creating a better future

Amin Fatoni <aminfatoni@unsoed.ac.id>

A manuscript number has been assigned to Glucose Biosensor based on Activated Carbon – NiFe2O4 Nanoparticles Composite modified Carbon Paste Electrode - [EMID:c74f311d3ad96014]

Results in Chemistry <em@editorialmanager.com> 13 May 2022 at 18:35
Reply-To: Results in Chemistry <support@elsevier.com>
To: Amin Fatoni <aminfatoni@unsoed.ac.id>

CC: "Wahyu Widanarto" aminfatoni@gmail.com, "Mekar Dwi Anggraeni" mekar.anggraeni@unsoed.ac.id, "Dian Windy Dwiasi" aminfatoni@hotmail.com

Dear Dr. Fatoni,

Your submission entitled "Glucose Biosensor based on Activated Carbon – NiFe2O4 Nanoparticles Composite modified Carbon Paste Electrode" has been been assigned the following manuscript number: RECHEM-D-22-00293.

You will be able to check on the progress of your paper by logging on to Editorial Manager as an author.
The URL is https://www.editorialmanager.com/rechem/.

Thank you for submitting your work to this journal.

Kind regards,

Reeshma Paraman
Administrative Support Agent – ASA
Results in Chemistry

#AU_RECHEM#

To ensure this email reaches the intended recipient, please do not delete the above code

creating a better future

Amin Fatoni <aminfatoni@unsoed.ac.id>

Your Submission - [EMID:e052c395e2f95c9b]

2 messages

Results in Chemistry <em@editorialmanager.com> 30 May 2022 at 12:36
Reply-To: Results in Chemistry <support@elsevier.com>
To: Amin Fatoni <aminfatoni@unsoed.ac.id>

Ref.: Ms. No. RECHEM-D-22-00293
Glucose Biosensor based on Activated Carbon – NiFe2O4 Nanoparticles Composite modified Carbon Paste Electrode
Results in Chemistry

Dear Dr. Fatoni,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript.

Your revision is due by Jun 20, 2022.

To submit a revision, go to https://www.editorialmanager.com/rechem/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Yi-Lun Ying, Ph.D.
Editor-in-Chief
Results in Chemistry

Comments from the Editors and Reviewers:

Reviewer #1: The authors presented a modified GOx-AC- NiFe2O4/CPE electrode for glucose biosensing. They investigated the loading ratio of carbon and NiFe2O4 nanoparticles as well as the concentration and pH of the buffer solution. After incorporating the glucose oxidase enzyme, the as fabricated glucose sensor showed similar performance in the detection of glucose in blood samples as the standard methods. The comments are shown below.

Major points to address:
1. As our journal specifically asks for new knowledge presented in the article, I didn't see the description of what new contribution the authors made to the community. As far as I know, there have been reports on the use of activated carbon for the application in biosensor. What's the difference?

2. The authors claimed that the presence of NiFe2O4 nanoparticles improve the electron transfer rate. However, the authors didn't study the NiFe2O4 alone. What about the performance of NiFe2O4 without active carbon? The authors may want to add control experiments here.

Minor points to address:
1. In the study of buffer concentration, from the figure 6b, it seems that the buffer concentration from 0-200 mM does not have much impact on the oxidative peak current. The authors may want to double check if their conclusion remains valid.

2. Even though the authors compare the performance of their biosensor with the standard method and the results are quite similar. I was wondering what about other parameters like stability and cost when compared with well established methods.

Based on the comments above, therefore I recommend this manuscript to be published after major revisions.

Reviewer #2: In this work, the authors proposed a new glucose biosensor. Although the main idea of the work is visible, but manuscript is poorly written and prepared and the overall quality of the manuscript is low. The motivation and

novelty of the work are poorly indicated, the introduction and literature review are scarce, the experimental part inconsistent, and the results and discussion are presented implicitly, and their description is scarce and inconsistent. The authors need to do a lot of work on highlighting the motivation of the work and the novelty of the proposed results, as well as more clearly present the results of the manuscript. In present form, it cannot be recommended for publication. The following is a list of major issues and concerns, as well as minor issues, which must be eliminated before further consideration of the possibility of accepting the article.

The list of major issues and concerns:

* It is necessary to give the principle of electrochemical analysis for glucose content and indicate the gross reactions. it is also necessary to describe the device of such sensors, their composition, indicating the role of each component.
* It is necessary to give examples of existing sensors and indicate the motivation for this work - indicate why certain components of the device are used and what are its advantages and novelties over analogues.
* It is necessary to more accurately describe the description of cyclic voltammetry (equipment and technique). It is necessary to indicate the concentration of KCl in which the electrode is immersed, since this affects its potential. it is also necessary to indicate the geometric dimensions of both the working and counter electrodes (active surface area).
* On cyclic voltammograms, it is traditionally customary to give not the absolute value of the current, but the current density normalized to the area of the electrode. The potential is given not just in volts, but in volts relative to a given reference electrode.
* It seems like the maximum current range of the potentiostat used in this work is 1 mA. Presenting cyclic voltammograms with current surpassing device range (Figure 4) is unacceptable. It is necessary either to measure them again in a different range, or to remove a part of the curve that goes beyond the instrument sensitivity range. Another option is to use electrodes with a smaller active area in further works.
* In figure 5, it is impossible to distinguish individual curves, and there is no legend on it. Also on the voltammogram, you can indicate the area at which the current was selected.
* Probably, when analyzing the obtained cyclic voltammograms, it makes sense to provide the current not at a specific potential, but at the peak of the redox reaction. If the registration conditions change, the reversibility of the redox-reaction may change, which will appears in the form of an increase in the potential difference between the peaks.
* In Figure 6, you can also show the current at the peak of the curve. It is also necessary to draw clearer conclusions on this graph about the conditions for the applicability of the sensor.
* The statement "The concentrations of the buffer solution indicated the number of ions involved in the electrochemical reaction" is unclear.
* The authors state that oxidation peaks are more sensitive to glucose concentration. But there is no clear reduction peak on the cyclic voltammogram. Therefore, it is obvious that the current at positive potentials will be more sensitive than at negative potentials.
* The cyclic voltammogram in Figure 7 is not very informative. It is desirable to increase it and mark the areas of applicability and inapplicability of the analysis. Graphs d,c and d don't much informative and they could be replaced by one graph with two y-axes (for positive and negative currents at different potentials).
* It is necessary to specify the conditions of applicability of the sensor and provide the requirements for sensors for real-life applications.
* The conclusions are sparse. It is necessary to clearly describe the advantages of the described approaches, the measurement accuracy and the conditions for the applicability of the sensor, including in comparison with analogues.

The list of minor issues and comments:

* The sequence of references in the manuscript is incorrect.
* Abbreviations are used without description.
* Some points of the experimental part are redundant due to the description of the motivation of the experiments. For example, in paragraphs 2.10 and 2.11, it would be enough to indicate in the paragraph about cyclic voltammetry to write that the curves were measured for different values of pH and at different buffer concentrations.
* The temperature for burning coconut shells is incorrect.
* If the results of the glucose concentration analysis are compared with a standard method, then a reference to this method or standard should be provided.

