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Research advances in SERS-based sensing platforms for multiplex mycotoxin detection in feed

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Mycotoxins in feed can pose significant risks to the health of livestock and poultry, leading to reduced economic returns and impaired production efficiency, thereby impeding the sustainable development of the livestock industry. Consequently, the exploration of highly sensitive, simple and rapid detection methods for trace mycotoxins in feed is crucial for ensuring feed safety and promoting industrial sustainability. Surface-enhanced Raman spectroscopy (SERS), a rapid detection method characterized by high sensitivity, ease of operation, and resistance to water interference, has gained substantial traction in mycotoxin detection within feed matrices in recent years. This review systematically summarizes the enhancement mechanisms, substrate types, and detection technologies of SERS, with a focus on analyzing the application cases and limitations of different substrates in the detection of mycotoxins in feeds. Additionally, it explores the joint application strategies of SERS with other technologies. Future research should focus on the development of low-cost substrates, anti-interference design for complex matrices, and integration of portable devices, so as to promote SERS technology to become the core solution for on-site rapid detection of feed safety.

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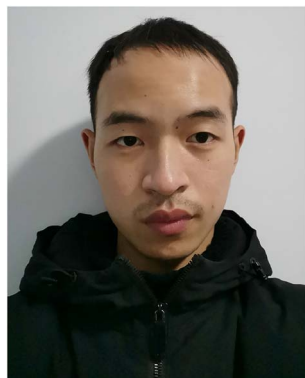
1 Introduction

Mycotoxins are toxic secondary metabolites produced by filamentous fungi. They are typically generated in warm and humid

environments and are widely present in feed raw materials. Common mycotoxins include ochratoxins (OTs), deoxynivalenol (DON), T-2 toxin (TS), aflatoxins (Afs), zearalenone (ZEN), and fumonisins (FBs).¹ Once mycotoxins enter the animal body, they can trigger a series of adverse reactions, negatively affecting the health and production performance of animals.² Typically, in feed raw materials, there often exists a phenomenon where two or more mycotoxins co-exist. Their synergistic or additive effects

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may pose greater harm to animals.³ According to recent survey results, 30% to 100% of food and feed samples are globally contaminated by multiple mycotoxins.⁴ Mycotoxin contamination results in annual economic losses exceeding tens of billions of US dollars. The significant economic losses incurred by the livestock industry due to mycotoxin contamination have positioned this as a critical feed-safety issue garnering global attention. Currently, the treatment methods for mycotoxins in feed mainly include physical reduction, chemical reduction, and biological reduction.⁵ However, most mycotoxins are stable compounds and are difficult to be completely removed during the actual crop processing.⁶ Therefore, to reduce the harm of mycotoxins to animals, countries have begun to introduce a series of policies to test feed raw materials and finished feeds to ensure that their mycotoxin content is within the standard values. Currently, conventional detection methods for mycotoxins mainly include high-performance liquid chromatography (HPLC),⁷ high performance liquid chromatography-mass spectrometry (HPLC-MS),⁸ and thin-layer chromatography (TLC),⁹ with chromatographic techniques being the most widely applied. These methods have become essential for laboratory detection of mycotoxins due to their high accuracy and sensitivity. However, they exhibit significant limitations in practical applications: on one hand, they rely heavily on large precision instruments, which not only occupy substantial space and incur high purchase and maintenance costs, but also require complex operational procedures that can only be performed by professional technicians in laboratory settings. On the other hand, the detection cycle from sample collection, submission to obtaining final results is lengthy, causing detection outcomes to lag behind feed production, circulation, and usage processes. Once mycotoxins in feed exceed the standard, toxins are highly likely to enter the farming stage along with the feed during the long detection period. This can potentially lead to animal poisoning, reduced production performance, and even threaten human health through the food chain, thereby triggering food safety crises. Therefore, on-site rapid detection technology for mycotoxins in feeds is of paramount importance.

Currently, on-site rapid detection methods for mycotoxins in feeds mainly include colloidal gold immunochromatography assay (GICA) and portable ELISA test kits. GICA offers advantages such as simple operation and no requirement for instruments, enabling rapid determination of toxin presence *via* colorimetric bands, which makes it suitable for batch screening. However, this method only allows qualitative or semi-quantitative analysis, failing to provide precise quantitative data.¹⁰ Portable ELISA test kits, capable of both qualitative and quantitative detection of mycotoxins with detection limits reaching those of laboratory ELISAs, suffer from operational limitations. Their procedures involve multiple steps including sample extraction, incubation, washing, and colorimetry, requiring training for on-site personnel. This significantly restricts the efficiency and convenience of on-site testing, still falling short of meeting the practical needs for field detection.¹¹

