

Submission ID #: 68896

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Title: NiO Nanoflowers for Non-Enzymatic Amperometric Detection of Glucose

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

✓ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **11/10/2025**

When you are ready to submit your video files, please contact our Content Manager, [Utkarsh Khare](#).

Current Protocol Length

Number of Steps: 25

Number of Shots: 55 (14 SC)

Introduction

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

- 1.1. **Piyush Choudhary:** The objective of this research is to demonstrate the effective processes involved in non-enzymatic electrochemical glucose detection.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

~~What are the most recent developments in your field of research?~~

- 1.2. **Piyush Choudhary:** Transition Metal-Oxide nanoparticles have been an upcoming trend in modern glucose sensors thus giving rise to an era of non-enzymatic glucose sensors.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.3.1*

CONCLUSION:

~~What advantage does your protocol offer compared to other techniques?~~

- 1.3. **Piyush Choudhary:** Almost all current glucometers in the market rely on enzymes for the detection of glucose. The NiO nanoflower catalyst is among the best materials for replacing enzymes in glucose sensing.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.2.1*

~~How will your findings advance research in your field?~~

- 1.4. **Piyush Choudhary:** This video article paves the way for better understanding of procedures specially for beginners and researchers starting afresh in the field of biosensors.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 6.2.1*

Protocol

2. NiO Nanostructured Flower Synthesis

Demonstrator: Piyush Choudhary

2.1. To begin, prepare a 6 millimolar solution of nickel chloride dihydrate in 10 milliliters of ethanol [1].

2.1.1. **WIDE:** Talent adding nickel chloride dihydrate to a clean beaker.

2.2. In a separate beaker, prepare 10 milliliters of a 3 percent polyvinylpyrrolidone or PVP solution in deionized water [1]. Ultrasonicate the solution for 5 minutes at 40 kilohertz and 50 watts [2], then mechanically stir it for an additional 5 minutes [3-TXT].

2.2.1. Talent adding deionized water to a beaker containing PVP.

2.2.2. Talent placing the beaker in the ultrasonic bath and setting the frequency to 40 kilohertz and power to 50 watts.

2.2.3. Talent mechanically stirring the solution for 5 minutes using a magnetic stirrer.
TXT: Repeat the ultrasonication and stirring cycle 2x

2.3. Once the PVP is completely dissolved, use a pipette to add 10 milliliters of the PVP solution dropwise into 10 milliliters of the nickel precursor solution under vigorous stirring [1]. Continue stirring the mixture for 30 minutes after the PVP addition [2].

2.3.1. Talent using a pipette to add the PVP solution dropwise into the nickel precursor solution while stirring continuously.

2.3.2. Talent placing the solution on a magnetic stirrer.

2.4. Next, add 10 milliliters of an 18 millimolar urea solution, prepared in a 1 to 1 mixture of deionized water and ethanol, dropwise to the stirred mixture [1]. Ensure that the total volume of the resulting solution is 30 milliliters [2].

2.4.1. Talent pipetting the urea solution dropwise into the beaker containing the nickel–PVP mixture.

2.4.2. Close-up shot showing the total solution volume reaching 30 milliliters mark on the beaker.

- 2.5. Stir the complete solution for 10 minutes [1]. Then, transfer it into a polytetrafluoroethylene vessel and seal the vessel inside a stainless steel autoclave [2].
 - 2.5.1. Talent increasing the speed of the magnetic stirrer.
 - 2.5.2. Talent carefully pouring the mixed solution into a polytetrafluoroethylene vessel and sealing it securely within the stainless steel autoclave.
- 2.6. Place the sealed autoclave in an electric oven set at 160 degrees Celsius and maintain it for 6 to 8 hours [1]. After completion, allow the autoclave to cool naturally to room temperature [2].
 - 2.6.1. Talent placing the sealed autoclave into an electric oven and setting the temperature to 160 degrees Celsius.
 - 2.6.2. Shot of turning off the oven.
- 2.7. Now, centrifuge the resultant product at 26.88 *g* to collect the green-colored, gelatinous nickel hydroxide precipitate [1]. Decant the clear supernatant solvent [2] and wash the precipitate with ethanol or deionized water at least three times [3]. Dry the washed precipitate at 70 degrees Celsius for 10 hours in an electric oven [4].
 - 2.7.1. Talent loading the centrifuge tubes into the centrifuge.
 - 2.7.2. Talent decanting the clear solvent from the centrifuged mixture.
 - 2.7.3. Talent adding the green precipitate with ethanol or deionized water.
 - 2.7.4. Talent placing the washed precipitate in an electric oven set at 70 degrees Celsius for 10 hours.
- 2.8. Then, place the dried powder in a box furnace set at 450 degrees Celsius for 4 hours to anneal [1] and obtain the pure-phase nickel oxide nanoflakes, which appear grey in color [2].
 - 2.8.1. Talent placing the dried powder sample into a box furnace.
 - 2.8.2. Close-up Shot of the grey nanoflakes.

