

Review

Aptamer/Nanozyme Chemical Sensors for On-Site Glyphosate Determination in Agricultural Runoff: Classification, Operating Principles, and Analytical Applicability

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Abstract

This critical perspective review first classifies glyphosate-sensing platforms and then evaluates their analytical applicability to agricultural runoff. Platforms are divided at the primary level into optical and electrochemical transduction, because these families measure different physical signals and have different sources of matrix interference. They are then grouped by the process that produces selectivity or signal change: direct interaction or metal coordination, affinity recognition by aptamers, antibodies, or molecularly imprinted polymers, catalytic modulation by enzymes or nanozymes, and separation or preconcentration before detection. This hierarchy distinguishes recognition chemistry from transduction method and device configuration. The review next defines four intended analytical applications—trace surveillance, runoff event screening, spill triage, and laboratory-adjacent confirmation—and compares them in terms of matrix, target concentration range, sample preparation, reporting metrics, and quality control requirements. Glyphosate occurs in dissolved and particle-associated forms, degrades mainly to AMPA, and coexists with phosphate, glufosinate, divalent cations, natural organic matter, and suspended sediment. Consequently, the lowest reported LOD is rarely the sole criterion for selecting a method. Matrix-matched calibration, spike recovery, selectivity, response time, storage stability, reader requirements, and invalid result rules determine whether an assay is suitable for a specified analytical application. The most defensible near-term approach combines matrix-specific sample preparation, platform-specific controls, and LC-MS/MS confirmation when results are regulatory, contested, or close to a decision threshold.

Keywords: glyphosate; agricultural runoff; aptamer; nanozyme; colorimetric sensor; electrochemical sensor; paper-based device; field detection; AMPA; chemical sensors



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1. Introduction

Glyphosate remains the most widely used herbicide because it is effective, inexpensive, and compatible with broad-acre weed control. Global use expanded after the adoption of glyphosate-tolerant crops and after use in non-crop land management, which made glyphosate a routine chemical input rather than an occasional treatment [1–3]. The same use pattern creates a monitoring problem. Glyphosate is strongly polar, zwitterionic over much of the environmental pH range, and usually detected with its degradation product aminomethylphosphonic acid (AMPA). It can adsorb to mineral surfaces, bind metals, and move with eroded particles during rainfall events [4–13]. For agricultural runoff, the relevant exposure window is often short. A storm can move dissolved glyphosate, particle-bound glyphosate, AMPA, phosphate, humic matter, and suspended clay into drainage

ditches within hours. Conventional laboratory methods can quantify low concentrations, but sample shipment, derivatization, matrix cleanup, and instrumental access slow the feedback loop [14–20]. Risk classification, residue regulation, and water quality thresholds also vary across agencies and jurisdictions, which makes transparent field screening useful even when confirmatory chromatography remains the reference method [21–24].

This review addresses chemical sensors intended to shorten that analytical feedback loop. For clarity, a sensing platform is described through three separate layers: the recognition process that interacts with glyphosate, the optical or electrochemical transduction method that converts that interaction into a measurable signal, and the device configuration used to handle the sample and read the response. This distinction is important because an aptamer, molecularly imprinted polymer (MIP), or nanozyme is not itself an optical or electrochemical category; the same recognition or catalytic element can be coupled to either signal domain. Glyphosate remains a difficult small-molecule target because it lacks a large hydrophobic surface, contains phosphonate and carboxylate groups that bind common metal ions, and competes with phosphate, AMPA, glufosinate, glycine, and humic ligands. Many assays therefore use indirect signal generation: glyphosate inhibits a catalytic reaction, changes a metal-mediated optical probe, occupies an imprinted cavity, or alters an electrode interface [25–29].

The practical aim of on-site sensing differs from that of trace determination under controlled laboratory conditions. The lowest reported LOD is not automatically the best choice for runoff analysis; the method must also tolerate suspended solids, phosphate, divalent cations, variable pH, natural organic matter, and changing ambient conditions. The review therefore progresses from general concepts to specialized chemistry. Section 2 introduces the primary optical/electrochemical classification, the secondary recognition and signal generation mechanisms, and a stepwise method selection rule. Section 3 defines the intended analytical applications, sample matrices, target concentration ranges, sample preparation choices, and performance criteria. Section 4 then examines target chemistry, aptamers, MIPs, antibodies, enzymes, and nanozymes. Sections 5 and 6 evaluate optical and electrochemical evidence, respectively, and Section 7 treats paper-based and portable device configurations without presenting them as a separate transduction principle. Section 8 summarizes which approaches are appropriate, why they are selected, and under which matrix and validation conditions they can support on-site glyphosate determination. Figure 1 provides the classification and reading sequence used throughout the review.

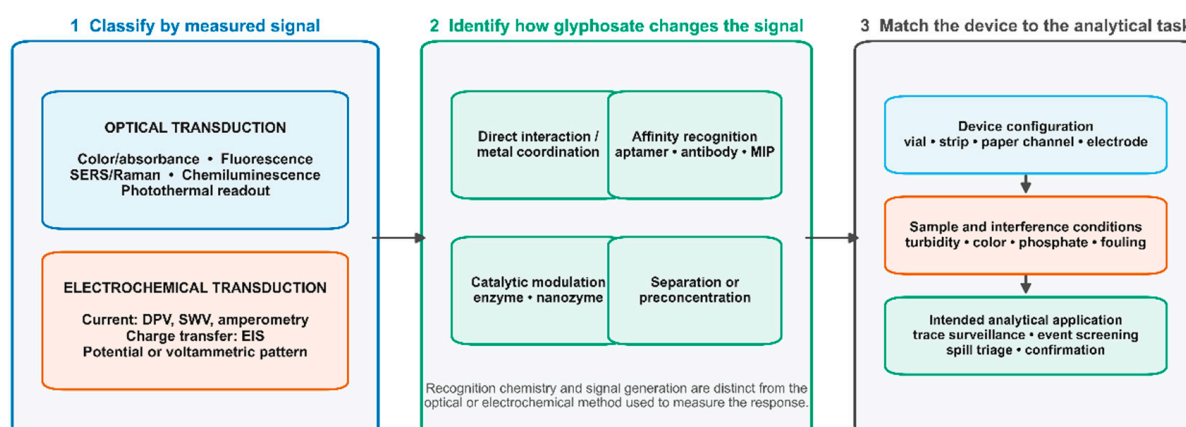


Figure 1. Two-level classification of glyphosate-sensing platforms. Platforms are first classified by optical or electrochemical transduction and then by the process that produces selectivity or signal change: direct interaction or metal coordination, affinity recognition, catalytic modulation, or separation/preconcentration. Device configuration is evaluated separately against sample and interference conditions and the intended analytical application.

2. Classification and Operating Principles of Glyphosate-Sensing Platforms

2.1. Primary Classification by Optical or Electrochemical Transduction

A useful classification begins with the physical quantity measured by the instrument. Recognition, transduction, and device configuration answer different questions. Recognition describes how glyphosate is distinguished from other species; transduction describes how that interaction becomes a measurable optical or electrical change; device configuration describes how the sample, reagents, and reader are arranged. Separating these layers prevents categories such as aptamer sensor, nanozyme assay, paper device, and electrochemical sensor from being treated as equivalent [29–34]. Figure 1 uses this hierarchy as the educational map for the review.

Optical platforms measure a change in light intensity, spectrum, scattering, or heat generated by absorbed light. Colorimetric methods measure absorbance or reflected color, fluorescence methods measure emission after excitation, surface-enhanced Raman scattering (SERS) measures vibrational signatures near an enhancing surface, and chemiluminescent or photothermal methods measure light produced by a reaction or heat produced after irradiation. Glyphosate is usually detected indirectly because it has weak intrinsic optical absorption in convenient spectral regions. The analyte instead changes metal coordination, catalytic conversion of a chromogenic substrate, fluorophore quenching or recovery, or access to a SERS-active surface. Optical methods can be simple and visually interpretable, but sample color, turbidity, ambient illumination, and optical substrate variability can bias the measured signal [35–47].

Electrochemical platforms measure current, charge transfer resistance, potential, or a voltammetric pattern at an electrode. Differential pulse voltammetry (DPV), square wave voltammetry (SWV), and amperometry quantify current produced by oxidation, reduction, adsorption, or an enzyme-coupled reaction. Electrochemical impedance spectroscopy (EIS) detects changes in interfacial charge transfer and capacitance after target binding or surface blocking. Potentiometric and electronic tongue approaches use potential or multielectrode response patterns. These methods can provide low detection limits with compact instrumentation, but their response depends on electrode composition, ionic strength, surface history, and fouling by organic matter or suspended particles [30–33,48–57].

2.2. Secondary Classification by Recognition and Signal Generation Mechanism

Within either transduction family, platforms can be grouped by how glyphosate changes the signal. First, direct interaction and metal coordination methods detect adsorption, complex formation, or a surface reaction without a separate biological receptor. Second, affinity methods use an aptamer, antibody, or MIP to preferentially bind glyphosate. Third, catalytic modulation methods measure inhibition, activation, or recovery of an enzyme or nanozyme reaction. Fourth, separation or preconcentration methods isolate glyphosate from AMPA or the sample matrix before optical or electrochemical measurement. This secondary classification identifies where chemical selectivity is generated and therefore which control experiments are required. A metal coordination assay requires tests against phosphate and other ligands; an aptamer assay requires binding and immobilization controls; a MIP requires template removal and nonspecific binding controls; and a catalytic assay requires substrate, pH, and inhibitor controls [25,29,35–37,58–74].