Surface-enhanced Raman spectroscopy (SERS), as a novel rapid detection technique, can enhance the intensity of Raman signals by a factor of 10^{10} to 10^{15} through enhancement substrates of different materials,¹² overcoming the drawbacks of conventional Raman spectroscopy such as low sensitivity and weak signal intensity. Compared with other detection techniques, SERS also has advantages such as rapid detection, non-destructive testing, and immunity to the influence of water molecules.¹³ With the continuous development of nanotechnology and new materials, various novel SERS substrates with higher enhancement factors have been emerging. When these substrates are sufficiently contacted with mycotoxin samples for testing and combined with portable Raman spectrometers, an on-site rapid detection system can be constructed to achieve instant analytical detection of mycotoxins. This paper briefly introduces the SERS technique and its detection methods. It focuses on reviewing the applications of different SERS detection substrates for the detection of mycotoxins in feed. Moreover, it summarizes the existing problems and prospects for future development, providing a theoretical basis and technical support for the future application of SERS in the detection of mycotoxins in feed.



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2 Overview of SERS

Raman spectroscopy is a type of scattering spectroscopy, which is formed when incident light irradiates the surface of a substance and causes a scattering effect. Due to the fact that substances with different structures generate distinct molecular vibrations and rotations, a unique "fingerprint spectrum" is formed, which can serve as a basis for substance identification.¹⁴ However, its Raman signal is relatively weak and difficult to detect, thus limiting its application. In the 1970s, researchers discovered that rough noble metal surfaces have the effect of enhancing Raman signals, which attracted extensive attention from scientists. A large number of studies were carried out on this enhancement mechanism and explanations were provided, leading to a breakthrough in the research of Raman spectroscopy. The Raman spectrum obtained from this enhancement effect is called SERS.^{15,16}



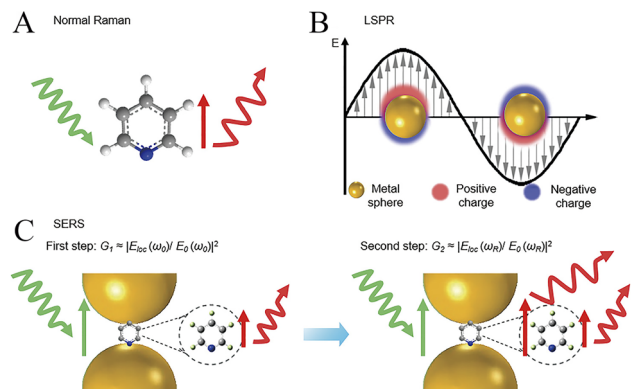


Fig. 1 Schematic diagrams of the electromagnetic enhancement mechanism of SERS.²⁴ (A) Normal Raman scattering, (B) LSPR enhancement mechanism, and (C) electromagnetic enhancement.

2.1 The enhancement mechanism of SERS

Although the SERS technique has been widely applied in many fields, its complex enhancement mechanism has not been thoroughly studied yet. Currently, there are two mechanisms widely recognized by the scientific community: the physical enhancement mechanism and the chemical enhancement mechanism.¹⁷ The physical enhancement mechanism, also known as the electromagnetic field enhancement mechanism, means that when light irradiates the metal surface, it causes the collective oscillation of free electrons on the metal surface. When the frequency of the electromagnetic wave approaches the oscillation frequency of free electrons, surface plasmon resonance (SPR) occurs. This phenomenon enhances the intensity of the electromagnetic field and improves the Raman signal of molecules adsorbed on the metal surface. When this resonance is confined to a nanoscale region, it is termed localized surface plasmon resonance (LSPR) (Fig. 1A and B), and its enhancement effect can reach 10^{14} .^{18,19} In addition, the nanogap between plasmonic nanostructures, where a significant enhancement of the local electromagnetic field can occur, is also referred to as a “hot spot” (Fig. 1C). This can greatly increase the intensity of the molecular Raman signal.²⁰ Research shows that the number of molecules in the hot-spot region accounts for less than 1% of the total number of molecules, yet contributes more than 50% of the Raman signal.²¹ The mainstream view of the chemical enhancement mechanism is that it is achieved through charge transfer. Upon laser irradiation, charge transfer occurs between the SERS-active substrate and the molecules on its surface, thereby changing the polarizability of the system and enhancing the Raman signal (Fig. 2A and B).²² The enhancement of the Raman signal is generally considered to be the combined effect of physical and chemical enhancements, with physical enhancement often contributing more.²³

2.2 SERS substrates and enhancement mechanisms

The SERS detection substrate is of great importance for the Raman enhancement of molecules. However, for the same

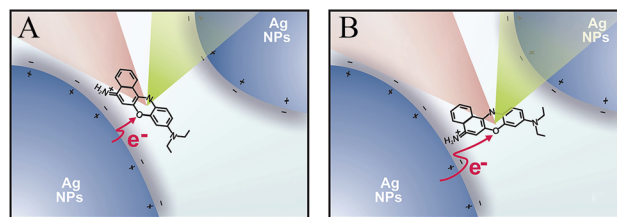


Fig. 2 Charge transfer enhancement mechanism of SERS.²⁵ (A) When the NB molecules are in close proximity to the surface of AgNPs, it facilitates charge transfer, resulting in a relatively small negative reduction potential. (B) When the NB molecules are farther away from the surface of AgNPs, the charge transfer efficiency decreases, and the reduction potential may become more negative.

