 2 (green) and 3 (yellow) weeks after the covering. Adopted from **Publication IV**. © 2020 IEEE.*

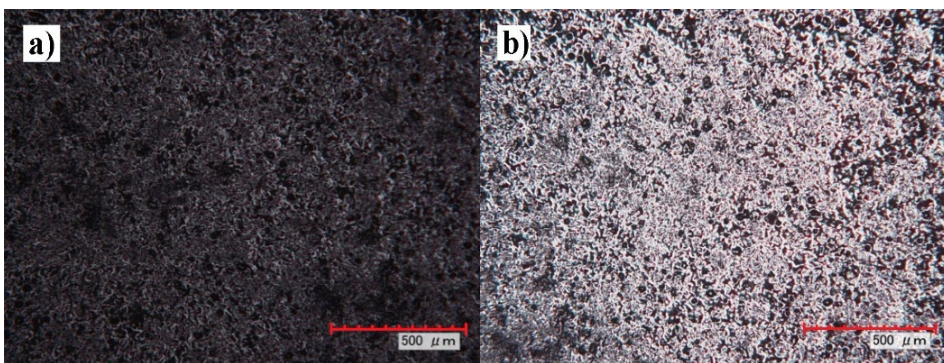


Figure 18. Digital optical microscopy images of RuO_2 (a) and $\text{RuO}_2\text{-Nf}$ (b) electrodes. The images were taken at the same light exposure. Reproduced from **Publication V**. © 2022 IEEE.

4.2.2 Number of Nafion layers (Publication II)

The thickness of Nafion is important from the point of real-life applicability: the pH electrodes that were used in a food sample should be thoroughly cleaned before the next use to avoid cross-contamination and false pH readings. Therefore, the Nafion membrane should withstand cleaning. That can be achieved by increasing the thickness of the Nafion membrane. The types of electrodes were fabricated to investigate the proper thickness of Nafion membrane: $\text{RuO}_2\text{-Nf}_1$, $\text{RuO}_2\text{-Nf}_3$ and $\text{RuO}_2\text{-Nf}_5$.

First, the physical appearance of the deposited membrane was evaluated using SEM (Figure 19). All the 3 electrode types had some defects in the Nafion membrane (cracks and pores), and the size and number of defects increased with the thickness of the Nafion membrane (Table 5). The size of the defects can be important from the point of application of the electrodes, since the defect can be bigger than the size of some sample components⁵ and, therefore, lead to contamination of the RuO_2 pH-sensitive layer that was observed in milk test for the $\text{RuO}_2\text{-Nf}_5$ electrodes.

Furthermore, the electrochemical characteristics of the fabricated electrodes were investigated. All the fabricated electrodes showed linear response with sensitivity values close to the theoretical Nernstian response of 58.4 mV/pH (at a temperature of 21 °C) with the sensitivity slightly decreasing with the increase of the thickness of the Nafion membrane Table 5. For the $\text{RuO}_2\text{-Nf}_5$ electrodes, the linearity was 0.942 and the hysteresis exceeded 20 mV. This can be due to the longer time needed for the ions to travel through a thicker Nafion membrane and agrees well with the findings of Lonsdale (Lonsdale, 2018).

The preliminary test in milk samples (Figure 20) showed excellent performance of $\text{RuO}_2\text{-Nf}_3$ electrodes even during the third application for measurement in milk. Therefore, the $\text{RuO}_2\text{-Nf}_3$ electrodes with the 7.47 μm thickness of the Nafion membrane were selected for further investigation.

⁵ For example, the size of fat globules is around 2-6 μm (Phadungath, 2005; Xu et al., 2016) that is smaller than defects in Nafion membrane for $\text{RuO}_2\text{-Nf}_5$ electrodes.

