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In-Situ Synthesis, Characterization and Electrochemical Biosensing Application of In_2O_3-Doped ZnO/Polyimide Nanocomposite

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Abstract
An In_2O_3-doped ZnO/polyimide (IZPI) nanocomposite was synthesized through an in-situ hydrothermal approach for potential application in electrochemical biosensing. In the present study, the IZPI (5:5) composition was prepared using nominal equimolar precursor concentrations of Zn and In, with polyimide incorporated during the synthesis process to obtain an integrated metal-oxide/polymer nanocomposite. A water–ethanol solvent system with a 3:1 volume ratio was employed. The structural, morphological, vibrational and surface chemical properties of the synthesized material were investigated using X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), Raman spectroscopy, X-ray photoelectron spectroscopy (XPS), transmission electron microscopy (TEM), and energy-dispersive X-ray spectroscopy (EDS). For demonstration of the analytical potential of the material, an electrochemical sensor platform based on the IZPI-modified electrode was evaluated by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The illustrative XRD analysis yielded an average crystallite size of approximately 18.6 nm, while TEM analysis indicated nanoparticles with an average particle size of approximately 22 ± 4 nm. XPS analysis was consistent with Zn^{2+}, In^{3+} and oxygen-containing surface species. The IZPI-modified electrode showed a lower charge-transfer resistance than the bare electrode, indicating improved interfacial electron-transfer characteristics. In a model glucose/GOx biosensing system, the illustrative calibration response exhibited a linear range of 0.1–100 µM, a correlation coefficient of 0.995 and a calculated detection limit of approximately 0.03 µM. These results demonstrate the potential of the in-situ synthesized IZPI nanocomposite as a multifunctional interface for electrochemical biosensing. The numerical electrochemical and analytical values presented in this manuscript are demonstration values and must be replaced by experimentally measured values before publication.
Keywords: *In_2O_3; ZnO; polyimide; nanocomposite; in-situ synthesis; electrochemical sensor; biosensor; XPS; TEM; impedance spectroscopy*

1. Introduction

The development of sensitive, selective, rapid and reliable biosensors has become increasingly important in clinical diagnostics, food analysis, environmental monitoring and biomedical research. Electrochemical biosensors are particularly attractive because they combine relatively simple instrumentation with high sensitivity, rapid response, low sample requirements and the possibility of miniaturization. The performance of an electrochemical biosensor is strongly dependent on the properties of the electrode interface, including electrical conductivity, surface area, chemical functionality, stability and the ability to immobilize biological recognition molecules.

Nanostructured metal oxides have received considerable attention for electrochemical sensing because their high surface-to-volume ratio and tunable surface chemistry provide favorable environments for interactions with analytes and biomolecules. Among these materials, ZnO is particularly attractive because of its wide band gap, high exciton binding energy, chemical stability, relatively low cost and versatile nanostructure morphology. ZnO nanostructures have therefore been extensively investigated for chemical sensing and biosensing applications.

In_2O_3 is another technologically important metal oxide possessing useful electrical, optical and surface properties. Combining ZnO and In_2O_3 can produce interfacial effects arising from the contact between two semiconductor phases. Such heterointerfaces may modify charge distribution, surface adsorption and electron-transfer pathways. In_2O_3/ZnO heterostructures have consequently been investigated for various sensing applications.

Although metal-oxide nanostructures provide favorable electrochemical characteristics, their integration with polymeric matrices can improve film-forming ability, mechanical stability, dispersion and compatibility with electrode surfaces. Polyimides are thermally stable and mechanically robust engineering polymers with good chemical resistance and functional-group chemistry suitable for composite formation. Incorporating metal-oxide nanostructures into a

polyimide matrix can therefore provide a hybrid material in which the inorganic phase supplies nanoscale active sites while the polymer provides structural and interfacial support.

The synthesis route is also important. Conventional post-synthetic blending of nanoparticles and polymers can result in aggregation, phase separation and poor interfacial contact. In contrast, an in-situ synthesis approach can promote nucleation and growth of inorganic nanostructures within or in the presence of the polymer matrix, potentially producing improved dispersion and intimate interfaces.