analyte, the enhancement factor (EF) of different detection substrates varies, being controlled by the material, shape, and spacing of the substrate.²⁶ Currently, the materials used to fabricate SERS substrates can be classified into noble metal nanomaterials and non-metal nanomaterials. Due to the fact that the plasmon resonance regions of noble metal gold and silver nanomaterials cover most of the visible and near-infrared wavelength ranges, they are the most widely studied materials in SERS substrates.²⁷ Moreover, noble metal nanoparticles in different morphologies have been reported to exhibit significant SERS enhancement, including gold nanospheres, gold nanoprisms, gold nanostars, *etc.*^{28–30} Non-metal materials are generally semiconductor materials or two-dimensional materials.^{31,32} However, their enhancement effects are relatively weak, and these materials are often not used alone. Additionally, different methods are employed to fabricate ideal and controllable SERS substrates, such as nanosphere lithography, electrochemical deposition, self-assembly techniques, *etc.*^{33–35}

Commonly used SERS substrates can be broadly classified into colloidal substrates and solid substrates. Colloidal substrates possess advantages such as simple fabrication, high sensitivity, and low cost.³⁶ The tips and intervals of different nanoparticles can give rise to significant SERS enhancement. Therefore, to improve the detection intensity of sol-based nanoparticles, we often control the salt concentration to induce the aggregation of nanoparticles, thus shortening the distance between them, or fabricate nanoparticles with tips and edges to achieve enhancement and lower detection limits.³⁷ However, this aggregation is uncontrollable, which can lead to non-uniform hotspots and variations in the number of hotspots, thereby reducing the detection stability and reproducibility.³⁸ In addition, colloidal substrates are also susceptible to environmental factors such as temperature, pH, and ionic concentration. To address these issues, scientists have attempted to immobilize nanoparticles on solid substrates to enhance the stability and reproducibility of SERS substrates.³⁹ Commonly used nanoparticle assembly techniques include chemisorption, physisorption, capillary-driven assembly and surface-energy-driven assembly, *etc.*⁴⁰ The forces between the surface functional groups of nanostructures and nanoparticles are utilized to control the formation of highly ordered, dense, and reproducible nanoarrays. Compared with colloidal substrates, solid substrates possess advantages such as high sensitivity, good uniformity, and strong stability. However,



they are more difficult to prepare and still have some problems to be resolved. Therefore, designing controllable high-density hot-spot substrates, confining analytes within the hot-spot range, and enhancing Raman signals through highly dynamic hot spots are of great significance for the development of SERS technology.⁴¹

2.3 SERS-based detection techniques

The detection techniques of SERS can be classified into direct detection techniques and indirect detection techniques. The principle of direct detection techniques is that the analyte directly approaches or contacts the enhanced substrate to obtain an enhanced Raman signal, which is then analyzed based on the resulting spectrum. It has advantages such as simple preparation and convenient operation. However, it is vulnerable to interference from other factors in the medium, thus affecting the analysis and judgment of the results.⁴² To address this issue, indirect detection techniques are often employed. The principle involves modifying SERS substrates with signal molecule and conjugating them with specific recognition elements such as antibodies, aptamers, or molecularly imprinted polymers to synthesize SERS nanotags. These nanotags enable specific recognition of target toxins, thus achieving highly sensitive and selective detection of mycotoxins. This approach effectively minimizes interference from other substances in samples, significantly enhancing the accuracy and reliability of detection.⁴³ However, challenges such as difficulty in nanotag preparation and high costs remain key issues to be addressed for on-site mycotoxin detection in feeds.

3 Research on the application of SERS in the detection of mycotoxins in feedstuffs

3.1 The direct detection technique of SERS

The direct detection technique of SERS has been widely applied in the field of mycotoxin detection due to its advantages such as

a simple fabrication process, low cost, and the ability to directly detect the signals of analytes. Generally, SERS substrates are mainly classified into two categories: colloidal substrates and solid substrates. Both achieve Raman signal enhancement through direct contact with or proximity to the surface. This section describes two types of substrates and summarizes them in Table 1. Although SERS enables single-molecule detection, it cannot directly detect the target analyte from complex samples and is susceptible to signal interference from other feed components.