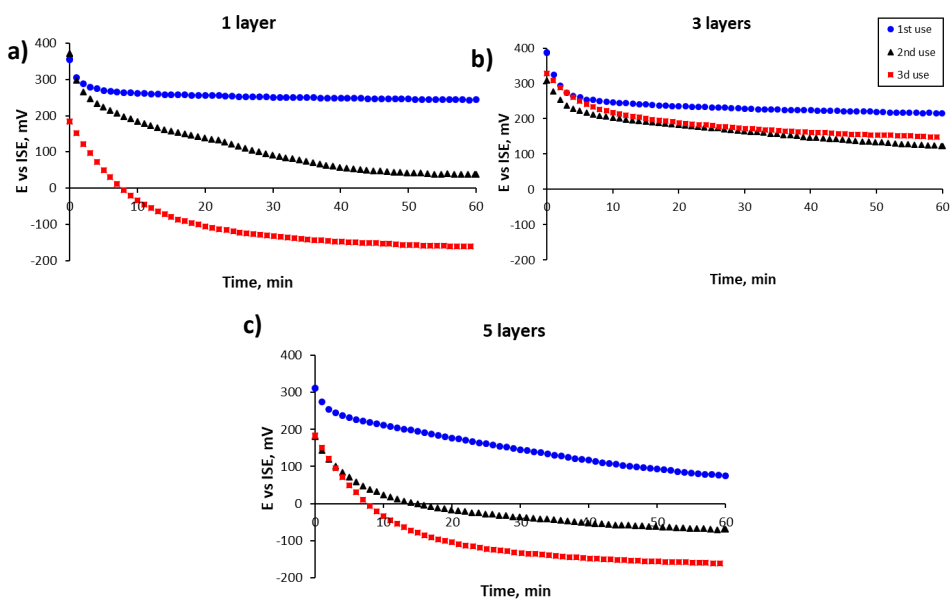


Figure 20. Performance of the RuO₂-Nf electrodes ((a) – RuO₂-Nf₁, (b) – RuO₂-Nf₃, (c) – RuO₂-Nf₅) was evaluated by placing them in a milk sample and continuously measuring the electrochemical potential for 1 hour. Furthermore, the electrodes were used 3 times (blue – first use, black – second use, red – third use) to evaluate whether the electrodes can be used repeatedly. RuO₂-Nf₃ showed the best performance. Reproduced from **Publication II**.

4.2.3 Time needed for deposition of one layer (Publication II)

Next, the time necessary for the drying of one layer of the Nafion membrane was investigated. As can be seen from the data presented in Table 6, the best characteristics of the RuO₂-Nf₃ electrodes were achieved when the Nafion layers were allowed to dry for 2 hours in between the layer deposition. In this case, more uniform values of the sensitivity were achieved with better linearity of the pH response.

Table 6. Characteristics of the RuO₂-Nf₃ electrodes with different drying time. Reproduced from **Publication II**.

Characteristic	Drying time of one layer of Nafion		
	0.5 h	1 h	2 h
S, mV/pH	53.1 ± 0.5	56.2 ± 10.9	60.5 ± 2.1
E ⁰ , mV	601.5 ± 131.7	549.0 ± 90.3	680.9 ± 10.3
R ²	0.981	0.978	0.984
Hysteresis A, mV	10 ± 2	11 ± 4	4 ± 2
Hysteresis B, mV	24 ± 6	21 ± 17	25 ± 4
Drift, mV/h	25-35	30-35	20-25

4.2.4 Drying temperature (Publication II)

It was previously reported (Kusoglu & Weber, 2017), that pre-heating and annealing of the Nafion membrane allow improving the proton conductivity of the membrane. Furthermore, thermal treatment of the membrane allows increasing mechanical stability of the membrane (Kusoglu & Weber, 2017), which is important for real-life application of the RuO₂-Nf electrode from the point of their susceptibility to cleaning. Xu and co-authors previously reported (K. Xu et al., 2016) enhanced mechanical and thermal

properties of the Nafion membrane dried at 80 °C. However, no explanation was given regarding why the temperature of 80 °C was selected. Hence, in this study, the range of temperatures from room temperature to 80 °C and its influence on the properties of the Nafion membrane was investigated.

The electrochemical characteristics of the RuO₂-Nf₃ electrodes dried at different temperatures are presented in Table 7. The RuO₂-Nf₃ electrodes dried at 60 and 80 °C showed good performance and provided better protection to the RuO₂ screen-printed layer (Figure 21). Apparently, the thermal treatment improved the mechanical stability of the Nafion membrane allowing for better protection of the screen-printed RuO₂ layer underneath due to the increased entanglement of polymer chains and coalescence of Nafion particles (Kim et al., 2015; Mauritz & Moore, 2004).

In real-life situations, it can be necessary to measure pH in samples of not room temperature, but elevated temperatures. For instance, acid-induced milk coagulation is carried out at a temperature of 40 °C (Phadungath, 2005). However, it is worth mentioning that the proton conductivity of the Nafion membrane is known to decrease with the temperature (Alberti et al., 2013). Therefore, the performance of the RuO₂-Nf₃ electrodes was performed at temperatures 25, 30, 35 and 40 °C (Table 8). The best performance was achieved for the RuO₂-Nf₃ electrodes dried at 80 °C (smaller hysteresis and drift, and a more linear increase of the sensitivity with temperature).