The two classification levels cross rather than replace each other. For example, an aptamer can produce fluorescence, SERS, or an electrochemical response; a nanozyme can generate a colorimetric, chemiluminescent, or electrode-based signal; and a paper channel can contain either optical reagents or printed electrodes. Paper, cartridges, vials, and handheld readers are therefore device configurations, not independent signal transduction

classes. This distinction allows studies to be compared first by operating principle and then by how the principle is implemented [35,36,58,59,75–85].

2.3. Selecting a Platform for an Analytical Application

Method selection should proceed in a fixed order. The analyst first defines the measurand—dissolved glyphosate, particle-associated glyphosate, or an operationally defined total—and the sample matrix. The expected concentration range and decision threshold then determine whether trace sensitivity, rapid high-range screening, or confirmatory accuracy is required. The analyst next selects a transduction method compatible with the dominant interference: optical methods require explicit control of color and turbidity, whereas electrochemical methods require control of conductivity, electrode fouling, and baseline stability. Recognition chemistry and device configuration are selected only after these conditions are specified. This sequence explains when a simple colorimetric assay is sufficient, why an electrochemical or multimode method may be preferred near a decision threshold, and under which conditions either result requires chromatographic confirmation.

3. Analytical Application Framework: Matrices, Target Concentration Ranges, and Performance Criteria

3.1. Intended Analytical Applications and Matrix-Specific Target Concentration Ranges

Before comparing sensors, the intended analytical application and target concentration range must be defined. Table 1 uses four stable codes throughout the review: A1, trace surveillance; A2, runoff event screening; A3, spill triage; and A4, laboratory-adjacent confirmation. These applications do not require the same LOD, linear range, sample volume, response time, or quality control burden. Drinking water, surface water, soil extracts, food extracts, and biological samples also have different regulatory or practical thresholds. Representative water limits illustrate the scale problem: U.S. drinking water regulation has used a glyphosate maximum contaminant level of 0.7 mg/L, whereas the EU drinking water framework sets a generic parametric value of 0.1 µg/L for an individual pesticide and 0.5 µg/L for total pesticides; food and soil decisions are matrix- or crop-specific rather than governed by one universal glyphosate concentration [21–24]. Every reported sensor should therefore state its target matrix, defined measurand, and intended application before the LOD is interpreted. An assay with a moderate LOD may be appropriate for A2 or A3 when it is rapid, tolerant of turbidity, and paired with an invalid result rule, whereas A1 requires a lower matrix-verified LOD/LOQ, blank control, recovery, and holding time evidence. A4 places greater weight on calibration, selectivity, uncertainty, and agreement with a reference method.

Table 1. Intended analytical applications and reporting priorities for glyphosate sensors.

Intended Analytical Application	Typical Matrix	Target Concentration Range or Decision Threshold	Most Important Reporting Metrics	Analytical Implication
A1. Trace surveillance	Drinking water, surface water, groundwater	Jurisdiction-specific water guideline or monitoring trigger	LOD/LOQ in matrix, blank drift, recovery, holding time	Low LOD is useful only when matrix blanks and spike recovery are demonstrated.
A2. Runoff event screening	Storm runoff, drainage ditch water	Post-application event threshold or site-specific action level	Response time, turbidity tolerance, filtration/extraction choice, spike recovery	A medium-sensitivity assay can be valuable if it works during the short runoff window.

Table 1. Cont.

Intended Analytical Application	Typical Matrix	Target Concentration Range or Decision Threshold	Most Important Reporting Metrics	Analytical Implication
A3. Spill triage	High-load runoff, tank mix or near-field water	Rapid high-concentration trigger	Simple reader, wide range, dilution tolerance, invalid test rule	Visual or phone assays may be sufficient when the goal is rapid prioritization.
A4. Laboratory-adjacent confirmation	Filtered water, soil extract, food extract	Method validation target for the matrix	Calibration model, matrix-matched standards, selectivity panel, reproducibility	Portable sensors should indicate which samples require LC-MS/MS or GC-MS confirmation.

3.2. Environmental Fate, Degradation Products, and Structurally Related Interferents

Glyphosate is transported as both dissolved and particle-associated material because its phosphonate and carboxylate groups bind mineral surfaces and metal ions. Microbial and environmental degradation mainly forms aminomethylphosphonic acid (AMPA), which retains the phosphonate group and can therefore respond in metal coordination, electrochemical, and some affinity formats. Glufosinate should be introduced early because it is a structurally related phosphinic herbicide used in similar agricultural contexts and can be tested in the same monitoring programs. Other matrix constituents, including phosphate, carbonate, humic substances, clay fines, divalent cations, and reducing organic matter, can cause false positive responses, signal suppression, signal enhancement, or reduced selectivity depending on the transduction pathway [4,5,7,12,14–20]. Table 2 organizes the main chemical and physical interferences that must be considered before comparing sensor performance. AMPA and glufosinate test molecular specificity, phosphate/carbonate and metal ions test coordination selectivity, while humic matter and suspended solids test optical, electrode, and paper-format robustness. The practical implication is that selectivity should be reported as a matrix-specific control panel rather than as a single interferent experiment.

Table 2. Common interferents and matrix constituents relevant to glyphosate sensing.

Group	Representative Species or Material	Likely Interference Mechanism	Most Affected Formats	Control or Mitigation
Degradation product	AMPA	Shared phosphonate chemistry can mimic glyphosate in metal-binding or surface recognition assays.	Aptamer, MIP, colorimetric, electrochemical	Report AMPA selectivity and, when possible, chromatographic or electrophoretic separation.
Related herbicide	Glufosinate	Structural similarity and phosphinic acid functionality can reduce chemical selectivity.	Affinity, MIP, metal coordination assays	Include glufosinate in selectivity panels at realistic co-occurrence levels.
Inorganic anions	Phosphate, carbonate	Compete with glyphosate at metal nodes, oxide surfaces, and catalytic centers.	Nanozyme colorimetry, fluorescence, electrochemical	Use phosphate-matched blanks and matrix spikes.
Metal ions	Ca ²⁺ , Mg ²⁺ , Fe ³⁺ , Cu ²⁺	Complex glyphosate or alter catalytic and electrochemical surfaces.	Fluorescence, colorimetry, electrochemical	Report hardness/ionic strength and test divalent cation tolerance.
Natural organic matter	Humic and fulvic substances	Quench fluorescence, reduce chromogens, foul electrodes, and bind paper fibers.	Optical, SERS, electrochemical, paper devices	Use filtered/standard addition controls and invalid image or baseline rejection rules.
Suspended solids	Clay, silt, organic particles	Scatter light, retain particle-bound glyphosate, and block electrode or paper surfaces.	Camera assays, paper microfluidics, electrochemical strips	State filtration pore size; distinguish dissolved from total event glyphosate.

3.3. Sample Preparation, Extract Quality, and Unit Reporting

Sample preparation should be matched to the defined measurand and analytical scope. If the target is dissolved glyphosate, filtration is appropriate, but the pore size, filter material, potential filter adsorption, and filtrate recovery should be reported. If the target is total glyphosate transported during a runoff event, the procedure must address particle-associated glyphosate through desorption or extraction from retained solids. Soil, food, and plant extracts require additional pH, ionic strength, and color/turbidity checks before their results are compared with water samples. Extract quality should be documented by matrix-matched blanks, spike recovery, replicate extraction, and, when available, LC-MS/MS or GC-MS confirmation. To improve unit consistency, the comparison tables identify LOD and linear range in the originally reported units, and the text interprets values by matrix rather than converting all nM, pM, $\mu\text{g/L}$, $\mu\text{g/mL}$, and mg/kg values into a single unit when molecular form, density, or matrix basis would make the conversion misleading.

Each platform should report a minimum set for evaluating on-site analytical suitability: LOD and LOQ in the relevant matrix, usable linear range, total analysis time including sample preparation, sample volume, reader and power requirements, storage stability, recovery and RSD, selectivity against AMPA, glufosinate, phosphate, divalent cations, and humic matter, and a predefined rule for invalid results. Establishing these criteria before platform-specific discussion allows later sections to focus on chemistry-specific limitations, such as aptamer binding verification, nanozyme substrate interference, optical substrate uniformity, MIP swelling, electrode fouling, or paper flow failure.

4. Recognition Chemistry and Nanozyme Signal Generation

4.1. Target Chemistry and Matrix Constraints

The classification in Section 2 identifies the measured signal and the source of selectivity before specialized recognition chemistry is considered. Glyphosate creates a chemical mismatch with many portable recognition systems because it is small, highly polar, and rich in oxygen donors. These features promote water solubility and metal binding but provide little hydrophobic surface for classical host–guest recognition. In aptamer assays, signal generation usually depends on target-induced changes in nucleic acid conformation or surface binding, so ionic strength, divalent cations, and immobilization can alter apparent affinity. In MIP sensors, rebinding within a charged polymer cavity changes the signal, but swelling, template leakage, and nonspecific uptake can shift the baseline. In nanozyme and metal coordination assays, glyphosate modulates catalytic activity or metal ligand fluorescence, so phosphate, AMPA, glufosinate, carbonate, and humic ligands can produce chemically plausible false responses. In electrochemical assays, adsorption or complexation changes current, impedance, or potential, while particles and organic matter can foul the electrode. Environmental samples therefore challenge each mechanism through both molecular cross-reactivity and matrix-dependent signal transfer [4,5,7,12].

