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Advances in ferritin biosensors: rapid and cost-effective iron level assessment

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Abstract

Iron is an essential element crucial for oxygen transport in the bloodstream; however, deviations from its normal physiological levels can result in health complications. Iron deficiency is a widespread condition and a leading cause of anemia globally. Conversely, excessive iron accumulation can cause severe liver damage, such as cirrhosis, by exacerbating oxidative stress. Consequently, assessing iron status through biomarkers like ferritin, which is a protein that reflects the body's iron stores, is critical for diagnosing iron-related disorders accurately.

The ferritin test, which measures the concentration of ferritin protein in the blood, is a standard method for iron analysis. However, frequent hospital visits for such testing can be both time-intensive and expensive. This has driven significant interest in developing rapid and cost-effective methods for ferritin detection. To meet this demand, a variety of biosensors have been designed using diverse techniques to enable convenient ferritin analysis.

Despite considerable advancements in biosensor technologies, a comprehensive classification and analysis of these devices remain lacking, leaving researchers without a unified understanding of their comparative strengths and limitations. This review addresses this gap by systematically categorizing ferritin biosensors based on their underlying mechanisms. The review explores electrochemical biosensors, such as those based on graphene, microfluidics, and ZnO–Mn_3O_4 nanocomposites, as well as electrical biosensors using quantum dots or gold nanorods. Additionally, optical biosensors, including photonic crystal sensors, surface plasmon resonance systems and magnesium sulfide nanosheets are discussed in detail. Unconventional and immunoassay-based biosensors, such as nanodiamond-based magnetic sensors and nanowire FETs, are also examined to highlight the breadth of detection strategies available.

With the rising prevalence of iron-related disorders and the limitations of traditional diagnostic tools, this review underscores the critical need for portable, rapid, and cost-effective ferritin biosensing technologies, offering insights relevant to both clinical practice and future research.

Introduction

Iron deficiency, defined by a marked reduction in healthy erythrocyte density due to inadequate iron levels, is a critical health issue. If untreated, this deficiency can advance to iron deficiency anemia, a condition that, while more prevalent among women and children, can affect individuals of all ages. Iron deficiency is particularly concerning as it not only impedes cognitive development from infancy through adolescence but also heightens susceptibility to various diseases (Oshin et al. 2020). Conversely, excessive iron levels can foster the proliferation of certain tumors and pathogens, underscoring the importance of accurate assessment of iron stores in the body. Among the available diagnostic methods, serum ferritin

measurement is the most reliable for evaluating iron reserves (Cui et al., 2003). Beyond its role as an iron storage protein, ferritin is actively released from infected tissues during infections. Recent findings indicate a significant increase in serum ferritin levels in patients with SARS-CoV-2, which may serve as a prognostic marker for COVID-19, correlating with cytokine storm severity (Selvarajan et al., 2021). Additionally, ferritin is crucial in diagnosing chronic conditions such as anemia, liver diseases, and neurodegenerative disorders like Parkinson's and Alzheimer's. While ferritin levels typically remain within a normal range in healthy individuals, deviations often signal underlying pathologies (Yen et al., 2016). Traditional diagnostic methods, such as enzyme-linked immunosorbent assays (ELISA) and radioimmunoassays (RIA), although effective, are costly, require specialized materials and expertise, and are impractical for widespread use in field settings (Peterson et al., 2014). The biosensor concept has advanced, defined by some as a standalone device that selectively and reversibly detects chemical species based on their density or activity in biologic materials. It converts molecular recognition by its bioactive layer into an electrical signal via electrochemical, thermal, optical, or mass-based transduction (Coulet, 1991). For this review article, the main issue is biosensors that are developed for ferritin detection. Therefore, there is increasing interest in the development of ferritin biosensors, which offer enhanced sensitivity, practicality, and cost-effectiveness due to their innovative nanostructures. Numerous studies focus on developing and evaluating these biosensors to overcome the limitations of conventional diagnostic methods (Kartalov et al., 2008; Lu et al., 2017).

Ferritin Biosensors and Mechanisms

Electrochemical Biosensors

Graphene-Based Biosensor

This section begins with a pioneering study on ferritin detection using graphene.

Biosensor design

In this research, a field-effect transistor biosensor, featuring a single-layer graphene conductive channel functionalized with anti-ferritin antibodies, achieved an impressive detection limit of approximately 10 fM. Biomolecule immobilization was achieved by treating the graphene surface with 1-pyrenebutanoic acid succinimidyl ester (PASE), followed by introducing an antibody solution, where PASE's succinimidyl groups covalently bonded with the antibody's amino groups to form stable amide bonds (Figure 1). The sensor's remarkable sensitivity was attained using a cost-effective shadow mask technique for electrode creation, making it a significant advancement in sensor technology (Oshin et al. 2020).

Mechanism of detection

The potential for non-invasive ferritin detection, possibly through saliva, was highlighted due to the biosensor's extraordinary performance. Graphene-based field-effect transistors (GFETs) configured with liquid-gated measurement setups (LG-FET) are widely used in biosensor applications, including the detection of ferritin, due to their high sensitivity and low operating voltage requirements. In such a setup, the sample containing the target analyte, ferritin in this case, acts as the liquid medium. A gate voltage is applied to the graphene channel through a reference electrode submerged in the liquid, establishing an electrical double layer (EDL) at the graphene-liquid interface. The capacitance of this EDL, denoted as C_{EDL}, combines with the air-gap capacitance (caused by graphene's hydrophobic properties) and graphene's inherent quantum capacitance to form the total gate capacitance of the device. This configuration allows the GFET to operate effectively at a low gate voltage, typically under 1 V. The thickness of the EDL is governed by the Debye length (λ_D), which depends on the ionic strength of the electrolyte. High ionic strength solutions limit detection due to charge screening. To overcome this, 0.01 × phosphate-buffered saline (PBS) (1.5 mM, $\lambda_D \approx 7.3$ nm) is used, ensuring the binding of ferritin-antibody complexes remains within the detectable range. Functionalization of the graphene surface adds a height (~ 4 nm), but this still fits within the adjusted Debye length. Ferritin detection is based on the modulation of the graphene channel's electrical properties as a result of antigen-antibody binding on the sensor surface. For p-type GFETs, which operate with a higher density of holes than electrons, the binding of negatively charged biomolecules like ferritin (474 kDa) introduces additional holes into the graphene channel. This results in an increase in drain-source current and a reduction in the channel's resistance. Conversely, positively charged molecules would reduce the drain-source current in p-type devices. This modulation of electrical properties occurs due to the localized electrostatic gating effect caused by the binding event. For ferritin detection, a working potential of 0.05 V is applied, and the changes in current are monitored as a direct response to biomolecular immobilization. Real-time detection of ferritin is achieved by gradually increasing its concentration in the sensing bath, starting from as low as 10 ng/L and reaching up to 8 µg/L. The device demonstrates high sensitivity, as even the smallest tested concentration resulted in a significant rise in drain current. This indicates that the detection limit of the GFET biosensor is below 10 ng/L (Oshin et al. 2020).

Advantages of the graphene-based biosensor

Furthermore, the response time of the sensor is remarkably fast, with measurable changes in current occurring within 10 seconds of adding ferritin to the sensing medium. This rapid detection capability highlights the potential of GFETs for real-time

monitoring of ferritin and other biomolecules in low-ionic-strength environments (Oshin et al. 2020).This study underscores the biosensor's advantages, including simplicity, rapid results, and cost-effectiveness, attributed to the use of graphene (Chen et al., 2021).

Limitations of the GFET biosensor

First; even though the suggested GFET biosensor has a high sensitivity (10 fM), a number of drawbacks could prevent practical use. The Debye length was extended by measurements in 0.01× PBS; however, physiological fluids, such as serum or saliva, have a higher ionic strength (~150 mM), which may impair detection because of charge screening. Second, despite graphene's affordability and commercial availability, its reproducibility and scalability may be impacted by variability introduced by chemical vapor deposition graphene and manual transfer procedures. Third, there was no testing done on the sensor's stability in biological samples or over an long-term storage. Furthermore, even though environmental changes can cause electrical drift in FETs, calibration procedures were not rigorously discussed.

Conclusion and future directions

With a record detection limit of 10 fM, this work introduces the first graphene-based FET biosensor for ferritin detection. Technical challenges still exist in spite of this achievement, such as device-to-device variation, potential nonspecific binding, and validation under physiological conditions. Standardization of signal readouts and tests using actual biological fluids are crucial for clinical translation. Although automation would be required for regulatory compliance, the shadow mask-based fabrication process offers a low-cost and scalable solution. Usability may be enhanced in the future by combining this sensor with digital interfaces and microfluidic handling. Given ferritin's limitations as an acute-phase reactant, expanding detection to other iron-related or inflammatory markers may also improve diagnostic specificity for iron-deficiency anemia and increase clinical relevance.

While the GFET biosensor demonstrates exceptional sensitivity and rapid response, its practical translation is challenged by device variability and sensitivity to ionic strength, which can limit its application in real physiological samples. Compared to optical and immunoassay-based sensors, GFETs offer unparalleled detection limits but face greater obstacles in operational stability.

Overall, graphene-based FET sensors fill the need for ultra-sensitive, label-free detection, particularly in research or settings requiring detection of extremely low ferritin concentrations, and they set a benchmark for sensitivity among electrical biosensors. Moving forward, comparison with more robust or clinically validated platforms—such as microfluidic or optical sensors—will be crucial for determining their practical clinical value.

Microfluidic-Based Biosensor

Mechanism of detection

Continuing this exploration of ferritin biosensors, this review turns to microfluidic-based electrochemical immunosensing, addressing the limitations of existing methods regarding portability and response times. An innovative laboratory-on-a-chip approach integrates nanotechnology, electrochemistry, and microfluidics, utilizing an electrochemically active screen-printed electrode. The electrode's surface was modified with amine-functionalized graphene oxide (NH_2-GO) to enhance antibody adhesion. This setup allows for continuous ferritin detection and is designed for use by non-governmental organizations and health monitoring entities in non-urban areas. Electrochemical methods are increasingly favored in clinical diagnosis due to their efficiency, minimal sample requirements, and ease of data evaluation. Currently, there is growing interest in applying electrochemical techniques to detect ferritin using static systems. Thanks to continuous flow systems, patients' samples can be measured in real-time and sequentially in clinical settings. Therefore, the primary objective of this study was to electrochemically analyze ferritin in a continuous flow setup using a laboratory-on-a-chip-based method with a microfluidic flow cell featuring an integrated but replaceable electrode (Garg, Christensen, et al., 2020).