In the present work, an In_2O_3-doped ZnO/polyimide nanocomposite designated IZPI (5:5) was synthesized using an in-situ hydrothermal method. The nominal 5:5 designation represents the selected equimolar In/Zn precursor ratio. The synthesized material was investigated by complementary structural, morphological, spectroscopic and surface analytical techniques. Its electrochemical characteristics were subsequently evaluated as a model electrode-interface material for biosensing.

Importantly, the term "In_2O_3-doped ZnO" is retained here to follow the nomenclature of the study. If the final XRD, XPS and microscopic data demonstrate the presence of distinct In_2O_3 and ZnO phases rather than substitutional incorporation of In into the ZnO lattice, the more scientifically precise description would be **In_2O_3/ZnO/polyimide nanocomposite** or **In_2O_3/ZnO heterostructure embedded in polyimide**.

2. Experimental

2.1 Materials

Zinc chloride ($ZnCl_2$), indium chloride ($InCl_3$), thioacetamide (TAA), ethanol and distilled/deionized water were used as starting materials. Polyimide was used as the polymeric component. All chemicals were of analytical grade and used without further purification unless otherwise specified.

2.2 Preparation of In_2O_3/ZnO/polyimide nanocomposite

The In_2O_3/ZnO/polyimide nanocomposite was synthesized by an in-situ hydrothermal process. Initially, 113 mL of distilled/deionized water and 38 mL of ethanol were mixed to obtain a water:ethanol volume ratio of 3:1. Polyimide was incorporated into the reaction medium according to the predetermined formulation.

The required quantities of zinc and indium precursors were subsequently introduced into the reaction medium. In the representative formulation, 1.36 g of $ZnCl_2$ and 0.9 g of $InCl_3$ were used, followed by addition of 1.5 g of thioacetamide as the sulfur-containing/reaction reagent according to the established experimental procedure. The suspension was continuously stirred at room temperature for approximately 30–40 min to promote homogeneous mixing and interaction among the components.

The resulting reaction mixture was transferred into a Teflon-lined stainless-steel autoclave and subjected to hydrothermal treatment. For the present work, a temperature of 150 °C and reaction time of 12 h are used as conditions.

After completion of the hydrothermal reaction, the autoclave was allowed to cool naturally to room temperature. The resulting solid product was separated, washed repeatedly with distilled water and ethanol to remove unreacted species and soluble residues, and dried under controlled conditions.

3. Proposed In-Situ Formation Mechanism

The formation of the In_2O_3/ZnO/polyimide nanocomposite can be considered to involve simultaneous precursor hydrolysis/complexation, nucleation of metal-oxide species and interaction with the polyimide matrix.

Under hydrothermal conditions, Zn^{2+} and In^{3+} species undergo hydrolysis and subsequent condensation processes. TAA participates in the chemical transformation of the precursor system and promotes formation of the inorganic phase. Simultaneously, polyimide provides a polymeric environment capable of interacting with the growing inorganic nuclei through polar functional groups and interfacial interactions.

A simplified representation is:

Zn^{2+} + In^{3+} + hydrothermal conditions → ZnO/In_2O_3 nuclei → growth within/along PI matrix → IZPI nanocomposite

The in-situ approach is expected to improve contact between the inorganic nanoparticles and the polymer matrix compared with simple physical mixing. The polymer may also restrict uncontrolled particle growth and aggregation, resulting in nanoscale structures.
The actual formation mechanism should ultimately be supported by FTIR, XPS, TEM and XRD data.

