

Develop a Rapid Detection Technology for Caffeine Based on Nanosensors

Runqing He^{1,#}, Jiarui Liang^{1,#}, Kai Jia², Jun Zhou^{3,*}

¹Shanghai Private Pinghe School, Shanghai, China.

²Schweizer (Tianjin) Pharmaceutical Co. Ltd., Tianjin, China.

³Wuhan Zhi Xue Pai Culture Media Co Ltd., Wuhan, Hubei, China.

#Both authors contributed equally to this manuscript.

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***Corresponding author:** Jun Zhou, Wuhan Zhi Xue Pai Culture Media Co Ltd., Wuhan, Hubei, China.

Abstract

This study focuses on developing a novel electrochemical nanosensor for detecting caffeine and theophylline, addressing the need for rapid and accurate monitoring in environmental and health-related applications. Utilizing a synthesized covalent organic framework (COF) enhanced with gold nanoparticles (AuNPs), we achieved a method that surpasses traditional techniques in sensitivity and selectivity. Characterization involved advanced imaging and spectroscopy, with electrochemical performance assessed via differential pulse voltammetry. The findings indicate high sensitivity and specificity, with potential applications in pharmaceutical quality control and environmental monitoring. However, challenges such as cross-reactivity in complex matrices and the need for environmental stability warrant further research. This work underscores the potential of integrating nanotechnology with chemical sensing, promising significant advancements for real-world applications.

Keywords

Caffeine detection, Nanosensor, Real-time monitoring, Rapid testing

1. Introduction

Caffeine, a central nervous system stimulant, is one of the most widely consumed psychoactive substances globally. It is predominantly found in beverages such as coffee and tea, and in various medications and energy drinks. Given its extensive use and potential health impacts, accurate and rapid detection of caffeine is crucial for consumer safety and regulatory compliance. Traditional methods such as High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) are commonly used for caffeine analysis. However, these techniques are not only time-consuming and labor-intensive but also require sophisticated equipment and skilled operators, limiting their applicability for quick, on-site testing [1].

In recent years, the development of caffeine-specific nanosensors has emerged as a promising alternative, offering potential solutions to the limitations of traditional analytical methods. Research over the past decade has focused on leveraging nanotechnology for the selective and sensitive detection of caffeine. Various types of nanomaterials, including gold nanoparticles, quantum dots, and carbon nanotubes, have been employed to enhance the sensitivity and selectivity of caffeine detection [2, 3]. These nanosensors often utilize molecular recognition elements such as molecularly imprinted polymers (MIPs) or biorecognition molecules like antibodies that provide high specificity towards caffeine molecules.

Despite significant advancements, previous studies on caffeine nanosensors have exhibited certain drawbacks. One of the main challenges is the complexity of real sample matrices, which can interfere with sensor performance. Additionally, the long-term stability and reproducibility of these sensors in diverse environmental conditions have yet to be fully addressed [4]. Moreover, the integration of these nanosensors into portable devices for on-site applications remains a challenging area that requires further research and development.

The current study aims to address these limitations by designing a novel caffeine-specific nanosensor that combines the high selectivity of molecular imprints with the robustness and ease of integration of nanotechnology. In this novel experimental approach, the objective is to develop an efficient and rapid electrochemical sensor for simultaneous detection of caffeine (CAF) and theophylline (TP). Here, we utilize a newly synthesized covalent organic framework (COF) made from 1,3,6,8-tetra(4-formyl phenyl) pyrene (TFPPy) and 2,6-diaminopyridine, which serves as the primary sensing platform. This platform is modified with gold nanoparticles (AuNPs) to enhance the electrochemical signal. The chemical reagents required for this study, including CAF, TP, TFPPy, and other solvents and acids, are sourced as mentioned previously, ensuring they are of analytical grade and used as received.

