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17β-Estradiol electrochemical biosensors: recognition elements, platforms, and analytical validation

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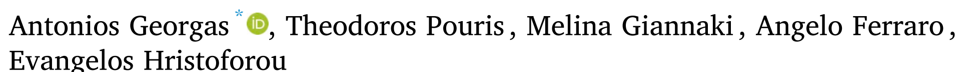

A R T I C L E I N F O

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A B S T R A C T

17β-Estradiol (E2) is a vital endogenous estrogenic hormone considered a potent endocrine-disrupting compound as its widespread presence in aquatic environments and biological fluids may lead to adverse effects for both aquatic ecosystems and human health. Electrochemical biosensing has evolved into an ideal approach for E2 detection and quantification, due to its ability to satisfactorily combine high sensitivity with speed, simplicity and the prospect of portability. This review introduces endocrine-disrupting compounds (definitions, mechanisms, exposure, assessment) and provides an overview of electrochemical strategies reported for E2 sensing focusing on how biorecognition design and electrode/nanomaterial engineering determine analytical performance and real-sample readiness. We examine primary recognition routes (including affinity-based molecules and synthetic receptors), emphasize the utilization of electrode platforms and surface modifications are used to enhance signal transduction as well as interface stability, and summarize measurement formats used to quantify E2. Finally, we review the ongoing challenges arising in transitioning E2 electrochemical sensors from laboratory prototypes to robust applications, emphasizing matrix effects, interference testing, comparability of reported figures of merit, and reporting practices that support reproducibility. Overall, the review seeks to establish a systematic synthesis of existing literature into a structured framework guiding the design of future electrochemical biosensors capable of addressing real-world requirements.

1. Introduction

1.1. Endocrine-disrupting compounds (EDCs): definitions, mechanisms, exposure, and assessment

Endocrine-disrupting compounds (EDCs) are commonly defined as external factors or mixtures that modify the function(s) of the endocrine system leading to adverse implications in an intact organism, its progency, or (sub)populations [1]. The definitions of the International Programme on Chemical Safety (IPCS), the United Nations Environment Programme (UNEP), and the World Health Organization (WHO) are aligned with this formulation which is reiterating in broad overviews addressing occurrence in aquatic environments and human exposure [2]. According to reports centered on public health, EDCs may intrude on hormone biosynthesis, metabolism, or action, thereby deviating from normal homeostatic control and/or reproduction [3].

The contrast between endocrine activity and endocrine disruption is a practical and significant distinction in both research and governance [4]. According to European Union (EU) guidance for identifying endocrine disruptors, a substance is concluded to have endocrine-disrupting properties only when three conditions are fulfilled: (i) an adverse effect, (ii) endocrine activity, and (iii) a biologically plausible link showing that the adverse effect is a consequence of the endocrine activity [4]. This tripartite structure is echoed in evidence-integration proposals for EDC evaluation, which similarly prioritize structured appraisal of evidence for effects, endocrine activity, and plausibility of a causal link [5].

EDCs compose a chemically varied range of compounds, that include encompassing industrial chemicals, plastics and plasticizers, pesticides, pharmaceuticals, metals, and persistent organic pollutants [6]. Nearly 800 substances are known or suspected to interfere with hormone receptors, hormone synthesis, or hormone conversion according to global assessments, which also indicate that existing lists are still insufficient [1]. Although discussions of endocrine disruption frequently focused on estrogenic impacts, multiple reviews highlight that mechanisms expand

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beyond a narrow receptor-centric view and beyond a limited set of pathways, supporting the adoption of wide, mechanism-aware screening and assessment approaches [7].

Mechanistically, the dysregulation of several EDCs in endocrine homeostasis by affects hormone synthesis and/or receptor binding, thereby altering endocrine signaling and downstream physiology. Receptor systems implicated include nuclear hormone receptors such as estrogen, androgen, progesterone, thyroid hormone, and retinoid receptors. Nonclassical pathways are also described, including actions via nonsteroid receptors, transcriptional coactivators, and enzymatic pathways involved in steroid biosynthesis and metabolism. In contrast to geonomic responses which can genomic responses which can be very time-consuming certain EDCs can operate through membrane-associated signaling and produce quick non-genomic responses in a matter of minutes [7].

Broader biological responses complicate interpretation while increasing the range of potentially relevant endpoints. The mechanistic repertoire of endocrine disruption is examined in relation to epigenetic alterations, which may have transgenerational implications. These modifications include DNA methylation, histone modifications, and noncoding RNAs [7]. According to scientific statements and mechanistic reviews such effects are associated with delayed or latent outcomes, including the “developmental basis of adult disease” concept [3]. Aquatic occurrence and exposure overviews similarly mention epigenetic mechanisms (e.g., histone modifications and DNA methylation/acetylation) in the context of endocrine disruption [2].

Dose-response behavior remains a major concern in endocrine disruption research and assessment. Global assessments and scientific statements explicate non-linear dose-response relationships, including non-monotonic dose responses (NMDR), alongside attention to low-dose effects and the possibility that low doses can be potent in certain contexts [1]. EU hazard-identification guidance explicitly considers low-dose effects and NMDR within weight-of-evidence and mode-of-action analysis [4]. However, other reports consider low-dose and NMDR as highly controversial, indicating ongoing methodological and interpretive challenges [2].

Differentiating between direct endocrine-mediated effects and endocrine endpoint changes that are secondary to generalized stress or systemic toxicity is a significant hurdle [8]. If causative pathways are not thoroughly examined screening assays may detect endocrine-related alterations that arise indirectly through neuroendocrine stress cascades or through liver toxicity influencing hormone clearance, raising the risk of misclassification [8]. This concern is consistent aligns with identification frameworks requiring a biologically plausible connection between endocrine activity and adverse outcomes rather than relying on endpoint perturbation alone [4].

EDCs originate from a variety of sources and release pathways, including pesticides, flame retardants, plastic additives, cosmetics, pharmaceuticals, and other industrial applications; releases from products containing EDC-containing products contribute to environmental contamination [1]. The term “multi-route” is regularly associated with human exposure which includes dietary uptake and ingestion of contaminated food, dust, and water; inhalation of gases and particles; dermal contact for selected compounds; and biological transmission across the placenta and through mother’s milk [7]. The significance of context-specific exposure profiles is further reinforced by the emphasis on occupational exposure for those who work with industrial chemicals, fungicides, and pesticides [3].

Aquatic environments are frequently emphasized as crucial environmental compartments for EDC occurrence and exposure. Wastewater discharge, agricultural runoff, combined sewer overflows, and leaching from consumer and industrial products are examples of inputs [2]. When human and animal excretion enters water bodies through inadequately treated wastewater, runoff, and disposal or leakage into groundwater steroidal estrogens are commonly described as “environmental estrogens” and emergent micropollutants in water [9]. In European aquatic environments, sanitary and agricultural sewage are described as dominant contributors to estrogen loads, including domestic sewage, wastewater treatment plant (WWTP) outflows, and slurry/manure used as fertilizer [10].

Hormonally active compounds are frequently claimed to be incompletely removed by conventional wastewater treatment; estrogens and other estrogenic contaminants are characterized as not totally eliminated in WWTPs and as exhibiting variable removal across treatment types [11]. For steroidal estrogens specifically, the lack of a single process capable of complete elimination in wastewater treatment plants has been stated explicitly, reinforcing concern about persistent low-level discharges [9]. The bulk of examined samples in Europe have reported environmental concentrations for estrogens between 0.1 and 10 ng/L, and bebates focused on the EU address estrogenicity benchmarks and environmental quality standards [10]. More broadly, aquatic habitats and drinking water have been shown to contain EDCs, often at ng/L to μg/L levels, with drinking water concentrations spanning wide ranges across studies and locations [2].

Compounds created during treatment may potentially be involved in exposure to water. In situations such as drinking water, showering/bathing, and swimming pools, disinfection byproducts (DBPs) are produced when disinfectants react with organic/inorganic matter in source water, leading to ingestion, inhalation, and dermal exposures [12]. Water-focused reviews additionally address persistent contaminants such as per- and polyfluoroalkyl substances (PFAS), their origins in diverse consumer and industrial uses and their contribution to localized drinking water contamination near facilities, landfills, and wastewater treatment plants; persistence and bioaccumulation are highlighted as major concerns [12].

Health and ecological concerns span multiple endpoints and life stages. Across reviews, EDCs are associated with reproductive, metabolic, neurological, cardiovascular, thyroid-related outcomes, and hormone-sensitive malignancies, with susceptibility often framed as highest during early development [1]. Formative effects may occur at lower levels than in adults during developmental exposure windows, which are referred to as periods of increasing sensitivity from fertilization through fetal development and nursing [1]. Disease-endpoint reviews similarly connect EDC exposures to reproductive, metabolic, neurologic, cardiovascular, and cancer endpoints, including developmental and transgenerational implications [7].

Reproductive effects are prominent and multifaceted. Folliculogenesis, steroidogenesis, ovulation, fertilization, and gestation are all reported to be interfered with by EDCs; early gestation exposure has been discussed as capable of disrupting implantation and uterine receptivity [7]. Cryptorchidism, hypospadias, reduced semen quality, and infertility are among the male reproductive consequences that are mentioned in several sources Testicular dysgenesis syndrome is associated with decreased androgen activity during fetal development [7]. Concerns including low semen quality in some countries and an increase in genital malformations have also been noticed in population-level evaluations, although attribution to specific chemicals remains complex [1].

Obesity, diabetes, and liver-related outcomes such as Non-alcoholic fatty liver disease (NAFLD) are among the metabolic consequences that are frequently studied, including mechanistic framing via altered lipid handling processes [7]. Overviews also refer to compounds that may alter energy metabolic homeostasis and increase the risk of weight gain as “obesogens” [2]. Global assessments for neurodevelopmental and thyroid-related endpoints emphasize thyroid-disruption-associated neurobehavioural disorders and developmental neurotoxicity linked with specific contaminants (e.g., Polychlorinated biphenyls (PCBs) and metals), while also noting that epidemiologic results can be conflicting for certain exposures and outcomes [1]. The importance of structured evidence integration and careful causal inference is reinforced by such discrepancies [6].

Ecological implications are described as extensive and

mechanistically instructive given conservation of endocrine pathways across taxa [3]. The effects of wildlife on growth and reproduction, including fish feminization/intersex and reduced reproductive success linked to estrogenic sewage effluents are summarized in global assessments [1]. Estrogen-focused reviews add observations across groups, including Vitellogenin biomarker induction, sex ratio imbalances, and reproductive disruption in fish and amphibians are among the cross-group observations added by estrogen-focused reviews; conflicting views are reported for invertebrate endpoints [10]. The significance of real-world exposure mixes is further supported by the emphasis on mixture effects, which include evidence for synergistic estrogenic potency in assay systems and additive or supra-additive estrogenic activity from combined exposures [1].

Although monitoring and assessment frameworks are evolving, scope and coverage continue to provide challenges. According to multiple sources monitoring is restricted in comparison to the likely scale of the problem, with measurement frequently concentrated on a narrow subset of chemicals [1]. European assessments focused on estrogen also highlight the need for analytical methods that can detect extremely low quantities in addition to monitoring gaps, notably in Eastern Europe [10]. EU guidance documents articulate a formal hazard-identification process using weight-of-evidence and mode-of-action analysis, focusing primarily on estrogenic, androgenic, thyroidal, and steroidogenic (EATS) modalities, and linking decisions to the Organisation for Economic Co-operation and Development (OECD) conceptual frameworks and test guidelines [4]. These documents also clarify that the guidance addresses hazard identification rather than full risk characterization[4]. According to systematic reviews frameworks internationally validated test methods may capture only a limited spectrum of endocrine-disrupting effects, with implications for estimating disease risk [5]. Regulatory coverage is described as uneven in water-related discussions: many Disinfection By-Products (DBPs) have been identified but only a small portion is regulated in some frameworks, and for PFAS the absence of maximum contaminant levels (at the time of writing) has been observed [12]. In Europe, estrogens have been placed on watch lists for Union-wide monitoring and environmental quality standards are reported for selected compounds [10]. Taken together, these themes encourage targeted attention to particular, high-potency endocrine-active chemicals that recur in aquatic environments, among which E2 is commonly used as a reference compound and remains of particular concern.

1.2. E2: identity, key properties, sources, and environmental behaviour

E2 is the primary natural (endogenous) steroidal estrogen, E2 is commonly employed as a high-potency reference estrogen in toxicological and environmental research [13]. of cholesterol derivation with a tetracyclic ring structure consisting of an aromatic A ring with a phenolic hydroxyl group and a second hydroxyl group at C17 in the β orientation(17β) [14]. Chemical identifiers frequently reported in environmental literature include the formula $C_{18}H_{22}O_2$, molecular mass of ≈272.38 g/mol, and CAS 50-28-2, are chemical identifiers reflecting common practice in sourcing analytical standards for laboratory work [15].

The physicochemical properties of E2 affect its transport, distribution, and persistence in both natural and artificial systems. E2 is highly hydrophobic with a high octanol-water partition coefficient of approximately 2.6–4.0. Various researchers have found a narrower range of 3.5–4.0, which is at the upper end of the range of possible values for E2. E2 is described as having low volatility and low solubility in water in comparison to conjugated estrogens, favoring particulate matter, sediments, and sludge over volatilization [14,16]. The pKa of 10.46 indicates that E2 is a very weak acid and is predominantly in a non-ionized form in environmental samples, which is a function of typical environmental pH. This is an important property for E2 in environmental samples, since a compound in a non-ionized form is more easily absorbed into a lipophilic medium [17].

E2 is largely endogenously produced by vertebrates and is excreted mainly through urine and feces, thereby acting as a natural occurring E2 source in the environment [14]. In the larger context of steroidal estrogens and endocrine-active chemicals, human activity and infrastructure are still significant drivers for environmental pathways and emission routes. Some major anthropogenic emission sources identified through studies are WWTP influent and effluent, biosolids, and concentrated animal feeding operations (CAFO), where animal manure and waste are hormone-rich and often land-applied or discharged to waters [18]. Agricultural slurry and animal manure are again highlighted as significant contributors, and both slurry and manure solids are identified to concentrate estrogenic activity compared to the aqueous phase [19].

Some transport pathways for E2 and other estrogens from these sources are direct discharge of effluent, treated or untreated, into rivers; runoff and tile drain flows from manured fields; leaching to groundwater through preferential flow paths in sediments; and sorption-mediated transport linked to colloid and organic matter transport [18]. These routes help to shed light on the occurrence of E2 downstream of these wastewater and agricultural sources and are also in line with the larger definition of aquatic compartments acting as receivers for endocrine-active chemicals.