#AU_RECHEM#

To ensure this email reaches the intended recipient, please do not delete the above code

---

Amin Fatoni <aminfatoni@unsoed.ac.id> 30 May 2022 at 13:51
To: Results in Chemistry <support@elsevier.com>

Dear Editor of Results in Chemistry,

Yi-Lun Ying, Ph.D.

Thank you for your email. I will revise and submit to the system before the deadline.

Best regards,
Dr. Amin Fatoni
[Quoted text hidden]

creating a better future

Amin Fatoni <aminfatoni@unsoed.ac.id>

Track the status of your submission to Results in Chemistry

1 message

Track your Elsevier submission <no-reply@submissions.elsevier.com> 2 July 2022 at 15:32
To: aminfatoni@unsoed.ac.id

Manuscript Number: RECHEM-D-22-00293
Manuscript Title: Glucose Biosensor based on Activated Carbon – NiFe2O4 Nanoparticles Composite modified Carbon Paste Electrode
Journal: Results in Chemistry

Dear Amin Fatoni,

Your submitted manuscript is currently under review. The peer review process can take a while, so we are trying out a new service that allows you to track the peer review status of your submission in more detail. You can access the service here:
https://track.authorhub.elsevier.com?uuid=5e60ce88-32b7-42aa-8191-abb3b4842073

This page will remain active until the peer review process for your submission is completed. You can visit the page whenever you like to check the progress of your submission. The page does not require a login, so you can also share the link with your co-authors.

We hope you find this service useful.

Kind regards,
Journal Office of Results in Chemistry
Elsevier B.V.

Please note this is an automated message and will only be sent once.
For more information and support, please visit https://service.elsevier.com/app/answers/detail/a_id/5971/supporthub/publishing/

creating a better future

Amin Fatoni <aminfatoni@unsoed.ac.id>

Submission Confirmation for RECHEM-D-22-00293R2 - [EMID:7bbee46f9b11b0d6]

1 message

Results in Chemistry <em@editorialmanager.com> 30 June 2022 at 20:08
Reply-To: Results in Chemistry <support@elsevier.com>
To: Amin Fatoni <aminfatoni@unsoed.ac.id>

Ref.: Ms. No. RECHEM-D-22-00293R2
Glucose Biosensor based on Activated Carbon – NiFe2O4 Nanoparticles Composite modified Carbon Paste Electrode

Dear Dr. Fatoni,

Results in Chemistry has received your revised submission.

You may check the status of your manuscript by logging onto Editorial Manager at (https://www.editorialmanager.com/rechem/).

Kind regards,

#AU_RECHEM#

To ensure this email reaches the intended recipient, please do not delete the above code

creating a better future

Amin Fatoni <aminfatoni@unsoed.ac.id>

Your Submission - [EMID:b7d83e0d21d3512c]

2 messages

Results in Chemistry <em@editorialmanager.com> 4 July 2022 at 16:00
Reply-To: Results in Chemistry <support@elsevier.com>
To: Amin Fatoni <aminfatoni@unsoed.ac.id>

Ref.: Ms. No. RECHEM-D-22-00293R2
Glucose Biosensor based on Activated Carbon – NiFe2O4 Nanoparticles Composite modified Carbon Paste Electrode
Results in Chemistry

Dear Dr. Fatoni,

I am pleased to tell you that your work has now been accepted for publication in Results in Chemistry.

It was accepted on Jul 04, 2022

Comments from the Editor and Reviewers can be found below.

Thank you for submitting your work to this journal.

With kind regards

Yi-Lun Ying, Ph.D.
Editor-in-Chief
Results in Chemistry

Comments from the Editors and Reviewers:

Reviewer #1: The manuscript has been improved a lot.

Reviewer #2: All of my comments to the Authors have been taken into account. Now this manuscript can be accepted for publication.

One comment to the authors on reference list issue (that I mentioned in the previous revision): even though the Authors used automatic reference manager, the order of references appearance is somehow failed; for example, in the second sentence of the Introduction - "According to WHO, approximately 43% of the 3.7 million deaths caused by diabetes mellitus occur before the age of 70 years, with the percentage of these deaths being higher in developing countries [28] ." - reference number should be [1], not the [28]. I recommend to authors to fix this inconsistency before the article production. Probably, if you erase all the references mentions, reinsert it and refresh the reference list, the problem will be fixed.

#AU_RECHEM#

To ensure this email reaches the intended recipient, please do not delete the above code

---

Amin Fatoni <aminfatoni@unsoed.ac.id> 4 July 2022 at 21:17
To: Results in Chemistry <support@elsevier.com>

Dear Yi-Lun Ying, Ph.D.
Editor-in-Chief
Results in Chemistry

Thank you for the good news.
I am sorry about the inline citation error, since the trouble while editing the manuscript using Mendeley changed to Zotero in different laptop. However, now I have fixed the problem by changing the style to name-year and change back to elsevier vancouver. The revised manuscript attached.

Best regards<
Amin Fatoni
[Quoted text hidden]

---

manuscript - revised - Rev2 - Clean.docx
972K

creating a better future

Amin Fatoni <aminfatoni@unsoed.ac.id>

Proofs of [RECHEM_100433]

1 message

Elsevier Ltd, Editorial-Production Department <Corrections.esch@straive.com> 6 July 2022 at 23:39
To: aminfatoni@unsoed.ac.id

PLEASE DO NOT ALTER THE SUBJECT LINE OF THIS E-MAIL ON REPLY

Dear Dr. Amin Fatoni,

Thank you for publishing with Results in Chemistry. We are pleased to inform you that the proof for your upcoming publication is ready for review via the link below. You will find instructions on the start page on how to make corrections directly on-screen or through PDF.

https://elsevier.proofcentral.com/en-us/landing-page.html?token=d949e028a75641decf0e73db7cb3a3

Please open this hyperlink using one of the following browser versions:
. FIREFOX 61.0+
. CHROME 68.0+
. SAFARI 11.0+
. Microsoft Edge 79.0+

We ask you to check that you are satisfied with the accuracy of the copy-editing, and with the completeness and correctness of the text, tables and figures. To assist you with this, copy-editing changes have been highlighted.

You can save and return to your article at any time during the correction process. Once you make corrections and hit the SUBMIT button you can no longer make further corrections.

Please review the proof and submit any corrections within 48 hours to help us publish your article as quickly and accurately as possible.

We very much look forward to your response.

Yours sincerely,

Elsevier

E-mail: Corrections.esch@straive.com

For further assistance, please visit our customer support site at https://service.elsevier.com. Here you can search for solutions on a range of topics. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.

Thank you for the valuable comment and suggestions. The response of reviewers comments are as follows:

Reviewer #1: The authors presented a modified GOx-AC- NiFe2O4/CPE electrode for glucose biosensing. They investigated the loading ratio of carbon and NiFe2O4 nanoparticles as well as the concentration and pH of the buffer solution. After incorporating the glucose oxidase enzyme, the as fabricated glucose sensor showed similar performance in the detection of glucose in blood samples as the standard methods. The comments are shown below.

Major points to address:
1. As our journal specifically asks for new knowledge presented in the article, I didn't see the description of what new contribution the authors made to the community. As far as I know, there have been reports on the use of activated carbon for the application in biosensor. What's the difference?
The activated carbon used in this research was the local source of activated carbon from coconut shell with special treatment of customized milling process, which showed the change of amorphous to crystalline with fullerene-C70 peak and various advantages reported in the published article (Widanarto, et.al 2022). The specification of the activated carbon used and their advantages has been add in the discussion session.

