Analytical Science Advances

WILEY-VCH

Chemistry Europe
European Chemical Societies Publishing

REVIEW OPEN ACCESS

Cost-Effective Nanosensor Solutions for Ultra-Sensitive Detection of Metronidazole

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Received: 19 November 2024 | **Revised:** 12 January 2025 | **Accepted:** 18 January 2025

Funding: The authors received no specific funding for this work.

Keywords: antibiotic | biosensors | electrochemical | metronidazole

ABSTRACT

Metronidazole (MNZ) is a widely used imidazole antibiotic effective against bacterial and protozoal infections, including giardiasis, trichomoniasis, bacterial vaginosis, and antibiotic-associated colitis. However, prolonged and excessive use of MNZ can lead to serious side effects, such as peripheral neuropathies, toxicity, and optic neuropathy. Therefore, the accurate detection and removal of MNZ present significant technical challenges. This manuscript introduces novel approaches for the development and integration of precise and cost-effective sensors specifically designed for the accurate measurement of MNZ levels. We explore cutting-edge nanotechnology strategies for detecting MNZ, with a particular focus on innovative nanobiosensors, including photodynamic-based biosensors, acousto dynamic sensors, and electrochemical biosensors. Additionally, we delve into the unique challenges and opportunities associated with multiphysics biometric biosensors and related nanotechnologies in the detection of MNZ. This review not only provides insights and scientific evidence regarding the application of nanobiosensors for the accurate measurement of MNZ but also highlights recent advancements in sensor technology that represent a significant leap forward in this field. By emphasizing these novel contributions, we aim to pave the way for future research and development in this critical area. Ultimately, our findings underscore the importance of reliable detection methods in mitigating the risks associated with MNZ use and improving patient safety.

1 | Introduction

Antibiotics have been used since ancient times to treat and prevent bacterial infections in humans and animals [1, 2]. They have saved millions of lives and ended many of the infectious diseases that have plagued humanity for centuries. On the other hand, despite the significant increase in the population in the past decades, antibiotics have improved the quality of human life in the whole world while reducing mortality [1, 2]. Antibiotics have significantly increased life expectancy. Metronidazole (MNZ) is a nitroimidazole-based drug used as an antiprotozoal and antibacterial agent. It is commonly used to treat mange, anti-anaerobic infections, and acne diseases. Long-term use of this drug can lead to drug resistance, posing a health risk to

consumers [3, 4]. The detection of MNZ is crucial across various clinical and environmental contexts, given its widespread use as an antiprotozoal and antibacterial agent. Initially introduced to combat infections in humans and animals, MNZ has proven effective in treating conditions ranging from bacterial infections to skin disorders. However, its extensive application has raised significant concerns regarding safety, particularly with the illegal use of MNZ in cosmetics and its potential for inducing drug resistance. This duality—its therapeutic benefits versus its risks—highlights the urgent need for reliable detection methods. However, due to its high antibacterial efficacy and low price, MNZ is still used illegally as an additive in cosmetic ingredients by many commercial companies [3, 4]. Accumulation of MNZ in the human body can cause toxic effects such as peripheral neuropathy and seizures, which are harmful to human and animal health. Therefore, the sensitive and specifying detection of MNZ can be very important [5, 6]. In clinical settings, the accumulation of MNZ can lead to serious health issues such as peripheral neuropathy and seizures, jeopardizing patient safety and complicating treatment regimens. Similarly, the environmental ramifications of MNZ runoff from agricultural and cosmetic applications can disrupt ecosystems and contribute to antimicrobial resistance, an escalating global health threat. As such, establishing effective monitoring systems for MNZ is not only vital for safeguarding public health but also for protecting environmental integrity. Traditional analytical methods, like high-performance liquid chromatography (HPLC), have long been the gold standard for detecting MNZ due to their sensitivity and specificity. However, these methods often come with drawbacks such as high costs, complexity, and lengthy processing times, which can hinder timely detection. To address these limitations, nanomaterial-based sensing technologies have emerged over the past two decades, offering innovative solutions that enhance detection capabilities while reducing costs and improving user-friendliness [7, 8]. Nanomaterials possess unique chemical and optical properties—such as high photon efficiency, broad excitation spectra, and photostability—that make them particularly suited for developing advanced sensors and biosensors for MNZ detection. These technologies have the potential to revolutionize monitoring practices in both clinical and environmental settings, enabling more sensitive and rapid assessments of MNZ presence [9]. This article aims to summarize recent advancements in MNZ nanosensors developed in the last decade, emphasizing their significance and performance while also highlighting the limitations of conventional detection methods. By understanding the landscape of MNZ detection technologies, we can better appreciate the importance of ongoing innovation in this critical area.

2 | Metronidazole

MNZ is an antimicrobial agent commonly used in the treatment of certain protozoal and anaerobic infections, making it a critical component in the management of various medical conditions. Originally developed to target *Trichomonas vaginalis*, the causative agent of trichomoniasis, its therapeutic applications have expanded to include infections caused by *Giardia lamblia* and *Entamoeba histolytica*, both of which can lead to severe gastrointestinal illnesses. Additionally, MNZ is effective against a range of anaerobic bacteria, which are often implicated in polymicrobial infections, particularly those occurring in the abdominal cavity, pelvic region, and among individuals with compromised immune systems. The versatility of MNZ is underscored by its role in treating conditions such as bacterial vaginosis and certain dental infections, where anaerobic bacteria thrive. Furthermore, its utility extends into surgical prophylaxis, especially in colorectal surgeries, to mitigate the risk of postoperative infections. This broad spectrum of activity makes MNZ an essential drug in both outpatient and inpatient settings, contributing significantly to patient care. Despite its efficacy, the increasing reliance on MNZ raises important concerns about the emergence of drug resistance. Prolonged or inappropriate use can lead to treatment failures, complicating the management of infections and posing significant public health risks. Additionally, the drug's potential for adverse effects, including peripheral neuropathy and central nervous system disturbances, necessitates careful monitoring and responsible prescribing practices. In summary, MNZ serves as a vital therapeutic agent in the fight against protozoal and anaerobic infections, but its widespread use must be balanced with vigilance against resistance and the management of potential toxicities. This highlights the importance of reliable detection methods for MNZ in clinical and environmental settings to ensure safe and effective use. MNZ is an antimicrobial agent commonly used in the treatment of certain protozoal and anaerobic infections [10, 11]. The drug is generally well tolerated but has occasionally been associated with the development of serious neurological side effects, including visual impairment, peripheral neuropathy, cochlear toxicity, ataxia, cerebellar dysfunction, vestibular toxicity, dysarthria, seizures, and encephalopathy (MNZ encephalopathy, MIE) [10, 11] (Figure 1).

