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Progress in smartphone-enabled aptasensors

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ABSTRACT

Despite their high analytical performance, conventional analytical biosensor devices are usually difficult to handle, time-consuming, bulky and expensive. As a result, their applications remain restricted to resource-limited environments. In particular, the transportation of conventional analytical equipment is challenging for the proper *in-situ* point of need (PON)/point of care (POC) detection of biomolecules. In this context, smartphones, the most widely utilized cutting-edge mobile gadgets, continue to be a favored option because of their ease of portability and revolutionary sensing capabilities. On the other hand, aptamers are molecular recognition units consisting of nucleic acids with highly sensitive and selective recognition capabilities towards their respective targets. The coupling of smartphones with aptamers have led the development of advanced, user-friendly, portable, and cost-effective in-situ PON/POC biosensors for the detection of biomolecules. Such sensors are well-suited for a variety of applications, including food safety, environmental monitoring, and disease diagnostics. Herein, for the first time, achievements made between 2017 and 2022 in the concept and design of the smartphone-enabled aptasensors for biosensing applications are reviewed. The review covers different fabrication strategies and the discussion of several operating systems, underlying programs, and related software. At the end, the important merits, challenges, and future prospects of smart phone-driven aptasensors are presented. This report intends to assist scientists and engineers in comprehending the fabrication of smartphone-based aptasensors and their underlying sensing principles, as well as stimulating the future developments in the direction of affordable, portable, simple, and readily available sensing devices.

1. Introduction

Biosensors are highly efficient instruments to monitor biochemical or biological operations that are fundamental in a variety of sectors, notably food safety, environmental monitoring, and disease diagnostics (Qi et al., 2022). Despite their accurate and reliable analytical performances, the conventional analytical biosensors are hard to handle, expensive, and relatively large in size (Huang et al., 2018; Zhu et al., 2017, 2019). The actual price and bulkiness of the readout devices have become additional obstacles in the fabrication of economical and portable biosensor detection systems, thus limiting their miniaturization. To address these challenges, researchers have been trying to use smartphones in biosensors and bioelectronics to reduce the overall cost and size of the device (Zhang and Liu, 2016), in addition to their enhanced portability. Smartphones are by far the most recent evolution of mobile phones that are similar to mini-computers in terms of the state-of-the-art built-in components that are highly beneficial and can be used for other electronic devices, including biosensors. For example, light sensors, micro-USB ports, high-definition (HD) cameras, storage memory, multiple communication features (e.g., Bluetooth and Wi-Fi),

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touch screens, headphone jacks, global positioning system (GPS), near-field communication (NFC), and so forth (Younis et al., 2020) are the important parts of smartphones to display the detection results. The leading innovations in the smartphone technologies during the last decade have made smartphones internationally ubiquitous. Because of their widespread use and robust built-in features and components, such as networking potential, sensors, and other multiple interfaces, the utilization of smartphones is increasing in mobile sensing applications for health tracking, POC diagnostics, and environmental monitoring (Xu et al., 2017), particularly in the countries with limited resources.

Smartphone-based biosensing technology has largely replaced time-consuming detection techniques that typically require expensive equipment and professional operators. The smartphones have received considerable attention. This is reflected by the increasing number of studies about the use of smartphone biosensors to pinpoint clinically meaningful analytes, including tuberculosis (TB) and cancer causative agents, along with performing point-of-need tests. While the potential of smartphone-based biosensors have been demonstrated, there are untapped opportunities for improving POC diagnostics and prognostic tools. This is gradually changing the way people think about mobile health and the biosensor market (Younis et al., 2020; Quesada-González and Merkoçi, 2017).

Aptamers are short, single-stranded oligonucleotide fragments (either RNA or DNA) that bind to a broad range of target molecules with great affinity and specificity. The targets include proteins, drugs, organic or inorganic entities (Jayasena, 1999; Luzi et al., 2003). Aptamers were initially discovered through a repeated rounds-based method termed "Systematic Evolution of Ligands by Exponential Enrichment" (abbreviated as "SELEX") by two laboratories independently, Tuerk and Gold and Ellington and Szostak (Ellington and Szostak, 1990; Tuerk and Gold, 1990). Many different SELEX procedures, such as graphene oxide SELEX, capillary electrophoresis SELEX, FluMag SELEX, etc., have been proposed to screen high-affinity aptamers quickly and easily (Gopinath, 2007; Kim and Gu, 2013). The SELEX method mainly involves binding, partitioning/elution, amplification, and separation phases, which are repeated to enrich the sequences bound to the target, and, subsequently, the aptamer is screened (Kim et al., 2019, Zahra et al., 2022a). Over 1000 well-characterized aptamers against a wide range of targets, from small molecules to whole cells and tissues have been published in the literature so far (Qi et al., 2022, Zahra et al., 2022a). Aptamers have been extensively used in the field of biosensing due to their unique binding characteristics (Kim et al., 2019). Although a number of reviews related to smartphone-based biosensors have been published over the last decade (Roda et al., 2016; Zhang and Liu, 2016; Li et al., 2016, 2022; Liu et al., 2019; Preechaburana et al., 2014), the progress made in the smartphones-driven aptamer-based biosensors remains to be reviewed despite the availability of a large number of recently published original research articles in this direction. The aptamers have several advantages over conventional antibodies, including flexibility, availability, high chemical stability, affinity, and specificity (Liu et al., 2009). The biosensors, that use aptamer as a bioreceptor (aptasensors), have emerged as efficient methods (Nguyen et al., 2017; Kim et al., 2010). When aptamers bind to their specific targets, they fold upon themselves into a well-defined three-dimensional configuration (Mayer, 2009). In aptamer-based biosensors, the recognizing ability of aptamers is converted into readable signals through pre-programmed algorithms. Significant amount of research are available, which focused on the use of aptamers in biosensors (Qi et al., 2022). All achievements in the concept, design, and advancements of smartphone-based aptasensors for various sensing applications are reviewed and presented in this article.

2. Smart phone-based aptasensors

Different hardware and software linked to the smartphone significantly expanded the phone's potential, turning it into a sensor, data processor, and system controller (Rezazadeh et al., 2019). Aptasensors can be classified on the basis of their signal transduction mode, with optical and electrochemical aptasensors presumably the most prevalent (Zahra et al., 2021a,b). The fundamentals of the design of optical and electrochemical aptasensors are discussed earlier by Zahra et al. (2022a, b). Such aptasensors can be integrated with smartphones for colorimetric, fluorescent, electrochemical and other applications. Table 1 summarizes the efficiency of smartphone-based aptasensors as well as their role in analytical methodologies including various operating mechanisms involved, apps, the limit of detection, and linear concentration ranges. In this section, we will discuss smartphone-based optical, electrochemical, and other aptasensors.

2.1. Optical aptasensors

Aptamers serve as bio-recognition factors in optical aptasensors, while a variety of techniques serve as signal transduction factors (Sadeghi et al., 2018). An optical aptasensor can be characterized by its light absorption and luminescence alterations resulting from interactions with various analytes. Many of these sensor systems feature simple labeling, cost-effectiveness, and low reagent requirements (Zahra et al., 2021b). Various smartphone-based biosensing devices rely primarily on the high quality camera lenses of smartphones. Almost all optical-based analytical techniques, such as reflectance, surface plasmon resonance (SPR), imaging, fluorescence, absorbance, and so forth, can be constructed using smartphone photo cameras as a "smart detector," complementary metal oxide semiconductor (CMOS) image sensors as a "smart recorder," and specific optical APPs (applications) as a "smart readout" (Wang et al., 2017). Colorimetric detection, in particular, can be achieved with only a smartphone by instantly processing the colored images acquired by its camera, making it the most attractive method for PON analysis (Liu et al., 2017). Depending on the analytical target, either a smartphone and its commercial apps can be chosen, or a specific program can be developed. Some examples of smartphone-based colorimetric, fluorescent, and other aptasensors for the detection of different targets, such as heavy metals, antibiotics, toxins, pesticides, bacteria, viruses, disease biomarkers, and hormones, are as follows:

(a) Colorimetric aptasensors

The use of gold nanoparticles (AuNPs) as signal transducers in aptamer-based quantification opens up new possibilities for the detection of numerous target analytes at low concentrations (Cheng et al., 2018; Zahra et al., 2021b). Using AuNPs for color signal production in colorimetric assays has been commonly used because of the noticeable color change that can be seen with the naked eye when nanoparticles agglomerate. Aptamers and AuNPs-based colorimetric detection approaches have recently been demonstrated to be more controllable and flexible (Ma et al., 2018). The salt-induced non-crosslinking aggregation (Ma et al., 2018) and the aggregation caused by the reduced distance generated by linkage of probes marked on AuNPs are the two main forms of color changes provided by AuNP aggregation (Bui et al., 2015). Due to their distinct aggregation mechanisms, the salt-induced non-crosslinking aggregation is considerably faster than crosslinking aggregation (Gu et al., 2020). It has been demonstrated that quantitative image analysis using a smartphone or digital camera is possible by reading out color information, specifically the red, green, and blue (RGB) values of the detection zone. Depending on the greyscale value or RGB channel, the RGB data is helpful in providing information about the concentration of the target (Kim et al., 2017).

Heavy metals, such as arsenic, mercury, lead, and cadmium, represent a stable group of environmental contaminants with limited biodegradability. Heavy metals build up in the environment and living organisms, leading to adverse consequences, including human diseases and environmental implications (Mehta et al., 2016). Siddiqui et al. (2020) estimated arsenic (As(III)) ions in purified soil using AuNPs and

Table 1
Performance summary and important parameters of various smartphone-based aptasensors since 2017 to April 2022.

Sensing mechanism	Detection target	Sample	Smart phone model used	Technology/ Accessories/ Software/Hardware	Application area	Linear range	Limit of detection	Reference
Colorimetric	Antibiotics Streptomycin (STR)	honey, milk and tap water	Honor 3C (Huawei, China)	battery-powered opto-sensing accessory attached to the camera, high-resolution digital camera and advanced multi-core pro-cessor, SBP-App	Food safety	–	2.3 nM	Liu et al. (2017)
Fluorescence- colorimetric (dual-functional)	Antibiotics Streptomycin (STR)	chicken and milk	–	Camera, RGB, The Touch Color APP, Digital image colorimetry	Food safety	0.1–100 μM	94 nM	Lin et al. (2018)
Fluorescence resonance energy transfer (FRET)	Antibiotics Kanamycin (KAN)	Milk	Redmi Note 4 camera-phone (Xiaomi)	200 × external macro lens, compact battery powered class III green laser (Amazon, Inc), polyacrylic sample slide cradle	Food safety	50–500 nM	28 nM	Umrao et al. (2019)
Electrochemical (EC)	Antibiotics Kanamycin (KAN)	Milk	Samsung Galaxy SIII	portable PalmSens instrument, wireless Bluetooth	Food safety	0.05–30 pM	30.0 fg/ mL or 0.05 pM	Yao et al. (2019)
Colorimetric	Antibiotics Chloramphenicol (CAP)	Milk and chicken	STF-AL10, Huawei, Shenzhen, China	Camera, Touch Color app	Food safety	–	7.65 nM and 5.88 nM	Wu et al. (2019)
Colorimetric	Antibiotics Tetracycline (TET) and Chloramphenicol (CAP)	Chicken and milk	–	Camera, color scanner APP (Color Grab)	Food safety	0.05–3.0 μM 0.05–1.8 μM	32.9 nM and 7.0 nM	Wu et al. (2020)
Colorimetric	Antibiotics Sulfadimethoxine (SDM)	Food samples	–	Camera, touch color app (RGB analysis) or Photoshop app	Food safety	–	0.023 ppm	Zhang et al. (2021)
Electro-optical (SPR-POF based) (dual-functional)	Antibiotics β-lactams Ampicillin (AMP)	water	–	potentiostat (PalmSens), spectrometer	Food safety	–	–	Galatus et al. (2017)
Lateral flow strips based	Antibiotics Enrofloxacin (ENR)	Skimmed milk powder, surimi homogenate, and honey	Huawei Honor View 20	Camera, Android 9.0 operating system (OS), Android Studio 3.5.3, debugged	Food safety	–	0.1 μg/L	Tian et al. (2020)
Colorimetric	Acetamiprid	cabbage, cucumber, and river water	Honor V40	Microplate Reader with SparkControl Software,	Food and environmental safety	–	1.74 μM	(Xu et al., 2022a)
Colorimetric	Acetamiprid	cabbage, cucumber, and river water	Honor V40	Microplate reader	Food, environmental safety	25–300 μM	3.81 μM	(Xu et al., 2022b)
Fluorescence	Pesticides chlorpyrifos, diazinon, and malathion	maize, long bean, cauliflower, eggplant, oyster mushroom, shiitake mushroom, apple, orange, tomato, blueberry, spinach, lettuce, and cabbage	iPhone 5	three conventional lasers (405 nm, 532 nm, and 632.8 nm), power, sample cascade, and fluoresence imaging optics (external lens, long-pass filter, and transmission diffraction grating), ImageJ and Origin software	Food and environmental safety	–	0.73 ng/ mL, 6.7 ng/mL, and 0.74 ng/mL	(Cheng et al., 2018)
Colorimetric-Electrochemical (dual-functional)	Mycotoxins Fumonisin B_1 (FB_1)	Corn		Camera, ImageJ software	Food safety	10^{-3}–10 ng/mL	–	Zheng et al. (2020)
Electrochemiluminecence (ECL)	Mycotoxins Aflatoxin M1 (AFM1)	milk	Samsung S4	Camera, Image J software	Food safety	10–200 ng/ mL	0.05 ng mL^{-1}	Khoshfetrat et al. (2018)
Fluorescence	Mycotoxins Anatoxin-a (ATX)	real lake water	–	portable blue laser diode, green emission	Food safety	–	1.2 nM 2.0 nM	Li et al. (2019)

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Table 1 (*continued*)