Previous efforts in laboratory-on-chip tools have aimed to detect various analytes, but challenges in electrode modification and chip design persist. This study successfully integrated a laboratory-on-chip device with a screen-printed electrode coated with electrochemically active carbon, making it cost-effective for point-of-care applications. Screen-printed electrodes are cost-effective for performing electrochemical measurements, particularly when paired with portable potentiostats for point-of-care and field applications (Mao et al., 2015). These electrodes are ideal for bulk production, offering minimal batch-to-batch variations. Researchers have leveraged these benefits to integrate screen-printed electrodes with flow cells, enabling the electrochemical detection of various analytes. However, many flow cell designs require electrodes to be discarded after a single use, contributing to waste. Conventional flow cells can also be complex and limit control over fluid distribution on the electrode surface. Thus, to measure biomarkers such as ferritin, there is a need for a simplified flow cell design that includes integrated screen-printed electrodes, allows for easy electrode replacement, and enables precise control of flow over the electrode surface (Ma et al., 2023). This study has therefore employed a magnetic coupling mechanism and simple flow cell design, facilitating the sequential passage of ferritin antigens across the electrode. This research represents an unprecedented use of a graphene-based 2D material for continuous electrochemical ferritin

detection within a straightforward flow cell design (Garg, Christensen, et al., 2020).

Electrode functionalization and stability

The electrode offers a working area of 11.34 mm². Amine-functionalized graphene oxide was integrated via drop-casting, and cyclic voltammetry was used for both ferritin (Fer) detection and electrochemical characterization. The immobilization of ferritin antibodies (Fer_{Ab}) was done onto the amine-functionalized graphene oxide-modified surface of the carbon-coated screen-printed electrode. This approach leverages the interaction between the amine groups in graphene oxide and the carboxyl groups in the antibodies, enabling stable and efficient antibody fixation to the electrode surface. Throughout the electrode modification processes, including plain carbon-coated screen-printed electrodes (SPCEs), NH_2-GO@SPCE, Fer_{Ab}/NH_2-GO@SPCE, and Fer/Fer_{Ab}/NH_2-GO@SPCE, the electrochemical response of carbon-coated screen-printed electrodes was consistently monitored to confirm the electrodes maintained their activity (Garg, Christensen, et al., 2020).

Ferritin concentrations were prepared in a redox probe-buffer solution and introduced into the microfluidic device at an optimized flow rate of 10 µL/min, determined through prior optimization studies. The electrode surface was washed with phosphate buffer between measurements. Additionally, the effects of buffer pH and interfering substances on the cyclic voltammetry signal were explored. Simulations using COMSOL Multiphysics 5.2 analyzed changes in liquid concentration and flow profiles within the flow cell. The stability of the amine-functionalized graphene oxide coating on the electrode surface was tested through continuous pumping and 48 consecutive scans, showing a stable response until the 25th scan, after which a gradual decline occurred. By the 48th scan, the current decreased by 6.7%, indicating it might be useful as a biosensor for long-term and detailed analyte detection (Garg, Christensen, et al., 2020).

It's worth mentioning that the stability of Fer_{Ab}/NH_2-GO was not assessed, likely due to the formation of covalent bonds between the amine groups in the amine-functionalized graphene oxide and the carboxyl groups in the antibody. This robust binding interaction negates the need for stability testing after antibody immobilization. To investigate the impact of functionalization and antibody immobilization, cyclic voltammograms were recorded for each consecutively modified electrode, including bare SPCE, NH_2-GO@SPCE, Fer_{Ab}/NH_2-GO@SPCE, and Fer/Fer_{Ab}/NH_2-GO@SPCE. Following the modification of the bare SPCE with amine-functionalized graphene oxide, an increase in electrochemical response was observed. This is attributed to the involvement of the lone electron pair associated with the nitrogen atom, which actively participates in electron transfer on the electrode surface, thereby enhancing system conductivity. Conversely, when ferritin antibodies were immobilized, a reduction in current was noted due to their insulating properties. Although the team could not quantify antibody density, previous studies indicate high densities can be achieved. The authors of this publication employed charge transfer resistance values recorded before and after antibody immobilization to calculate the extent of antibody distribution across the electrode surface. Upon antigen introduction, an immune complex forms between the antigen and antibody, resulting in a notable decrease in current, further impeding flow (Garg, Christensen, et al., 2020).

Performance of the microfluidic device

The study evaluated a microfluidic flow cell for the quantitative electrochemical analysis of ferritin, using the Fer/Fer_{Ab}/NH_2GO@SPCE in the flow cell. Increased ferritin concentration led to a current reduction due to the insulating immune complex formed by ferritin and its antibody, achieving a linear range of 7.81 to 500 ng/mL, suitable for clinical applications. The R^2 value was 0.996, and the detection limit was 0.413 ng/mL (Garg, Christensen, et al., 2020).

To assess the reproducibility, responses from five electrodes prepared similarly showed consistent results with a relative standard deviation of 0.74%. The immunosensor's selectivity for ferritin was confirmed by testing responses to other proteins like hemoglobin and myoglobin, with minimal interference observed. The sensor's reusability was demonstrated through five regeneration cycles, resulting in an 11% decrease in response, indicating the device's capability for multiple uses before requiring electrode replacement (Garg, Christensen, et al., 2020).

Furthermore, ferritin was added to human serum at different concentrations. According to quantative readings, the results varied by 5% to 10%, but the system showed promise for optimization. Comparisons with commercially available ELISA kits revealed that while ELISA had a broader linear range (0 to 1500 ng/mL), it relies on secondary antibodies and chromogenic substrates. Also, ELISA's dependence on a microplate reader increases its cost. In contrast, the lab-on-a-chip device employed primary antibodies specific to ferritin and utilized a handheld potentiostat for cost-effectiveness and portability (Garg, Christensen, et al., 2020).

Comparison with existing technologies

The performance of this immunosensor can be compared to other microfluidic electrochemical sensors for ferritin. Ko et al. developed a PDMS/PDMMA-layered microfluidic device with a gold electrode, but it lacked reusability, making it unsuitable for point-of-care applications. In another study by Reymond et al., an electrochemical microchip with eight channels was developed for a microfluidic-based ferritin immunoassay. The microchip was modified using magnetic and non-magnetic microspheres to immobilize

antibodies (Wang et al., 2009). However, their approach fell short of covering the clinical range required for ferritin measurements. Additionally, Song et al. (2017) used three antibody sets and a cotton thread as a microfluidic circuit, achieving a wide linear range of 5-5000 ng/mL, though the system's complexity and multiple antibodies increased costs. A recent paper-based electrode system for ferritin detection outperformed this study in linear range and limit of detection but required multiple adjustments and could not accommodate electrode changes within the same device. The literature on microfluidic and electrochemical ferritin detection highlights challenges in electrode functionalization, chemistries for antibody immobilization, and difficulties in replacing electrodes in existing systems (Garg, Christensen, et al., 2020).

Advantages and future applications

When compared to other reports on immunosensors for ferritin detection, the immunosensor (Zhang et al., 2006) under discussion here offers several advantages. It effectively covers the clinical ferritin range and simplifies the functionalization and immobilization processes into a single step. Amine-functionalized graphene oxide serves as both the immobilization matrix and electrode modifier, enabling continuous electrochemical detection. The microfluidic channels in the top chip allow for easier electrode replacement, enhancing usability. This dynamic ferritin determination method shows promise for clinical use and could be suitable for small clinics operated by personnel without advanced training. With further design advancements, it could evolve into portable bedside technology. Additionally, the system's versatility allows for the detection of various analytes through different bioreceptors (Garg, Christensen, et al., 2020).

Limitations of the microfluidic-based biosensor

The proposed microfluidic electrochemical immunosensor for ferritin offers significant potential but faces key challenges. Stability under physiological conditions remains uncertain, as the NH_2-GO coating showed durability over 48 scans, yet its long-term performance in clinical samples with varying pH and ionic strengths requires further testing. The cost of nanomaterials, particularly NH_2-GO, must be addressed for large-scale production. The need for frequent calibration is critical to maintain accuracy after prolonged use or storage. Electrode reusability is limited, with performance dropping by ~11% after five cycles of regeneration. Additionally, while the sensor showed good selectivity against interferents like hemoglobin and myoglobin, further validation with diverse real-world clinical samples is necessary. Lastly, scalability and regulatory approval require addressing reproducibility, biocompatibility, and compliance with clinical standards.

Conclusion

This immunosensor represents a promising step toward point-of-care diagnostics, offering sensitivity, selectivity, and real-time detection in clinically relevant ranges. However, challenges such as improving stability in physiological conditions, reducing electrode degradation, and enabling cost-effective NH_2-GO production must be resolved. The sensor's design could be enhanced for better flow dynamics and portability. Future work should validate performance in larger, diverse sample sets while exploring multi-biomarker detection to broaden its applications. With these advancements, this sensor type holds potential for scalability and regulatory readiness, ultimately contributing to more accessible, efficient diagnostics in resource-limited settings.

This immunosensor represents a promising step toward point-of-care diagnostics, offering sensitivity, selectivity, and real-time detection in clinically relevant ranges. However, challenges such as improving stability in physiological conditions and reducing electrode degradation must be resolved. While microfluidic-based electrochemical sensors offer practical advantages in continuous monitoring and portability, their detection limits are generally not as low as those of GFETs, and reusability remains a concern compared to SPR platforms.

To summarize, microfluidic immunosensors fill the need for quick and convenient ferritin testing, featuring moderate sensitivity and straightforward operation. They connect the advantages of highly sensitive electrical systems with the limitations of optical methods that lack portability. This makes them especially attractive for decentralized or resource-limited clinical settings.

Ferritin Detection by Utilizing ZnO-Mn_3O_4 Nanocomposite

In another study mentioned in this review, an electrochemical biosensor was developed for real-time detection of ferritin during biomedical analysis. This research introduces a new biosensor designed to detect ferritin quantitatively and selectively using just a drop of blood plasma (Gautam et al., 2023).

