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Electrochemical biosensors for the detection of protozoan parasite: a scoping review

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ABSTRACT

The development of rapid, accurate, and efficient detection methods for protozoan parasites can substantially control the outbreak of protozoan parasites infection, which poses a threat to global public health. Idealistically, electrochemical biosensors would be able to overcome the limitations of current detection methods due to their simplified detection procedure, on-site quantitative analysis, rapid detection time, high sensitivity, and portability. The objective of this scoping review is to evaluate the current state of electrochemical biosensors for detecting protozoan parasites. This review followed the most recent Preferred Reporting Items for Systematic Review and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) recommendations. Using electrochemical biosensor and protozoan parasite keywords, a literature search was conducted in PubMed, Scopus, Web of Science, and ScienceDirect on journals published between January 2014 and January 2022. Of the 52 studies, 19 were evaluated for eligibility, and 11 met the review's inclusion criteria to evaluate the effectiveness and limitations of the developed electrochemical biosensor platforms for detecting protozoan parasite including information about the samples, biomarkers, bioreceptors, detection system platform, nanomaterials used in fabrication, and limit of detection (LoD). Most electrochemical biosensors were fabricated using conventional electrodes rather than screen-printed electrodes (SPE). The range of the linear calibration curves for the developed electrochemical biosensors was between 200 ng/ml and 0.77 pM. The encouraging detection performance of the electrochemical biosensors demonstrate their potential as a superior alternative to existing detection techniques. On the other hand, more study is needed to determine the sensitivity and specificity of the electrochemical sensing platform for protozoan parasite detection.

KEYWORDS

Protozoan; parasites; electrochemical; biosensor; diagnostic

1. Introduction

Despite developments in healthcare facilities and services, protozoan parasites pose a significant threat to global public health, affecting hundreds of millions of individuals especially in low- and middle-income countries. In July 2020, Malaysia experienced a *P. malariae* outbreak, disrupting the National Malaria Elimination Programme during a government-imposed Movement Control Order (MCO). This outbreak imposed significant healthcare costs and redirected resources to affected areas [1]. Additionally, the Indigenous communities in Malaysia are at risk of *E. histolytica* infections (amoebiasis) due to poor sanitation practices, leading to gastrointestinal symptoms. While indigenous and rural communities are highly vulnerable to protozoan infections, urban and metropolitan populations are also significantly affected by protozoan parasites like *T. vaginalis* due to sexual activities [2]. On the other hand, high-income countries are not immune to the impact of protozoan parasite infections. In the United States, *N. fowleri* infections have been documented with a mortality rate ranging from 90–95% [3,4]. Furthermore, Spain and France have observed a modest increase in *E. histolytica* cases, attributed in part to elevated migration rates and instances of male-to-male transmission [5]. In addition, the data underscores the concerning trend of asymptomatic cases, which often go undetected and contribute to ongoing transmission dynamics.Unlike major bacterial and viral pathogens, parasitic protozoan infections cannot be prevented with established and readily accessible vaccines [6]. With the emergence of drug-resistant protozoan parasite strains, the global burden of protozoan parasite diseases has become more severe, necessitating the development of practical diagnostic tools for early and accurate detection.

For decades, the identification of protozoan parasites through microscopic examination and morphological analysis of clinical samples has been considered the gold standard for diagnosing parasitic infections in the laboratory. Despite its widespread use, this diagnostic assay necessitates a highly trained operator, which developing and underdeveloped countries lack [7]. Saidin et al. [8] mentioned this approach has limited sensitivity and specificity, posing challenges in

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distinguishing between the pathogenic Entamoeba species, *E. histolytica*, and the nonpathogenic species, *E. dispar* and *E. moshkovskii*, due to their morphological similarities. Immunoassays, such as enzyme-linked immunosorbent assay (ELISA), present challenges due to their reliance on costly and time-intensive monoclonal antibodies with high affinity [9]. Moreover, utilizing immunoassay for real-time infection screening is compromised by the enduring presence of antibodies in samples, potentially undermining accuracy [10]. Wong et al. [11] further highlighted the limited availability of parasitic antigens for various protozoan infections, impeding their clinical screening applicability. The employment of molecular testing has drawn significant interest as an alternative to conventional microscopy-based methods for identifying protozoan parasites. Nonetheless, a few challenges limit progress in this area, such as the high cost of reagents and equipment, the requirement for sophisticated facilities, and a lack of available commercial testing options [11,12]. Furthermore, the effectiveness of molecular testing is constrained by the risk of false positives, which can arise from the amplification of non-viable protozoan parasites [10]. Considering the limitations associated with conventional and advance molecular methods for protozoan parasite detection, there has been a pronounced shift toward the exploration of biosensors. These biosensors offer the potential for enhanced sensitivity and specificity, obviating the necessity for specialized technical expertise or dedicated facilities.

Biosensors were introduced by Leland C. Clark in the 1950s and have significantly reshaped diagnostic methodologies. As outlined by the International Union of Pure and Applied Chemistry (IUPAC), a biosensor utilizes biochemical reactions, primarily involving enzymes, immune systems, or cells, to detect a readable signal, commonly through electrical, thermal, magnetic, or optical means. Central to its structure is a three-component system: (1) the specific biomarker (bioanalyte), (2) a receptor or recognition element interacting with the biomarker (bioreceptor), and (3) the apparatus converting this interaction into a detectable signal (signal transduction) [13]. Moreover, this technology underpins point-of-care (POC) testing, extending clinical evaluations beyond conventional healthcare environments. A biosensor's efficacy depends on four pivotal parameters: (i) Specificity, indicating the bioreceptor's ability to recognize the target in the sample interferences; (ii) Reproducibility, reflecting consistent outcomes across multiple tests; (iii) Stability, measuring the biosensing system's sensitivity to environmental perturbations; (iv) Sensitivity, denoting the minimum detectable analyte concentration [14,15]. Significantly, electrochemical biosensors exhibit advantages over other types, owing to their capability to function at ambient temperatures without external heating, thereby often necessitating minimal or no power due to their self-sustainability [16].