A defensible analytical method separates enrichment, selective recognition, and signal transduction into stages that can be verified independently. Laboratory LC-MS/MS provides a reference example because derivatization or direct injection, chromatographic retention, and mass selection can be validated separately [14–20]. LC-MS/MS nevertheless requires trained operation, sample transport, costly instrumentation, and controlled maintenance. Portable sensors compress the same analytical logic into fewer steps and must reveal matrix effects through blanks, spikes, interferent checks, and confirmation rules. This compression explains the appeal of metal coordination and nanozymes but also their limitation: Cu(II), Fe(III), Co(II/III), and lanthanide nodes can bind phosphonate groups and amplify signals, yet they also respond to phosphate and humic ligands unless selectivity is tested explicitly [60–64].

4.2. Aptamers, Imprints, and Antibody-Derived Recognition

Aptamers offer a modular route to small-molecule recognition because nucleic acid sequences can be selected *in vitro* and then coupled to optical or electrochemical labels [86–91]. For glyphosate, aptamer-based assays are attractive because the recognition layer can be regenerated and immobilized on FeMOFs, electrodes, or nanoparticles [35]. The practical issue is that glyphosate offers few binding handles, and many reported aptamer formats depend on assay architecture as much as on intrinsic affinity. Salt concentration, divalent cations, truncation, and surface density can change the apparent response. An aptamer sensor suitable for on-site analysis should therefore report not only K_d or LOD but also sequence, immobilization density, regeneration cycles, and performance in phosphate-rich water.

Molecularly imprinted polymers (MIPs) solve a different part of the problem. They can create cavities matched to glyphosate geometry and charge and can be formed on gold, carbon, or screen-printed electrodes [65–70]. MIPs often deliver strong apparent selectivity and low LODs, including picomolar or even lower values in EIS reports [66,70]. These numbers should be interpreted with care. Template removal, nonspecific swelling, ionic strength shifts, and polymer aging can create baseline drift. In agricultural runoff, a MIP layer also faces fouling by humic matter and clay fines. The best MIP designs are therefore those that pair low LOD with regeneration data, blank runoff controls, and comparison against AMPA, phosphate, and glufosinate.

Antibody and enzyme-based recognition remains useful for comparison. Immunosensors and immunoassays can distinguish small structural differences when antibodies are well characterized [25,28]. Enzyme inhibition or enzyme-coupled detection can be simple, but enzymes impose cold chain and shelf-life constraints that nanozymes were designed to reduce [71–73]. These biological systems still define two benchmarks for application to on-site analysis: a portable assay must be stable without laboratory storage, and it must include enough controls to detect false positives from other phosphonate or phosphate-containing species.

4.3. Nanozyme Amplification and Signal Logic

Nanozymes are used in glyphosate sensing mainly in three signal logics. In inhibition assays, glyphosate reduces peroxidase-like or phosphatase-like activity, lowering the rate of chromogen oxidation or substrate hydrolysis [36,37,74]. In activation or recovery assays, glyphosate competes with a quencher or restores fluorescence by altering a metal ligand complex [38,39]. In multimode assays, one material gives color, fluorescence, SERS, or thermal signals that cross-check each other [35,40,41]. These modes differ in field value. Inhibition assays are simple and can be transferred to paper or phone imaging, but they can be affected by pH and by other ligands. Multimode assays reduce false confidence, but they also add instrumentation and calibration steps.

The catalytic layer also changes the meaning of response time. A 10 min nanozyme reaction can be acceptable if it replaces derivatization and laboratory shipment. A 30 min binding and washing process may still be suitable for a local monitoring station but less suitable for direct field screening. The same distinction applies to LOD. A material that reports 0.001 pg/mL in an optimized EIS cell is not necessarily more useful than a 0.1 µg/mL smartphone assay if the former lacks runoff validation and the latter gives recoveries near 90–100% in river samples [36,66]. The comparison in later sections therefore treats LOD as one metric within a broader operating envelope.

4.4. Recognition-Specific Controls and Immobilization Stability

Recognition-specific controls should be interpreted against the analytical application framework in Section 3. Aptamer systems should report sequence, target-binding verification, immobilization density, regeneration, and response in phosphate-rich water. MIP systems should report template removal, swelling or aging behavior, blank runoff controls, and comparison against AMPA, phosphate, and glufosinate. Nanozyme systems should report pH, peroxide or substrate concentration, metal ion competition, and matrix spike recovery. These controls determine whether an observed signal reflects glyphosate recognition, matrix-dependent contrast, or a change in transduction chemistry.

The same logic applies to calibration. External calibration in buffer is useful for material screening, but it does not prove that a runoff sample can be measured. Matrix-matched calibration is stronger, although true runoff blanks can be difficult to obtain because background glyphosate and AMPA may already be present. Standard addition is often the most defensible near-site approach. It requires more steps than a single reading, but it compensates for matrix suppression in colorimetric, fluorescent, and electrochemical systems. For a portable analytical kit, the compromise can be a two-point spike: one blank channel and one channel spiked near the decision threshold. This approach does not create a full calibration curve, but it can reveal whether the matrix suppresses or amplifies the signal beyond an acceptable tolerance.

Immobilization chemistry also deserves more attention than it receives in many proof-of-concept papers. Aptamers can be immobilized through thiol–gold chemistry, biotin–streptavidin, electrostatic adsorption, or covalent coupling. Each route changes orientation, surface density, nonspecific adsorption, and regeneration. Nanozymes can be dried on paper, dispersed in solution, embedded in polymer films, or deposited on electrodes. Dry storage may change particle aggregation and catalytic activity. MIP films can crack, swell, or retain template. These are not minor fabrication details. They decide whether a sensor works only on the day of synthesis or can be used after storage in a portable analytical kit. A publication aimed at environmental deployment should report storage duration, storage temperature, response after rehydration, and at least three independently prepared batches.

Standard sample diversity is therefore a core validation issue rather than a supplementary detail. A practical validation set should include reagent water standards, matrix-matched surface water or runoff standards, soil or sediment extracts when particle-bound glyphosate is claimed, and at least one colored or organic-rich extract that tests optical and electrochemical matrix tolerance. For every extract, reports should state the extraction solvent or buffer, pH adjustment, filtration or centrifugation step, extract dilution, spike level, recovery acceptance window, and whether the extract was checked by a reference method. Without these details, a low LOD in buffer cannot be translated into a reliable claim for soil, runoff, or food-derived matrices.

The recognition layer also interacts with sampling strategy. If the aim is dissolved glyphosate, filtration before sensing is justified, but the report should state filter pore size and whether the filter adsorbs glyphosate. If the aim is glyphosate transported in both dissolved and particle-associated forms, the analytical procedure must include extraction or desorption from particles. This distinction is often blurred. A ditch water assay that filters out suspended sediment may under-report the event load, whereas an unfiltered optical assay may over-report because particles scatter light. Particles can also block an electrode surface. The review therefore uses the term on-site detection rather than definitive total load quantification unless a study explicitly addresses particle-associated glyphosate.

5. Colorimetric and Other Optical Platforms

5.1. Colorimetric Nanozyme Assays

Colorimetric assays remain the most direct route to on-site analysis because the signal can be read by eye, a small photometer, or a phone camera. The main glyphosate formats use TMB, ABTS, p-nitrophenyl phosphate, or related chromogenic substrates. In the Cu(II)-TMB format, glyphosate suppresses Cu(II)-promoted TMB oxidation, giving a 95.6 ng/mL LOD and a 2–20 µg/mL range in water samples [42]. In the Fe₃O₄@C7/PB nanozyme assay, glyphosate inhibits peroxidase-like activity; spectrophotometric reading gives a 0.10 µg/mL LOD, while smartphone reading gives 0.50 µg/mL over 0.50–15 µg/mL [36]. These values are less low than the best SERS or electrochemical reports, but the method uses a short reaction and a reader that already exists in most field settings.

The trade-off is selectivity under runoff chemistry. Chromogenic oxidation is affected by pH, peroxide concentration, dissolved organic matter, reducing agents, and particles that scatter light. A phosphate-rich runoff sample can also compete with glyphosate for metal nodes. For this reason, colorimetric assays need matrix spikes more than they need another decimal place in LOD. Table 3 compares representative optical systems by reporting LOD, range, response time, reader, recovery, and the main field limitation. The table shows a broad spread: SERS and fluorescence can reach pM or nM levels, whereas visual color assays often operate in the high nM to mM range. This spread does not map directly onto suitability for on-site analysis.

5.2. Fluorescence, SERS, and Multimode Readouts

Optical readouts extend the sensitivity window. The FeMOF aptasensor reported three channels: 0.05 nM by SERS, 0.50 nM by RRS, and 1.00 nM by fluorescence [35]. The difference among channels is informative. SERS provides the lowest LOD, but it depends on substrate uniformity and Raman access. Fluorescence gives simpler instrumentation, but metal ion and dissolved organic matter quenching can be difficult in runoff. RRS is useful as a cross-check, although it is less common in field laboratories. These comparisons support multimode sensing as a quality control strategy rather than as a simple race to the lowest LOD.

The g-C₃N₄/Cu²⁺ fluorescence assay reached 0.01 µg/mL and 0.02–6 µg/mL in water, with recoveries around 96.5–104.4% [39]. The hemin SERS method reached 9.59 pM in honey and covered 0.1 nM–10 µM [44]. A multimode N-CDs/FMOF-Zr platform reported nM-level detection through color, fluorescence, and photothermal readouts [40]. These systems demonstrate that glyphosate can be made optically visible by metal coordination, surface enhancement, or nanozyme modulation. They also show why optical methods intended for on-site use require a matched sample preparation and calibration procedure. Honey, fruit extract, drinking water, and runoff contain different optical interferents, so validation in one matrix cannot be transferred without new calibration.

Table 3. Quantitative comparison of representative colorimetric and optical glyphosate sensors. LOD and linear range are retained in the original reported units to avoid unsupported cross-matrix conversion.