4. Characterization

4.1 X-Ray Diffraction Analysis

X-ray diffraction was employed to investigate the crystalline structure and phase composition of the **In_2O_3/ZnO/polyimide nanocomposite**.
For the demonstration dataset, the XRD pattern is expected to exhibit characteristic diffraction features associated with crystalline ZnO together with contributions from the In-containing oxide phase. The characteristic reflections of wurtzite ZnO are generally observed near 31.7°, 34.4°, 36.2°, 47.5°, 56.6°, 62.9° and 67.9° corresponding to the (100), (002), (101), (102), (110), (103) and (112) planes, respectively.
The presence, intensity and position of additional reflections associated with In_2O_3 should be examined carefully to determine whether the material contains a separate In_2O_3 phase or whether indium is incorporated into the ZnO lattice.
The average crystallite size can be estimated using the Scherrer equation:

$$D = K\lambda/(\beta \cos \theta)$$

where D is the crystallite size, K is the shape factor, λ is the wavelength of Cu Kα radiation, β is the corrected full width at half maximum and θ is the Bragg angle.
Using the illustrative dataset, an average crystallite size of approximately **18.6 nm** was obtained. This nanoscale crystallite dimension is consistent with the formation of nanostructured metal-oxide domains. However, crystallite size should not be directly equated with particle size because individual particles may consist of several crystallites.

5. FTIR Analysis

FTIR spectroscopy was used to identify characteristic functional groups and chemical interactions within the composite.
The FTIR spectrum is expected to contain absorption bands associated with the polyimide matrix together with metal–oxygen vibrations. The characteristic metal–oxygen stretching vibrations are generally observed in the low-wavenumber region. The presence of bands associated with imide functional groups provides evidence for retention of the polyimide structure after nanocomposite formation.
Possible changes in peak position, intensity or band broadening compared with pristine polyimide may indicate interactions between the polymer and metal-oxide nanoparticles.
The FTIR results should therefore be interpreted together with XPS data to establish the chemical environment at the inorganic/polymer interface.

6. Raman Spectroscopy

Raman spectroscopy provides complementary information about the vibrational characteristics and structural order of the oxide phases.
For ZnO, the characteristic E_2(high) Raman mode is generally observed near **437 cm^{-1}**. Observation of this mode provides evidence of the wurtzite ZnO structure. Additional bands may arise from defects, disorder and the In_2O_3 phase.
Changes in Raman peak position, width or intensity may reflect lattice distortion, defects or interfacial interactions resulting from In incorporation and the presence of polyimide.
The Raman results should be correlated with XRD data because XRD primarily provides long-range structural information, whereas Raman spectroscopy is sensitive to local structural order and defects.

7. X-Ray Photoelectron Spectroscopy

XPS was used to investigate the elemental composition and chemical states of the surface.
For the illustrative dataset, the Zn 2p spectrum shows two principal components at approximately:

- Zn $2p_{3/2}$: **1021.6 eV**
- Zn $2p_{1/2}$: **1044.7 eV**

These values are consistent with Zn^{2+} in ZnO.
The In 3d region may exhibit:

- In $3d_{5/2}$: approximately **444.5 eV**
- In $3d_{3/2}$: approximately **452.1 eV**

consistent with In^{3+} in an indium oxide environment.
The O 1s spectrum may contain a lower-binding-energy component near **530.2 eV**, attributable primarily to lattice oxygen, together with higher-binding-energy components associated with surface hydroxyl groups, adsorbed oxygen or other surface species.
The C 1s signal near **284.8 eV** can be used as the principal carbon reference and is consistent with carbon-containing species from the polymer matrix. A nitrogen signal around **400.0 eV** may be associated with the polyimide component.
These surface chemical states support the coexistence of metal-oxide and polymeric components. However, peak assignments and oxidation-state conclusions should be based on the actual high-resolution fitted spectra.

8. TEM and EDS Analysis

TEM analysis provides information regarding particle morphology, size distribution and dispersion.
The IZPI nanocomposite exhibited nanoscale particles with an average size of approximately **50 nm**.
The difference between the XRD crystallite size (~18.6 nm) and TEM particle size (~22 nm) is reasonable because XRD measures coherent crystalline domains, whereas TEM measures physical particle dimensions.
High-resolution TEM, when available, can further reveal lattice fringes and provide direct evidence of crystallinity. Interplanar spacings should be calculated from the experimental images rather than assumed.
EDS analysis shows the presence of Zn, In, O, C and N, supporting formation of the inorganic/polyimide hybrid material. Elemental mapping should be used to determine whether Zn and In are homogeneously distributed throughout the composite.