Table 7. Characteristics of the RuO₂-Nf₃ electrodes dried at different temperatures. Reproduced from Publication II.

Characteristic	Drying temperature, °C			
	21	40	60	80
S, mV/pH	60.5 ± 2.1	48.9 ± 1.0	52.0 ± 2.0	52.2 ± 2.1
E ⁰ , mV	680.9 ± 10.3	554.0 ± 23.9	667.3 ± 19.9	485.7 ± 21.2
R ²	0.984	0.995	0.994	0.983
Hysteresis A, mV	4 ± 2	22 ± 2	10 ± 3	8 ± 5
Hysteresis B, mV	24 ± 1	21 ± 3	19 ± 10	14 ± 4
Drift, mV/h	20-25	10-20	10-20	20-30

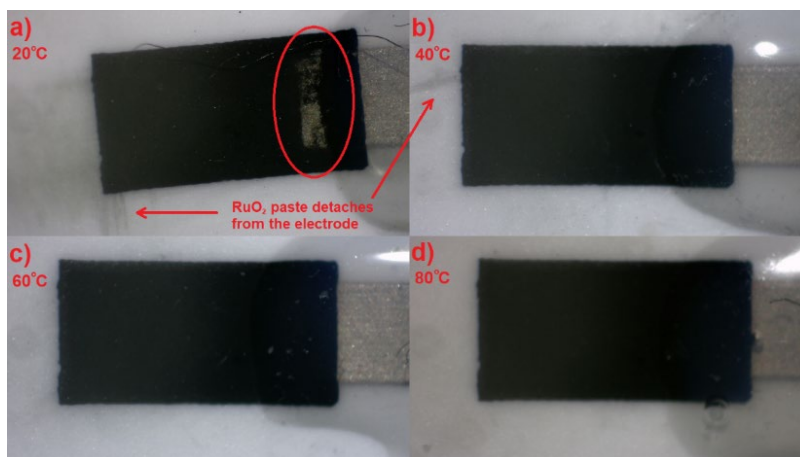


Figure 21. Images of the state of the RuO₂-Nf₃ electrodes after use as pH -electrodes (Nafion membrane is removed) for the Nafion membrane dried at (a) 20, (b) 40, (c) 60 and (d) 80 °C. The electrodes dried at higher temperatures exhibited better protection of the RuO₂ screen-printed layer from the decay. Reproduced from Publication II.

Table 8. Electrochemical characteristics of the RuO₂-Nf electrodes tested in heated samples. Adopted from **Publication II**.

Characteristic	Temperature of the sample, °C			
	25	30	35	40
RuO₂-Nf₃ electrodes dried at 60 °C				
S, mV/pH	52.3 ± 2.0	54.5 ± 2.1	54.7 ± 6.8	56.4 ± 10.7
E ⁰ , mV	641.6 ± 8.8	556.3 ± 29.9	596.8 ± 17.4	645.6 ± 11.5
R ²	0.997	0.972	0.952	0.956
Hysteresis A, mV	12 ± 7	11 ± 6	17 ± 8	28 ± 10
Hysteresis B, mV	12 ± 5	23 ± 9	26 ± 2	32 ± 10
Drift, mV/h	15-25	10-15	5-15	5-15
RuO₂-Nf₃ electrodes dried at 80 °C				
S, mV/pH	53.5 ± 2.3	54.0 ± 0.8	54.5 ± 2.2	54.7 ± 2.2
E ⁰ , mV	640.7 ± 75.7	548.1 ± 16.3	506.2 ± 41.9	583.6 ± 58.2
R ²	0.985	0.979	0.985	0.995
Hysteresis A, mV	8 ± 1	15 ± 6	12 ± 2	15 ± 7
Hysteresis B, mV	20 ± 6	17 ± 1	15 ± 9	20 ± 10
Drift, mV/h	5-20	5-15	10-20	10-15

4.2.5 Stability of the readings of the RuO₂-Nf electrodes (Publication II)

Solid-state electrodes require initial conditioning in water or buffer solutions that is needed to reach stable Ru(IV)/Ru(III) ratio in the fabricated electrodes (Manjakkal et al., 2014; Zhuiykov, 2009). The fabricated RuO₂-Nf electrodes reached stable sensitivity values on the third week of conditioning (Figure 22), while RuO₂ electrodes required over one month of conditioning in water (Figure 22).