3. Electrode Fabrication

- 3.1. Polish the glassy carbon electrode with 0.05 micrometer alumina nanoparticle slurry on a clean electrode polishing pad by moving it repeatedly in the shape of the numeral eight [1]. Rinse the electrode immediately with deionized water [2] and ultrasonicate it using a bath sonicator in isopropyl alcohol and deionized water for 2 minutes each at

40 kilohertz and 50 watts [3].

3.1.1. Talent rubbing the glassy carbon electrode with 0.05 micrometer alumina nanoparticle slurry on a polishing pad in a figure-eight motion.

3.1.2. Talent adding deionized water to the polished electrode.

3.1.3. Talent placing the electrode in a bath sonicator containing isopropyl alcohol and deionized water.

3.2. Next, add a known mass of nickel oxide nanoflake powder, ranging from 3 to 15 milligrams, to 3 milliliters of deionized water to prepare a nanoparticle suspension [1]. Adjust the amount of powder so that the nanoparticle concentration ranges between 1 and 5 milligrams per milliliter [2].

3.2.1. Talent weighing nickel oxide nanoflake powder in a beaker.

3.2.2. Talent adding water to the beaker with nanoflake powder.

3.3. Place the mixture in a bath ultrasonicator for 10 to 20 minutes to achieve a uniform dispersion [1-TXT].

3.3.1. Talent placing the beaker containing the nanoparticle suspension into a bath sonicator. **TXT: Ensure that no precipitates or settled particles remain**

3.4. Now, cast 10 microliters of the aliquot by drop-casting it onto the cleaned surface of the glassy carbon electrodes [1]. Label the electrodes as NiO (*N-I-O*) NF 1 to NiO NF 5 and allow the modified electrodes to dry at room temperature overnight for 10 to 12 hours [2].

3.4.1. Talent pipetting and drop-casting 10 microliters of nanoparticle suspension onto the surface of the cleaned electrodes.

3.4.2. ~~Talent labeling the electrodes sequentially as NiO NF 1 through NiO NF 5.~~ **NOTE: Not filmed, VO merged with the next shot**

3.4.3. Talent placing the coated electrodes on a clean bench surface.

3.5. Use a diluted binder solution containing 0.5 weight percent in ethanol to compactly bind the nanoparticles onto the surface of the electrodes [1]. Carefully drop-cast 6 microliters of the binder solution onto each modified electrode [2] and let them dry in air at room temperature for 30 minutes [3]. Keep the prepared electrodes in a desiccator until further use [4].

3.5.1. Talent mixing the diluted binder solution in ethanol.

- 3.5.2. Talent drop-casting 6 microliters of the binder solution onto each electrode.
- 3.5.3. Talent placing the electrodes on work bench.
- 3.5.4. Talent placing the dried electrodes carefully inside a desiccator and closing the lid securely.

4. Electrochemical Detection Process

4.1. Take 20 milliliters of 0.1 molar sodium hydroxide solution in the electrochemical cell to prepare the electroanalytical solution [1]. Use a three-electrode setup to analyze the glucose concentration [2]. Insert the silver or silver chloride electrode with 3 molar potassium chloride as the reference electrode and the platinum wire as the counter electrode [3].