2. The authors claimed that the presence of NiFe2O4 nanoparticles improve the electron transfer rate. However, the authors didn't study the NiFe2O4 alone. What about the performance of NiFe2O4 without active carbon? The authors may want to add control experiments here.

My research objective was use the cheap activated carbon as biosensor electrode. However, the electron transfer rate was not good enough, therefore, NiFe2O4 nanoparticles has been added for improving electron transfer rate. The research result showed the carbon active electron transfer was better with addition of the nanoparticles, however, we assume that the use of nanoparticles should be minimized at maximum of 10%, the reduce the cost of the future application. The results was also been added the carbon active without the nanoparticle addition, and we can see, the nanoparticle addition was improve the oxidation peak of the fabricated carbon paste electrode.

Minor points to address:
1. In the study of buffer concentration, from the figure 6b, it seems that the buffer concentration from 0-200 mM does not have much impact on the oxidative peak current. The authors may want to double check if their conclusion remains valid.
The buffer concentration from 25 to 100 mM showed slightly increase the oxidation current, however the higher buffer concentration did not much impact to the oxidative peak current, therefore, I select the smallest concentration of buffer used with the highest oxidation peak of 100 mM buffer. The selection of low buffer concentration was also due the following reason (1) to eliminate the possible interference of buffer capacity in electroanalysis and (2) to reduce the use of relatively expensive buffer as electrolyte. The information was also been added in the discussion session.
2. Even though the authors compare the performance of their biosensor with the standard method and the results are quite similar. I was wondering what about other parameters like stability and

cost when compared with well-established methods.
The main advantages of this research was the use of local activated carbon which huge production and low economic value. So knowing the prospect of the application of activated carbon for biosensor application could improve the value of activated carbon. In another side, the fabrication of sensor in the future using low cost of activated carbon would resulting a low cost biosensor. The biosensor fabrication could be increasing with the introducing of nanoparticle, and in this condition, we uses as low as possible of nanoparticle added but still have improvement of the original activated carbon alone as carbon paste electrode.
Based on the comments above, therefore I recommend this manuscript to be published after major revisions.

Reviewer #2: In this work, the authors proposed a new glucose biosensor. Although the main idea of the work is visible, but manuscript is poorly written and prepared and the overall quality of the manuscript is low. The motivation and novelty of the work are poorly indicated, the introduction and literature review are scarce, the experimental part inconsistent, and the results and discussion are presented implicitly, and their description is scarce and inconsistent. The authors need to do a lot of work on highlighting the motivation of the work and the novelty of the proposed results, as well as more clearly present the results of the manuscript. In present form, it cannot be recommended for publication. The following is a list of major issues and concerns, as well as minor issues, which must be eliminated before further consideration of the possibility of accepting the article.

The list of major issues and concerns:
* It is necessary to give the principle of electrochemical analysis for glucose content and indicate the gross reactions. it is also necessary to describe the device of such sensors, their composition, indicating the role of each component.
The principle of glucose determination using electrochemical has been added to the discussion session
* It is necessary to give examples of existing sensors and indicate the motivation for this work - indicate why certain components of the device are used and what are its advantages and novelties over analogues.
The novelty of the fabricated carbon paste electrode was the use of local coconut shell activated carbon with special treatment which showed several advantages. The information has been added in the discussion.
* It is necessary to more accurately describe the description of cyclic voltammetry (equipment and technique). It is necessary to indicate the concentration of KCl in which the electrode is immersed, since this affects its potential. it is also necessary to indicate the geometric dimensions of both the working and counter electrodes (active surface area).
The detail information has been added to the method session.
* On cyclic voltammograms, it is traditionally customary to give not the absolute value of the current, but the current density normalized to the area of the electrode. The potential is given not just in volts, but in volts relative to a given reference electrode.
The potential of the cyclic voltammograms has been revised relative to the reference electrode of Ag/AgCl.
* It seems like the maximum current range of the potentiostat used in this work is 1 mA.

Presenting cyclic voltammograms with current surpassing device range (Figure 4) is unacceptable. It is necessary either to measure them again in a different range, or to remove a part of the curve that goes beyond the instrument sensitivity range. Another option is to use electrodes with a smaller active area in further works.
The measurement has been repeated with the smaller concentration analyte, and the new Figure 4 has been updated.

* In figure 5, it is impossible to distinguish individual curves, and there is no legend on it. Also on the voltammogram, you can indicate the area at which the current was selected.
Figure 5 has been revised according to the reviewer suggestion.

* Probably, when analyzing the obtained cyclic voltammograms, it makes sense to provide the current not at a specific potential, but at the peak of the redox reaction. If the registration conditions change, the reversibility of the redox-reaction may change, which will appears in the form of an increase in the potential difference between the peaks.
Some peak showed the change of redox peak with the increasing analyte concentration. However, we select one point of applied potential for easy application in the future, when the use of amperometric detection, where the applied potential should fix in one value for easy application and instrumentation setting for portable device.

* In Figure 6, you can also show the current at the peak of the curve. It is also necessary to draw clearer conclusions on this graph about the conditions for the applicability of the sensor.
Additional information has been added for the conclusion selection of buffer concentration in the discussion session according to the result presented in Figure 6.

* The statement "The concentrations of the buffer solution indicated the number of ions involved in the electrochemical reaction" is unclear.
The hydrogen peroxide determination using electrochemical technique has been reported depends on the phosphate buffer concentration, using mechanism of phosphate mediated binding site of phosphate free precursor site. The discussion about the effect of buffer concentration has been revised.
* The authors state that oxidation peaks are more sensitive to glucose concentration. But there is no clear reduction peak on the cyclic voltammogram. Therefore, it is obvious that the current at positive potentials will be more sensitive than at negative potentials.
There is a reduction peak in CV, but it was not sharp enough. The desired oxidation and reduction area has been marked in the Figure 7.
* The cyclic voltammogram in Figure 7 is not very informative. It is desirable to increase it and mark the areas of applicability and inapplicability of the analysis. Graphs d,c and d don't much informative and they could be replaced by one graph with two y-axes (for positive and negative currents at different potentials).
Figure 7 has been revised according to the suggestion.
* It is necessary to specify the conditions of applicability of the sensor and provide the requirements for sensors for real-life applications.
The information has been added in the discussion session.

* The conclusions are sparse. It is necessary to clearly describe the advantages of the

described approaches, the measurement accuracy and the conditions for the applicability of the sensor, including in comparison with analogues.
The advantages of the research has been added to the conclusion.

The list of minor issues and comments:
* The sequence of references in the manuscript is incorrect.

The sequence of references have been revised, using Mendeley reference manager software

* Abbreviations are used without description.
Description of the abbreviations have been added.