Advantages of electrochemical detection methods

The electrochemical biosensing platform utilized in this study employs a semiconductor-based nanostructured screen-printed electrode. The electrochemical detection methods, recognized for their simplicity, rapidity, cost-effectiveness, and heightened sensitivity, have gained considerable attention in recent times. Among the approaches mentioned, those utilizing screen-printed electrodes are particularly illuminating, offering various advantages over traditionally fabricated electrodes. These screen-printed electrodes are not only ideal for single-use

applications and easily transportable, but they are also produced through screen-printing technology, allowing the integration of multiple electrodes on a single substrate. This capability facilitates the simultaneous analysis of numerous samples, making these electrodes well-suited for high-throughput screening and field-based applications. Additionally, the precision with which the screen-printing technique deposits electrode materials results in well-defined electrode surfaces and enhanced reproducibility (Gautam et al., 2023).

Mechanism of detection

Electrochemical ferritin detection involves binding ferritin to recognition elements like antibodies or aptamers on the electrode surface and measuring electrochemical signals from redox or enzymatic reactions. Correlating these signals with ferritin concentrations in the sample is straightforward. Recent developments in electrochemical sensors for ferritin in blood have included work by Garg et al. (2020) and Boonkaew et al. (2020), all focusing on antibody capture. This research introduced hydrothermally produced ZnO-Mn_3O_4 nanomaterials for specific ferritin detection in blood plasma, enhancing the catalytic properties of the electrodes. The ZnO-Mn_3O_4 composite offers superior sensitivity, stability, and electrocatalytic performance compared to separate ZnO and Mn_3O_4 materials, making it a robust method for detecting ferritin, and monitoring iron metabolism-related conditions. Moreover, working area of the screenprinted electrode was modified using the drop-casting technique to apply the ZnO-Mn_3O_4 nanocomposite. Techniques such as XRD, FESEM, and SPM, which are well-suited for material characterization, were employed to verify the successful formation of the ZnO-Mn_3O_4 material via hydrothermal processes. The developed mechanism is fully portable and user-friendly, allowing for accurate on-site determination of ferritin concentrations in real blood samples (Gautam et al., 2023).

Material analysis

FESEM and XRD were used to analyze the ZnO-Mn_3O_4 nanocomposite's phase and morphology, revealing nanotube and spherical structures. EDX peak analysis confirmed composition, while XRD clarified purity and crystal structure. Notably, the particle size found through FESEM analysis perfectly matched the crystallite size of the ZnO and Mn_3O_4 materials determined by XRD. Lattice tension was estimated using modified Williamson-Hall analysis and the uniform deformation model. The lattice stress within the ZnO-Mn_3O_4 nanocomposite was calculated to be 2.16 × 10-3. Additionally, Scanning Probe Microscopy (SPM) data for the surface of the ZnO-Mn_3O_4 material were gathered as part of the research. The findings from FESEM were supported by the surface topography of the ZnO-Mn_3O_4 nanolayer, confirming the successful formation of the ZnO-Mn_3O_4 nanocomposite (Gautam et al., 2023).

Electrochemical properties

Furthermore, the study examined the electrochemical relationship between the ZnO-Mn_3O_4 modified electrode and plasma samples, as well as phosphate buffer at pH 7, using cyclic voltammetry. It was observed that the phosphate buffer at pH 7 and the screen-printed electrode equipped with a semiconductor nanostructure exhibited no electrochemical activity upon contact. However, upon diluting the initial plasma sample with phosphate buffer at pH 7, a noticeable and irreversible redox peak at -0.76 V, along with a slight oxidation peak at -0.18 V relative to the reference electrode, emerged. The increase in cathodic current was directly proportional to the increase in plasma volume, as Fe^{3+} ions in the plasma were reduced to Fe^{2+} ions at an $E_{1/2}$ value of -0.29 V relative to the reference electrode. The large surface area of the screen-printed electrode modified with ZnO-Mn_3O_4 and its amorphous properties enhanced electron transfer, contributing to increased cathodic current for ferritin detection. Control experiments with a bare screen-printed electrode showed no detectable current, affirming the modified electrode's catalytic performance (Gautam et al., 2023).

After the irreversible reduction reaction between the semiconductor nanostructured screen-printed electrode and plasma ferritin during cyclic voltammetry, amperometric measurements are required. Amperometric responses were recorded at a standard redox potential of -0.76 V for a 0.25 mL plasma sample in phosphate buffer at pH 7. A notable difference in response was observed with the added plasma, indicating a strong electrochemical relationship between the ZnO-Mn_3O_4 surface and plasma ferritin. Amperometric results showed that the cathodic current increased with ferritin concentration, and a calibration graph illustrated a significant linear correlation with an R^2 value of 0.9578. The sensor had a detection limit of 0.04 µg/dL and a rapid response time of 0.1 seconds. However, the strip degraded after three consecutive analyses, suggesting that multiple assays on the same strip are not recommended for accurate physiological detection. Following one experiment, degradation occurs on the modified surface responsible for sensing, making it more suitable for biofluid testing (Gautam et al., 2023).

Validation and stability studies

In the relevant research, electrochemical impedance spectroscopy (EIS) was conducted to assess the interactions between the semiconductor nanostructured electrode and plasma ferritin at various concentrations. The semicircles observed at varying frequencies indicated charge transduction on the ZnO-Mn_3O_4 plasma interface surface. Varied concentrations of plasma ferritin influenced charge transduction on the ZnO-Mn_3O_4 surface. Increased ferritin concentrations led to significant reductions in charge-carrying resistance, facilitating faster interactions between

plasma ferritin and the electrode surface (Gautam et al., 2023).

Specificity and robustness

The robustness and specificity of the electrochemical sensor were evaluated using a cyclic voltammetry curve generated for a specific concentration of ferrous ascorbate tablets. A redox voltage of −0.76 V for ferrous ascorbate tablets matched that of plasma samples, demonstrating high selectivity and specificity of the semiconductor screen-printed electrode for ferritin detection. In contrast, when plasma was tested on a bare screen-printed electrode, no oxidation or reduction peaks were observed, indicating that carbon does not interact electrochemically with plasma (Gautam et al., 2023).

Morphological validation

FESEM images showed no morphological changes in the ZnO-Mn_3O_4 nanocomposite after electrochemical interaction with ferritin, highlighting the material's role in catalyzing these interactions. Scanning Probe Microscopy (SPM) measurements indicated a surface roughness of approximately 47 nm, similar to the initial measurement (Gautam et al., 2023).

Comparison with enzymatic sensors

Unlike many existing ferritin detection sensors that rely on enzymatic methods, this study's sensor does not require immobilization of ferritin antibodies, which can be costly and demand specialized laboratory setups (Gautam et al., 2023).

Validation using biochemical analyzers

Furthermore, validation of the biosensor was conducted by comparing its performance with an automated biochemical analyzer. A calibration plot using plasma ferritin at various concentrations yielded a correlation coefficient of 0.978 , indicating a successful linear correlation. The sensor had a detection limit of 0.04 µg/dL and a rapid response time of 0.1 seconds. Analyses of 20 blood samples showed that the sensor's measurements closely matched the gold standard values provided by the biochemical analyzer, with a correlation coefficient of 0.9998, confirming high predictive accuracy. The coefficient of variation percentage was also determined to examine the data's repeatability (Gautam et al., 2023).

Stability studies

Stability was assessed by analyzing ferritin levels three times daily over several days. Results showed minor deviations across samples, indicating high sensitivity. Furthermore, the electrochemical sensor's stability was evaluated by measuring currents obtained from two distinct ferritin densities over 20 different days. The observation revealed a notably low coefficient of variation for the currents obtained from each density on different days, providing strong evidence of the sensor's stability (Gautam et al., 2023).

Overall, the technique developed in this study eliminates the need for antibodies specific to determining ferritin density in real blood plasma samples. The semiconductor-based, nanostructured, screen-printed electrode, developed as part of this research, not only serves as a catalyst by accelerating the reaction rate but also enables specific ferritin detection. The sensor's specificity is evident through changes in the phase and morphology of the ZnO-Mn_3O_4 nanocomposite before and after biomolecule detection. Real-time validation of each analysis stage and the sensor development process was conducted using human blood plasma. The developed sensor is user-friendly, provides quick readings, is cost-effective, and has a superior detection limit. Laboratory studies indicated a technology readiness level of 4, suggesting potential for refinement and its competitiveness in the commercial market (Gautam et al., 2023).

Limitations of the sensor

While the ZnO-Mn_3O_4-based ferritin biosensor demonstrates excellent sensitivity, selectivity, and response time, several limitations need to be addressed for real-world applications. Although the synthesis process is described as straightforward, the scalability of the hydrothermal method and the potential cost implications of mass production are not discussed. Additionally, the issue of reusability is a limitation, as the sensing surface degrades after a single use. This makes the sensor suitable for single-use applications but raises concerns about waste generation and economic feasibility. Also, the need for calibration across batches to ensure consistent performance in clinical settings requires further standardization. Finally, the sensor's performance was validated with only 20 samples**,** limiting its clinical generalizability. Larger, multicenter studies are needed to assess robustness, user variability, and device performance in real healthcare settings.

Conclusion and future directions

The ZnO-Mn_3O_4-based biosensor offers rapid and sensitive ferritin detection, making it a strong candidate for point-of-care diagnostics. However, challenges such as limited number of uses for testing on one strip of the device and cost-effective scalability remain. For clinical translation, validation across diverse patient samples and regulatory testing are essential. Future work should also explore fabricating such platforms which can be used for much more tests quantatively and multiplexed detection capabilities to expand its clinical utility. With these advancements, this sensor could become a viable and scalable tool for ferritin monitoring in healthcare.

The ZnO-Mn_3O_4-based biosensor offers rapid and sensitive ferritin detection, making it a strong candidate for point-of-care diagnostics. However, the strip's limited reusability and the need for calibration across batches highlight challenges in sustainability and

standardization. Compared to antibody-based sensors, this approach eliminates the need for biological reagents, which can reduce costs and increase robustness, but it is less reusable and may lack the specificity of immunosensors in highly complex samples.