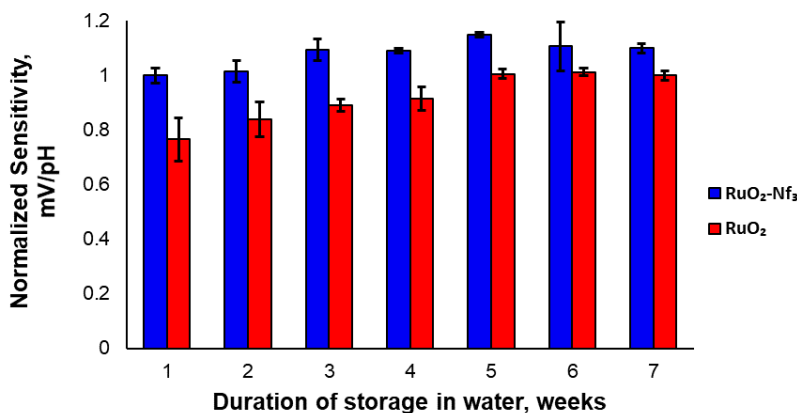


Figure 22. Change of sensitivity of the RuO₂ (red) and RuO₂-Nf₃ (blue) electrodes with time. The sensitivity was normalized against the value of the sensitivity on week 7. The RuO₂-Nf₃ reached stable sensitivity values in a shorter time compared to the RuO₂ electrodes. Adopted from **Publication II**.

4.2.6 Cross-sensitivity of the RuO₂-Nf electrodes (Publication III)

It was previously demonstrated, that only small positively charged ions can impact the performance of screen-printed RuO₂ electrodes (Manjakkal et al., 2020). Furthermore, Nafion membranes are known to have high ion mobility towards smaller cations and the negative charge of sulfonate groups in the Nafion backbone causes lower mobility towards anions (Kusoglu & Weber, 2017). Therefore, the cross-sensitivity of the fabricated RuO₂-Nf

electrodes was tested in presence of Na^+ , K^+ , Li^+ and NH_4^+ utilizing chloride salts of these cations since Cl^- ions were shown to not alter the sensitivity of the RuO_2 electrodes (Manjakkal et al., 2020). The results are presented in Figure 23. The presence of interfering cations in concentrations of 0.1 M did not significantly change the sensitivity of the RuO_2 -Nf electrodes except for NH_4^+ . The increase of sensitivity in presence of NH_4Cl was 2.83 mV/pH can be due to the presence of ammonia in the aqueous solution that can the composition of a double-layer on the surface of the RuO_2 electrodes (Kurzweil, 2009). Nonetheless, the linearity of the response of the RuO_2 -Nf electrodes toward the pH change was above 0.99 for all the investigated cases.

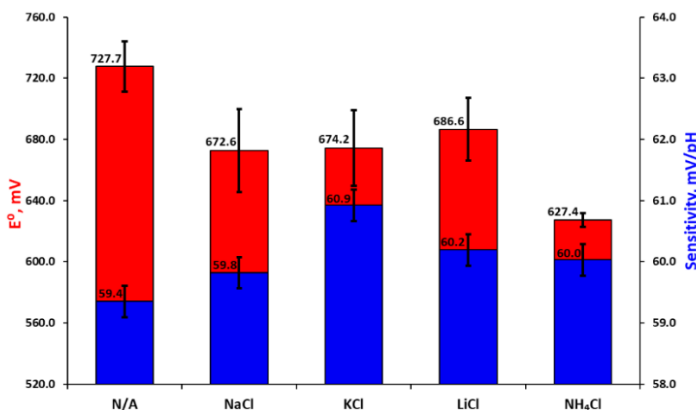


Figure 23. RuO_2 -Nf electrodes are not sensitive to the presence of cations in buffer solutions at concentrations up to 0.1 M. The E^0 of the fabricated electrodes (red, primary Y-axis) did not change much in the presence of interfering cations. The sensitivity of the fabricated electrodes (blue, secondary Y-axis) remained close to the theoretical Nernstian response indicating only negligible sensitivity towards most common interfering ions. Adopted from Publication III.

4.2.7 Reusability of the fabricated RuO_2 -Nf electrodes (Publication VI)

Next, the reusability of the RuO_2 -Nf electrodes after deterioration of Nafion membrane was investigated. Nafion membrane is a soft membrane, that is partially soluble in water and, therefore, might wear off with time. Here, the possibility of recover the RuO_2 electrodes and repeatedly cover them with new Nafion membrane was investigated. The results are presented in Table 9. It can be seen that all the electrochemical characteristics were worsening with the next usage: the sensitivity, R_2 and E^0 were decreasing, whereas hysteresis a and B were increasing. Nonetheless, at room temperature the RuO_2 -Nf electrodes exhibited acceptable characteristics and, therefore, can be used repeatedly but only at temperatures around 25 °C.