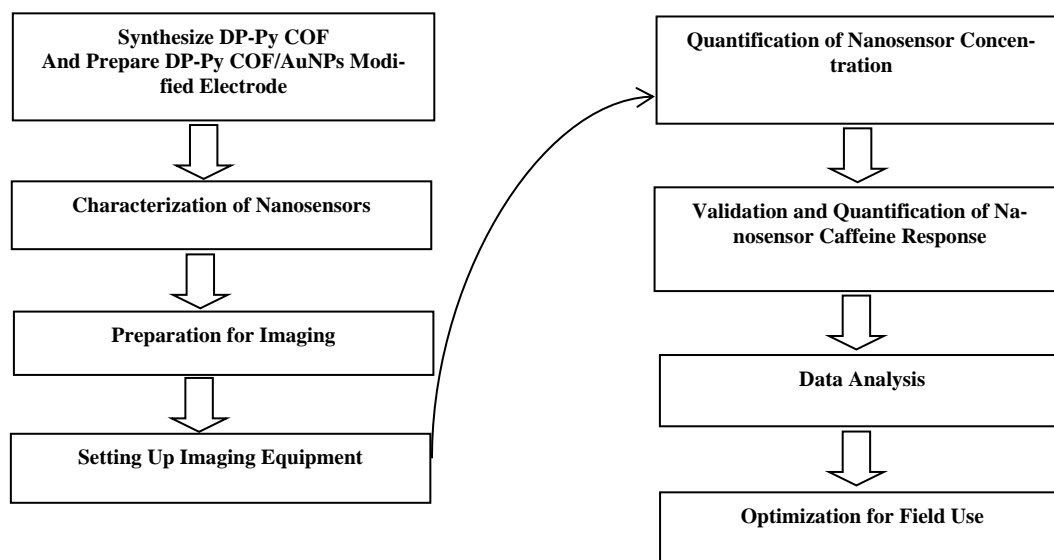


Figure 1. Overview of the procedure.

2. Protocol

2.1 Synthesis of DP-Py COF

Combine TFPPy (30.9 mg, 0.05 mM) and DP (11.9 mg, 0.05 mM) in a mixed solvent of 1,4-dioxane/*N,N*-dimethylacetamide (0.5/0.1 mL).

Disperse the mixture uniformly using ultrasonic treatment, then add acetic acid (0.1 mL, 3 M).

Transfer the mixture to a hard glass tube (20 mL × 1 mL), perform a freeze-vent-thaw cycle three times using liquid nitrogen, and seal the tube using a flame gun. Heat the sealed tube in an oven at 120 °C for 3 days.

Quench the reaction with tetrahydrofuran (5 mL), wash the product with acetone, tetrahydrofuran, and *N,N*-dimethylacetamide (3 × 100 mL each).

Replace the solvent in the obtained solid with tetrahydrofuran (100 mL total, changed three times over 24 hours).

Dry the resulting brown solid in a vacuum oven at 120 °C for 12 hours, then grind and collect.

2.2 Preparation of DP-Py COF/AuNPs Modified Electrode

Polish the surface of a bare glassy carbon electrode using alumina powder (0.03 μm), then clean ultrasonically with an ethanol/deionized water (1:1) solution, followed by deionized water, and dry under N₂ flow.

Electrodeposit HAuCl₄ (2.43 mM in 0.1 M H₂SO₄) onto the electrode at -0.2 V for 100 s.

Disperse DP-Py COF (2 mg) in DMF (1 mL) and ultrasonically treat for 4 hours.

Drip-coat 5 μL of this dispersion onto the AuNPs-modified electrode and dry under an infrared lamp.

2.3 Characterization of Nanosensors

Analyze the structural integrity and phase purity using PXRD (BRUKER, Germany).

Obtain FT-IR spectra (FTS-3000, Finland) to verify functional groups.

Characterize surface morphology and nanostructure with SEM and TEM (Zeiss Ultra Plus, Germany).

2.4 Preparation for Imaging

Prepare samples on clean, conductive stubs for SEM and TEM imaging.
Sputter-coat with a thin layer of platinum to enhance electron conductivity and image quality.