Sensing mechanism	Detection target	Sample	Smart phone model used	Technology/ Accessories/ Software/Hardware	Application area	Linear range	Limit of detection	Reference
	Cylindrospermopsin (CYN) Nodularin (NOD) Microcystin-LR (MC-LR)			filter, external lens (12 mm in diameter, 54 mm of focal length, Edmund Optics), smartphone reader device			2.8 nM 1.4 nM	
Colorimetric	ibuprofen enantiomers (S and R ibu)	Tap and river water	–	Camera, RGB, Android-based color analysis application	Environmental monitoring	–	1.24 and 3.91 pg/ mL	Ping et al. (2018)
Fluorescence	Allergens	Food	iPhone and iPad	Zero Spy camera, and a Li-Ion battery of 860 mAh. The camera, the display, the illumination circuit, and the power-off button, wireless Bluetooth	Food safety	–	–	Abdelli et al. (2019)
Colorimetric	Heavy metals Cadmium (Cd^{2+})	water	iPhone 4s	smartphone-based colorimetric system (SBCS), a 96-well microplate loading station, a wide-angle lens, a dark room, and a piece of low-power electroluminescent sheet	Environmental monitoring	2–20 μg/L	1.12 μg/L	Gan et al. (2020)
Colorimetric	Heavy metals Cadmium (Cd(II))	Water, rice	–	Camera, Color Assist app	Food, environmental safety	1–400 ng/ mL	1 ng/mL	Xu et al. (2019)
Colorimetric	Heavy metals Arsenic (As(III))	Soil	(LG-F470L, LG Electronics, Korea	Android operating system (Android 4.0.3, IceCreamSandwich), camera, 8-megapixel complementary metal oxide semiconductor (CMOS) image sensor with light emitting diode (LED) flash, removable optical unit (comprised of an adapter, cartridge holder, polarizer, and a test chip)	Environmental monitoring	–	14.44 ppb (aqueous samples), 1.97 ppm (field soil)	Siddiqui et al. (2020)
Fluorescence –Colorimetric (dual-functional)	Heavy metals Mercury ions (Hg^{2+})	Porphyra		ring ultraviolet lamp at 380 nm, circular UV lamp, camera, android application	Environmental monitoring	0.1–9.0 μM	4.92 nM	Shi et al. (2021)
Colorimetric-Electrochemical (dual-functional)	Adenosine triphosphate, and Mercury (ATP and Hg^{2+})	Rabbit serum and tap water	–	home-made electrochemical detection module, miniaturized workstation, Wireless Bluetooth	Disease diagnosis/ environmental monitoring	–	5.203 pmol L^{-1} and 1.597 pmol L^{-1}	Gu et al. (2020)
Fluorescence	influenza A (H1N1) virus	Human blood serum and buffer	–	Camera, UV LEDs (395 nm), Image Pro Plus software, inverted fluorescence microscope with a color CCD camera	Influenza virus/disease diagnosis	–	70 ng mL^{-1}	Lee et al. (2018)
Colorimetric	*Plasmodium falciparum* lactate dehydrogenase (PfLDH)	Human blood serum	iPhone 6s and iPad	POLARstar® Omega Plate Reader Spectrophotometer, Adobe Photoshop' 'Adobe Illustrator', 'Pymol' and 'Origin Pro', 'ImageJ' software, Preview App OSX′	Malaria/ Disease diagnosis	–	–	Fraser et al. (2018)
Plastic optic fiber (POF) based	Plasmodium falciparum glutamate	Human blood serum and buffer	Honor 7X (BND-AL10)	16 MP camera of f/ 2.2 aperture, 10 × macro lens, a light-emitting diode (LED)	Malaria/ Disease diagnosis	–	264 pM	Sanjay et al. (2020)

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Table 1 (*continued*)

Sensing mechanism	Detection target	Sample	Smart phone model used	Technology/ Accessories/ Software/Hardware	Application area	Linear range	Limit of detection	Reference
	dehydrogenase (PfGDH)			flash, four-channel smartphone slip-on accessory, 30 cm long optic fiber, ImageJ software				
Colorimetric	Bacteria Mycobacterium tuberculosis (TB)	Human sputum	Huawei honor 3C (H30–C00)	rear camera, color analysis apps	TB/Disease diagnosis	–	10^4 CFU/ mL	Li et al. (2018)
Electrochemical (EC)	Bacteria *Listeria* spp.	Irrigation water	–	Smartphone-based potentiostat	Food, environmental safety	10^2–10^6	23 CFU mL^{-1}	Sidhu et al. (2020)
Colorimetric	Pathogenic bacteria Escherichia coli O157:H7	Milk	–	Light-emitting diode, a mobile power pack, NUPACK	Food safety	10^3–10^6 cfu/mL	5.24 × 10^2 cfu/ mL	Yang et al. (2021)
Fluorescence	Pathogenic bacteria *Staphylococcus aureus* (*S. aureus*)	peanut milk	Samsung Galaxy S6	Camera, ImageJ software, smartphone-fluorescence microscope developed by 3D printing of core body attached with a smartphone. white light emitting diode, 10x objective (Nikon Finite), dichroic mirror, eye piece (working distance 13.97 mm) with 10x magnification	Food safety	–	–	Shrivastava et al. (2018)
Microfluidic paper-based colorimetric	Pathogenic bacteria*E. coli* O157: H7 and *S. Typhimurium*	pear juice, buffer	iPhone 11 Pro Max	photo studio booth, Adobe Illustrator (CC2019), Whatman Grade 1 filter paper	Food safety	10^2 CFU/ mL - 10^8 CFU/mL)	10^3 CFU/ mL and 10^2 CFU/ mL	Somvanshi et al. (2022)
Fluorescence	17-β-Estradiol	Wastewater	Samsung Galaxy S4 smartphone,	digital camera, mobile fluorescence reader	Environmental monitoring, food safety	–	1 pg/mL	Lee et al. (2017)
Surface plasmon resonance (SPR)	25-hydroxyvitamin D (25OHD)	Human blood serum	Apple iPhone 6s	Camera, Polymer chip, SPR sensors, Flashlight LED,	Vitamin disorders/ Disease diagnosis	0–100 nM	0.19 RIU	Walter et al. (2020)
Fluorescence resonance energy transfer (FRET)	Endocrine disrupting chemical Diethylstilbestrol (DES)	River water, prawns, and fish	Mate 20, Huawei Technologies Co., Ltd., China	portable 980 nm laser, IR 750 shortwave pass filter (infrared filter), app	Food safety	–	0.024 ng/ mL	Wu et al. (2021)
Microfluidic-Colorimetric	K^+ ions	Whole blood and serum	–	power supply, an image analysis box, a WiFi chip, voltage switch and voltage adjustment buttons, an LED, CMOS camera, self-developed RGB color code APP, paper microchip	chronic kidney disease/ Disease diagnosis	0.05–9 mM	0.01 mM	Tseng et al. (2022)
Electrochemical (EC)	Thrombin and Vaspin	Human saliva, buffer	–	iOS-based data displaying software, wireless mini-potentiostat	Periodontal disease/ Disease diagnosis	0–15 nM	0.02 nM and 1 nM in buffer and saliva	Joe et al. (2022)
Electrochemical	Glucose and insulin	Human saliva	–	wireless biochip, micro device with Bluetooth, multichannel potentiostat, self-developed app	Prediabetes/ diabetes/ Disease diagnosis	0.1–50 mM 0.05–15 nM	0.08 mM 0.85 nM	Liu et al. (2022)
Aptameric graphene-based field effect transistor **(GFET)**	Interleukin-6 (IL-6) cytokine biomarkers	Human saliva	–	Wireless Bluetooth/ cloud sever through Wi-Fi connection, on-board analyzer (ADS 1274, Texas Instruments) and controller (STM 32F4, STMicroelectronics)	cytokine disorders/ Disease diagnosis	10 pM–20 nM	12 pM	Hao et al. (2019)

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Table 1 (*continued*)

Sensing mechanism	Detection target	Sample	Smart phone model used	Technology/ Accessories/ Software/Hardware	Application area	Linear range	Limit of detection	Reference
Colorimetric	Cancer biomarker, Osteopontin (OPN)	Buffer and commercial human serum	–	Camera, ImageJ software	Cancer/disease diagnosis	5–1000 ng/ mL	5 ng/mL	Pereira et al. (2022)
Colorimetric	Carcinoembryonic antigen (CEA)	Human blood serum	–	Adobe Camera	Cancer/Disease diagnosis	4–25 ng mL^{-1}	0.19 ng mL^{-1}	Yang et al. (2022)
Lateral flow strip based Thermal detection	Exosomes (Early-stage cancer diagnostic biomarkers)	Human blood serum	iPhone Xs (Apple, U.S. A)	IOS 12.2 operating system, Excitation laser (808 nm laser light), smartphone accessible thermal detector (72 mm (L), 50 mm (W), and 60 mm (H), total weight 56.4 g)	Cancer/Disease diagnosis	–	1.4×10^4 exosomes/ μL	Cheng et al. (2019)
Electrochemical (EC)	COVID-19 nucleocapsid protein (Np)	Tap water, milk, or human blood serum		miniature Palmsens potentiostat, Wireless Bluetooth	COVID-19/ Disease diagnosis	50 pg mL^{-1} - 100 ng mL^{-1}	16.5 pg mL^{-1}	Han et al. (2021)
Electrochemical (EC)	Neutrophil gelatinase-associated lipocalin (NGAL)	urine	Huawei Mate 20 lite	audio and microphone channels, Google Play link, potentiostat, Epson XP 15000 printer	Acute kidney injury/Disease diagnosis	3–300 nM	3 nM	Rosati et al. (2022)

an aptamer-based colorimetric method with a smartphone equipped with a portable optical unit. The testing chip was made with polydimethylsiloxane (PDMS), which consisted of two reaction zones: one for testing and the other for controlled samples. The average RGB pixel values were computed from the acquired digital photograph, and the blue to red (B/R) ratio was examined and linked to the target concentration. Five different concentrations of a standard target solution were measured in field soil samples from different areas contaminated with waste. In another study, using AuNPs and aptamer in combination with a smartphone-based colorimetric readout, Xu et al. devised a simple approach for the detection of cadmium Cd(II) (Xu et al., 2019). AuNPs were dependent on the free cationic polymer polydiene dimethyl ammonium chloride solution (PDDA) as a signal element. In the absence of cadmium ions in the solution, PDDA and the aptamer were joined by electrostatic force. AuNPs remain as free particles, and the solution color appears to be wine red. When a small amount of the target was added, some aptamers bound to Cd(II), while others bound to PDDA, resulting in an aggregation of excessive PDDA and AuNPs. The light intensity of the solution diminished, and the absorption peak wavelength shifted to the blue. The color of the solution changed to a crimson hue. As the target concentration was further increased, the solution color changed to blue. This was rationalized in terms of fact that an increase in the concentration of target can attract and bind more aptamers, concurrently generating free PDDA that caused the agglomeration of AuNPs. The R values of the solution were recorded with a smartphone and investigated using the ColorAssist app.

The term "small molecule" refers to organic entities with a molecular weight of less than 1000 Da (Peltomaa et al., 2018). Many small molecules have been identified as pollutants in food, feed, and other agricultural goods. Pharmaceuticals, molecular markers, antibiotics, pesticides, and toxins are also included in the category of small molecules. In environmental monitoring, food safety, medical diagnostics, and various other fields, there is a growing need for the fast, simple, selective, and sensitive detection of a broad range of small-molecule targets (Yao et al., 2018). Zhang et al. constructed a smartphone-based colorimetric aptasensor that relies on AuNPs aggregation for fast and reliable sulfadimethoxine (SDM) quantitative detection. The aptamers desorb from the AuNP surface to bind with SDM. Subsequently, the AuNPs clumped together in high-salt conditions, generating a red-to-blue color transformation. An app examined the R, G, and B values of photos captured with a smartphone camera for quantitative bioanalysis, and the R value showed an excellent linear relationship with target concentrations. In the absence of the target, however, no AuNPs agglomeration or color swatches (a spectrum with many different tints of the same color) occurred. This common type of smartphone-based colorimetric strategy allows for simple, straightforward, and quantitative detection of several targets without the need for expensive and cumbersome instrumentation, which is incredibly beneficial in resource-constrained situations like PON and POCT (Zhang et al., 2021). Relying on an ssDNA component that coordinately controls AuNPs aggregation, Wu et al. built a POCT colorimetric aptasensor for multiplex antibiotic detection. The property of multifaceted aptamer, which acts as a molecular switch and a binding element for antibiotics, was tailored to be immobilized on the surface of AuNPs. In the presence of an antibiotic, the aptamer component specifically binds to it and dissociates AuNPs, while the non-specific component of the same aptamer synergistically controls AuNP agglomeration under high-salt circumstances. As a result, the generation of multiple color variations in the AuNPs mixture was employed as a smartphone-based autonomous signal readout (Fig. 1A). For the independent sensing of chloramphenicol (CAP) and tetracycline (TET), the aptasensor demonstrated exceptional sensitivity and selectivity (Wu et al., 2020). Wu et al. designed a highly sensitive and label-free colorimetric aptasensor for the detection of CAP using AuNPs agglomeration and smartphone imaging. Subtly, a classical reaction in which La^{3+} (added as a trigger agent) strongly combines with the phosphate groups of aptamer in AuNPs-aptamer probe in the absence of CAP (target), stimulates AuNPs aggregation and color change of the solution. However, in the vicinity of CAP, the aptamer undergoes structural switching as a result of the CAP-aptamer complex formation. The complex failed to bind with the surface of the AuNP. As a result, the cross-linking function of La^{3+} remained ineffective and AuNP agglomeration was not observed (no color changes). As the concentration of CAP increased, the agglomeration of AuNPs decreased, which was indicated by a color transition from blue to violet-red. This obvious color shift was captured by a smartphone-based imaging app to get the RGB data from digital photos. Because of the instability of trigger component La^{3+} in storage and its low biocompatibility, *in-situ* use of the suggested sensor may be restricted. Moreover, the analytical effectiveness in terms of sensitivity and selectivity needs improvement in future investigations (Wu et al., 2019).