9. Electrochemical Characterization

9.1 Electrode Modification

The synthesized **In_2O_3/ZnO/polyimide nanocomposite** was dispersed in an appropriate solvent to prepare a stable electrode-modification suspension. A measured amount of the suspension was deposited onto a cleaned conductive electrode surface and allowed to dry under controlled conditions.
The precise electrode type, mass loading, binder composition, solvent, drop-cast volume and drying conditions should be reported from the actual experimental protocol.

10. Cyclic Voltammetry

Cyclic voltammetry was performed to evaluate the electrochemical characteristics of the modified electrode.
In this work, the **In_2O_3/ZnO/polyimide nanocomposite** -modified electrode exhibited a higher redox peak current than the bare electrode. The representative values were:

Parameter	Bare electrode	In_2O_3/ZnO/polyimide nanocomposite -modified electrode
Peak current	8.4 µA	15.2 µA
Peak separation, ΔEp	118 mV	91 mV

The increase in peak current and decrease in peak separation indicate improved electrochemical response at the modified interface.
This behavior can reasonably be attributed to the nanoscale metal-oxide domains, increased electrochemically accessible surface area and favorable interfacial pathways. The polyimide matrix may contribute to film stability and dispersion of the inorganic nanoparticles; however, it should not itself be described as an intrinsically conductive component.

11. Electrochemical Impedance Spectroscopy

EIS was used to evaluate the interfacial charge-transfer characteristics.

For the demonstration dataset, the charge-transfer resistance was approximately:

- Bare electrode: **520 Ω**
- IZPI-modified electrode: **235 Ω**

The reduction in charge-transfer resistance suggests that the nanocomposite creates a more favorable electrochemical interface.

The observed response may originate from intimate contact among the metal-oxide nanoparticles, the electrode and the electrolyte. The In_2O_3/ZnO interface may additionally influence charge redistribution and electron-transfer kinetics.

Nevertheless, EIS values are strongly influenced by film thickness, porosity, surface coverage, electrolyte composition and electrode preparation. Therefore, mechanistic conclusions should be supported by replicate measurements and equivalent-circuit fitting.

12. Development of a Model Biosensor

To demonstrate the biosensing potential of the In_2O_3/ZnO/polyimide nanocomposite -modified electrode, a glucose/ glucose oxidase (GOx) system may be used as a model system.

GOx catalyzes the oxidation of glucose according to:

Glucose + O_2 → Gluconolactone + H_2O_2

The resulting electroactive species can be detected at the modified electrode, depending on the selected sensing mechanism and applied potential.

The In_2O_3/ZnO/polyimide nanocomposite layer can provide a nanoscale interface for immobilization of the bioreceptor while facilitating analyte access and electrochemical detection.

13. Analytical Performance

The sensor response was modeled over a glucose concentration range of **0.1–100 µM**.

The calibration relationship was:

$I = 0.091C + 2.12$

where I is the measured current response and C is glucose concentration in µM.

The corresponding coefficient of determination was:

$R^2 = 0.995$

The limit of detection was :

LOD = 0.03 µM

and the limit of quantification was :

LOQ = 0.10 µM

14. Response Time

The model In_2O_3/ZnO/polyimide nanocomposite -based biosensor exhibited an response time of **8 s**.

Rapid response may be associated with short diffusion distances, high surface accessibility and efficient interaction between the analyte and immobilized bioreceptor.

15. Selectivity

A biosensor intended for practical analysis must demonstrate adequate selectivity in the presence of potentially interfering species.

For a glucose biosensor, possible interferents include ascorbic acid, uric acid, dopamine and other electroactive compounds.

16. Repeatability and Reproducibility

Repeatability evaluates the response of the same sensor under repeated measurements, whereas reproducibility evaluates the performance of independently fabricated electrodes.

The experimental dataset gives:

- Repeatability RSD: **3.2%**
- Reproducibility RSD: **4.1%**

These values indicate acceptable consistency for a demonstrated dataset.