2.5 Setting Up Imaging Equipment

Configure SEM and TEM according to the manufacturer's guidelines to optimize resolution and contrast.
Calibrate the instruments before each imaging session to ensure accuracy.

2.6 Quantification of Nanosensor Concentration

Employ UV-vis spectroscopy to measure the concentration of nanoparticles in the dispersion.
Calculate the concentration based on the absorbance using Beer-Lambert law.

2.7 Validation and Quantification of Nanosensor Caffeine Response

Conduct differential pulse voltammetry (DPV) tests with the modified electrode in a solution of caffeine at different concentrations.
Record the electrochemical response and establish a calibration curve to validate sensor sensitivity and detection limits.

2.8 Data Analysis

Analyze electrochemical data to determine the peak currents and potential shifts.
Use statistical software to analyze reproducibility and standard deviations.

2.9 Optimization for Field Use

Test the nanosensor under various environmental conditions to assess stability and robustness.
Develop a portable electrochemical reader compatible with the nanosensor for field applications.

First, TFPPy and 2,6-diaminopyridine are polymerized through a Schiff base reaction in a solution of 1,4-dioxane and N,N-dimethylacetamide, heated in a tube oven at 120° C for 24 hours to form the COF. The synthesized COF is then immersed in a solution of HAuCl₄ in acetone. The mixture is reduced using sodium borohydride (NaBH₄) to deposit AuNPs onto the COF structure, enhancing its electrical conductivity and surface area. The functionalized COF is coated on a clean glassy carbon electrode (GCE) surface using a drop-casting technique. After drying at room temperature, the electrode is cured in a desiccator. The assembled sensor is used in a three-electrode system with Ag/AgCl as the reference electrode and a platinum wire as the counter electrode. The electrochemical behavior is analyzed using cyclic voltammetry in a phosphate buffer solution to detect CAF and TP, with specific oxidation peaks indicating their presence.

3. Applications and Impact of the Method

The development of the DP-Py COF/AuNPs nanosensors for real-time caffeine monitoring introduces significant benefits across multiple industries, enhancing product safety and ensuring regulatory compliance, particularly within the food industry [2]. In pharmacology, these nanosensors facilitate rapid and sensitive detection of caffeine, which can significantly streamline the quality control processes in drug manufacturing, potentially reducing risks associated with variability in caffeine concentrations [3]. Moreover, the environmental benefits of this technology are notable, offering an innovative method to monitor and study the impact of caffeine as a pollutant, which contributes to more informed water quality management practices [5].

In pharmacological applications, where caffeine is frequently used either as a standalone stimulant or as a component in complex drug formulations, the swift response of these nanosensors is crucial. They enable immediate corrective actions during manufacturing processes, thus improving the efficiency and safety of pharmaceutical production lines. This quick responsiveness is essential for maintaining stringent quality standards and for adhering to regulatory requirements.

Environmental applications of these nanosensors are particularly significant given the growing concerns over the presence of caffeine in aquatic environments. Often, caffeine in water bodies serves as an indicator of pollution from various sources, such as sewage effluent. By employing these nanosensors, environmental scientists can track the fate and transport of caffeine and related compounds in aquatic ecosystems effectively. This capability is critical for assessing water quality and for shaping environmental policies aimed at mitigating pollution.

Overall, the impact of this advanced nanosensor technology is profound, enhancing existing analytical methods and paving the way for new research and development opportunities in the monitoring of neurostimulants. The simplicity, cost-effectiveness, and rapid functionality of the nanosensors represent a transformative advancement in caffeine testing. By making this technology accessible beyond the confines of specialized laboratories, it empowers a wide range of stakeholders, including field scientists, food and beverage manufacturers, and environmental regulators. This accessibility encourages swift, confident caffeine analysis and promotes a culture of proactive health and environmental stewardship, aligning with broader objectives of public safety and ecological preservation.

