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Colorimetric Biosensors for Rapid Biological Detection: Principles, Nanomaterial Advances, Portable Platforms, and Challenges

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Abstract

Colorimetric biosensors offer the advantages of being simple, low cost, portable and the response being visually interpretable, and have thus become potential platforms for rapid, biological detection without the need for complex laboratory instrumentation. In this review, recent progress in colorimetric biosensing is discussed, focusing on the principles and mechanisms behind colour generation, such as localized surface plasmon resonance, aggregation of nanoparticles, enzymatic reactions, and nanozyme-catalysis. Enhance analytical signals by nanomaterials, especially by the use of nanoparticles of noble metals, nanozymes and advanced nanostructures, is discussed. Recent developments on paper-based platforms, as well as smartphone-assisted colour analysis, are also emphasized for point-of-care and decentralised testing. Applications included pathogen, nucleic-acid, protein, biomarker, food, and environmental detection, highlighting the wide range of system applications. But there are still problems concerning sensitivity, colour interpretation, biological interference, nanomaterial reproducibility, quantitative analysis, standardization, and real world validation. The practical application of colorimetric biosensors can be further enhanced by further integration with nanotechnology, digital imaging, and portable platforms.

Keywords; Colorimetric biosensors; Rapid biological detection; Nanomaterials; Nanozymes; Smartphone-assisted sensing

INTRODUCTION

The need for rapid and convenient biological analysis has stimulated the development of biological sensing technologies that could deliver reliable results without the time, cost and instrumentation demands of traditional laboratory-based diagnostic tools. This need is especially crucial for early disease diagnosis, pathogen identification, food safety and point-of-care testing where biological targets might be needed to be detected at the point of interest. Clinically and analytically traditional methods are frequently based on bulky and expensive instruments, trained staff, and relatively complex procedures that may limit its use where resources are limited or in decentralized settings [1]. In this context, colorimetric biosensors have become simple analytical platforms able to transform a specific biological recognition event into a measurable and/or visible colorimetric change. The process of recognition can include antibodies, nucleic-acid probes, aptamers, enzymes or any other biorecognition elements, and the resulting colour signal can represent the presence or concentration of the target. Colorimetric biosensors may be used for the detection of pathogens, proteins, nucleic acids, enzymes and clinically significant disease markers depending on the sensing strategy [2].

One of the significant benefits of colorimetric biosensing is that the analytical response may be easily detected visually, without requiring advanced optical equipment for a speedy screening. These systems are particularly appealing for biological detection at the point-of-care and on-site due to their ease of use, portability, low-cost and quick response [3].

Their capabilities have been further widened by recent research that has integrated them with nanomaterials and nanozymes, that can help amplify the optical or catalytic signal and increase the analytical performance [4]. Meanwhile, paper-based analytical devices have offered low-cost, compact sensing formats, and smartphone cameras and image processing have made it possible to take digital photos of colour signals and quantitatively analyze them beyond the confines of traditional laboratories. The developments are progressing colourimetric biosensors from simple visual assays to integrated, portable diagnostic platforms [5]. Hence, this review highlights the technological developments on colorimetric biosensors and its application in rapid biological detection where nanomaterials are used for sensing, nanozymes as signal amplification, paper based platforms and smart phone based colorimetric detection.

PRINCIPLES AND DETECTION MECHANISMS OF COLORIMETRIC BIOSENSORS

The colorimetric biosensors are based on a change in colour that occurs when a specific interaction takes place between a biological recognition element and its target. The colour response can either be a change in the optical properties of the nanomaterials or it can be a chemical reaction that produces a coloured product. In this regard, gold nanoparticles (AuNPs) are significant among the most explored methods because of their localized surface plasmon resonance (LSPR) which is highly sensitive to particle size; shape; aggregation; and the surrounding environment. Another significant basis for colour generation is the enzyme and nanozyme mediated reactions which catalyse reactions of chromogenic substrates. In this way the basic principles of colorimetric biosensing can be generalized into two categories, depending on whether the optical signal is generated by a physical or chemical process [6][7].