4.3 Performance of the fabricated RuO_2 -Nf electrodes in food samples (Publications III and VII)

Notwithstanding the number of papers dealing with the development and fabrication of new pH sensors published every year, only a few of them report application on other samples than buffer solutions. This is due to the fact that solid-state electrode face difficulties when measuring in real-life samples (Figure 16). The same applies to the CGE: not only electrode requires extensive cleaning, but also should be properly used for accurate pH measurement (Galster, 1991).

Table 9. Electrochemical characteristics of the RuO₂-Nf electrodes, covered with Nafion 3 times and tested in samples of varying temperature. Reproduced from **Publication VI**. © 2020 IEEE.

Characteristic	Temperature of the sample, °C			
	25	30	35	40
First coating				
S, mV/pH	53.5 ± 2.3	54.0 ± 0.8	54.5 ± 2.2	54.7 ± 2.2
E ⁰ , mV	640.7 ± 75.7	548.1 ± 16.3	506.2 ± 41.9	583.6 ± 58.2
R ²	0.985	0.979	0.985	0.995
Hysteresis A, mV	8 ± 1	15 ± 6	12 ± 2	15 ± 7
Hysteresis B, mV	20 ± 6	17 ± 1	15 ± 9	20 ± 10
Drift, mV/h	5-20	5-15	10-20	10-15
Second coating				
S, mV/pH	49.5 ± 5.8	40.4 ± 6.8	47.8 ± 5.0	33.3 ± 4.8
E ⁰ , mV	537.9 ± 23.4	350.7 ± 18.1	425.9 ± 18.1	259.6 ± 80.4
R ²	0.994	0.952	0.977	0.876
Hysteresis A, mV	24 ± 7	9 ± 4	20 ± 7	28 ± 1
Hysteresis B, mV	9 ± 2	11 ± 3	20 ± 4	8 ± 7
Drift, mV/h	0-15	0-20	0-20	0-15
Third coating				
S, mV/pH	53.7 ± 1.5	27.7 ± 7.4	28.1 ± 5.8	13.7 ± 4.2
E ⁰ , mV	522.8 ± 22.3	342.6 ± 117.6	260.5 ± 51.0	67.7 ± 64.8
R ²	0.975	0.966	0.941	0.831
Hysteresis A, mV	20 ± 4	19 ± 1	24 ± 4	22 ± 5
Hysteresis B, mV	4 ± 1	7 ± 4	20 ± 4	10 ± 2
Drift, mV/h	10-20	5-15	10-20	10-20

4.3.1 Measurement in dairy samples (Publication III)

The RuO₂-Nf electrodes, together with RuO₂-CuO-Nf electrodes were tested for accurate pH measurement in different samples, including juices, carbonated and caffeinated beverages in the [MSc thesis of Iuliia Vetik](#) and will not be discussed here. In this study, the main attention was paid to the application of the fabricated electrodes to one of the most challenging samples – dairy samples that have denser textures and contain fats and proteins that interfere with pH measurement. The results of the pH measurement in dairy samples are presented in [Figure 24](#). It is worth mentioning that response time also increased from 5 minutes for milk and yoghurt to 10 minutes for sour cream, cottage and melted cheese.

It can be seen that for milk, yoghurt, sour cream and cottage cheese the difference in pH readings from the CGE was below 0.5 pH units and only for the melted cheese sample that had the densest texture the pH difference was around 3 pH units. For the second CGE that was used as a reference, the pH difference was reaching 0.1 pH units for denser samples. However, the difference of 0.5 pH units is still considered good as it is similar to those observed by other researchers for different sensing materials used in different samples (section 1.1). This finding correlated well with the recent investigation by Chawang et al. (Chawang et al., 2022), where the authors have demonstrated the influence of the viscosity of the sample on the performance of the iridium oxide electrode.