Electrochemical biosensors are emerging as robust diagnostic tools, offering the capability to identify biomarkers within bodily fluids without compromising its sensitivity and specificity. Tancharoen et al. [17] demonstrated that their electrochemical biosensor was capable to detect as low as 10–250 RNA copies/mL of zika virus in serum sample. Another example is the work of Ilkhani and Farhad [18], that describes a novel electrochemical DNA biosensor was fabricated for the detection of Ebola virus, which they successfully detect at 4.7 nM of Ebola virus with high reproducibility, sensitivity, and selectivity. Nonetheless, electrochemical biosensors can simultaneously detect multiple biomarkers or pathogens. Wan et al. [19] demonstrated this by developing a multiplexing electrochemical biosensor for cancer biomarkers, achieving high sensitivities of 5 $pg{\cdot}mL^{-1}$ for prostate-specific antigen and 8 $pg{\cdot}mL^{-1}$ for Interleukin 8, enhancing diagnostic information comprehensiveness. Integration of machine learning (ML) methodologies enhances the reliability of electrochemical biosensors, facilitating improved accuracy in both detection and classification tasks. As an example, De Castro et al. [20] utilized ML methodology, Sure Independence Screening and Sparsifying Operator (SISSO), to rectify false negative outcomes in their multiplexed electrochemical biosensor designed for COVID-19 detection, resulting in an accuracy rate devoid of false positives and negatives.

Number of studies have been conducted on the development of electrochemical biosensors for the detection of protozoan parasites. Accordingly, this review seeks to provide a comprehensive overview of contemporary trends in electrochemical biosensor advancements tailored for protozoan parasite detection. Critical parameters, encompassing protozoan parasite types, sensor specifications, surface modifications, electrochemical detection methodologies, and limits of detection (LoD), are systematically discussed to evaluate the efficacy and constraints of current electrochemical biosensor platforms for protozoan parasite detection.

2. Methodology

This study was performed as a scoping review to provide a concise overview of the currently available studies on an electrochemical biosensor for the protozoan parasites. The review adopted the latest Preferred Reporting Items for Systematic Review and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) guidelines.

2.1. Search scheme and keyword

A comprehensive search was conducted using the specified keywords in three databases (PubMed,

Scopus, Web of Science, and ScienceDirect) in January 2023. The keywords used for the search are 'electrochemical biosensor' and 'protozoan parasite'. These keywords were combined with the Boolean operators AND producing the search string '(electrochemical biosensor) AND (protozoan parasite)'. The search string was used for all four databases without any filters. Furthermore, the list of references from the retrieved literature was manually screened as an additional search in the protocol.

2.2. Inclusion and exclusion criteria

Articles were excluded if (i) the studies were not relevant to the development of electrochemical biosensors for the detection of the protozoan parasites; (ii) the studies were published in languages other than English or Malay; (iii) the studies were reported in the form of the book chapter, encyclopedia articles, conference proceedings, mini-reviews, systematic reviews, or review articles. Only research articles were selected in this scoping review.

2.3. Selection of studies

A 4-member reviewer's team participated in the article selection process. The collected research titles and abstracts from the above-mentioned electronic databases were screened independently by 2 reviewers (S. Y.S, N.H.J). The other 2 reviewers (N.H.O, H.H.H) screened the articles published from the conference proceedings, citation searching and thesis abstracts. The mentioned above inclusion and exclusion criteria were used for article selection. The article's title and abstract with chosen keywords were scrutinized before going through the full text of the article. If any difference of opinion arose among reviewers, a discussion and consensus meeting was conducted to conclude.

2.4. Data extraction

Details of the included studies were extracted and summarized in Table 1. The following data were extracted: types of protozoan parasites, targets, samples, platform of detection system, nanomaterials used for fabrication, detection methods, and LoD.

3. Results

3.1. Search result

A total of 52 studies was identified in three databases (Figure 1). From 52 studies, 10 were discarded, resulting in 39 studies were being subjected to the title screening. After screening the titles, a total of 19 studies that were unrelated to the electrochemical biosensor or protozoan parasite were removed. The remaining 23 studies were screened for abstract eligibility, and 4 irrelevant studies were excluded because that were related to the protozoan parasite but did not involve electrochemical biosensors. In the end, 19 studies were evaluated for eligibility, with 2 being inaccessible, being unavailable, and 6 lacking human samples. Eleven papers were included in the final analysis and one article was included after a more thorough and in-depth search. The collected studies demonstrate the publications of the development of electrochemical biosensors targeting protozoan parasites from January 2014-January 2022. Table 1 summarizes the key characteristics of the included studies: type of protozoan parasite, targets, samples, type of bioreceptor, sensor specification, platform technologies for fabrication, detection methods, and LoD.

3.2. Type of protozoan parasite

Figure 2 demonstrates the protozoan parasites commonly studied to develop electrochemical biosensors. The review included 11 studies: four on electrochemical biosensors for *Plasmodium falciparum* detection, three for *leishmania*, two for *Trichomonas vaginalis*, and one each for *Entamoeba histolytica*, *Toxoplasma gondii*, *Trypanosoma cruzi* detection. Therefore, this scooping review will discuss diverse electrochemical biosensor applications, specifically highlighting the advancements made in enhancing the identification of protozoan parasitic infections.

3.3. Sample matrix and type of Bioreceptor

All the studies included in the analysis primarily focus on detecting protozoan parasites in human-related samples, such as blood, stool, saliva, and vaginal discharge, as indicated in Table 2. Among these studies, one specifically utilizes cell culture obtained from patients for analysis. The predominant source for identifying protozoan parasites is blood samples, followed by vaginal discharge, while stool, saliva, and cell culture are less frequently used sources. The review revealed that most studies utilized antibodies and nucleic acids as bioreceptors in electrochemical biosensors, while antigens were the least commonly employed element (Figure 3).