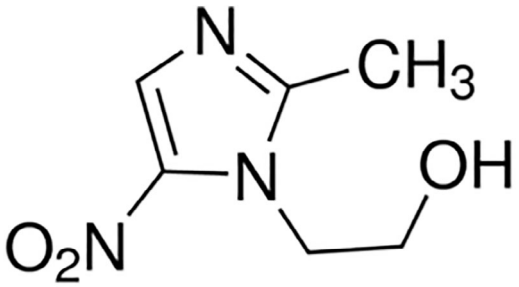

FIGURE 1 | Chemical structure of metronidazole.

MNZ is widely used as an antibiotic but has difficulty surviving in the aquatic environment. MNZ residues have caused serious concerns due to their adverse effects on environmental safety and human health [12, 13]. The relative removal of MNZ was examined by UV/peroxydisulfate (UV/PDS), UV/hydrogen peroxide (UV/H_2O_2), and UV/chlorine processes [12, 13]. The overview of MNZ is tabulated in Table 1.

3 | Conventional Methods in the Determination of MNZ

As the importance of MNZ, numerous methods have been applied for the determination of MNZ. Supercritical fluid chromatography [16], thin-layer chromatography (TLC) [17], polarography [18], HPLC, Figure 2 [19], gas chromatography (controlled pore glass) [20], flow injection analysis [21], spectrophotometry, and

TABLE 1 | Metronidazole overview.

Used for	Pelvic inflammatory disease, endocarditis, vaginosis, giardiasis, trichomoniasis, amebiasis, dracunculiasis, clostridium difficile colitis, pelvic inflammatory disease, antiprotozoal	[14, 15]
Side effect	Nausea, a metallic taste, loss of appetite, thrombophlebitis, headaches, occasionally seizures or allergies, hypersensitivity, leucopenia, neutropenia, peripheral neuropathy, central nervous system toxicity	
Pharmacokinetics	Distributed widely and having low protein binding (<20%), metronidazole given orally is absorbed almost completely, bioavailability >90% for tablets, and absorption is unaffected by infection. Rectal and intravaginal absorption are 67%–82% and 20%–56% of the dose, respectively	
Drug interaction	Dronabinol, flibanserin, lomitapide, barbiturates, tinidazole, fluorouracil, disulfiram, live typhoid vaccine	

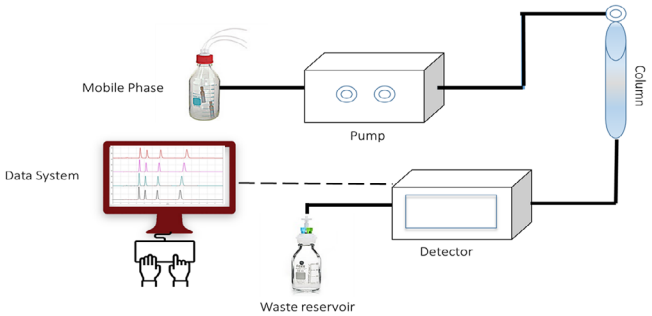

FIGURE 2 | HPLC working principle. HPLC, high-performance liquid chromatography.

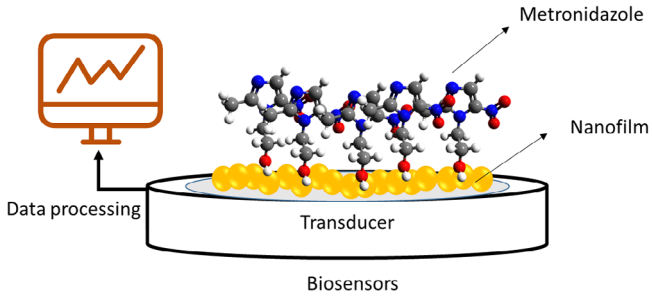

FIGURE 3 | Schematic illustration of biosensor methodology.

derivative spectrophotometry (Spec) [22, 23] are the extensively used methods in determination of MNZ.

These conventional methods have several limitations, including the need for heating or extraction, being time-consuming, having a limited range of determinations, reliance on non-aqueous systems, prolonged reaction times, low sensitivity, and issues with the stability of the resulting colored product [24–26]. Developed systems showed acceptable sensitivity and selectivity despite some drawbacks [25, 27, 28]. An effective method for the real-time determination of MET, hydroxymetronidazole (HMET), and neospiramycin (NSPY) in animal models, based on HPLC with UV detection (HPLC-UV), has been established [29]. HPLC technique was used for sensitive and simple detection of MET in human serum. The created system presented good analytical features, but, in clinical states, other substances may be co-administered, and possible interfering drugs would have to be inspected [30]. A reversed-phase HPLC (RP-HPLC) technique with UV detection was approved for real-time detection of MET and miconazole [24]. In a research study, a chromatographic technique was developed to detect MNZ in tablets. According to the obtained results, the created technique showed good accuracy, specificity, and suitable analytical characteristics [31]. HPLC and UV were fabricated for the simple determination of MNZ in the dermatological formulation. The planned method showed suitable sensitivity and was applied to clinical and pharmaceutical approaches [32]. Routine methods in the determination of MNZ are summarized in Table 2.

