



Detection of pH and Urea with an Extended Gate Field-Effect Transistor-Based Microsensor

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Abstract

Extended gate field-effect transistor-based (EGFET) microsensors have been developed for highly sensitive detection of pH and urea. EGFET was made by integrating with a microelectrode. Briefly, the EGFET-based pH microsensor was developed by modifying the surface of a microelectrode with polypyrrole (PPy) via electropolymerization and integrating it with a small, simple, and inexpensive metal oxide semiconductor field-effect transistor (MOSFET). As for the EGFET-based urea microsensor, urease enzyme was immobilized in PPy. The measurements of pH and urea were made in solutions at different pH values and the urea concentrations, respectively. The results showed that the pH microsensor had a very good sensitivity of 67 mV/pH in a pH range of 5-12. Similarly, the EGFET-based urea microsensor showed a linear response range of 10^{-9} to 10^{-5} M urea with sensitivities of 35.5 mV/decade urea and 10 μ A/decade urea. The reported EGFET-based pH and urea microsensors have great potential for use in the biomedical field, especially in applications where local analysis is required.

Keywords: EGFET, pH, urea, urease, polypyrrole, electropolymerization, microsensor

Genişletilmiş Kapı Alan Etkili Transistör Tabanlı Mikrosensör ile pH ve Üre Tespiti

Öz

pH ve ürenin yüksek hassasiyette tespiti için genişletilmiş kapı alan-etkili transistör tabanlı (EGFET) mikrosensörler geliştirilmiştir. EGFET, bir mikroelektrot ile entegre edilerek oluşturulmuştur. Kısaca, EGFET-tabanlı pH mikrosensörü, mikroelektrot yüzeyine elektropolimerizasyon yoluyla polipirol (PPy) modifiye edilerek ve buna küçük, basit ve ucuz yarı iletken metal oksit alan etkili transistör (MOSFET) entegre edilmesiyle geliştirilmiştir. EGFET-tabanlı üre mikrosensöründe ise, üreaz enzimi PPy içerisine mobilize edilmiştir. pH ve üre ölçümleri sırasıyla farklı pH değerlerine ve üre konsantrasyonlarına sahip solüsyonlarla yapılmıştır. Sonuçlar pH mikrosensörünün pH 5-12 aralığında 67 mV/pH gibi çok iyi bir hassasiyete sahip olduğunu göstermiştir. Benzer şekilde, EGFET-tabanlı üre mikrosensörü, 35.5 mV/decade üre ve 10 μ A/decade üre hassasiyetleriyle birlikte 10^{-9} - 10^{-5} M üre aralığında doğrusal bir yanıt gösterdi. Bildirilen EGFET pH ve üre mikrosensörleri, biyomedikal alanda, özellikle yerel analizin gerekli olduğu uygulamalarda kullanım için büyük potansiyele sahiptir.

Anahtar Kelimeler: EGFET, pH, üre, üreaz, polipirol, elektropolimerizasyonu, mikrosensör

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1. Introduction

pH defined as a logarithmic measure of hydrogen ion concentration is a basic analytical measure of the mechanism of chemical or biological reactions (Li et al., 2020, Şen et al., 2016). Proper measurement and control of pH is essential for many industrial applications and living organisms due to its relevance to many chemical and biochemical processes (Vonau & Guth, 2006). For example, urea, acetylcholine, and L-arginine can be detected by sensing pH change (Fenoy et al., 2020). Among them, urea has taken great attention from researchers, and for detection of urea, many techniques have been improved. As a result of protein metabolism, ammonia is converted to urea in the kidneys and liver. Since urea is a product of protein metabolism, balancing it in the human body is important for renal health. The urea level is considered an important marker for monitoring kidney functions. Measuring urea for medical diagnosis can show malnutrition, renal functioning, increased catabolism of proteins, high protein diet, pregnancy, dehydration, inhibition in urinary system, shock, and stress (Jakhar et al., 2018). The urea level in a healthy human should be in the range of 8-20 mg/dL (Ahuja et al., 2008).