3.4. Working electrode and fabricated nanomaterials

The limit of detection (LoD) heavily influenced by sensor specification of the electrochemical biosensor. This parameter reflects the sensor's sensitivity in detecting protozoan parasites. A lower LoD indicates higher sensitivity, enabling the detection of protozoan parasites even at the early stages of infection. Working electrode

Table 1. Characteristics of the developed electrochemical biosensors that have been identified and analyzed in this scoping review.

Type of protozoan parasite	Year of Study	Type of bioreceptor	Sample	Detection system	Working electrode	Type of Nanomaterial	Shape of nanomaterials	Size/Surface area of Nanomaterials	Detection method	Limit of detection (LoD)	Equivalent LoD	Ref.
Plasmodium falciparum	2017	Antibody	Saliva	Conventional three electrode system	Indium tin oxide	Platinum nanoparticles	None	31.2nm	Chronocoulometry & Cyclic voltammetry	2.2pg/ml	2.2 pg/ml	[21]
Plasmodium falciparum	2017	Antibody	Blood	Screen printed electrode	Gold	Gold nanoparticles	None	40nm	Chronoamperometry	40pg/ml	40 pg/ml	[22]
Plasmodium falciparum	2018	Aptamer	Blood	Conventional three electrode system	Gold	None	None	None	EIS	0.77pM	0.77 pg/ml	[23]
Plasmodium falciparum	2021	Aptamer	Blood	Conventional three electrode system	Gold	None	None	None	SWV	3.73nM	3,730 pg/ml	[24]
Trichomonas vaginalis	2018	Nucleic acid (DNA)	Vaginal discharge	Conventional three electrode system	Gold	Gold nanoparticles	Anisotropic	0.19cm^2	DPV	48.1ng/μL	48,100 pg/ml	[25]
Trichomonas vaginalis	2022	Nucleic acid (DNA)	Vaginal discharge	Conventional three electrode system	Gold	Gold nanoparticles	Non-spherical	77 ± 2nm	DPV	68.9ng/ml	68,900 pg/ml	[26]
Trichomonas vaginalis	2022	Nucleic acid (DNA)	Cell culture	Conventional three electrode system	Glassy carbon	Poly(ortho-aminophenol)	None	None	DPV	1.0pg/μL	1,000 pg/ml	[27]
Leishmania	2016	Nucleic acid (DNA)	Blood	Conventional three electrode system	Polycrystalline gold	Gold nanoparticles	Nanoleaves	None	DPV	0.07ng/μL	70 pg/ml	[28]
Leishmania	2020	Antibody	Blood	Screen printed electrode	Carbon	Gold nanoparticles	None	None	Cyclic voltammetry	200ng/ml	200,000 pg/ml	[29]
Entamoeba histolytica	2014	Antibody	Stool	Screen printed electrode	Gold	Gold nanoparticles	None	None	EIS	10pg/ml	10 pg/ml	[30]
Toxoplasma gondii	2020	Antigen	Blood	Screen printed electrode	Gold	None	None	None	EIS	0.31IU/mL	24,924 pg/ml	[31]
Trypanosoma cruzi	2016	Antigen	Blood	Screen printed electrode	Carbon	Gold nanoparticles	None	None	Amperometry	3.03ng/mL	3030pg/ml	[32]

DPV = differential pulse voltammetry, EIS = electrochemical impedance spectroscopy, SWV = square wave voltammetry.

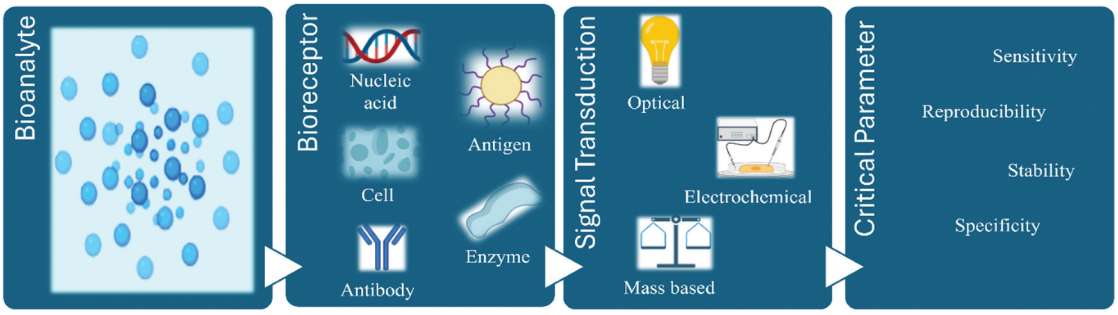

Figure 1. Three-component system of biosensor and its critical parameter.