3.1.1 Colloidal substrate. Due to the advantages of metal colloids, such as low cost, ease of preparation, easy modification, and high SERS activity, they have been widely used as SERS substrates.^{44,45} Among them, gold and silver nanoparticles are the most widely applied. Lee *et al.*⁴⁶ synthesized silver nanoparticle sols as SERS substrates for the rapid detection of Afs in corn. By combining the *k*-nearest neighbor algorithm and the multiple linear regression model, the limit of detection (LOD) was determined to be 13–36 $\mu\text{g kg}^{-1}$, and the limit of quantification (LOQ) was 44–121 $\mu\text{g kg}^{-1}$. Similarly, Liu *et al.*⁴⁷ utilized gold nanoparticle sols as SERS detection substrates. By optimizing the sample extraction method, they achieved rapid on-site detection of aflatoxin B1 (AFB1), with a LOD of 0.85 $\mu\text{g kg}^{-1}$, and the entire process took approximately 10 minutes. It is worth noting that the pH during the synthesis of metal nanoparticles can affect the SERS activity, which may be related to the roughness of the particles formed at different pH values. Studies have revealed that Ag nanoparticles synthesized under alkaline conditions exhibit higher SERS activity, enabling successful pg-level detection of ochratoxin A (OTA) and AFB1.⁴⁸ Additionally, other studies have revealed that the intensity of Raman signals is also related to the degree of nanoparticle aggregation. Yuan *et al.*⁴⁹ controlled the aggregation of Ag nanoparticles by using sodium chloride, generating ideal SERS activity and successfully detecting DON in corn, kidney bean, and oat samples. Likewise, Rojas *et al.*⁵⁰ found that the choice of

Table 1 Summary of direct detection technologies for SERS

Substrate type	Mycotoxins	SERS substrates	Sample	Limit of detection	Rate of recovery	Analysis time	Ref.
Colloidal substrate	AFs	Ag nanosphere	Maize	13–36 $\mu\text{g kg}^{-1}$	—	—	46
	AFB1	Au nanoparticle	Wheat, corn, protein feed	0.85 $\mu\text{g kg}^{-1}$	—	10 min	47
	OTA; AFB1	Ag NP@pH-11	Cocoa beans	2.6 (OTA), 4.2 (AFB1) pg mL^{-1}	98.58–108.44% (OTA), 96.96–109.22% (AFB1)	4.07 s (CARS-PLS), 2.5 min (GA-PLS)	48
	DON	Ag NPs	Corn, bean, oat	0.1, 1, 100 μM	—	—	49
	OTA	Ag NPs	Wine, wheat	—	—	—	50
	DON	Ag NCs@PDA	Pig feed	0.243 pg L^{-1}	—	—	51
Solid substrate	AFs	Ag nanorod array	Standard solution	5×10^{-5} (AFB1), 1×10^{-4} (AFB2), 5×10^{-6} (AFG1, AFG2) M	—	—	54
	AFB1	CuO@Ag microbowl array	Corn	4 pg kg^{-1}	—	—	55
	AFB1; ZON; DON	3D-Nanocauliflower (AuNPs@PDMS@AAO)	Maize	1.8 (AFB1), 47.7 (ZON), 24.8 (DON) ng mL^{-1}	94–110% (AFB1), 97.8–104% (ZON), 93–120% (DON)	—	56
	AFB1	AuNBPs-AAO	Peanuts	0.5 $\mu\text{g L}^{-1}$	103–111%	1 min	57



extractant could affect the aggregation of nanoparticles. When detecting OTA in wheat, compared with using a combination of salt and chloroform or ethyl acetate, employing pure chloroform as the extraction solvent can enhance the SERS signal of OTA in wheat samples. Generally, there are often many non-target analytes in a normal colloidal detection matrix. Bare metal nanoparticles are susceptible to the influence of other components in the colloid, which can compromise their enhancement performance. Therefore, we utilize a core-shell structure to coat non-metal materials on the nanoparticles, forming a protective layer to improve their stability and enhancement performance. It has been reported that Tegegne *et al.*⁵¹ coated a layer of ultrathin polydopamine on silver cubes (Ag NCs@PDA) to serve as a SERS substrate for the quantitative detection of DON in pig feed. Its EF was as high as 1.82×10^7 , and the LOD was as low as 0.243 pg L^{-1} , which was 1.8 times lower than that of bare Ag NCs. Moreover, it had good temporal stability and reproducibility. Even after being stored for three months, it could still maintain 88.24% of the original Raman intensity. Generally speaking, the development of colloidal substrates has great potential. However, the reproducibility and uniformity of SERS signals remain one of the major challenges to be overcome for colloidal substrates.

3.1.2 Solid substrate. To overcome the drawbacks of colloidal SERS substrates, we fabricated ordered nanoarrays through self-assembly on solid materials. This approach not only ensures the controllability of SERS hotspots but also enhances the uniformity and reproducibility of the enhancement substrates while achieving high sensitivity. The fabrication of solid SERS substrates can be accomplished either through direct deposition of colloidal nanoparticles onto solid substrates, or by template synthesis and nanofabrication of nanostructures directly on solid platforms.⁵² Oblique angle deposition is a novel thin-film deposition technique. By controlling the tilt and rotation of the substrate, special nanostructures can be obtained.⁵³ Previous studies have utilized oblique angle deposition (OAD) to fabricate AgNR array substrates and combined this with density functional theory (DFT) calculations to obtain SERS spectra of aflatoxins (AFs), successfully detecting AFB1, aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2). The substrates prepared by this method feature uniform nanostructures, minimal variations in binding sites, and high signal reproducibility, with inter-batch differences of less than 15%.⁵⁴ Additionally, the array is deposited under vacuum conditions, ensuring strong adhesion between the nanostructures and solid substrate, thus enabling reliable detection across diverse environmental conditions. However, the preparation of such substrates typically requires expensive vacuum deposition equipment and suffers from low vacuum efficiency, failing to meet the demands of high-throughput detection. Therefore, further optimization of this method is necessary to adapt it to on-site mycotoxin detection scenarios. Thus, researchers have turned their attention to cost-effective template-assisted methods. This approach primarily employs template materials as scaffolds, followed by forming SERS substrates with tailored morphologies through techniques such as nano-metal infiltration or deposition within