Major Principles

1. **Localized Surface Plasmon Resonance (LSPR):** Noble-metal nanoparticles, particularly AuNPs and silver nanoparticles, exhibit collective oscillation of surface electrons when exposed to light. Binding of a target or changes in nanoparticle aggregation can alter the LSPR response, producing a visible colour change.
2. **Enzyme-Mediated Color Generation:** Natural enzymes can catalyse reactions involving chromogenic substrates, producing coloured reaction products. This principle is widely used where a biological target regulates or initiates an enzymatic reaction, allowing its presence to be translated into a measurable colour intensity.
3. **Nanozyme-Mediated Catalysis:** Nanozymes are nanomaterials with enzyme-like catalytic activity and can replace or supplement natural enzymes in colorimetric assays. Their relatively high stability, tunable catalytic properties, and low-cost preparation make them useful for signal amplification.

Major Detection Mechanisms

1. **Nanoparticle Aggregation/Disaggregation:** Target-induced aggregation changes the interparticle distance and LSPR coupling of nanoparticles, commonly shifting the colour of AuNPs from red/burgundy toward blue or purple. Conversely, target-induced dispersion can restore the original colour.
2. **Chromogenic Substrate Oxidation:** Enzymes or nanozymes catalyse oxidation of substrates such as TMB, ABTS, or OPD, generating coloured products. The resulting intensity can be correlated with the concentration of the biological target.
3. **Nanoparticle Deposition, Growth and Etching:** Changes in nanoparticle size, morphology, or surface composition caused by deposition, growth, or etching modify their optical response and consequently produce a detectable colour variation.
4. **Surface-Modification-Based Detection:** Target binding to functionalized nanoparticle surfaces changes the local chemical or optical environment, which can modify the colour or LSPR signal. This mechanism allows biological recognition elements such as antibodies, aptamers, or nucleic-acid probes to be coupled with colorimetric transduction.

NANOMATERIAL-BASED ADVANCES FOR SIGNAL ENHANCEMENT

The use of nanomaterials has significantly contributed to the development of colorimetric biosensors, by affording them an enhanced optical property, high surface area, catalytic activity, and easily tunable surface chemistry. These properties can enhance the interaction among recognition elements and the analytes, and enhance the colour response during detection. It has been found that the usage of nanomaterials in biological analysis of colorimetric systems is especially important for enhancing the sensitivity and practical application of these systems, with noble-metal nanoparticles, nanozymes, carbon-based materials, metal oxides and other hybrid nanomaterials being studied.

1. Noble-Metal Nanoparticles

The localized surface plasmon resonance (LSPR) of the nanoparticles that release strong and easily observable optical signals, is the reason for the widespread use in colorimetric biosensors, especially of nanoparticles of noble metals such as gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs). Optical properties of the nanoparticles can be modified by the induced aggregation, dispersion or modification of surface environments, resulting in different colour changes. Their surface can also be easily modified with antibodies, aptamers, DNA probes, and other recognition molecules, making them applicable for the detection of nucleic acids, proteins, and biomarkers and pathogens [8].

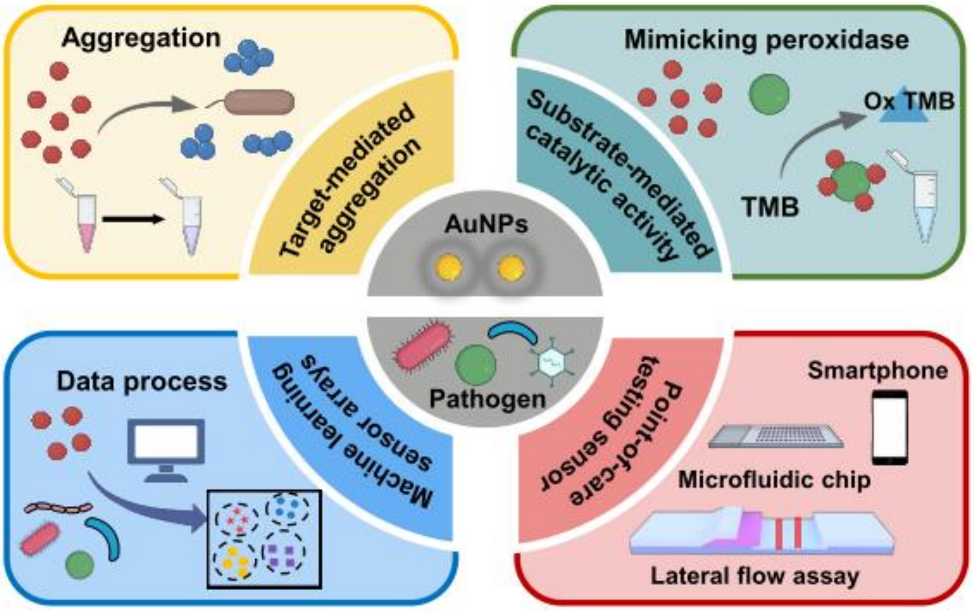

Figure 1: Schematic representation of colorimetric sensors for pathogen identification based on functionalized AuNPs [8]