Whether E2 is converted into active form again or converted into another form, this depends on transformation and biodegradation processes. Biodegradation processes, which involve microbial oxidation of E2 into less potent estrone (E1), deconjugation of sulfate and glucuronide conjugates, and mineralization under favorable conditions, are generally considered to be major removal processes in both natural and engineered systems [14]. The matrix, microorganisms, and treatment conditions are known to significantly impact reported degradation kinetics. References are made to half lives of E2 in river water ranging from hours to days (0.2–9 days in river water E2 biotransformation studies), and sediment-containing systems are known to have faster removal rates than water-based systems; cultures such as Rhodococcus sp. ED55 is reported to have the ability to mineralize mg/L concentrations of E2 in just hours (half life ≈1.06 h in mineral salts medium with ED55) [14,20,21].

Abiotic changes can also have an impact on exposure patterns. For example, oxidation by disinfectants can cause halogenation. For chlorine-based WWTP disinfection processes, oxidation by disinfectants can cause halogenation, and photolysis is described as rapid for halogenated compounds, while free E2 photolysis is generally slower; these changes can cause changes in sorption and biodegradation patterns [20]. Partitioning to solids is another pattern seen throughout this data. For example, sorption to sediments, sludge, and humic materials (as represented by high Kd and Koc values) can limit E2 mobility in water but can also provide E2 with protection from rapid biodegradation and can cause it to persist in these solid materials [14,22].

Based on these sources and processes, E2 can be found in wastewater influent and effluent, surface waters, and agricultural slurries over a wide range of concentrations depending on proximity to sources and sample matrix[16,19]. The environmental range of E2 in surface and groundwater, as represented in overviews, can vary from sub ng/L to tens or hundreds of ng/L, while manures and slurries have significantly higher concentrations (μg/L to mg/L equivalents) [16,19].

Ecotoxicologically, E2 is described as a highly potent compound with documented effects on fish and other vertebrates at low ng/L concentrations. For example, endpoints such as vitellogenin induction and feminization have been documented at ng/L concentrations [23,24]. E2 equivalents (EEQ) are often employed in monitoring and evaluation to account for the additive effects of mixtures and metabolites. E2 is viewed as a priority compound with regard to evaluating the risk of endocrine disruption, treatment efficacy, and management of agricultural and municipal sources of E2 due to its potency and presence of E2 in treated effluent and manures [21].

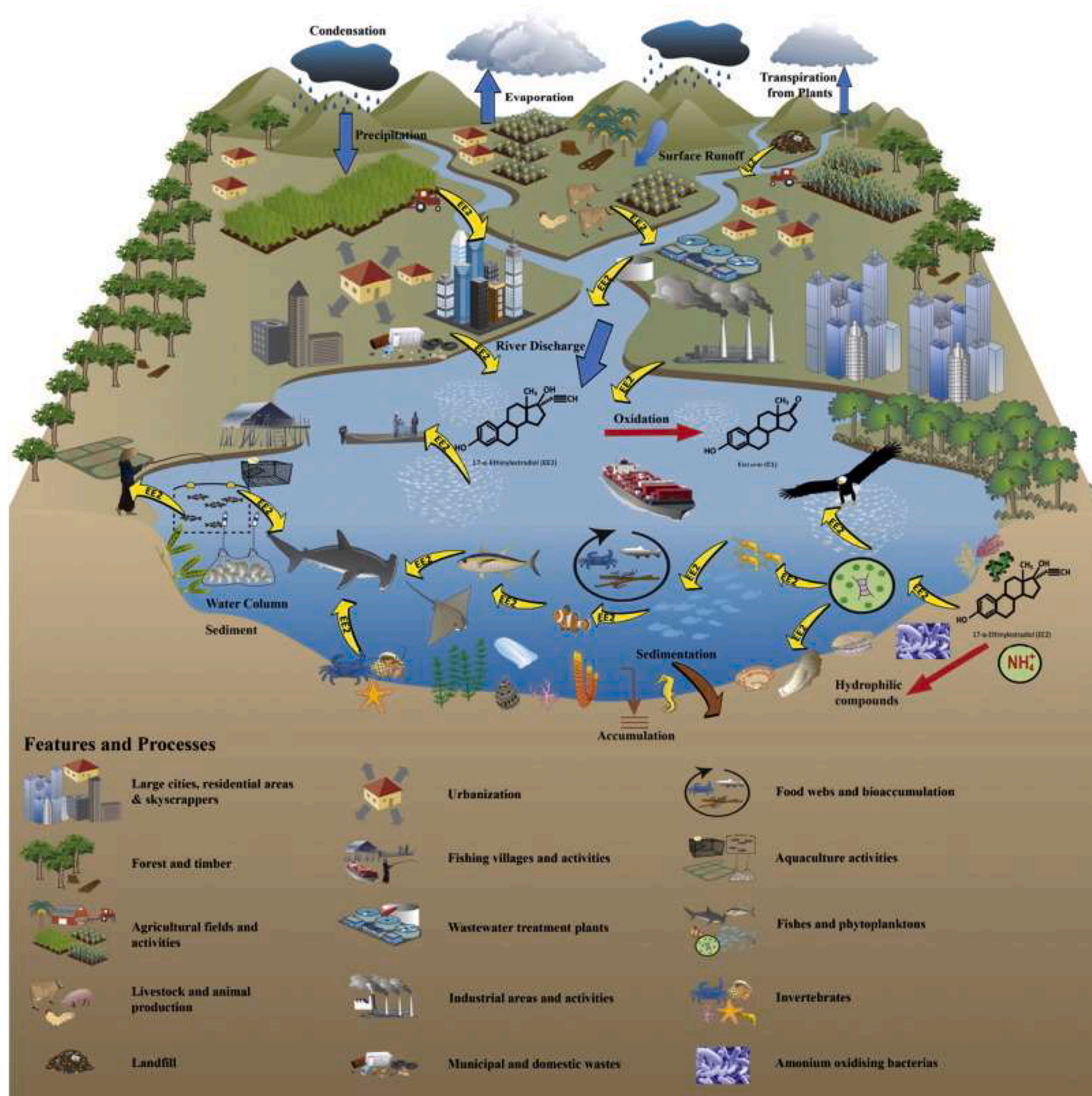

Fig. 1. Fate and transport of E2 in the aquatic environment (reproduced from [25] with permission).

These aspects of E2 influence the analytical strategy. For example, monitoring often includes free E2 and its conjugates and metabolites and transformation products. This is because deconjugation of E2 may regenerate free E2 during treatment or after discharge to surface waters. In addition, metabolites such as E1 (Estrone) retain estrogenicity, although at a lower potency [14]. Solid phases are often included with water samples in monitoring strategies. Sediments and biosolids often have higher concentrations of E2 compared to overlying water. Bioassay-based EEQ screening is also discussed as an additional strategy to target chemical analysis [19,22]. When designing monitoring programs and risk assessments, parent compounds and transformation products must be taken into account due to the variability in degradation kinetics, which can range from rapid microbial mineralization in specialized cultures to persistence associated with sorbed phases or halogenated derivatives [20,21]. Fig. 1 provides an overview of the main pathways through which estrogens enter and move through aquatic environments, highlighting the complexity of monitoring them in real water systems.

The justification for fast, sensitive, routine detection in water becomes more apparent in light of this chemical and environmental context: exposure characterization and risk management are complicated by low-effect concentrations, continual emissions and transformation processes.

1.3. Environmental and human health impacts of E2 and related estrogens: why sensitive water monitoring is needed

There is an emerging interest in the environmental and clinical detection procedures of environmental E2 and other estrogens like E1 and Estriol (E3) due to their broad recognition of their potency as EDCs, capable of interfering with natural physiological processes in both wild and human life [26]. Concerns about the continuous exposure hazards to aquatic systems and their users There are increased concerns about the continuous exposure hazards to aquatic systems and their users due to the persistence of E2, coupled with its continuous discharge from human sources, owing to its association with detrimental biological consequences to species, even in trace amounts [27]. Besides natural estrogens, 17α-ethynylestradiol (EE2), a synthetic hormone used for birth

control, derived from natural E2, is employed for the treatment of both environment and human health. Similar to natural estrogens, EE2 is the principal contributor to estrogenic activity, dominated by effluent discharge from urban WWTPs, hospital effluent, and livestock activities [25].

Such estrogens are said to produce a variety of ecotoxicological effects on aquatic ecosystems, which include reproductive problems, feminization of males, abnormalities, and population-level effects on fish and other vertebrates. Several declines in wildlife populations have been linked to the presence of estrogens in the environment, with the recorded effects including life cycle and generational effects on the populations of the organisms exposed to the estrogens [28]. Studies on the exposure of teleosts to exogenous E2 have shown physiological problems, which include inhibition of spermatogenesis, decreased gonadosomatic index, liver histopathology, and decreased growth, which indicate potential ecological risks, particularly with regard to wild and cultured fish populations [29]. Top predators and aquatic biota consumers are of concern because estrogens are said to be sufficiently persistent to bioaccumulate in sediments and groundwater and biomagnify in the food web, as suggested in reference [28].

The endpoints reported vary from cellular effects to population effects and include sterility, spawning failure, feminization, thyroid hormone disorders, and developmental alterations. Complete spawning failure has been observed at 0.2 ng/L in experimental studies on *Gobiocypris rarus*, and other studies reported lowest observed effect concentrations (LOECs) and no observed effect concentrations (NOECs) at low ng/L for sensitive fish species [30]. Biomarker response: In fish, biomarkers such as lipid peroxidation, hydroperoxide content, and protein carbonylation are reported to increase after E2 exposure in laboratory bioindicator studies, along with antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), indicating E2-induced cellular stress response [31]. In addition, hepatic lesions, decreased sperm motility, and decreased reproductive indices are reported to be associated with regulated E2 treatment, linking biochemical and histopathological effects with decreased reproductive performance [29].

1.4. Human health considerations

The literature has existing evidence on the correlation of natural and synthetic estrogens with human health outcomes, which include the disruption of reproductive balance, development timing, and the possibility of hormone-sensitive cancers such as breast and endometrial cancers [28]. In the clinical and biomonitoring settings, the need for ultralow detection limits cannot be overemphasized, as changes from physiological levels of E2 are significant biomarkers for conditions such as precocious puberty and endocrine disorders [32]. Although the carcinogenic and development effects are recognized as issues, the reviews and assessments include the potential for endocrine disruption with exposure to synthetic estrogens and offer conflict data on direct relationships with exposure levels [30].

1.5. Why routine, sensitive monitoring is needed (and what it demands analytically)

There is considerable evidence to underscore the importance of monitoring E2 and related EDCs at low levels in aquatic environments. First of all, in order to conduct risk assessments, it is essential to achieve analytical sensitivity at low levels since ecotoxicological effects such as spawning failure and reproductive disorders have been observed at sub-ng/L levels of E2 and related EDCs [30]. Secondly, it is argued that regulations such as the EU WFD identify E2 as a priority substance and set EQS that rely heavily on monitoring to assess compliance and status in the environment [26]. Third, it is argued that monitoring of E2 and its metabolites is necessary since it is not certain that conventional WWTPs can remove EDCs completely and since treatment technologies may generate EDCs with unknown or even greater toxicity than the original compound [31]. Finally, the presence of EDCs across multiple matrices (surface water, wastewater, sediments, biota, and some foodstuffs) and the potential for bioaccumulation are used to justify systematic detection to protect ecosystem and human health [28].

Meeting these monitoring needs imposes demanding analytical requirements because relevant effects occur at very low concentrations and environmental matrices are complex. Ultrahigh sensitivity is described for some clinical and environmental applications, with targets down to the pg/ml (and lower) range for clinical diagnostics and environmental trace detection [32]. Conventional chromatographic and mass-spectrometric approaches can deliver high sensitivity but are associated with practical limitations, including high capital and operational costs, extensive sample preparation, and constraints on routine field deployment [28]. Specific analytical limitations have also been reported experimentally, such as situations where High-performance liquid chromatography (HPLC) detection limits constrained the ability to generate degradation profiles at 1 ng/L spiking levels [31]. Emerging biosensor and aptasensor approaches are presented as promising for portability, reduced cost, and rapid results, but they face challenges including reproducibility, matrix interferences, aptamer immobilization, and the need for amplification strategies to reach environmental and clinical decision limits [26]. In parallel, scalable biodegradation or remediation approaches are described as needing to avoid generating toxic by-products and to be assessed for safety, efficacy, and feasibility at scale [27].

Overall, the studies synthesized across these sections converge on a coherent motivation for this review's focus: E2 and related estrogens combine high potency at low concentrations with sustained entry into aquatic systems, incomplete removal in conventional treatment, and complex transformation pathways, all of which make robust, sensitive detection in water central to environmental assessment and health-protective monitoring [28].

2. Biorecognition strategies for E2

This chapter is organized around recognition strategy, namely aptamers, antibodies (immunosensors), and molecularly imprinted polymers (MIPs), and follows established taxonomies and foregrounds on how the choice of a biorecognition element shapes the analytical performance, materials selection, and device formats [26,33].

To keep this discussion focused, the papers considered for this review have been gathered from significant scientific databases by combining keywords like endocrine disrupting compounds, 17β-estradiol, electrochemical sensing, biosensors, aptamers, immunosensors, and molecularly imprinted polymers. Special emphasis was given in peer-reviewed articles that not only discussed the sensor, but also included analytical performance and, if available, evaluation of complex matrices. Landmark papers that were published earlier have also been considered, in cases that they were marked as significant for the proper understanding of how this field evolved and how the current strategies of sensing have developed.

Aptamer-based sensors (Section 2.1) are treated first because aptamers have been highlighted as a particularly promising recognition class for E2 owing to their high specificity, stability, and adaptability, and because much recent work has focused on sequence engineering (split, truncated, SELEX-derived) and nucleic-acid amplification strategies to push limits of detection (LODs) into the femto- to nanomolar range [26]. At the same time, the very amplification schemes and nanomaterial integrations that enable ultralow LODs introduce reproducibility and stability challenges that require careful consideration in device design and real-sample validation [26].

Section 2.2 examines immunosensors, where antibody-based competitive and label-based electrochemical formats have demonstrated ng/L sensitivity and a clear pathway to on-site measurement through combinations of magnetic beads and screen-printed electrodes, but also face issues such as steric hindrance on immobilization as well as

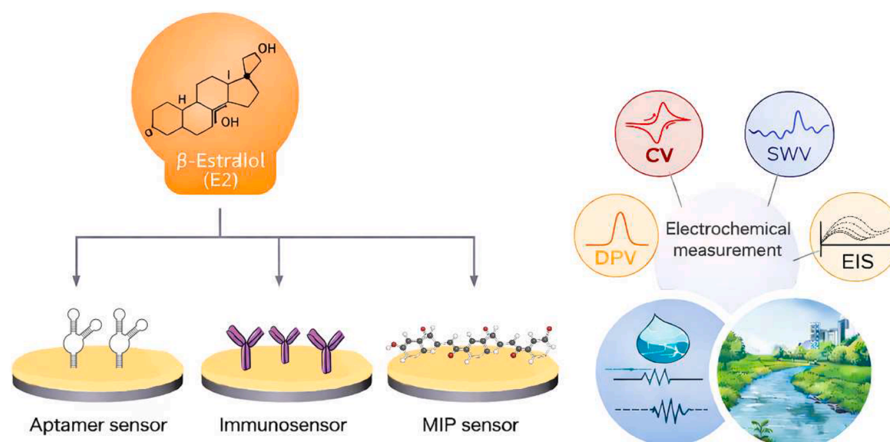

Fig. 2. Graphical overview of the electrochemical biosensing pathways for 17β-estradiol (E2). The schematic portrays the target analyte (E2) and the three distinct surface-modified platforms of interest: aptamer sensors, immunosensors, and molecularly imprinted polymer (MIP) sensors. Subsequent signal transduction is depicted through electrochemical measurement techniques, specifically cyclic voltammetry (CV), square wave voltammetry (SWV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS). The analytical workflow concludes with an illustration of environmental water monitoring, demonstrating the translation of these sensing mechanisms to real-world matrices such as rivers and surface waters.

limited operational stability of prepared electrodes over weeks [34].