4 | Biosensors

Biosensors are analytical instruments that transform biological responses into electrical signals [33, 34]. They are designed to exhibit high specificity and should function independently of various physical conditions, such as temperature and pH levels, while also being recyclable. The concept of a "biosensor" was introduced by Cammann, and its formal definition was established by the International Union of Pure and Applied Chemistry (IUPAC) [35–37]. The development of biosensors, including their transduction mechanisms, materials, and immobilization techniques, necessitates a collaborative approach that integrates knowledge from biology, chemistry, and engineering [35–37]. The materials utilized in biosensors can be categorized into three main groups based on their operational mechanisms: the bio-affinity group, which encompasses antibodies and nucleic acids; the bio-catalytic group, which includes enzymes; and the microbial group, which consists of various microorganisms [35–37].

On the basis of transducers, biosensors are categorized into three main groups, including electrochemical, optical, and mass-based biosensors (Figure 3, 4).

An electrochemical biosensor is a specific type of biosensor that translates biological data, such as the concentration of an analyte recognized by a biochemical receptor, into an electric current or voltage [38, 39]. These biosensors serve as valuable diagnostic tools capable of identifying biological markers present in various

TABLE 2 | Routine methods in determination of Metronidazole (MNZ).

Methods	Linearity μg g^{-1}	LOD μg g^{-1}	Comments	Refs.
Spec	—	1–32	Simple, fast, accurate, and precise, highly reproducible	[28]
Spec	1–20	0.27	Simple, acceptable sensitivity, and selectivity	[24]
HPLC	0.005–1.000	0.005	Ability to detect metronidazole, HMET, and NSPY simultaneously and their metabolite	[29]
HPLC	1 and 10	10	Applicable for human serum, drug interaction would have to be inspected	[30]
RP-HPLC	1–20	0.27	Applicable for pharmaceutical dosage forms, good sensitivity	[24]
Chromatography	50.0–6030.0	3.1	Accurate, precise specific, and could be applied to the simultaneous quantitative analysis of MIZ and MNZ in tablets	[31]
HPLC/UV	35–46	1.8	Simple and fast method for determination of MNZ in dermatological formulation	[32]

Abbreviations: HMET, hydroxymetronidazole; LOD, limits of detection; NSPY, neospiramycin; RP-HPLC, reversed-phase high-performance liquid chromatography.

bodily fluids, including sweat, blood, stool, and urine. The effectiveness of these biosensors is enhanced by the integration of suitable immobilization methods with efficient transduction mechanisms [38, 39]. On the other hand, optical biosensors provide notable benefits compared to conventional analytical methods, as they facilitate real-time, direct, and label-free detection of a wide range of chemical and biological substances. Their key advantages include high sensitivity and specificity, compact size, and cost-effectiveness [40, 41]. Optical biosensors are the most commonly referenced type of biosensor. They operate by utilizing the interaction between optical fields and biological recognition elements for detection. Optical biosensing can be categorized into two primary modes: label-free and label-based. In the label-free mode, the detection signal is produced directly through the interaction between the analyte and the transducer [40, 41]. Mass-based biosensors, on the other hand, exhibit changes in oscillation frequency as the mass of the sensor increases. These biosensors are classified into four main types: surface acoustic, microcantilevers, piezoelectric quartz, and magnetoelastic biosensors [42, 43]. A summary of the advantages and disadvantages of various biosensors can be found in Table 3.

5 | Developed Biosensors for Determination of MNZ

Molecularly imprinted polymers (MIPs) are proper candidates to expand the selectivity of fluorescence detection [50, 51]. Molecular recognition sites in synthetic MIPs can precisely bind target molecules [50]. MIP biosensor based on carbon dots (CDs) was fabricated for selective detection of MET in human serum samples [52]. Due to high specific electrical conductivity and good surface area, $CuCo_2O_4$ nanoparticles modified with nitrogen-doped carbon nanotubes ($CuCo_2O_4$/N-CNTs) were used as the electrochemical platform for detection of MET. The developed sensor, Figure 5, was able to quickly detect MNZ in blood samples, serum, and tablets [53].

Linear sweep voltammetry (LSV) was used as an electrochemical technique for the sensitive detection of MET in human urine samples. Molybdenum carbide with functionalized carbon nanofiber (Mo_2C/f-CNF) was used as the proper nanocomposite [54]. Fullerene (C60), reduced graphene oxide (rGO), and nafion (NF) (C60-rGO-NF/screen-printed carbon electrode [SPE]) as a nanocomposite with SPEs was applied for specific determination of MET in human urine samples [55]. Dysprosium vanadate Dy(VO4) oxidized with carbon nanofiber (f-CNF) was used on the disposables SPE for selective detection of MET in urine, blood, and lake water samples. The designed system had a simple and low-cost structure, biocompatible with appropriate specificity and repeatability [56]. A simple and highly reproducible sensor was engineered for ultra-sensitive detection of MET in biological and pharmaceutical samples. Polydopamine/carboxylic multi-walled carbon nanotubes (MWCNTsCOOH) nanocomposites modified glassy carbon electrodes (GCEs) were used to reach optimum sensitivity [57]. Graphene oxide (GO) has been the interest of researchers and industry due to its polar and two-dimensional (2D) layered structure. The low concentration of MNZ antibiotic in drug samples could be measured using graphene nanocomposite with excellent analytical properties [58]. Crump-like nickel manganous oxide nanoparticles decorated partially rGO (NiMnO@pr-GO) nanocomposite showed excellent analytical properties in the identification of MET. The created platform, Figure 2, exhibited acceptable selectivity and wide-range linearity