2. Nanozymes

Nanozymes are nanomaterials with enzyme-like catalytic activity and have emerged as promising alternatives of the biological enzymes for colorimetric sensing. The peroxidase-like and oxidase-like nanozymes can catalyse chromogenic reactions to yield coloured products for enhanced visual signals. Nanozymes are usually more stable, more easily tunable in their catalytic activity, and more readily prepared than natural enzymes and are thus promising for the use in rapid biological detection. In recent years, the research on the use of the nanozymes made of metal, metal oxide, carbon-based, and single atoms has been carried out to enhance the colorimetric sensitivity [9].

3. Other Advanced Nanomaterials

There are other advanced nanomaterials that offer further opportunities to enhance colorimetric sensing such as graphene based materials, metal oxides, magnetic nanomaterials, and hybrid nanostructures. The modifiable surface, high surface area, adsorption capacity and catalytic properties can make them suitable for target capture and the generation of signals. Magnetic nanomaterials can also be used for separation and enrichment of analytes; the engineered nanocomposites can integrate recognition, catalytic, and optical properties on a single sensing platform [10].

4. Signal Amplification

In the case of biological targets that are present at very low concentrations, signal amplification is crucial. Nanoparticles can enhance the number of recognition sites and nanozymes can enhance the colour formation by catalytic conversion of multiple molecule substrate. Other approaches involve the use of nanoparticle aggregation, catalytic amplification and multiple nanomaterials in hybrid sensing systems. These approaches can enhance the colorimetric biosensors' capabilities in detecting biological agents quickly and with greater sensitivity [11].

PAPER-BASED AND SMARTPHONE-INTEGRATED COLORIMETRIC BIOSENSORS

Combining colorimetric sensing and the development of paper-based analytical devices (PADs) with smartphones has been an effective pathway to portable and decentralized biological detection. The simple substrate of paper allows the attachment of reagents and biorecognition elements without any external pumps to drive the flow. Paper is also porous enough to transport samples through capillary action. Colour changes are more easily detected on a white paper background making colourimetry more suitable for paper based platforms. These properties enable the fabrication of devices that are both low cost and light, disposable, and appropriate for fast and outside-of-the-laboratory testing. A new research trend has thus emerged to integrate paper based sensing with digital image acquisition for the objective and quantitative interpretation of colour signals [12].

1. Paper-Based Colorimetric Biosensors

Paper-based colourimetric biosensors feature a paper-based sensing platform (functionalized paper) and provide biological recognition elements and chromogenic reagents in clearly defined reaction areas. The presence of the target analyte causes a biochemical or nanomaterial reaction that generates a colour change which can be interpreted visually or digitally. They are especially useful for point-of-care/point-of-need applications due to their low material usage, ease of fabrication, portability, and disposable properties. In recent years, multiplex paper devices have been developed that can detect multiple targets simultaneously by having different sensing areas.

2. Smartphone-Assisted Detection

Smartphone-assisted detection is the practice of using the camera of the smartphone to read the produced colour, and software to process the parameters of color such as RGB or other colour space information, thereby improving paper-based colourimetry. This can decrease the need for subjective visual interpretation and can yield quantitative or semi-quantitative results. Illumination, optical accessories, fixed imaging distance, background correction and image-processing algorithms can be used to improve the image quality. The smartphone can thus be used as an optical reading device as well as a computational device, making it a suitable tool for portable biological analysis.

3. Advantages for Point-of-Care Testing

The benefits of the combination of paper and smartphones for point-of-care testing come from the complementary attributes of both. The paper-based devices need only a small amount of sample, they are cheap and disposable, and the smartphone offers ubiquitous imaging, processing, data storage and communication. They are able to perform a fast test using a small amount of lab equipment and also carry out a decentralized analysis in a resource limited environment. Recent studies are also investigating multiplex detection, more efficient image processing techniques, and integration of a smart phone–paper platform for biomedical applications [13].