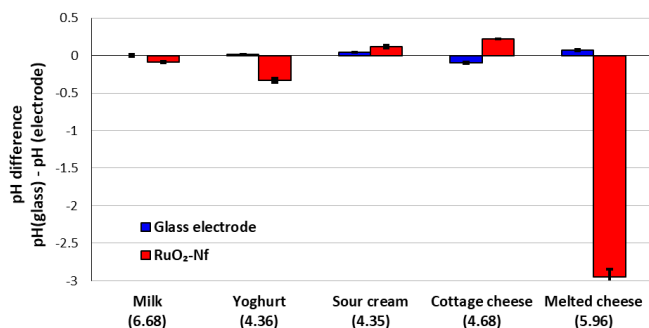


Figure 24. Results of pH measurement in dairy samples. The fabricated RuO₂-Nf electrodes had error below 0.5 pH units in all samples except for melted cheese where the error was 2.95 pH units. Adopted from **Publication III**.

4.3.2 Continuous measurement in milk during coagulation

Monitoring the pH during the milk coagulation allows producing cheese with better quality (e.g., texture, taste) (Fox et al., 1990; Lucey et al., 2003). However, at present, there is no method that would allow continuous measurement of pH. Currently, pH is measured using the point method, when the pH of a sample is measured, e.g., every hour with cleaning in between the measurements (Rosca et al., 2019). Developing an electrode that would allow measurement of pH during the milk coagulation could allow more precise determination of cheese cutting time. Therefore, the fabricated electrodes were tested for applicability for pH measurement during milk coagulation. As demonstrated in Figure 16, RuO₂-Nf electrodes exhibit behavior similar to the CGE when measuring in milk for one hour. In this study, an enzyme was used to induce milk coagulation. Since during enzymatic coagulation pH value should remain the same, it was expected to observe the constant response from the fabricated electrodes during milk coagulation. However, the pH measured with the fabricated RuO₂-Nf electrode was gradually increasing by 2.76 and 3.15 pH units during the 35 min of measurement for electrodes 1 and 2 respectively (Figure 25). At the same time, the change of the measured pH with the CGE was only 0.06 pH units. Thus, even though RuO₂-Nf electrodes show good performance in milk, they are not yet suitable for measurement during milk coagulation and once again indicated the inapplicability of the RuO₂-Nf electrodes for denser samples.

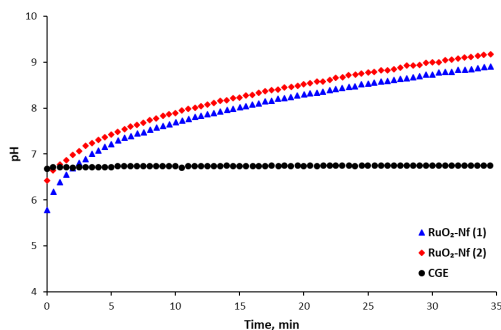


Figure 25. Change of pH value measured with the CGE (black) and 2 RuO₂-Nf (blue and red) electrodes in parallel during milk coagulation. While the CGE showed stable pH readings, the fabricated RuO₂-Nf electrodes failed to accurately measure the pH.

4.3.3 Cleaning after measurement in dairy samples (Publication VII)

The reusability of the RuO₂-Nf electrodes was discussed in section 4.2.7, however, actual reusability of any electrode implies a correct cleaning procedure that allow to maintain the active surface of an electrode in-between the measurement. Therefore, here the cleaning of RuO₂-Nf electrode after the usage in milk is discussed below. Four approach that are commonly advised for the cleaning of pH electrodes were investigated: (i) [removal of fats and oils] mechanical cleaning with surfactants: (ii) [removal of protein residues] cleaning with acidic pepsin solution; (iii) [removal of mineral deposits] acidic cleaning. The results are presented in Figure 26. The sensitivity of RuO₂-Nf electrodes improved after any of the cleaning approaches, however, the sensitivity of the RuO₂-Nf electrodes in all the cases was changing during first 24 hours after cleaning, indicating that conditioning of the RuO₂-Nf electrodes is necessary prior to the next usage (similarly to the CGE). This conditioning is necessary to allow the double layer, consisting of H⁺ and OH⁻ ions to form on the surface of the electrodes (Manjakkal et al., 2020). The smallest change in sensitivity was observed for the RuO₂-Nf electrodes cleaned with the acidic pepsin solution, hence, making this cleaning approach the most favourable once amongst investigated options.