template pores. A study has proposed a simple and cost-effective method to fabricate photocatalytically active CuO@Ag microbowl arrays. The approach involves first performing colloidal lithography on a Cu foil, followed by sputtering Ag onto the array surface to form an Ag layer (Fig. 3A). The resulting array enables self-cleaning of adsorbed analytes under visible-light irradiation, allowing repeated SERS detections. When applied to detect AFB1 in corn samples, this method achieved a detection limit of 4 pg kg^{-1} .⁵⁵ Another low-cost template is anodic aluminum oxide (AAO), which is typically prepared from aluminum foil at low cost and enables large-scale fabrication. Li *et al.*⁵⁶ poured polydimethylsiloxane (PDMS) into an AAO membrane, forming PDMS@AAO after curing. Following removal of the aluminum substrate, Au nanoparticles were sputtered onto the array surface to create a unique 3D nanobroccoli SERS substrate, featuring a large contact area and uniform distribution of SERS hotspots (Fig. 3B). This substrate successfully detected three mycotoxins—AFB1, ZEN, and DON—in corn samples, with detection

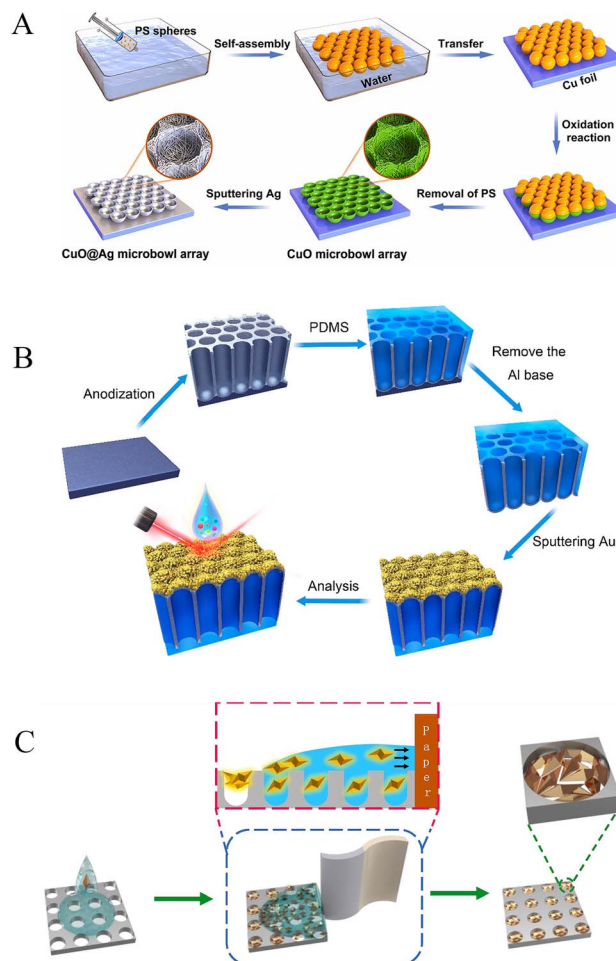
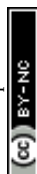


Fig. 3 Schematic illustrations for the preparation of partial solid SERS substrates. (A) Illustrates the process of constructing SERS substrates by direct deposition of colloidal nanoparticles onto solid substrates.⁵⁵ (B) and (C) show the preparation procedures for constructing SERS substrates via template-assisted methods.^{56,57}