- 4.1.1. Talent pouring 20 milliliters of 0.1 molar sodium hydroxide solution into the electrochemical cell.
- 4.1.2. Talent assembling the three-electrode system on the electrochemical workstation.
- 4.1.3. Close-up shot showing the silver/silver chloride reference electrode and platinum wire counter electrode properly positioned in the electrochemical cell.

4.2. Activate the nickel oxide nanoflake-modified glassy carbon electrode by stabilizing it with 15 cycles of cyclic voltammetry [1-TXT]. Set the potential window between 0 and 0.8 volts at a scan rate of 200 millivolts per second [2]. Observe that the nickel(III)/nickel(II) (*nickel 3 to 2*) reduction peak potential typically appears around 0.4 to 0.6 volts [3].

- 4.2.1. Talent placing the NiO NF@GCE electrode into the electrochemical cell. **TXT: NiO NF@GCE: Nickel Oxide Nanoflake-Modified Glassy Carbon Electrode**
- 4.2.2. SCREEN: Screen-4.2.2.mp4 00:08-00:20.
- 4.2.3. SCREEN: Screen-4.2.3.mp4

4.3. After stabilization, run chronoamperometry for real-time glucose detection using the glassy carbon electrode [1]. Firstly, establish the baseline by running chronoamperometry at the nickel reduction potential or glucose oxidation potential for 100 seconds [2]. Once the base current is established, pause the chronoamperometric experiment by selecting the **Pause** button in the software [3].

- 4.3.1. SCREEN: Screen-4.3.1.mp4

4.3.2. SCREEN: Screen-4.3.2.mp4 00:01-00:05 AND-00:20-00:24.

4.3.3. SCREEN: Screen-4.3.3.mp4 00:08-00:15.

4.4. Next, prepare a 2 molar glucose stock solution for analysis [1].

4.4.1. Talent mixing the glucose stock solution by inverting the tube.

4.5. Lift the electrode stand and add 10 microliters of the glucose stock solution to the electroanalytical solution to achieve a final glucose concentration of 1 millimolar [1-TXT]. Stir the solution for 30 seconds to achieve uniform mixing [2].

4.5.1. Talent lifting the electrode stand and pipetting 10 microliters of glucose stock into the electrochemical cell. **TXT: Ensure negligible total volume change**

4.5.2. Talent stirring the cell solution gently.

4.6. Place the electrodes back into their previous positions and resume the chronoamperometric measurement after 5 seconds by pressing the **Resume** button in the software [1]. Choose a 30-second analysis window for the chronoamperometric detection of different glucose concentrations [2].

4.6.1. SCREEN: Screen-4.6.1.mp4 .

4.6.2. SCREEN: Screen-4.6.2.mp4 *Video editor: Please speed up.*

4.7. Repeat the process of glucose addition and measurement to gradually increase the glucose concentration up to 15 millimolar and record the corresponding chronoamperometric responses [1].

4.7.1. Talent adding glucose aliquots to the cell.

5. Optimization of Electrochemical Process Parameters

5.1. Using a low loading concentration of the nanomaterial, optimize the potential window where the Faradaic reaction occurs [1-TXT]. Perform an initial broad scan from negative 1 volt to positive 1 volt using cyclic voltammetry [2].

5.1.1. SCREEN: Screen-5.1.1.mp4. **TXT: Use ~1mg/mL nanomaterial**

5.1.2. SCREEN: Screen-5.1.2.mp4.

5.2. Identify a smaller potential range where the Faradaic reaction occurs by locating the reduction and oxidation peaks in the cyclic voltammogram [1-TXT]. Collect the cyclic voltammograms for the electrochemical process within the selected potential range [2].

5.2.1. SCREEN: Screen-5.2.1.mp4. **TXT: Use 0.1 - 0.8 V window for NiO NF@GCE based electrodes in 0.1 M NaOH**

5.2.2. SCREEN: Screen-5.2.2.mp4.

5.3. Now, add a small amount of glucose, typically 1 millimolar, to the electrochemical solution to generate distinct redox peaks [1]. Observe that the glucose addition enhances the peak corresponding to glucose oxidation, helping to resolve multiple peaks in the voltammogram [2].