On the other hand, non-biological recognition tools, such as MIPs, are highlighted in Section 2.3. Although these reagentless methods are of significant interest due to their robust nature, they are not as selective as bioreceptors. Hence, it is vital to incorporate them with transducers that are of high specificity, in order to prevent interfering species with redox properties from interfering with the generated signals [33].

With this format of reviewing, it is possible to compare and contrast the basic trade-offs that are typically highlighted in literature, such as analytical specificity, robustness in complex matrices, and practicality of field applications. Moreover, this format creates a foundation that can lead to further discussion on various transduction modes, functional materials, and the significant gaps in technology that are required to bridge the gap from a laboratory-based device to a point-of-care E2 sensor [26,33].

Fig. 2 is a summary of all the analytical concepts that are covered in this review. It presents a detailed illustration of the target molecule, denoted as E2, in conjunction with various recognition tools, such as aptamers, immunosensors, and MIPs, as well as various electrochemical transduction modes that are highlighted in this paper.

2.1. Aptamer-based electrochemical biosensors for E2

2.1.1. Introduction

Aptamer-based electrochemical sensors have emerged as a versatile class of bioaffinity devices for detection of the small steroid E2. Reports in the literature illustrate a diverse set of assay architectures, from simple surface-immobilized direct-binding formats to split-aptamer sandwiches and enzyme- or nucleic-acid-amplified schemes, and a wide range of electrochemical transduction modes including impedance, voltammetry, electrochemiluminescence (ECL) and photoelectrochemical (PEC) readouts. Reviews and comparative studies catalogue this breadth and the enabling roles of nanomaterials and redox reporters in pushing analytical sensitivity into the sub-picomolar and lower regimes [35,36].

2.1.2. Principles and assay formats for small-molecule E2

Aptamer assays (aptasensors) for E2 use multiple recognition and reporting topologies that are adapted to the small, nonpolar target. One common approach is direct binding of E2 to a surface-immobilized aptamer: target binding induces steric blockage of a solution redox probe or a change in interfacial impedance that is read out label-free [37,38]. Conformational-change and structure-switching formats exploit aptamer folding on target binding to modulate the distance or accessibility of a redox tag or to alter charge-transfer pathways; these have been implemented as both impedance “signal-on” sensors and as redox-tagged voltammetric assays[36]. Fig. 3 schematically shows the basic principle of aptamer-based electrochemical E2 sensing, in which target recognition is translated into a measurable change at the electrode interface.

Competitive and hybridization-mediated configurations are another widespread class: an aptamer immobilized on the electrode surface is competed by a guanine-rich complementary oligonucleotide or cDNA that carries a redox tag, so that E2 binding inhibits cDNA hybridization and reduces the reporter signal [39]. Split-aptamer sandwich designs use two short aptamer fragments that only assemble in the presence of

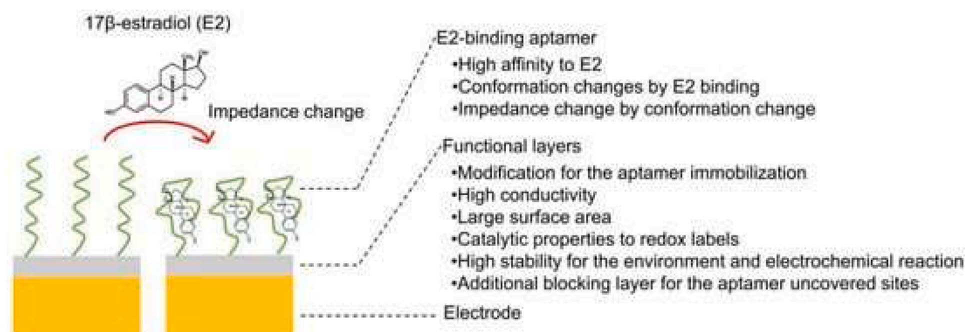

Fig. 3. Simplified structure of estradiol aptasensors based on impedance change [36].

E2, creating a sandwich complex that can block or recruit redox reporters and is compatible with disposable electrodes and ratiometric measurements [40,41]. When coupled with signal amplifications strategies, such as enzyme-assisted cycling or nucleic acid cascades like the hybridization chain reaction (HCR), these binding interactions are significantly magnified, allowing for attomolar to femtomolar LODs [36, 40]

2.1.3. Surface functionalization and aptamer immobilization

Immobilization chemistry critically affects and assay's background current, stability and reusability. Numerous validated surface strategies have been applied to E2 aptasensors design. Self-assembled monolayers (SAMs) relying on thiol-gold (Au-S) interactions are widely used to enable covalent anchoring of thiolated aptamers directly to gold surfaces. This step is commonly followed by a passivation step with short alkanethiols such as 6-mercapto-1-hexanol (MCH) to minimize non-specific binding [38,39]. Streptavidin-biotin coupling on carboxyl-terminated SAMs provides an alternative route to immobilize biotinylated aptamers, with defined protocols including SAM formation with 3,3′-dithiodipropionic acid, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)/ N-hydroxysuccinimide (NHS) activation of carboxyl groups, streptavidin coupling, and ethanolamine blocking reported for gold microelectrode chips [37]

Electrode surfaces are diversified by nanomaterial coatings that increase electroactive area and facilitate immobilization: electrodeposited gold nanoparticles (AuNP) on glassy carbon and two-dimensional transition-metal sulfide nanosheets (e.g., CoS) have been used as composite supports for thiol-aptamers, while polypyrrole nanowires and carboxylated polymer microspheres enable covalent coupling of amino-modified aptamers on carbon screen-printed electrodes [39,41]. Split-aptamer and HCR constructs may be attached to AuNP-modified indium tin oxide (ITO) via Au-S bonds or to electropolymerized carboxylic copolymers via EDC/NHS, depending on the chosen transduction modality [35,40]. Broad surveys note that immobilization strategies also include electropolymerized copolymers, host-guest chemistries, and π-π adsorption on graphene derivatives, reflecting the wide toolbox available for surface design [36].

2.1.4. Signal strategies and amplification

Electrochemical readouts in E2 aptasensors fall into several principal families. Label-free impedimetric sensing monitors changes in electron-transfer resistance or capacitance after target binding, typically using the ferricyanide/ferrocyanide redox couple as the solution probe; such electrochemical impedance spectroscopy (EIS) assays have achieved sub-picomolar sensitivities in optimized configurations [35,38]. Redox-probe-mediated "signal-off" voltammetric assays use the same ferri/ferrocyanide couple: aptamer-target complexes block probe access and lower peak currents measured by differential pulse voltammetry (DPV) or square-wave voltammetry (SWV) [37,41].

Redox-tag strategies place an electroactive reporter directly on an oligonucleotide that participates in the assay. Methylene blue (MB) has been widely used both as a direct redox label on guanine-rich cDNA reporters and as a multifunctional probe that also sensitizes photoactive quantum dots in dual-mode PEC/EC assays; MB-based competitive and HCR-amplified schemes have produced fM-level analytical sensitivity in some reports [39,40]. Ratiometric dual-signal designs combine two electrochemical or photoelectrochemical currents (for example an MB redox current and a reference ferri/ferrocyanide signal) to normalize for environmental or device-to-device variability and improve quantitative reliability [40].

Enzyme and nanoparticle amplifications further enhance sensitivity. Glucose oxidase and DNase I cycling have been used to convert single binding events into amplified electron-transfer signatures or catalytic turnovers, while in situ deposition of nanoparticle redox probes such as nickel hexacyanoferrate (NiHCF) provides strong signal gains for DPV readouts [35,36]. Photoelectrochemical transduction that couples aptamer recognition to photocurrent from CdTe (Cadmium telluride) or hematite-based photoanodes achieves very low LODs when combined with nanostructured electrodes and sensitizers [35].

Representative performance metrics demonstrate how these strategies translate into analytical capability. A straightforward label-free, ferri/ferrocyanide-mediated SWV assay on a streptavidin-coated gold microelectrode reported a linear range of 0.01–1 nM and a practical detection limit of 0.1 nM [37]. Competitive hybridization using MB-labeled guanine-rich cDNA on CoS (Cobalt sulfide) nanosheets with electrodeposited AuNPs achieved a reported linear range down to 10ˆ−12 M and an LOD of 7.0 × 10ˆ−13 M, with successful analysis of diluted human urine [39]. Split-aptamer sandwich DPV sensors on carbon SPEs (Screen Printed Electrodes) reached a 4.8 × 10ˆ−13 M LOD and were validated in spiked lake water with HPLC comparison [41]. Platinum-enhanced photoelectrocemical/electrochemical ratiometric HCR systems combining MB and CdTe QDs (Quantum Dots) reported ultralow LODs (0.06 and 0.02 pg/ml for electrochemical and PEC ratios, respectively) and extensive water-sample validation [40]. Broad surveys report that reported LODs across electrochemical E2 aptasensors span approximately 10ˆ−15 to 10ˆ−8 M, depending on assay format, nanomaterial amplification and transduction mode [35,36].

2.1.5. Performance trends and validation in real matrices

A strength of the E2 aptasensor literature is the number of studies that include real-sample testing and orthogonal validation. Urine has been the most frequently reported biofluid for validation, often after substantial dilution: a CoS/AuNP DPV sensor analyzed urine diluted 100 × by standard addition with recoveries between 94.4% and 104.0% and relative standard deviations (RSDs) $<$3.4% [39], while a label-free EIS sensor using thiolated aptamers on Au reported successful detection in urine diluted 1000 × with recoveries near 92–101% across volunteers and a regeneration protocol enabling $\geq$10 cycles [38]. Environmental water matrices have likewise been tested: split-aptamer DPV sensors and ratiometric PEC/EC HCR sensors reported recoveries ≈97–102% in spiked lake-water samples, with HPLC (High-Performance Liquid Chromatography) or LC-MS/MS (Liquid Chromatography-Tandem Mass Spectrometry) as reference methods in those studies [40,41].

Reproducibility and short-term stability data are provided in several implementations: CoS/AuNP sensors reported intra-assay RSDs of 1.6–3.4% and retained ~95% of signal after one week at 4 °C [39], and ratiometric PEC/EC devices showed RSDs of ~2.5–3.8% for stability and reproducibility measures over seven days or across multiple sensors [40]. Reviews summarizing many studies emphasize that matrix effects, required dilutions, and the need for orthogonal confirmation (HPLC/LC-MS/MS) are recurring practical considerations when moving from buffer to biological or environmental samples [35,36].

2.1.6. Practical limitations and translational opportunities

Despite numerous reports of impressive analytical sensitivity, significant practical restrictions persist. To reach such strict detection limits, a multi-step surface modification, longer incubation phases, or nucleic-acid amplification steps are involved, contributing to longer analysis times and increased complexity. For example, specific thiolated-aptamer EIS sensors require up to 4 h of equilibration for binding experiments [38], and split-HCR protocols require a 120 min E2 incubation period to optimize MB adsorption [40]. Moreover, non-specific background signals can be suppressed by utilizing assembly protocols such as the formation of carboxyl-SAM, EDC/NHS activation, streptavidin coupling and blocking, at the expense of a more complex fabrication procedure as we as an increased reagent consumption [37].

The selectivity of these platforms is adequate when tested against structurally related steroidal compounds and common environmental interferents. Frequently examined interferents include estrone (E1), estriol (E3), bisphenol A (BPA), ethinylestradiol (EE2), progesterone (P4), naphthalene derivatives and 1-aminoanthraquinone, with many studies reporting insignificant cross-reactivity at 10- to 100-fold excess

Table 1
Aptamer-based biosensors.

Aptamer sequence & modification	Linear range (M)	LOD (M)	Selectivity panel	Stability	Matrix / validation	Ref
5′-Biotin-GCT TCC AGC TTA TTG AAT TAC ACG CAG AGG GTA GCG GCT CTG CGC ATT CAA TTG CTG CGC GCT GAA GCG CGG AAG C-3′	1.0×10^{-11} - 1.0×10^{-9}	1.0×10^{-10}	1-aminoanthraquinone; 2-methoxynaphthalene	Not reported	Buffer only	[37]
5′-SH-GCT TCC AGC TTA TTG AAT TAC ACG CAG AGG GTA GCG GCT CTG CGC ATT CAA TTG CTG CGC GCT GAA GCG CGG AAG C-3′	1.0×10^{-11} - 1.0×10^{-8}	2.0×10^{-12}	1-aminoanthraquinone; 2-methoxynaphthalene	Reusable ≥10 cycles	Human urine (diluted 1000 ×)	[38]
5′-SH-GCT TCC AGC TTA TTG AAT TAC ACG CAG AGG GTA GCG GCT CTG CGC ATT CAA TTG CTG CGC GCT GAA GCG CGG AAG C-3′	1.0×10^{-14} - 1.0×10^{-9}	5.0×10^{-15}	Estriol; Bisphenol A; Nonylphenol; Diethyl phthalate; Resorcinol; Atrazine	Regenerable with 50 mM EDTA	River water	[42]
5′-SH-(CH2)6-TTT T GCT TCC AGC TTA TTG AAT TAC ACG CAG AGG GTA GCG GCT CTG CGC ATT CAA TTG CTG CGC GCT GAA GCG CGG AAG C-3′	5.0×10^{-13} - 5.0×10^{-9}	6.0×10^{-14}	Bisphenol A; naphthalene; 1-aminoanthraquinone	Retained 95.2% after 1 week at 4 °C	Human urine	[43]
5′-SH-(CH2)6-GCT TCC AGC TTA TTG AAT TAC ACG CAG AGG GTA GCG GCT CTG CGC ATT CAA TTG CTG CGC GCT GAA GCG CGG AAG C-3′	1.0×10^{-12} - 1.0×10^{-9}	7.0×10^{-13}	1-aminoanthraquinone; naphthalene; PCB; bisphenol A; phthalic acid ester; testosterone; cholesterol	Retained 94.8% after 1 week at 4 °C	Human urine	[39]
5′-NH2-(CH2)6-AT ACG AGC TTG TTC AAT ACG AAG GGA TGC CGT TTG GGC CCA AGT TCG GCA TAG TGT GGT GAT AGT AAG AGC AAT C-3′	1.0×10^{-15} - 1.0×10^{-6}	1.0×10^{-15}	Progesterone; Bisphenol A	Not reported	Tap water & human urine	[44]
Apt1: 5′-SH-(CH2)6-GCT TCC AGC TTA TTG AAT TAC ACG CAG AGG GT-3′ Apt2: 5′-AGC GGC TCT GCG CAT TCA ATT GCT GCG CGC TGA AGC GCG GAA GC-SH-3′	1.5×10^{-12} - 1.0×10^{-10}; 1.0×10^{-10} - 7.0×10^{-0}	5.0×10^{-13}	Estriol; Progesterone; Testosterone; DBP; BSA; Atrazine; BPA	Not reported	Tap water, Milk, Serum	[45]
5′-GCT TCC AGC TTA TTG AAT TAC ACG CAG AGG GTA GCG GCT CTG CGC ATT CAA TTG CTG CGC GCT GAA GCG CGG AAG C-3′	1.8×10^{-15} - 5.5×10^{-11}	5.9×10^{-16}	AA; UA; Glutamate; BPA; DES	96.0% retention after 2000 s continuous operation	Tap water & Milk	[46]
5′-SH-(CH2)6-GCT TCC AGC TTA TTG AAT TAC ACG CAG AGG GTA GCG GCT CTG CGC ATT CAA TTG CTG CGC GCT GAA GCG CGG AAG C-3′	1.0×10^{-13} - 2.0×10^{-10}	5.3×10^{-14}	Ethinylestradiol; Estriol; 4-nonylphenol; Atrazine; BPA; Diethyl phthalate	~95% retention after 7 days at 4 °C	Wastewater	[47]
Apt1: 5′-AAG GGA TGC CGT TTG GG-3′ Apt2: 5′-CCC AAG TTC GGC ATA GTG-3′	3.7×10^{-14} - 1.8×10^{-9}	2.3×10^{-14}	Estriol; Bisphenol A; Diethylstilbestrol	Stable over 7 days (RSD 0.8%)	Pond water	[48]