limits of 1.8 ng mL^{-1} , 47.7 ng mL^{-1} , and 24.8 ng mL^{-1} , respectively. Another simpler “drip-dry” method involves self-assembling gold nanobipyramids into AAO (Fig. 3C), enabling the detection of mycotoxins in peanut extracts within 1 minute. While this approach has a detection limit of $0.5 \mu\text{g L}^{-1}$,⁵⁷ limiting its capability for low-concentration toxin analysis, its detection efficiency significantly surpasses that of traditional ELISA. This offers a reliable strategy for the development of on-site detection methods for feed mycotoxins. Additionally, nanolithography techniques can precisely fabricate solid SERS substrates by irradiating photoresists with light or electron beams to form patterns, followed by constructing periodic nanostructures *via* etching or deposition processes. SERS substrates prepared by this method exhibit excellent uniformity and enable precise regulation of hotspot distribution. However, no relevant studies have been reported on the direct detection of mycotoxins using solid SERS substrates. Similar to the oblique angle deposition method, this technique suffers from the drawback of requiring high-cost instruments for substrate preparation, thus rendering it inapplicable for current on-site mycotoxin detection. In summary, direct detection using solid SERS substrates demonstrates unique advantages in mycotoxin analysis, leveraging exceptional sensitivity, stability, and uniformity. When integrated with portable instruments, this approach facilitates the development of on-site rapid detection technologies for feed mycotoxins. However, direct detection with solid SERS substrates remains suboptimal for on-site mycotoxin testing. Lacking specific recognition elements, these substrates rely primarily on physical adsorption, which may lead to false positives due to nonspecific binding of structurally similar compounds. Moreover, the lack of Raman signal molecule modification in this structure often necessitates the use of complex chemometric methods for toxin detection, posing significant challenges to the on-site operation of mycotoxin detection and data processing capabilities.

3.2 Indirect detection technique of SERS

In a typical SERS detection process, to ensure the accuracy of detection, we usually need to go through a cumbersome sample-handling procedure. The target analyte has to be extracted before SERS analysis can be carried out. This process is time-

consuming and seriously hinders the development of rapid detection techniques. Therefore, to overcome the drawback of being unable to directly detect the target analyte in the sample, we modify or functionalize SERS substrates with capture media such as antibodies, aptamers, and molecularly imprinted complexes to fabricate SERS probes, thus enabling direct detection and analysis in complex samples. This section respectively describes the above-mentioned SERS sensors based on three types of recognition elements and summarizes them in Table 2. Due to the fact that previous studies on indirect detection have often focused excessively on sensitivity while paying less attention to detection time, the analysis time is not included in Table 2 for the time being. Theoretically, indirect detection methods require longer analysis time than direct methods. This is because they involve a necessary capture process for specific recognition elements, which is clearly disadvantageous for on-site mycotoxin detection. However, owing to direct detection methods are susceptible to interference from other matrices in the sample, indirect detection clearly holds greater advantages in terms of multiplex detection and sensitivity. But as shown in Tables 1 and 2, the low sensitivity of direct detection methods can be compensated for by preparing SERS substrates with high enhancement performance. However, based on a comprehensive consideration of previous studies, indirect detection remains the primary choice for on-site detection of mycotoxins.

3.2.1 Antibody-based SERS sensor. Antibodies, as a type of immunoglobulin, can specifically bind to antigens. By functionalizing nanoparticles, SERS immunoassays based on antigen–antibody reactions can be achieved, which significantly improves the selectivity and sensitivity of SERS detection. This type of SERS sensor is typically based on two types of immunoassays, namely the competitive immunoassay and the sandwich immunoassay. However, most mycotoxins are small-molecule compounds with limited antibody binding sites, making them suitable for competitive detection. Compared with the sandwich assay, the competitive method requires fewer antibodies, reducing antibody costs by 50%, which better meets the needs of on-site mycotoxin detection.

Liu *et al.*⁵⁸ developed a competitive SERS immunoassay for detecting ZEN in feed. The method involves labeling Au

Table 2 Summary of indirect detection technique of SERS

Recognition element	Mycotoxins	Signal molecule	SERS substrates	Sample	Limit of detection	Rate of recovery	Ref.
Antibody	ZEN	4,4'-Dipyridyl	AuNPs	Feed	1 pg mL^{-1}	99–105.2%	58
	AFB1; OTA; ZEN	Nile blue A	AuNPs	Rice wheat, corn	0.82 (AFB1), 1.43 (OTA), 1.00 (ZEN) pg mL^{-1}	70.35–118.04% (average)	59
Aptamer	OTA; ZEN	4-Mercaptobenzoic acid; 5,5'-dithiobis-(2-nitrobenzoic acid)	Au@Ag CS	Wheat, corn	0.018 (OTA), 0.054 (ZEN) ng mL^{-1}	$96.0\% \pm 3.1\%$ – $110.7\% \pm 4.2\%$ (OTA), $96.9\% \pm 2.6\%$ – $107.4\% \pm 3.9\%$ (ZEN)	61
	OTA; ZEN	4-Mercaptopyridine, 4-mercaptobenzonitrile	Au@AgNPs	Corn	0.94 (OTA), 59 (ZEN) ng mL^{-1}	92.9–106.6% (OTA), 93.13–118.38% (ZEN)	62
	FB1	Cyanine 5.5	AuNR	Corn	3 pg mL^{-1}	92–107%	63
MIPs	AFB1	Rhodamine 6G	TA-AgNPs MF	Peanut	$0.1 \mu\text{g L}^{-1}$	93–102%	64