Recognition Mode	Nanozyme/Probe	Signal	Matrix	LOD (Reported Unit)	Linear Range (Reported Unit)	Time	Reader	Recovery/RSD	Field Limitation	Refs
Aptamer/FeMOF	FeMOF nanozyme and metal–organic fluorescence	SERS	Wastewater	0.05 nM	0.1–14 nM	ca. 30 min	Raman spectrometer	92.1–97.5% recovery	Best analytical LOD but instrument-dependent	[35]
Aptamer/FeMOF	FeMOF	RRS	Wastewater	0.50 nM	1–10 nM	ca. 30 min	Fluorometer/RRS	93.4–98.6% recovery	Useful confirmation channel, less common in field kits	[35]
Aptamer/FeMOF	FeMOF	Fluorescence	Wastewater	1.00 nM	2–10 nM	ca. 30 min	Fluorometer	91.8–99.2% recovery	Aptamer batch stability must be controlled	[35]
Cu(II) coordination	Cu(II)-TMB oxidation suppression	Colorimetry	Tap water, lake water	95.6 ng/mL	2–20 µg/mL	23 min	UV-vis	90.3–103.7% recovery	Simple chemistry but less sensitive than aptamer/optical modes	[42]
Nanozyme inhibition	Fe ₃ O ₄ @C7/PB peroxidase-like nanozyme	ABTS colorimetry	River and tap water	0.10 µg/mL	0.125–15 µg/mL	10 min reaction	UV-vis	89.4–97.1% recovery	Good phone compatibility after color normalization	[36]
Nanozyme inhibition	Fe ₃ O ₄ @C7/PB peroxidase-like nanozyme	Smartphone RGB	River and tap water	0.50 µg/mL	0.50–15 µg/mL	10 min reaction	Smartphone	89.4–97.1% recovery	Camera and illumination control dominate uncertainty	[36]
Phosphatase-like nanozyme	Co ₃ O ₄ nanoplates	p-NPP colorimetry	Apple, cucumber, soil	0.175 mg/kg	0.4–10 mg/kg	10 min	UV-vis/visual	85.0–108.0% recovery	Food matrices need extraction and pH control	[74]
Chelation probe	Rhodamine derivative	Fluorescence	Water and strip tests	4.1 nM	0.01–30 µM	2 min	Fluorometer/strips	95.2–104.6% recovery	Reagent synthesis and solvent fraction affect portability	[43]
Cu(II)-mediated quenching	g-C ₃ N ₄ nanosheets	Fluorescence recovery	Lake water, tap water	0.01 µg/mL	0.02–6 µg/mL	10 min	Fluorometer	96.5–104.4% recovery	Metal competition can alter response in hard water	[39]
Hemin affinity	Hemin SERS probe	SERS	Honey	9.59 pM	0.1 nM–10 µM	20 min	Portable Raman possible	Most recoveries 92–135%	High sensitivity but substrate and Raman access limit routine use	[44]
MOF/nanocarbon	N-CDs/FMOF-Zr	Color/fluorescence/photothermal	Environmental water	0.21 nM	0.5–800 nM	15 min	UV-vis/fluorescence/thermal	91.0–106.0% recovery	Multimode readout improves confidence but increases workflow	[40]
Phosphatase-like nanozyme	Ce-based nanozyme	Chemiluminescence	Vegetable extract	0.12 µg/L	0.5–100 µg/L	12 min	Luminometer	87.6–105.2% recovery	Sensitive but less paper-ready than colorimetry	[37]

Table 3. Cont.

Recognition Mode	Nanozyme/Probe	Signal	Matrix	LOD (Reported Unit)	Linear Range (Reported Unit)	Time	Reader	Recovery/RSD	Field Limitation	Refs
Metal porphyrin MOF	AgNPs/Cu-TCPP	Color/fluorescence/photothermal	Fruit and water	0.67 μ M	2–100 μ M	18 min	UV-vis/thermal	88.5–107.4% recovery	Useful for residue screening near high thresholds	[41]
Host–guest/DNA cluster	Beta-CD@DNA-CuNCs	Colorimetry	Water and food extracts	0.03 μ M	0.1–20 μ M	15 min	UV-vis	92.0–108.5% recovery	Cyclodextrin inclusion helps selectivity but matrix color remains a risk	[45]
Nanozyme array	Pt nanozyme array	Visual colorimetry	Tea and water	0.08 μ M	0.1–50 μ M	20 min	Image analysis	90.0–106.0% recovery	Array response needs calibration transfer	[46]
Cu(II) inhibition	Cu(II)-TMB	Label-free colorimetry	Water	0.31 μ M	1–25 μ M	20 min	UV-vis	92.3–104.1% recovery	Chemistry overlaps with phosphate-rich interferents	[47]

The mechanism-level comparison in Figures 2 and 3 emphasizes where selectivity and amplification enter optical sensors. Figure 2a illustrates receptor-controlled FeMOF aptamer amplification, Figure 2b shows metal ligand suppression of TMB oxidation, and Figure 2c shows nanozyme inhibition coupled to phone imaging. Figure 3a uses g-C₃N₄/Cu²⁺ fluorescence recovery, Figure 3b places SERS sensitivity on a hemin surface, and Figure 3c uses color, fluorescence, and photothermal signals as cross-checks. This contrast shows that no optical principle is universally better. Each principle places the burden of selectivity in a different location: receptor sequence and surface density for aptamers, metal competition for Cu(II) assays, substrate uniformity for SERS, and cross-channel calibration for multimode systems.

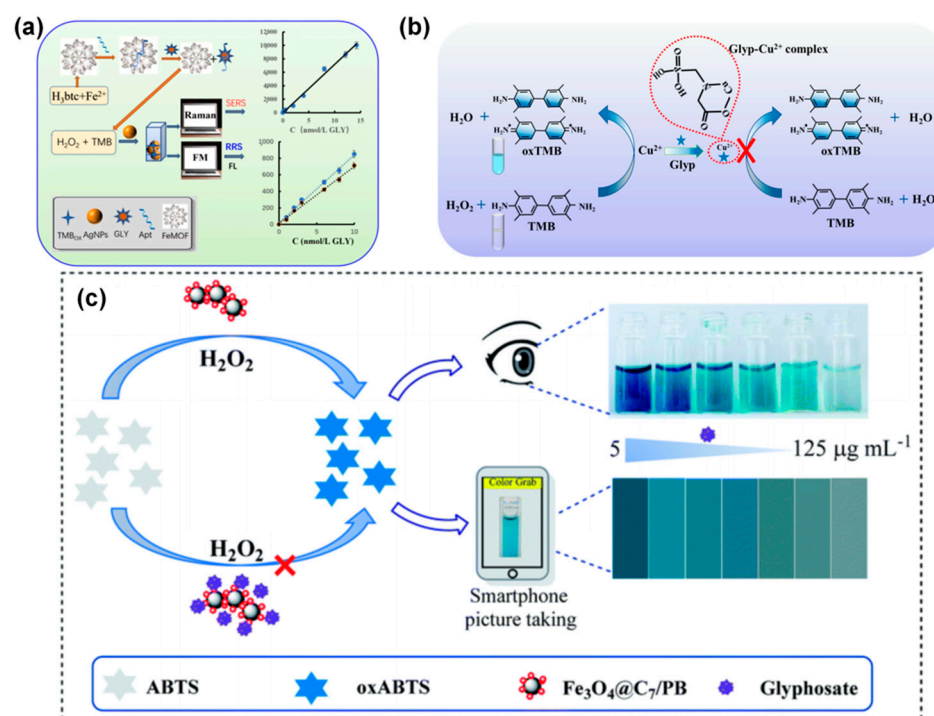


Figure 2. Colorimetric and nanozyme glyphosate chemistry. (a) FeMOF trimode aptasensor concept from [35]. (b) Cu(II)-modulated TMB oxidation principle from [42]. (c) Fe₃O₄@C₇/PB peroxidase-like nanozyme inhibition and smartphone readout from [36].

5.3. Critical Comparison for Runoff Analysis

The most useful colorimetric systems for runoff are not always the most sensitive systems in buffer. A phone-readable 0.5 µg/mL assay is close to practical screening if the field question is whether a runoff event contains elevated glyphosate after application [36]. It is less suitable for low-background watershed surveillance. A pM SERS system may support trace confirmation but will need a stable substrate, fixed sampling geometry, and clear rules for particle removal [44]. Fluorescence systems sit between these ends. They can reach low µg/L or nM levels, but they require correction for quenching and turbidity. A portable analytical kit should therefore pair an optical sensor with a turbidity flag, a color reference, and a matrix spike control.

The best use of optical nanozyme chemistry may be as a front-end screen. Samples above a trigger value can be sent for LC-MS/MS confirmation, while samples below the trigger can be archived with field metadata. This tiered strategy fits runoff monitoring because event frequency is high and laboratory capacity is limited. It also matches the uncertainty of glyphosate chemistry. A color or fluorescence signal should not be treated as legal proof of

concentration unless the method has undergone matrix-specific validation; it can, however, guide sampling density and identify events that deserve confirmatory analysis.