concentrations [37,39,41]. Regeneration and reuse are achievable in some label-free EIS formats (e.g., EDTA rinsing for at least 10 reuse cycles) but not universally reported across sensor type0s [38].

There is considerable scope for further translational development of these technologies. The incorporation of ratiometric dual-mode sensing and split aptamer-based constructs offers considerable resilience against changes in sample matrix composition and device stability, thereby making them more appropriate for low-volume single-use devices [40,41]. Furthermore, as part of an effort to develop ultra-high sensitivity assays, it is possible to propel the LODs to sub-picomolar levels using various combinations of nanomaterial-based surface area enhancement and signal amplification cascades based on enzyme and nucleic acid-based signal generation [35,36]. Finally, as part of validating these biosensors, it is evident that rigorous sample preparation, including appropriate sample dilution and reference-based methods, offers considerable scope to validate aptamer-based E2 electrochemical biosensors in matrices such as urine and environmental water samples [38–40].

One additional limitation that should be noted about aptamer sensors is that their performance is often more sensitive to experimental conditions than the reported analytical figures may initially suggest. Since target recognition depends on the aptamer maintaining its correct three-dimensional structure, even relatively small changes in pH, ionic strength, buffer composition, or sample matrix can affect folding, binding efficiency, and ultimately the measured signal. This is particularly important in real samples, where salts, organic matter, proteins, and other coexisting species may disturb interfacial conditions and reduce assay robustness. For this reason, beyond achieving low detection limits, greater emphasis should also be placed on stability, reproducibility, and reliable performance under analytically relevant conditions.

A brief overview of selected aptamer-based electrochemical E2 biosensors is provided in Table 1, which includes details of their assay configuration and essential analytical metrics.

2.2. Electrochemical immunosensors for E2

2.2.1. Introduction

Electrochemical immunosensors, which are intended for the detection of E2, utilize the specificity of the interaction between antibodies and antigens over a wide range of electroanalytical techniques. Literature indicates the majority of the work has been focused on the detection of small molecules, particularly with competitive formats. These immunosensors utilize a wide range of signal transduction mechanisms, which include traditional enzymatic, nanoparticle-based quenching, and label-free capacitive methods. In addition, researchers strategically utilize various electrode materials and immobilization chemistries, to achieve the necessary balance sensitivity, robustness and real-world applicability. The following sections synthesize these approaches with particular attention to assay design, transduction strategies, electrode construction and reported analytical performance.

2.2.2. Why immunoassays dominate for small molecules? competitive formats

Small molecules such as E2 lack the two distinct epitopes typically required for conventional sandwich immunoassays; as a result, competitive formats predominate in the electrochemical literature for E2. Many groups implemented direct competitive assays in which sample E2 competes with a labelled hapten or labelled antibody for limited capture sites on the electrode-bound recognition element, permitting a concentration-dependent inverse signal suitable for low-molecular-weight analytes [49–57]. Variants on the competitive

Fig. 4. Schematic representation of an E2 electrochemical immunosensor (reproduced from [58] with permission).

theme include directly labelled small-molecule conjugates (e.g., enzyme-labelled E2 or hapten-protein conjugates), labelled nanoparticle haptens, and competition schemes that exploit enzymatic co-substrate competition in solution rather than a labelled antigen on the electrode [49,50,54,57,58]. These implementations illustrate how competition architectures remain the practical default for electrochemical immunosensing of E2. Fig. 4 schematically shows the working principle of an electrochemical immunosensor for E2, in which antibody recognition is translated into a measurable signal through HRP-based electrochemical detection.

2.2.3. *Labelled versus label-free immunosensors: signal strategies*

A broad palette of signal strategies has been demonstrated. Enzyme labels (primarily alkaline phosphatase (ALP) and horseradish peroxidase (HRP)) are widely used to convert electroinactive substrates into electroactive products for amperometric or voltammetric measurement; examples include ALP-labelled reagents with p-aminophenyl phosphate → p-aminophenol detection by amperometry [49], HRP-estradiol conjugates detected via H_2O_2/HQ catalysis and DPV or amperometry [51,53,54,58]. Enzymatic formats have delivered sub-pg/ml sensitivity in many cases, for example LODs in the low-pg/ml range for competitive HRP or ALP assays on disposable electrodes [49,54,56,58].

Parallel with enzymatic detection, nanoparticle labels and hybrid materials have been employed to push LODs and introduce alternative transduction mechanisms. Nanoparticle-based haptens (e.g., Polyaniline (PANI)@AuNPs-BSA-E2) have been used as labelled competitors that act as efficient quenchers in an ECL system, providing extremely wide dynamic ranges and ultralow LODs (reported femtogram-per-mL sensitivities) when combined with cathodic ECL emitters[50]. Metal-ion-loaded nanoparticle labels paired with stripping voltammetry provide another route to ultralow detection limits by converting a biorecognition event into distinct metal stripping peaks; this approach enabled detection limits down to the 10–2 pg/ml level for E2 using Pb^{2+}/Cd^{2+}-loaded Fe_3O_4 labels with Square-Wave Anodic Stripping Voltammetry (SW-ASV) readout [59]. Mixed signal modalities have also been explored: ratiometric and dual-mode sensors combining an internal metal reference (Cd^{2+}) with Metal-Organic Framework-based (MOF) catalytic reporting of a labelled hapten were demonstrated to improve robustness and provide complementary DPV and chronoamperometric outputs [57].

Label-free electrochemical immunosensors, principally non-faradaic capacitive/EIS platforms, have been developed as well, offering very rapid analysis without the need for labelled reagents. Two examples used covalent antibody immobilization on miniaturized wire electrodes or ZnO-nanostructured Ag wires to achieve low-pg and even sub-pg LODs by measuring antigen-induced changes in interfacial capacitance or impedance; one reported an LOD of 1 pg/ml and rapid 10-minute assays in packaged water [60], while another achieved reported femtogram-per-mL LODs using ZnO nanorods with capacitive EIS readout [61]. These label-free formats eliminate the need for labels, but instead demand strict control over surface chemistry and noise control.

2.2.4. *Electrode platforms and immobilization approaches*

The selection of electrode materials includes screen-printed carbon electrodes (SPCEs), glassy carbon electrodes (GCEs), polycrystalline gold discs or chips, and low-profile wire electrodes (Ag wires). To expand the sensing areas and also improve electron transfer efficiency, these base platforms are frequently functionalized with nanoscale modifiers. Disposable printed carbon platforms were used in multiple enzyme-labelled competitive assays, with passive adsorption of coating antigens or capture antibodies to yield convenient, low-cost sensors suitable for water and serum testing [49,54–56]. Glassy carbon electrodes served as a basis for more heavily modified surfaces employing POM-ZrO2 ECL emitters or MXene/polydopamine/Au composites for antibody immobilization and internal referencing [50,57]. Gold electrodes and electrodeposited AuNPs were used to exploit thiol-chemistry and orientation control of immobilized antibodies through Protein G or thiolated Protein G scaffolds [52,53,58].

Immobilization chemistries reflect a trade-off between simplicity and oriented, stable attachment. Passive adsorption of coating antigen or antibody is common on disposable carbon platforms for ease of fabrication and acceptable stability in short-term assays [49,55]. Covalent grafting strategies, electrochemical grafting of p-aminobenzoic acid followed by streptavidin-biotin capture [54], propanethiol SAMs with Cu underpotential deposition (UPD) and Dithiobis(succinimidyl propionate) (DTBP)-thiolated Protein G scaffolds [52], and cysteamine SAMs with AuNPs for antibody chemisorption [58], were employed to improve orientation and reduce non-specific binding, often improving reproducibility and enabling reuse. Nanostructured supports such as graphene-polyaniline composites, Polyamidoamine (PAMAM)-Au, Ti_3C_2 MXene, ZnO nanorods, and AuNPs have been introduced both to increase the electroactive surface and to provide chemical handles for

Table 2
Immunosensors.

Biorecognition Element	Label / amplification	Linear range (M)	LOD (M)	Selectivity panel	Stability	Matrix / validation	Ref
Mouse anti-estradiol mAb (2F9) immobilized via rabbit anti-mouse IgG	ALP-E2 conjugate	9.2 × 10^−11 - 1.8 × 10^−9	1.8 × 10^−10	Not reported	Not reported	Extracted human serum	[55]
Monoclonal anti-estradiol Ab (m-E2)	Alkaline phosphatase (AP) label (direct-labelled antibody; p-APP substrate)	9.0 × 10^−12 - 9.2 × 10^−9	9.2 × 10^−13	17α-estradiol, estrone, estriol, progesterone	21 days at 4 °C (≈75% signal retained)	Buffer, distilled water, river water, tap water	[49]
Rabbit anti-estradiol Ab (Polyclonal)	Estradiol-alkaline phosphatase (AP) conjugate	1.1 × 10^−10 - 5.5 × 10^−10	5.5 × 10^−11	Testosterone, methyltestosterone, progesterone	8 days at 4 °C (with preservative)	Non-extracted bovine serum	[56]
Monoclonal anti-estradiol Ab	HRP-labeled estradiol	9.2 × 10^−11 - 5.5 × 10^−9	2.2 × 10^−11	Not reported	Signal decreased 44% after 2 weeks at 4 °C	Spiked human blood serum	[53]
Monoclonal mouse anti-estradiol Ab	Estradiol-BSA conjugate	7.3 × 10^−11 - 8.1 × 10^−9	4.4 × 10^−11	17-ethynylestradiol, progesterone, testosterone	Not reported	Tap water, river water (spiked)	[52]
Biotinylated monoclonal anti-estradiol Ab (mouse)	HRP-labeled estradiol	3.7 × 10^−12 - 9.2 × 10^−10	2.8 × 10^−12	Progesterone, testosterone, cortisol, estrone	~9 days at 8 °C (humid)	Human serum, urine	[54]
Anti-estradiol Ab (monoclonal)	HRP-GO-Ab conjugate (competitive immunoassay)	1.5 × 10^−10 - 2.6 × 10^−8	7.3 × 10^−11	1-aminoanthraquinone, 2-methoxynaphthalene	83% retained after 28 days at 4 °C	Water, milk	[51]
Anti-estradiol Ab (monoclonal)	Cu2+-labeled antigen (E2-BSA-Cu)	1.8 × 10^−13 - 3.7 × 10^−7	5.5 × 10^−14	Diethylstilbestrol (simultaneous), progesterone, estriol	Good stability over repeated measurements	Water, milk	[59]
Anti-estradiol Ab (monoclonal)	Unlabeled antigen / unlabeled antibody (label-free HRP-H2Q-H2O2 co-substrate system)	1.98 × 10^−15 - 5.00 × 10^−11	3.1 × 10^−15	Progesterone, estrone, estriol	Good repeatability; electrode reusable ~100 cycles	Bovine serum	[58]
Anti-17β-estradiol Ab (monoclonal)	None (label-free capacitive immunosensor)	1.8 × 10^−14 - 3.7 × 10^−12	3.7 × 10^−15	Stigmasterol (18%), BPA	~70% response retained after 14 days at 4 °C	Packaged drinking water	[60]
Anti-estradiol Ab (monoclonal)	Label-free (THI redox mediator on MWCNTs/AuNPs)	3.7 × 10^−14 - 3.7 × 10^−10	3.7 × 10^−14	FSH, LH, Glu, AA, UA, NSE, CEA	Good stability; repeatability CV = 2.82%	Clinical serum	[62]
Anti-17β-estradiol Ab (monoclonal)	None (label-free capacitive ZnO-nanorod immunosensor)	3.7 × 10^−14 - 7.3 × 10^−10	3.7 × 10^−14	Not reported	Stable; %RSD = 4.35	Buffer	[61]
Anti-estradiol Ab (monoclonal)	PANI@AuNPs-BSA-E2 quencher (dual-mechanism ECL quenching: energy + electron transfer)	3.7 × 10^−17 - 7.3 × 10^−7	3.7 × 10^−18	DES, estriol, estrone, EE, BPA, DDT, TCDD	>90% ECL retained after 7 days	Lake water, milk	[50]
Anti-estradiol Ab (monoclonal)	Cd2+ (reference) + Cu-MOF-E2-BSA (indicator); ratiometric DPV and i-t dual-mode	3.7 × 10^−15 - 3.7 × 10^−8	1.7 × 10^−15	Nonylphenol, estrone, DES, BPA, glucose, uric acid	Good operational stability (ratiometric RSD = 3.2%)	Lake water, tap water	[57]

antibody attachment or mediator incorporation [50–52,57,61].