Thus, $ZnO\text{-}Mn_3O_4$ nanocomposite-based biosensors fill a unique niche by providing a user-friendly, rapid, and label-free alternative for ferritin measurement, particularly where low-cost, one-time testing is prioritized over repeated use or multiplexing.

Optical Biosensors

Photonic crystal biosensor

Additionally, this section discusses a unique application of iron oxide nanoparticles (IONPs) in a photonic crystal (PC) optical biosensor for measuring serum ferritin. This approach significantly enhances sensitivity, offering an alternative to traditional biomarkers like ferritin and hemoglobin in diagnosing iron deficiency anemia, especially in resource-limited settings (Lee et al., 2016).

Mechanism of detection and analysis methods

Photonic crystals exhibit selective reflection of specific wavelengths when exposed to broad-spectrum light, with biomolecule binding altering the dielectric permeability and shifting the reflected wavelength. Unlike ELISA tests, which rely on enzyme-labeled antibodies, the photonic crystal sensor utilizes the dielectric permittivity of the analyte, allowing for label-free detection (Boonkaew et al., 2020). The intrinsic dielectric permittivity of the target analyte is utilized by the PC biosensor to facilitate detection. When exposed to broadband light at normal incidence, the interaction between the analyte and the sensor surface induces a refractive index change. This change is measured as a shift in the wavelength of reflected light (in nanometers), enabling the quantification of analytes bound to the sensor. The platform supports both label-free detection and sandwich assays without requiring tagged enzymes. This research employed three analysis methods: label - free, sandwich with a secondary antibody, and a secondary antibody functionalized with IONPs (Peterson et al., 2014).

The findings from these three analyses were obtained using the Biomolecular Interaction Detection (BIND) system. In the label-free analysis, which utilized only a monoclonal antibody, the ferritin detection limit was established at 2.43 µg/mL, with a dynamic range from 2.43 µg/mL to 4.0 µg/mL. Incorporating a secondary polyclonal antibody in the sandwich assay improved sensitivity, reducing the detection limit to 0.38 µg/mL and maintaining a dynamic range between 0.38 µg/mL and 4.0 µg/mL. However, the most significant enhancement was observed when using immunofunctionalized IONPs, achieving an impressive detection limit of 26 ng/mL and extending the dynamic range from 26 ng/mL to 2000 ng/mL (Peterson et al., 2014).

Comparison to ELISA and sensitivity performance

The results demonstrate that iron oxide nanoparticles functionalized with secondary antibodies significantly enhance the sensitivity and performance of the biosensor compared to label-free and sandwich assays. Wavelength shifts in biomolecule detection were notably greater with this approach, while the responses in label-free and sandwich assays remained stable due to their lower dielectric permittivity. The PC biosensor with functionalized antibodies showed double the sensitivity of label-free analyses. It achieved a ferritin detection limit of 26 ng/mL, which is within the typical ELISA range of 5-50 ng/mL (Peterson et al., 2014).

Clinical relevance and future potential

According to World Health Organization (2020), the cutoff values for ferritin to differentiate between iron deficiency and non-deficiency are 30 ng/mL in the presence of inflammation and 15 ng/mL in its absence. Although the PC biosensor's detection limit exceeds the non-inflammatory ferritin cutoff, the results are promising for future enhancements. This biosensor also provides a broader dynamic range than ELISA platforms, allowing for more flexibility in analysis timing and kinetics. This wider detection range reduces the need for multiple dilutions, minimizing variability in the final test results. Thus, this study highlights the effectiveness of the photonic crystal biosensor, combined with immune-functionalized iron oxide nanoparticles and the BIND system, in delivering accurate serum ferritin assessments compared to commercial ELISA tests (Peterson et al., 2014).

Real-world limitations of the PC biosensor

Although the PC biosensor utilizing IONPs demonstrates encouraging potential for ferritin detection as an iron deficiency biomarker, several practical limitations must be considered for its widespread implementation. One key issue is stability under physiological conditions, as the presence of non-specific binding from interfering serum proteins can compromise detection accuracy. Furthermore, the antibody conjugation process to nanoparticles, as outlined in the referenced study, is intricate and may lead to increased production costs and challenges in large-scale manufacturing. The assay also requires precise calibration due to factors such as variability in antibody affinity and steric effects related to nanoparticle size, which can influence overall performance. Despite showing promising sensitivity and reproducibility, the absence of comprehensive optimization studies addressing nanoparticle dimensions and assay parameters suggests that further refinement is necessary to achieve reliable outcomes across varied clinical and environmental settings. Lastly, reliance on specialized instrumentation, such as the

BIND system, raises concerns regarding the feasibility and affordability of deploying this biosensor technology in resource-constrained environments.

Conclusion

The PC biosensor with IONPs represents a significant advancement in ferritin detection, offering enhanced sensitivity and performance compared to traditional ELISA methods. However, technical challenges remain, including the need for improved stability in complex matrices, optimization of IONP size to minimize steric hindrance, and simplification of the conjugation process to reduce production costs. For clinical translation, the development of calibration-free protocols and robust methods to mitigate non-specific binding are critical. Also, regulatory readiness will require extensive validation with diverse population samples, beyond the control sera used in this study. Future directions include integrating the PC biosensor with portable readout systems, such as smartphone-based spectrometers mentioned in the article, to enable point-of-care applications. Additionally, expanding its capabilities to measure multiple biomarkers simultaneously could enhance its utility in diagnosing iron deficiency and related conditions. These advancements will be crucial for transitioning this promising technology from proof-of-concept to widespread clinical adoption.

The PC biosensor with IONPs represents a significant advancement in ferritin detection, offering enhanced sensitivity and performance compared to traditional ELISA methods. However, technical challenges remain, including the need for improved stability in complex matrices, optimization of IONP size to minimize steric hindrance, and simplification of the conjugation process to reduce production costs. While these optical platforms provide broader multiplexing potential than most electrochemical sensors, they typically require more sophisticated instrumentation.

Surface Plasmon Resonance Biosensor

Mechanism of detection

A significant advancement in surface plasmon resonance (SPR) technology has enabled the quantification of ferritin concentrations in HBS-EP buffer and serum for the first time. Monoclonal antibodies were immobilized on a gold surface modified with carboxymethyl dextran using an amine coupling technique, facilitating real-time observation of antigen-antibody interactions. SPR technology, particularly that provided by BIAcore, has proven effective for biospecific interaction analysis, including kinetic data analysis and concentration determination. A sandwich development strategy was employed to enhance sensitivity for detecting biomarkers like IgE and human chorionic gonadotropin. This SPR-based assay is innovative for real-time, label-free immunological testing of serum ferritin. It requires minimal time and can be reused over 50 times. To minimize non-specific protein adsorption, fetal bovine serum was diluted with HBS-EP buffer during measurements. Ferritin was introduced via a 20 µL injection, followed by 0.5 mg/mL ferritin polyclonal antibodies to amplify the response signal. A pH 2.0 glycine-HCl solution was then injected to refresh the surface. Responses were quantified by assessing SPR signal changes relative to the baseline. Each concentration was tested in triplicate, and the detection limit was established based on significant differences compared to controls. Maximizing the quantity of immobilized antibody is crucial for an optimal response, and electrostatic attractions within the dextran layer enhance binding efficiency. Variables such as pH, ionic strength, and protein density were optimized before immobilization. The monoclonal antibody was successfully immobilized, ferritin solution was introduced and subsequently polyclonal antibody injection was used to amplify the signal, resulting in greater sensitivity and specificity (Cui et al., 2003).

Sensorgram observations

The BIAcore system recorded real-time sensorgrams of antigen-antibody interactions. The signal intensified as ferritin bound to the immobilized monoclonal antibody. Following the injection of 0.5 mg/mL polyclonal antibody, the response signal increased further, and the surface was renewed with pH 2.0 glycine-HCl to remove bound ferritin and antibodies (Cui et al., 2003).

Optimal amplification concentration

The concentration of the amplifying antibody is crucial for effective signal amplification in the sandwich strategy. The study assessed different concentrations of ferritin polyclonal antibody post-immunoreaction with 100 ng/mL of ferritin. Polyclonal antibodies' injection concentrations of 0.1, 0.2, 0.5, and 0.8 mg/mL.resulted in amplification factors of 0.86, 1.34, 2.22, and 2.58, respectively, showing that amplification significantly depends on antibody concentration (Cui et al., 2003).

Calibration and detection limits

Using 0.5 mg/mL for the amplification immunoassay minimized non-specific adsorption and reagent use. Calibration curves for ferritin in HBS-EP buffer ranged from 20 to 1000 ng/mL, demonstrating the SPR sensor's capability for measuring ferritin concentrations. The primary response exhibited a linear range over 20-200 ng/mL, with a detection limit of 15 ng/mL. After amplification, the linear range extended, revealing a detection limit of 18 ng/mL. The sandwich assay significantly improved sensitivity and specificity, but the amplified response showed higher relative standard deviations than the primary response. Notably, the detection limit increased from 15 ng/mL to 18 ng/mL post-amplification, likely due to non-specific adsorption of the amplified antibody on the sensor surface (Cui et al., 2003).

Serum ferritin quantification

In the same study, ferritin in fetal bovine serum was also quantified, ranging from 20 to 800 ng/mL. The sensorgram for serum displayed distinct behavior, with a sharp rise and fall in the primary response phase, attributed to the refractive index shift from high protein concentration. Although serum was diluted to reduce non-specific adsorption, it remained elevated. Calibration curves were constructed for ferritin in the serum concentration range of 40-800 ng/mL. In the primary response, the linear range was observed above 40-300 ng/mL ferritin, with a detection limit of 37 ng/mL. The amplified response demonstrated a linear range above 30-300 ng/mL and a detection limit of 28 ng/mL. The differences in calibration curves between serum and HBS-EP buffer were significant, with the amplified curve in serum showing lower values than the primary curve, likely due to non-specific adsorption of proteins during the primary immunoassay. Nonetheless, the amplified response demonstrated greater sensitivity, indicating improved specificity due to the selective interaction between antibodies and the target analyte. The high protein concentration, which may lead to surface blockage, in serum may also reduce non-specific adsorption rates. The relative standard deviation in serum tests was lower in the amplified response compared to the primary response. While the detection limit of 28 ng/mL is higher than the 3 ng/mL limit of particle-enhanced turbidimetric immunoassay, it remains within the normal serum ferritin range (15-300 ng/mL). This study highlights the SPR biosensor's effectiveness for rapid, direct detection of serum ferritin, with enhanced sensitivity and specificity through secondary amplification (Cui et al., 2003).