APPLICATIONS IN RAPID BIOLOGICAL DETECTION

Colorimetric biosensors have gained widespread use for biological detection purposes due to the fact that their optical changes can be read visually or through the use of a simple portable instrumental device, which eliminates the need for very complicated instrumentation, unlike that used by many traditional methods. They are used in clinical diagnostics, food safety, environmental monitoring, and recently, they are integrated with nanomaterials, functional nucleic acids, enzymatic reactions, and portable platforms to enhance the sensitivity and selectivity [14].

1. **Pathogen Detection:** One of the primary uses of colorimetric biosensors is pathogen detection, especially for bacteria and viruses. One example of a visible colour change in a nanoparticulate system would occur when it was aggregated, accumulated or catalysed after it recognized a pathogen. Aptamers, antibodies and DNA-based recognition elements have been examined to detect bacterial and viral targets in clinical, food and environmental samples in real time with the aid of functionalized gold nanoparticles. These methods are appealing for point-of-care testing, as they offer easy and quick visual results.
2. **Nucleic-Acid Detection:** Nucleic-acid detection is a technique used to identify certain DNA or RNA sequences that are linked with a disease or pathogen. The use of sequence-specific recognition combined with amplification methods like isothermal amplification and subsequent aggregation of nanoparticles or enzymatic colour

generation is possible with colorimetric assays. These can enhance the sensitivity of visual detection, without resorting to complicated instrumentation.

3. **Protein and Biomarker Detection:** Another significant application is detection of protein and disease biomarkers, in which the target specificity is achieved by the use of antibodies, aptamers, enzymes, etc. Colorimetric platforms have been explored for the detection of proteins and clinically relevant biomarkers, and signal enhancement of weak signals in low target concentrations can be achieved by using nanomaterials for signal amplification.
4. **Food and Environmental Monitoring:** The colorimetric biosensors are also used in food and environmental monitoring for detection of bacterial contamination and other biological hazards in food and water. The speed at which they can be viewed visually, low expense and portability make them useful for screening in situations where lab analysis may be less feasible.

Table 1: Applications of Colorimetric Biosensors in Rapid Biological Detection

Application	Major Targets	Typical Colorimetric Approach	Key Benefit
Pathogen detection	Bacteria, viruses	AuNP aggregation, aptamer/antibody recognition, nanozyme catalysis	Rapid and visual screening
Nucleic-acid detection	DNA, RNA	Sequence recognition, amplification, nanoparticle-based colour change	Sensitive target-specific detection
Protein and biomarker detection	Proteins, disease biomarkers	Antibody/aptamer recognition, enzyme or nanozyme reactions	Low-concentration detection
Food and environmental monitoring	Foodborne bacteria, biological contaminants	Nanoparticle-based colorimetry and paper-based assays	Portable and low-cost screening

EMERGING CHALLENGES IN RAPID BIOLOGICAL DETECTION

Although extensive research has been carried out on colorimetric biosensors, there are still some issues that hinder these sensors from being scaled up to a reliable real-world biological detection tool. Analytical sensitivity, colour interpretation, interference from complex samples, stability of the samples and reproducibility are the major concerns. Additional variations in the camera, illumination, image-processing techniques, and the measuring conditions can influence the analytical response in smartphone-integrated systems. Recent studies have thus placed a great emphasis on the need for improved standards, controlled imaging conditions and validation with realistic samples prior to actual use in practice [15].

1. **Limited sensitivity:** More sophisticated laboratory methods may be used to achieve greater sensitivity than colorimetric methods, especially at low target concentrations. For trace biological detection, therefore, signal amplification strategies are needed.
2. **Subjective colour interpretation:** Detection with the naked eye is dependent on the observer and is not a good method for distinguishing slight colour differences, resulting in less quantitative accuracy.
3. **Complex biological samples:** Proteins, cells, salts, and other components in real samples can interfere with recognition or colour generation, affecting selectivity and accuracy.
4. **Nanomaterial stability and reproducibility:** Variations in nanoparticle size, morphology, surface modification, and storage stability can influence colour responses and make reproducible sensor fabrication difficult.
5. **Quantitative analysis:** Converting colour intensity into reliable concentration values requires controlled reaction and measurement conditions rather than simple visual observation.
6. **Smartphone standardization:** Differences among smartphone cameras, lighting conditions, imaging distance, and image-processing algorithms can produce inconsistent results, highlighting the need for standardized imaging protocols.
7. **Scale-up and validation:** Many reported systems remain at laboratory or proof-of-concept stages. Large-scale manufacturing, long-term stability, regulatory evaluation, and validation using real clinical or environmental samples remain important requirements for practical deployment.