2.2.5. *Performance trends and validation in real matrices*

Reported analytical performance spans many orders of magnitude in sensitivity and dynamic range depending on label strategy and transduction. Enzyme-amplified competitive assays on disposable screens and modified electrodes routinely achieved LODs in the sub-pg to low-pg/ml range; examples include an ALP-labelled screen-printed competitive assay with LOD 0.25 pg/ml and robust recovery in river and tap water [49], and an HRP-based SPCE platform with LOD 0.77 pg/ml validated in serum and urine [54]. Label-free capacitive EIS designs reported comparably low LODs and very short analysis times: 1 pg/ml (10 min assay) for an 11-mercaptoundecanoic acid (11-MUA) SAM Ag-wire capacitive sensor [60] and a fg-level LOD for a ZnO-nanorod EIS sensor [61]. Ultrahigh sensitivity into the fg/ml regime was demonstrated with nanoparticle-based ECL quenching (LOD 3.7 fg/ml) and metal-ion stripping approaches (LOD 0.015 pg/ml for E2) when combined with carefully engineered transduction schemes [50,59]. Ratiometric and dual-mode approaches (e.g., Cd^{2+} internal reference with Cu-MOF label) were used to enhance reliability and provide orthogonal readouts, achieving low-pg/ml LODs and successful recovery in lake water, milk and serum [57].

Validation in complex matrices is a recurring focus. Several studies performed recovery experiments in environmental waters, milk or serum and reported recoveries generally within 85–106% and acceptable RSDs, demonstrating practical applicability after minimal sample preparation in many cases [49,51,53,54,57,59]. Comparisons with reference methods were also reported: a disposable SPE alkaline phosphatase (AP)-assay showed agreement with LC-APCI-MS/MS for bovine serum analysis[56], and a paper-based label-free immunosensor was compared to a commercial ECL system with modest relative deviations [62].

2.2.6. *Practical limitations and opportunities*

The reviewed literature emphasizes various practical considerations that govern sensor design. For example, in terms of enzyme assays, although there is considerable signal amplification with enzyme assays, there is gradual deterioration of enzyme activity. For example, in one study involving flow cell analysis of HRP activity, it was observed that there were considerable losses in HRP activity over two weeks, indicating limitations in terms of stability without cold storage and stabilizers [53]. Alternatively, disposable coated electrodes offer variable shelf lives, with one SPCE format retaining ≈75% signal after 21 days in refrigerated storage, though longevity remains dependent on storage conditions and surface chemistry [49]. In terms of cost considerations for individual tests, reusable sensors with robust regeneration protocols

would be more practical. For example, in one study involving an AuNP-cysteamine SAM sensor, it was observed that the sensor retained its analytical performance over 100 reuse cycles [58].

Practical considerations in terms of utility of assays are heavily dependent upon assay time and complexity. Although in terms of label-free assays involving capacitance measurements, assays can be performed within minutes [60,61], in terms of assays involving more complex protocols such as nanoparticle conjugation and MOF labeling and overnight incubation, overall assay times may be compromised [50, 51,57]. For point of care devices, the cost of the assay still plays an important role, although optimized paper-based microfluidic devices have shown the possibility of developing such devices for as low as $0.30 per device [62].

Looking to the future, there are several possibilities for optimization of the sensor design. Ratiometric internal reference systems, combined with dual mode readouts, may improve robustness to matrix effects [57]. ECL and nanoparticle quenching may extend the dynamic range of the assay and push the sensitivity to the fg/ml range[50]. Moreover, further optimization of the methods for oriented immobilization of antibodies (providing scaffolds for protein G, thiolated protein G, streptavidin-biotin chemistries) may help to improve reproducibility and reduce non-specific responses [52–54]. Combination of such advanced strategies for stabilization and labeling of antibodies appears to be essential for low LODs, sample applicability, and reasonable assay throughput.

A summary of electrochemical immunosensors for E2 is given in Table 2, including the implementation of competitive assay designs, immobilization and labeling strategies, signal transduction mechanisms, and analytical metrics.

2.3. Molecularly imprinted polymer (MIP)-based electrochemical sensors for E2

2.3.1. The reasons for choosing MIPs in E2 detection

MIPs have been extensively explored for use in synthetic recognition for E2 detection, owing to their high selectivity and robustness under electrochemical detection. Various studies have utilized electropolymerized MIPs for small-molecule E2 detection by using a thin, conformal recognition layer directly on the electrode surface, which enhances fast electron transfer and integration with voltammetry-based detection schemes [63–66]. Other studies have utilized bulk-based MIPs, such as sol-gel films, magnetic core-shell MIPs, and polymer nanoparticles, for shape- and functionality-specific recognition, allowing for a wide range of electrode configurations, from GCEs to SPEs to CPEs [67–70]. As such, from the literature above, it is evident that the use of MIPs for small-molecule E2 detection is based upon their high selectivity for small-molecule E2 detection and their robustness for continuous use under moderate chemical and thermal stress [65,67].

2.3.2. MIP fabrication strategies

To address diverse practical requirements, the surveyed studies employ a spectrum of polymerization methodologies. Specifically, these include electropolymerization for surface-confined recognition, thermal- or UV-initiated bulk polymerizations for the synthesis of particulate MIPs, and sol-gel techniques designed to yield rigid, inorganic-organic hybrid layers.

Electropolymerized films: Multiple groups electropolymerized conducting monomers directly onto electrodes to form MIP films. Polypyrrole electropolymerization by cyclic CV was used to imprint E2 on a screen-printed gold electrode (SPGE), producing a polypyrrole MIP film that supported linear sweep voltammetry detection of E2 [63]. Similarly, Bai et al. used CV to electropolymerize pyrrole onto a GCE modified with a CuxO/flower-like MXene composite, forming a surface MIP for DPV detection [64]. Electropolymerized poly-imidazole MIP layers have also been deposited on graphene oxide (GO)/Ag nanoparticle-modified GCEs for SWV-based sensing with fM sensitivity [65]. The electropolymerization route enables thin, adherent films amenable to rapid template removal and direct electrochemical interrogation [63–65].

Thermal and UV-initiated bulk polymerizations and surface imprinting: Thermal free-radical polymerization yielded MIP particles used in carbon paste electrodes (CPEs); for example, thermal polymerization with 4,4′-Azobis(4-cyanopentanoic acid) (ACPA) initiator produced MIP particles for incorporation into carbon paste-based sensors that were read by adsorptive stripping voltammetry [68]. Surface imprinting on magnetic Fe_3O_4@SiO_2 cores via thermal polymerization generated Magnetic Molecularly Imprinted Polymer (MagMIP) particles that were drop-cast into CPE matrices for Differential Pulse Adsorptive Stripping Voltammetry (DPAdSV) detection of E2 [69]. UV-initiated solution/suspension polymerization (2,2′-Azobis(2-methylpropionitrile)initiation) was used to cast methacrylic acid (MAA)/ ethylene glycol dimethacrylate (EGDMA) MIP films on GCEs for broad-range electrochemical detection in serum [71].

Sol-gel approaches and hybrid films: Sol-gel chemistry was applied to create rigid MIP films for E1 sensing, where phenyltrimethoxysilane and methyltrimethoxysilane served as functional monomers and Tetraethyl orthosilicate (TEOS) as crosslinker; these films were compatible with ECL assays because of the stable immobilization of tris(2,2′-bipyridine) ruthenium(II) on a Nafion/Multi-walled carbon nanotubes (MWCNT)-COOH platform beneath the sol-gel layer. Sol-gel MIPs thus offer thermal/chemical robustness and an inorganic network that can stabilize embedded reporters and improve film durability [67].

Template removal procedures vary with polymer type: methanol/ acetic acid washes were used for magnetic surface-imprinted MIPs[69], hot water rinsing for a sol-gel E1 MIP [67], and electrochemical monitoring was reported during template removal in at least one thermal-bulk MIP study[68]. Some electropolymerized systems relied on CV-driven extraction or solvent washes to expose binding sites [63, 66].

2.3.3. Conductive composites and nanomaterial-enhanced MIPs

A recurring theme across the reports is the deployment of conductive nanomaterials and composites to enhance transduction, increase electroactive surface area, and provide internal reference signals.

MXene and mixed-valent metal oxides as ratiometric platforms: Bai et al. anchored a mixed-valent Cu_xO/Cu_2O—CuO composite onto flower-like Ti_3C_2Tx MXene on a GCE to create a dual-peak ratiometric sensor in which the Cu_xO signal provides an internal reference (ICu_xO) while the MIP-modulated E2 signal (IE2) is measured by DPV; the ratiometric $IE2/ICu_xO$ strategy delivered both high sensitivity and improved stability against signal drift [64].

Carbon nanomaterials and metallic nanoparticles: Graphene oxide combined with electrodeposited Ag nanoparticles supported an electropolymerized poly-imidazole MIP that gave fM detection limits and a wide linear range when interrogated by SWV [65]. Gold-functionalized carboxylated multi-walled carbon nanotubes (Au@MWCNT-COOH) were used as a conductive scaffold beneath an electropolymerized molecularly imprinted membrane on GCE, supporting SWV quantification of E2 in complex matrices [66]MWCNT-COOH and Nafion were used to immobilize Ru(bpy)3 for an ECL sol-gel MIP sensor for estrone, with MWCNT-COOH contributing both conductivity and increased binding site density [67].

Nanoparticle MIPs and composite pastes: Magnetic Fe_3O_4@SiO_2 cores enabled facile separation and concentration when incorporated into carbon paste electrodes as MagMIP-modified CPEs [69]. Surfactant-free emulsion polymerization produced MIP nanoparticles grafted to MWCNT-GMA that were incorporated into Nafion-coated SPCEs for agricultural wastewater analysis [70]. Carbon black inclusion in a carbon paste matrix (CPE-MIP-CB) was exploited to increase active surface area for adsorptive stripping voltammetry detection of E2 [68].

These material strategies collectively demonstrate how conductive

Table 3
MIP-based biosensors.

Polymerization approach	Functional monomer (s) / crosslinker	Extraction / rebinding conditions	Matrix	Linear range (M)	LOD (M)	Selectivity	Stability	Ref
Electropolymerization	Imidazole (monomer) + GO + AgNP (nanocomposite modifiers)	Extraction: PBS: acetone (4:1), 12 min; Rebinding: 9 min	River water (spiked)	1.0 × 10ˆ−14 - 2.5 × 10ˆ−7	3.01 × 10ˆ−15	Phenol, catechol, cholesterol, hydroquinone	~90% retained after 25 days; 89.65% after 45 days	[65]
Bulk / Surface Imprinting	Methacrylic acid (MAA) / Ethylene glycol dimethacrylate (EGDMA)	Extraction: Methanol/Acetic acid (9:1), Soxhlet; Rebinding: 10 min	River water (spiked)	5.0 × 10ˆ−7 - 1.4 × 10ˆ−5	1.3 × 10ˆ−7	Estrone, hydroquinone, carbendazim	Repeatability RSD 3.2%	[69]
Bulk polymerization MIP particles used in carbon paste electrode	Methacrylic acid (MAA) / Ethylene glycol dimethacrylate (EGDMA)	Extraction: MeOH/ AcOH (9:1) + MeOH + water; Rebinding: not specified as a timed step	River water (spiked)	51.0 × 10ˆ−7 - 2.3 × 10ˆ−5	3.0 × 10ˆ−8	Hydroquinone, uric acid, carbendazim; EE2	River water spikes (triplicate): RSD 0.10–0.18; recovery 103–105%	[68]
Emulsion Polymerization	N-methacryloyl-L-cysteine (MAC) / HEMA / EGDMA	Extraction: 1 M NaCl; Rebind: Flow conditions	Artificial urine & serum	1.0 × 10ˆ−11 - 3.0 × 10ˆ−9	2.3 × 10ˆ−10	Cholesterol, stigmasterol, estradiol hetero-forms	Reusable up to 4 times	[70]
Electropolymerization	L-arginine (L-Arg) / no crosslinker	Extraction: Ethanol/Acetic acid (4:1), 8 min; Rebinding: 150 s	Water, milk, pork, human serum	1.0 × 10ˆ−10 - 1.0 × 10ˆ−6	3.33 × 10ˆ−11	Estrone, estriol, DES, dopamine, vitamin C, glucose, uric acid, Na+, K+	~90% retained after 7 days	[66]
Electropolymerization	Pyrrole (Monomer)	Extraction: Methanol/Acetic acid (9:1); Rebinding: 15 min	Tap water, urine, milk	1.0 × 10ˆ−11 - 5.0 × 10ˆ−8	4.0 × 10ˆ−12	Progesterone, bisphenol A, cortisol	Retained 94.5% after 30 days	[71]
Solution / Suspension Polymerization (drop-cast MIP film)	Methacrylic acid (MAA) / Ethylene glycol dimethacrylate (EGDMA)	Extraction: Methanol washing; Rebinding: Not specified	Human serum	1.0 × 10ˆ−9 - 1.0 × 10ˆ−4	1.0 × 10ˆ−10	Bisphenol A, progesterone	~98% reproducibility; stable for ~4–5 days	[64]

and nanostructured supports can amplify MIP-based recognition into robust electrochemical signals across voltammetric and ECL readouts [64,65,67]

2.3.4. Analytical performance trends and validation in real samples

MIP-based electrochemical sensors for E2 span many orders of magnitude in sensitivity and have been validated in diverse matrices.

Sensitivity and dynamic range: Reported LODs vary dramatically depending on the transduction chemistry and nanomaterial enhancements. The most sensitive electrochemical MIP system among the surveyed works was an electropolymerized poly-imidazole MIP on GO-AgNP-modified GCE with an LOD of 3.01 fM and a linear range of 10 fM to 250 nM [65]. He et al. reported an electropolymerized L-arginine molecularly imprinted membrane (MIM) on Au@MWCNT-COOH/GCE with a linear range from 1 × 100 to 1 × 10ˆ−6 M and a low LOD of 3.33 × 10ˆ−11 M [66]. On the other end, bulk and particle-based MIPs incorporated into paste electrodes produced micromolar LODs: MagMIP-modified CPE had an LOD of 0.13 μM [69], while carbon paste MIP-carbon black electrodes achieved an LOD of 0.03 mmol/L (30 μM) in a DPAdSV assay[68]. Electropolymerized polypyrrole on SPGE gave an intermediate performance with LOD 0.00836 ppm (≈8.36 μg/L) and linear range 0.5–10.0 ppm [63]. A ratiometric MXene/Cu_xO-supported polypyrrole MIP achieved a low-picomolar LOD (6.9 pM) with linearity from 20 pM to 10 nM [64]. UV-initiated MAA/EGDMA MIP films reported an LOD of 0.1 nM with an extremely wide linear range (0.001–100 μM) when applied to serum [71].

Validation and recoveries in real matrices: Several studies validated sensors in environmental and biological samples with acceptable recoveries. Bai et al. validated their MXene-Cu_xO MIP sensor in artificial urine, real female urine, whole milk, and fetal bovine serum with recoveries from 92.8% to 102.6% and RSDs below 4.45% [64]. He et al. applied an electropolymerized MIM to spiked water, milk, pork and human serum with recoveries of 96.6–111.1% and RSDs ≈4.7–8.6% [66]. Sol-gel MIP ECL work targeted E1 and was validated against river water and LC-MS/MS, showing strong agreement (relative deviations 0.1–4.1%) [67]. Magnetic-MIP CPEs and nanoparticle-MIP SPCEs were applied to river water and agricultural wastewater, respectively, with recoveries around 96–105% [69,70]. The carbon paste MIP detector demonstrated spiked river water recoveries of 103–105% at high concentrations used in the study [68].