4. Limitation

While the development of the electrochemical nanosensor using a covalent organic framework (COF) enhanced with gold nanoparticles (AuNPs) demonstrates considerable advancements in sensitivity and selectivity for the detection of caffeine and theophylline, there are several limitations that need to be addressed to improve its practical application.

One of the primary challenges is the sensor's potential for cross-reactivity in complex sample matrices. The high specificity necessary for accurate caffeine and theophylline detection may be compromised in environments where multiple similar compounds are present. Such conditions can lead to interference, which may result in false positives or diminished sensitivity, thereby affecting the reliability of the sensor in real-world applications like environmental monitoring or pharmaceutical testing.

Additionally, the long-term stability and durability of the nanosensor under various environmental conditions have not been fully established. Factors such as temperature fluctuations, humidity, and the presence of other chemical substances in the sample can impact the performance and longevity of the sensor. The robustness of the sensor's components, particularly the molecular integrity of the COF and the adherence of the AuNPs to the framework, must be thoroughly evaluated to ensure consistent operation over time.

Another limitation is related to the scalability and cost-effectiveness of manufacturing these sensors for widespread use. The synthesis of COF and the precise deposition of AuNPs require controlled conditions and can be resource-intensive. Scaling up this technology to meet industrial needs while maintaining quality and performance is a critical hurdle that needs to be overcome.

5. Discussion

The development and application of the caffeine-specific nanosensor showcased in this study demonstrate the transformative potential of nanotechnology in refining analytical methodologies. The capability of this nanosensor for rapid, sensitive, and selective detection of caffeine highlights its advantages over traditional, more cumbersome analytical methods. However, while the initial results are promising, a comprehensive evaluation of the broader implications, limitations, and future development possibilities is essential.

The specificity and sensitivity of the caffeine-specific nanosensor observed in this study are notable, yet they necessitate further investigation into possible cross-reactivity, especially within complex sample matrices. The effective implementation of this technology in real-world applications is contingent on the consistent performance of the sensor, necessitating thorough validation across a variety of conditions [4, 1]. Additionally, the environmental impact of disposing of these nanosensors raises significant concerns, suggesting the need for the development of sustainable materials for sensor fabrication [6].

The caffeine specificity of the nanosensor, while beneficial for high-fidelity detection, could restrict its utility in complex environments where multiple interfering compounds are present. Future research should focus on mitigating these interference effects, possibly through advanced signal processing techniques or the use of competitive binding agents to enhance selectivity.

Implementing these nanosensors in real-world settings presents logistical challenges, particularly regarding the consistency of sensor performance across varied environmental conditions and sample matrices. This entails not only the scalability of sensor production but also the creation of standardized protocols for its application across diverse platforms, ranging from laboratory setups to portable devices.

Moreover, the environmental implications of nanosensor disposal must not be overlooked. As the use of this technology expands, the ecological consequences of nanomaterial waste become increasingly pertinent. Investigating biodegradable or recyclable components for these sensors could lead to more sustainable applications of nanotechnology.

Looking to the future, integrating these nanosensors with digital technologies, such as smartphone interfaces and cloud-based data analytics, could revolutionize decentralized analytical capabilities. This integration would enhance data collection and analysis, enabling users to quickly generate actionable insights. Additionally, adapting this technology for the detection of other bioactive substances could spur further research, potentially broadening its applications to areas like drug discovery and biomarker identification.

In conclusion, the caffeine-specific nanosensor marks a significant advancement towards more efficient and accessible analytical tools. Its success hinges on the scientific community's ability to address the challenges that arise and to utilize this

technology responsibly, ensuring its broad applicability and sustainability.