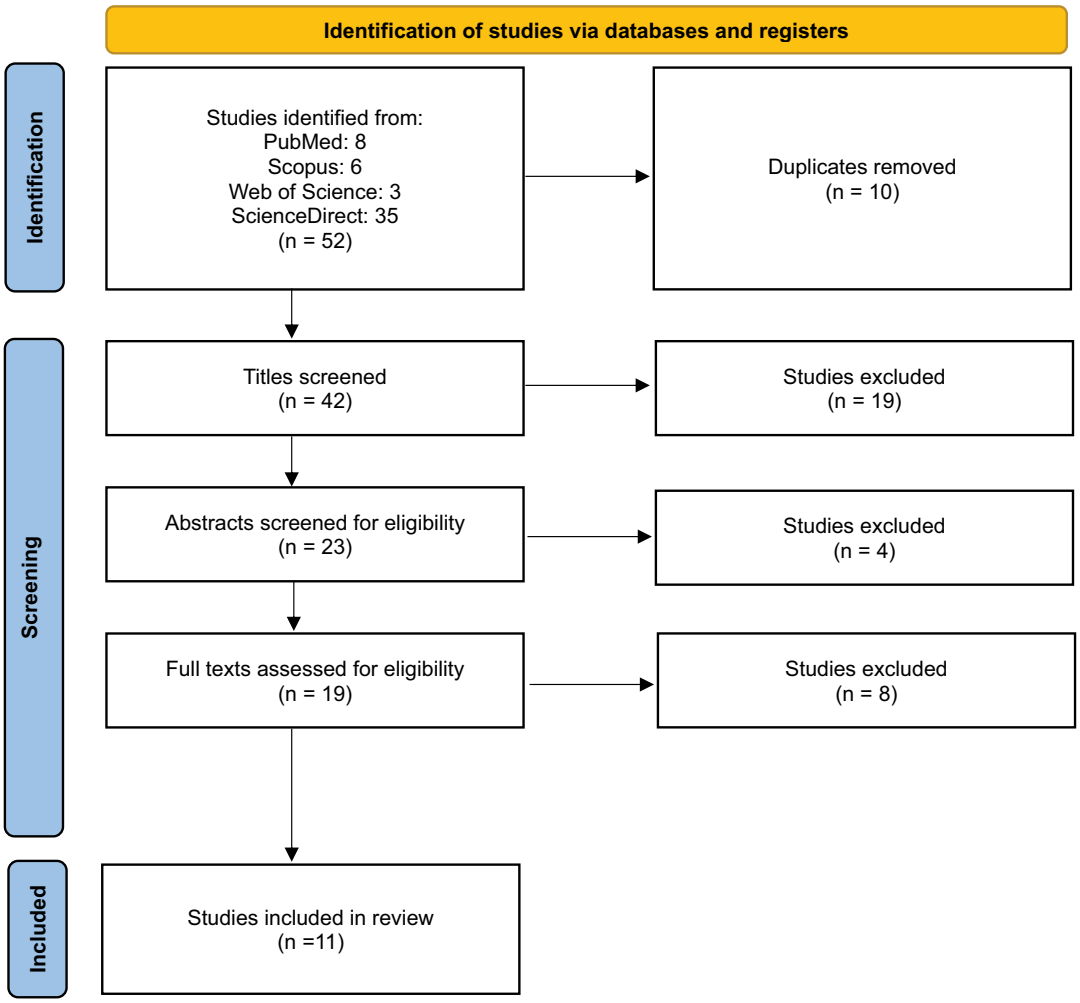

Figure 2. PRISMA-ScR flowchart of the scoping review process.

Table 2. The samples used to detect the protozoan parasite using developed electrochemical biosensors.

Sample matrix	Number of studies
Saliva	1
Stool	1
Cell culture	1
Vaginal discharge	2
Blood	7

and various nanomaterials used aim to improve the sensor's performance in terms of sensitivity and accuracy for effective management of parasitic infections. Table 3 shows that most studies use gold electrodes as the working electrode in electrochemical biosensors for detecting protozoan parasites. Similarly, Table 4 indicates that gold nanoparticles are the most studied nanomaterials in these biosensors. These findings highlight a strong connection between using gold-based components in this area of research. Figure 4,

3.5. Platform of detection system

In this review, the development of electrochemical biosensors for detecting protozoan parasites involved two main types of detection systems: the conventional

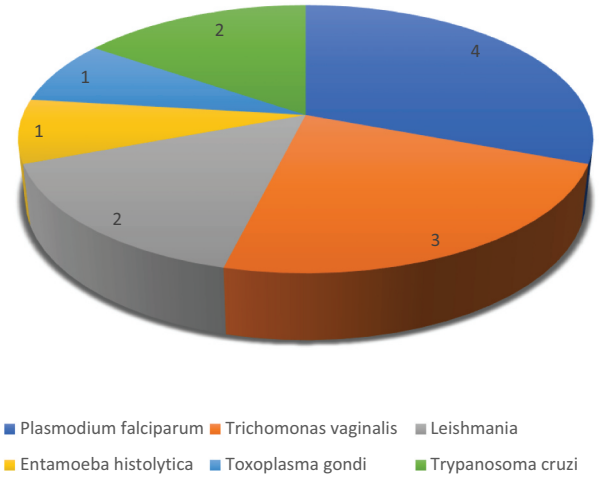

Figure 3. Protozoan parasites involved in the development of electrochemical biosensor.

Table 3. Nanomaterials used to modify the working electrode surfaces to improve electrochemical biosensors to detect the protozoan parasite.

Type of Nanomaterials	Number of Studies
Platinum nanoparticles	1
Gold nanoparticles	7
poly(ortho-aminophenol)	1

Table 4. Each study that used unit concentration different from pg/ml was then converted to its equivalent LoD.

Protozoan Parasite	Obtained LoD	Equivalent LoD	Reference
Plasmodium falciparum	2.2 pg/ml	2.2 pg/ml	[21]
Plasmodium falciparum	40 pg/ml	40 pg/ml	[22]
Plasmodium falciparum	0.77pM	0.77 pg/ml	[23]
Plasmodium falciparum	3.73 nM	3,730 pg/ml	[24]
Trichomonas vaginalis	48.1 ng/µl	48,100 pg/ml	[25]
Trichomonas vaginalis	68.9 ng/ml	68,900 pg/ml	[26]
Trichomonas vaginalis	1.0 pg/µl	1,000 pg/ml	[27]
Leishmania	0.07ng/µl	70 pg/ml	[28]
Leishmania	200ng/ml	200,000 pg/ml	[29]
Entamoeba histolytica	10pg/ml	10 pg/ml	[30]
Toxoplasma gondii	0.31IU/ml	24,924 pg/ml	[31]
Trypanosoma cruzi	3.03ng/ml	3030 pg/ml	[32]

three-electrode system and the screen-printed electrode. Despite the advantages of the screen-printed electrode, which allows for miniaturization of detection, most of the studies still preferred to use the bulky three-electrode system.