Liu et al. (2017) designed a cost-effective aptasensor strategy for food safety. This approach utilized battery-powered optosensing

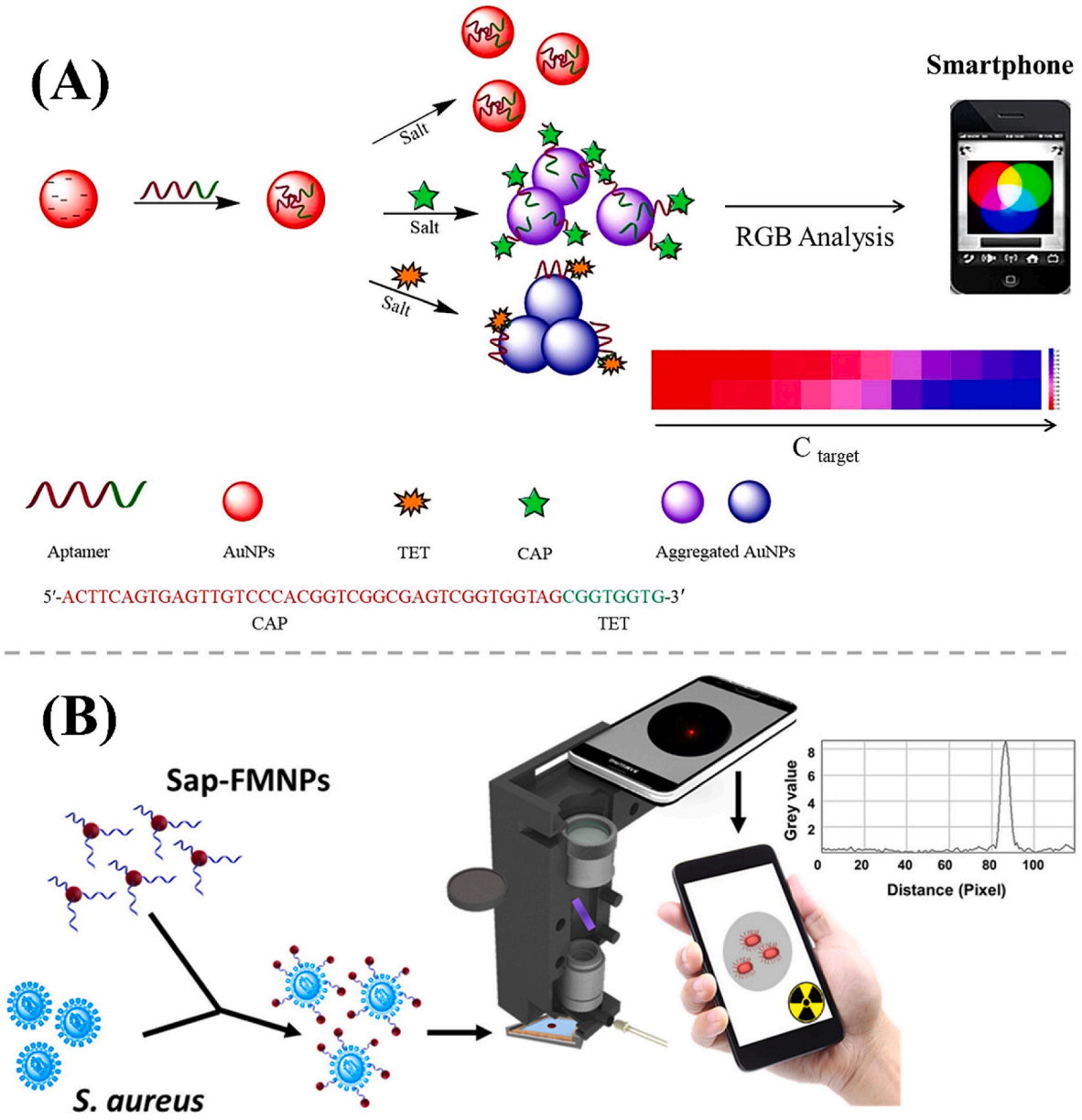

Fig. 1. **(A)** Schematic of CAP/TET detection based on colorimetric *aptasensors* with AuNPs. The aptamer plays a role as a molecular switch regulating the aggregation of AuNPs. When antibiotic targets take away their particular aptamer fragment from the surface of AuNPs, unstable AuNPs get agglomerated to different degrees depending upon the concentration of the target in high-salt conditions. It results in color variations that can be identified by UV-spectroscopy and smartphone technology, respectively (Wu et al., 2020). **(B)** Smartphone-dependent quantitative imaging in presence of the aptamer and AuNPs for the detection of the S. aureus. The targeted pathogens were captured selectively via a bacterial recognition cassette through aptamer conjugated fluorescent magnetic nanoparticles (Sap-FMNPs). The smartphone-based fluorescence microscopy imaging was executed by using an LED and a computer supported image analysis and processing to quantify bacterial contents in the minimally processed liquid foodstuffs (Shrivastava et al., 2018).

equipment linked to a smartphone camera to estimate the concentration of targets via a colorimetric assay based on aptamer-conjugated AuNPs. The small and simple optosensing equipment was prototyped and built utilizing simple and cost-effective three dimensional (3D) printing technology. It involved light-emitting diodes (LEDs) of 520 and 625 nm for a two-color ratiometric detection scheme. An easy-to-use Android app (SBP-App) was designed for the automated image analysis of the digital images, as well as to report and share results showing the efficiency of SBP. For the colorimetric depiction of ibuprofen (Ibu) enantiomers, Ping et al. (2018) for the very first time established a facile and selective quantitative enantiomer analysis via android-based color analysis software. An enantiomeric composition of ((R)-(−)-ibuprofen (R-Ibu) and (S)-(+)-ibuprofen (S-Ibu) was identified using AuNPs capped with IBU binding aptamers in real water samples. Zheng et al. (2020) constructed a colorimetric aptasensor to detect the most common mycotoxin, fumonisin B1 (FB1). It was achieved by controlling the proportion of bubbles on the surface of a closed bipolar electrode (BPE) device, which was made by linking a platinum cable (anode) inside the reporting cell to a glassy carbon electrode (GCE, cathode) inside the sample cell via an electric cable. Bipolar electrodes (BPE) served as an electric connection between reporting and sensing solutions to enhance ECL and EC signals. The FB1 binding aptamer was adsorbed on the AuNPs surface (aptamer@AuNPs) and the captured DNA on Fe_3O_4 NPs (cDNA@Fe_3O_4) to create recognition probes. Both recognition probes were coupled to the GCE (Glassy carbon electrode) cathode after depositing silver particles on AuNPs (as

Aptamer@Au-silver/Fe_3O_4NPs@cDNA/GCE) that stimulated H_2O_2 decomposition into oxygen bubbles. The quantity of Prussian blue (PB) deposited at the diving electrode (ITO electrode) in the reporting cell was significantly lowered by the adsorption of bubbles on GCE, as could be seen by the amount of PB deposited at the diving electrode (ITO electrode). Due to the strong affinity between aptamer and FB1 target, AuNPs-aptamer probe was released from the recognition probe surface in the presence of target (FB1), resulting in a decreased quantity of Ag particles placed on GCE. As a result, a few bubbles were seen adhering to the surface of GCE. The current recovery that was seen by a color shift in PB = with the naked eyes can be exploited by ImageJ software on a smartphone to report the concentration of FB1.

Biomarkers are biomolecular signs of both normal and abnormal biological processes, as well as pharmacological and physiological responses to drug therapy (Sahab et al., 2007). Pereira et al. (2022) developed an efficient cellulose-based colorimetric aptasensor for osteopontin (OPN, a cancer biomarker) quantification in POC cancer diagnostics. To accomplish this, the cellulose paper was chemically modified with (mercaptopropyl) methyldimetoxisilane to immobilize the thiolated aptamer as a biological sensing layer. The surface modification was confirmed by thermogravimetric analysis (TGA) and Fourier transform infrared (FTIR) spectroscopic techniques. A typical staining solution, called Bradford reagent, was used for colorimetric detection. Color development was carried out using a dye, Bio-Rad Protein Assay Dye, suited for protein analysis. The digital photographs of the color transformation on the standardized test-strips were captured using a smartphone camera in moderate light, and thus the RGB color coordinates were examined using ImageJ software. In general, this aptasensor exhibited better sensitivity for applications in cancer monitoring.

Infectious diseases are caused by pathogens that cannot be seen by the naked eye,.These pathogens can be transmitted directly or indirectly from one person to another through food, water, contaminated air, and human contact (Jiang et al., 2020). Some common infectious diseases are tuberculosis, hepatitis, malaria, influenza, lower respiratory infections, and SARS CoV-2 (Jiang et al., 2020). Li et al. proposed a reliable, truly portable, and cost-effective latent tuberculosis infection (LTBI) monitoring scheme based on chemically-stable enzyme-linked aptamers (Li et al., 2018). Two dot-blot assays (indirect and direct) for PON TB diagnosis were established using a biotin-labeled aptamer that can specifically identify mannose-capped lipoarabinomannan (Man-LAM) on the cell wall of Mycobacterium tuberculosis (*M. tb* or *M. TB*). A mobile application (M. tb-app) was used to analyze the digital images obtained by a smartphone rear camera and compile diagnostic reports, which were then shared on the internet. This application helped in TB diagnosis without requiring any expensive equipment or specialized training. The analysis revealed that the designed dot-blot tests employing the aptamer for *M. tb* assessment in spiked clinical and sputum samples had excellent specificity and sensitivity. Moreover, the results were comparable to those obtained with the standard TB detection techniques, such as microbiological culture and acid-fast stain. Somvanshi et al. revealed the design and construction of aptamer-functionalized AuNPs coated on polystyrene microparticles (PS), enabling paper-based simultaneous identification of *S. Typhimurium* and *E. coli* O157:H7 via colorimetric signal interpretation (Somvanshi et al., 2022). The digital patterns were created using Adobe Illustrator. To create the paper-based device, a Xerox ColorQube wax printer was used to imprint wax patterns on Whatman Grade 1 filter paper. In combination with a salt-based aggregation process, the AuNPs coated PS (PS–Au) improved the colorimetric signal sensitivity. Using a smartphone digital camera, an image analysis approach was used to evaluate the outcomes. The suggested image analysis consisted of three important steps: extraction of the region of interest (ROI), color retrieval of the sensing zone, and data processing of the colorimetric signals. An optical system recorded the colorimetric impulses in the monitoring domains (a photo studio booth). Furthermore, image processing was used to enable the devices to provide not only qualitative positive/-negative results but also quantitative detection via color intensity correlation with target concentration. This method can be extended to the detection of other pathogens as well. Using aptamers coated on the surface of magnetic beads for magnet-guided capturing, washing, and identification of the disease biomarkers in serum, Fraser et al. developed an innovative idea for malaria testing (Fraser et al., 2018). The colorimetric analysis of *Plasmodium falciparum* lactate dehydrogenase (PfLDH) using an equipment-free magnet-guided system was made possible through an aptasensor by integrating three distinct microfluidic chambers. To obtain reduced inter-chamber diffusion rates and improved sensitivity, as well as to provide the ability to evaluate samples at POC testing, prototypes of microfluidic aptasensors were designed. The PfLDH affirmative purple signal apparent to the bare eyes was objectively quantified using sensors embedded into a mobile tele-medical system. A small amount of blood (5 μL) and reagent (20 μL) allowed the Aptamer-Tethered Enzyme Capture (APTEC) biosensor to produce quantifiable signals in about 20 min after generation. The minimum parasite content spotted on the microfluidic aptasensor using pixel area metrics was 250 parasites/μL. This value is within the clinical sensing range and significantly more specific/sensitive than those of clinical samples from malaria patients or in vitro cultured parasite samples when detecting PfLDH. The approximate cost of the current prototype sensor is 3 USD/cassette.

(b) Fluorescent aptasensors

Fluorescent detection through a smartphone is a promising biosensing approach that can potentially contribute to on-site analyses (Umrao et al., 2019). For instance, Shi et al. (2021) fabricated a ratiometric fluorescent biosensor based on aptamer-modified copper and gold nanoclusters (apt-Cu@AuNCs) to quantify mercury ions (Hg^{2+}) in Porphyra. Without Hg^{2+}, the apt-Cu@Au NCs were widely disseminated in solution. The Hg^{2+} ions interacted with thymine to form a thymidine–Hg–thymidine structure, which induced the aggregation between AuNCs and CuNCs. The aggregation led to variations in their fluorescence emission due to FRET. Noticeably, the bright color variations were discernible to the bare eyes. Furthermore, a lightweight and efficient device was fabricated using a microfluidic chip and a smartphone for colorimetric measurement of Hg^{2+} in the range of 0.5–7.0 M. The method has huge potential for determining the amount of Hg^{2+} in aquatic samples.

For the on-site rapid and simultaneous analysis of pesticides such as malathion, diazinon, and chlorpyrifos, Cheng et al. produced a handheld multimodal platform by integrating a smartphone spectrum reader and a fluorescent apta-LFB (lateral flow biosensor) (Cheng et al., 2018). With a zero-background signal, the detection technique combined the benefits of fluorescence spectrum analysis, 3D printing technology, LFB testing, and DNA aptamers. Gold nanostars and quantum dot nanobeads (as fluorophore-quencher nano-pair) were effectively utilized as “turn-on” signals rather than “turn-off” signals. The researchers made a smartphone-based spectrum reader to examine the fluorescent apta-LFB. The device was also tested for spiked spinach samples and found to be stable, reliable, portable, and easy to use. The antibiotic residues present in food can be detected effectively using on-site fluorescent and visual methods in everyday life. Lin et al. integrated fluorescence analysis with smartphone-based digital image colorimetry to develop a rapid and sensitive POCT approach for antibiotic testing (Lin et al., 2018). The target streptomycin (STR) was selectively identified by the STR binding aptamer, and the remaining aptamer interacted with the complementary DNA (cDNA) to generate double-stranded DNA (dsDNA). Because SYBR Green (a dye) binds with dsDNA, it gives a visible green fluorescence emission. The fluorescence emission was found to decrease with increasing streptomycin concentration owing to a lower proportion of dsDNA. The smartphone camera was used to take the digital images of fluorescence from the samples, and the Touch Color app on the phone

was used to get the RGB values of the images. Li et al. (2019) devised a differential fluorescence sensor module comprised of single-stranded DNA (ssDNA) dyes and aptamers for integrated sensing and differentiation of four major cyanotoxins using an average smartphone around 5 min after reaction completion. The test materials were preloaded and desiccated on a microfluidic chip that had a 60-day shelf life. When one or more cyanotoxins were added to the aptasensors, the green fluorescence was reduced, resulting in pattern-based fluorescent signatures that allowed precise quantification and detection of each target species in a complex mixture. A custom-designed smartphone reader device took a photograph of the microchip-based fluorescence sensor array, which produced quantifiable digital signals as a function of target concentrations. A smartphone app interface was introduced for on-site data analysis and result dissemination.