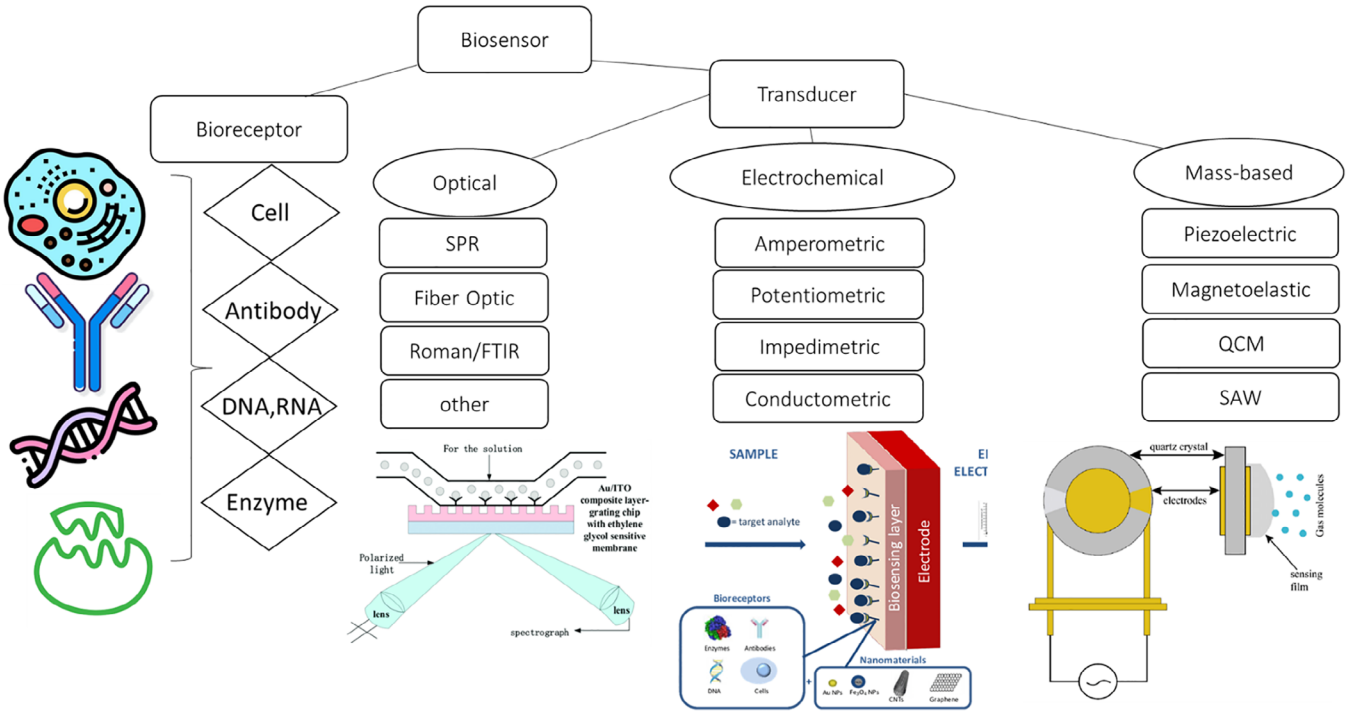

FIGURE 4 | Biosensors classification based on transducer type. FTIR, Fourier transfer infrared; QCM, quartz crystal microbalance; SPR, surface plasmon resonance.

TABLE 3 | Advantages and disadvantages of different biosensors.

Biosensor type	Advantages	Disadvantages	Refs.
Electrochemical	Speed, low detection limits reaching as low as picomoles, and the use of inexpensive equipment for sensing are key advantages. Electrochemical sensors are available in various formats, ranging from advanced wearable devices to fully integrated systems. They offer real-time insights into the chemical composition of the environment and can respond reliably and reversibly without altering the sample	• Narrow or limited temperature range, short or limited shelf life • Cross-sensitivity of other gases Increased exposure to the target gas leads to a reduced lifespan, unstable voltage and current, and lower power efficiency	[44, 45]
Optical	High sensitivity and selectivity; real-time measurement; good reproducibility, simple, low cost, anti-interference ability; wide detection range	Time consuming; limited application (small molecules); low selectivity; low reproducibility; low stability; low selectivity	[46, 47]
Mass-based	This method leverages the optical properties of the medium, enabling a sensitive, rapid, accurate, and linear response. These characteristics make it particularly well-suited for mass production and capable of detecting changes in viscosity and viscoelasticity within the solution and at the biointerface	Regardless, these sensors suffer from poor selectivity and interference from other electroactive substances. Additionally, they have a limited lifespan due to device degradation, sensor drift, and the need for component replacements	[48, 49]

and was applicable for real and tablet samples [59]. An advanced system based on Pt nanospheres/polyfurfural film-modified GCE was fabricated for the detection of MET. The developed biosensor was able to detect MET in human serum samples appropriately [60]. MNZ concentration was analyzed in pharmaceutical samples by ZnCo-based metal-organic frameworks (MOFs) based on ZIF-8 and ZIF-67 systems properly. The established system presented acceptable selectivity with wide-range linearity [61]. For sensitive and simple detection of MET in pharmaceutical and human urine samples, the continuous pulsed-potential (CPP)

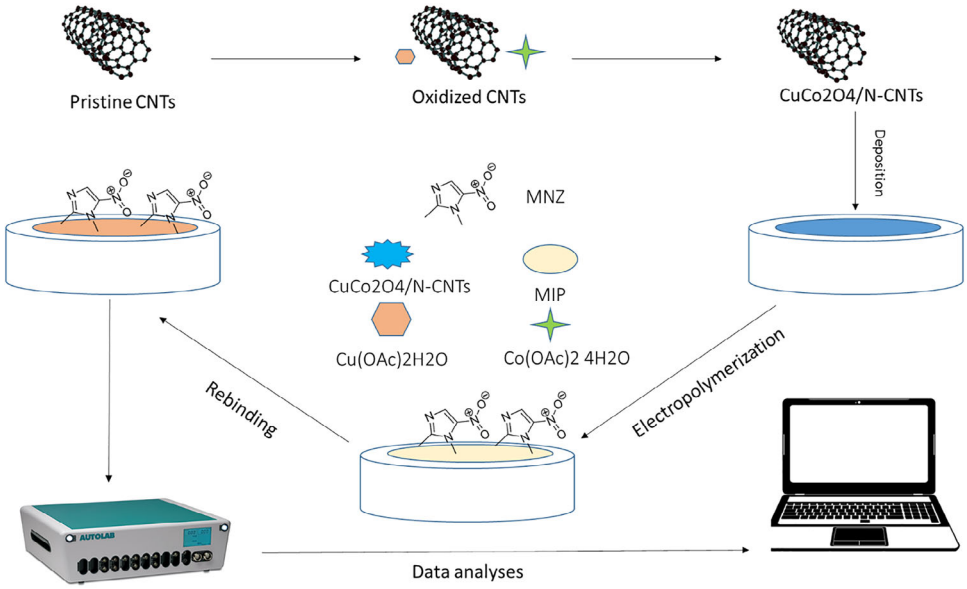

FIGURE 5 | Schematic diagram for preparation of $CuCo_2O_4$/N-CNTs/MIP/GCE and electrochemical determination of metronidazole. $Co(OAc)_2$ $4H_2O$ $CuCo_2O_4$/N-CNTs, adapted from ref [53]. MNZ, metronidazole; GCE, glassy carbon electrode; MIP, molecularly imprinted polymer.