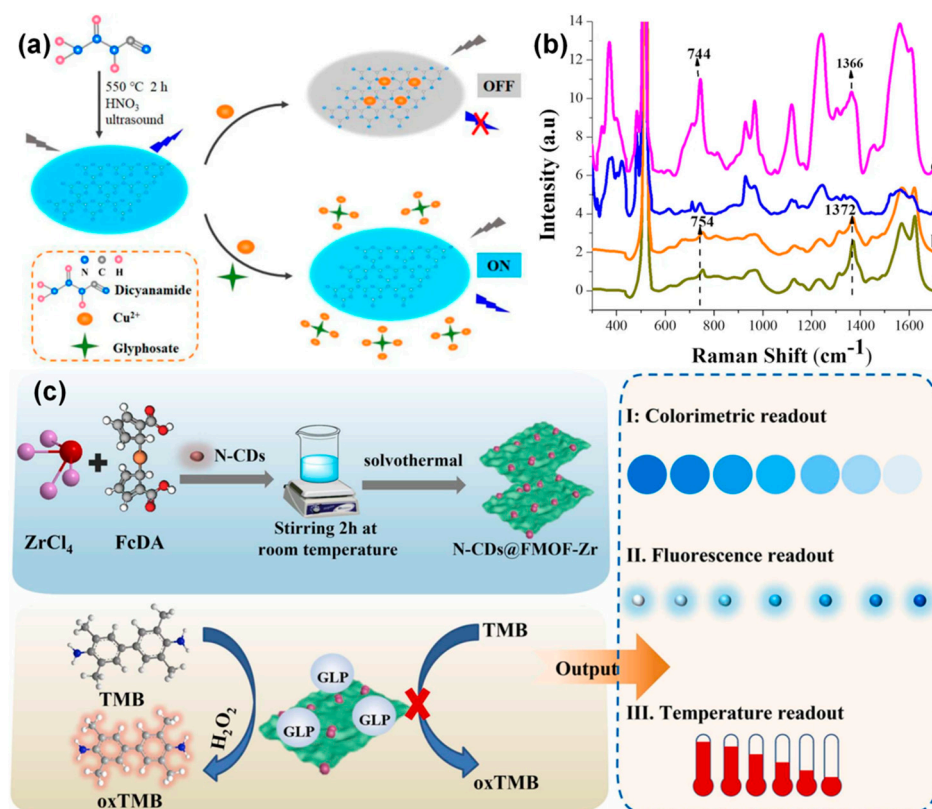


Figure 3. Optical glyphosate readouts beyond simple colorimetry. (a) g-C₃N₄ nanosheet/Cu²⁺ fluorescence sensing principle from [39]. (b) Hemin-based SERS recognition and analytical response from [44]. (c) N-CDs/FMOF-Zr multimode colorimetric, fluorescence, and photothermal platform from [40].

5.4. Camera-Based Quantification and Optical Error Sources

Smartphone colorimetry converts an analytical chemistry problem into a combined chemistry and imaging problem. The chemical reaction may be reproducible, but the image intensity depends on white balance, exposure, lens shading, ambient light, viewing angle, and the color space used for analysis. A phone-based glyphosate assay should therefore include a reference patch or internal color standard in the same image as the assay zone. Reporting only RGB changes from one phone model is insufficient for application to on-site analysis. A better study design uses at least two phone models, fixed and ambient illumination tests, and an image processing rule that is defined before validation. This requirement is especially important for agricultural runoff, where sample color can vary after rain, fertilizer application, and sediment transport.

Optical sensors also differ in how they fail. A TMB or ABTS colorimetric assay may give a lower signal if reducing matter consumes oxidized chromogen. A fluorescence assay may give a lower signal if humic substances absorb excitation or emission light. A SERS assay may give a false low response if glyphosate does not reach the enhancing surface or if the nanoparticle substrate aggregates in salts. A photothermal assay may be affected by background absorption and heat dissipation. These failure modes are not interchangeable, which is why multimode optical systems are promising. When a sample produces a positive colorimetric response but no fluorescence recovery, the disagreement itself contains information about matrix interference. Multimode designs should report discordant samples rather than averaging channels into a single favorable result.

The field value of optical sensors can be estimated by comparing their operating range with plausible decision tasks. A sub-nM SERS or aptamer-MOF assay can support low-level monitoring if the instrument and substrate are available [35,44]. A 0.1–0.5 µg/mL nanozyme assay is better matched to rapid post-application or spill screening [36]. A high-µM visual assay can still be useful where the question is gross contamination, but it should not be described as a trace environmental monitor [59]. These distinctions make the table more than a list of LODs. They show that optical sensors should be grouped by intended decision: trace surveillance, event screening, spill triage, or laboratory-adjacent confirmation.

Selectivity claims in optical papers should also be read quantitatively. It is not enough to state that common ions do not interfere. The tested interferent concentration should be related to runoff chemistry. Calcium and magnesium can be present at millimolar levels in hard water. Phosphate can rise after fertilizer application. Humic substances may vary by orders of magnitude across watersheds. A glyphosate sensor that tolerates 10-fold excess glycine but is not tested against phosphate cannot be considered fully evaluated for agricultural runoff. Future colorimetric and fluorescence studies should include an interference panel with phosphate, AMPA, glufosinate, glycine, nitrate, calcium, magnesium, iron, humic acid, and turbidity. The response should be reported as percent signal change relative to a glyphosate standard, not only as a qualitative bar chart.

6. Electrochemical Platforms

6.1. Electrode Materials and Recognition Layers

Electrochemical glyphosate sensors are attractive because they separate signal measurement from sample color and because portable potentiostats are now inexpensive. Screen-printed carbon electrodes, gold electrodes, carbon paste electrodes, laser-induced graphene, and molecularly imprinted films have all been used [30,48–57,65–70,72,73,92–98]. The central design question is how the electrode converts a non-electroactive or weakly electroactive molecule into a measurable change. Some systems rely on metal–glyphosate complexation at copper, gold, MOF, or clay-modified surfaces. Others use MIP cavities to concentrate glyphosate and change charge transfer resistance. A third group uses enzyme inhibition or nanocomposite-mediated adsorption.

Table 4 shows how strongly the reported LOD depends on the electrode and signal mode. Zn-ZIF-67/montmorillonite modified carbon paste electrodes reached 0.003 nM in water and 0.009 nM in soil extract [51]. Gold nanoparticle–graphene screen-printed electrodes reached 6.6 ng/L in one Nafion-containing configuration [50]. MIP nanoparticle sensors and MIP films reported ng/mL to pg/mL or lower values [66,68]. These values are much lower than many optical colorimetric assays. The limitation is that electrochemical LODs are often measured in cleaner matrices with controlled electrolyte, while runoff introduces natural organic matter, suspended solids, divalent cations, and changing conductivity.

Table 4. Quantitative comparison of representative electrochemical glyphosate sensors. LOD and linear range are retained in the original reported units, with matrix context shown in the adjacent column.

Electrode	Modification	Recognition	Technique	Matrix/Condition	LOD (Reported Unit)	Linear Range (Reported Unit)	Sensitivity	Time	Matrix Validation	Portability Note	Refs
SPCE	AuNPs-Gr(DMF)	None; direct oxidation/complexation	DPV	Buffer and wine	0.30 µg/L	1–50 µg/L	0.149 µA per µg/L	5 min	Wine recovery reported	Portable potentiostat compatible	[50]
SPCE	AuNPs-Gr(NAF)	None; direct oxidation/complexation	DPV	Buffer and wine	6.6 ng/L	20–100 ng/L	0.627 µA per ng/L	5 min	Wine recovery reported	Lower LOD but narrower range	[50]
CPE	Zn-ZIF-67/montmorillonite	Coordination on MOF/clay	DPV	Water	0.003 nM	0.01–1 nM	34.7 µA/nM	10 min	Recovery 99.4–101.4%	High analytical performance; paste electrode reproducibility must be watched	[51]
CPE	Zn-ZIF-67/montmorillonite	Coordination on MOF/clay	DPV	Soil extract	0.009 nM	0.03–1 nM	31.2 µA/nM	10 min	Recovery 98.6–102.3%	Soil matrix increases LOD three-fold	[51]
SPE	MIP nanoparticles	MIP cavity	SWV	Water	1.94 ng/mL	2.5–350 ng/mL	NR	15 min binding	Tap water recovery 96–103%	Strong selectivity, but imprint aging needs routine controls	[68]
Portable SPE	Electrochem-SENSE electrode	Direct electrochemical signal	SWV/DPV	Drinking water	10 ng/mL	10–10,000 ng/mL	NR	<5 min	Blind sample testing reported	Designed for small-volume field operation	[52]
Laser-induced graphene	rGO/LIG	Direct adsorption	DPV	Drinking water	0.144 nM	1–1000 nM	NR	3 min	Spiked water recoveries reported	Substrate fabrication is scalable	[53]
Gold electrode	MIP film	Molecular imprint	EIS	Water	0.04 nM	0.1–100 nM	NR	20 min	Tap water validation	EIS gives low LOD but longer acquisition	[69]
QCM/Au	Polypyrrole MIP	MIP cavity	DPV/QCM	Aqueous buffer	1 pM	1 pM–1 nM	NR	15 min	Selectivity over AMPA evaluated	Picomolar performance from model solutions needs runoff proof	[69]
Glassy carbon	MIP chitosan	MIP/biopolymer	EIS	River water	0.001 pg/mL	0.01–100 pg/mL	NR	30 min	River recovery reported	Very low reported LOD requires independent replication	[66]
Gold electrode	MIP	MIP cavity	DPV	Water and soil	0.25 ng/mL	1–1000 ng/mL	NR	20 min	Soil extract tested	Polymer regeneration is a practical constraint	[67]

Table 4. Cont.