* Some points of the experimental part are redundant due to the description of the motivation of the experiments. For example, in paragraphs 2.10 and 2.11, it would be enough to indicate in the paragraph about cyclic voltammetry to write that the curves were measured for different values of pH and at different buffer concentrations.
The method in 2.10 and 2.11 has been revised according to the reviewer suggestion

* The temperature for burning coconut shells is incorrect.
The temperature for coconut shells burning has been corrected to 300 ^{0}C

* If the results of the glucose concentration analysis are compared with a standard method, then a reference to this method or standard should be provided.
The reference of the standard method has been added to the discussion session

Int. J. Electrochem. Sci., 17 **(2022)** Article Number: xx, doi: 10.20964/

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Glucose Biosensor Development using Carbon Paste Electrode Made from Activated Carbon – $NiFe_2O_4$ Nanoparticles Composite

Amin Fatoni[1,], Wahyu Widanarto[2], Mekar Dwi Anggraeni[3], Dian Windy Dwiasi[1]*

[1] Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Jenderal Soedirman, Purwokerto, Indonesia
[2] Department of Physics, Faculty of Mathematics and Natural Sciences, Universitas Jenderal Soedirman, Purwokerto, Indonesia
[3] Department of Nursing, Faculty of Health Sciences, Universitas Jenderal Soedirman, Purwokerto, Indonesia
[*]E-mail: aminfatoni@unsoed.ac.id

Received: 1 xxx 2021 / *Accepted:* 1 xxx 2021 / *Published:* 1 xxx 2021

Carbon-based materials continue to pique the interest of many scientists due to their desirable characteristics such as large surface area, high electrical conductivity, and stability. This study aimed to describe the use of local coconut shell-based activated carbon (AC) to produce carbon paste electrodes used in the development of glucose biosensor. Subsequently, the performance of the carbon paste electrode was enhanced by using $NiFe_2O_4$ nanoparticles (NiFe-nps) to improve the electron transfer and redox potential behavior. The results showed that the best carbon paste electrode contains an activated carbon-paraffin oil ratio of 2:0.75 b/b, with 8% of NiFe-nps added to the activated carbon. The detection of hydrogen peroxide using an AC-NiFe2O4/CPE electrode showed an oxidation peak at 0.35 V and reduction peak at -0.5 V, with the optimum operational condition using 100mM phosphate buffer and optimum pH of 7.5. The glucose oxidase enzyme (GOx) was immobilized on the AC-NiFe2O4/CPE electrode for glucose determination, and the modified GOx-AC-NiFe2O4/CPE showed a linear response to detect glucose in both the oxidation (0.12V) and reduction (-0.4V) peaks. This analysis was conducted using cyclic voltammetry under optimal conditions. The fabricated glucose biosensor did not reveal any significant difference in detecting glucose in blood samples when compared to the standard method used in the hospital.

Keywords: activated carbon, carbon paste electrode, coconut shell, glucose biosensor, metallized carbon

1. INTRODUCTION

Diabetes mellitus has recently emerged as the most studied and lethal degenerative disease. According to WHO, approximately 43% of the 3.7 million deaths caused by diabetes mellitus occur before the age of 70 years, with the percentage of these deaths being higher in developing countries [1] . According to the *International Diabetes Federation* (IDF), the global prevalence of diabetes mellitus was 424.9 million people in 2017 and is expected to reach 628.6 million in 2045 [2]. Furthermore, diabetes mellitus was one of the top four comorbid diseases among Covid-19 patients in Indonesia, in 2020. The percentages of COVID-19 patients who were infected with the disease and those who died from it were 34.5% and 11.6%, respectively [3].

Early detection of blood glucose is one of the most essential measure for reducing diabetes mellitus, and the detection is best achieved using biosensors [4]. The biosensor is an analytical method that combines the biologically active compound with a transducer to convert biological interactions into readable signals [5]. Glucose biosensors mostly utilize the enzyme of glucose oxidase as biological sensing and coupling with electrochemical detection. Biosensors are still worth developing because they offer several advantages such as being easy to prepare in a small portable system, being relatively inexpensive, having high sensitivity and selectivity and allowing *real-time* measurements [6].

Biosensors are typically developed to increase their sensitivity, selectivity, stability and effectiveness, as well as reduce their production costs. The performance improvement strategies could be done in the biological sensing elements (enzymes, antibodies, cells), supporting materials for sensing immobilization or developing new detectors. Alternatively, several new materials, such as carbon nanoparticles [7], gold nanoparticles [8] and nickel nanoparticles [9] have been claimed to improve the performance of biosensors that use electrochemical detection,.

Carbon-based modified materials have recently received attention due to their numerous benefits such as large specific surface area, good electrical conductivity, and stability. Furthermore, some of the carbon materials that have been explored included nanotubes [7], graphene [10], mesoporous [11], carbon black [12] and activated carbon [13]. Preliminary study shows that the activated carbon gotten from local coconut shells has a high porosity, surface area, and high electronic conductivity, making it promise to be applied in various technological innovation.

This study examined the design of a glucose biosensor using activated carbon as a base material, which is derived from coconut shells. The aim was to use the activated carbon to produce a carbon paste incorporated with $NiFe_2O_4$ nanoparticles. There have also been a few reports of the use of activated carbon from local coconut shells, specifically for biosensor applications.

2. EXPERIMENTAL

2.1. Materials

Activated carbon was prepared from local coconut shell, glucose oxidase from *Aspergillus niger* (type II, >= 15000 U/g solid, Sigma) $NiCl_2 .6H_2 O$ (Merck, Germany), $FeCl_3.6H_2O$ (Merck, Germany), glutaraldehyde 25% (Sigma), albumin from bovine serum (BSA) (Sigma), glucose anhydrous (≥ 98.0%)

(Sigma), hydrogen peroxide 30% (Merck, Germany), sodium hydroxide (Merck, Germany) , disodium hydrogen phosphate (Merck, Germany) and sodium dihydrogen phosphate (Merck, Germany).

2.2. Apparatus and measurements

The morphology of carbon paste electrodes was examined using Scanning Electron Microscopy (SEM) (JSM-6510 LA, JEOL, Japan), operating at 15 kV. A three-electrode system was used for electrochemical analysis, along with an Ag/AgCl reference electrode, a carbon paste electrode as a working electrode, and a stainless-steel rod as a counter electrode. Furthermore, a potentiostat was used to conduct the electrochemical measurements (Rodeostat, IORodeo Smart Lab Technology, US).

2.3. Coconut shell activated carbon preparation

The carbonization process was used to create activated carbon (AC), which involved burning coconut shells at 80°C for an hour in an oxygen-depleted environment to remove the organic elements found in the shells. Consequently, this loss of organic molecules led to the formation of carbon pores and the physical activation of the carbon from charred coconut shells. The severance of carbon chains from organic molecules was enabled by intense heat and water vapor, which was used to remove impurities and impure hydrocarbons from the activated carbon during the physical activation process. The resulting carbon was heated to a temperature of 800-900°C after the water vapor streaming. Usually, carbon monoxide, carbon dioxide, and hydrogen are produced when water vapor reacts with carbon. Lastly, the activated carbon was pulverized using a mill shaker for 100 minutes to obtain micrometer-sized particles.