Stability of sensor surface

Multiple SPR immunoassays can be conducted on the same immobilized surface, but exposure to harsh chemicals may desorb antibodies and antigens adhered to the sensor surface. In the study, surface regeneration was achieved using glycine-HCl buffer. On the same immobilized surface, an amplifying immunoassay for ferritin at 80 ng/mL was performed over 50 cycles, showing that the stability of the immobilized antibody surface remained intact. This research thoroughly examined antibody immobilization, electrostatic adsorption, immune reactions, and regeneration using real-time SPR methods. The ferritin amplification immunoassay demonstrated the potential of this biosensor for clinical serum protein detection (Cui et al., 2003).

Limitations of the SPR-based biosensor

SPR-based biosensors for ferritin detection exhibit notable challenges for real-world applications. Non-specific adsorption of serum proteins, as highlighted in the study, can undermine accuracy and reproducibility. Additionally, the cost of materials, such as monoclonal antibodies and gold-coated surfaces, limits scalability for routine diagnostics. Another critical issue is the need for repeated calibration**.** Although the sensor surface was shown to be reusable for more than 50 cycles, baseline drift and gradual signal loss suggest that periodic recalibration would be necessary to maintain accuracy over time. This could introduce additional complexity in clinical settings. Furthermore, reliance on a laboratory-based BIAcore system and specific reagents restricts portability and point-of-care usability, posing a barrier to clinical adoption.

Conclusion and future directions

SPR biosensors hold great promise for ferritin detection, offering real-time analysis with high sensitivity. However, challenges such as non-specific adsorption, signal drift, and cost constraints must be addressed to enable clinical translation. Improved surface chemistries, cost-effective materials, and robust regeneration protocols are critical. Additionally, integrating SPR sensors into portable platforms and validating them in clinical trials will be necessary for scalability and regulatory approval. Future research should explore nanostructured surfaces and automated calibration systems to enhance sensitivity and usability, paving the way for widespread diagnostic applications.

SPR biosensors hold great promise for ferritin detection, offering real-time analysis with high sensitivity. However, challenges such as non-specific adsorption, signal drift, and cost constraints must be addressed to enable clinical translation. Compared to other optical sensors like photonic crystals, SPR offers superior reusability and rapid analysis but generally requires specialized equipment and is less adaptable to portable or disposable formats than microfluidic or nanocomposite-based sensors.

In this context, SPR biosensors effectively bridge laboratory immunoassay methods and rapid clinical diagnostics, excelling in kinetic analysis and repeat testing, and highlighting the trade-offs between sensitivity, operational complexity, and practical deployment.

Fluorescent Detection of Ferritin Using Two Dimensional (2D) Magnesium Sulfide Nanosheets

For the third example of optical biosensors, below an up-to-date study is explained, which utilizes two dimensional fluorescent nanosheets for detecting ferritin .

Synthesis and characterization

In this study; a stable, fluorescent, greenish-blue hexagonal magnesium sulfide (MgS) nanosheet (NS) was developed via an ultrasonic probe approach. Stirring and ultrasonic techniques were used to synthesize the 2D MgS nanosheets in hexagon shape. Transmission electron microscopy (TEM) morphological study verified the hexagonal nanosheet structure and transparent properties (Babulal et al., 2024).

Photoluminescence properties

When excited at 370 nm, the 2D MgS nanosheets' photoluminescence (PL) intensity peaked at 435 nm after being optimized across a range of wavelengths. This suggests that the excitation affects the material's fluorescence intensity. Further evaluations of the fluorescence intensity were conducted for MgS nanosheets, pure ferritin, and their combination. When ferritin was added, the greenish-blue fluorescence that the nanosheets displayed under UV light decreased in intensity (Babulal et al., 2024).

Interaction with ferritin

A stock solution of ferritin at 0.4 µM in deionized water was prepared and added to a 1 mL cuvette in order to measure the PL intensity as a function of concentration in order to assess the interaction between ferritin and the fluorescent 2D MgS nanosheets. As ferritin concentration rose from 10 nM to 200 nM, a notable decrease in fluorescence intensity for MgS NS was observed. A linear increase in the Stern–Volmer plot, indicative of a static quenching mechanism, was produced by ferritin's significantly stronger fluorescence quenching effect on the nanosheets. The limit of detection was determined as 9 Nm (Babulal et al., 2024).

Selectivity against interfering analytes

Other biomolecules such as albumin, cholesterol, glutathione, lipase, phenylalanine, and tryptophan were compared to ferritin's interaction with the 2D MgS nanosheets. These analyte concentrations were double that of ferritin. Ferritin and MgS were found to interact more strongly, resulting in a fluorescence quenching efficiency of roughly 77%. Ferritin's quenching effect outweighed the other analytes', even at twice the concentration (Babulal et al., 2024).

Application in blood serum analysis

To evaluate the practical applicability of the synthesized MgS nanosheets, they were tested with blood serum samples employing a standard addition method for real sample analysis. The research illustrated the efficacy of the 2D MgS nanosheets in detecting ferritin among diverse interfering agents, including salts, vitamins, and proteins. The PL quenching response was analyzed in spiked serum samples and a linear relationship was found using the Stern-Volmer equation for ferritin concentrations between 10 and 200 nM. The correlation coefficient was very high ($R^2 = 0.9912$). These results suggest that the developed fluorescent sensor utilizing MgS nanosheets has potential as a diagnostic instrument for evaluating ferritin concentrations in clinical environments (Babulal et al., 2024).

Limitations and conclusion

Despite the promising sensitivity and selectivity of the 2D MgS NS-based biosensor, some limitations must be addressed for real-world applications. The stability under physiological conditions is a concern, as the study demonstrates short-term stability, but long-term behavior in complex biological matrices remains unexplored. The cost of nanomaterials and reliance on pure reagents (e.g., $MgCl_2$ and 1-methylimidazole) may hinder scalability, despite the simplicity of the synthesis process. Additionally, the biosensor requires precise calibration. Lastly, although selectivity tests showed minimal interference from other biomolecules,further validation in varied biofluids is essential for robust clinical performance.

The 2D MgS NS biosensor offers a sensitive, selective, and innovative approach for detecting ferritin, but challenges remain for clinical translation. Stability in physiological conditions must be enhanced, and the synthesis process optimized to reduce costs. Regulatory-readiness will require standardized production methods that meet safety guidelines. Future efforts should aim to improve the sensor's robustness in complex biofluids, broaden its detection range, and integrate it into portable diagnostic systems. Alternative nanomaterials or doping strategies could enhance fluorescence performance and reduce costs. These advancements will enable the biosensor's scalability and establish its potential as a practical diagnostic tool for ferritin-related diseases.

The 2D MgS NS biosensor offers a sensitive, selective, and innovative approach for pdetecting ferritin, but challenges remain for clinical translation. Stability in physiological conditions must be enhanced. Compared to other optical biosensors, MgS-based fluorescent sensors offer simpler, label-free detection with high specificity.

Overall, fluorescent nanosheet biosensors expand the toolkit for ferritin measurement, providing a rapid and visually interpretable platform, particularly valuable where ease of use is desired, yet still need further development to match the robustness and versatility of other established biosensor classes.

Unconventional Biosensors

Ferritin detection by using nanodiamonds

The paper further explores the use of color centers in nanodiamonds for ferritin detection. These nanometer-sized diamonds, equipped with nitrogen-vacancy defect centers (NV), exhibit exceptional biocompatibility, intense fluorescence, and impressive magnetic sensitivity, making them promising nanosensors for use within living organisms (Noah & Ndangili, 2022). The use of color centers in nanodiamonds has gained traction as a research avenue, offering applicability within multicellular organisms while overcoming the limitations of conventional agents like dyes, fluorescent proteins, and quantum dots, which are often used to visualize internal cellular structures (Garg, Chatterjee, et al., 2020).

Mechanism and experimental approach

The detection mechanism leverages the magnetic noise generated by the paramagnetic iron core of ferritin molecules as a contrast feature. When ferritin molecules are present on the surface of nanodiamonds (Figure 2), their magnetic properties interact with the NV centers, leading to a pronounced decrease in both coherence time and relaxation time. These measurable reductions serve as indicators of ferritin detection. The NV centers' fluorescence, governed by their spin state, enables optical readout and precise manipulation even at am, positioning them as sensitive atomic-scale magnetic sensors. Experimental results demonstrated that ferritin induces spin decoherence through thermally driven fluctuations in its unpaired electron spins, significantly diminishing the spin stability of nearby NV centers. Compared to traditional methods such as atomic force microscopy and immunoassays—often constrained by complexity, cost, or potential biomolecular damage—this approach offers a non-invasive, highly sensitive alternative. The study further explored the potential of nanodiamonds functionalized for biomolecular binding, facilitating ferritin adsorption through electrostatic interactions, thereby establishing a foundation for intracellular biosensing using NV centers (Ermakova et al., 2013).

Instrumentation and measurements

Fluorescence and optically detected magnetic resonance were measured using a custom-built confocal microscope, with a 532 nm excitation wavelength. For accurate measurements, nanodiamonds were spin-coated onto a glass coverslip, with microstrip lines created through photolithography for microwave application. The Hahn echo pulse sequence was used to assess electron spin coherence times, while an adapted inversion recovery technique measured relaxation times (Ermakova et al., 2013).

Results and sensitivity

In this study, 64 nanodiamonds were divided into two groups: one with free, uncoated nanodiamonds and the other with ferritin-coated nanodiamonds. It was found that the electron spin coherence and relaxation times of ferritin-conjugated nanodiamonds were about seven times shorter than those of free nanodiamonds. Specifically, ferritin-conjugated nanodiamonds had a relaxation time of 5.7 µs and a coherence time of 1.6 µs, compared to 41.8 µs and 6.0 µs for free nanodiamonds. These findings demonstrate the effectiveness of nanodiamonds as nanoscale magnetic field sensors, showing that even small amounts of ferritin significantly affect nitrogen-vacancy spin properties. Remarkably, this sensitivity allows for the detection of a single ferritin molecule near the nanodiamond sensor. The observed magnetic field fluctuations result from interactions between the electron spins of the NV center and Fe^{3+} ions, which reduce relaxation time, while coherence time is affected by longitudinal interactions (Ermakova et al., 2013).