Selectivity assessment: Selectivity tests vary in scope. Bai et al. conducted a comparatively broad anti-interference evaluation including structural analogues diethylstilbestrol (DES) and common small interferences (Bisphenol A-BPA, Bisphenol S-BPS) and ions, showing robust selectivity at 10 nM competitor concentrations against 1 nM E2 [64]. Regasa et al. evaluated common phenolic and steroidal interferents and reported good discrimination down to the fM target range [65]. Some particle-based MIPs show notable cross-reactivity: the MagMIP CPE responded strongly to E1 (95.7%) of E2 response [69], while thermal-bulk MIP particles exhibited high response to EE2 (97.3%) [68]. Electropolymerized polypyrrole on SPGE reported selectivity against testosterone in the tested conditions [63]

2.3.5. Practical issues: template leakage, fouling, regeneration, reproducibility

Practical deployment of MIP-based sensors raises recurring concerns that are addressed to varying degrees in the studies.

Template removal and leakage: Template extraction procedures were explicitly reported in some works, with solvent washing (methanol/acetic acid) for MagMIP particlesv [69], hot water rinsing for sol-gel films [67], and electrochemical/solvent procedures for electropolymerized films [63,68]. However, not all studies reported detailed removal protocols, and none of the summaries included a quantitative evaluation of residual template leakage over time, leaving this as an open operational consideration [63,71].

Fouling and regeneration: Several electrode architectures permit simple regeneration. Carbon paste and SPEs with particle/MIP layers can be renewed by polishing or replacing the paste; da Silva et al.

Table 4
Electrode platforms and measurement techniques.

Recognition type	Electrode / Substrate	Nanomaterials / dopants	Immobilization strategy	Electrochemical technique	Ref
Aptamer	Au	—	Biotin-Streptavidin Affinity (non-covalent)	SWV, CV	[37]
Aptamer	GCE	Au, CoS	Au-S Interaction (covalent)	DPV, EIS	[39]
Aptamer	Au	—	Au-S Interaction (covalent)	EIS	[38]
Aptamer	BDD	Au	Au-S interaction (covalent)	EIS	[42]
Aptamer	GSE	CuS, Au	Au-S interaction (covalent)	CV, DPV	[43]
Aptamer	GSE	—	EDC/NHS (covalent)	EIS	[44]
Aptamer	Au	—	Au-S interaction (covalent)	CV, DPV	[45]
Aptamer	ITO; Bioanode: CNC/AuNP/ITO Biocathode: CNC/AuNP/ITO	CNCs, AuNPs, PMNPs	Drop-casting	CV, EIS, E_OCV	[46]
Aptamer	Au	AuNPs	DNA Hybridization	EIS, CV, DPV	[47]
Aptamer	LIG	Au, ZIF-8	Au-S interaction (covalent)	EIS, CV, SWV	[48]
Immunosensor	SPCE	—	Passive adsorption	CV	[49]
Immunosensor	GCE	AuNPs, PANI	Adsorption	ECL, EIS, CV	[50]
Immunosensor	GCE	GO, Graphene, PANI	Covalent	DPV, EIS	[51]
Immunosensor	Au	AuNPs	Affinity/SAM	Amperometry, CV, SWV	[53]
Immunosensor	Au	—	Affinity/SAM	SWV	[52]
Other	SPCE	rGO, GO, Graphene	Adsorption	SWV, CV	[75]
Immunosensor	Au	AuNPs	Adsorption	SWV, CV	[58]
Immunosensor	SPCE	—	Covalent	amperometry	[54]
Immunosensor	SPCE	—	Passive adsorption	DPV, CV	[55]
Immunosensor	Silver wire (diameter = 0.25 mm) pair, two-electrode setup; (7.85 mm2 effective surface area)	—	Covalent	EIS	[60]
Immunosensor	Silver (Ag) wire with ZnO nanorods (Ag-ZnONRs)	—	Covalent	EIS	[61]
Immunosensor	SPE	—	Passive adsorption	DPV	[56]
Immunosensor	SPCE	AuNPs,MWCNTs, THI	Adsorption	DPV, CV, ECL	[62]
Immunosensor	GCE; Au	MOF	Affinity/SAM	DPV, EIS, amperometry, chronoamperometry	[57]
Immunosensor	GCE	Graphene	π-π stacking	EIS	[59]
MIP	GCE	FL-Mxene, CuxO	Electropolymerization	DPV (ratiometric signal IE2/ICuxO), CV, EIS	[64]
MIP	Au; CPE	Carbon black	other	DPV	[68]
MIP	GCE	MWCNT, CNT, Au	Electropolymerization	SWV, CV, ECL	[66]
MIP	GCE	AgNPs, GO, Graphene	Electropolymerization	SWV, CV	[65]
MIP	CPE	—	Adsorption	Differential pulse adsorptive stripping voltammetry (DPAdSV), CV, EIS	[69]
MIP	GCE	—	Drop-casting	DPV, CV, amperometry	[71]
MIP	SPCE	MWCNT, Nafion	Adsorption	DPV, CV	[70]

specifically note the electrode surface can be renewed by polishing "more than ten times" without loss of performance [69]. Electropolymerized thin films can be electrochemically or solvent-cleaned to reuse electrodes; some electropolymerized sensors demonstrated multiple reuses (e.g., polypyrrole MIP reused at least seven times with modest performance loss) [63]. Conversely, some MIP layers show limited long-term mechanical stability: a UV-cast MIP layer began to deteriorate after four days of ambient storage in one report [71].

Stability and reproducibility: Storage stability and inter-electrode reproducibility were addressed in a number of works. Bai et al. reported 92.9% signal retention after 30 days for their MXene/Cu_xO MIP system [64]. Regasa et al. observed >90% performance retention after eight extraction/rebinding cycles and ~89–90% retention over multi-week storage windows has been reported in other studies [65,66]. Reported RSDs for validated matrices were generally acceptable: e.g., Bai et al. RSDs <4.45% across matrices and He et al. RSDs ≈4.7–8.6% across complex samples [64,66].

Table 3 provides an overview of MIP-based electrochemical E2 sensors, comparing imprinting/fabrication routes, electrode modifiers, measurement mode, and the resulting sensitivity/selectivity and real-sample applicability.

3. Electrode platforms and nanomaterials engineering

3.1. Scope and chapter roadmap

This chapter surveys electrode substrates and nanomaterials engineering leveraged in electrochemical biosensing of E2, emphasizing platform classes, materials roles, interfacial chemistries, antifouling/matrix strategies, and practical fabrication guidance for robust sensing in complex samples [72].

We integrate a generic platform/nanomaterial taxonomy (e.g., SPEs on plastic and paper, lithographic Au chips/disks, glassy carbon, ITO, flexible/wearable and miniaturized wire formats, carbon nanomaterials, noble metals, 2D materials such as MXenes/graphene, and MOF hybrids) with E2-specific case studies compiled in Table 3 to connect technology options with demonstrated implementations and performance [73].

The chapter proceeds from a consolidated landscape (Table 4) to platform-specific sections, a nanomaterials toolbox and mechanistic roles, interfacial immobilization routes, antifouling/matrix handling and stability, and finally practical engineering workflows and integration pathways to guide design choices [74]. A consolidated view of the common surface-engineering architecture used across E2 biosensors is presented in Fig. 5.

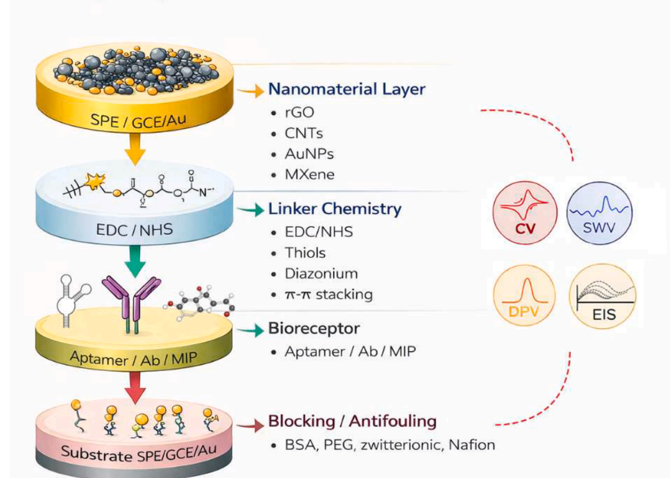

Fig. 5. Typical surface-engineering "stack" used to construct electrochemical biosensors for β-estradiol (E2) and the measurement formats used for readout. The schematic summarizes a common build-up sequence starting from the electrode substrate (e.g., SPE, GCE, Au), followed by a nanomaterial layer (e.g., rGO, CNTs, AuNPs, MXene) to enhance surface area and charge transfer, and linker/anchoring chemistry (e.g., EDC/NHS coupling, thiol self-assembly, diazonium grafting, π-π interactions) to enable stable functionalization. A biorecognition layer (aptamer, antibody, or MIP) is then introduced, and blocking/antifouling coatings (e.g., BSA, PEG, zwitterionic layers, Nafion) are applied to mitigate non-specific adsorption and improve performance in complex matrices. Subsequent evaluation of the resulting interface is typically achieved using CV for electrochemical characterization, alongside DPV/SWV/EIS for the measurement of analytical signals.

3.2. Consolidated landscape of E2 electrode platforms

To enable a side-by-side evaluation of platform designs and engineering trade-offs across immunosensors, aptasensors, and MIPs, Table 4 summarizes the electrode substrates, nanomaterial modifiers, stated roles, immobilization chemistries and the electrochemical measurement techniques, used in each surveyed E2 study.

This table allows readers to map substrate-modifier pairings such as SPCE with Eu_2O_3@rGO, Au chips with SAM/streptavidin aptamer scaffolds, GCE with graphene-polyaniline/PAMAM-Au and enzyme labels, and ITO with AuNP/DNA/QDs assemblies to their distinct analytical purpose. These roles include enhancing conductivity, increasing the active sensing surface area, providing stable anchoring for bioreceptor immobilization, generating internal reference signals, and promoting electrocatalytic processes under readouts spanning DPV, SWV, EIS, ECL, and PEC.

3.3. Screen-printed and paper-based platforms

SPEs, encompassing SPCEs and SPGEs (Screen Printed Gold Electrodes), interdigitated microelectrodes (SP-IDMEs), multi-working-electrode arrays, paper-microfluidic integrations, and flexible/wearable variants, rely on proprietary manufacturer parameters for ink composition and curing to ensure batch reproducibility [73].

Paper-based platforms utilize cellulose substrates combined with screen-printed, laser-scribed, or pencil-drawn carbon tracks. These can be integrated into foldable origami or pop-up microfluidic designs, where capillary flow enables pump-free handling, making them suitable for portable use [74].

E2 studies on screen-printed and paper configurations span carbon SPCE immunosensors built by passive adsorption and blocking [49,55, 56], SPCEs modified with Eu_2O_3@rGO to catalyze E2 oxidation [75], SPCE aptasensors combining polypyrrole nanowires with EDC/NHS-activated polymer microspheres for covalent aptamer attachment [41], a SPGE with electropolymerized polypyrrole MIP [63], and paper-integrated screen-printed working electrodes modified with MWCNT/Thionine(THI)/AuNP nanocomposites for label-free immunodetection [62].

Pretreatments commonly include electrochemical activation of carbon SPEs and localized modification by drop-casting or electrodeposition to achieve reproducible electroactivity and spatially confined functionalization on disposable formats [73].

3.4. Lithographic Au chips, Au disks, and wire-based configurations

Gold microelectrode chips fabricated by sputtering/photolithography have been functionalized with carboxyl-terminated SAMs, EDC/NHS-coupled streptavidin, and biotinylated aptamers, with ethanolamine blocking and SWV/EIS readouts in ferro/ferricyanide mediators [37].

Au disk immunosensors employ AuNP scaffolds on thiolated interlayers (e.g., cysteamine SAM + chemisorbed AuNPs) to restore and enhance electron transfer and support antibody immobilization, with antifouling via protein blocking and regeneration in acidic media [53, 58].

Underpotentially deposited Cu monolayers stabilized by propanethiol on Au minimize nonspecific adsorption and provide strong affinity to thiolated Protein G scaffolds for oriented antibody presentation [52].

Miniaturized two-electrode wire sensors include Ag wires bearing 11-MUA SAMs with EDC/NHS antibody coupling for capacitive sensing, and Ag wires with ZnO nanorods modified by phosphonate SAMs for EIS/capacitive transduction in small volumes [60,61].

3.5. Glassy carbon and oxide/2D-modified electrodes

On glassy carbon electrodes, graphene-polyaniline and PAMAM-Au composites have been used as conductive transducers with carboxylated GO supporting high-loading HRP-GO-Ab catalytic labels, leveraging GO—COOH for enzyme/antibody conjugation [51].

Aptamer transducers on 2D cobalt sulfide nanosheets with electrodeposited AuNPs employ MCH backfilling and MB readouts to capitalize on increased electroactive area and Au-thiol immobilization [39].

MIP composites have been constructed by sequential AgNP deposition, GO electrodeposition, and electropolymerization of imidazole with E2 templating, followed by solvent elution to generate selective recognition at the GCE interface [65].

MXene-based 2D hybrids include Cd^{2+}/Au/pDA/Ti_3C_2 composites that serve both as supports and internal references paired with Cu-MOF labels for ratiometric DPV and chronoamperometry, and flower-like Ti_3C_2Tx MXene with Cu_xO nanoparticles beneath a pyrrole MIP layer for dual-peak ratiometric detection [57,64].

Metal-oxide ECL emitters have been drop-cast on GCE and paired with PANI@AuNP quenchers conjugated via EDC/NHS to assemble dual-mechanism quenching immunosensors [50].

3.6. Carbon paste and bulk-modified electrodes

Bulk-modified carbon paste electrodes incorporate both MIPs and carbon black into the paste (CPE-MIP-CB), with modifier loadings tuned to balance charge-transfer resistance against access to imprinted cavities [68]

Magnetic MagMIP-CPEs prepared by mixing Fe_3O_4@SiO_2-supported MIPs into the paste offer selective recognition and renewable surfaces by simple polishing [69].

Solution-synthesized MIPs can also be drop-cast as films on polished GCEs to provide straightforward imprinted recognition coatings [71].

3.7. Nanomaterials toolbox and mechanistic roles

Carbon nanomaterials including graphene/rGO, Carnob Nanotubes (CNTs), carbon black, and graphene quantum dots increase electroactive surface area, bioreceptor loading capacity, and electron-transfer kinetics across multiple electrode platforms [73].

Metallic nanostructures such as AuNPs, Ag, Pt and Pd provide conductive pathways, Au-thiol immobilization sites, and catalytic enhancement, while mediator nanoparticles like Prussian Blue enable low-potential operation particularly suited to disposable SPE and paper contexts [74].