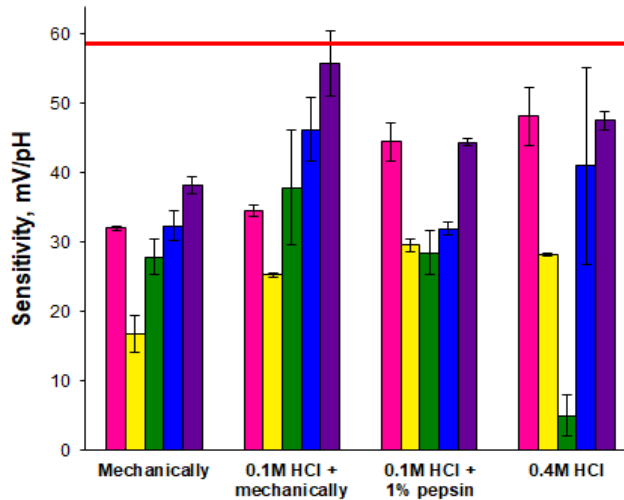


Figure 26. Change of the sensitivity of the RuO₂-Nf electrodes with cleaning: initial sensitivity (pink), sensitivity after using in milk (yellow), sensitivity straight after cleaning (green), sensitivity 12 hours after cleaning (blue) and sensitivity 24 hours after cleaning (purple). Red line indicates theoretical Nernstian sensitivity. Reproduced from Publication VII. © 2022 IEEE.

Conclusion

In this dissertation, solid-state RuO₂ are brought forward in order to replace fragile and expensive conventional glass electrode in the pH measurement of food samples.

The aims declared in the beginning of the thesis were reached with the following outcome:

Aim 1 Find the fabrication parameters for the screen printing of ruthenium(IV) oxide pH electrodes.

To reach the first aim of the thesis, the RuO₂ electrodes were fabricated by screen printing and the influence of the sintering temperature on the performance of the fabricated electrodes was discussed. Sintering temperature of 850 was selected as optimal for the fabrication of the RuO₂ electrodes with the best characteristics.

Aim 2 Find fabrication parameters for the deposition of the protective Nafion membrane. Investigate the properties of the ruthenium(IV) oxide pH electrodes covered with the Nafion membrane.

To reach the second aim, a protective membrane of Nafion™ was deposited and demonstrated to allow pH measurement in milk. Therefore, the properties of the Nafion™ membrane were investigated next. The conditions for the deposition of the Nafion™ membrane (thickness, drying time and temperature) were selected from the point of their effect on the electrochemical characteristics of the RuO₂ electrodes. Furthermore, it was demonstrated that the Nafion™ membrane improves the durability of the RuO₂ electrodes.

Aim 3 Investigate the performance of the fabricated electrodes in food samples and during food processing.

To reach the third aim, the fabricated electrodes were used for the pH measurement of dairy samples. The fabricated electrodes covered with Nafion™ protective membrane showed good performance in dairy samples and only failed to accurately measure pH in melted cheese. Apparently, not only the composition but also the texture of the samples can affect the performance of the screen-printed RuO₂ electrodes. Next, an attempt was made to use the fabricated electrodes for continuous measurement during milk coagulation. The experiment demonstrated, that even though the fabricated electrodes can be used for continuous monitoring of milk over 15 hours, they yet fail to measure pH during milk coagulation. Nevertheless, the reported electrodes can be successfully employed for pH measurement of different real-life samples and are suitable for continuous pH measurement that make them of potential interest for food researchers and food industry for on-line and in-line pH monitoring.

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Abstract

Screen-printed pH sensors based on ruthenium(IV) oxide for measurement in food samples

The quality of water and food on its basis (dairy, meat and fish products) is a matter of great concern. One of the most essential food quality parameters is pH. It can be used to monitor the quality of fermentation processes, evaluate freshness and reveal impurities in food samples. At present, pH of food products is determined by a standard potentiometric method based on the measurement of the electrochemical potential change between a pH-sensitive and a reference electrode immersed into the test solution. The glass electrodes in conventional pH meters are a combination of pH-sensitive and reference electrodes incorporated in one glass body. However, due to its high cost, fragility of the glass body of the electrode, possible contamination of samples and the reference junction, as well as inability of continuous measurement, glass electrode cannot be implemented for monitoring of on-line industrial processes. Therefore, novel materials and methods are thoroughly investigated.

One of the most investigated alternatives to conventional glass electrode is electrochemical sensors based on metal oxides. These sensors are easy to fabricate, exhibit good sensitivity and linear response, with some of them being compatible with conventional pH meters. Among the metal oxides, suitable for pH measurement, ruthenium(IV) oxide (RuO_2) has proven itself to be the most beneficial one, due to its optimum performance characteristics, such as excellent pH sensitivity, low drift and hysteresis, consistent performance even in bacteria-rich environment. However, even though the RuO_2 -based pH sensors exhibit excellent performance in aqueous media, the actual challenge is to make these sensors applicable to real-life samples due to the complexity of their composition.