Lee et al. (2018) prepared a fluorescent probe-based turn 'ON–OFF' aptasensor to detect the influenza A (H1N1) virus. The virus can be viewed simply by using a portable, low-cost, home-made system (with an overall value of less than $20 USD) integrated with a smartphone camera. A mixture containing dark quencher-labeled guard DNA (G-DNA) and streptavidin-functionalized quantum dot (Qdot)-aptamer beacons remains in an "OFF" state in the absence of the H1N1 virus. Aptamer binds to H1N1 viruses preferentially, releasing G-DNA that restores the fluorescence and consequently turns "ON" the aptasensor. Without requiring complicated and expensive accessories, the handheld detection system enables end users to access fluorescence intensity from any location. As a result, information technology devices, such as smartphones and online databases, can be used to transfer and evaluate data in real time for assessment and diagnosis. This can be used in a variety of important fields, such as pharmaceutical, military, and medical settings. Shrivastava et al. devised a rapid, potential, and culture-free lab-on-smartphone infrastructure that used dual functionalities of magnetic nanoparticles (FMNPs) for instant on-chip identification and quantification of bacteria using smartphone imaging (Shrivastava et al., 2018). Additionally, the smartphone-based gadget utilized for this reason was constituted with a white LED and a bacterial detection cassette installed temporarily on the smartphone, resulting in a small system that consumes very little energy to operate. Using aptamer-functionalized FMNPs, multidrug-resistant *Staphylococcus aureus* colonies were preferentially trapped on a manufacturer's bacterial sensing cassette in slightly processed liquid samples (Fig. 1B). Bacterial capture on-chip from food samples took roughly 10 min. Then, the fluorescence from the FMNP-tagged bacterial colonies was photographed with a smartphone camera and processed by image processing to quantify bacterial loads. This smartphone-based technique is capable of detecting even a single bacterium without any complex sample preparation procedures. This method allows for a wide range of important applications in the fields of diagnostic microbiology, environmental monitoring, and food safety (Shrivastava et al., 2018).

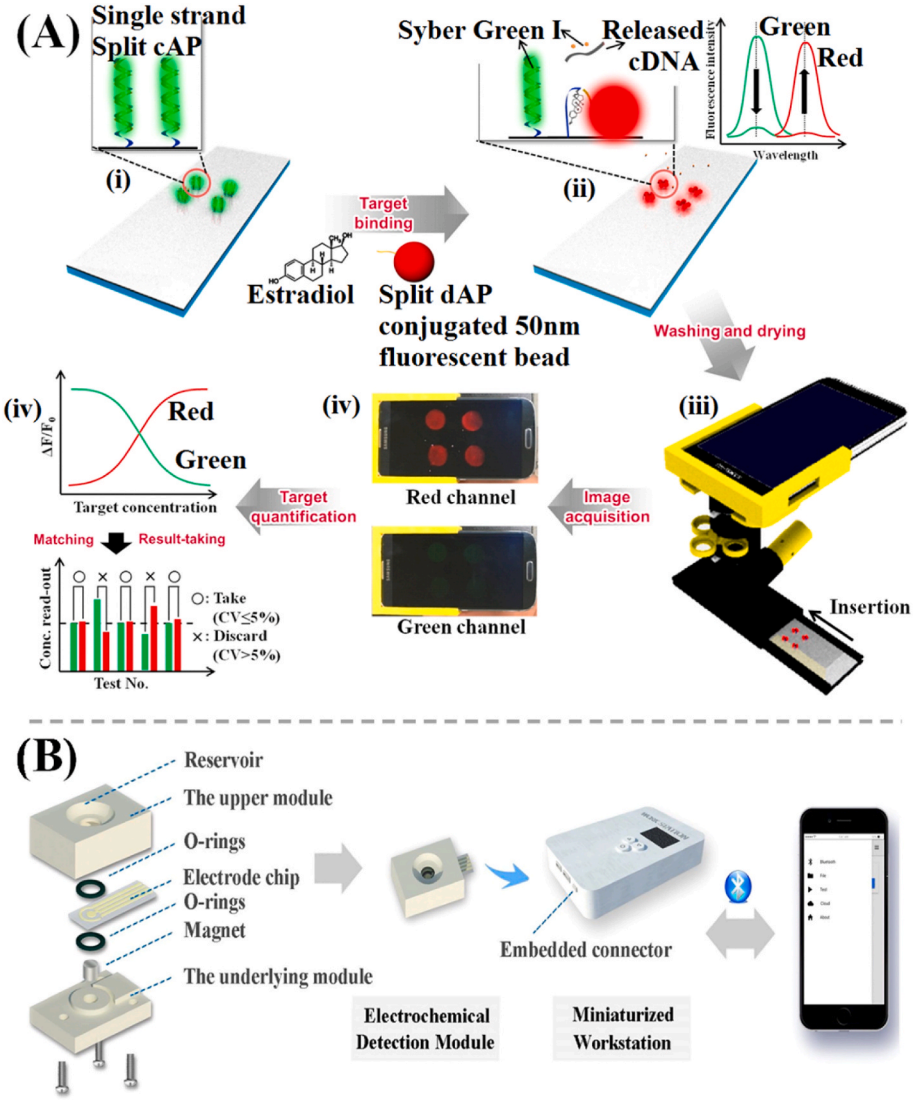

Fig. 2. (A) Representation of the steps involved in the fluorescence imaging aptasensor based on a smartphone for the quantification of 17-β-Estradiol in wastewater. (i) The micro-spots of cAP (complimentary aptamer) and cDNA (complimentary DNA) hybrid, labeled with SGI, after printing and cross-linking over a microarray (fluorescence-enhanced substratum). (ii) Upon target interaction with dAP-coupled fluorescent beads on the microarray, cAP diminishes green fluorescence signals, liberating SGI and cDNA, consequently increasing red fluorescence via dAP due to target binding. (iii) The glass substratum along with the microarray was inserted into a mobile reader. (iv) The fluorescent photographs of the green and red channels were captured. (v) The transformation in fluorescence signals for each channel was examined and a corresponding method for selection of the valid results was chosen. A test result with the matched ratio within a specific CV value was taken for the accurate quantification (Lee et al., 2017). **(B)** Schematic of a home-made electrochemical set-up and a miniaturized workstation that were used for the detection of ATP and Hg^{+} ions (Gu et al., 2020).

By combining a fluorescent imaging-based aptasensensor with a smartphone, Lee et al. reported the quantitative analysis of 17-β-estradiol in sewage water (Lee et al., 2017). The authors used an aptamer-based microarray alongside fluorescent imaging on two channels (red and green) of a mobile reader to accomplish smartphone imaging-based fluorescence microscopy. This technique employed a customized 3D-printed fluorescent microscope as a smartphone fluorescence reader, and then created an appropriate mobile biosensor on a microarray boosted by fluorescence using a pair of separated estradiol probe aptamers. Each fragment of one target analyte produced two distinct fluorescence signals, which were then compared for a signal change (Fig. 2A). A biosensing analysis was carried out with spiked sewage water, and the efficiency of the method was successfully compared with the gold standard enzyme-linked immunosorbent assay (ELISA). Wu et al. devised a flexible regenerative up-conversion-luminescence aptasensor for the quantification of diethylstilbestrol (DES) using fluorescence resonance energy transfer (FRET) between up-conversion nanoparticles (UCNPs) and dabcyl (Wu et al., 2021). The aptasensor device enabled dual readout via a fluorescence spectrophotometer and a smartphone. The cross-linking glutaraldehyde technique was employed to assemble aptamer complementary DNA sequences (cDNA) and UCNPs. Following that, the dabcyl-labeled DES binding aptamer was covalently linked to cDNA to construct a FRET platform by quenching the up-conversion luminescence (UL) of UCNPs. The UL was steadily activated while the DES (target) was co-incubated with the adaptable biosensor. Dabcyl was dislodged from the surface of the biosensor as a result of the preferential binding of the aptamer to DES, ultimately disrupting the FRET circuit efficiently. As a result, the signals from the biosensor were received as an image using a spectrum from a fluorescence spectrophotometer or smartphone. The biosensor can be regenerated using a dabcyl-labeled aptamer. Finally, for the biosensor, a smartphone-based handheld device with an Android app was built.

2.2. Electrochemical aptasensors

Smartphones have been connected to handheld equipment as tools to control, capture, and display electrochemical data (Zhang and Liu, 2016). Electrochemical sensing methods have a number of advantages, including low cost, fast response, high controllability, and sensitivity. Such sensors have been acknowledged as one of the best ways to quickly and easily find (bio)chemical targets in biological, food, and environmental samples (Yao et al., 2019). Various smartphone-based electrochemical sensing bioassays have developed in recent years. In most cases, the smartphone is linked to an additional electrochemical unit. Some common electrochemical techniques, including potentiometry, voltammetry, impedance, and amperometry, are employed for smartphone data capture and interpretation (Rezazadeh et al., 2019). A dual-signal electrochemical-colorimetric aptasensor for on-site assessment of Hg^{2+} and adenosine triphosphate (ATP) was reported by Gu and co-workers (Gu et al., 2020). Split aptamer functionalization and magnet-driven procedures were two of the most important operational aspects of this study. Dual-signal detection was achieved by integrating an electrochemical aptasensor with naked-eye colorimetry for on-site target detection. A handheld compact workstation was integrated with a self-designed magneto-electrochemical sensing system to perform the electrochemical quantification, which can be operated by a smartphone interface (Fig. 2B). The magnet-driven immobilization and binding in the presence of the magnetic Fe_3O_4 nanoparticles resulted in a hue, and the intensity changed in AuNP dispersion and current signals following variation in target concentration. The suggested method provides a low-cost, quick-response, portable, and sensitive analysis tool. The aptasensor can be adapted to detect different targets by using compatible aptamers.

Galatus et al. (2017) described a hybrid sensing system capable of sensitive and complementary quantification of bactericidal β-lactam antibiotics, (i.e. ampicillin). They used methylene blue as a marker to demonstrate the thiolated aptamer's immobilization on the electrode surface and its ability to bind with the target (ampicillin). The molecular binding of the aptamers with their target on the Au chip developed the resonance wavelength. The refractive index of the samples was analyzed in relation to the binding of non labeled molecules on the sensor surface to detect various targets in aqueous solution. Red fluorescent fiber was used as an emitter solution. The optical curves were used to estimate the reaction kinetics. Experimental hybrid sensor testing results demonstrate the practicality of the suggested optical method. Relying on smartphone analysis of data, this technique might be utilized to create a portable and cost-effective hybrid fiber optic SPR biosensing system. Kanamycin (KAN) was detected wirelessly with a potentiometric ultrasensitive enzymatic aptasensor constructed by Yao et al. using the freestanding graphene paper (GNP) (Yao et al., 2019). For efficient aptamer deposition via π-π-stacking interactions, freestanding GNP was employed as a biocompatible substrate. There has been an increase in sensitivity throughout the binding event between immobilized aptamer and KAN following the addition of nuclease (DNase I) (Fig. 3A). In the detection cell, DNase I was employed as an unaltered nuclease to cleave the detached aptamer and liberate KAN back into the binding catalytic reaction. The potentiometric sensor was linked wirelessly to a smartphone, which captured the real-time data. This simplified the detection system's design and reduced its cost dramatically.

Han et al. (2021) introduced an electrochemical aptasensor derived from CRISPR/Cas12a for the ultrasensitive, simplified, fast, and cost-effective detection of COVID-19 nucleocapsid protein (Np). To begin with, an arching probe was fused with an activator strand and the COVID-19 Np specific aptamer (Np-A48). When the COVID-19 Np target was present, the aptamer preferably attached to it, releasing the activator strand, which was combined with crRNA to trigger the DNase function (viz. the trans-cleavage reaction of Cas12a). Following that, the polyA-methylene Blue electrochemical reporters got cleaved off the surface of the electrode, resulting in a decreased electrochemical signal. Without Np, the arching probe inhibits the activator strand from joining the Cas12a-crRNA complex, hence impeding Cas12a′s DNase activation and generating a strong electrochemical signal. As a result, the aptasensor may be used to assess Np as a POCT for early disease diagnosis, food safety analysis, and water quality testing, without the need for time-consuming sample processing. Due to the advancement of wireless communication technology, the ubiquity of smartphones, and the portability of the suggested Palmsens miniature potentiostat for the connectivity of results on a smartphone, the suggested aptasensor has enormous potential for future industrialization. Joe et al. reported an aptamer duo-based handheld electrochemical aptasensor that could wirelessly disseminate obtained electrochemical information to smartphones, enabling periodontal disease diagnostics using the Odontogenic Ameloblast-associated Protein (ODAM) biomarkers (Joe et al., 2022). By conducting chronoamperometry and cyclic voltammetry on the screen-printed gold electrodes (SPGE), the efficiency of the sandwich-type aptasensor was validated using two model protein biomarkers, e.g., thrombin and vaspin. The SPGE was immobilized with the primary aptamers for each biomarker (i.e., V1APT and TBA15) via Au–S (gold-thiol) bonding, which was employed as the main capturing probe for the target binding (Fig. 3B). Horseradish peroxidase conjugated secondary aptamers (i.e., V49APT and TBA29) were used as amperometric signaling probes. Peak currents were dramatically reduced following the addition of target biomarkers, indicating that the target had been successfully bound to the aptamer. The peak current was further reduced when the 2nd Apt@HRP bonded to the target and formed a sandwich complex, indicating that the wireless mini-potentiostat aptasensing sandwich method was successful.