2D materials including MXenes and graphene derivatives offer high conductivity, tunable surface terminations for immobilization, and mechanical flexibility compatible with microfluidic and wearable integrations [76].

Metal-organic frameworks and MOF-derived composites contribute high surface area for pre-concentration, porous binding environments, electrocatalytic centers, and stabilization of embedded nanoparticles against aggregation or dissolution [72].

Within the graphene family, rGO restores conductivity while oxygenated sites on GO and linker chemistries (e.g., 1-Pyrenebutyric Acid N-hydroxysuccinimide Ester (PBASE), EDC/NHS) support-controlled immobilization; blocking with BSA, Polyethylene Glycol (PEG), and Tween-20 reduces nonspecific adsorption in portable/readout systems [77].

3.8. Antifouling, matrix handling, stability, and reproducibility

Antifouling strategies span ternary SAMs/MCH passivation on Au, polydopamine coatings, BSA blocking, ethanolamine quenching of residual COOH, and PVA blocking on SPCEs to mitigate nonspecific adsorption in serum, urine, and water matrices [73].

Paper/origami microfluidics leverage capillary flow and reagent stacking to mitigate matrix effects without pumps, while mediator selection and device geometry allow low-potential operation to suppress interferents in portable formats [74].

MOF/carbon composites and MIP layers deliver selectivity but can introduce nonspecific adsorption or aqueous-stability considerations, and cross-matrix validation in serum, urine, and water is routinely reported to establish applicability [72].

Stability and reuse examples in Table 3 include disposable SPEs with short-term storage, regenerable Au-based immunosensors, reusable aptamer EIS electrodes, MIP interfaces capable of multiple elution/rebinding cycles, and multi-week storage figures for modified electrodes across EC/ECL/PEC modes.

3.9. Practical engineering guidance and integration pathways

Screen printing enables low-cost mass production of reproducible, single-use sensors, with ink formulation and curing conditions governing batch reproducibility and with printing best suited to flat substrates [73].

Paper-based engineering draws on laser scribing, inkjet/digital dispensing, stencil and 3D printing with distinct trade-offs in resolution and waste; capillary action removes the need for pumps and supports smartphone/mini-potentiostat connectivity for point-of-care and wearable deployments [74].

MXene films are readily formed by solution processing (drop-cast, spin-coat, spray), support EDC/NHS bioconjugation, and benefit from anti-oxidation stabilization via grafting/polymer coatings, with storage of delaminated colloids and vacuum-sealed devices recommended for stability [76].

Graphene workflows typically follow device cleaning, linker functionalization (PBASE, EDC/NHS, 3-Aminopropyltriethoxysilane (APTES)), and blocking (BSA/PEG/Tween-20), and are compatible with portable readouts and microfluidic integration [77].

Ratiometric and dual-mode designs, using internal references such as Cd^{2+} or Cu_xO, or combining electrochemical and photoelectrochemical signals on ITO/GCE, enhance reproducibility and matrix tolerance, as shown in Table 3 [40,64].

4. Electrochemical transduction

4.1. Readout formats in E2 sensing

In the reviewed E2 literature the electrochemical readout strategy dictates the assay architecture, reagent selection, and electrodes requirements. For example, label-free impedimetric and mediator-blocking voltammetric formats use outer-sphere redox probes (most commonly ferri/ferrocyanide) to report changes in interfacial electron transfer, whereas enzyme- or metal-labelled immunoassays exploit catalytic turnover or stripping peaks to generate amplified current or peak signals. These distinctions determine assay complexity, incubation/readout times and the kind of validation data typically reported.

4.2. Pulse voltammetry (DPV/SWV) in E2 assays

DPV and SWV are widely used as primary analytical readouts across aptamer, immunosensor and MIP studies. DPV is commonly paired with redox labels or enzymatic reporters: for instance, a classic ALP-based competitive immunosensor measured the ALP product 1-naphthol by DPV to reach picogram-level sensitivity [55]. DPV is also used to read redox-indicator tags: an aptasensor used MB bound to a guanine-rich cDNA and measured MB peak current by DPV as the analytical signal, while using ferri/ferrocyanide only for electrode characterization [39].

SWV is frequently used where mediator-blocking or direct oxidation/reduction of an enzymatic product is the reporting mechanism. An early aptamer chip measured current suppression in a 5 mM ferri/ferrocyanide mediator by SWV (signal decrease on target binding) [37]. Immuno- and enzyme-assisted competitive formats also use SWV: Monerris et al. detected enzymatically generated o-benzoquinone by SWV following HRP/H_2O_2 chemistry [58]

Beyond simple peak-current detection, several studies implement ratiometric or multi-peak DPV strategies to improve robustness. Examples include dual-peak internal-reference DPV using a Cu_xO/MXene MIP platform (IE2/ICu_xO ratiometric readout) and a dual-mode DPV reporting Cd^{2+} and Cu-MOF oxidation peaks as internal reference and reporter, respectively [57,64]. Molecularly imprinted sensors also employ adsorptive stripping variants DPAdSV or DPV for template removal/analysis) to enhance sensitivity for non-enzymatic targets [68,69].

4.3. Impedimetric readout (EIS) in label-free interfaces

EIS is the principal readout for label-free, affinity-based assays. Aptamer sensors have used EIS to measure changes in charge-transfer resistance (Rct) upon target binding (a "signal-on" or blocking response) with ferri/ferrocyanide as the faradaic probe for fitting to a Randles circuit [38]. Reviews reinforce that label-free EIS and voltammetric mediator-blocking formats are recurring approaches in aptasensing of E2 [35].

Impedimetric or capacitive non-faradaic readouts are also used in antibody sensors: miniaturized two-electrode capacitive immunosensors report direct capacitance changes without redox mediators, enabling rapid, low-volume assays [60,61]. EIS is commonly applied both as a primary readout (label-free detection) and as a characterization tool for stepwise electrode modification, typically using $[Fe(CN)_6]^{3-/4-}$ as the redox probe for interface diagnostics [38,57].

4.4. Amperometry and enzyme-linked formats

Amperometric readouts and enzyme labels remain central to many

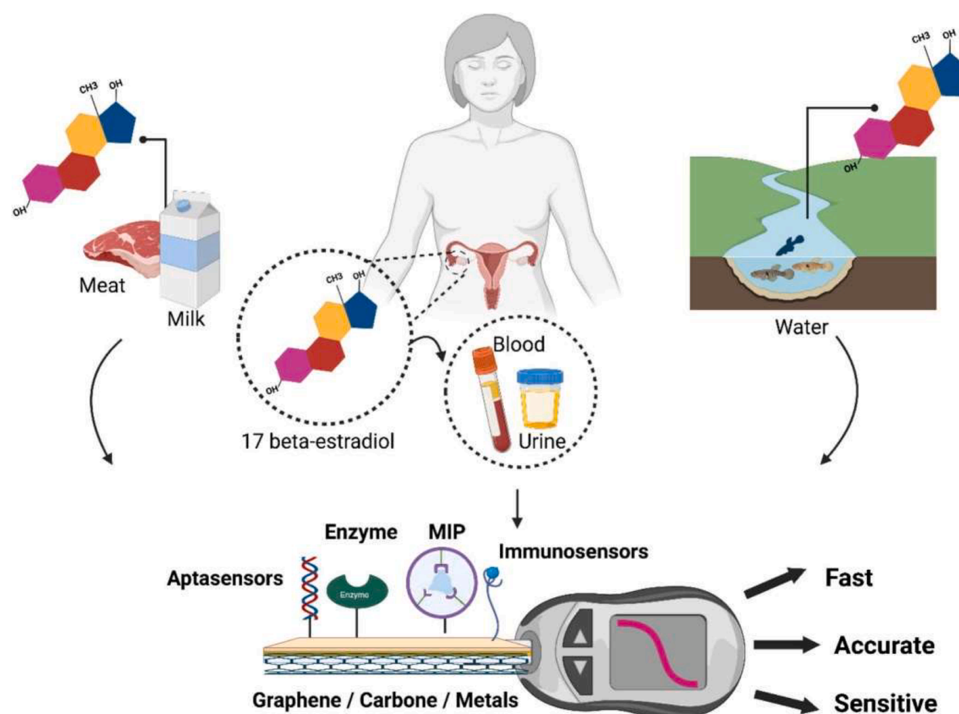

Fig. 6. The figure illustrates diverse biosensor strategies, including aptasensors, immunosensors, enzyme-based sensors, and sensors based on molecularly imprinted polymers (MIPs), graphene, carbon nanomaterials, and metal-based nanostructures. These biosensors demonstrate high sensitivity and specificity, enabling rapid detection of 17β-estradiol across biological and environmental samples such as blood, urine, milk, meat, and water (reproduced from [26] with permission).

immunosensor designs in the dataset. Disposable screen-printed amperometric immunosensors used alkaline phosphatase (AP) or HRP labels with enzymatic substrates that produce electroactive products measured as steady-state currents (e.g., AP → p-aminophenol measured at +300 mV; HRP → benzoquinone reduction measured amperometrically) [49,53]. Competitive formats that keep enzyme conjugates in solution or immobilized exploit DPV/SWV to quantify the enzymatic product (e.g., 1-naphthol from ALP detected by DPV) [55,56].

Hybrid electrochemical modes are present: chronoamperometric i-t measurements are paired with catalytic nanomaterials (e.g., Cu-MOF electrocatalysing H_2O_2) to provide time-resolved currents as an analytical signal, while DPV captures concomitant metal-ion oxidation peaks for ratiometric readout [57].

4.5. Practical comparability

A few practical patterns are evident across the Table 4: ferri/ferrocyanide remains the most frequently cited characterization probe for CV/EIS, and MB, thionine and other redox indicators are recurrent as covalently or non-covalently attached reporting tags in aptamer assays. Ratiometric and dual-mode strategies (electrochemical/ photoelectrochemical or internal-reference DPV peaks) are explicitly used in recent reports to improve stability and anti-interference performance [40,64]. Many studies include the specific supporting electrolyte and mediator concentrations, voltammetric pulse parameters, or incubation times, underlining that such experimental details are essential for cross-study comparison and reproducibility [37,39,41].

Within the reviewed E2 biosensing studies, voltammetric pulse techniques (DPV/SWV) and impedimetric measurements (EIS or capacitive readouts) dominate as primary analytical formats; DPV/SWV are frequently paired with enzymatic or redox tags for amplified current/peak signals, while EIS and non-faradaic capacitance measurements are favoured for label-free affinity detection. Amperometry and chronoamperometric catalytic readouts continue to play an important role in enzyme-labelled and electrocatalytic nanoparticle assays [49,53, 57]. Recent works show increasing use of ratiometric and dual-mode readouts to enhance robustness and reduce interference, highlighting a current direction for method development in electrochemical E2 sensing [40,64]

5. Discussion: real-sample analysis, interferences, comparability, and outlook

5.1. Real-world matrices: what is being validated, and how

Environmental waters dominate real-sample demonstrations across formats, spanning river, tap, lake, wastewater and generalized “water” samples in both immunoassay and (bio)recognition-free electroanalytical designs [39–41,44,49,61]. Filtration-only workflows are common in water testing, as illustrated by lake water filtration prior to dual-modal aptamer measurements and centrifugation/filtration for river water prior to SWV or DPAdSV readouts [39,57,61,63]. Some water assays avoid pretreatment altogether, exemplified by a disposable SPE amperometric immunosensor used directly in river and tap waters and by screen-printed protocols that rely on-electrode blocking and competition chemistry [49,54]. Conversely, solid-phase extraction of water prior to analysis is also reported, underlining the diversity of sample preparation choices even within the same matrix class [52]. Fig. 6 summarizes the main electrochemical recognition strategies used for E2 sensing and highlights their application in representative real-sample matrices.

Biological matrices are well represented but unevenly distributed across formats and preprocessing intensity, with “serum,” “urine,” and “milk” featuring frequently alongside spiked matrices and certified controls [36,50,54,55,58]. Notably, non-extracted bovine serum has been measured directly by DPV or competitive AP immunoassay, with confirmation against LC-MS/MS or HRP-quenching methodologies, while early DPV on SPCE required serum extraction by diethyl ether prior to measurement [55,56]. Human serum has been validated either via certified materials or clinical samples compared to reference

methods, illustrating clinically oriented adoption of label-free and labeled electrochemical readouts [54,62]. Urine appears in both aptamer and MIP formats, with dilution factors of 10ˆ2–10ˆ3 reported to mitigate matrix effects in impedance and DPV measurements [38,39,64]. Milk has been widely used as a test medium for both immunosensors and MIPs, underscoring the importance of E2 detection in food safety contexts [50,51,57,64]. Alternative biofluids, such as saliva, have also been successfully analyzed. In particular, a SWV electrocatalytic Eu_2O_3@rGO/SPCE system only required simple dilution for sample preparation, highlighting the viability of alternative biofluids, operated in acidic supporting electrolytes [75].

The current literature reveals two dominant methodological patterns. First, the analysis of water samples typically requires minimal pretreatment or simple filtration or dilution. On the contrary, biological matrices often necessitate either rigorous extraction (e.g., ether extraction for SPCE-DPV) or the development of specialized protocols that rely on surface blocking and competition chemistries [52,54–56]. Second, ratiometric and internal-reference strategies increasingly appear in water and milk/serum validations as a means to suppress matrix-induced variability without extensive sample workup [40,50,57,64].

5.2. Interference and selectivity: what is routinely tested, and what is not

Interference panels strongly reflect the chemistry of co-occurring steroidal and phenolic species, with E1, E3, EE2, P4, testosterone, and BPA repeatedly used to assess cross-reactivity across antibody-, aptamer-, and MIP-based designs [41,49,50,52,57,64]. These panels often stress structural similarity by including DES and nonylphenol, and in some cases compounds are assessed at high fold-excess to probe specificity margins in complex backgrounds [41,49,57]. Selectivity tests also include non-steroidal aromatics and dyes, such as 2-methoxynaphthalene or 1-aminoanthraquinone, as negative controls, particularly in early aptamer works and in mixed label-free readouts [38,39,51].

Beyond molecular analogs, several reports examine electroactive interferents, cations/anions, and biomolecular background species that are likely present in real waters or biofluids, including ascorbic acid, uric acid, dopamine, common ions, and proteins, with tolerable signal shifts reported at 10-fold excess in non-biological electrocatalytic platforms and selective MIP layers [65,66,69,75]. However, many studies assess single interferents rather than mixtures, and interference testing frequently occurs in buffer or simplified matrices even when the sensor is ultimately applied to real samples, indicating a gap between selectivity screening conditions and the compositional complexity of the target matrix [51,54,62].

Blocking and passivation steps, such as BSA, milk proteins, MCH or ethanolamine, are widely used to manage nonspecific adsorption, but few works quantify antifouling performance longitudinally in real matrices, despite successful demonstrations of stable signals over days to weeks in storage or repeated use [39,54,58]. The breadth of interferents tested is nonetheless expanding, with ratiometric internal references and selective imprinted cavities increasingly leveraged to suppress ambiguous responses from co-existing phenolics and redox-active species [40,57,64,65].