Ion sensitive field-effect transistor (ISFET) is a special type of metal oxide semiconductor field-effect transistor (MOSFET) and used for measuring ion concentration. The metal gate of MOSFET is replaced by an ion-sensitive membrane, electrolyte solution, and a reference electrode in ISFET. The working principle of ISFET is based on change in current caused by a change in ion concentration. The advantages of ISFET are low power consumption, low cost, ease of operation, small size, fast response time, significant durability, easy storage, and lower temperature dependence (Avolio et al., 2020). In addition, ISFETs are highly sensitive, and can potentially be miniaturized (Ghoneim et al., 2019). Despite the various advantages, ISFETs suffer from the need for reference electrodes, complex gate encapsulation procedures, and costly cleanroom processes (Mokhtarifar et al., 2019). As an alternative, extended gate field-effect transistor-based (EGFET) sensors have been intensively studied for chemical sensing including pH measurement (Pan et al., 2003). Unlike ISFET the sensing membrane of an EGFET-based sensor is placed away from the field-effect transistor (FET) device which has no direct contact with the chemical environment. The working principle of an EGFET-based sensor relies on the change in the response of the sensing membrane to varying concentrations of analytes. For example, the change in the conductivity of a sensing membrane such as polypyrrole (PPy) at different pHs modulates the potential applied through the gate and thus changes the electric current flowing between drain and source electrodes (Ghoneim et al., 2019). Physically separating the sensing membrane and protecting the gate region ensures that the activity of the target analyte makes an additional chemical contribution to the threshold voltage (Pullano et al., 2018). EGFET-based sensors possess advantages such as high transconductance, improved threshold voltage shift, stability, improved responsiveness, low noise, low mass production cost, and being disposable (Pullano et al., 2019).

In recent years, popularity of conducting polymers are increasing in the field of sensors due to their low cost, robustness, and versatility (Avolio et al., 2020). Conductive polymers offer a practical matrix, especially for the encapsulation of enzymes in a one-step procedure (Ahuja et al., 2008). Entrapment of enzymes

in conducting polymers not only increases the lifetime of the enzyme but also reduces the cost of measurement. Entrapment of enzymes in conducting polymers permits the localization of biologically active molecules on electrodes of different geometry/size and increases the possibility of precisely polymer coating over conductive microspheres with complex geometry (Ahuja et al., 2008; Cosnier & biotechnology, 2000). Entrapment of enzymes in conductive polymers can be achieved via chemical or electrochemical polymerization. Electropolymerization is often preferred due to its advantages such as better controllability of film thickness and morphology. The electropolymerization process takes place on the electrode surface by anodic oxidation of the corresponding monomer in the electrolyte solution.

PPy is a biocompatible conducting polymer and can be used as a sensing membrane in pH sensors. It has high chemical stability, easy oxidation, low monomer cost, high biocompatibility, very good thermal, mechanical and electrical properties (Vandana Bisht et al., 2005; Pandey et al., 2018). In addition, pyrrole (Py) monomer has the advantage of being easily oxidized, water-soluble, and commercially available (Sadki et al., 2000). There are many studies in the literature where PPy was applied to pH sensing. For example, Lakard et al. developed a potentiometric pH sensor based on PPy. The sensor had a quasi-linear response between pH 2 and 11 with a pH sensitivity of -51 mV/pH (Lakard et al., 2007). In a similar study, the effect of PPy film thickness and scan rate on pH sensitivity was measured and the highest response slope was reported to be 53.0 mV/pH at a scan rate of 25 mV/s (Mo et al., 2003). In another work, Naser et al. designed an electrochemical flow cell pH sensor for aqueous solutions based on EGFET. Two different sensitivities were obtained on days 1 and 15 in the pH range of 8.00 to 10.80. The sensitivity on day 1 (52.31 mV/pH) decreased to 42.65 mV/pH on day 15 (Mokhtarifar et al., 2019). PPy has also been applied for the electrochemical detection of urea. For example, Rajesh et al. designed a potentiometric urea biosensor by electrochemically preparing a conducting copolymer *poly(N-3-aminopropyl pyrrole-co-pyrrole)* (PAPCP) film onto an indium-tin-oxide (ITO) coated glass plate. The urease enzyme was covalently immobilized on the prepared polymer. In the study, both pH and urea measurements were carried out. The pH and urea sensitivity of the sensor were 47 mV/pH and 27.5 mV/decade urea, respectively (V Bisht et al., 2005). In a different study, a potentiometric urea biosensor was developed by electropolymerization of PPy, p-toluene sulphonic acid and BSA onto an ITO coated glass plate. The urease enzyme was covalently linked to the prepared conducting PPy. The sensor showed a linear response in the range of 6.6×10^{-6} to 7.5×10^{-4} M with a sensitivity of 17.3 mV/decade (Ahuja et al., 2008).