The variation of nanodiamonds' sizes

Nanodiamonds vary in size, complicating the localization of NV centers. This variation causes differences in the distance between ferritin molecules and the NV center, leading to fluctuations in the magnetic field's magnitude. However, the study accounted for these variations by assuming random placement of 8-15 ferritin molecules bound to nanodiamonds. This research highlights the potential of NV centers for detecting individual ferritin molecules, showcasing the sensitivity needed for single protein detection and facilitating cellular studies. With further development, this technique could be instrumental in analyzing critical biological processes, including electron transfer (Ermakova et al., 2013).

Challenges and future potential

Despite their potential, NV center-based nanodiamond sensors face several real-world limitations. One challenge is their stability under physiological conditions, as noncovalent binding of ferritin molecules to nanodiamonds may be disrupted by pH or ionic changes in biological environments. Additionally, the cost of nanomaterials could hinder scalability, given the complex synthesis methods described in the study. Another limitation is the need for calibration, as the variability in the distance and orientation between ferritin and NV centers affects measurement accuracy. Moreover, signal variability resulting from the variety in nanodiamond size and characteristics presents challenges for reproducibility. These issues highlight the need for robust, cost-effective, and standardized approaches to enhance the practicality of these sensors.

NV center-based nanodiamond sensors offer a breakthrough in single-molecule detection, but technical challenges remain. Variability in nanodiamond properties and ferritin adsorption requires better surface functionalization to improve stability and reproducibility in physiological environments. For clinical translation, standardized protocols for nanodiamond fabrication and calibration are essential. Future research should focus on cost-effective production methods and exploring covalent surface modifications to enhance stability. Expanding the range of detectable biomolecules and advancing multi-NV center systems could improve sensitivity and enable broader applications in diagnostics and biomedical research. These advancements would bring NV center-based sensors closer to clinical and regulatory readiness, paving the way for transformative impacts in healthcare.

NV center-based nanodiamond sensors offer a breakthrough in single-molecule detection, but technical challenges remain. Variability in nanodiamond properties and ferritin adsorption requires better surface functionalization to improve stability and

reproducibility in physiological environments. While these sensors achieve unmatched sensitivity at the molecular level—surpassing even GFETs—they are currently less practical for routine diagnostics due to complexity and cost, as compared to more established immunoassay or electrochemical platforms.

Thus, nanodiamond-based sensors fill a unique research gap, enabling quantum-level detection and single-molecule studies, but are best suited for specialized settings where ultimate sensitivity outweighs throughput.

Immunoassay-Based Biosensors

Horn-like polycrystalline-silicone nanowire field-effect transistor

Another study examined ferritin detection using a distinctive horn-like polycrystalline-silicon nanowire field-effect transistor (poly-Si NW FET) biosensor. This sensor utilized a tailored dummy gate structure with a trapezoidal configuration to craft nanowires resembling horns (Yen et al., 2016).

Mechanism of field-effect transistor biosensors

FET biosensors, including carbon nanotube and nanowire variants, excel in converting molecular interactions at their surfaces into electrical signals. These biosensors operate on the principle of conductivity changes within the transistor channel, primarily driven by analyte binding to receptors on the sensor gate. The presence of a molecular dipole layer modifies the oxide surface's work function, while the effective charges carried by the molecules induce surface band bending. These combined effects shift the transistor's flat band voltage, consequently altering the threshold voltage. FETs are highly sensitive to analyte binding, making them versatile and efficient tools for detecting a wide array of biomolecules. However, carbon nanotubes face limitations such as inefficiency, short tube lengths, high impurity levels, irregular diameters, and challenges in controllability and scalability (Ertaş et al., 2024). In contrast, nanowire field-effect transistors have shown the ability to detect label-free electrical flow and facilitate biological recognition (Yen et al., 2016).

Challenges and advancements in nanowire transistor fabrication

The electrical detection of biomolecular interactions on NW FET surfaces is gaining traction for developing affordable, portable sensors that require no specialized training. However, fabricating silicon-based NW FETs for bio-detection remains challenging, especially with "bottom-up" or "top-down" lithography methods. Researchers are focused on simple, cost-effective techniques to produce poly-Si NW FETs for medical applications. Using a polycrystalline silicon sidewall spacer technique allowed for nanoscale pattern identification, aligning with commercial semiconductor processes and avoiding costly beam lithography for mass production (Yen et al., 2016).

Features of horn-like nanowire transistors

Horn-like poly-Si NW FETs are notable for their selectivity, sensitivity, real-time response, and label-free detection, making them promising sensors for biological environments. These nanowires are created using a tetraethyl orthosilicate etching method concealed by a dummy gate design, which simplifies fabrication and offers cost savings through efficient lithography. The study found that these corroded horn-like nanowires had a higher orderliness than those grown via vapor-liquid-solid methods. Their vertical configuration allows for potential innovations in 3D integration (Yen et al., 2016).

Surface functionalization for ferritin detection

The devices detect serum ferritin using anti-ferritin antibodies as recognition agents functionalized on the sensor surface. Effective detection requires surface functionalization of polycrystalline silicon nanocables. This involves attaching specific recognition agents to achieve selectivity. The silicon oxide on the nanocable's surface is crucial during silanization, where silanol groups are converted into amines using 3-aminopropyltriethoxysilane. A bifunctional linker, glutaraldehyde, is then employed for the secure immobilization of anti-ferritin, enhancing binding affinity (Yen et al., 2016).

Electrical mechanism of antigen detection

The detection mechanism of an immuno-field effect transistor biosensor is based on the interaction between biological receptors and their target analytes. In this system, specific biological receptors, such as antibodies, are chemically immobilized onto the gate oxide surface of the sensor. These receptors exhibit a strong and selective binding affinity toward their corresponding target analytes, such as antigens, within a buffered environment. When an antigen binds to the antibody on the gate oxide surface, this interaction induces changes in the surface potential of the semiconductor channel. The altered surface potential modulates the channel's conductance, which, in turn, leads to variations in the drain current of the device. This current change is directly linked to the nature and strength of the antigen-antibody interaction, providing a means to detect the presence of the target analyte. The type of semiconductor material used in the FET biosensor determines the direction of the current change. For instance, in n-type semiconductors, where electrons are the majority charge carriers, the current response reflects the charge polarity of the antigen. Conversely, in p-type semiconductors, which rely on holes as the majority carriers, the response similarly depends on the charge associated with the analyte. The magnitude of the current change correlates with the binding strength between the antigen and the antibody,

allowing for quantification of the interaction. The detection process assumes that antigen-antibody binding at the gate oxide surface alters the charge distribution perpendicular to the sensor surface. This redistribution of charges is a key principle enabling the immuno-FET biosensor to convert biomolecular interactions into measurable electrical signals with high sensitivity and specificity (Yen et al., 2016).

Debye length challenges in field-effect transistor biosensors

A challenge arises from the electrical double layer, or Debye length, which limits charge detection to a few nanometers under physiological ionic strength. Since proteins, like antibodies, are about 10 nm in size, the Debye length can hinder the field-effect transistor's charge detection capability. In a 0.1 × PBS solution, the Debye length is approximately 2.3 nm and about 7.3 nm in a 0.01 × PBS solution. The height of antibodies immobilized on the sensor was around 4 nm, necessitating the use of a 0.01 × PBS buffer to ensure adequate Debye length. Thus, stable antigen-antibody interactions must involve two molecules under these conditions (Yen et al., 2016).

Ferritin detection results and sensor performance

Understanding the Debye length is crucial for the performance of sensitive polycrystalline silicon nanowires during detection. When antigens bind strongly to the antibody-modified surface, detection is achieved by monitoring the threshold voltage of the polycrystalline nanowire. In this study, ferritin detection utilized a horn-like polycrystalline silicon nanowire biosensor in a PBS buffer solution. After introducing 50 pg/mL of ferritin, a significant decrease in drain current was noted due to increased negative charge from antigen-antibody binding. The sensor's sensitivity was further validated by varying concentrations of serum ferritin from 50 pg/mL to 500 ng/mL, demonstrating impressive linearity (R^2 = 0.9997) across this range. The biosensor successfully detected ferritin concentrations as low as 50 pg/mL (Yen et al., 2016).

Sensor advantage

In biomedical diagnostics, the initial step involves modifying the nanowire surface with the appropriate ferritin antibody before detection. The exceptional sensitivity of the ferritin antibody-functionalized horn-like polycrystalline silicon nanowire sensor stems from strong binding affinity between the antigen and antibody. Furthermore, this sensor features a 2 µm long channel, a lower threshold voltage of 1.1 V, and a significantly larger on-off current ratio (I_{on}/I_{off}) of 3.47 × 10^5. It also exceeds the Nernst limit with a sensitivity of 133.47 mV/pH, thanks to a high capacitive-coupling ratio. This study represents the first application of a horn-like polycrystalline silicon nanowire FET biosensor for ferritin detection, holding promise as a diagnostic device for general clinical examinations (Yen et al., 2016).

Real-world limitations of the biosensor

The horn-like poly-Si NW FET biosensor faces several real-world challenges. Stability under physiological conditions is a concern, as the sensor operates optimally in low ionic-strength solutions (e.g., 0.01× PBS), which differ from the high ionic-strength environments of human serum. Additionally, while the fabrication process avoids expensive lithography techniques, the cost of nanomaterials and thermal processes may hinder scalability. Furthermore, calibration requirements may increase complexity in clinical settings, as variability in human samples can affect measurement accuracy. These limitations must be addressed for successful real-world applications.

Conclusion and future outlook

The horn-like poly-Si NW FET biosensor offers high sensitivity (133.47 mV/pH) and low detection limits (50 pg/mL ferritin), making it promising for diagnostics. However, stability in physiological conditions and calibration protocols remain critical challenges. For clinical translation, validation in real human samples and integration into portable diagnostic platforms are needed. Future research should focus on enhancing membrane stability, developing multiplex detection, and enabling real-time wireless monitoring. These advancements will help this sensor transition from a prototype to a clinically viable technology.