3.6. Detection technique

The detection technique is one of the critical components in the development of a highly sensitive electrochemical biosensor platform for detecting protozoan parasites. Like sensor specification, this factor will determine the LoD of the developed electrochemical biosensor. As shown in Figure 5, the developed electrochemical biosensor mainly employed differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) for electrochemical biosensor detection of protozoan parasites. Figure 6,

3.7. Assessment of study outcomes

Based on LoD of the developed biosensors, the developed electrochemical biosensor's sensitivity was assessed. All studies expressed the LoD in concentration units ranging from pico to nano scales. For better comparison, the LoD measurements are then changed to their equivalents in pg/mL, and the data are summarized in Table 3.

4. Discussion

Electrochemical biosensors recognize changes in surface properties in response to biological interactions, primarily facilitated through redox reactions. These alterations are subsequently quantified through parameters such as current, potential, or etc, yielding in a detectable sensor output [33]. As previously elucidated, the electrochemical biosensor encompasses the analyte, bioreceptor, and signal transducer, with each component serving defined functions within the biosensor system. According to Huang et al. [34], electrochemical biosensor can be classified into four types based on the way the analyte is measured: amperometric, potentiometric, impedimetric and voltametric. Fundamentally, amperometric measures analyte concentration using electrical current, potentiometric uses potential differences, and impedimetric assesses through system impedance. On the other hand, voltametric measures analyte concentrations by applying series of potential to an electrode and analyzing the current produced. Electrochemical biosensors offer high sensitivity, lower detection limits, automation, cheaper testing costs, and disposable instruments and methods that function with minimal sample volumes [35]. The promising trajectory of electrochemical biosensor development not only offers enhanced diagnostic precision but also introduces the potential for high-throughput screening. The present scoping review sought to evaluate the performance of electrochemical biosensors for detecting protozoan parasites. (Figure 7)

This review has identified a substantial gap in the type of protozoan parasites that have been encompassed within its scope. Among the four types of protozoan parasites, *Plasmodium falciparum*, known to cause malaria, was the most extensively studied in terms of detection using electrochemical biosensors, as evidenced by the analysis of nine published research articles spanning from 2014 to 2022. Malaria affects 200–400 million people worldwide and kills nearly 400,000 [36]. According to the WHO's 2021 report, the COVID-19 epidemic caused 13 million malaria cases and 63,000 malaria deaths. Multidrug-resistant strains have

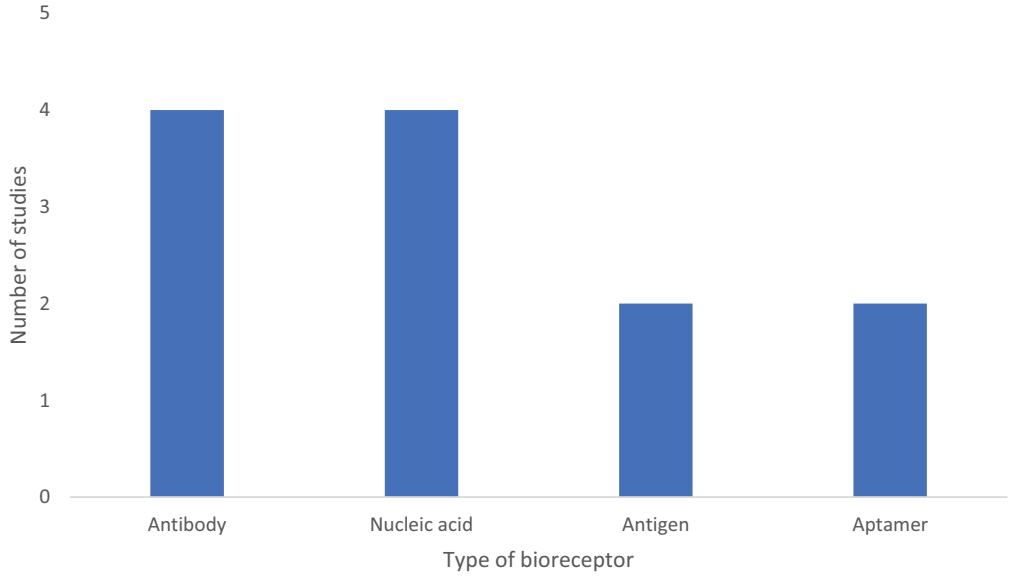

Figure 4. The selection of the bioreceptor determines the specific biomarker targeted for detection.

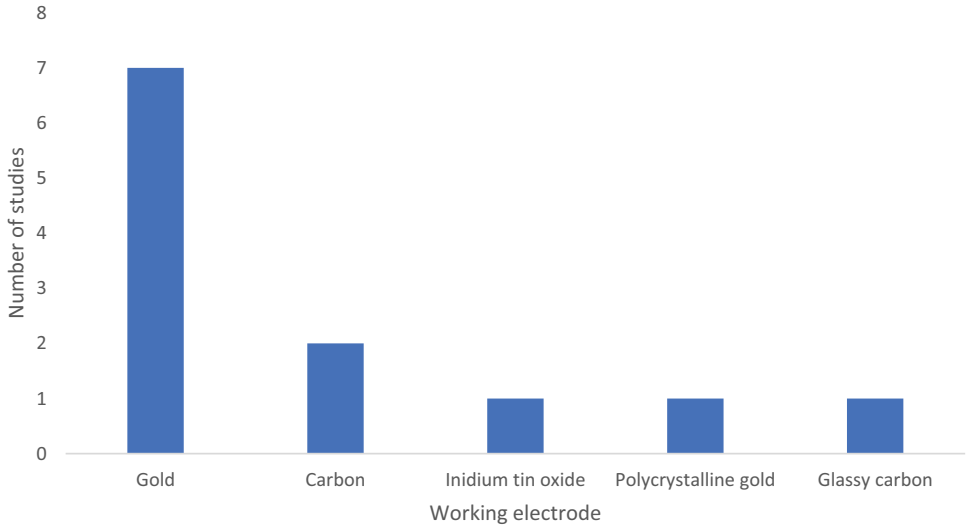

Figure 5. Working electrode, one of the factors that may contribute to the improvement of performance of electrochemical biosensor.