The aptasensor platform designed by Rosati et al. can efficiently measure clinically important levels of neutrophil gelatinase-associated lipocalin (NGAL), an acute kidney injury biomarker, using a rapid and simple smartphone readout (Rosati et al., 2022). The "plugin and print"

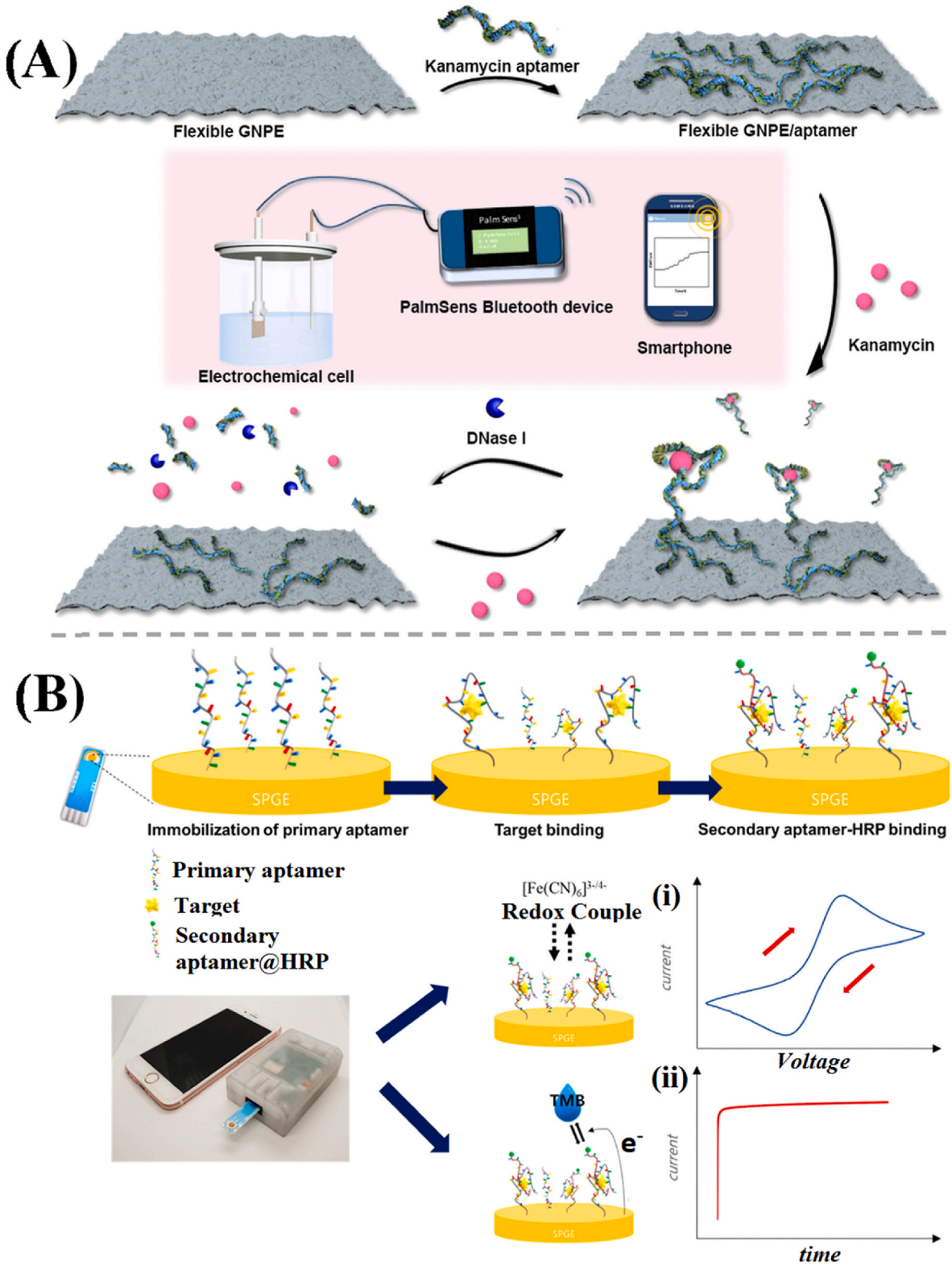

Fig. 3. **(A)** Representative sketch to show the principle of operating a flexible freestanding GNP-based potentiometric enzymatic aptasensor with nuclease (Dnase I) based amplification quantification of kanamycin for food safety (Yao et al., 2019). **(B)** The layout to show the functionalization method of a screen-printed gold electrode (SPGE) with aptamer duo to detect periodontal disease biomarkers (two different detection methods); i) cyclic voltammetry (CV) and ii) Chronoamperometry (CA) (Joe et al., 2022).

strategy to device construction ensured scalability and high versatility without the use of highly technical equipment. A commercially available app (Google Play link) together with a simple custom-made electronic circuit ($5 cost) were employed to link these gadgets to the smartphone and perform electrochemical impedance spectroscopy (EIS) measurements. Steps involved in the fabrication have been shown in the figure (Fig. 4A). Finally, NGAL aptamers immobilized devices were employed to detect NGAL in artificial urine and PBS successfully.

2.3. Other aptasensors

Khoshfetrat et al. devised a robust electrochemiluminescence (ECL) aptasensor for the monitoring of aflatoxin M1 (AFM1) using BPE (Khoshfetrat et al., 2018). The thiolated AFM1 binding aptamer was anchored on magnetic Fe_3O_4 nanoparticles coated with AuNPs (Apt-GMNPs). Luminol activated silver nanoparticles-decorated graphene oxide (GO-L-AgNPs) gets involved in π-π interactions with the immobilized aptamer's unpaired bases (Apt-GMNPs-GO-L-AgNPs). Following the introduction of Apt-GMNPs-GO-L-AgNPs into an Au anodic BPE array, the specific electrodes were exposed to varying concentrations of AFM1. The GO-L-AgNPs dissociate from the aptamer upon interaction with AFM1, and the ensuing ECL of H_2O_2 and luminol at the anodic poles were recorded using a smartphone or photomultiplier tube (PMT), and the digital photographs were processed using ImageJ software. This protocol offers a new avenue for analyte detection in POCT that does not require complex instrumentation and may be powered by a battery.

Cheng et al. improved exosome detection sensitivity by using an aptamer nanoflower augmented lateral flow strip (ANAN-LFS) and Au@Pd nanopopcorn with a thermal signal output (Cheng et al., 2019). The Au@Pd nanopopcorn-anchor conjugates were deposited upon a conjugate pad to flow through the control and test lines in the absence of

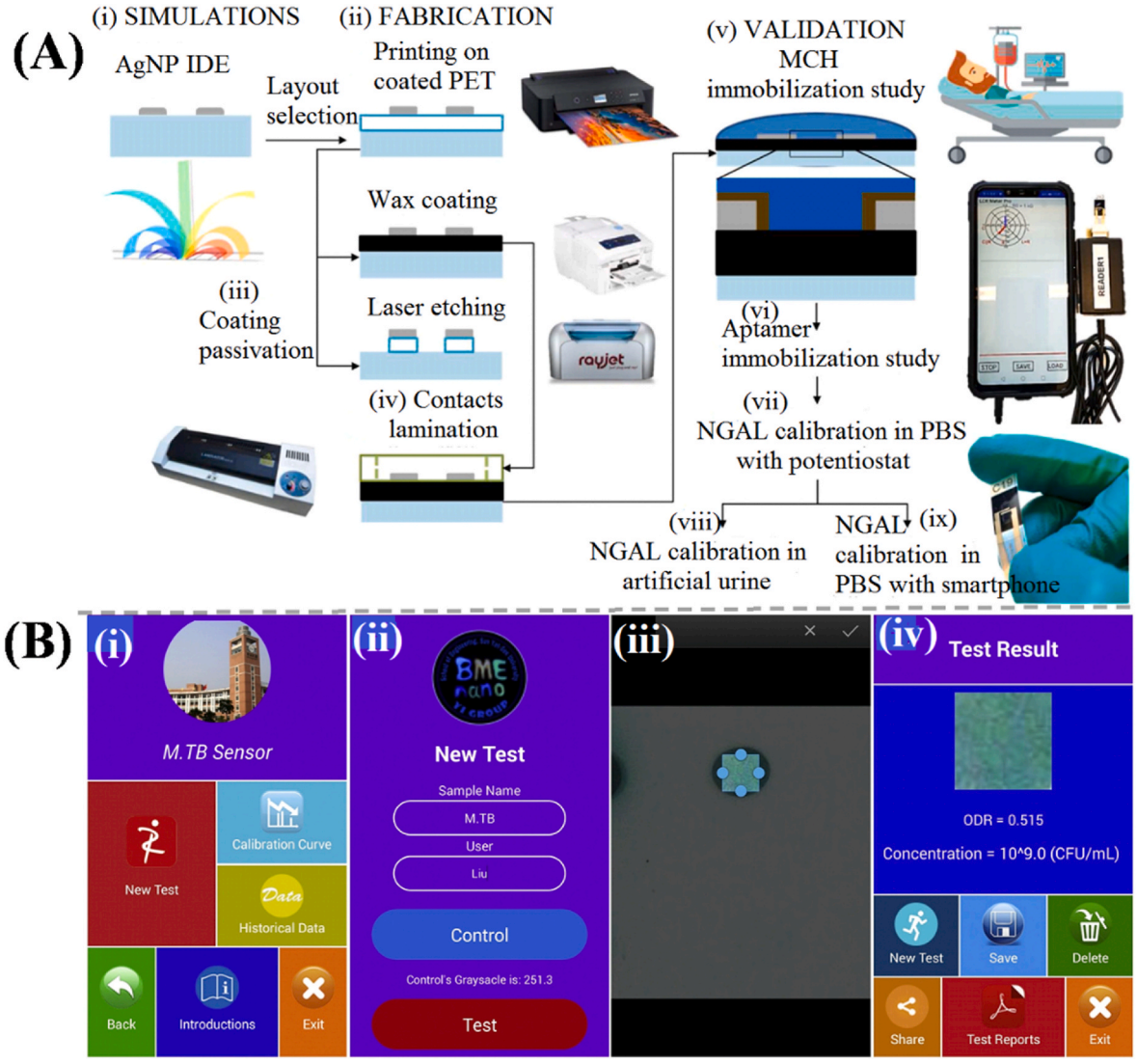

Fig. 4. **(A)** The layout of the process involved in the inkjet devices' simulation, assembly, optimization, and authentication is shown. (i) The interdigitated electrodes (IDE) design was finalized involving the simulations and (ii) inkjet-printing with silver nanoparticles (AgNP) ink on coated substrate for room temperature sintering, (iii) Two coating passivation approaches were tested and the devices were then (iv) laminated using plastic sheets which had patterns (for contacts insulation), (v) Bare wax and laser etched devices were tested with mercaptohexanol (MCH) self-Assembled Monolayer immobilization to choose the most efficient passivation process, (vi) Wax passivated equipment were functionalized using NGAL Aptamers, and (vii) via potentiostat, employed to identify NGAL in Phosphate Buffered Saline (PBS) and (viii) artificial urine. (ix) Smartphone-based quantification was executed using same devices for the calibration of NGAL in PBS (Rosati et al., 2022). **(B)** The user-friendly interface of the APP designed for M.TB aptasensor. (i) Main menu of the interface, (ii) Sample and user data interface: Contextual grayscale data was acquired by clicking the "Control" button prior to sample testing. (iii) The interface with captured color photographs and the selection areas was presented. (iv) Interface with processed image and the outcome after interpolation. The application was validated using a Huawei Honor 3C (H30–C00) smartphone (Li et al., 2018).

exosomes (negative). When exosomes were present (positive), the conjugates were deposited into the lipid bilayer of the exosome membranes via hydrophobic interactions, leading to the formation of Au@Pd nanopopcorn-anchor-exosome complexes. The above complexes were then placed on the conjugate pad and allowed to migrate. Because of its binding efficiency and affinity, a CD63 aptamer was used as an additional bio-recognition component for CD63 aptamer nanoflower development. The nitrocellulose membrane was infused with the produced CD63 aptamer nanoflower, which performed as the test line. In a typical quantification, the Au@Pd nanopopcorn-anchor-exosome complexes might be identified on the test line by a strongly affinitive reaction between the CD63 aptamer nanoflowers and exosomes, where laser stimulation prompted by the deposition of Au@Pd nanopopcorns would produce a strong thermal signal and a distinct black stripe. Excessive Au@Pd nanopopcorn-anchor conjugates then diffused into the control area and were trapped by the streptavidin biotin-labeled probes through hybridization, resulting in the formation of another black stripe. The thermal signal on the test line was created for quantification by a self-assembled smartphone-based thermal reader that was supplied with a slew of components such as a smartphone, a smartphone-accessible thermal detector, an excitation laser, and a 3D-printing holder. Critically, the thermal imaging displayed the temperature disparity on the test line was intuitively recorded by a preinstalled Seek Thermal program. The technology has a lot of potential for early cancer detection and monitoring in regular clinical care facilities. The use of mobile devices as detection platforms enabled Sanjay et al. to design an inexpensive fiber-optic-based sensor for POC malaria diagnosis (an alternative to the conventional SPR system) (Sanjay et al., 2020). This approach monitors any changes in refractive index generated by PfGDH binding to the aptamer, much like a commercial SPR platform. A four-channel smartphone slip-on adapter was created to connect one end of the optic fiber to the smartphone camera and the opposite end to the smartphone flashlight. To detect PfGDH, optical fiber probes were constructed using Au-sputtered U-bent plastic optic fibers and functionalized using PfGDH binding aptamer. This set-up was used to acquire images of the fiber's light spots at predetermined time intervals following incubation with mock malaria samples. The ImageJ program was used to look at the blue histogram readings (B-values) of the light spots in the captured photos. Using a planar-optical waveguide framework incorporated onto a polymer chip, Walter et al. devised the first all-optical surface plasmon resonance (SPR) sensing strategy (SmartSens) (Walter et al., 2020). For smartphones with SPR biosensors, a planar-optical waveguide design is suitable to fit the entire optical setup into one polymer sensor chip, resulting in a layout of less than a few square centimeters. Because the suggested sensor system uses polymer chips, it can be produced commercially and at a cheap cost. Aptamers were used to test the model analyte 25-hydroxyvitamin D (25OHD). Due to the sensor's waveguide structure, it is possible to simultaneously detect several biomarkers using the sensor's high degree of parallelization and miniaturization. Its multiplexing capabilities, along with its ability to be read out using a regular smartphone, might make the sensor system suitable for household diagnostic applications, such as monitoring long-lasting diseases and for diagnosis in limited resource environments. Hao et al. (2019) introduced a portable, fully integrated graphene-based nanosensing device for specific and sensitive on-line monitoring of salivary cytokine biomarkers, including interleukin-6 (IL-6). To accomplish the processing and transduction of information that indicates cytokine IL-6 levels, the system used an aptameric graphene-based field effect transistor (GFET) nanosensor with on-line signal processing circuits and a buried-gate configuration. The signal may be shown on the inbuilt liquid crystal display (LCD) device as well as wirelessly transmitted to a smartphone via an on-board Wi-Fi adapter, enabling on-line visualization of the cytokine concentration variation patterns. Importantly, with a Wi-Fi connection, this data may be sent to a cloud server, allowing clinicians to remotely control the health problems of patients. The sensor system is about the size of a smartphone and can detect changes in IL-6 levels in saliva, gargle solution, and 1X PBS buffer solution in a few minutes. This makes it ideal for detecting illnesses in salivary cytokine biomarkers at an early stage.