Electrode	Modification	Recognition	Technique	Matrix/Condition	LOD (Reported Unit)	Linear Range (Reported Unit)	Sensitivity	Time	Matrix Validation	Portability Note	Refs
Pencil graphite	Double-template imprinted polymer/AuNP/MWCNT nanofilm	MIP	DPASV	Soil and human serum	0.35 ng/mL	3.98–176.23 ng/mL	NR	NR	Simultaneous glyphosate/glufosinate analysis demonstrated in real samples	Nitrosation and template removal steps add complexity	[65]
Electronic tongue	Metal electrodes	Pattern recognition	Voltammetry	Water with interferents	Classification mode	0.5–50 mg/L	NR	few min	Interferents discriminated	Good screening concept, not a trace method	[54]
Copper electrode	Cu surface complexation	SWV	Water	0.02 mg/L	0.05–5 mg/L	NR	10 min	Tap water test	Simple electrode but copper surface fouling likely		[54]
Glassy carbon	CuAl-LDH/graphene nanocomposite	Cu-glyphosate complexation and stripping current inhibition	DPV	Surface water	1 nM	2.96 nM–1.18 µM	NR	NR	Spiked surface water recovery 97.64–108.08%	Langmuir-type response and electrode fabrication require control	[57]
Pencil graphite/hollow fiber	MWCNT-ionic liquid/CuO composite	Extraction/preconcentration followed by glyphosate oxidation	DPV	Soil and river water	1.3 nM	5 nM–1.1 µM	NR	NR	Real sample results compared with a standard method	Hollow-fiber fabrication and preconcentration increase workflow complexity	[96]

6.2. MIP, MOF, and Carbon Composite Performance

MIP-based sensors dominate the lowest reported electrochemical LODs. In principle, imprinting is well matched to glyphosate because it creates a cavity for a molecule that is difficult to bind by hydrophobic affinity. In practice, the imprinting chemistry must be judged by more than selectivity factors. Template leakage can create false low-level signals, and incomplete removal can narrow the working range. EIS formats amplify small interfacial changes, which explains sub-pg/mL reports [66]. The same amplification makes them vulnerable to fouling and ionic strength changes. A MIP sensor intended for runoff should report blank drift over repeated exposures, recovery after rinsing, AMPA selectivity, and performance after storage.

MOF and clay composites take a different route. The Zn-ZIF-67/montmorillonite platform gives a useful example because the LOD changed from 0.003 nM in water to 0.009 nM in soil extract [51]. The three-fold difference is small compared with many matrix effects, but it is a reminder that clay and organic matter do not behave like buffer. Graphene and gold nanoparticle composites improve conductivity and surface area [50,53]. Their field value is strongest when they are printed, disposable, or compatible with simple pretreatment. A laboratory-polished electrode with a very low LOD may be less useful than a slightly less sensitive screen-printed electrode that can be replaced after each runoff event.

The electrochemical contrast in Figure 4 is governed by both material chemistry and device manufacturability. Figure 4a represents a manufacturable screen-printed AuNPs-graphene path, Figure 4b represents high-sensitivity Zn-ZIF/MMt nanocomposite design, and Figure 4c represents synthetic selectivity from MIP nanoparticles. Taken together, these panels clarify why electrochemical platforms are strong candidates for quantitative field monitoring but also highly sensitive to surface history.

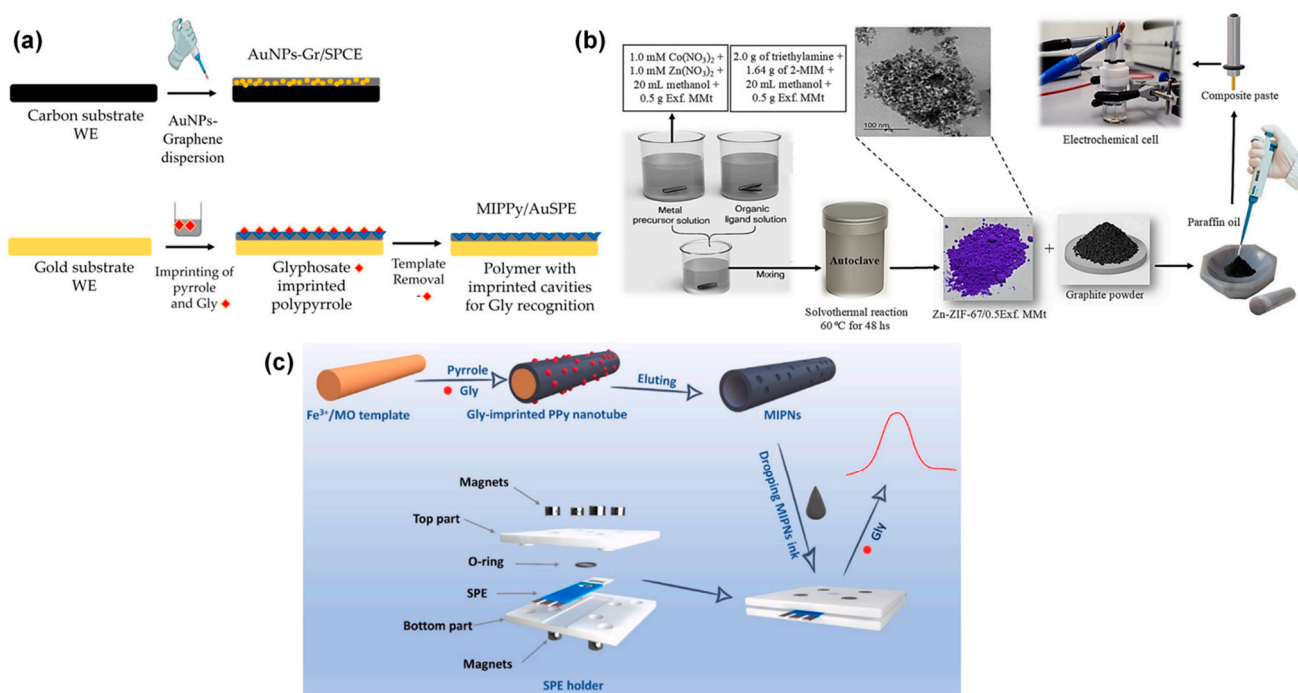


Figure 4. Electrochemical glyphosate platforms. (a) Gold nanoparticle–graphene-modified screen-printed electrode design from [50]. (b) Zn-ZIF-67/montmorillonite carbon paste sensor design and response concept from [51]. (c) Molecularly imprinted nanoparticle electrochemical sensor design [68].

6.3. Critical Comparison with Optical Assays

Electrochemical systems often outperform optical colorimetry in LOD, but they do not automatically outperform it in field reliability. An optical assay can fail because the sample is colored or turbid. An electrochemical assay can fail because the electrode surface fouls, the supporting electrolyte changes, or dissolved oxygen and metal ions shift the baseline. These failure modes are different, so they can be paired. A practical portable analytical kit could use a colorimetric nanozyme test as a low-cost screen and an electrochemical strip as a quantitative follow-up. Agreement between the two channels would increase confidence; disagreement would trigger filtration, dilution, or laboratory confirmation.

The strongest electrochemical evidence is not the lowest single number. It is the combination of disposable electrodes, small sample volume, short assay time, matrix recovery, and calibration stability. ElectrochemSENSE illustrates this integrated analytical design with a small-volume portable format and a 10 ng/mL LOD in drinking water [52]. The value is higher than several nanocomposite reports, but the device concept is closer to field use. Conversely, an EIS sensor reporting 0.001 pg/mL can be important for mechanistic sensitivity, but it needs independent runoff testing before it can be considered suitable for on-site analysis [66]. This distinction should guide future comparative papers: every electrochemical glyphosate sensor should report an analytical LOD and a field operating level.

6.4. Signal Normalization and Electrode Failure Modes

Electrochemical sensors need method-specific quality controls because the signal is surface dependent. A screen-printed electrode can vary in carbon ink composition, binder content, surface roughness, and printed geometry; a gold or graphene modification adds another source of variation. For a disposable sensor, batch-to-batch variation is the main concern; for a reusable sensor, fouling and incomplete regeneration are the main concerns. Both cases require normalization. Ferricyanide or another outer-sphere redox probe can track baseline electron transfer changes, but it may not be compatible with every on-site analytical procedure. Internal standard addition can compensate for matrix suppression but increases the number of measurements. These controls should be evaluated during method development rather than introduced only after poor recovery appears.

The difference between DPV, SWV, and EIS is also practical. DPV and SWV give peak currents that are relatively easy to interpret and can be implemented on handheld potentiostats. EIS can detect subtle interfacial changes and often gives low LODs, but it requires fitting, frequency selection, and stable open-circuit conditions. In runoff, conductivity and suspended matter can change the impedance spectrum independent of glyphosate. This does not make EIS unsuitable; it means EIS papers should report equivalent-circuit fitting quality, blank drift, and matrix-matched spectra. A single Nyquist semicircle in buffer is not enough evidence for field use.

Electrode fouling is the most likely practical failure mode in agricultural samples. Humic substances can adsorb to carbon and gold, proteins or biofilms may be present in drainage water, and clay particles can physically cover the electrode. Filtration can reduce fouling but may remove particle-bound glyphosate. Anti-fouling coatings can protect the electrode but may slow mass transfer. A useful future protocol would compare unfiltered runoff, filtered runoff, and filtered-plus-spiked runoff on the same sensor. The report should state how much signal is lost after one exposure and whether a rinse restores the baseline. These details decide whether the device is a disposable strip, a reusable probe, or a laboratory-only electrode.

Electrochemical application to on-site analysis will also depend on reader software. Portable potentiostats can store raw voltammograms, calibration curves, and metadata, but many papers report only processed peak currents. For environmental monitoring,

raw data retention is important because later confirmation may require checking baseline, peak shape, and noise. A field application should record electrode batch, sample pH, conductivity, temperature, filtration status, calibration date, and operator. These metadata are not burdensome if they are captured automatically by a phone or handheld reader. They are the difference between a one-time demonstration and a defensible monitoring record.