LITERATURE REVIEW

Liu et al., (2025)[16] Infectious diseases have a wide range of significant characteristics with complex clinical manifestations. The incidence rate and mortality rate remain high, seriously endangering human health. Therefore, rapid identification and sensitive diagnosis of pathogens are crucial for patient recovery. In order to overcome the limitations of traditional cultivation methods, such as long detection time and limited microbial species, various new detection technologies have rapidly developed in recent years. This not only greatly shortens the detection time, but also effectively improves the accuracy and sensitivity of pathogen detection. This article reviews the rapid detection methods for pathogens that are currently in the research process or early application stage in recent years, including detection technologies in molecular biology, immunology, biosensors, and microfluidics. It focuses on summarizing the sample pretreatment method, detection principles, mechanisms, and advantages of various technologies, and analyzes the current application status of various technologies in pathogen detection in clinical biological samples, in order to provide technical references for rapid detection of different clinical biological samples.

Jin et al., (2024) [17] In this investigation, an intelligent paper-based biosensor was produced by utilising filter paper as a matrix to create boronate affinity paper-based materials. The paper was both cost-effective and readily available. Horseradish peroxidase (HRP) from crude horseradish extract was immobilised on the surface of the material. This intelligent paper-based biosensor was ultimately built using a cold assembly technique. When paired with a smartphone APP, it made it simple, accurate, and simultaneous to detect the levels of glucose (GLU), uric acid (UA), and sarcosine (SAR) in urine, greatly improving the ease and usefulness of real-time monitoring. In order to confirm the accuracy of the detection results, UV spectroscopy was also used as a support method. The biosensor showed linear ranges of 2–200, 2–500, and 2–500 μmol L–1 for the detection of GLU, UA, and SAR, respectively, with detection limits (LODs) of 1 μmol L–1 for all analytes. With relative standard deviations less than 3.5%, the recovery rates of GLU, UA, and SAR identified by the intelligent paper-based biosensors in real-world urine sample analysis fell within a tolerable range. This clearly illustrates the intelligent paper-based biosensor's great accuracy and dependability. A new method for the detection of H2O2-related biomarkers has been developed with the invention of this inexpensive, portable biosensor.

Rawat et al., (2025) [18] The basic features of nanoparticles are examined in this study, including their high surface area-to-volume ratio, distinctive optical, electrical, and magnetic properties, and functionalisation for enhanced selectivity and biocompatibility. The usefulness of these technologies is demonstrated by notable case studies on the detection of cancer biomarkers using gold nanoparticles (AuNPs), glucose monitoring using graphene-based electrodes, and multiplexed pathogen detection utilising nanomaterial-enhanced microfluidic chips. Notwithstanding their benefits, problems including the toxicity of nanomaterials, repeatability, and large-scale production continue to be significant barriers. Additionally, the use of nanomaterial-based biosensors in environmental sensing—such as air quality monitoring and heavy metal detection—is investigated. The creation of scalable, affordable, and AI-integrated biosensing systems for next-generation applications is emphasised in future visions. Nanomaterial-based biosensors have the potential to transform precision medicine, environmental sustainability, and disease diagnostics by overcoming present constraints and using transdisciplinary developments.

Jayan et al., (2020) [19] Microorganism biomarkers and bio-recognition ligands that are often employed in detection assays are discussed. Recent advances in bio-recognition-based detection methods for assessing microbe activity in complicated food matrices are reviewed, and the molecular interaction between biomarkers and ligands is critically assessed. Nucleic acids, proteins, antigens, and metabolic products are examples of particular biomarkers of microorganisms that may be used to identify the microbial activity in food. The selectivity of cell detection from complicated food matrices can be enhanced by the use of modern bio-recognition ligands in detection methods such biosensors, lateral flow assays, and microfluidic devices. The industry may use the new bio-recognition-based techniques to assure food safety since they close the gap between culture-dependent enumeration and molecular techniques. To evaluate each developing method's effectiveness in actual samples, a validation process must be developed.

Celik et al., (2023) [20] To begin the appropriate therapy, it is essential to diagnose infection-causing bacteria utilising sensitive, quick, selective, and affordable diagnostic tests. These tests can stop the spread of diseases. Furthermore, antimicrobial resistance detection contributes significantly to public health. Alternatives to the conventional diagnostic tests used in clinics have been developed in recent years. Particularly, colorimetric tests—which reduce the requirement for an instrument—have benefits because of their affordability, quick reaction time, naked-eye detection, and usefulness. In this research, we paid particular attention to pH indicators and colorimetric assays based on nanomaterials for the detection of antibiotic resistance and infection-causing microbes.