Here, a novel EGFET-based pH and urea microsensor was developed. Briefly, the microsensors were constructed by integrating PPy-modified microelectrode with a small, simple and inexpensive MOSFET. First, PPy was electrochemically deposited on a gold microdisk electrode as a sensing membrane for pH measurement. The electrochemical deposition of PPy was carried out in galvanostatic mode. After optimizing the deposition time, the sensor was tested in phosphate buffered saline (PBS) at various pHs. Next, urease enzyme was entrapped in PPy to construct an EGFET-based urea microsensor, which is new to the best of our knowledge. Results showed that the EGFET-based pH and urea microsensors detected pH and urea successfully with good sensitivity. The reported microsensors have great potential

for use in the biomedical field, especially in applications where local analysis is required.

2. Materials and Methods

2.1. Materials

Phosphate buffered saline (PBS) (Sigma-Aldrich, USA), sodium hydroxide (NaOH) (Sigma-Aldrich, USA), hydrogen chloride (HCl) (Sigma-Aldrich, USA), Py (Sigma-Aldrich, USA), lithium perchlorate (LiClO₄) (Sigma-Aldrich, USA), perchloric acid (HClO₄) (Supelco, USA), urea (Sigma-Aldrich, USA), urease (Sigma-Aldrich, USA), silver paste (Sigma-Aldrich, USA), n-type MOSFET (IRFZ44N, International Rectifier, USA)

2.2. Microchip fabrication

A glass slide was spin coated with S1818 positive photoresist at 2000 rpm for 30 sec. After prebaking, the photoresist was patterned via contact lithography. The exposed areas of the photoresist were removed using a developer solution (CD26, Microchem, USA). Titanium and gold films were sputtered on the glass slide with a patterned photoresist. Lastly, the remaining photoresist was removed using acetone to define the gold (Au) patterns of the microchip (Şen et al., 2013; Şen et al., 2012; Şen et al., 2014). Next, SU-8 photoresist was spin coated, prebaked and patterned using photolithography. After post baking, the unexposed regions were removed to define the diameter of the microdisk electrode (40 µm). Prior to use, the SU-8 layer was hard-baked at 180 °C for 30 min. A silver (Ag) electrode was printed on the microchip as a reference electrode using Ag paste.

2.3. PPy electropolymerization and pH detection with an EGFET-based pH microsensor

PPy solution was prepared using 0.2 M Py 0.2 M lithium perchlorate (LiClO₄) and 0.1 M perchloric acid (HClO₄). A two-electrode electrochemical cell was used for electropolymerization. Electropolymerization of the PPy solution was carried out in galvanostatic mode by applying a current of 0.1 µA to the working electrode (gold electrode) for different time intervals (1, 3, 4, 5 and 10 min) by potentiostat/galvanostat (PGSTAT204, Metrohm, Switzerland). The measurement capacity and characterization of the EGFET-based pH microsensors were performed in PBS solutions at varying pH values using a FET analysis unit (B2901A Precision Source/Measure Unit) (Aykaç et al., 2021). While the source and the drain of the n-type MOSFET were connected to the source and drain inputs of the FET analysis unit, the gate is connected to the EGFET-based pH microsensor. In order to make the measurement