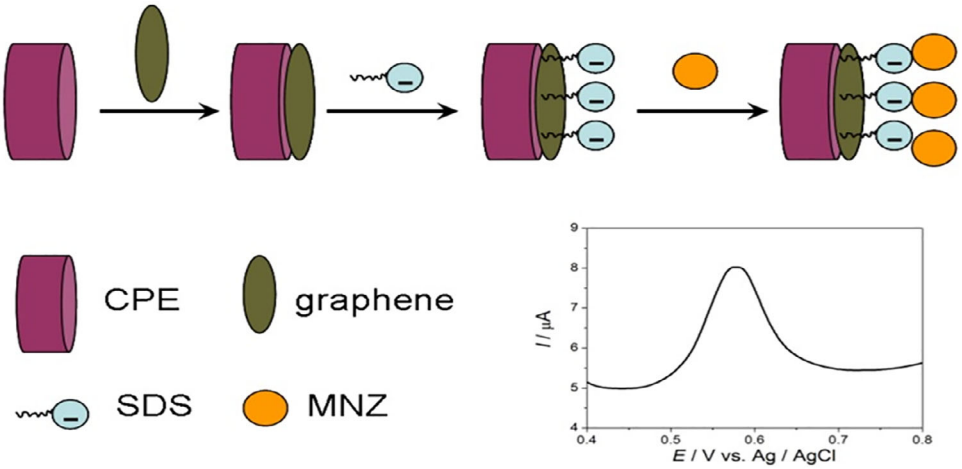

FIGURE 6 | Schematic illustration of the preparation processes of the electrode for the determination of MNZ [63]. CPE, carbon past electrode; MNZ, metronidazole; SDS, sodium dodecyl sulfate.

method was applied correctly. Organized biodevices revealed satisfactory analytical results such as high sensitivity and selectivity [62]. The good reproducibility, wider linear range, high sensitivity, and minimal surface fouling on the modified electrode were described from a developed biosensing tool for the determination of MET in real samples. Figure 6 presents the preparation processes of the electrode for the determination of MNZ [63].

To create a simplistic technique with high selectivity and sensitivity for MNZ determination, fluorescent CDs were used correctly. CDs received huge consideration for their beneficial features, such as exceptional optical properties, special aqueous, easy accessibility, good photostability, low toxicity, and good biocompatibility [64, 65]. An innovative fluorescence biosensor based on CDs was developed for rapid and selective determination of MNZ. The planned bio-system revealed satisfactory linear range and sensitivity and is applicable in pharmaceutical samples [66]. In a novel research work, gold nanoparticles (Au NPs) decorated on poly (diallyldimethylammonium chloride) (PDDA) functionalized graphene hydrogel (Au NPs@PDDA/GH) nanocomposites were assembled for ultra-sensitive detection of MNZ in the human urine sample. The developed tool showed rapid operation and wide-range linearity, high stability with a cost-effective and simple structure [67]. Surface plasmon resonance (SPR) biosensors, Figure 7, are a spectroscopic method that quantitatively measures binding events in real-time without labeling the interacting molecules. First established in 1983, it measures the refractive index changes when the molecules interact at the sensing surface [68].

Förster resonance energy transfer also known as FRET is a multipurpose and sensitive device for quantitative and qualitative analysis of biological processes and interactions. Easy-to-use access to a wide range of fluorescent materials and sophisticated

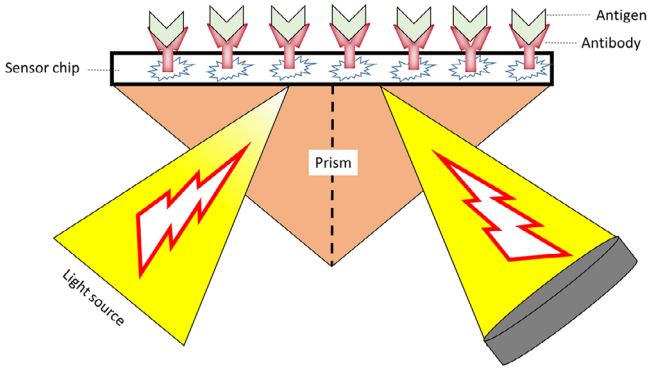

FIGURE 7 | SPR-based biosensor working principle. SPR, Surface plasmon resonance.

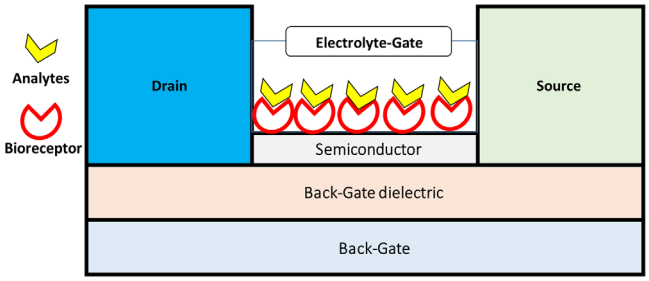

FIGURE 8 | Schematic illustration of the FRET. FRET, Förster resonance energy transfer.

microscopes and spectrometers have made FRET a noticeable procedure for biosensing [69, 70]. A schematic illustration of the FRET biosensor is presented in Figure 8.