made malaria worse, as dihydroartemisinin-piperaquine treatment failures [37]. Despite the substantial threat posed by protozoan parasites like *Trichomonas vaginalis* and *Entamoeba histolytica*, there is a burgeoning interest in the development of point-of-care (POC) biosensors. Furthermore, in severe or late treatment, these infections result in severe medical conditions, including cancer, vital organ damage and death [38]. Moreover, the genus Trypanosoma, which includes species such as *Trypanosoma brucei* and *Trypanosoma cruzi*, the causative agents of African sleeping sickness and Chagas disease respectively, represents a significant concern in global health. Despite the critical need, the development of electrochemical biosensors for the detection of Trypanosoma spp. remains in its nascent stages [39]. These devices hold particular significance for Southeast Asian, Indian, and African countries, where limited resources and economic constraints intensify the issue's impact [40,41]. This review points out the need for more investigation into the development of electrochemical biosensors to detect a broader spectrum of protozoan parasites, such as *Cryptosporidium*, *Entamoeba histolytica*, *Toxoplasma gondii* and *Trypanosoma cruzi*. The preceding comparison draws a correlation to the WHO's initiative to alleviate the prevalence and eradication of parasitic diseases through the implementation of POC devices, which have the potential to yield significant advantages.

This scoping review assesses the performance of an electrochemical biosensor developed to detect protozoan parasites. The evaluation was conducted based on the biosensor's limit of detection (LoD) and specificity. The developed electrochemical biosensors to detect protozoan demonstrated a limit of detection (LoD) that varied between 200 ng/ml and as little as 0.77 pM. All the studies included in the review

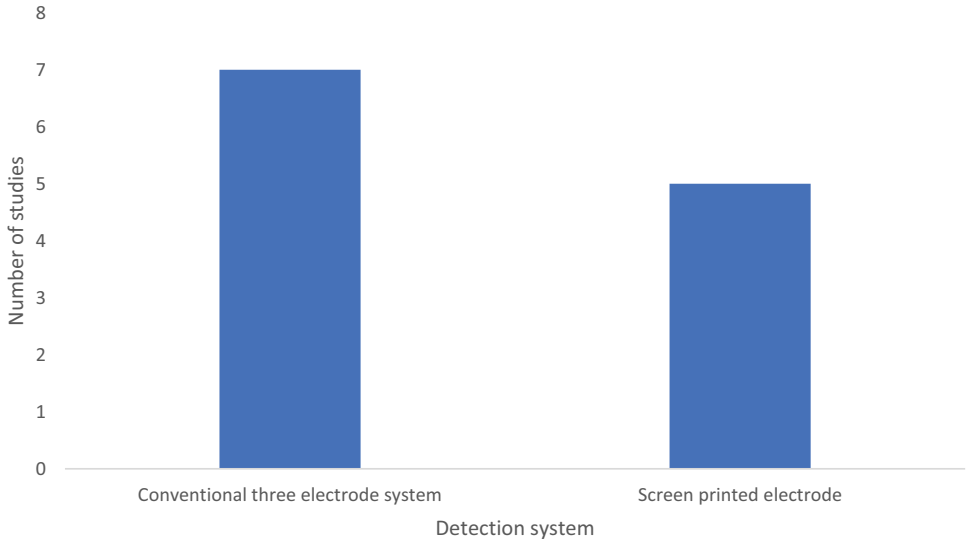

Figure 6. Two types of electrochemical detection systems commonly used in developing electrochemical biosensors to detect protozoan parasites.

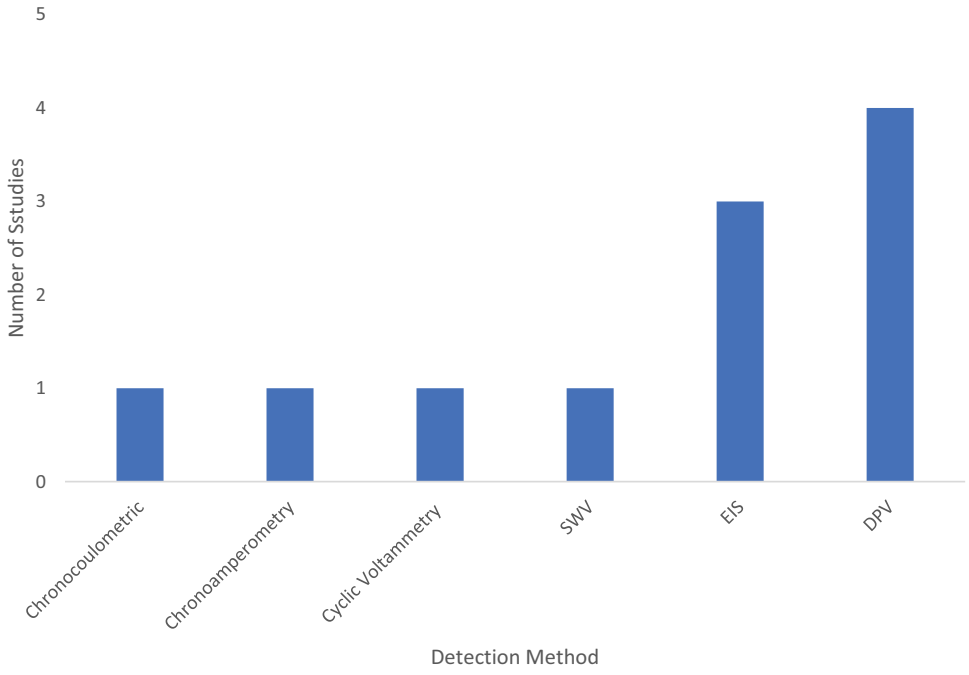

Figure 7. Detection method applied for development of electrochemical biosensors. DPV = differential pulse voltammetry, EIS = electrochemical impedance spectroscopy, SWV = square wave voltammetry.