more sensitive and stable, pH measurements were carried out using an Ag electrode printed on the microchip. PBS solutions at different pH (5.53 - 7.08 - 7.96 - 8.57 - 9.82 - 12.07) were used to determine the pH sensitivity of the EGFET pH microsensor. I_{DS}-V_{GS} curves were obtained by scanning the gate potential from 0 to + 2 V while applying a constant potential of + 0.5 V between the source and the drain electrodes. The effect of different time intervals on pH sensitivity was calculated.

2.4. PPy/urease electropolymerization and urea detection with an EGFET-based urea microsensor

A Py-urease solution was prepared by dissolving 4 mg of urease in 75 µl of PPy solution. Electropolymerization of the Py-urease solution was carried out in galvanostatic mode by applying a current of 0.1 µA for 180 seconds by potentiostat (Aydin & Şen, 2017). The sensor was tested in deionized water containing varying concentration of urea (10⁻⁹ mol/L, 10⁻⁸ mol/L, 10⁻⁷ mol/L, 10⁻⁶ mol/L, 10⁻⁵ mol/L, 10⁻⁴ mol/L, 10⁻³ mol/L, 10⁻² mol/L, and 10⁻¹ mol/L). Both I_{DS}-V_{GS} and I_{DS}-V_{DS} curves were obtained. In the case of I_{DS}-V_{GS}, the potential applied to the gate was scanned from 0 to + 2 V, while a constant potential of + 0.5 V was applied between the source and the drain electrodes. As for the I_{DS}-V_{DS} curves, the potential applied to the drain was scanned from 0 to + 2 V, while a constant potential of + 0.5 V was applied between the gate and the source electrodes.

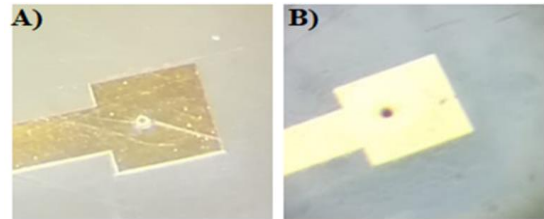


Figure 1. Optical images of an Au microelectrode before (A) and after electropolymerization of PPy/urease (B).

3. Results and Discussion

PPy is a biocompatible conducting polymer and can be used as a sensing membrane in pH sensors. Basically, PPy was electrochemically deposited on an Au microelectrode for high sensitive detection of pH which was then applied for the detection of urea. Microelectrodes have several advantages over traditional macroscale electrodes, e.g. fast response, high current density, high signal-to-noise ratio, low I-R drop, small double layer capacitance.

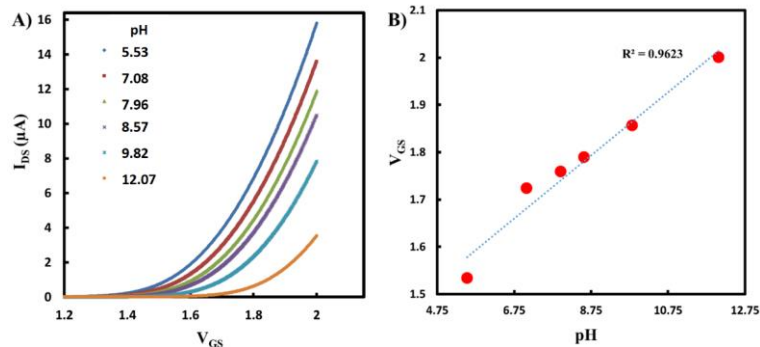


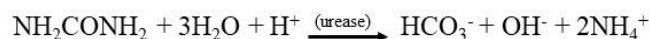
Figure 2. I_{DS}-V_{GS} curves obtained at varying pH values (A) and a calibration curve based on a constant I_{DS}.