2.4. $NiFe_2O_4$ nanoparticles (NiFe-nps) preparation

The coprecipitation method [14] was used to synthesize NiFe-nps with $NiCl_2.6H_2O$ and $FeCl_3.6H_2O$ as ion provider Ni^{2+} and Fe^{3+}. The mole fraction ratio used was 1:2, by dissolving 1.188 grams of $NiCl_2.6H_2O$ in 20 mL distilled water and 2,701 grams of $FeCl_3.6H_2O$ in 20 mL of distilled water in a separate glass beaker. The two solutions were then homogeneously mixed, and NaOH precipitation agent was then dropped into the mixture of Ni and Fe while stirring (1000 rpm) at 85 ^{0}C for 60 minutes. The resulting nanoparticles were then precipitated continued by washing distilled water for approximately 7 times of 50 ml. The retrieved precipitated nanoparticles were dried at 90 ^{0}C and the brown nanoparticles powder was then used for further procedures.

2.5. Carbon paste electrode manufacture

The carbon paste electrodes were made by mixing AC and paraffin oil 5:4 w/w ratio [15]. Subsequently, the mixture was homogenized with a mortar and pestle for 30 minutes, and the resulting composite was placed in a 5mm inner diameter polylactic acid (PLA) tube. The PLA tube was

then added with a 5mm graphite rod as a connector, and the lower side of the electrode was polished with HVS paper until a flat and shiny surface was observed, which indicated that the electrode (AC/CPE) was ready for use.

2.6. Electrode testing on hydrogen peroxide

This test was conducted to determine the sensitivity of the carbon paste electrode to detect hydrogen peroxide without any modification. The electrochemical method used was cyclic voltammetry to easily observe the oxidation and reduction peaks of hydrogen peroxide. The hydrogen peroxide solution was prepared in 50 mM phosphate buffer with pH of 7.0 and concentrations of 2,4,6,8 and 10 mM.

2.7. Carbon paste electrode composite optimization

The composition of the carbon paste electrode was optimized by adding NiFe-nps to improve the electrical conductivity. This optimization was carried out in the same way as the manufacture of electrode paste, with the addition of nanoparticles at various concentrations of 2, 4, 8, and 10% w/w. The modified electrodes (AC-NiFe2O4/CPE) along with various nanoparticle compositions were then tested with hydrogen peroxide and compared to carbon paste without NiFe-nps.

2.8. Modified electrode detection test on hydrogen peroxide

This test was conducted to determine the sensitivity of the modified electrode tested to hydrogen peroxide. Consequently, the Cyclic Voltammetry method was employed, and the test solution was hydrogen peroxide in 50 mM phosphate buffer pH 7. The hydrogen peroxide concentration used was 0-10 mM.

2.9. Optimization of the cyclic voltammetry method

Cyclic voltammetry optimization was conducted to determine the optimal condition of the electrochemical cell with a scan rate. Determination of the optimal condition of the electrolyte solution was carried out with the concentration of 5mM H_2O_2 solution in phosphate buffer pH of 7. The scan rate used was 0.05 to 0.2 V/s to see the optimum oxidation-reduction potential current. Then measurements were made using the CV method with the potential used between 1 to -1 Volt, every 3 repetitions.

2.10. pH optimization

This test was conducted to determine the optimal conditions for electrochemical cells with varying buffer pH. This test is carried out to determine whether the modified electrode performs best in

the slightly acidic, neutral or slightly alkaline environments. This optimization used a pH range of 6, 6.5, 7, 7.5, and 8 with the optimized CV from previous procedures.

2.11. Optimization of buffer concentration

This test was conducted to determine the optimal condition of the electrochemical cell using a buffer solution. The buffer used was phosphate buffer pH of 7, with concentrations of 50, 100, 150, 200 and 250 mM, and measurements were made with the best conditions from the previous steps using CV method.

2.12. Testing of modified electrodes with GOx enzyme at various glucose concentrations

This test was conducted to determine the sensitivity of the modified electrode containing the glucose oxidase (GOx) enzyme after an electrochemical test on a glucose standard solution. The GOx was immobilization on the AC-NiFe2O4/CPE was performed according to the previous study [16]. In brief, 25 µl glucose oxidase enzyme (5 U/µl) was mixed with 7.5 µL (5 mg/250µL) of bovine serum albumin (BSA) in phosphate buffer and 10 µL of 2.5% glutaraldehyde. The mixture was then dropped onto the surface of the activated carbon electrode and allowed to dry at room temperature. The excess of glucose oxidase enzyme was rinsed with phosphate buffer and the resulting GOx-AC-NiFe2O4/CPE was kept at the refrigerator at 4 ^{0}C for later use. Glucose solution with concentrations ranging from 1 to 10 mM was tested. The linearity, limit of detection and limit of quantification were further calculated from the responses of the glucose standard solution.

2.13. Glucose determination in blood samples

Blood plasma as real samples were collected from the Wijayakusuma hospital, Purwokerto, Indonesia. Before blood analysis, a standard curve of glucose solution was prepared. The blood plasma samples were then dissolved five times in 50 mM phosphate buffer pH 7.0 to reduce the possibility of matrix effect. The plasma samples were then analyzed using the optimal GOx-AC-NiFe2O4/CPE condition, and the oxidation peak of 0.12 V was used to determine the glucose concentration. The results of glucose determination of plasma samples using the modified electrode were statistically compared to the results of the hospital's standard spectrophotometric method using Wilcoxon Signed-Rank test.

3. RESULTS AND DISCUSSION

3.1. $NiFe_2O_4$ nanoparticles preparation

The co-precipitation method was used in this study to synthesize NiFe-nps, with $FeCl_2$, $FeCl_3$ as precursors and NaOH as precipitation agent [17]. The $NiFe_2O_4$ nanoparticles should be formed in the alkaline condition [18], therefore, NaOH was added dropwise until the pH reached 11. The resulting $NiFe_2O_4$ nanoparticles were brown powder.

3.2. Carbon paste electrode preparation

The carbon paste electrode (AC/CPE) was created by mixing AC (**Fig. 1a**) with paraffin oil. The AC was molded into a paste using paraffin oil as an adhesive. The greater amount of paraffin oil used, the softer the carbon paste and the lower the electrical conductivity. However, the lower of paraffin oil ratio, the greater the electrical conductivity produced, but the more difficult it would be obtaining the paste. Therefore, the formation of carbon paste formation requires accurate proportion to get the best carbon paste. The ratio of AC and paraffin oil used were 2:1.5, 2:1, and 2: 0.75. The best condition was a ratio of 2:0.75 (b/b), whereas the lower ratio was too dry and difficult to make the paste. The carbon paste electrode was prepared using an insulator with a diameter of 10 mm, with a hole of 5 mm for the electrode, and the thickness of the carbon paste of 2 mm. The connector used is graphite with a diameter of 5 mm (**Fig. 1b**).

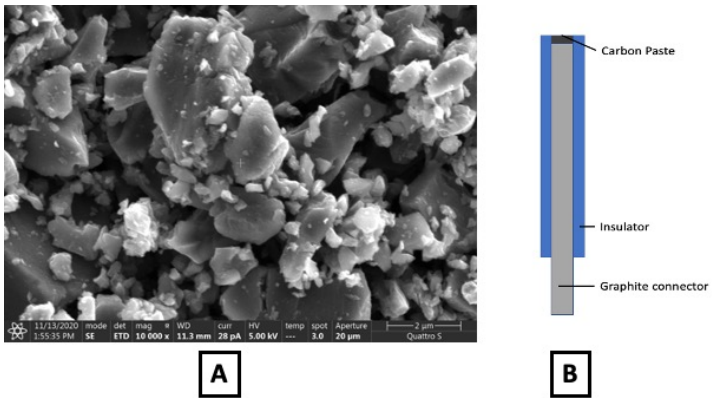

Figure 1. The morphology of activated carbon from coconut shell (AC) was observed using a scanning electron microscope (a). The design of carbon paste electrode for glucose biosensor using an insulator from polylactic acid (PLA) printed using a 3D printer and a graphite rod as a connector (b).