Choi et al., (2018) [21] To stop the spread of pandemic illnesses, reduce economic and social losses, and enable appropriate clinical therapy, rapid, sensitive, selective, easy, and affordable pathogen diagnosis is crucial. Many sensor-based diagnostic systems that outperform traditional pathogenic diagnostic tests have been developed in recent years. Among them, colorimetric biosensors that can identify target molecules with the unaided eye have garnered a lot of interest because of their affordability, ease of use, and simplicity. Nanomaterials have recently been used as a flexible signal transduction and amplification method for the quick and accurate detection of viruses and dangerous microorganisms. This article reviews current developments and trends in the use of colorimetric biosensors that use different nanomaterials to identify and diagnose harmful bacteria and viruses. Furthermore, how nanomaterials and bioreceptors might be more effectively combined to create a sensitive and quick colorimetric detection system in the future is described.

Zaman et al., (2021) [22] ascertain the significance of quick BI monitoring for sterile surgical tool quality control. From April 1, 2021, to July 8, 2021, this study was carried out at the Labaid Cardiac Hospital in Bangladesh. This study was carried out using an auto reader incubator and 100 steam and 100 ethylene oxide (EO) fast BIs. The auto reader was utilised once a day to evaluate the quick BI of steam and EO. To validate the fast BI test findings from the auto reader, all of the tested BIs were subsequently incubated for 48 hours in a traditional incubator.

The BI was found to be positive twice during steam sterilisation, while all of the EO BI findings were negative. The surgical supplies from the two cargoes were re-sterilized. Once more, the goods were released following a review of the BI outcome. After 48 hours of incubation, all BIs—aside from positive steam fast BIs—were discovered to have no growth in order to cross-check the auto reader results. After 48 hours of incubation, growth was observed in the positive fast BI of steam. If fast BI tests were not conducted at the time of sterilisation failure, process recall would not have been feasible. Therefore, the patient can avoid serious SSI by integrating a quick BI test with an auto reader.

DISCUSSION

The papers analyzed show that colorimetric biosensors have been evolving from simple visual assays to more and more complex platforms for rapid biological detection. Their main advantage is that they allow the recognition of a specific event in a biological process into an easily recognizable colour change, thus eliminating the need for expensive instrumentation. A key feature of this development has been the use of nanomaterials to achieve improved optical responses, catalytic activity and signal amplification. In particular, the use of noble-metal nanoparticles and nanozymes for pathogen, nucleic-acid, protein, and biomarker detection has been widely explored. Paper-based platforms and smartphone-based analysis are also shown to be practically feasible in recent studies. In one instance, glucose, uric acid and sarcosine were detected simultaneously using a smartphone-based spot-on analysis system, for example, showing the potential for portable monitoring of biomarkers. In the literature, colorimetric methods are also highlighted as the rapid and cost-effective screening of microorganisms. However, issues such as sensitivity, matrix interference, reproducibility, quantitative colour interpretation and validation using real samples are still important concerns. Thus, standardization of the nanomaterials, controlled imaging, signal amplification and extensive real world validation are needed for future development.

CONCLUSION

Colorimetric biosensors are one of the promising methods for fast biological detection due to their ease of use, visual output, low cost and portability, which are suitable for point-of-care applications. The advancement of noble-metal nanoparticles, nanozymes, graphene-based materials, magnetic nanomaterials, and hybrid nanostructures has increased signal generation and detection capabilities for the various biological targets. By combining inexpensive sensing substrates and readily available digital imaging technologies, paper-based devices and smartphone-assisted analysis have further enhanced the potential for decentralized and resource-limited testing.

They have been shown to be broadly applicable to detection in the fields of pathogens, nucleic-acids, proteins, biomarkers, food and environment. However, sensitivity, complicated biological matrices, stability of nanomaterials, subjective colour interpretation, quantitative measurement, standardization are all limitations that hinder the broader use of nanomaterials in practical applications. There is a need, therefore, to focus future research work on reproducible fabrication, improved signal amplification, standardised analysis by smartphone, multiplex detection and validation with real samples. The developments can aid in bringing colorimetric biosensors from laboratory demonstrations to reliable rapid-detection technologies.

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