This work presents research dedicated to improve the performance of RuO_2 electrodes. The reliable pH measurement with the screen-printed RuO_2 electrodes was achieved by covering them with a protective Nafion™ membrane. This manuscript is tackling on the (i) fabrication and properties of the RuO_2 electrodes, (ii) fabrication and properties of the Nafion™ membrane, as well as (iii) application of the developed electrodes for measurement in food samples.

Lühikokkuvõte

Ruteenium(IV)oksiidil põhinevad siiditrükiga pH-andurid toiduproovide mõõtmiseks

Vee ja sellel põhineva toidu (piima-, liha- ja kalatooted) kvaliteedi kontroll on toidutööstuses väga oluline. Üks oluline toidukvaliteedi parameeter on pH. Seda saab kasutada käärimisprotsesside kvaliteedi jälgimiseks, toidu värskuse hindamiseks ja lisandite tuvastamiseks toiduproovides. Tavaliselt määratakse tänapäeval toiduainete pH standardse potentsiomeetrilise meetodiga, mis põhineb elektrokeemilise potentsiaali muutuse mõõtmisel pH-tundliku ja katselahusesse sukeldatud võrdluselektroodi vahel. Klaaselektroodid tavalistes pH-meetrites koosnevad pH-tundlikest ja võrdluselektroodidest, mis on ühendatud ühte klaasist korpusesse. Klaaselektroodi ei saa kasutada *on-line* tööstusprotsesside jälgimiseks kuna neil on kõrge hind, elektroodi klaaskeha on habras, and saastuvad kiiresti ja ei ned saa kasutada pideva mõõtmise režiimis. Seetõttu uuritakse põhjalikult uudeid alternatiivseid materjale ja meetodeid pH mõõtmiseks toidutööstuses.

Üks enim uuritud alternatiive tavapärasele klaaselektroodile on metalloksiididel põhinevad elektrokeemilised andurid. Neid andureid on lihtne valmistada, neil on hea tundlikkus ja lineaarne vastus ja mõned neist ühilduvad ka tavapäraste pH-meetritega. Ruteenium(IV) oksiid (RuO_2) on osutunud kõige kasulikumaks metallioksiidiks pH mõõtmise jaoks tänu oma headele mõõtmis omadustele, nagu suurepärase pH tundlikkus, madal triiv ja hüsterees ja ühtlane töö isegi bakteririkkas keskkonnas. Kuigi RuO_2 -põhistel pH-anduritel on vesikeskkonnas suurepäraseid omadused on neid seni olnud väga keeruline rakendada kuna päriselu proovide jaoks tööstuses.

Selles doktoritöös pühenduti RuO_2 elektroodide töökindluse parandamisele toidutööstuse jaoks. Usaldusväärne pH mõõtmine siiditrüki meetodil valmistatud RuO_2 elektroodidega saavutati kui and olid kaetud kaitsva NafionTM membraaniga. See käsikiri käsitleb (i) RuO_2 elektroodide valmistamist ja omadusi, (ii) NafionTM membraani valmistamist ja omadusi, samuti (iii) väljatöötatud elektroodide kasutamist toiduproovide analüüsil.

Appendix 4

Publication IV

M. Lazouskaya, M. Tamm, O. Scheler, K. Uppuluri, and K. Zaraska, "Nafion as a protective membrane for screen-printed pH-sensitive ruthenium oxide electrodes," *Proceedings of IEEE Biennial Baltic Electronics Conference (BEC)*, V. 2020-October, P. 18–21, 2020, Tallinn, Estonia.

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Appendix 5

Publication V

K. Uppuluri, M. Lazouskaya, D. Szwagierczak, and K. Zaraska, "Influence of temperature on the performance of Nafion coated RuO₂ based pH electrodes," in *IEEE International Conference on Flexible and Printable Sensors and Systems (FLEPS)*, 2021, Manchester, United Kingdom.

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Appendix 6

Publication VI

M. Lazouskaya, O. Scheler, K. Uppuluri, K. Zaraska, and M. Tamm, "Reusability of RuO₂-Nafion electrodes, suitable for potentiometric pH measurement," in *IEEE International Conference on Flexible and Printable Sensors and Systems (FLEPS)*, 2022, Vienna, Austria.

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Appendix 7

Publication VII

M. Lazouskaya, I. Vetik, O. Scheler, K. Uppuluri, N. Razmi, K. Zaraska, and M. Tamm, "Cleaning procedure for the screen printed RuO₂-based pH electrodes," in *IEEE Sensors Conference, 2022*, Dallas, Texas, USA.

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