The horn-like poly-Si NW FET biosensor offers high sensitivity (133.47 mV/pH) and low detection limits (50 pg/mL ferritin), making it promising for diagnostics. However, stability in physiological conditions and calibration protocols remain critical challenges. While these field-effect devices rival GFETs in sensitivity and outperform many optical sensors in miniaturization, they still face limitations in operation within complex biological matrices and in large-scale manufacturing.

In summary, poly-Si NW FETs bridge the gap between academic proof-of-concept and potential clinical deployment and are poised to contribute substantially once operational issues are resolved.

Other Electrical Ferritin Biosensors

The paper also briefly discusses electrical ferritin biosensors, which utilize quantum dots or golden nanorod reporter probes as well as a paper-based ferritin biosensor. Features of the sensors described in this section are briefly mentioned in order to demonstrate to the reader what other different types of ferritin sensors there are, how much research has been done in this field, and the variety.

The first sensor discussed is an electrochemical device using tungsten disulfide quantum dots to identify human serum ferritin, boasting a linear range of 10 to 1500 ng/mL and detection limits of 6.048 ng/mL in cyclic

voltammetry and 3.800 ng/mL in direct pulse voltammetry (Selvarajan et al., 2021).

The second sensor, developed by Garg et al. (2021), utilizes transition metal chalcogenides, specifically hexagonal boron nitride quantum dots, for serum ferritin determination. These dots, known as white graphene, improved the sensor's linear range and detection limit, with a linear range of 10 to 2000 ng/mL and a detection limit of 1.306 ng/mL. These quantum dots, recognized as 2D layered materials, enhanced the device's analytical performance. Their thermal stability, chemical resistance, and mechanical strength make them well-suited for integration into electrical biosensor platforms (Selvarajan et al., 2021).

In another study, Song et al. (2017) introduced a sensitive electrochemical method for detecting human serum ferritin, utilizing a natural cotton thread-integrated gold nanorod reporter probe.

This sensor boasts a detection limit of 1.58 ng/mL within 30 minutes and a broad linear range of 5 to 5000 ng/mL, making it suitable for detecting elevated ferritin levels in various clinical scenarios and for application within COVID-19 electrical biosensing platforms (Selvarajan et al., 2021).

Lastly, Boonkaew et al. (2020) developed a paper-based analytical device for serum ferritin detection, incorporating graphene oxide. This device has a linear range of 1–1000 ng/mL and an exceptionally low detection limit of 0.19 ng/mL, maintaining stability for up to three weeks. This handcrafted paper-based sensor was employed to quantify ferritin levels in individuals diagnosed with iron deficiency anemia (Selvarajan et al., 2021).

Conclusion

This review has examined the current landscape of ferritin biosensor technologies, highlighting significant advances in material science and sensor design that could address persistent challenges in iron-related diagnostics. Innovative platforms—including graphene-based field-effect transistors (GFETs), photonic crystal biosensors, nanodiamond quantum sensors, and microfluidic- or nanocomposite-based electrochemical devices—demonstrate substantial promise for both research and future clinical applications. For example, the GFET-based biosensor discussed herein achieved a record detection limit of <10 ng/L for ferritin, markedly surpassing the sensitivity of standard ELISA assays and enabling the potential for non-invasive, real-time monitoring. Likewise, microfluidic immunosensors and $ZnO–Mn_3O_4$ nanocomposite electrodes offer rapid, cost-effective detection with detection limits as low as 0.04 µg/dL, and surface plasmon resonance (SPR) platforms have demonstrated reliable reusability and specificity in buffer and serum.

However, it is important to emphasize that, despite these advances, most ferritin biosensor technologies remain at the proof-of-concept or early laboratory validation stage. Many platforms—including GFETs and nanodiamond-based sensors—face significant hurdles related to stability in physiological conditions, device-to-device reproducibility, calibration requirements, and large-scale manufacturability. The majority of reported performance data derive from controlled samples or spiked matrices, and robust validation using diverse, real-world clinical samples is still lacking. Furthermore, the operational complexity and cost of some optical and nanomaterial-based systems may limit their immediate adoption in point-of-care or decentralized settings.

Looking forward, the clinical translation and widespread implementation of ferritin biosensors will require further standardization, large-scale clinical validation, and the establishment of regulatory protocols. With further optimization—such as enhancing sensor robustness in complex biological fluids, expanding multiplexing capabilities, and reducing production costs—these technologies could eventually complement or even improve upon current gold standards in iron status assessment. Pending successful clinical trials and regulatory approval, next-generation biosensors hold the potential to enhance early detection and monitoring of iron deficiency and related disorders, improve accessibility to diagnostics in resource-limited settings, and support more personalized approaches to patient care.

In summary, while ferritin biosensors represent a rapidly evolving area of research with considerable potential, their clinical impact will ultimately depend on rigorous validation, comparative studies with established methods, and demonstration of real-world benefits in healthcare delivery.

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Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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Table 1. Advantages, disadvantages and clinical applications of the ferritin biosensors.

Type of the Biosensor	Advantages	Disadvantages	Clinical Applications

Graphene- Based Biosensor	• The production of graphene is straightforward, and it is both affordable and readily available on the market (Oshin et al. 2020).
	• Graphene's two-dimensional (2D) flat surface makes it highly suitable for functionalization (Oshin et al. 2020).
	• It enables the most sensitive detection of ferritin among all reported sensors (Oshin et al. 2020).
	• Employing anti-ferritin specific antibodies allows for the creation of a highly selective biosensor that exclusively targets ferritin (Oshin et al. 2020).
	• Supports real-time monitoring and analysis.
	• Offers the possibility of detecting ferritin through non-invasive methods (Oshin et al. 2020).
		• The resolution of the Silhouette Cameo cutting machine limits the minimum sizes achievable for electrode patterning (Oshin et al. 2020).
		• Charge changes on the FET surface are detectable only within the Debye length, as charges beyond this distance are shielded by electrostatic effects (Oshin et al. 2020):
		  ○ High ionic strength shortens the Debye length, reducing the graphene field-effect transistor's (GFET) detection range. Low-ionic-strength solutions improve detection but may not accurately reflect physiological conditions (Oshin et al. 2020).
		  ○ The ~4 nm antibody immobilization layer affects detection sensitivity by adding height which can fall within the Debye length (Oshin et al. 2020).
			• GFETs hold great promise for non-invasive, ultra-sensitive detection of iron deficiency, with extremely low detection limits (Oshin et al. 2020).

Microfluidic-Based Biosensor	• The microfluidic flow cell-based electrochemical detection method is highly sensitive and selective for ferritin measurement (Garg, Christensen, et al., 2020). • The lab-on-chip with NH_2-GO-functionalized SPCE detects ferritin in the 7.81–500 ng/mL range, covering clinically relevant levels and maintaining stability against pH changes and interferents (Garg, Christensen, et al., 2020). • Amine-functionalized SPCE simplifies the process by enabling both electrode modification and immuno-sensing on a single platform (Garg, Christensen, et al., 2020). • The sensor remains stable, with only a 6.7% reduction in current after 48 continuous cycles (Garg, Christensen, et al., 2020). • The device is adaptable for detecting other biomarkers by replacing the bio-receptor (Garg, Christensen, et al., 2020).	• The sensor's reusability declines over time, showing an 11% reduction in current response, five regeneration cycles later (Garg, Christensen, et al., 2020). • Spiked serum sample tests showed 5–10% deviation in ferritin measurements, indicating the need for further optimization (Garg, Christensen, et al., 2020). • The detection range is more limited compared to ELISA, and the system falls short of ELISA's upper detection limit of 1500 ng/mL (Garg, Christensen, et al., 2020).	• Screen-printed electrodes offer a low-cost, consistent platform for electrochemical measurements and are compatible with portable potentiostats for point-of-care and field use. • The device is suitable for small clinical setups and can be operated by minimally trained technicians (Garg, Christensen, et al., 2020). • Continuous flow systems provide efficient real-time analysis of multiple patient samples in clinical environments (Garg, Christensen, et al., 2020). • The device's flexibility enables its use for detecting a variety of biomarkers, enhancing clinical applications (Garg, Christensen, et al., 2020).

	• The handheld potentiostat is both portable and affordable (Garg, Christensen, et al., 2020).		

Ferritin Detection by Utilizing ZnO-Mn_3O_4 Nanocomposite	• Combining ZnO and Mn_3O_4 as a nanocomposite exhibits synergistic effects that significantly improve its electrochemical performance, resulting in being more sensitive, selective, and stable for ferritin detection compared to the use of individual nanomaterials (Gautam et al., 2023).
	• This composite provides an expanded surface area for ferritin attachment thanks to the interconnected nature of its nanoparticles (Gautam et al., 2023).
	• The inclusion of Mn_3O_4 increases electrocatalytic efficiency, thereby enhancing the sensor's sensitivity and lowering its detection limit (Gautam et al., 2023).
	• Mn_3O_4 contributes to the stability of the composite structure and does not allow ZnO nanoparticles to aggregate, guaranteeing the sensor's reproducibility and consistent performance over time (Gautam et al., 2023).
	  • The sensor has a detection limit of 0.04 µg/dL and responds rapidly in just 0.1 seconds (Gautam et al., 2023).
	  • The semiconductor nano-structured decorated screen-printed electrode (SND-SPE) functions as a catalyst, facilitating faster reactions while precisely detecting ferritin (Gautam et al., 2023).
	  • The SND-SPE excels in cost-effectiveness, user-friendliness,providing quick readout and enhanced detection limits compared to other sensors (Gautam et al., 2023).
		• The sensor strip shows degradation after three usages, suggesting that it is appealing to conduct multiple tests on the same strip for accurate physiological readings (Gautam et al., 2023).
		• The current laboratory study indicates a technology readiness level (TRL) of 4. To reach a higher TRL, future research should explore a broader range of input parameters and include comprehensive material characterization (Gautam et al., 2023).
			• The ZnO-Mn_3O_4 layer effectively catalyzes the reduction of Fe3+ ions, resulting in a device that is robust, quick, stable, affordable, and biodegradable—ideal for point-of-care applications (Gautam et al., 2023).
			• The entire setup is fully portable and can be conveniently used for precise, on-site measurement of plasma ferritin levels in actual blood samples (Gautam et al., 2023).
			• This sensor shows promise for precise and dependable detection of ferritin in blood plasma, aiding in the early diagnosis and tracking of various diseases and disorders associated with iron metabolism (Gautam et al., 2023).