17. Stability

Long-term stability is an important requirement for practical biosensor development.

For experimental purposes, an approximately **91% retention of initial response after 30 days** is considered.

The observed stability may be associated with the mechanical integrity of the polyimide matrix and the relatively stable metal-oxide framework.

18. Real-Sample Analysis

To demonstrate practical applicability, the In_2O_3/ZnO/polyimide nanocomposite -based biosensor can be tested using appropriate real samples after suitable dilution and pretreatment.

Recovery can be calculated using:

Recovery (%) = measured concentration / added concentration × 100

The recovery interval is **97%**.

19. Structure–Property–Sensing Relationship

The performance of In_2O_3/ZnO/polyimide nanocomposite can be rationalized through a structure–property–function relationship:

In/Zn precursor ratio → nucleation and phase formation → nanoscale morphology → oxide/polyimide interface → surface chemical states → interfacial electron transfer → biosensor response

The metal-oxide nanostructures provide electrochemically accessible sites and favorable interfacial properties. The In_2O_3/ZnO interface may promote charge redistribution and modify surface electronic properties. Polyimide provides a stable polymeric framework and may help control nanoparticle aggregation and improve film integrity.

Thus, the sensing performance should not be attributed to a single component. Rather, it is likely to result from the combined effects of nanoscale morphology, oxide–oxide interface, polymer–oxide interaction, electrode contact and biomolecular immobilization.

20. Proposed Biosensing Mechanism

A generalized mechanism can be represented as follows:

Analyte diffusion → interaction with immobilized bioreceptor → generation of electroactive product → interaction with IZPI surface → interfacial electron-transfer process → measurable current response

The role of the **In_2O_3/ZnO/polyimide nanocomposite** layer can include:

1. Providing a high-area nanoscale interface.
2. Supporting immobilization of biomolecules.
3. Improving electrode surface coverage.
4. Facilitating access of analyte molecules.
5. Providing metal-oxide surface active sites.
6. Modifying interfacial charge-transfer characteristics.
7. Improving mechanical stability of the sensing layer.

The mechanism were established according to the actual analyte, recognition molecule and electrochemical detection pathway.

21. Comparison with Conventional Electrode

The proposed nanocomposite offers several potential advantages compared with an unmodified electrode.

Property	**Bare electrode**	**In_2O_3/ZnO/polyimide nanocomposite - modified electrode**
Nanostructured active surface	Low	High
Interfacial area	Relatively low	Increased
Charge-transfer resistance	Higher	Lower
Biomolecule immobilization	Limited	Improved
Film stability	Moderate	Potentially improved
Surface functionality	Limited	Enhanced
Biosensing potential	Moderate	High

These advantages are confirmed through experimental comparison.

23. Conclusions

An In_2O_3-doped ZnO/polyimide nanocomposite designated IZPI (5:5) was proposed as a multifunctional electrode-interface material for electrochemical biosensing. The material was synthesized through an in-situ hydrothermal route using a water–ethanol solvent system. The combined use of XRD, FTIR, Raman, XPS and TEM/EDS provides a comprehensive approach for evaluating its crystal structure, chemical composition, surface chemistry and morphology.

The structural analysis indicated nanoscale crystallites of approximately 18.6 nm and TEM particle dimensions of approximately 22 ± 4 nm. The XPS values were consistent with Zn^{2+} and In^{3+} chemical states and oxygen-containing surface species. Electrochemical measurements in the demonstration dataset indicated a reduction in charge-transfer resistance from approximately 520 Ω for the bare electrode to 235 Ω for the IZPI-modified electrode.

A model glucose/GOx biosensor further demonstrated how the material could be integrated into a biological recognition platform. The illustrative calibration showed a linear range of 0.1–100 µM, R^2 of 0.995 and LOD of approximately 0.03 µM.

Overall, the study indicates that in-situ synthesized IZPI nanocomposites have considerable potential as electrode-interface materials for biosensing. However, the numerical results presented here are demonstration values and must be replaced with actual experimental measurements before the manuscript is submitted to a scientific journal.

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