3.3. Modification of AC-NiFe2O4/CPE

The nanoparticles used in this study were added to the carbon paste to increase its conductivity. The nanoparticles added were NiFe-nps at concentration of 2,4,6,8 and 10% w/w. The results showed that NiFe-nps of 2 to 8% had a significant increase in conductivity significantly, while higher concentration did not.

The morphology of the carbon paste and carbon paste electrodes containing NiFe-nps was observed using an electron microscope. There was not much difference between carbon paste and carbon paste with NiFe-nps. This could be due to the small proportion of addition of nanoparticles, only about 10% and because both, activated carbon and NiFe-nps, were conductive materials with varying sizes (**Fig. 2**).

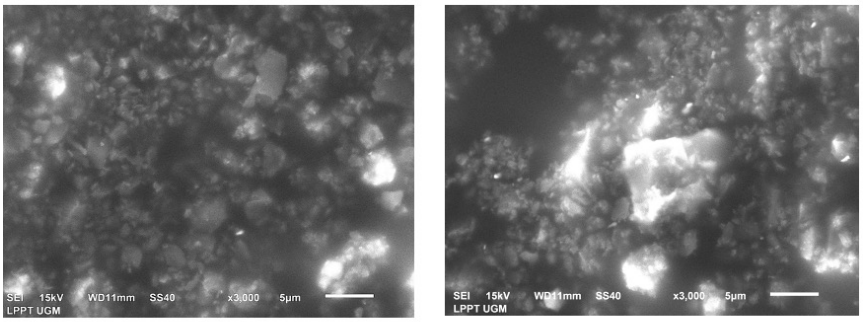

Figure 2. Scanning electron microscope image of carbon paste electrode (A) and Carbon paste with NiFe-nps modified electrode (B).

The modified AC/CPE electrode with and without NiFe-nps were determine the electrochemical properties using hydrogen peroxide. The AC-NiFe2O4/CPE electrode showed a higher oxidation peak with a higher background current compared to the activated carbon paste electrode without NiFe-nps (**Fig. 3**). Therefore, the nickel ferrite nanoparticles were improved the electron transfer rate of the modified electrode.

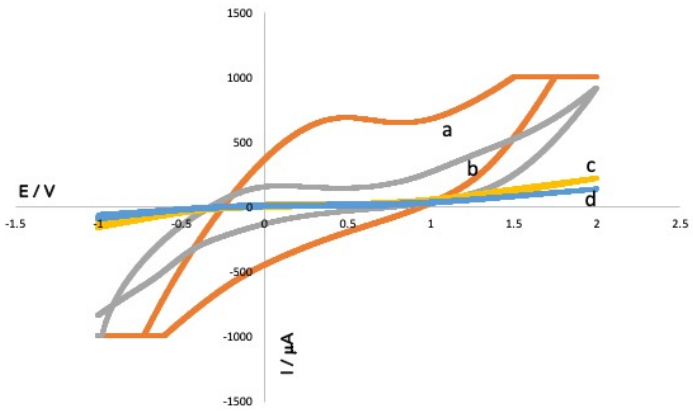

Figure 3. AC/CPE modified electrode with NiFe-nps for hydrogen peroxide determination (a) and without the addition of NiFe-nps (b). The baseline without hydrogen peroxide was shown in c and d voltammogram respectively.

3.4. Modified electrode for hydrogen peroxide determination

The carbon paste electrodes were tested for performance in various comparisons using hydrogen peroxide at a concentration of 1 mM in a 5 mM phosphate buffer solvent with a pH of 7.0.

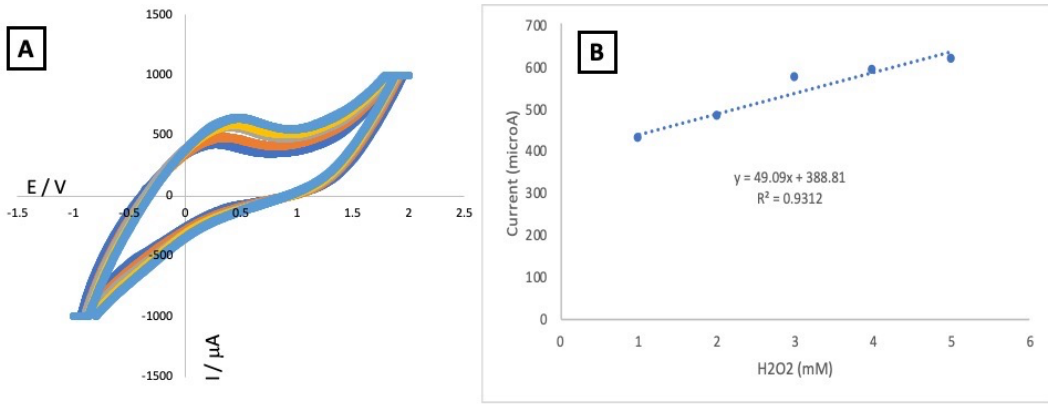

Figure 4. Cyclic voltammogram of carbon paste electrodes in various concentrations of hydrogen peroxide (1-5 mM) in phosphate buffer (A). The linearity of increasing oxidation peak with the hydrogen peroxide concentration at 0.35V (B)

The test was carried out using a cyclic voltammetry method with a potential range of -1 to 2 V, scan rate of 0.1 V/s and three-times scanning. The carbon paste electrode showed an increasing oxidation peak with increasing of hydrogen peroxide concentration (**Fig. 4**). The oxidation peak was observed at about 0.35 V and the reduction peak decreased by about -0.5V.

3.5. Buffer optimization

The buffer pH and concentration were optimized to achieve the best conditions for the determination of hydrogen peroxide. The pH of the solution affects the behavior of electroactive compounds on the oxidation and reduction reactions in electrochemical systems. In this study, a variation of pH 6 to 8. The 6 to 8 range has been selected for further application of the modified electrode using glucose oxidase enzyme. Buffer pH of highly acidic or alkaline could destroy the enzyme structure, thus resulting in a low response of glucose oxidase. The results showed that pH 7.5 had the highest change in oxidation peak change compared to other pH values, while measuring the oxidation of hydrogen peroxide at a concentration of 5 mM (**Fig. 5a**). The concentrations of the buffer solution indicated the number of ions involved in the electrochemical reaction. In this study, 25 to 200 mM was used. The results further showed that an increase in buffer concentration from 25 to 100 mM indicating an increase in the difference in oxidation peak currents of hydrogen peroxide. However, the higher buffer concentrations did not result in an increase in the oxidation peak (**Fig. 5b**).