3. Roles of smartphones in aptasensing

New apps and improvements in smartphone technology have inspired research towards the application of smartphones in the varieties of biosensors. There are two types of smartphone-based equipment: the smartphone as (1) an interface and (2) a detector. In the first scenario, a smartphone is connected to equipment that has miniaturized versions of instrumental components. Connecting cellphones to bioanalytical devices through Wi-Fi, Bluetooth, or a micro-USB connector is another approach of using them for analytical purposes. As a consequence, the gadget executes the measurement while the smartphone controls the experimental system and displays the test results on the screen like a mini-computer. In some instances, commercially available applications are used as they are considerably easier to design as compared to fully integrated platforms (Roda et al., 2016).

The digital camera in smartphones is an essential feature. Modern smartphones incorporate at least two digital cameras, at front and rear, and the latter is usually of higher quality. It is possible to capture 3D images with the multiple rear cameras of some smartphones. A smartphone camera, on the other hand, is far more than its lens: it seems to have night-vision filters, ambient light sensors, optical zoom equipment, stabilizers, and a slew of other image-enhancing electronics. In terms of megapixels, the existing smartphone cameras capture high-resolution quality images with billions of tiny monochromatic pixels. Nevertheless, camera quality is not solely determined by a megapixel count; better sensors and lenses can produce quality videos and images even with a lower megapixel count, as long as the lenses are better at controlling exposure time, contrast, ambient light, and so on (Quesada-González and Merkoçi, 2017). Smartphones are increasingly deemed as exceptional sensing tools, with developer's excitement in designing apps, especially for the Android operating system. In certain cases, the created applications also included capturing capabilities, allowing them to directly snap a digital photo of the sample. Nevertheless, some apps lack this option and are only capable of analyzing the received images. It is very obvious from the literature that most of the cited aptasensors have used imageJ software (benchtop reader-not currently available in Android) simultaneously along with various other smartphone apps for the analysis of digital images captured by a smartphone or to compare the results between the two platforms (benchtop and smartphone readers). Nevertheless, ImageJ and other benchtop readers are not within the scope of this review. In this section, we will take a closer look at the most commonly used smartphone applications, accessories, interfaces, software/hardware, and other technical advancements on the way to incorporating them into the latest aptasensors.

3.1. 3D printed smartphone-based platform (SBP-App)

Simple Android software was developed to analyze photographs taken using rear camera of a smartphone, report on findings, and share them with others. The aptamer functionalized AuNPs solution was photographed using the back camera of a smartphone. The RGB information was extracted and converted into quantitative data using customized software "SBP-App," which was designed in Java using the Android Developer tools. As a result, an algorithm that measures the correlation between the solution color and the concentration of the target analyte was first created and then included in the SBP-App. Lin et al. used SBP-App to display and share wirelessly the target concentration of the sample on the screen (Liu et al., 2017). The aptamer functionalized AuNPs can be utilized as a colorimetric signal for the quantification of biomarkers related to food safety, which entails the use of a smartphone for target detection and data processing.

3.2. Smartphone-based fluorescence reader

Umrao et al. simplified a FRET aptasensor based on a spectrophotometer by substituting (i) the xenon flash lamp with a rechargeable battery-powered green laser diode pointer as the excitation source, (ii) multiple pairs of optics with a single external collection lens, and (iii) the commercial monochromator with two acrylic long-pass filters. This reduced the initial cost of the aptamer FRET biosensor to 23 USD + the cost of the smartphone. The samples in this biosensor were irradiated with a green laser diode, and photos of the fluorescence were taken with a smartphone (Umrao et al., 2019).

3.3. M. TB sensor App

The *M. TB* sensor was developed for the Android platform (version 4.2.2) and deployed on a Huawei Honor 3C (H30–C00) phone. The program offers a user-friendly layout (Fig. 4B i & ii) for executing sensing measurements. The app was used to analyze images saved for testing sputum samples by direct or indirect dot-blot methods. The major role of the app is to collect RGB data using JPEG digital images received from other sources (i.e., scanners) or photographs shot with a smartphone's built-in camera. As a result, an algorithm based on the correlation between dot darkness and *M. TB* concentration was initially built. It has previously been stated that the RGB data obtained from digital images may be transformed into grayscale values using two algorithms: the averaged RGB method and the weighted RGB technique. The optical darkness ratio (ODR) generated by Adobe Photoshop was used as a reference in this study to compare two algorithm techniques. For that purpose, a square frame (Fig. 4B–iii) was used to select an area of interest within a dot, and the color information of each pixel within the selected region was captured without any external color reference. The average scores of the R, G, and B channels were computed, and quantified using mathematical algorithms (Fig. 4B–iv). Using the calibration curve contained in the APP, the bacterial concentration can be estimated in CFU/mL (Li et al., 2018).

3.4. Processing color of the digital images

Color variables, such as saturation (S), hue (H), blue (B), red (R), gray (Gr), green (G), and brightness (V), of the standard target mixtures with their corresponding aptamers need to be analyzed by capturing and processing their digital images. The smartphone apps that are used to process the color information of the digital images have been grouped together as under:

(a). RGB App

Even basic home studies show that mobile phone cameras are capable of detecting the slightest variations in color scheme. To exploit the potential of smartphones connected to optical biosensors, an app that can ascribe quantifiable metrics to such tonal variations may be deployed (Quesada-González and Merkoçi, 2017). The first software for performing colorimetric analysis on various biosensors and aptasensors was developed in 2014 for both iOS and Android phones. It was defined on the basis of primary color detection, e.g., RGB. The conventional RGB scale allocates a whole-number value between 0 and 255 to each of these three primary colors in a particular tone, with [0, 0, 0] denoting pure black and [255, 255, 255] indicating absolute white (Yetisen et al., 2014). An average high-definition camera ought to be able to differentiate up to 16777216 colors. The phone app employs the complementary metal–oxide–semiconductor (CMOS) sensors inside the phone's camera to measure electromagnetic radiation from the colored testing zones. The algorithm interprets this data in terms of analyte concentrations in each testing zone, and the app provides the relevant points on the screen of a smartphone. The susceptibility of a colorimetric measurement is determined by the number of calibration points, the color consistency of the colorimetric events, and the integrity of the CMOS camera sensor. However, reliable colorimetric tests need meticulous control over variables, such as the distance between the sample and the camera, temperature, and ambient light (Yetisen et al., 2014). This user-friendly app has been used extensively in various colorimetric aptasensors reported in the last five years.

(b). Digital image colorimetry (DIC)

DIC is a new variant of the colorimetric technique that has recently emerged (Choodum et al., 2017). Image capture and color readouts are mainly involved in DIC (Peng et al., 2017). In this approach, an image capturing instrument is employed to capture a sample image, and then image analysis software is used to evaluate the colors of the captured image. Since the information in DIC is delivered through imaging software, the effect of the naked human eye is diminished, significantly enhancing the reliability of the detection results. Images can be captured using a camera, scanner, computer camera, or smartphone. Smartphones are regarded as the greatest tools for image acquisition due to their exceptional photographic competence, diverse image processing applications, and accessibility. DIC image analysis is mostly dependent on RGB color space. Any color can be split into three primary colors and afterwards read out via image analysis software. The value of each primary color is an integer from 0 to 255. The RGB score is comparable to the color luminosity, with the large values indicating significant illumination. The Touch Color app is color-capture software that is universally used on smartphones. It is capable of automatically transforming any captured color into RGB values (Lin et al., 2018).

(c). Android-based color analysis application

Ping et al. (2018) designed the first Android-based color analysis app for smartphones to allow the quantitative colorimetric depiction of Ibu enantiomers. The camera on the smartphone was utilized for image capture (capturing and evaluating the RGB spectrum of the images) and processing of image information (calibration plot fitting) through the designed software. The reaction events were accomplished in 1 min. The operator first picks an area illustrative of using the on-screen "construct new sensor" and thereafter labels the experiment name and selects "Load image" or "Take photo" to obtain calibration scores. It then generates an average RGB value (from 100 pixels of reference colors) to define the color of sample. Linear RGB values are obtained from exponential RGB values by the software. Data points are recorded in the database for the next computation. A calibration curve is constructed and the shortest distance between the target data values and the calibration point is calculated by this app. A usual approach is to select "collect," then click "OK" when all collection has been completed and the square icon has been moved to the photo. The user is informed about "less point" if an error is made via a dialog box. Finally, click "Analytical Results" after selecting a sample. The analysis outcomes are shown on the smartphone screen when the computation is completed. Overall, the mobile phone analysis results were superior to those of the UV-Vis approach in both tap and river water samples. This app could potentially be used to detect other trace pollutants utilizing the Android platform.

(d). Smartphone-based colorimetric system (SBCS)

A SBCS was self-developed and used to study the colorimetric changes for cadmium evaluation (Gan et al., 2020). The SBCS was validated using an iPhone 4s as a detection gadget and a compact attachment as an illumination source. The compact attachment is made up of a low-power electroluminescent sheet and a dark chamber. It also has a loading station for 96-well microplates and a wide-angle lens. In the dark chamber, the phone camera and the wide-angle lens were in direct contact. An electroluminescent sheet provides the illumination

required for detection in a dark chamber. An objective-C language-based software was installed on the smartphone for analyzing and extracting image data. Fig. 5A shows the detection procedure. To begin, the 96-well microplate was mounted on the loading station, and a smartphone was placed in the appropriate enclosure to capture a colorimetric image. Afterwards, the image was processed using an image analysis algorithm. To determine the administered dose of Cd^{2+}, a standard curve of Cd^{2+} concentration and color was created. In the mean pixel intensity computation, the image-analyzing algorithm uses the RGB color model. Gan et al. retrieved the red and blue channel pixels from the color model and analyzed the image information using the ratio of AR to AB (AR/AB) (the difference in pixel value of the red and blue channel pixels with the blank hole, respectively) (Gan et al., 2020). On the basis of the calibrated working curve, a linear relation between the Cd^{2+} concentrations and the measured values was established and employed for quantitative analysis. Unlike bulky and expensive multi-microplate readers, the SBCS is compact and convenient to use without any extra equipment and has huge potential for specific detection of Cd^{2+}.

3.5. Pixilation

Pixilation is the method of transforming the color, luminosity, and other visual features of a sample into digital output. Pixilation can be employed for simplified digital image processing in optical sensing if the existing software is unable to support RGB or fluorescent analysis. Usually, a monochromatic filter or the selection of only one RGB channel is recommended. The images can then be turned into grayscale so that the pixels cannot be counted as a concentration-sensitive variable with different software (Rezazadeh et al., 2019).

3.6. Smartphone spectrum reader and multiple accessories

Using a high definition (HD) camera on the iPhone 5, Cheng et al. reported a fluorescence apta-LFB spectrometer with a simple cascade, excitation laser source, power, and fluorescence imaging optics (long pass filter, external lens, transmission diffraction grating) that can be used anywhere (Cheng et al., 2018). An orientation of 47° on the transmission diffraction grating (1200 lines/mm) allowed for the compactness of this spectrometer equipment for imaging numerous apta-LFBs. The dispersed photons and the auto fluorescence of the nitrocellulose membranes were integrated with the direct reflexion excitation light, which was then projected onto the mobile phone's sensing device. A long-pass filter with a blocking wavelength of 500 nm was utilized to suppress the shorter wavelengths to further remove the background noise and decrease dispersed excitation photons and auto fluorescence. The grating scattered radiated light onto the smartphone's

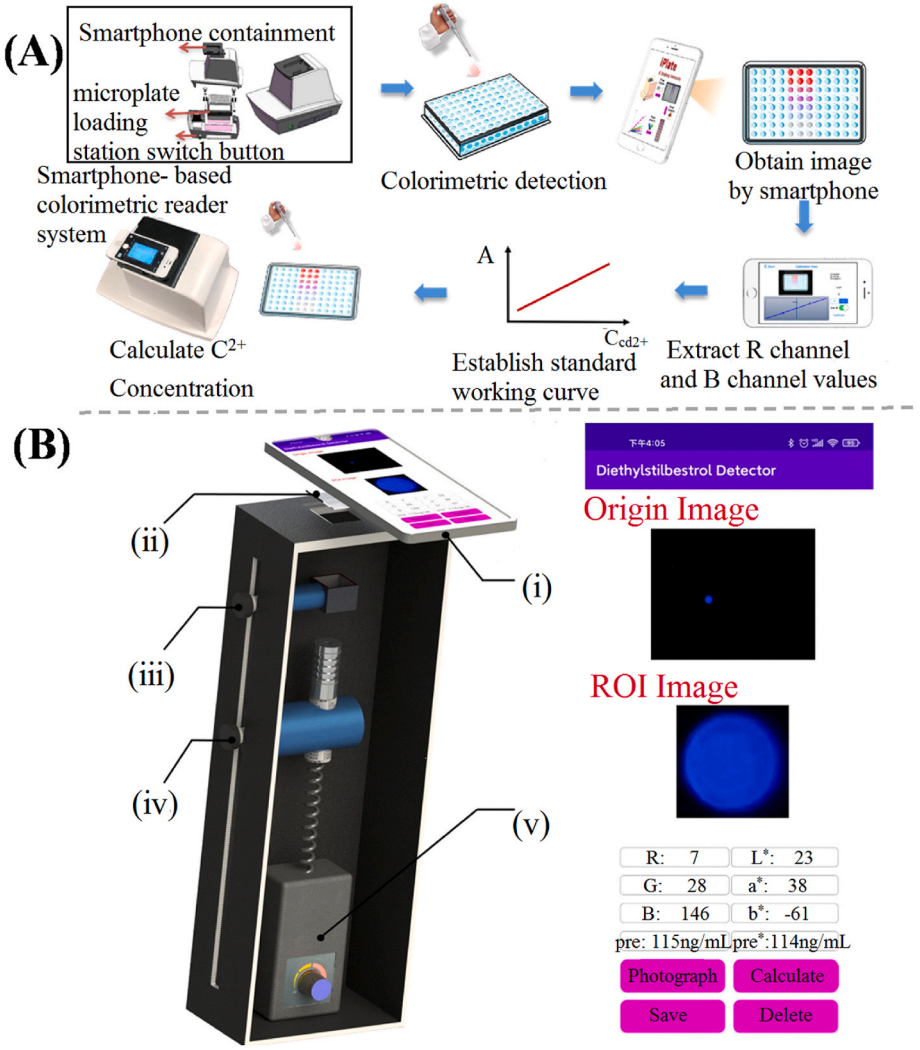

Fig. 5. **(A)** Schematic to show the quantification of cadmium ions using a smartphone-based colorimetric reader system (Gan et al., 2020). **(B)** A handheld device and software: Portable equipment consists of (i) a smartphone, (ii) an IR 750 shortwave pass filter (infrared filter), (iii) a flexible biosensor support platform, (iv) a knob for adjusting the focal length of the laser, and (v) a portable 980 nm laser. Interface of smartphone application based on Android platform (Wu et al., 2021).