The FRET method was applied for the detection of MNZ by self-assembly via 0D/2D N–C QDs/gC_3N_4 nanocomposites appropriately. The developed tool is easy to work with and has high analytical properties and wide-range linearity [71]. Original electrochemical synthesis of copper oxide nanoparticles decorated graphene-b-cyclodextrin (GR-b-CD/CuO NPs) composites was assembled for nanoscale detection of MNZ. The established system is selective, highly sensitive, and robust in the existence of a range of possibly interfering electroactive compounds [72]. $SrMoSe_2$ nanosheets modified GCE ($SrMoSe_2$/GCE) showed excellent properties in the efficient electrochemical system for sensitive identification of MNZ [73]. Rod-like SrV_2O_6 was used in biodevice structures for selective and ultra-sensitive detection of MNZ in environmental and biomedical samples [74]. Biphenylene nanosheet (BPNS) and its doped counterparts (B- and Al-BPNS) were employed as sensors for the identification of MNZ based on the density functional theory (DFT). The findings indicated MNZ was detectable promptly and with acceptable limits of detection (LOD) and wide-range linearity [75]. Bismuth-doped carbon quantum dots (Bi-CQDs) derived from walnut shells in rice morphology with an average size of 3–4 nm were used for highly selective detection of MNZ in nM through the internal filtration effect (IFE) [76]. The resulting g-C_3N_4/MnO_2/ZnO composite was used to expertise an electrochemical sensor that facilitates swift MNZ determination in food. The result displays that the g-C_3N_4/MnO_2/ZnO composite shows extraordinary electrocatalytic capability good sensitivity [77]. A new sensor specifically designed for MNZ was created by integrating fluorescent molecularly imprinted polymers (MFMIP) with magnetic components, alongside a non-imprinted reference composite (MFNIP). The MFMIP composite, which includes CdTe@TGA quantum dots and Fe_3O_4@TEOS magnetite nanoparticles, demonstrated interactions tailored to MNZ due to its unique cavities, a finding that was validated through SPR analysis [78]. A new MOF based on zinc (Zn(II)) was developed as a highly sensitive and selective sensor for the fluorescent detection of MNZ and aromatic nitrophenols [79]. Nitrogen-doped CDs that emit blue light were created using citric acid and urea via the hydrothermal method, achieving a fluorescence quantum yield of 35.08%. Research indicated that these nitrogen-doped CDs (N-CDs) possess strong fluorescence stability and resistance to chemicals. When used for detecting MNZ, our N-CDs demonstrated a rapid response time, along with high selectivity and sensitivity, while also exhibiting low cytotoxicity [80]. MoS2/graphite-like carbon nitride composite-modified GCE was advanced for detection of MNZ. The planned electrochemical biosensor showed that an exceptional LOD with a good linear dynamic range of 2–125 μM [33]. The surface of gold nanoparticles AuNPs was modified with melamine (MA) to create a colorimetric probe that does not rely on enzymes for sensing. Subsequently, the MA-functionalized AuNPs (MA@AuNPs) were attached to polyamide 6 nanofiber membranes (PA6 NFMs). The resulting membranes exhibited a colorimetric reaction when exposed to MTZ, attributed to the interaction between MA and MTZ, which causes the AuNPs to clump together. These colorimetric strips can be utilized for easy detection with standard smartphone cameras, allowing for low detection limits in a portable format [81]. Biosensor technology can be compared to other nanomaterial-based methods, such as the synthesis of molybdenum disulfide (MoS_2) and bismuth trisulfide (Bi_2S_3) particles. In this approach, Bi_2S_3 is loaded onto MoS_2 nanosheets and characterized using various techniques. The degradation of MTZ and cefalexin (CFX) is then investigated using MoS_2, Bi_2S_3, and MoS2/Bi2S3 particles under near-infrared (NIR) light irradiation. Additionally, the electronic structures and molecular geometries of the MoS_2 monolayer, as well as the MoS_2/MTZ and MoS_2/CFX complexes, are analyzed using DFT [82]. In summary, while the N-CDs biosensor method is centered on the detection of MNZ through fluorescence, the MoS2/Bi_2S_3 approach focuses on the environmental remediation of MNZ through photocatalytic degradation. Both methods highlight the versatility of nanomaterials in addressing pharmaceutical challenges, but they serve different purposes—one for sensing and the other for degradation. (Table 4)

Biosensor detection methods and nanomaterial-based detection methods are both crucial in analytical chemistry and biomedical applications, yet they differ significantly in principles, mechanisms, and applications. Biosensors are analytical devices that convert biological responses into electrical signals. They typically consist of a biological recognition element, such as enzymes, antibodies, or nucleic acids, coupled with a transducer. These devices utilize specific biological interactions, such as antigen–antibody or enzyme–substrate interactions, for target detection. They achieve high sensitivity due to the specific binding of the biological element to the target analyte, and they offer high selectivity for specific biomolecules, minimizing interference from other substances. Many biosensors enable real-time monitoring

TABLE 4 | Developed biosensors for determination of metronidazole.