improved sensitivity compared to standard clinical methods and commercially available kits. *Plasmodium falciparum* was detected using an electrochemical biosensor that utilized aptamer as the bioreceptor and electrochemical impedance spectroscopy (EIS) as the detection technique. Significantly, the working electrode was not developed using any nanoparticles. The review revealed that the studies included exhibited a notably high level of sensitivity to the approach employed. On the other hand, the detection strategy that employed nanoparticles onto the working electrode fabrication showed the lowest LoD reading. The prior study conducted by Grieshaber et al. [42] highlights that the efficacy of electrochemical biosensors is influenced by various factors, such as the material of the working electrode and the methods employed for electrochemical detection. Evaluating variables that impact the efficacy of electrochemical biosensors is a crucial step toward the advancement of the electrochemical biosensors with enhanced sensitivity and specificity.

Bioreceptor element is crucial in developing an electrochemical biosensor for protozoan parasite detection, as it directly influences the biosensor's sensitivity and specificity. The choice of bioreceptor in an electrochemical biosensor for detecting protozoan parasites is influenced by the type of target bioanalyte. Table 1 indicates that antigen is the predominant

target, suggesting that antibodies or aptamers are more suitable bioreceptors for such detection. Despite the growing use of aptamers as bioreceptor elements in sensor-related studies, the developed electrochemical biosensors for detecting protozoan parasites still mainly rely on time-consuming and expensive antibodies. Aptamers, with their smaller size, offer a significant advantage over antibodies, leading to higher surface coverage density and increased binding sites in electrochemical biosensors. Furthermore, aptamers demonstrate reduced batch-to-batch variability and improved thermostability, enhancing the reusability and reproducibility of the developed electrochemical biosensor [43,44]. Nucleic acid (DNA) also gets a great attention to be fabricated as bioreceptor especially for detection of *Trichomonas vaginalis*. The preference for nucleic acid as a bioreceptor can be attributed to its simplicity, cost-effectiveness, and, most importantly, its ability to enhance current molecular detection methods like the polymerase chain reaction (PCR). Morales & Halpern [45] noted that conventional nucleic acid bioreceptors face limitations in sensitivity due to steric hindrances. As a result, studies employing nucleic acids as bioreceptor elements may exhibit higher LoD readings, indicating a reduced ability to detect lower concentrations of target analytes. While not all studies in this review quantitatively report specificity, those that do categorize their analysis into two distinct approaches: (1) assessing non-target biomarker interactions with the bioreceptor, and (2) measuring the proportion of true negatives among individuals without the condition. The electrochemical biosensors under review exhibit high specificity, a testament to the careful selection of bioreceptor elements, underscoring their potential for further development into precise, on-site diagnostic tools for protozoan parasites.

The electrode is a crucial component of the electrochemical biosensor, serving as a transducer to convert the biochemical signal into an electrical signal for analysis. Electrochemical biosensors employ three types of electrodes: working, reference, and counter. However, conventional electrodes used in point-of-care (POC) devices are hindered by their bulky size and the requirement for larger sample and reagent quantities. The adoption of miniaturized electrodes, like screen-printed electrodes (SPE), has facilitated the transition to more practical and dependable POC devices [15]. In this review, most studies (seven) utilized the conventional three-electrode setup, while four studies employed the screen-printed electrodes (SPE) approach. The cost-effectiveness of SPE allows for inexpensive mass production while maintaining sensor reproducibility and reliability. This makes SPE a highly beneficial choice for on-site analysis [15]. Modifying the composition of inks during electrode printing in the manufacturing process allows for easy adjustment and enhancement of sensitivity and specificity. Given the advantages offered by SPE for the development of electrochemical biosensors, this research field should prioritize the utilization of SPE over conventional bulky electrodes [46,47].

One approach to enhance the performance of electrochemical biosensors for detecting protozoan parasites is to modify the working electrode surface using nanomaterials. Nanomaterials possess unique physical and chemical properties, such as diffusivity, solubility, optics, thermodynamics, color, and magnetism [48]. Table 4 clearly indicates that gold nanoparticles (AuNPs) have been widely employed in developing electrochemical biosensors for the detection of protozoan parasite. The primary benefit of using AuNPs is their capacity to stabilize the immobilization of biomolecules while preserving their activity. Additionally, their large surface area relative to volume, increased surface energy, capability to reduce the distance between proteins and metal particles, and their role in enhancing electron flow between redox proteins and electrodes are key factors that aid in facilitating efficient electron transfer in electrochemical systems [16]. According to Young et al. [49], the electrochemical properties are significantly influenced by the structure, composition the structure, composition the structure significantly influence the electrochemical properties, composition significantly affect the electrochemical properties, and the interface between the nanoparticles and the electrode. Three studies from this review showed that structure modifications (nanoleaves, anisotropic and non-spherical structures), can improve AuNP efficacy and lower the limit of detection [50]. Apart from AuNPs, PtNPs, and polymer-based materials are also utilized in electrochemical biosensors for detecting protozoan parasites. PtNPs offer synthesis flexibility and a microscopic tunneling effect, leading to lower LoD readings, while polymer-based materials are environmentally friendly and suitable for mass production [51]. Therefore, further research on utilizing PtNPs and polymer-based materials in electrochemical biosensors is warranted to enhance the detection performance.