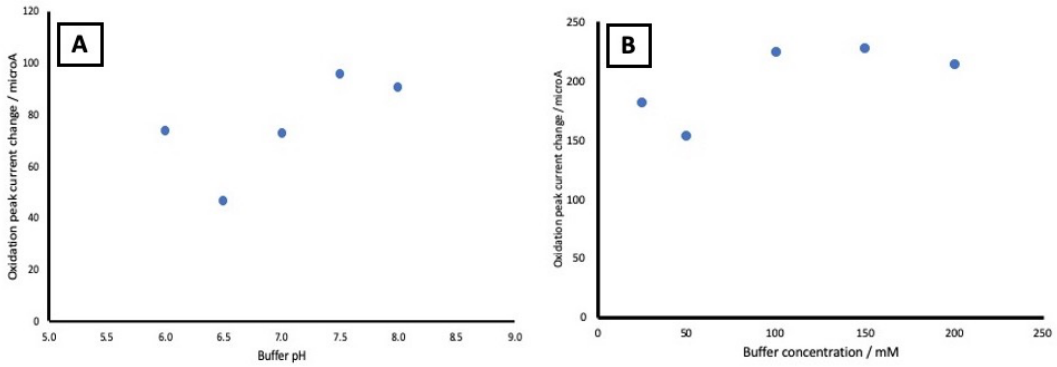

Figure 5. Effect of buffer pH (a) and buffer concentration (b) in the oxidation peak change of 5 mM hydrogen peroxide.

3.6. Glucose measurement

Glucose oxidase (GOx) has been immobilized on the AC-NiFe2O4/CPE electrode using glutaraldehyde as a crosslinker and bovine serum albumin as a stabilizing agent. The use of albumin and glutaraldehyde has been previously reported to offer excellent maintenance the glucose oxidase immobilization in the glucose biosensor [7]. The glucose standard solution was measured using cyclic voltammetry under previously obtained optimal conditions.

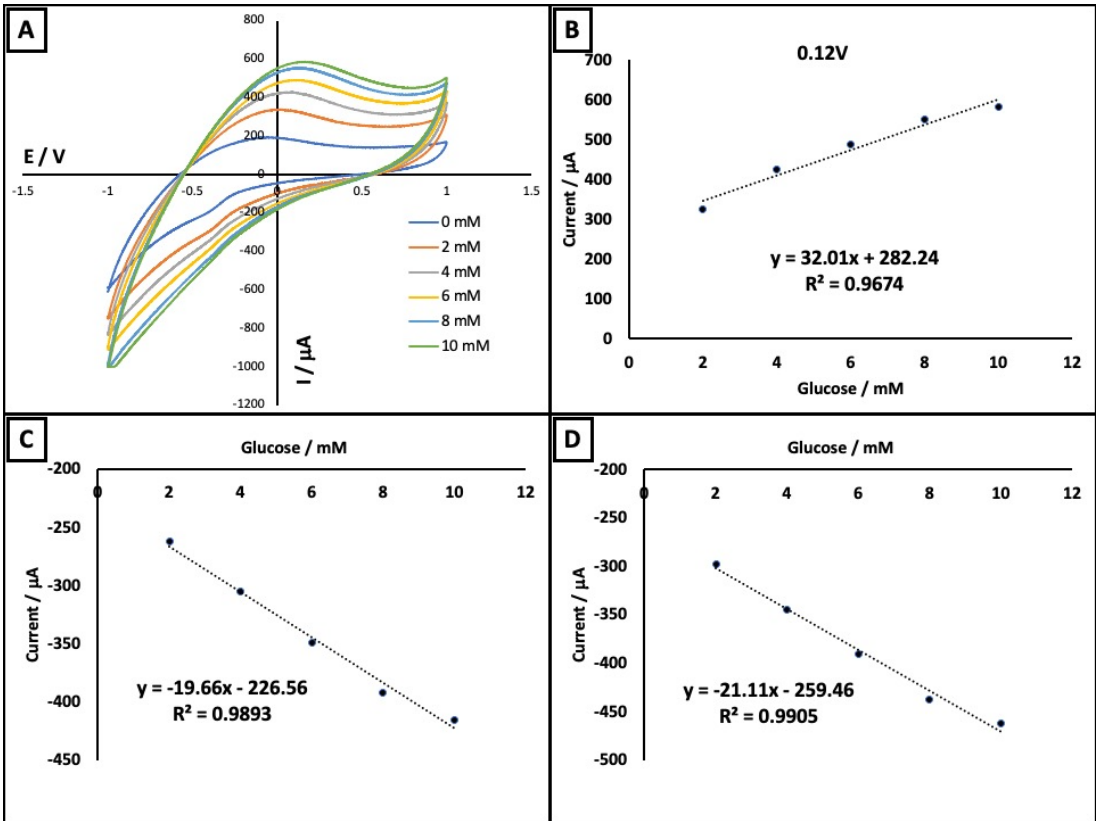

Figure 6. Glucose determination using GOx- AC-NiFe2O4/CPE electrode showed increasing oxidation and reduction peaks by cyclic voltammetry (A). The increasing peaks with the glucose concentration showed at 0.12V for oxidation (B), -0.35V and -0.4V for reduction potential.

Fig. 6A shows the cyclic voltammogram of the modified electrode used to detect standard glucose solution ranging from 2 to 10mM. Both oxidation and reduction peaks increase with the addition of glucose concentration. Furthermore, the oxidation peak at 0.12V had the highest sensitivity with a slope of 32.01x (**Fig. 6B**) compared to reduction peaks at -0.35V and -0.4V (**Fig. 6C-D**). However, reduction peaks are preferable in glucose biosensors development to avoid the common interference that appear at the oxidation potential, such as 4-acetaminophen at 0.6V [19], ascorbic acid at 0.4V and uric acid at 0.5V against Ag/AgCl reference electrode [20].

The reduction peaks were then analyzed at -0.35V and 0.4V to get the best equation needed to determine glucose. The reduction potential of -0.35V was chosen for potential a lower reduction potential close to zero volts to avoid the possible electroactive interferences. The presence of $NiFe_2O_4$ nanoparticles in the carbon paste electrode improves the electrocatalytic performance of the modified working electrode. The nanoparticles and activated carbon of the carbon paste electrode provided a large number of molecules for electron transfer of hydrogen peroxide detection with a redox potential close to zero, which is similar to the previously reports on metallized carbon [21] to avoid the unwanted electrochemical reactions of common interfering substances. Additionally, the reduction potential at -0.4V was also analyzed because the CV voltammogram showed that the larger peak increased with an increase in the reduction potential. Thereafter, the reduction peaks changes at -0.4V with increasing glucose concentration showed better sensitivity and coefficient of determinant with the regression equation of $y = -21.11x – 259.46$ and coefficient of determination of $R^2 = 0.9905$. The calculated limit of detection and limit of quantification were 1.1 mM and 3.7 mM respectively. Table 1 compares the fabricated glucose biosensor to previously reported carbon paste electrode / activated carbon-based amperometry glucose biosensor. Most of modified electrode with activated carbon or carbon paste electrodes had relatively high applied potentials of about 0.4-0.55V, while this study had a low applied potential of 0.12 V or negative applied potential of -0.35 to -0.4 V. The low applied potential in the amperometric glucose biosensor has a great advantage of avoiding the common electroactive interferences such as uric acid and ascorbic acid.