Methods	Samples	Nanocomposite	Electrode	Linear range (μM dm3)	LOD (nmol dm3)	Refs.
MIP	Serum	Carbon dot		50–1200	17.4	[52]
MIP/DPV	Blood/tablet	$CuCo_2O_4$/N-CNTs	GCE	0.005–0.1	0.48	[83]
LSV	Urine	Mo2C/f-CNF	GCE	0.04–647.7	0.002	[54]
SWV	Urine	(NF (C60-rGO-NF/SPE)	SPE	2.5×10^{-7} to 34×10^{-6}	2.1×10^{-7}	[55]
LSV/CV	Blood/urine	Dy(VO4)/f-CNF	SPCE	1.5–1036.9	6	[56]
CV/DPV	Biological and pharmaceutical	MWCNTs-COOH	GCE	5–5000	0.25	[57]
Amperometric	Real samples	GO@AgNPs-GCE	GCE	0.09–4594	0.069	[58]
FTIR/Raman	Real samples/tablets	NiMnO@pr-GO	GCE	90	1.22	[59]
CV/DPV	Human serum	Pt nanospheres/polyfurfural film	GCE	2.5–500	50	[60]
LSV/CV	Pharmaceutical	ZnCo-MOFs	GCE	0.05–100	17	[61]
LSV/CV	Real samples/tablets	HCF	Gold	20.0–800.0	0.15	[62]
DPSV	Real samples/tablets	SDS	CPE	0.08–200	8.5	[63]
FTIR, XPS	Pharmaceutical	CDs	—	3.3×10^{-6}–2.4×10^{-4}	1.2×10^{-7}	[66]
LSV/CV	Real samples/urine	AuNPs@PDDA/GH	GCE	0.4–656.4	0.097	[67]
FRET	Biological	N–C QDs/g-C3N4	—	0–2.6×10^{-5} mol/L	0.66 μM	[71]
Electrochemical	Real samples/tablet	GR-b-CD/CuO NPs	GCE	0.002–210.0 mM	0.6 nM	[72]
DPV	Urine sample	$SrMoSe_2$/GCE	GCE	0.05–914.92 μM	0.001 μM	[73]
CV	Environmental and biomedical	SrV_2O_6/GCE	GCE	0.01–207 μM	4 nM	[74]
NA	NA	Graphene	NA	NA	NA	[75]
Optical	Pharmaceutical	Bi-CQDs	—	200 nM–2 μM	15.72 nM	[76]
Electrochemical/CV	Food	g-C_3N_4/MnO_2/ZnO	—	2–250 μM	0.21 μM	[77]
Fluorescent, SPR	Pharmaceutical	Fe_3O_4@TEOS	—	5–60 μM	5.00 μM	[78]
Fluorescent	Water solutions	Zn-MOF	—	—	0.66 ppm	[79]
Colorimetric	Pharmaceutical	MA@AuNPs	—	2–100 nM	2 nM	[81]
Electrochemical/CV	Pharmaceutical	MoS_2/gCN/GCE	GCE	2–125 μM	0.099 μM	[33]
Fluorescent	Pharmaceutical	N-CDs	—	0–179 μM	0.25 μM	[80]

Abbreviations: CPE, carbon past electrode; $CuCo_2O_4$/N-CNTs, $CuCo_2O_4$ nanoparticles modified with nitrogen-doped carbon nanotubes; DPSV, differential pulse stripping voltammetry; DPV, differential pulse voltammetry; Dy(VO_4)/*f*-CNF, dysprosium vanadate Dy(VO_4) oxidized carbon nanofiber (*f*-CNF); FTIR, Fourier transfer infrared; GCE, glassy carbon electrode; GO@AgNPs, Ag nanoparticle-decorated graphene oxide; HCF, hexacyanoferrate; LOD, limits of detection; LSV, linear sweep voltammetry; MIPs, molecularly imprinted polymers; Mo2C/f-CNF, molybdenum carbide/functionalized carbon nanofiber; MWCNTs-COOH, multi-walled carbon nanotubes; NF (C60-rGO-NF/SPE, fullerene (C60), reduced graphene oxide (rGO) and nafion, SWV, square wave voltammetry; NiMnO@pr-GO, nickel manganous oxide nanoparticles decorated partially reduced graphene oxide; SDS, sodium dodecyl sulfate; SPE, screen-printed carbon electrodes; SrV_2O_6, rod-like strontium vanadate; ZnCo-MOFs, ZnCo-based metal-organic frameworks.

of analytes, making them particularly valuable in medical diagnostics, environmental monitoring, and food safety. Common types of biosensors include enzymatic biosensors, immunosensors, DNA biosensors, and cell-based biosensors. In contrast, nanomaterial-based detection methods leverage nanomaterials, such as nanoparticles, nanowires, and nanotubes, to enhance the detection of various analytes through physical or chemical interactions. These methods benefit from the unique optical, electronic, and catalytic properties of nanomaterials, which can significantly enhance detection sensitivity and specificity. They are versatile and can be applied to a wide range of analytes, including small molecules, proteins, and nucleic acids. Signal amplification is a key feature of these methods, as nanomaterials can amplify signals through mechanisms like SPR, fluorescence enhancement, or electrochemical signal amplification. Additionally, nanomaterial-based detection methods can be integrated into various platforms, including electrochemical sensors, optical sensors, and field-effect transistors. Their applications are

TABLE 5 | Comparison of biosensor detection methods and nanomaterial-based detection methods.

Feature	Biosensor detection methods	Nanomaterial-based detection methods
Recognition mechanism	Biological interactions	Physical/chemical interactions
Sensitivity	High, due to specific binding	Can be very high, often enhanced by nanomaterials
Selectivity	Very high, specific to target biomolecules	Varies, can be enhanced with functionalization
Real-time monitoring	Often possible	Possible, depending on the design
Applications	Primarily in biological and medical fields	Broader range, including electronics and materials science
Complexity	Can be complex due to biological components	Varies, but often simpler in terms of biological components

similar to those of biosensors, extending to medical diagnostics, environmental monitoring, and food safety, as well as areas like drug delivery and imaging. Common types of nanomaterial-based sensors include nanoparticle-based sensors (e.g., gold nanoparticles), carbon nanotube sensors, quantum dot sensors, and nanowire sensors. In summary, whereas both biosensor and nanomaterial-based detection methods play vital roles in various fields, biosensors are characterized by their biological specificity and real-time capabilities, whereas nanomaterial-based methods are distinguished by their versatility and enhanced signal detection capabilities. The choice between these methods depends on the specific requirements of the application, including the type of analyte, sensitivity needed, and detection environment. Comparison of biosensor detection methods and nanomaterial-based detection methods is summarized in Table 5.