The comparison with optical assays suggests a practical hybrid. A disposable screen-printed electrode can provide a quantitative result near the decision threshold, while a colorimetric channel can identify strong matrix color or gross contamination. The two channels need not have the same LOD. Their value comes from different failure modes. If both channels classify a sample as above the trigger, the result is more credible. If one channel is invalid or discordant, the sample can be diluted, filtered, or sent to LC-MS/MS. This type of decision logic is common in field diagnostics but remains underused in pesticide sensing papers.

7. Paper-Based and Portable Analytical Devices

7.1. Paper, Microfluidics, and Portable Readers

Paper-based devices change the evaluation criteria because they integrate sampling, reagent storage, flow control, and disposal. General paper microfluidics has matured through wax printing, nitrocellulose patterning, electrochemical uPADs, phone-based optical reading, and multiclass residue procedures that inform validation practice [75–85]. Glyphosate-specific paper devices are less mature, partly because glyphosate is small and highly polar and partly because runoff requires particle control. Recent platform reviews and environmental monitoring studies agree that portable formats must be judged by sampling and calibration as much as by transducer material [99–101]. Paper e-microfluidic isotachopheresis separates glyphosate and AMPA and gives a visual range of about 100–400 μM [58]. This is not a low-level environmental monitor, but it addresses a problem many simple assays ignore: AMPA and glyphosate may need to be separated before detection.

The systems most relevant to on-site analysis compress reader and chemistry. The paper-based urine sensor reached a 75 $\mu\text{g/L}$ LOD over 100–700 $\mu\text{g/L}$ with a portable potentiostat [102]. The handheld GlyphoSense biosensor reported a 0.028 $\mu\text{g/mL}$ LOD and a 0.016–12.5 $\mu\text{g/mL}$ range with relative standard error of 1.2–5.8% [103]. The smartphone $\text{Fe}_3\text{O}_4\text{@C7/PB}$ assay reached 0.5 $\mu\text{g/mL}$ through phone imaging [36]. These systems occupy a realistic range for near-site decisions: not all are trace monitors, but all reduce sample volume, transport, and laboratory dependence. Table 5 compares this deployment range quantitatively by placing LOD, assay time, sample volume, reader power, recovery or error, and matrix-handling burden in the same row-level framework.

Application codes in Table 5 refer to Table 1: A1, trace surveillance; A2, runoff event screening; A3, spill triage; and A4, laboratory-adjacent confirmation. A suitability score describes operational practicality in the originally tested matrix and does not by itself establish validation for every matrix within the coded application.

Table 5. Quantitative comparison of paper-based and portable glyphosate-sensing devices. Original units are retained and interpreted against the stated matrix. The final column reports an operational suitability score and the corresponding intended application code(s) from Table 1.

Format	Sample Type	Pretreatment	Sample Volume	Reader/Power	Assay Time	LOD (Reported Unit)	Range (Reported Unit)	Recovery/Error	Matrix Handling	Score; Application Code	Refs
Paper e-microfluidics	Glyphosate/AMPA standards	Isotachophoretic preconcentration	ca. 10 μ L	Power supply, visual readout	8 min	100 μ M visual threshold	100–400 μ M	Model system	Separation handles AMPA but LOD is high for surface water	3/5; A3	[58]
Capillary ITP reference	Glyphosate/AMPA standards	Capillary isotachophoresis	μ L scale	UV detector	8–12 min	1–2 μ M	2–100 μ M	Model system	Better sensitivity than paper variant, less field simple	2/5; A4	[58]
Paper electrochemical device	Human urine	Dilution/filtration	50 μ L	Portable potentiostat	10 min	75 μ g/L	100–700 μ g/L	Urine recovery 94–106%	Biological matrix shows device ruggedness but not runoff specificity	4/5; A4	[102]
Cotton bud assay	Water	No extraction	Drop scale	Visual/colorimetric	5 min	0.12 mM	0.25–2.5 mM	Water spiking	Usable for high-level spill screening only	5/5; A3	[59]
Chemiluminescent disposable assay	Water	No extraction	Drop scale	Camera/luminometer	10 min	0.45 μ M	2–500 μ M	Water spiking	Lower LOD than visible cotton format but needs light control	4/5; A2/A3	[59]
Handheld enzymatic biosensor	Water and beverage models	Minimal dilution	Small-volume	Dedicated handheld reader	1 min	0.028 μ g/mL	0.016–12.5 μ g/mL	RSE 1.2–5.8%	Strong usability claim; enzyme storage remains central	5/5; A2/A4	[103]
Electrochem-SENSE	Drinking water	No derivatization	5 μ L	Handheld potentiostat	<5 min	10 ng/mL	10–10,000 ng/mL	Blind samples	Small sample demand suits field kits	5/5; A1/A4	[52]
Smartphone nanozyme assay	River and tap water	Filtration optional	100 μ L	Phone camera	10 min	0.5 μ g/mL phone	0.5–15 μ g/mL	89.4–97.1%	Needs fixed illumination and color card	4/5; A2/A3	[36]
MOF microfluidic paper	Food extract/water	Extraction and dilution	50–100 μ L	Phone/UV-vis	15 min	sub- μ M reported	μ M range	Food recovery reported	Promising integration but field runoff not yet central	4/5; A2/A4	[104]
Hinge-like paper dual channel	Water/food	Extraction/dilution	Drop scale	Phone/fluorometer	15 min	low- μ M reported	μ M range	Recovery reported	Dual channel adds confirmation but complicates calibration	4/5; A2/A4	[105]
Liquid crystal interface	Water	Surfactant interface assembly	10–20 μ L	Polarized optical viewer	10 min	μ M-level	μ M–mM	Water test	Label-free but optics are less common for farmers	3/5; A3	[106]

Table 5. Cont.

Format	Sample Type	Pretreatment	Sample Volume	Reader/Power	Assay Time	LOD (Reported Unit)	Range (Reported Unit)	Recovery/Error	Matrix Handling	Score; Application Code	Refs
Paper microfluidic template	Model analyte	Wax-patterned channels	μL scale	Visual/electrochemical	Varies	Platform paper	Platform paper	General platform	Provides manufacturing route rather than glyphosate chemistry	4/5; A2	[85]
Electrochemical uPAD	Model analytes	None or filtration	μL scale	Portable potentiostat	Minutes	Platform dependent	Platform dependent	General platform	Establishes electrode integration for future glyphosate assays	4/5; A2/A4	[81]
Phone microplate reader	Color/fluorescence assays	Assay dependent	Well-plate	Cellphone optics	Minutes	Assay dependent	Assay dependent	Clinical proof	Transferable optics, not yet runoff-specific	3/5; A2/A4	[75]
ECL paper sensor	Model analytes	None	μL scale	Voltage supply/camera	Minutes	Low-μm platform	Platform dependent	Model validation	Good for dark signal assays but power burden remains	3/5; A2/A4	[77]

7.2. Device Configuration, Matrix Effects, and Sample Pretreatment

Device configuration should be separated from recognition chemistry and transduction principle. A colorimetric, fluorescent, electrochemical, or aptamer-based assay can be arranged as a cartridge, paper channel, vial kit, disposable electrode, or handheld reader system. The analytical question is whether that arrangement controls sample volume, fluid transport, reagent storage, reader alignment, calibration, and waste handling. Cartridges can protect reagents and standardize flow paths but increase fabrication complexity. Paper devices reduce sample volume and simplify disposal but introduce humidity, background adsorption, and wicking variability. Handheld readers improve quantitative traceability but require calibration, power, and raw data retention. The discussion below therefore evaluates chemistry-specific limitations separately from the physical arrangement used to perform the analysis.

Runoff is not a clean aqueous standard. A portable analytical kit must decide how to handle at least five matrix terms: sediment, pH, phosphate, divalent cations, and organic matter. Filtration removes particles but can also remove particle-bound glyphosate. Dilution reduces interference but raises the practical LOD. Acidification can desorb glyphosate but may disrupt aptamers or nanozyme activity. Chelators can suppress metal interferences but may destroy metal-mediated sensing. These trade-offs should be reported explicitly. A paper stating only that river water was spiked does not provide enough information unless it gives pH, conductivity, turbidity, dissolved organic carbon, or at least a description of filtration and storage.

The table also shows that paper and portable formats need a distinct set of evaluation criteria. A 100 μM visual threshold may be useful for A3 spill triage; a 10 ng/mL electrochemical kit may be suitable for drinking water screening; and a 0.028 $\mu\text{g/mL}$ handheld biosensor may support routine local monitoring [58,103]. These applications should not be collapsed into one ranking. The relevant question is whether the assay's operating range matches the decision threshold and whether a non-specialist can execute the sample preparation and analysis procedure without losing the analyte. Figure 5 follows directly from this comparison: Figure 5a presents paper isotachopheresis as a separation and pre-concentration method for glyphosate/AMPA, whereas Figure 5b presents disposable paper electrochemistry for compact quantification.

7.3. Future Analytical Directions

Future glyphosate sensors should be designed around an analytical objective rather than a single performance metric. The first priority is matrix-matched validation. Each new sensor should test buffer, tap water, surface water, and a real runoff or soil leachate sample. At minimum, the study should report recovery at three concentrations, a matrix blank, AMPA and phosphate responses, the validated pH interval, and a short storage test. The second priority is calibration transfer. Smartphone assays need fixed illumination, a color reference, and a model evaluated across phone types rather than a single camera. Electrochemical assays need electrode-to-electrode variation, batch reproducibility, and criteria for rejecting fouled electrodes. The third priority is paired confirmation. A portable analytical kit that combines a rapid colorimetric screen with a quantitative electrochemical strip can identify inconsistent results before an environmental management decision is made.