5.3.1. Talent pipetting 10 microliters of glucose solution into the electrochemical cell.

5.3.2. SCREEN: Screen-5.3.2.mp4.

5.4. Identify the anodic peak potential corresponding to glucose oxidation, which changes with glucose concentration [1]. Measure chronoamperometric responses slightly above this potential for accurate detection [2].

5.4.1. SCREEN: Screen-4.2.3.mp4. 00:07-00:09 **NOTE: Authors asked to reuse this clip**

5.4.2. SCREEN: Screen-5.4.2.mp4.

5.5. Finally, once the potential window and glucose oxidation potential are determined, optimize the loading concentration by varying the nanomaterial suspension concentration drop-cast onto the electrode [1].

5.5.1. Talent drop-casting nanomaterial onto the electrode.

Results

6. Results

6.1. The X-ray diffraction pattern of the nickel oxide nanoflowers showed distinct peaks corresponding to the 111, 200, 220, 311, and 222 planes of nickel oxide, confirming phase-pure formation [1].

6.1.1. LAB MEDIA: Figure 1A. *Video editor: Highlight the 5 peaks.*

6.2. The Raman spectrum of nickel oxide nanoflowers displayed characteristic peaks at 512, 692, 1073, and 1460 inverse centimeters, confirming the nickel oxide phase [1].

6.2.1. LAB MEDIA: Figure 1B. *Video editor: Highlight the four labelled peaks at 512, 692, 1073, and 1460 on the Raman spectrum.*

6.3. The low-magnification micrograph showed uniformly distributed flower-shaped nickel oxide particles [1] and the high-magnification micrograph revealed marigold flower-like spherical particles with an average diameter of approximately 0.91 micrometer [1].

6.3.1. LAB MEDIA: Figure 1C.

6.3.2. LAB MEDIA: Figure 1D.

6.4. The cyclic voltammograms for different loading concentrations of nickel oxide nanoflowers showed increasing peak currents up to 4 milligrams per milliliter, after which they saturated [1].

6.4.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the curve labelled 5 mg/mL and the curve labelled 4 mg/mL.*

6.5. Chronoamperometric responses obtained for different nanomaterial loadings demonstrated that electrodes with 4 milligrams per milliliter loading showed the highest current response to glucose [1].

6.5.1. LAB MEDIA: Figure 2B. *Video editor: Emphasize the purple trace graph corresponding to 4 mg/mL .*

6.6. The current response plotted against glucose concentration confirmed a higher slope for the 4 milligram per milliliter nickel oxide nanoflower electrode, indicating greater

sensitivity [1].

6.6.1. LAB MEDIA: Figure 2C. *Video editor: Highlight the line corresponding to 4 mg/mL NiO NF*

6.7. The cyclic voltammogram of nickel oxide nanoflower-coated electrodes recorded with and without 2 millimolar glucose revealed an enhancement of the nickel redox peak upon glucose addition [1].

6.7.1. LAB MEDIA: Figure 2D. *Video editor: Highlight the line corresponding to “nafion NiO NF4 @GCE+ 2mM glucose”.*

6.8. The chronoamperometric selectivity response indicated excellent discrimination against ascorbic acid, sucrose, fructose, and sodium chloride at 0.1 millimolar concentration [1].

6.8.1. LAB MEDIA: Figure 4A. *Video editor: Sequentially highlight each labelled spike corresponding to AA, sucrose, fructose, and NaCl.*

6.9. The chronoamperometric curves recorded across multiple cycles showed reproducible current responses, confirming electrode repeatability [1].