Table 1. Comparison of activated carbon / carbon paste electrode for amperometry glucose biosensor

Electrode structure	Linear range	Applied potential	Reference
GOx-PtNPs-PAA-aSPCEs	20 µM – 2.3 mM	0.2 V	[22]
GOx/MCPE	0.5 µM – 10 µM	0.4 V	[23]
AuNi@AC	0.41 µM – 1.7 mM	0.55 V	[24]
Ni-Pd@AC/GCE NCs	0.01 mM–1 mM	0.5 V	[25]
NiO-HAC/GCE	10 µM–3.3 mM	0.55 V	[26]
FMPS-Gly	0.5 mM – 10 mM	0.5 V	[16]
GOx-AC-NiFe2O4/CPE	2-10 mM	0.12 V, -0.35V and -0.4V	This work

GOx, glucose oxidase; PtNPs, platinum nanoparticles; PAA, poly azure a; MCPE, modified carbon paste electrode; AuNi, Au-Ni alloy; AC, activated carbon; Ni, nickel; Pd, palladium; GCE, glassy carbon electrode; NCs, nanocomposites; HAC, heteroatom-enriched activated carbon; FMPS, 4-formyl-3-methoxyphenoxymethyl)polystyrene; gly, glycine; Gr, graphite.

3.6. Glucose measurement in blood samples

The fabricated glucose biosensor using GOx-AC-NiFe2O4/CPE electrode was validated for glucose determination in blood samples. Subsequently, six blood plasma samples were collected from local hospital laboratory. The results showed the comparison of the glucose concentration of blood plasma samples obtained using fabricated glucose biosensor with the standard (spectrophotometric) method performed in the hospital laboratory (Table 2). The Wilcoxon Signed-Rank test also revealed that there was no significant difference between the fabricated glucose biosensor and the standard method ($P > 0.05$).

Table 2. Blood glucose concentration obtained by fabricated biosensor and standard spectrophotometric method performed in the hospital laboratory.

Sample	Fabricated biosensor (mg/dL) 3 replications	Standard spectrophotometric method (mg/dL)
1	137.5 ± 2.5	139
2	114.4 ± 0.7	114
3	97.5 ± 1.6	97
4	129.4 ± 2.5	128
5	287.1 ± 2.6	288
6	131.4 ± 1.6	131

4. CONCLUSION

The carbon paste electrode for the electrochemical glucose biosensor was made of composite AC and NiFe-nps. The optimal condition obtained were the AC – paraffin oil ratio of 2:0.75 and the addition of NiFe-nps of 8% w/w. The buffer phosphate used was optimum at a pH of 7.5 and a concentration of 100 mM. The modified electrode also detected was a success to detect standard glucose with a linear response at 2 mM to 10 mM. Furthermore, the fabricated glucose biosensor also produced similar responses in the detection of glucose in blood sample when compared to the standard method used in the hospital.

ACKNOWLEDGMENT
We would like to thank the Universitas Jenderal Soedirman, Indonesia Research Grant of "Riset Unggulan Perguruan Tinggi" Contract Number: T/592/UN23.18/PT.01.03/2021. We also would like to thank the Wijayakusuma Hospital in Purwokerto, Indonesia, for facilitating the validation method of glucose determination in blood samples.

References

[1] W. WHO, *Curr. Med. Res. Opin.*, (2016).

[2] International Diabetes Federation, (2017).

[3] K. Kemkes, *Kementrian Kesehatan Republik Indonesia*, 2020. https://www.kemkes.go.id/article/print/20101400002/13-2-persen-pasien-covid-19-yang-meninggal-memiliki-penyakit-hipertensi.html (accessed Sep. 26, 2021).

[4] E. H. Yoo and S. Y. Lee, *Sensors*, (2010), doi: 10.3390/s100504558.

[5] A. Koyun, E. Ahlatcolu, and Y. Koca, in *A Roadmap of Biomedical Engineers and Milestones*, (2012). doi: 10.5772/48824.

[6] D. Grieshaber, R. MacKenzie, J. Vörös, and E. Reimhult, *Sensors*, 8, (2008), 1400, doi: 10.3390/s8031400.

[7] A. Fatoni, A. Numnuam, P. Kanatharana, W. Limbut, C. Thammakhet, and P. Thavarungkul, *Sensors Actuators, B Chem.*, 185, (2013), doi: 10.1016/j.snb.2013.05.056.

[8] Y. Li, H. J. Schluesener, and S. Xu, *Gold Bull.*, (2010), doi: 10.1007/BF03214964.

[9] M. Tyagi, M. Tomar, and V. Gupta, *Biosens. Bioelectron.*, (2013), doi: 10.1016/j.bios.2012.07.062.

[10] A. Fatoni *et al.*, *Analyst*, 00, (2014), 1, doi: 10.1039/c4an01000k.

[11] K. Wang *et al.*, *Electrochim. Acta*, (2009), doi: 10.1016/j.electacta.2009.02.097.

[12] M. Cammarota *et al.*, *Electrochim. Acta*, (2013), doi: 10.1016/j.electacta.2013.07.129.

[13] J. H. Kim, S. Cho, T. S. Bae, and Y. S. Lee, *Sensors Actuators, B Chem.*, (2014), doi: 10.1016/j.snb.2014.02.054.

[14] T. Vigneswari and P. Raji, *J. Mol. Struct.*, 1127, (2017), 515.

[15] Y. Zhang, F. Li, X. Liu, J. Lu, and G. Zhang, *Electrochim. Acta*, (2017), doi: 10.1016/j.electacta.2017.05.020.

[16] S. Donmez, F. Arslan, N. Sarı, E. Hasanoğlu Özkan, and H. Arslan, *Biotechnol. Appl. Biochem.*, 64, (2017), 745.

[17] K. Maaz, S. Karim, A. Mumtaz, S. K. Hasanain, J. Liu, and J. L. Duan, *J. Magn. Magn. Mater.*, 321, (2009), 1838.

[18] S. H. Lafta, *Open Chem.*, 15, (2017), 53.

[19] A. Amani, S. Khazalpour, and D. Nematollahi, *J. Electrochem. Soc.*, 160, (2012), H33.

[20] A. Fatoni, A. Numnuam, P. Kanatharana, W. Limbut, and P. Thavarungkul, *Analyst*, 139, (2014), doi: 10.1039/c4an01000k.

[21] W.-Z. Jia, K. Wang, and X.-H. Xia, *TrAC Trends Anal. Chem.*, 29, (2010), 306.

[22] F. Jiménez-Fiérrez, M. I. González-Sánchez, R. Jiménez-Pérez, J. Iniesta, and E. Valero, *Sensors*, 20, (2020), 4489.

[23] O. Colak, H. Arslan, H. Zengin, and G. Zengin, *Int. J. Electrochem. Sci.*, 7, (2012), 6988.

[24] K. Arikan, H. Burhan, E. Sahin, and F. Sen, *Chemosphere*, 291, (2022), 132718, doi: https://doi.org/10.1016/j.chemosphere.2021.132718.

[25] Y. Koskun, A. Şavk, B. Şen, and F. Şen, *Anal. Chim. Acta*, 1010, (2018), 37, doi: https://doi.org/10.1016/j.aca.2018.01.035.

[26] Y. Ni, J. Xu, Q. Liang, and S. Shao, *Sensors Actuators B Chem.*, 250, (2017), 491, doi: https://doi.org/10.1016/j.snb.2017.05.004.