CMOS image sensor during imaging enabled separation of the spectrum components into a clearly viewable, colorful band. Commercial tools, such as ImageJ and Origin, can be used to produce phone images, which can later be quantified and used to make zero-background outputs.

Hao et al. built an on-site signal processing circuit for online analysis of IL-6 monitoring (Hao et al., 2019). With the help of the on-board analyzer and controller, the originating signal of the variation in signal transduction (Ids) that is used to quantify the IL-6 concentration was acquired, conditioned, and evaluated. Because of the controller's computational and serial communication capabilities, the signal can be continuously transduced to the relevant IL-6 levels using a standard calibration formula. The IL-6 concentration data was displayed on the integrated LCD screen or wirelessly transferred to the smartphone via the Wi-Fi adapter and was plotted as a function of time using an Android App.

Wu et al. (2021) combined a smartphone, a platform they built independently, a near-infrared filter, and a compact 980 nm laser (Fig. 5B–a). The focal length and the power were controlled to guarantee the optimal excitation performance of the fabricated aptasensor. In addition, a software program based on the Android system was created (as shown in Fig. 5B–b). The software captures digital photographs of the samples after recognition events are accomplished and automatically trims the region of interest (ROI) to recover image data (brightness and color) followed by calculation to measure the target quantification results.

Shrivastava et al. used fluorescent latex beads with an average diameter of 1 μm that were about the same size as the pathogens they wanted to quantify. This allowed them to optimize and compare the imaging efficiency of smartphone-based fluorescence microscopy as compared to traditional fluorescence microscopy (Shrivastava et al., 2018). After measuring the size of the fluorescent beads using confocal laser scanning microscopy, the same beads were employed to optimize the smartphone image processing and fluorescence microscope approach. The lateral resolution of the smartphone fluorescence microscope was further tested and validated by visualizing the 1951 USAF (United States Air Force) resolution standard target. The intensity of each pixel from the individual fluorescent bead inset of the figure (previously indicated as 1C) shows a significant peak in which the bead was situated. This peak can readily be distinguished by post-processing of the fluorescent image captured by the smartphone. The authors also employed an image segmentation algorithm to test the usefulness of the smartphone-based technology for precisely counting clusters of bacteria.

3.7. Smartphone-based fluorescence reader and microfluidic chips

Li et al. introduced a smartphone-based prototype readout system that integrates a star-shaped microfluidic chip with a 3D-printed imaging interface for digital examination of the fluorescence emission intensity (Fig. 6A–i & ii) (Li et al., 2019). A microfluidic chip sample holder, batteries, an emission filter, and a lens were included in this low-cost light-protective smartphone accessory (Fig. 6A–iii). The fluorescent sensor inside the microfluidic chip was illuminated with a blue laser wavelength (λex = 462 nm) at ~75° incidence angle with regard to the chip, and the generated green fluorescence (λem = 528 nm) was chosen via a band-pass filter and measured by the smartphone camera (Fig. 6A–iv). Four cyanotoxins introduced into the standard 1X PBS buffer at a broad concentration range (2×10^{-3} to 100 μM) were used to calibrate the smartphone reading. Each cyanotoxin was put into the central reservoir of a microfluidic chip at a specific concentration and then propagated into four reaction wells. The smartphone detector recorded and examined the decline of green fluorescence (i.e., ΔG/G0) in the reaction wells. The authors designed and validated a beta version of the smartphone software for instant processing of images on the device. Users can capture images with their smartphone camera or retrieve previously stored images of testing and control samples from the photo gallery. It then used the attenuated green fluorescence levels and incorporated calibration algorithms to quickly measure the levels of cyanotoxins of significance and display the measurement outcomes on the smartphone screen with time tags.

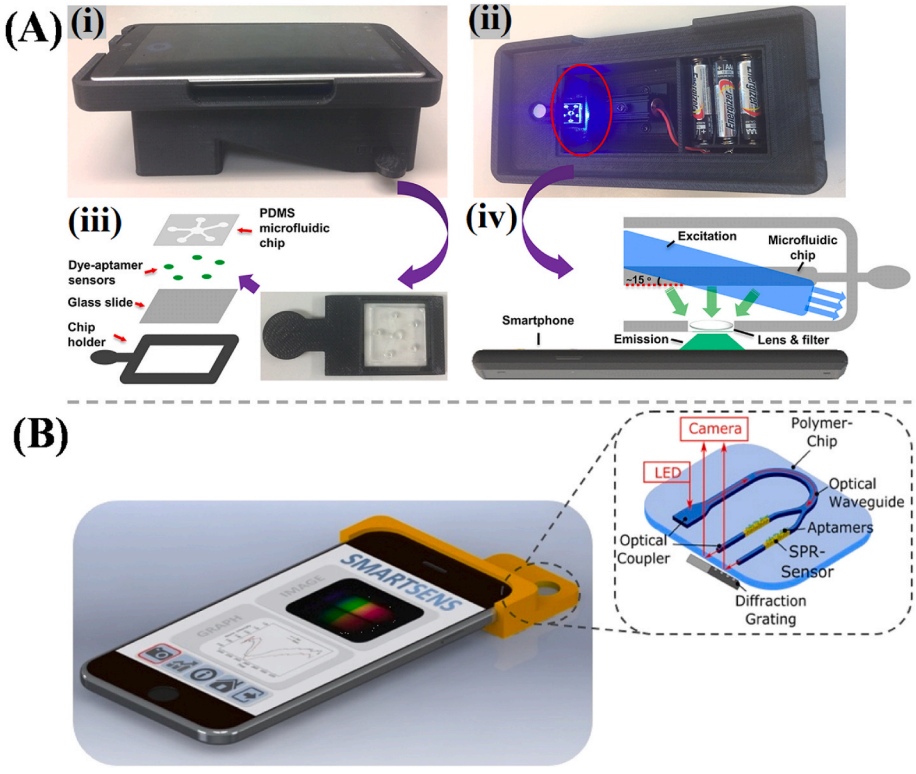

Fig. 6. **(A)** Schematic of the smartphone-based fluorescent sensor. (i, ii) Photographs of the actual smartphone reader device from different perspectives, (iii) microfluidic chip composed of polydimethylsiloxane (PDMS) material(the target inlet can be seen in the middle of the chip and attached to other reaction wells via five micro channels (5 × 0.2 mm)), (iv) schematic of the optical illumination structure (Li et al., 2019). **(B)** A theoretical scheme to show an all-optical planar polymer-based biochip aptasensor based on smartphones. As shown in the inset, a polymer chip comprising Surface Plasmon Resonance (SPR) and planar-optical waveguide sensors might be cross-examined using back camera of an average smartphone and flashlight LED (Walter et al., 2020).

3.8. Homemade system of microfluidic chip for image acquisition

The fluorescent color variations of the synthesized aptamer probe allowed the quantitative measurement of the Hg^{2+} ions. The fluorescent hues of the probe were acquired using a handmade device in which the color information was retrieved using image processing technologies on a smart phone. Furthermore, to enhance the accessibility and detection effectiveness of the system, a microfluidic chip was used as the signal cell for the interaction of the fluorescent probe with the Hg^{2+} target. The fluorescence colors were extracted and processed after the fluorescent images were captured by the smart phone, and the RGB data was acquired by image processing. Subsequently, a linear regression model using the ratio of "R" and "B" values (VR/VB) as the dependent variable and Hg^{2+} ion concentration as the independent variable was constructed (Shi et al., 2021).

3.9. SPR-based polymer chip and diffraction grating

An SPR sensing chip was inserted into a suitable sensor case, which aligns the sensor module in relation to the back camera of a smartphone and LED flashlight (Walter et al., 2020). When an image was captured with the smartphone, light from the smartphone flashlight LED was paired into the planar-optical waveguide structure of the sensor chip and redirected to an Au-coated SPR sensor area. The light combines with the surroundings, i.e., with the particular target material, at the SPR sensor module. This leads to a variation in light propagation, which would then be steered further to the smartphone camera through a diffraction grating positioned between the SPR sensor chip and the smartphone camera (Fig. 6B). The resultant image of the diffracted light was employed to compute the SPR signal and to evaluate the biomarker binding. Because the sensor chip is capable of including two or more SPR sensor components, simultaneous detection of biomarkers is possible (Walter et al., 2020).

3.10. Open source miniature potentiostat

To simplify on-site electrochemical sensing, a handheld potentiostat with wireless transmission to smartphones might be ideal solution. Such setting would allow evaluation at the point of use, without the need for computers or wired connectivity. Open-source potentiostats (Ding et al., 2018; De Oliveira et al., 2018) were originally designed for use with a computer through the USB connector, and hence did not had interface for smartphone connection. Ainla et al. developed the first open-source "universal wireless electrochemical detector" (UWED) miniaturized potentiostat detector that can be coupled to a smartphone (or tablet) via the "Bluetooth Low Energy" procedure (Ainla et al., 2018). The smartphone serves as a user interface to receive experimental information and display the results immediately, and a proxy to store, process, and transmit information and experimental procedures (Fig. 7). This method simplifies the design or the fabrication process and reduces both the cost and the size of the hardware. It also renders the UWED responsive to various analyses through simple software modifications. The UWED is capable of executing the most popular electroanalytical procedures, including square wave voltammetry, cyclic voltammetry, chronoamperometry, and potentiometry. The outcomes are comparable to tabletop commercial potentiostats. Since this UWED is simple, compact, made of low-cost materials, and totally wireless, it opens up new avenues for the creation of low-cost diagnostics, biosensors, and wearables (Ainla et al., 2018). During the last five years (already discussed in this review), various smartphone-based aptasensors have been reported to use potentiostat.

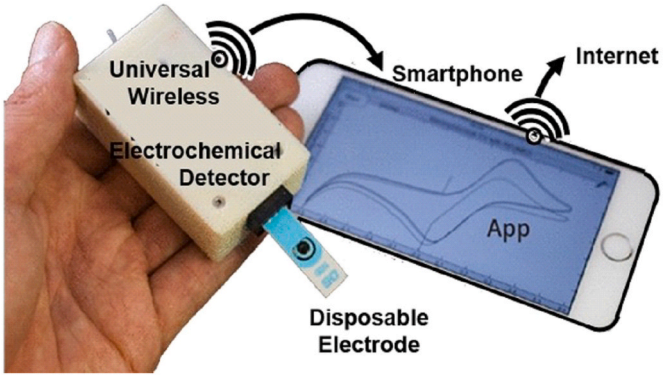

Fig. 7. An open-source "universal wireless electrochemical detector" (UWED) miniaturized potentiostat detector that can be coupled with a smartphone to create low-cost diagnostics, biosensors, and wearables (Ainla et al., 2018).

4. Advantages and limitations/challenges

The advantages and limitations of smartphone-enabled aptasensors are as follows.

4.1. Advantages

I. Smartphone-based aptasensors are affordable. For instance, the fabrication cost of SBP is ~13.0 USD per set (Liu et al., 2017), and the STR measurements can be performed for an additional ~5 USD per sample.

II. Colorimetric sensing is highly desired for both qualitative and quantitative smartphone-based aptasensors because of its ease of analysis and data interpretation. Since there is a direct relationship between the colorimetric signal and analyte concentration, both qualitative and quantitative analyses are feasible with this technique. The simplicity, portability, and accessibility of cellphones as quantification tools add great value to colorimetric sensing (Quesada-González and Merkoçi, 2017; Arumugam et al., 2020; Pereira et al., 2022).

III. Smartphone-based aptasensors are mostly simple and easy to fabricate; neither requires the use of large and complicated equipment, nor skilled operators (Lin et al., 2018). Furthermore, the existing sensors can be modified for the detection of other molecules simply by replacing the particular aptamer sequences.

IV. The exceptionally high availability of smartphones in metropolitan and remote regions, as well as the convenience of the operating procedure, can expedite the screening of active food toxicants and contaminants. This can substantially improve the global food safety management systems. In addition, this can enable the food industry as well as the individual consumers to monitor the entire food chain supply.

V. Several smartphone-based aptasensors are capable of performing quantitative imaging, on-the-spot detection, and rapid capture of bacteria from minutely processed food samples. For instance, (Shrivastava et al., 2018), devised a highly effective method for accurate detection of harmful bacteria (pathogens) through their spatial arrangements. Additionally, their platform incorporates a white LED into the smart phone without affecting the image quality due to background noise, as evidenced by the bacterial cell data count. Detection based on imaging by smartphone fluorescence microscopy might be an efficient and potential aptasensing technique for investigating clinically significant pathogenic microorganisms at extremely low concentrations.

VI. Smartphone-based aptasensors are user-friendly, reliable, and rapid: Smartphone-based readout sensing techniques enable the development of a thorough monitoring and implementation system which can be accessible to all. Such technologies are particularly helpful for underprivileged people with symptoms indicative of several infectious diseases.