6 | Overview of Advancements in Analytical MNZ Nanosensors

The advancements in biosensor technology for the detection of MNZ reflect a growing need for efficient, sensitive, and selective analytical methods across clinical and environmental contexts. The use of MIPs has emerged as a promising strategy to enhance the selectivity of fluorescence detection. By creating specific binding sites that mimic the target molecule, MIPs allow for precise identification and quantification of MNZ in complex biological matrices, such as human serum, where the presence of other substances can complicate analysis. The integration of nanomaterials in sensor design has significantly improved the performance of MNZ detection systems. For instance, the combination of $CuCo_2O_4$ nanoparticles modified with nitrogen-doped carbon nanotubes ($CuCo_2O_4$/N-CNTs) as an electrochemical platform has demonstrated rapid detection capabilities in blood, serum, and tablet samples. This highlights the effectiveness of nanocomposites in enhancing electrical conductivity and surface area, which are critical for electrochemical measurements. Various electrochemical techniques, such as LSV and differential pulse voltammetry (DPV), have been employed to achieve high sensitivity and selectivity in detecting MNZ in urine and blood samples. Notably, the use of molybdenum carbide-functionalized carbon nanofibers (Mo_2C/f-CNF) and other innovative nanocomposites has resulted in impressive LOD and wide linear ranges. This is particularly relevant given the low concentrations of MNZ that often need to be detected in biological samples, emphasizing the importance of these advanced sensing methods. The exploration of SPE with various nanocomposite materials, such as fullerene-rGO, has also shown great promise for detecting MNZ in urine. The ease of fabrication and cost-effectiveness of SPEs make them attractive options for point-of-care testing, allowing for rapid analysis in clinical settings. Furthermore, the versatility of techniques, such as FRET and SPR, underscores the breadth of approaches available for MNZ detection. FRET's capability for real-time analysis and SPR's label-free detection methods offer valuable alternatives that could be adapted for various applications, from laboratory research to field testing. The development of biosensors utilizing materials like gold nanoparticles decorated on graphene hydrogels showcases the trend toward combining multiple functional components to achieve ultra-sensitive detection. These innovative materials not only enhance sensitivity but also demonstrate excellent stability and biocompatibility, crucial for biomedical applications. The variety of nanocomposites explored, including bismuth-doped carbon quantum dots and g-C_3N_4/MnO_2/ZnO composites, indicates a rich landscape of options for designing sensors with tailored properties. The performance metrics of these sensors, such as reproducibility and specificity, further validate their potential for practical applications in both pharmaceutical and environmental monitoring. Additionally, the reproducibility of biosensors can significantly depend on the methods used for their fabrication. Variations in the synthesis of nanocomposites or the conditions under which MIPs are created can lead to inconsistencies in sensor performance. For instance, the uniformity of the molecular imprinting process directly affects how well the sensor can recognize and bind to MNZ. Therefore, standardized protocols for sensor fabrication are essential to ensure that each sensor produced under the same conditions yields similar results. Moreover, as biosensors for MNZ detection demonstrate promising sensitivity, selectivity, and reproducibility, it becomes increasingly important to consider their scalability for real-world applications. Scalability refers to the ability to produce biosensors in larger quantities while maintaining performance, affordability, and usability. Addressing scalability is crucial for the successful implementation of these sensors in clinical, pharmaceutical, and

environmental monitoring contexts. As the demand for rapid, accurate, and sensitive detection methods for MNZ continues to grow, the integration of nanotechnology and innovative biosensor designs will play a pivotal role in addressing these challenges. Ongoing research should focus on optimizing these biosensors to improve their robustness, miniaturization, and adaptability for a wide range of applications. Collectively, these advancements not only promise enhanced safety in pharmaceutical use but also contribute to broader efforts in environmental protection and public health.

7 | Conclusion and Future Prospective

The advancements in biosensor technology for the detection of MNZ have underscored the potential for rapid, sensitive, and selective analysis in both clinical and environmental contexts. Innovative materials, such as nanocomposites and molecularly imprinted polymers, have significantly enhanced the performance of these sensors. However, challenges related to reproducibility, scalability, and integration with existing diagnostic workflows remain critical barriers to widespread adoption. To address these challenges and optimize the functionality of MNZ biosensors, future research should emphasize the integration of multiple sensing technologies. Combining different modalities—such as electrochemical, optical, and mass-sensitive methods—could synergistically enhance sensitivity and selectivity. Multimodal approaches may facilitate simultaneous detection of MNZ alongside other relevant biomarkers, thus improving overall diagnostic capabilities in complex biological matrices. Another important direction for future studies is the development of portable and user-friendly devices suitable for point-of-care testing. By incorporating intuitive interfaces and simplified operational protocols, researchers can facilitate adoption by healthcare professionals and enable real-time monitoring in diverse settings. Exploring alternative materials is also crucial. Although current nanomaterials demonstrate promise, investigating new materials with unique properties may yield even more effective biosensors. This includes the search for biodegradable or environmentally friendly options that maintain high sensitivity and stability while reducing environmental impact. Improving the long-term stability and robustness of biosensors is essential for ensuring consistent performance across various environments. Future research should address issues related to material degradation, sensor fouling, and response variability over time, enhancing the reliability of these tools. Additionally, establishing regulatory and standardization frameworks will streamline the approval processes for new biosensor technologies. Engaging with regulatory bodies early in the design phase can help clarify safety and efficacy criteria, facilitating faster market entry for innovative solutions. Finally, conducting extensive pilot studies in real-world applications will provide valuable insights into the practical performance of MNZ biosensors. These studies can help identify potential challenges and inform design modifications to enhance usability and effectiveness. By focusing on these interconnected research directions, the field of biosensor technology can continue to evolve, leading to more effective, reliable, and accessible tools for detecting MNZ. Such advancements will not only improve patient safety and therapeutic outcomes but also contribute to ongoing efforts to mitigate the impact of antimicrobial resistance and protect public health.

Author Contributions

Ahmad Mobed: CRediT contribution not specified. **Mohammad Darvishi:** writing–original draft, writing–review & editing. **Vahid Aliverdiloo:** Writing–original draft, writing–review & editing. **Sara Ebrahimi:** writing–original draft, writing–review & editing. **Mobasher Hajiabbasi:** writing–original draft, writing–review & editing. **Hamidreza Hasanzadehkhanmiri:** conceptualization, supervision.

Acknowledgements

The authors would like to thank the Social Determinants of Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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