Microelectrodes are considered powerful tools for in vivo or on-site measurements in various fields such as physiology, microbial ecology, medicine, neuroscience, and environmental monitoring. After the PPy deposition on an Au microelectrode, the success of the process was first checked with a microscope (Top Ring Led Illuminated Stereo Microscope). As can be seen in Figure 1, the color of Au microelectrode changed from orange-yellow to black, confirming the successful coating of PPy.

EGFET-based pH microsensors use chemical protonation and deprotonation reactions causing a potential difference between the solution and the extended gate of the transistor. The EGFET-based pH microsensor was connected to an n-type MOSFET and I_{DS} - V_{GS} curves were obtained in PBS solutions at different pH values. As shown in Figure 2A, the obtained I_{DS} - V_{GS} curves changed with changing pH of the solution being measured. The change in the conductivity of the semiconductor PPy at different pH has modulated the potential applied to the channel between the source and the drain in the MOSFET. This situation resulted in different I_{DS} - V_{GS} curves at different pH values. According to the results, EGFET-based pH microsensor successfully detected pH in a relatively wide range of 5 to 12 with a pH sensitivity of 67 mV/pH. A calibration curve was also obtained based on V_{GS} at $I_{DS} = 3 \mu A$ with an R^2 value of 0.99, clearly showing that the relationship between pH and voltage values was linear (Figure 2B). The thickness of the PPy coating can greatly affect the sensitivity of the sensor.

In order to see the effect of electropolymerization time on the sensitivity, 0.1 μA current was applied to the working electrode for 1, 3, 4, 5 and 10 min in galvanostatic mode. It is known that the thickness of PPy can be roughly controlled by controlling the electropolymerization time (Adelaju et al., 1996). Long or short PPy deposition times greatly reduced the pH sensitivity of EGFET-based pH microsensor. For example, electrochemical deposition of PPy for 1 and 10 min resulted in pH sensitivities of 29 and 27 mV/pH, respectively. According to the results, 4 min deposition time yielded the highest sensitivity. In addition, incubating for 40 min at room temperature improved the

sensitivity by 27 mV/pH. In Table 1, the performance of the EGFET-based pH microsensor is compared with some recent reports in which pH sensitivity was determined electrochemically with various modified electrodes. The PPy-modified EGFET-based pH microsensor seemed to outperform most of these electrodes in terms of both pH sensitivity and linear range. In addition to pH sensing, the polymer can also be applied for the enzymatic detection of urea. Basically, urease catalyzes the conversion of urea and releases hydroxide ions, which in turn changes the local pH Eq.(1) (Syu et al., 2009). The local pH change can be easily associated with the level of urea in the environment.



Equation 1: Chemical equation of reaction between urea and urease (Syu et al., 2009)

The adopted strategy allowed the entrapment of the enzyme with PPy in a single step. The EGFET-based urea microsensor was tested in deionized water containing different concentration of urea ranging from 10^{-1} mol/L to 10^{-9} mol/L. As can be seen in Figure 3A_i, the I_{DS} - V_{GS} curves differed depending on the urea concentration of the solution being measured. The gate voltage of the MOSFET is affected by the potential of the EGFET and the potential of the sensing film may appear as the potential source. Since urease changes the local pH in the presence of urea, the working principle of EGFET-based urea microsensor is the same as the EGFET-based pH microsensor. V_{GS} shifts to a more positive direction with increasing urea concentration, which is reasonable as the ammonia produced by the enzyme has a pH causing the local pH to increase. The calibration curve demonstrating the relationship between V_{GS} and $-\log[\text{urea}]$ has a R^2 value of 98.63 (Figure 3A_{ii}). Although the sensor gave very good linear response between 10^{-9} mol/L and 10^{-5} mol/L with a sensitivity of 35.5 mV/decade, the same was not true for larger concentrations. This may be because the enzyme reaches saturation after a certain level of urea concentration. In addition to I_{DS} - V_{GS} curves, I_{DS} - V_{DS} curves were also obtained.