nanoparticles with 4,4'-bipyridine and conjugating them with ZEN antibodies to form SERS probes, followed by modifying capture substrates with ZEN-BSA (Fig. 4A). This approach is applicable to various naturally ZEN-contaminated feeds, achieving a detection limit of 1 pg mL^{-1} and a dynamic range of $1\text{--}1000 \text{ pg mL}^{-1}$. Subsequently, Sun *et al.*⁵⁹ developed a SERS sensor based on silica photonic crystal microspheres for the detection of three mycotoxins: AFB1, OTA, and ZEN (Fig. 4B). The detection limits were 0.82 pg mL^{-1} , 1.43 pg mL^{-1} , and 1.00 pg mL^{-1} , respectively. Notably, compared with direct detection, the conjugation of recognition elements and modification with Raman signal molecules significantly improved the detection sensitivity of SERS sensors, enabling detection limits in the picogram per milliliter range. However, antibody-based SERS sensors also have certain limitations. Variations in antibody sources and batches may occasionally lead to false-positive results, which is an unavoidable challenge for all antibody-based recognition sensors.⁶⁰ This is primarily attributed to poor specificity during antibody purification processes. Nevertheless, antibody-based SERS immunosensors remain one of the optimal choices for trace determination of mycotoxins in feed.

3.2.2 Aptamer-based SERS sensor. Aptamers are short, artificial DNA or RNA chains that can be selected from an

artificially constructed nucleotide library through a technique known as the systematic evolution of ligands by exponential enrichment (SELEX). They possess a high degree of affinity and specificity for target substances. Especially in recent years, research on SERS sensors has trended toward using aptamers as specific recognition elements.

Aptamer-based SERS sensors mostly employ competitive assay principles for detection. Chen *et al.*⁶¹ designed and fabricated a SERS aptasensor for simultaneous detection of ZEN and OTA in wheat and corn samples (Fig. 5A). Using AuNRs as capture carriers and Au@Ag nanoparticles as SERS substrates, the sensor achieved detection limits of 0.018 ng mL^{-1} and 0.054 ng mL^{-1} for ZEN and OTA, respectively. Results were consistent with ELISA, demonstrating excellent detection performance. Subsequently, Xue *et al.*⁶² prepared a rigid SERS substrate (ITO/AuNPs/GO) and combined it with aptamer-functionalized Au@AgNPs. Through a special “competitive” approach, they

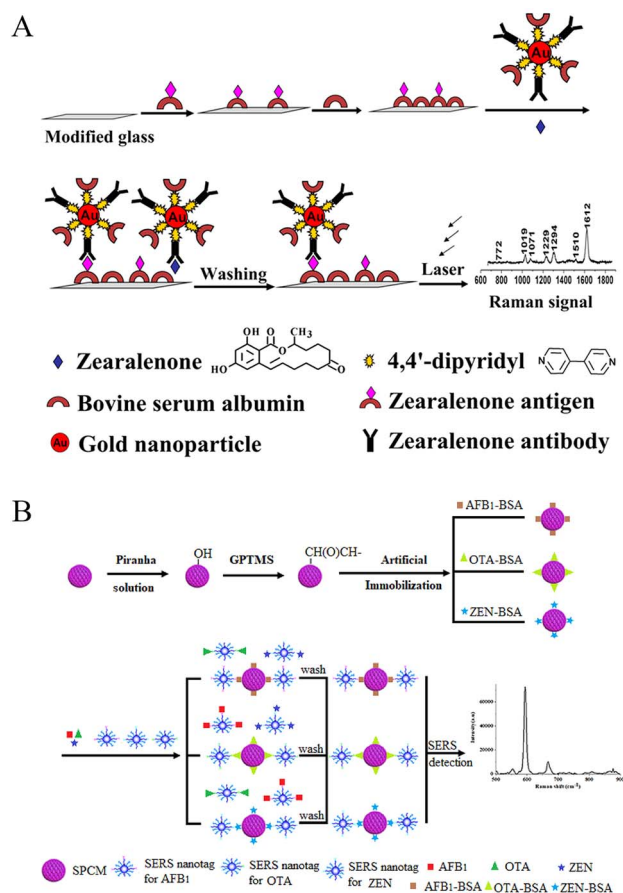


Fig. 4 Schematic diagram of mycotoxin detection using competitive immunoassay method. (A) Detection of single mycotoxin.⁵⁸ (B) Detection of multiple mycotoxins.⁵⁹