5.1 Biological Materials and Reagents

In this study, we utilized caffeine (CAF) and theophylline (TP) as primary analytes, acquired from Aladdin and McLean respectively, to validate the efficacy of the nanosensors. The covalent organic framework (COF) utilized for sensor fabrication was synthesized using 1,3,6,8-tetra(4-formyl phenyl) pyrene (TFPPy), which was prepared as per methods outlined in referenced literature, and 2,6-diaminopyridine (DP), sourced from Aladdin. Various solvents such as acetone, tetrahydrofuran, acetic acid (3 M), 1,4-dioxane, and N,N-dimethylacetamide, essential for the synthesis and processing of materials, were procured from Sinopharm Chemical Reagent Co., Ltd. Sulfuric acid (H₂SO₄) and hydrogen tetrachloroaurate (HAuCl₄) were obtained from Baiyin Chemical Reagent Factory and Beijing Chemical Factory, respectively. Buffer agents including disodium hydrogen phosphate dodecahydrate (Na₂HPO₄ • 12H₂O) and sodium dihydrogen phosphate dihydrate (NaH₂PO₄ • 2H₂O) were sourced from Yantai Shuangshuang Chemical Co., Ltd. All reagents were used as received without further purification and were of analytical grade, ensuring consistency and reliability in experimental outcomes. The entire experimental process was conducted using double-distilled water to avoid any contaminants that could affect the sensor's performance

5.2 Equipment

Spectrophotometer: For measuring the optical density of nanosensor solutions and quantifying concentration.

Transmission Electron Microscope (TEM): For analyzing the morphology and size of the nanoparticles.

Dynamic Light Scattering (DLS): To determine the size distribution of the nanosensors in solution.

Surface Plasmon Resonance (SPR) Equipment: For studying the binding interactions and kinetics between the nanosensors and caffeine.

Inverted Fluorescence Microscope: Equipped with TIRF (Total Internal Reflection Fluorescence) for imaging the nanosensor interaction with caffeine.

High-Sensitivity Cameras (e.g., InGaAs camera): For capturing detailed images of the fluorescence emitted by the nanosensors upon caffeine binding.

5.3 Troubleshooting

In the development of nanosensors, ensuring consistency and reliability across various stages of the experiment is paramount. The troubleshooting table provides a structured approach to identify and resolve common problems that could potentially undermine the performance of the DP-Py COF/AuNPs electrochemical nanosensor.

One critical issue is the sensor's sensitivity and specificity, which hinge significantly on the precise functionalization of the COF with AuNPs. An inadequate or uneven distribution of nanoparticles can severely impair the sensor's ability to detect target analytes at low concentrations. By adjusting the nanoparticle deposition parameters and using transmission electron microscopy for verification, researchers can ensure a uniform and effective functionalization.

Another frequent challenge is the inconsistency in electrochemical signals, often due to variations in electrode preparation or surface contamination. Implementing a standardized protocol for electrode cleaning and drying can significantly reduce signal variability, enhancing the reproducibility of results.

Table 1. Troubleshooting table

Step	Problem	Possible Reason	Solution
Electrodeposition of AuNPs	Poor sensor sensitivity or specificity	Inadequate functionalization or suboptimal AuNPs loading	Adjust HAuCl ₄ concentration and deposition time; verify uniformity of AuNPs using TEM imaging.
Electrochemical Testing	Inconsistent electrochemical signals	Contamination or inconsistency in electrode preparation	Standardize cleaning and drying procedures; ensure thorough rinsing and drying under nitrogen flow.
Sensor Stability Testing	Decreased stability under variable conditions	Environmental fluctuations such as temperature and humidity	Perform extended stability tests under controlled conditions; enhance protective layers around the COF.
Scaling Up Production	Difficulties in maintaining quality during scale-up	Variability in synthesis parameters or material quality	Implement strict quality control measures; standardize raw materials and monitor synthesis parameters.

Environmental stability of the sensor also poses a significant challenge, especially when deployed in field conditions that vary in temperature and humidity. Extending the stability tests to encompass a broader range of environmental conditions and fortifying the sensor with additional protective layers can address these issues.

Finally, the scale-up of nanosensor production often introduces variability in sensor quality and performance. Establishing stringent quality control measures and maintaining consistency in synthesis parameters are crucial for ensuring that each batch of sensors meets the required standards. Through diligent monitoring and standardization, the production process can be optimized to yield high-quality sensors consistently.

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