Photonic Crystal Biosensor	• The photonic crystal (PC) biosensor is highly sensitive, with the ability of sensing serum ferritin levels as low as 26 ng/mL (Peterson et al., 2014). • These biosensors demonstrate excellent reliability, with minimal inter-and intra-assay	• Non-specific binding in complex samples like serum can affect biosensor sensitivity (Peterson et al., 2014). • Functionalizing antibodies with nanoparticles adds complexity to	• PC biosensors with the Biomolecular Interaction Detection (BIND) system show potential as effective diagnostic tools for detecting iron

	variability (Peterson et al., 2014). • Iron-oxide nanoparticles (IONPs) improve the signal-to-noise ratio, outperforming conventional detection methods (Peterson et al., 2014). • Compared to enzyme-linked immunosorbent assay (ELISA) tests, the PC biosensor assay shows lower bias and tighter agreement for measuring ferritin in control sera (Peterson et al., 2014).	the preparation process (Peterson et al., 2014). • Despite promising results, PC biosensors require further validation compared to well-established ELISA methods (Peterson et al., 2014).	deficiency (Peterson et al., 2014). • Proof-of-concept studies confirm that iron-oxide nanoparticles improve the sensitivity of PC biosensors for detecting nutritional biomarkers (Peterson et al., 2014).
Surface Plasmon Resonance Biosensor	• This real-time method for ferritin detection is quick, does not require labeling, and allows reuse of the same surface over 50 times. • The assay is satisfactorily sensitive, specific and reproducable (Cui et al., 2003). • Sensitivity and specificity can be improved in surface plasmon resonance (SPR) biosensors by using a sandwich method with analyte-specific antibodies (Cui et al., 2003). • The detection limit for ferritin in serum is 28 ng/mL (amplified), making it suitable for detecting ferritin levels within the normal range of 15–300 ng/mL (Cui et al., 2003).	• The detection limit of particle-enhanced turbidimetric immunoassay was lower than that of this study's assay in serum (Cui et al., 2003). • In HBS-EP buffer, relative standard deviations (RSDs) rose from 0.1%–2.2% (primary) to 1.3%–10.7% (amplified) in the 20–1000 ng/mL ferritin range and a slight increase in limit of detection (LOD) was observed from 15 ng/mL to 18 ng/mL, likely due to amplified antibodies which were adsorbed on the sensor surface non-specifically (Cui et al., 2003). • The response signal decreased by approximately 15.6% after 50 measurement cycles (Cui et al., 2003).	• The amplified immunoassay with real-time SPR biosensors proves effective for rapid detection of serum proteins in clinical applications (Cui et al., 2003).
Fluorescent Detection of Ferritin Using 2D Magnesium Sulfide Nanosheets	Ultrathin 2D nanosheets offer high surface-to-volume ratio and sensitivity, making them ideal for biosensing (Babulal et al., 2024). Sulfur-based nanomaterials are non-toxic, water-dispersible, and possess strong optical and catalytic properties (Babulal et al., 2024). Metal sulfide nanostructures exhibit notable luminescence and photochemical activity (Babulal et al., 2024). MgS nanosheets demonstrate high selectivity for ferritin with 77% quenching, a 9 nM LOD, and a linear detection range of 10–200 nM (Babulal et al., 2024).	The disadvantages of this biosensor are not explicitly mentioned in its original article. So, we explain some possible disadvantages: • Synthesis via ultrasonication may result in inconsistencies in nanosheet size, shape, or surface chemistry, affecting reproducibility. • The long-term stability of the fluorescent MgS nanosheets under ambient or storage conditions is not established. • Detection relies on fluorescence spectrometry, which may not be readily available in all diagnostic settings.	Sensitivity of the fluorescent 2D nanosheets can be further enhanced for clinical ferritin detection (Babulal et al., 2024). The biosensor effectively detected ferritin in serum with strong linearity, showing promise for clinical use (Babulal et al., 2024).
Ferritin Detection by Using Nanodiamonds	• Nanodiamonds with nitrogen-vacancy (NV) centers are highly suited for biological sensing due to their non-toxic nature,	• Variations in nanodiamond size and the uncertain positioning of NV centers create inconsistencies in the distance to ferritin molecules,	• The method can be adapted to cellular environments (Ermakova et al., 2013).

	strong fluorescence, and sensitivity to magnetic fields under ambient conditions (Ermakova et al., 2013). • The fluorescence of NV centers is influenced by their electronic spin state, which enables detecting optically and manipulating individual spins at room temperature (Ermakova et al., 2013). • NV centers are excellent atomic-scale magnetic field sensors because they have long spin coherence times (Ermakova et al., 2013). • Even a small number of ferritin molecules significantly affect NV spin properties, proving the potential of nanodiamonds as nanoscale magnetic sensors (Ermakova et al., 2013). • This technique has the sensitivity to detect single proteins and can be adapted for use in cellular environments (Ermakova et al., 2013).	leading to fluctuating magnetic field measurements (Ermakova et al., 2013).	
Horn-Like Polycrystalline-Silicon Nanowire Field-Effect Transistor	• The horn-like polycrystalline-silicon nanowire field-effect transistor (poly-Si NW FET) sensor demonstrated enhanced sensitivity, achieving 133.47 mV/pH, which surpasses the Nernst limit (Yen et al., 2016). • A 2 µm channel length in the horn-like poly-Si NW FET device provided optimal performance, including a lower threshold voltage (1.1V) and a high Ion/Ioff ratio of 3.47×10^5 (Yen et al., 2016). • The sensor successfully detected serum ferritin concentrations as low as 50 pg/mL in a microfluidic channel (Yen et al., 2016). • The poly-Si NW FET device is highly potent for biosensing thanks to being extremely sensitive and specific, providing real-time response and label-free detection (Yen et al., 2016).	• Maintaining close proximity between biological species and the semiconductor surface is vital for high sensitivity at physiological salt concentrations, where the Debye length becomes short (Yen et al., 2016). • Adjusting the Debye length to match receptor height is essential to enhance detection sensitivity (Yen et al., 2016).	• This horn-like poly-Si NW biosensor shows promise as a diagnostic tool for routine clinical tests (Yen et al., 2016).

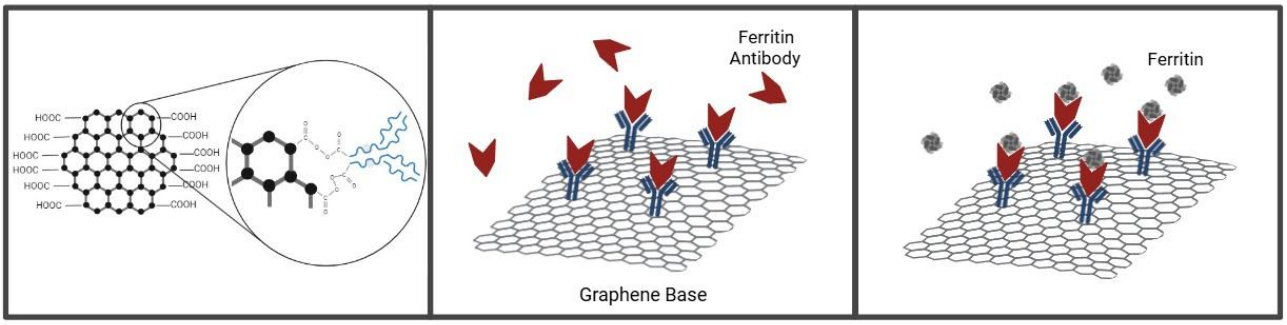

Figure 1. (A) Structure of graphene; (B) Immobilization of anti-ferritin antibodies on the graphene base; (C) Binding process of ferritti-ferritin antibodies.

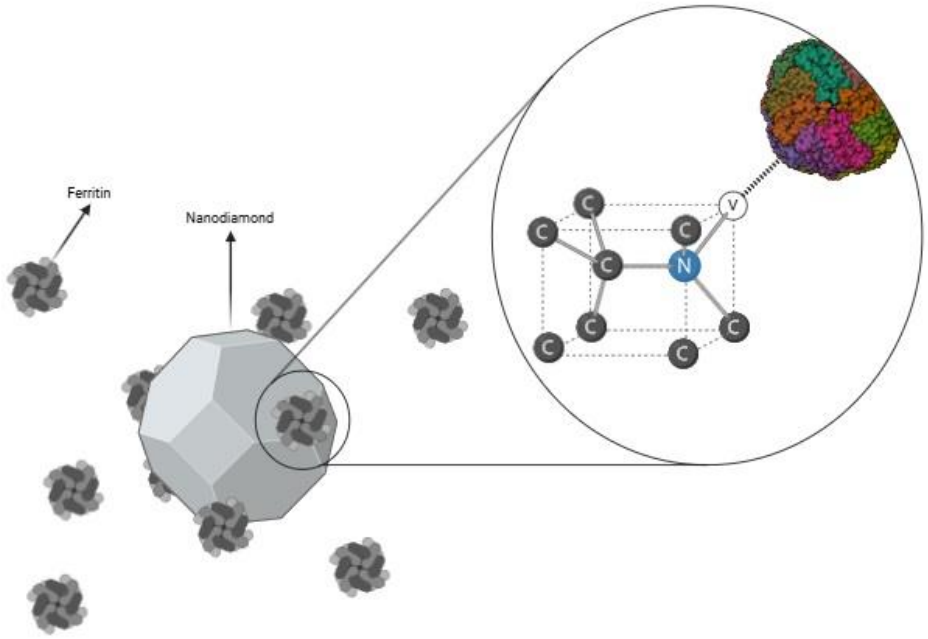

Figure 2. Binding of ferritin molecules to nanodiamonds (C=Carbon, N=Nitrogen, V=Vacancy center).