VII. Smartphone aptasensors are capable of detecting multiple analytes/biomarkers simultaneously. For instance, Walter et al. combined two SPR sensors with the devised sensor chip, which detected two biomarkers concurrently (Walter et al., 2020). The rate of multiplexing was further enhanced by integrating more sensor components. So, the sensor seems to offer quantitative multiplexing for the simultaneous estimation of numerous targets/disease biomarkers. The multiplexing and easy read-out capabilities with a standard smartphone make these sensors very useful for home testing applications, such as chronic illness monitoring, differential diagnoses in low-resource situations, etc.

VIII. Smartphone-based aptasensors involve a broad spectrum sensing approach for worldwide testing that meets the REASSURED requirements (real-time connectivity, ease of sampling, affordability, specificity, ease of use, speed and robustness, device flexibility, and deliverability).

IX. Rapid detection is preferred for real-time sensing applications. The identification system based on smartphone aptasensing is found to be time-efficient. For example, the sensing of ATP and Hg^{2+} was achieved within 2 min by a low-cost electrochemical smartphone-based aptasensor (Gu et al., 2020).

X. The set up for smartphone aptasensing is portable and efficient for on-site analyses as compared to the conventional biosensors or multi-microplate readers, which are huge and expensive. For example, the set-up for SBCS (Gan et al., 2020) is compact, easy to operate, and requires no additional equipment. It has enormous potential for high-throughput sensing of Cd^{2+}, and is extremely useful for simultaneous detection of a large number of samples. In another example, the POCT aptasensor designed by Han et al. (2021) does not require bulky apparatus and specialized facilities, which allows more convenient and flexible analyses. Interestingly, the entire test was completed within 30 min, and this could be a potential alternative for mobile and rapid detection of COVID-19. Instead of the RT-qPCR method, a CRISPR/Cas12a electrochemical aptasensor seems to be more convenient for the detection of COVID-19, as well as very useful in food safety, environmental monitoring, and other fields.

XI. The use of cellphone cameras, both as optical detectors and as a linkage between sensor and phone, is one of the most obvious advantages of phone-based optical sensors. It has the ability to profoundly minimize and simplify optical attachments in sensor systems. The devices can execute colorimetric measurements using biochemical test strips with only a mobile phone app without requiring other accessories. Images captured by aptasensor system's mobile-cellular camera phones are usually accompanied by important information, such as time, date, GPS location, etc. This data can be used to evaluate and predict prevalence of several infectious diseases. For example, the smartphone-based aptasensors designed by Fraser et al. (2018) was used to evaluate and predict the prevalence of malaria.

XII. POCT smartphone aptasensors with an on-board Wi-Fi device can communicate the data to a smartphone, which allows on-line monitoring of various biologically-important molecules. The aptasensor fabricated by Hao et al. (2019) for the on-line monitoring of cytokine levels is a good example in this context. Such smartphone-based aptasensors are specifically useful under special situations like COVID-19 lockdown, for instance, for the clinicians to monitor patients' health status from a distance by uploading data to a cloud server through Wi-Fi.

4.2. Disadvantages/Limitations

XIII. The smartphone's performance may not be as effective as laboratory tools, and various auxiliary components may be required to enhance and ensure optimal performance. Another disadvantage is the considerable disparities in smartphone camera technology, which cause noticeable differences in color sensitivity in output data among various cellphones.

XIV. SPR sensors based on smartphones were developed to provide accurate optical monitoring for assessing bio-molecular interactions. The sensors clearly possessed the benefits of reliable quantification with a low limit of detection for targets. The sensors, however, require optical interfacing modules or nanostructure grids to create SPR on the surface of the sensor. This can add a significant amount to the overall detection cost.

XV. Numerous smartphone apps have been developed and implemented for the quality assessment of different food samples. Yet, given the significant variations in phone, camera, and food samples' composition, such sensors require a specific calibration before usage.

XVI. Maintaining the uniformity and quality of the recorded images are other important challenges. To avoid non-standardized settings and undesirable inaccuracies, a reliable approach demands that the photographs be taken under the identical photographic conditions, such as magnification, pixels, distance, illumination, position, and so forth. Unfortunately, the captured digital images are unable to predict the detection results accurately because of a variety of illumination settings, including smartphone LED light, fluorescent light, sunlight, etc. Calibration techniques for smartphone photography, including parallel detection, mapping algorithms, and a black box (chamber), have been developed but require further improvement. As a result, optical biosensors based on smartphones are still recommended for qualitative analysis.

XVII. Uneven or uncontrolled light interference with the target solutions is one of the critical challenges of smartphone-based aptasensing, as it interferes with the target signals. Optical fibers or collimating lenses can be used to control light dispersion and improve detection sensitivity. However, the installation of these components may limit the detection platform to a single device type and limit its ability to quickly adopt new technology. Adoption of transferrable substitutes, such as computational algorithms or approaches, can minimize the constraints of secondary smartphone enhancements.

XVIII. Internet connectivity is another major challenge in resource-constrained localities because of a lack of inexpensive communication platforms and networks. Furthermore, the use of cloud-connected POCT devices may be retarded by infrastructure expenditures and cultural-language barriers.

XIX. Another significant challenge associated with heating or mixing the samples, data transfer/management, signal detection, and ongoing operations of POCT equipment is the power requirement. Internet connectivity, flashlights, GPS, cameras, and sensors, all require the use of inexpensive and widely available power sources in smartphone-based platforms. Extra auxiliary functions, in addition to built-in features, can be utilized to power POCT systems. Piezoelectric materials, body energy harvesters, lithium-ion/high-capacity rechargeable batteries, transportable biofuel cells, and wireless energy transmission can all be used to improve power constraints.

Given the aforementioned gaps and constraints, more research is required to overcome the challenges and issues associated with smartphone setups.

5. Future outlook

Advancements in both aptasensing and features of smart phones (portability, cameras, and connection) and their increasing coupling are enabling the development of user-friendly and portable analytical gadgets. Bioanalysis can now be performed by a non-expert at anytime and anywhere. Smartphone-based target detection provides one of the best

avenues for aptamer-based biosensing devices to be used outside of laboratories. Such technological advancements are considered to have a significant impact on society. When it comes to environmental monitoring, food safety, or disease diagnostics, smartphone-based aptasensors are believed to play a critical role. Some future prospects have been suggested as follows.

I. The current COVID-19 pandemic is posing numerous challenges to healthcare sectors across the globe. Because of the surge in diseases and social alienation during the COVID-19, the healthcare sector was put to the test and was under tremendous pressure. In this context, the POC devices can play an important role in the decentralized healthcare system or telemedicine because these devices can identify disease markers at the patient sites. Considering their accessibility, ease of use, reliability, and promptness, considerable attempts have been made to build POC devices using diverse platforms. Smartphones have made breakthroughs in POC diagnostic devices for use at doctors' clinics, offices, and at home. Given the current COVID-19 pandemic, smartphone-based aptasensors for handheld and POCT COVID-19 testing, like the one designed by Han et al. (2021), are expected to be extremely useful. The technique may offer a wide range of uses, including real-time surveillance for individualized diagnostics. Some efforts on smartphone-based POCT aptasensors have now been reported in recent years, and they are projected to have high business value.
II. The idea of smartphone-based aptasensors is relatively new as compared to smartphone-based biosensors at large, and the systems are typically demonstrated in laboratories under standard testing conditions. Efforts should be made to construct and demonstrate easy-to-use aptasensors on smartphones for POC analysis outside the laboratory environment with minimal user involvement. Efforts should be dispersed in several directions, including sensor manufacturing, transmitting data, and smartphone data processing, to boost the performance while maintaining the affordability and portability for the successful commercialization.
III. Since all smartphone-based aptasensors require a custom-built app, the excellence of the app quality is becoming the criterion to rate the developed methodology. The app ought to be easy-to-operate and accurate. For programmers, the app must fulfill technological and commercial regulations to facilitate future functional expansions and updates. Furthermore, the app should be capable of uploading patient data (age, gender, area, etc.) to a cloud server, which can assist health authorities in making prompt and correct decisions related to infectious disease outbreaks.
IV. The latest smartphone-based aptasensors should be endowed with a variety of potential innovations, including paper-based, flexible, and microfluidic technologies. Paper-based aptasensors can be promising for integration with smart phones. This is predominantly because of the benefits of paper, such as its low cost, recyclability, natural abundance, and simplicity in both its fabrication and usage. Importantly, the paper-based sensors have been demonstrated to be extremely portable; in most cases, these sensors do not require any additional equipment, reagents, and power source other than the smartphone itself. Furthermore, colorimetric sensing can be effectively conducted with a smartphone via photographing color variations in paper aptasensors, which provides a fascinating and practical option for quick monitoring via personalized operations. Yet, there is only one report on microfluidic paper-based smartphone aptasensors (Somvanshi et al., 2022) for multiplexed detection of pathogenic bacteria in water and food safety. Further efforts should be made to find and address the challenges associated with the fabrication and execution of paper-based smartphone aptasensors to further simplify the sensing.
V. Wearable technology is also seeing improvement with paper-based biosensors. Yet, one of the biggest disadvantages of paper-based sensors is humidity interference. Combining paper-based biosensors and encapsulating the constituents on the substratum with plastic films exhibits satisfactory results for sweat-based POCT. Paper-based smartphone aptasensors may also find important applications in wearable technology.
VI. The success of aptasensing through smartphones seems to be highly dependent on aptasensing innovations, which involve challenges for such spectacular integration. Although smartphone aptasensing is still in its infancy, some manufacturers are now selling hardware and software to enable aptasensing applications on smartphones. There are organizations involved in developing applications for extracting and processing data via smartphones, with each situation requiring customized software and hardware. Depending on the system requirements, researchers can either create their own software and hardware, or purchase it from companies, or use an already existing one. For example, the Merkoci group demonstrated that by integrating commercially available, cost-effective hardware accessories and open-source software with a smartphone, it is easier to build a handheld plate reader. This can facilitate optical biosensing and offer comparable results to expensive and bulky commercial readers (Bergua et al., 2022). The researchers explicitly demonstrated how the handheld plate reader can quantify bioluminescent, colorimetric, turbidity, and fluorescent signals. After that, the sensor was used to carry out gold-standard screening tests, such as an ELISA, for the identification of a SARS-CoV-2 N-protein.
VII. Each year, a large number of aptasensors are reported in the literature, claiming the detection of a broad range of targets. Yet, there are only a couple of aptasensors that are available on the market for commercial purposes. Currently, the aptamers are at a very early stage of commercialization as compared to the widespread use of antibodies. The commercialization of aptamers is limited predominantly by three factors: (i) the SELEX technique is not mature and consistent, and the aptamer screening is time-consuming, unpredictable, and suffers from reproducibility; (ii) the aptamer's three-dimensional folding is arbitrary, and the mechanism of aptamer-target association is unclear; and (iii) aptamers are synthesized in ideal buffer systems. They are frequently invalidated when utilized in complex analytical systems, leading to unsteady performance. With the advancements in the next generation of synchrotron radiation technology, free-electron spectroscopy technology will be able to investigate the single-stranded DNA structure and folding dynamics at the atomic level in the future. With the advancement of electron microscopy, it will be possible in future to look at the structure of aptamer-protein complexes. This would help in understanding how aptamers interact with their targets at the molecular level, which is important for the large-scale commercial use of aptamers. Moreover, aptamer screening should be performed under conditions which are similar to the real or practical testing conditions.
VIII. Lab-on-fibers may be connected to a variety of interfaces, including chips, or linked via cellphones utilizing handheld and engrained equipment.
IX. Significant progress has been made recently in the domain of portable food monitoring, particularly related to the miniaturization of analytical equipment for on-site analysis of food and water. This has been implemented to some level with the assistance of smartphones. However, there is still a gap between academic research and consumer requirements that demands our attention. Smartphones having wireless or wired connectivity (i.

e., Bluetooth, Wi-Fi, and 4G/5G) can be connected with food assessment systems to improve food safety. The inherent potential of smartphones to access the internet makes them perfect tools in the Internet of Things (IoT) for controlling, analyzing, and sharing data. The results can be shared on a cloud server for additional research and dissemination. Therefore, when paired with radio frequency identification (RFID) tags, which are at the frontline of IoT sensing technologies, the smartphone-based aptasensors have the ability to stimulate the growth of the IoT. However, food matrices change continuously throughout storage and transportation, demanding legitimate quality detection. The widespread use of smartphones strengthens the practical significance of sensing devices, which might be utilized by both professional inspectors and non-professionals (i.e., consumers). Together with detection tools, the consumers should be able to operate as a formidable protector of their food chain. The inexpensive, handheld, and consumer-based detection is suitable for integration with production lines and smart packaging, as well as for placement at sites of susceptibility throughout food supply chains. This will facilitate immediate at-home or online food safety inspection, and shed light on food hygiene monitoring 'from farm to fork.' Smartphones, as a vital connection between networks, sensing, and people, are likely to play an essential role in emerging analytical techniques. The most recent food inspection assays demand the collection of important data in a rapid, reliable, and timely manner. To comply with food safety regulations, the growing use of smartphone-based aptasensors will require more involvement from artificial intelligence, cloud computing, big data, and other emerging technologies.

It is almost impossible to imagine how widely smartphone-based aptasensors will be used in people's daily lives. It is clear now that researchers would need to be as determined and imaginative as Bill Gates, who once prophesied that "one computer on every desk and one in every home" would happen with smartphone-based aptasensor technology (considering individual smartphone users) when it comes to its all-in-one applications in disease diagnosis, food safety, and environmental applications.

6. Conclusions

The combination of smartphones and aptasensor technology represents a significant divergence from conventional sensing methodologies and equipment, providing healthcare diagnostics/POCT, food safety, and environmental monitoring without requiring many resources and facilities. Smartphone-based aptasensors are viewed as more affordable and user-friendly than the standard analytical biosensors. The review provided a detailed account of the development and applications of smartphone-based optical, electrochemical, and many other aptasensors, which offer portable sensing platforms for on-site assessment of complex environmental and biological samples. The specimen includes metal ions, viruses, proteins, disease biomarkers, antibiotic and pesticide residues, and so forth. In summary, the smartphone-based aptasensing technology has potential to revolutionize the detection systems. It has been shown to have both potential and opportunities to improve POC and PON diagnostic and prognostic methods, which has changed the way people think about mobile health and the biosensing market.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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