Signal processing is a crucial aspect in electrochemical biosensor development, converting the binding event between the target bioanalyte and bioreceptor on the working electrode into measurable data. This review indicates that differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) are predominantly used for signal processing in detecting protozoan parasites. While differential pulse voltammetry (DPV) relies on assessing current in relation to applied potential (voltametric), electrochemical impedance spectroscopy (EIS) is based on monitoring changes in impedance (impedimetric). A key benefit of

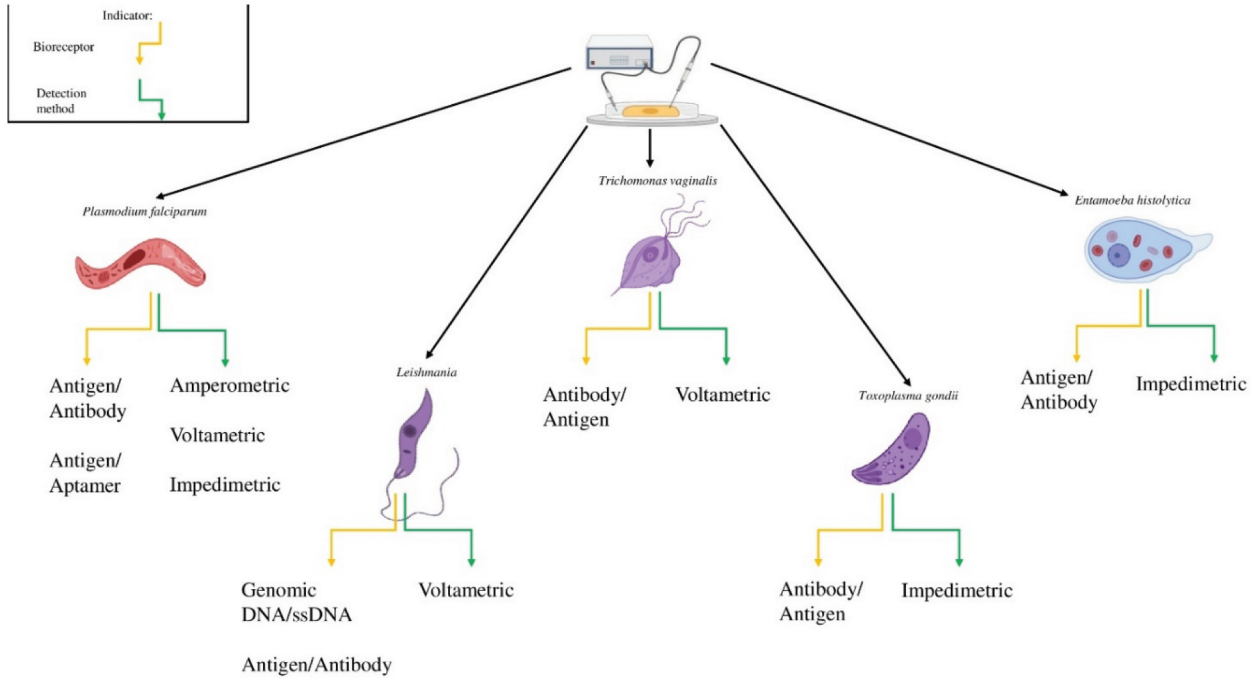

Figure 8. Summary of bioreceptor and detection methods of electrochemical biosensor that been employed to detect protozoan parasites.

employing both methods is their independence from labeled molecules or tags in analyte detection. As a result, these methods enable real-time monitoring of electrochemical reactions, yielding dynamic insights into bioanalyte behavior over time [52]. While this review indicates that square-wave voltammetry (SWV) is not the preferred choice for detecting protozoan parasites, it does highlight its superior sensitivity compared to other voltametric methods. This heightened sensitivity is attributed to the minimal impact of non-faradaic currents [53]. On the other hand, amperometric also been employed to detect protozoan parasite, chronocoulometry and chronoamperometry. According to Mortimer [54], chronocoulometry is favored over chronoamperometry due to its capacity to integrate the current signal, resulting in a reduced signal-to-noise ratio. This characteristic renders chronocoulometry more suitable for measuring the kinetics of chemical reactions due to electron transfer. (Figure 8)

5. Conclusion

This study evaluated the capacity of electrochemical biosensors for detecting protozoan parasites. The evaluation of the current electrochemical biosensor involved analyzing several components: bioreceptors, protozoan parasite types, biorecognition elements of electrochemical biosensors, working electrodes, fabricated nanomaterials for working electrodes, electrochemical detection methods, and limits of detection. The analysis indicated that the electrochemical biosensor focused more on designing to detect *Plasmodium falciparum*. The development of electrochemical biosensors is crucial for detecting other protozoan parasites, including *E. histolytica* and *Cryptosporidium spp*. These pathogens have been identified as significant contributors to the outbreak of foodborne diseases. This review reveals that the performance of the developed electrochemical biosensor in terms of limit of detection (LoD) was impacted by the bioreceptor and detection methods utilized. Despite the significant advantages of aptamers over antibodies, the majority of bioreceptors are antibody-based. Notably, aptamers are deemed the most sensitive in electrochemical biosensors, often employing AuNPs on the working electrode fabrication. The sensor specifications presented exhibit high sensitivity when utilized in conjunction with voltammetry detection methodologies. The results indicate that utilizing both detection methods can enhance the efficacy of the electrochemical biosensor. There is a pressing need for extensive research to be conducted on developing electrochemical biosensors utilizing SPCs. The future study is necessary to facilitate the application of electrochemical biosensors for on-site, portable, and real-time analysis.

Disclosure statement

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