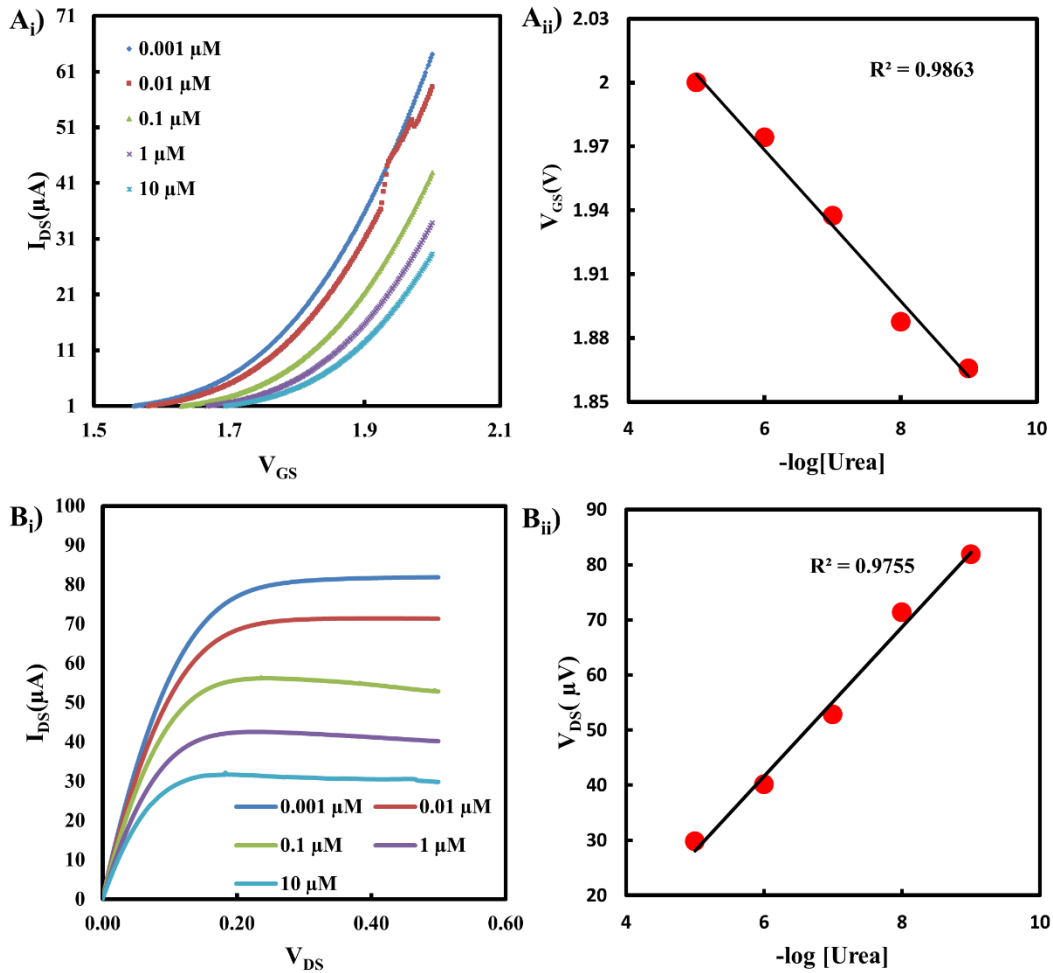


Figure 3. $I_{DS}-V_{GS}$ (A_i) and $I_{DS}-V_{DS}$ (B_i) curves obtained at varying urea concentrations along with respective calibration curves (A_{ii}, B_{ii})