5.3. Comparability of analytical figures of merit: why LODs and ranges are not apples-to-apples

Comparisons of analytical figures of merit of analytical methods, such as LOD and linear range, are not easily possible due to differences in sensor modalities and transduction chemistry and assay methodology in measuring a common analyte [38,50,51,75]. For example, the ultra-low LODs of label-free EIS aptamer sensors in urine samples (10ˆ3 dilution) or those of non-faradaic capacitance immunosensors in packaged/tap water samples are not comparable to those of DPV or ECL immunoassays in minimally diluted serum or milk samples [38,50,60,62]. Moreover, in competitive immunometric assays, differences in enzyme labels (e.g., AP and HRP) dictate differences in signal chemistry (e.g., 1-naphthol, hydroquinone/benzoquinone, or ECL quenching). Such differences in chemistry directly impact S/N ratios and, consequently, LODs, regardless of matrix effects and extraction methods [49,50,54,55].

The integration of ratiometric techniques introduces additional layers of complexity when evaluating analytical performance across studies. While dual-signal normalization to $[Fe(CN)_6]^{3-/4-}$, metal-ion references, or internal nanomaterial peaks effectively minimize signal variance, the resulting metrics do not map onto single-signal LOD frameworks, even under similar calibration conditions [40,57,64]. This is well exemplified by a ratiometric DPV immunosensor using Cd^{2+} as an internal reference and Cu-MOF as a catalytic label, which yielded distinct DPV and chronoamperometric LODs and linear ranges, highlighting the impact of signal normalization and readout methodology on apparent sensitivity [57]. In a similar manner, dual-modal PEC/EC aptamer sensors yielded ultra-low pg/ml LODs in filtered lake water, by utilizing normalization of both photocurrent and MB redox signals to ferri/ferrocyanide. However, this result cannot be directly benchmarked against standard single-mode DPV measurements in complex biological sera [40].

Reported analytical performance is further skewed by sample preparation and its accumulation methodology. For instance MIP platforms that utilize adsorptive stripping voltammetry, introduce a preconcentration step via surface accumulation potentials, while conversely, non-stripping DPV/SWV protocols depend solely on simple equilibrium binding [68,69]. Furthermore, pre-analytical modifications, such as the ether extraction of serum, the filtration of environmental waters, and the extensive dilutions of urine, result in LOD claims that hinder cross-study evaluation, by artificially suppressing matrix load and baseline noise [38,54,55]. Beyond sample handling, electrochemical parameters, such as the choice of redox mediator (e.g., MB, THI, ferri/ferrocyanide) and the applied potential window, cause variations in background currents and dynamic ranges [39,51,62].

Finally, recognition-free electrocatalytic SWV on Eu_2O_3@rGO/SPCE or ratiometric MIP platforms that monitor intrinsic E2 oxidation peaks reflect fundamentally different analytical paradigms compared to affinity-based assays, making cross-comparisons most meaningful within rather than across these categories [56,67].

Key experimental and reporting factors that limit cross-study comparability of LOD and dynamic range are summarized in Fig. 3.

5.4. Stability, reuse, and deployment: what supports translation

Reusability and operational stability are repeatedly demonstrated but vary widely by mechanism and surface chemistry, with several reports detailing multi-day storage and multi-cycle use under modest refrigeration [38,57,58,65]. An aptamer EIS sensor regenerated with EDTA showed at least 10 reuse cycles with reproducible Rct responses, while an unlabeled SWV immunosensor reported roughly 100 determinations using acidic glycine desorption and stable current responses over three weeks [38,58]. MIP films and composites exhibit sustained performance over weeks with multiple extraction-rebinding cycles, and polishing-enabled carbon-paste/MagMIP interfaces can be renewed over ten times without performance loss, suggesting practical routes to reuse in environmental monitoring [65,69]. Storage stability at 4–8 °C ranges from ~1–4 weeks at >80–90% of initial response across enzyme-labeled immunosensors, ratiometric immunosensors, and MIPs, although some platforms show steeper declines beyond two weeks, emphasizing the importance of shelf-life reporting for eventual deployment [49,57,63,65].

Manufacturability and portability are well served by screen-printed and paper-based devices, including disposable SPE immunosensors, SPCE-based competitive assays, and integrated paper microfluidic immunosensors that have been validated against clinical reference

methods [49,54,62]. The prevalence of SPCE/SPGE and on-chip designs across aptamer, immuno-, and MIP platforms indicates a consistent pathway toward point-of-need testing with modest instrumentation and low per-test costs, as demonstrated in a paper-based DPV immunoassay correlated to ECL hospital assays [62]. At the same time, some of the lowest LODs arise from benchtop ECL or dual-modal PEC/EC designs and may entail multi-hour incubations and specialized emitters or photoactive reagents, which can trade off simplicity for sensitivity in resource-limited settings [40,50]. Consequently, the primary barrier to clinical translation is no longer electrode fabrication, but rather the successful integration of long-term stability, robust antifouling strategies, and matrix-resilient signal transduction with rapid, preparation-free protocols [49,54,58,62].

5.5. Current interpretations of "selectivity" and "matrix validation"

Currently, "selectivity" is often defined by testing discrete interferents, at fixed or elevated concentrations within ideal buffer solutions. Meanwhile, "matrix validation" is evaluated through spike-recovery experiments using filtered or diluted real samples, rather than performing blind tests on native, unspiked specimens [38,51,57]. For example, DPV and EIS immunoassays analyzing milk and water samples, commonly screen against DES, E1, E3, BPA, and NP, and perform spike-recovery tests in processed samples. Even though some studies cross-validate the results with Enzyme-Linked Immunosorbent Assay (ELISA) or LC-MS/MS, specificity is not fully proven in real-world applications [50,51,56,57]. Similarly, aptasensors that test urine, heavily dilute the samples (10^2–10^3-fold) to measure changes in Rct or redox currents. Dilution yields high recovery rates, however it fails to establish performance in raw urine [38,39]. MIP sensors employing stripping or ratiometric dual-peak techniques, exhibit enhanced anti-interference capabilities and show good recoveries in spiked river or wastewater, but typically under filtered or diluted conditions [65,69,70].

Ultimately, the prevailing approach to defining "selectivity" relies on controlled interferent testing, while "matrix validation" is usually confined to standard-addition methods within simplified or pretreated real samples, with direct comparative analyses of clinical or environmental samples confirmed by orthogonal methods remaining scarce [55,56,62]. Nevertheless, certain studies set a higher standard by analyzing non-extracted bovine serum assays with LC-MS/MS, or by using ELISA or HPLC-FLD (-Fluorescence Detection) to benchmark untreated lake, serum, and milk samples, therefore solidifying claims of real-world applicability [56,57,64].

5.6. Outlook: evidence-backed priorities

1) Prioritize unextracted biofluid testing and explicitly disclose effective dilution factors. Although current literature establishes viability across matrices like serum, urine and saliva, the variations in dilution and extraction steps skew reported LODs and recoveries [38,54,56,75]. To establish matrix robustness, future research must conduct parallel tests at multiple dilution levels, with an emphasis on minimal dilution scenarios [62].
2) Standardize interference testing with realistic, mixed analyte panels. Rather than the common practice of testing concentrated single interferents in simple buffers, research should shift to mixed analyte challenges, incorporating naturally co-existing analogs (such as E1, E3, EE2, P4, testosterone, BPA and DES) and thus yielding more accurate cross-reactivity [41,49,52,57]. Additionally, bioaffinity assays should adopt practices from electrocatalytic and MIP studies, by integrating electroactive species and common ions, in order to evaluate redox interference and baseline drift [65,75].
3) Cross-verify native sample results against orthogonal methods. As spike-recovery data alone offers limited evidence of accuracy, studies must benchmark their sensors performance against established techniques like LC-MS/MS, HPLC-FLD, ELISA, or clinical ECL. Consequently, the method's reliability will be further validated, particularly as direct comparisons get extended to unspiked matrices [51,56,62,64].
4) Prioritize ratiometric and dual-mode sensing strategies to minimize matrix effects, ensuring quantitative comparison with single-signal baselines. The integration of internal reference signals, including ferri/ferrocyanide, heavy-metal references, or internal nanomaterial peaks, consistently improves reproducibility and lower LODs in water, milk and serum matrices. To fully understand the trade-offs for real-world use, future studies must benchmark these dual-mode systems against their conventional; single-signal counterparts [40,57,64].
5) Standardize sample preparation and accumulation reporting to enable transparent LOD comparisons. Apparent analytical sensitivity is frequently artificially inflated by the use of techniques, such as adsorptive stripping preconcentration, either extraction of serum, and extenmsive dilution. To resolve this issue, the accumulation parameters (time and applied potential), extraction protocols, and dilution factors should be directly disclosed alongside their reported analytical ranges and LODs [38,55,68,69].
6) Assess antifouling techniques using real-wolrd matrices over time. Despite the standard practice of utilizing BSA, milk, MCH or ethanolamine for surface blocking, quantitative reporting pf antifouling metrics in complex samples is inadequate. Implementing multi-day repeated assessments of both passivated and raw interfaces in real matrices is critical for optimizing the sensors' design [39,51,54,58].
7) Evaluate stability and reusability under field-relevant storage and operational conditions. Although current reports of 8–45-day storage capacities, 7–100 operational cycles, and renewable surfaces are encouraging, the lack of testing under field-relevant environments hinders comparative analysis. Future work should implement standardized protocols for accelerated aging and surface regeneration to accurately benchmark the shelf life and operational lifespan across platforms [38,58,65,69].
8) Adopt mass-producible electrodes and streamline assay protocols with the intended field applications. Although these sensors demonstrate a high level of correlation to established methods, their practical application is hindered by the level of operation complexity. For point-of-care screening to become a reality, scalable formats (SPCE, SPGE, and paper) must be combined with low incubation times and mediator-free readout strategies [49,54,62].

As a whole, this matrix of priorities promotes a practical validation of matrices, comparability, and robustness-by-design. With a focus on established methods such as ratiometric normalization and standardization, it is possible to quickly capitalize on the success that is being demonstrated in multiple formats of E2 sensors and transduction methods.

6. Conclusion

The advancement in the electrochemical sensor technology for E2 has been marked by diversification both in terms of recognition chemistry and electrode design. The basic problem remains the sensitive and selective measurement of the small hydrophobic molecule in the presence of complex matrices. A review of the literature suggests that the performance of the sensor is not dominated by any particular component but is achieved through the harmonious combination of the recognition chemistry, electrode design, and measurement format. Specifically, the recognition chemistry dominates the selectivity and binding properties, whereas the electrode and its nanostructural modifications dominate the efficiency of the signal transduction process, capacity, and antifouling properties. At the same time, the measurement format dominates the stability and nature of the analytical signal.

In order for these technologies to move forward from the laboratory bench to the field, there is a need for the focus to move beyond the

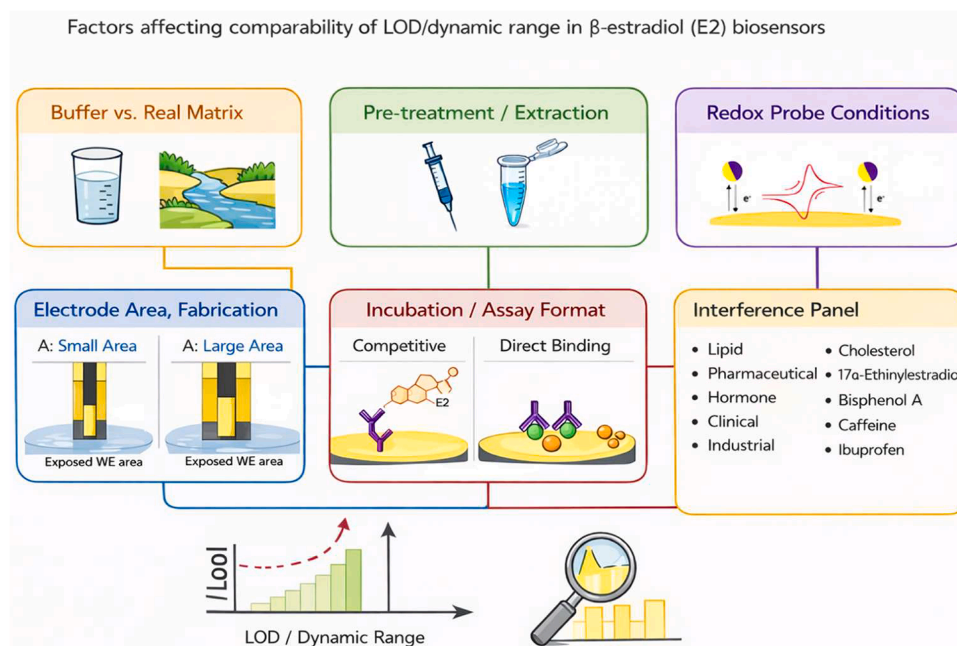

Fig. 7. Key factors that limit cross-study comparability of reported limit of detection (LOD) and dynamic range among electrochemical 17β-estradiol (E2) biosensors. The diagram outlines common sources of experimental variability, including (i) evaluations conducted in buffer as opposed to complex biological samples, (ii) variations in sample preparation protocols, (iii) differences in redox probe deployment and electrochemical parameters, (iv)inconsistencies in electrode area and fabrication (especially for printed and nanostructured electrodes), (v) divergencies in incubation protocols, as well as assay architecture (such as competitive versus direct-binding formats), and (vi) the stringency of selectivity assessments. These dependencies can significantly alter apparent analytical performance even in structurally similar sensor designs, underscoring the need for transparent reporting and validation in application-specific environments.

performance characteristics and into the utility of the technology. Specifically, there is a need for critical evaluation of the technology's performance in complex matrices, testing for interference relevant to naturally occurring hormone and contaminant matrices, and assessment for long-term stability and reproducibility appropriate to the intended use. The physical challenges of handling the real-world sample can be resolved by coupling these sensors with microfluidic devices. By automating fluid processing and ensuring consistent volume delivery, microfluidics eliminate the need for highly technical equipment, such as micropipettes, and highly trained personnel, thus bringing the gap to point-of-care sensing.

Alongside physical translation, the establishment of standardized analytical reporting is of utmost importance, as inconsistent reporting inhibits progress across the field. Variations across the calibration conditions, matrix handling and calculated metrics bottleneck our ability to directly compare sensor performance across studies.

Moving forward, advancing electrochemical E2 biosensing requires a tighter integration of rational electrode design choices with robust and realistic validation protocols. More consistent reporting of electrode fabrication, surface chemistry, stability, selectivity, and real-sample recovery will make the field easier to interpret and will accelerate convergence toward sensor designs that are not only sensitive, but also reproducible and practically deployable (Fig. 7).

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Data availability

No data was used for the research described in the article.

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