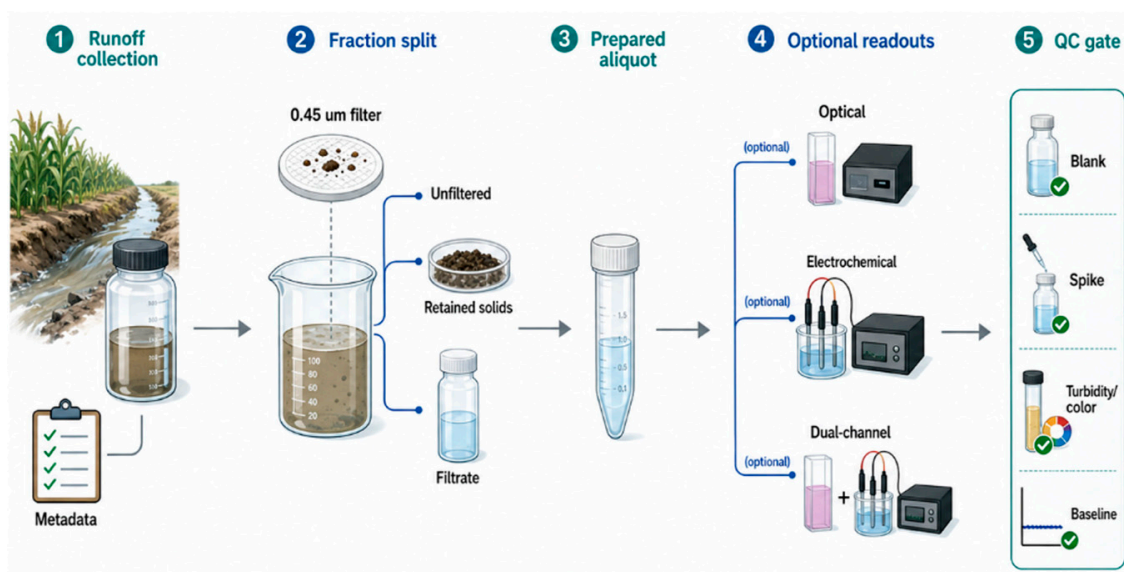


Figure 6. Matrix-specific analytical procedure for glyphosate in runoff. “Runoff sample acquisition” is the common sampling step for A1–A4 in Table 1 and is distinct from A2 runoff event screening. The procedure separates dissolved and particle-associated fractions, shows solids retained on a 0.45 µm filter, and treats optical and electrochemical measurements as alternative or sequential options according to the analytical application.

7.4. Intended Analytical Applications and Acceptance Criteria

A sensor for agricultural runoff should be evaluated against the same four intended analytical applications defined in Table 1. A1 trace surveillance seeks low-level, comparable measurements across sites or dates and therefore requires a low matrix-verified LOD/LOQ, blank stability, holding time evidence, and agreement with a reference method. A2 runoff event screening seeks rapid identification of elevated concentrations after herbicide application and rainfall; total analysis time, recovery near a site-specific trigger, turbidity tolerance, and an invalid result rule are central. A3 spill triage addresses high-concentration samples and prioritizes a wide range, dilution integrity, rapid response, and clear referral for confirmation. A4 laboratory-adjacent confirmation uses a portable or benchtop sensor close to controlled laboratory conditions and emphasizes matrix-matched calibration, selectivity, reproducibility, uncertainty, and comparison with LC-MS/MS or GC-MS. These four applications differ in analytical purpose but use the same names and codes throughout the text and Tables 1 and 5.

Acceptance criteria should follow the application rather than a single ranking of LOD. For A1, the report should state the matrix-verified LOD/LOQ, blank drift, inter-batch precision, holding time, and agreement with a reference method. For A2, it should state total analysis time, the invalid test rate, recovery near the trigger concentration, and the effect of suspended solids. For A3, it should demonstrate dilution integrity, upper-range performance, cross-reactivity, and documentation suitable for selecting confirmatory samples. For A4, it should report the calibration model, selectivity panel, repeatability and intermediate precision, measurement uncertainty when available, and the rule used to reconcile sensor and chromatographic results. The same platform may satisfy more than one application, but suitability must be demonstrated in the stated matrix rather than inferred from buffer calibration.

Paper devices also need analytical operation data that chemical studies often omit. Reagent zones should be tested after storage at realistic humidity and temperature. Flow time should be measured across paper lots. Sample volume tolerance should be reported because on-site users may not deliver an exact pipetted volume. Color zones should be

protected from ambient contamination and evaporation. Electrochemical paper devices should state whether the electrode remains wet, whether the reference electrode is printed or external, and how long the open-circuit potential stabilizes before measurement. None of these tests require expensive instrumentation, but together they distinguish a proof-of-concept paper device from a reliable on-site analytical test.

The analytical procedure in Figure 6 includes an invalid test output because invalid results are part of reliable monitoring. The procedure does not require every device to combine optical and electrochemical detection. A single-channel assay may be adequate for A3 spill triage or A2 rapid screening when its matrix controls pass, whereas a sequential two-method procedure is more useful near a decision threshold because optical screening can flag turbidity or color interference and electrochemical measurement tests an independent transduction pathway. An on-site device should refuse to report a concentration if turbidity exceeds the calibration range, the internal color reference is outside tolerance, the electrode baseline is unstable, or spike recovery fails. This rule reduces the number of reported values but increases confidence in the values retained.

Data handling is the final part of on-site analysis. A phone-connected or handheld reader can record time, location, weather notes, sample pretreatment, calibration batch, and raw signal. These records allow later comparison with rainfall, application date, and laboratory confirmation. They also allow a watershed program to distinguish sensor drift from real concentration changes. Future glyphosate sensors should therefore be designed as measurement systems rather than isolated materials. The sensing material remains important, but the complete analytical system includes sampling, pretreatment, controls, readout, metadata, and confirmation rules. This view is consistent with analytical validation guidance, biosensor definitions, electrochemical sensor reviews, and pesticide biosensor experience, all of which treat LOD as one part of a controlled measurement process rather than a complete method claim [31–34,71,107–111].

7.5. Reporting Benchmarks for Comparative Tables

The five tables use a common analytical reporting scheme. LOD is retained in the originally reported unit when direct conversion would hide matrix assumptions. Linear range is reported beside LOD because a low LOD with a narrow range may require dilution or repeat measurement for runoff samples collected after application. Reaction time is distinguished from total analysis time, which also includes filtration, dilution, incubation, imaging, and data processing. Recovery and RSD are treated as indicators of on-site analytical performance rather than supplementary values. A sensor with 95–105% recovery in real surface water is more convincing for that matrix than a lower-LOD sensor validated only in deionized water.

This benchmark logic should be extended in future glyphosate reviews. Each row should state the target concentration range or decision threshold for which the method is suitable: trace surveillance, event screening, spill triage, or laboratory-adjacent confirmation. Each figure of merit should be tied to a matrix. A LOD in buffer, a LOD in river water, and a LOD in soil extract are different claims. Each device should also report what happens when the assay fails. Optical assays need invalid image rules, electrochemical assays need baseline rejection rules, and paper devices need flow failure rules. These details make comparison less flattering but more useful.

The strongest future studies will publish not only calibration plots but also on-site analytical protocols. A useful protocol specifies sample bottle material, holding time, filtration choice, pH adjustment, calibration frequency, spike level, acceptance criteria, and confirmatory method. It includes real runoff samples collected during rainfall rather than only spiked tap water and reports negative observations such as matrix suppression

or electrode fouling. These details help other groups design stronger assays and make aptamer and nanozyme sensors credible to environmental analysts who require defensible data rather than materials performance alone.

8. Conclusions

Glyphosate sensing has moved beyond single-format proof-of-concept assays. Aptamer–MOF systems, nanozyme colorimetry, fluorescence recovery, SERS, MIP electrodes, MOF/clay composites, paper microfluidics, and handheld readers cover a wide range of LODs and operating formats [30,37–57,65–77,88–98]. No platform dominates all analytical criteria. Optical and colorimetric methods are simple and visible, but they require controls for turbidity, illumination, and metal competition. Electrochemical sensors offer low LODs and compact instrumentation, but electrode fouling, electrolyte dependence, and surface history remain major risks during on-site analysis. Paper-based devices can reduce sample volume and simplify disposal, but glyphosate/AMPA separation, reagent storage, and matrix-matched calibration still limit performance.

The strongest near-term path is a tiered analytical procedure rather than a universal sensor. A low-cost optical or colorimetric assay can identify high-risk samples during runoff events. A disposable electrochemical strip can quantify samples near a trigger range, and laboratory LC-MS/MS can confirm regulatory, contested, or near-threshold results. Aptamers and nanozymes reduce assay complexity within this procedure, but their results still require matrix spike validation, interference testing, and calibration transfer. Future studies should report both analytical figures of merit and operational figures of merit: LOD, range, total analysis time, recovery, RSD, sample volume, pretreatment, reader and power requirements, reagent stability, and invalid test criteria.

The next generation of glyphosate runoff sensors should be judged by whether they improve decisions during real runoff events. A method that produces a reliable moderate sensitivity result in 10 min can be more useful than one that reaches a lower LOD only in buffer. The analytical need is reliable screening, explicit uncertainty, and a path to confirmation. Sensors that combine matrix-matched sample preparation, independent readouts where justified, embedded quality controls, and transparent calibration are the strongest candidates for routine agricultural water monitoring.

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