The response of the EGFET-based urea microsensor examined in the saturation regime of the MOSFET. Figure 3B_i shows that the $I_{DS}-V_{DS}$ characteristics of the sensor changed accordingly with varying concentration of urea. Basically, the conductivity of PPy dropped at high pH caused by the enzymatic reaction of urease and thus I_{DS} got reduced with increasing urea concentration. It can be clearly seen in Figure 3B_i that the I_{DS} values became nearly constant after a certain V_{DS} in all test solutions at a fixed $V_{GS} = + 0.5$ V. A calibration curve was obtained the same as before. The relationship between I_{DS} and $-\log[urea]$ was linear with an R^2 value of 97.55 (Figure 3B_{ii}). The sensitivity of the sensor was calculated to be 10 $\mu A/decade$. In Table 2, the performance of the EGFET-based urea microsensor was compared to the analytical performance of different enzymatic urea sensors. The present urea microsensor seemed to have a good linear range and sensitivity. EGFET-based microsensors can be used in bioanalytical applications with advantages such as miniaturization, convenient integration into different micro-systems, stability to environmental changes such as light and temperature, high conductivity, and sensitivity.

4. Conclusions and Recommendations

The use of EGFET-based microsensors for pH and urea detection has been successfully demonstrated. PPy was preferred as a sensing membrane because of its pH sensitivity. Since the thickness of PPy greatly affects the pH sensitivity, polymer deposition time was used to control the thickness and the optimum deposition time was found to be 4 min. Incubation at room temperature also improved and stabilized the response of the sensor. The EGFET-based pH microsensor had a good linear range (pH 5-12) and pH sensitivity (67 mV/pH). Using the same approach as the EGFET-based pH microsensor, EGFET-based urea microbiosensor was fabricated quickly and easily in a single step, where the enzyme urease was encapsulated in PPy during electrochemical deposition. Urea microbiosensor gave a response similar to the pH microsensor. The sensor exhibited a linear response in the urea concentration range of 10^{-9} M to 10^{-5} M with a sensitivity of 35.5 mV/decade. In the light of the obtained results, it can be said that EGFET-based pH and urea microsensors have great potential to be used for bioanalysis, especially in applications where local analysis is required.

Table 1. Comparison of the analytical performance of different modified electrodes for pH measurements

Electrode type	Sensitivity (mV/pH)	Linear range (pH)	Linearity	Reference
PPy/ EGFET microsensor	67	5 - 12	0.9623	This work
InZn _x O _y -based EGFET	56.29	2 - 12	0.999	(Singh et al., 2019)
Co(1,2-C ₂ B ₉ H ₁₁) ₂]-Doped Pyrrole/ ITO	50	1 - 14	0.85	(Pruna et al., 2018)
AlN-gate ISFET based pH sensor	~ 33	4 - 10	0.98145	(Sinha et al., 2019)
Graphene oxide-modified carbon electrodes/pH	51	2 - 10	0.9912	(Neupane et al., 2021)
MWCNT/PANI-modified SPCE	20.63	2 - 11	0.99089	(Bao et al., 2019)
HQS-doped PPy pH sensor	54.67	2 - 12	0.995	(Prissanaroon-Ouajai et al., 2009)

Table 2. Comparison of analytical performance of different enzymatic urea sensors measurements

Electrode type	Sensitivity	Linear range (mM)	LOD(μM)	Reference
EGFET microsensor/PPy/Urease	35.5 mV/decade urea, 10 μA/decade urea	10 ⁻⁶ – 10 ⁻²	–	This work
Polyaniline/Urease for ammonium ion selective	14.7 ± 0.9 mV/decade urea	10 ⁻² – 10 ²	-	(Mello & Mulato, 2020)
Polyaniline/Urease for hydroxyl ion selective	7.4 ± 0.5 mV/decade	10 ⁻² – 10 ²	-	(Mello & Mulato, 2020)
Chitosan/Nitrocellulose/GA/Urs	23 mV/decade	0.002–0.08	1	(Jakhar et al., 2018)
(Cu)-doped/PPy/Pt	4.5 μA/decade	0.001–10	–	(Yun et al., 2006)
Urease/Copolymer poly(<i>N</i> -3-aminopropyl pyrrole-co-pyrrole) / ITO	27.5 mV/decade	6.3 · 10 ⁻³ – 4.07 · 10 ⁻¹	–	(V Bisht et al., 2005)

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