6.9.1. LAB MEDIA: Figure 4C. *Video editor: Display the overlapping traces for cycle 1, cycle 5, cycle 10, and cycle 20 to show consistent current steps.*

1. Millimolar

Pronunciation link: <https://www.merriam-webster.com/dictionary/millimolar>

IPA: /ˌmɪləˈmoʊlər/

Phonetic Spelling: mil·uh·moh·ler

2. Nickel chloride dihydrate

Pronunciation link: <https://www.merriam-webster.com/dictionary/nickel>

IPA: /ˈnɪkəl/

Phonetic Spelling: nik·uhl

(compound term; element pronunciation used as reference)

3. Polyvinylpyrrolidone

Pronunciation link: <https://www.merriam-webster.com/dictionary/polyvinylpyrrolidone>

IPA: /ˌpɑːliˌvʌnəlpɪˈroʊlɪˌdoʊn/

Phonetic Spelling: pol·ee·vy·nuhl·pih·roh·luh·dohn

4. Ultrasonic

Pronunciation link: <https://www.merriam-webster.com/dictionary/ultrasonic>

IPA: /ˌʌltrəˈsɑːnɪˌkeɪt/

Phonetic Spelling: uhl·truh·saa·nih·kayt

5. Kilohertz
Pronunciation link: <https://www.merriam-webster.com/dictionary/kilohertz>
IPA: /'kɪləˌhɜːts/
Phonetic Spelling: kil·uh·hurts
6. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /'sentrəˌfjuːdʒ/
Phonetic Spelling: sen·truh·fyooj
7. Polytetrafluoroethylene
Pronunciation link: <https://www.merriam-webster.com/dictionary/polytetrafluoroethylene>
IPA: /ˌpɑːliˌtetrəˌflʊroʊˈeθɪˌliːn/
Phonetic Spelling: pol·ee·teh·truh·floor·oh·eth·uh·leen
8. Autoclave
Pronunciation link: <https://www.merriam-webster.com/dictionary/autoclave>
IPA: /'ɔːtəˌkleɪv/
Phonetic Spelling: aw·tuh·klayv
9. Anneal
Pronunciation link: <https://www.merriam-webster.com/dictionary/anneal>
IPA: /əˈniːl/
Phonetic Spelling: uh·neel
10. Nanoflakes
Pronunciation link: <https://www.merriam-webster.com/dictionary/nano->
IPA: /'nænəʊˌfleɪks/
Phonetic Spelling: nan·oh·flayks
11. Nanoflowers
Pronunciation link: <https://www.merriam-webster.com/dictionary/nano->
IPA: /'nænəʊˌflaʊərz/
Phonetic Spelling: nan·oh·flow·erz
12. Glassy carbon electrode
Pronunciation link: <https://www.merriam-webster.com/dictionary/electrode>
IPA: /ɪˈleɪkˌtroʊd/
Phonetic Spelling: ih·lek·trohd
13. Chronoamperometry
Pronunciation link: <https://www.merriam-webster.com/dictionary/chronoamperometry>
IPA: /ˌkraːnoʊˌæmpəˈrɑːmətri/
Phonetic Spelling: kron·oh·am·puh·rah·muh·tree
14. Cyclic voltammetry
Pronunciation link: <https://www.merriam-webster.com/dictionary/voltammetry>
IPA: /ˌvoʊlˈtæmɪtri/
Phonetic Spelling: vohlt·am·uh·tree
15. Faradaic
Pronunciation link: <https://www.merriam-webster.com/dictionary/faradaic>
IPA: /fəˈreɪdɪk/
Phonetic Spelling: fuh·ray·dik

16. Anodic

Pronunciation link: <https://www.merriam-webster.com/dictionary/anodic>

IPA: /əˈnɑːdɪk/

Phonetic Spelling: uh·nod·ik

17. Raman

Pronunciation link: <https://www.merriam-webster.com/dictionary/Raman>

IPA: /ˈrɑːmən/

Phonetic Spelling: rah·muhn

18. Diffraction

Pronunciation link: <https://www.merriam-webster.com/dictionary/diffraction>

IPA: /dɪˈfrækʃən/

Phonetic Spelling: dih·frak·shuhn

19. Alumina

Pronunciation link: <https://www.merriam-webster.com/dictionary/alumina>

IPA: /əˈluːmɪnə/

Phonetic Spelling: uh·loo·mih·nuh

20. Nafion

Pronunciation link: <https://www.merriam-webster.com/dictionary/Nafion>

IPA: /ˈnæfɪ.ən/

Phonetic Spelling: naf·ee·on