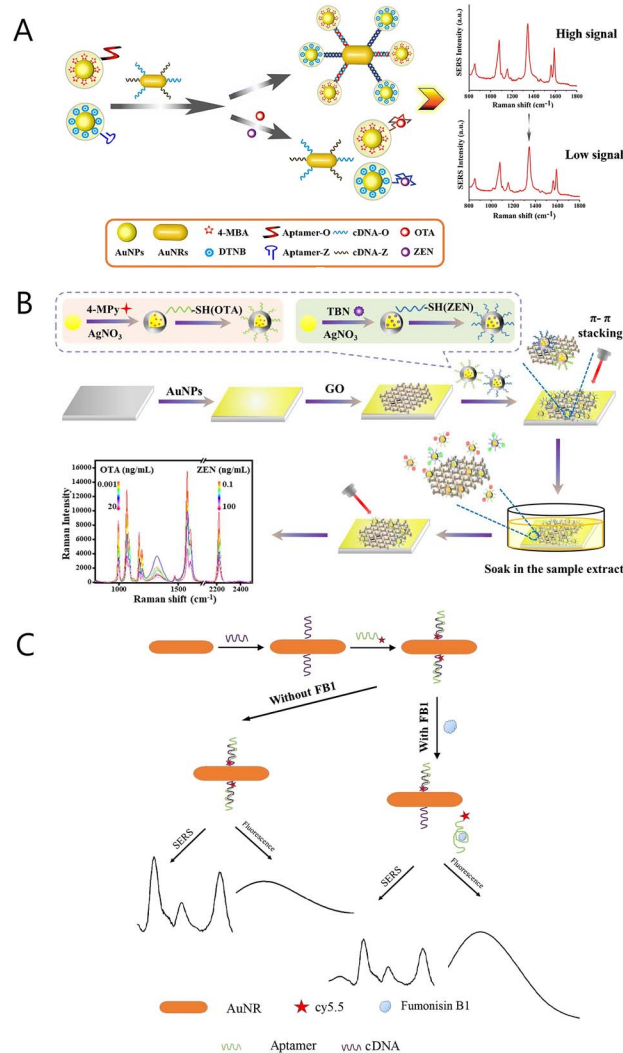


Fig. 5 Schematic diagram of aptamer-based SERS sensor for mycotoxin detection. (A) and (B) Employ competitive assay for detection,^{61,62} while (C) demonstrates dual-mode detection using both SERS and fluorescence methods.⁶³

achieved the detection of OTA and ZEN in corn. The principle is as follows: when mycotoxins are absent in the solution, aptamers on Au@AgNPs adhere to the GO surface *via* π - π stacking, generating strong SERS signals. When mycotoxins are present, they bind to their respective aptamers, leading to the dissociation of signal probes and weakening of SERS signals (Fig. 5B). The detection limits for OTA and ZEN were 0.94 pg mL^{-1} and 59 pg mL^{-1} , respectively. To ensure the accuracy of detection results, SERS aptasensors are often integrated with other methods to form dual-mode detection systems. For example, a combined SERS-fluorescence assay for FB1 detection involves immobilizing the complementary strand of the FB1 aptamer onto AuNRs, followed by the addition of Cy5.5-labeled FB1 aptamers. In the absence of FB1, the aptamers bind to their complementary strands, generating strong SERS signals and weak fluorescence. When FB1 is present, the aptamers dissociate from the complementary strands to bind the target, leading to a decrease in SERS intensity and an increase in fluorescence intensity that are dependent on concentration within a specific range (Fig. 5C). This enables quantitative determination of FB1 with a detection limit of 3 pg mL^{-1} .⁶³ When this method was used to determine FB1 in corn samples, the results showed no significant difference from those obtained by liquid chromatography/mass spectrometry (LC-MS) or mass spectrometry (MS). Compared with single-mode detection, this dual-mode assay exhibits higher reliability and accuracy. The detection limits of aptamer-based SERS sensors are comparable to those of antibody-based SERS sensors. However, aptamers maintain high specificity while offering advantages such as chemical stability and modifiability, making them particularly suitable for large-scale fabrication of portable and sensitive on-site detection sensors—advantages that antibodies lack. With the development of aptamer selection technologies and biosensors, their applications in on-site detection are expected to expand in the future.

3.2.3 SERS sensor based on molecularly imprinted polymers. Molecularly imprinted polymers (MIPs), emerging as specific recognition elements in mycotoxin detection in recent years, are artificially synthesized polymers prepared by mimicking the interactions of receptors and antibodies. Featuring multiple artificially created recognition sites, MIPs offer advantages such as high selectivity, good predictability, and versatility, demonstrating great potential in the capture and detection of target analytes.⁶⁴ Fan *et al.*⁶⁵ developed a molecularly imprinted-based SERS sensor for detecting AFB1 in feed. A molecular simulation method was selected to screen monomers for optimizing the synthesis of MIPs. *N*-Isopropylacrylamide (NIPAM) and 7-ethoxycoumarin (7-EOC) were used as monomers and a virtual template. With the aid of molecular design, the optimal monomers and the ratio of monomers to the template were obtained. Eventually, the resulting dummy molecularly imprinted solid-phase extraction (DMISPE) could be used for the separation and enrichment of AFB1. Moreover, a monolayer of lipioic acid-modified AgNPs constructed by self-assembly at the liquid-liquid interface was employed as the SERS-active substrate for quantifying DMISPE-eluted AFB1 (Fig. 6). When this sensor was used to detect AFB1 in peanuts,

high detection sensitivity was achieved. The linear concentration range was from $0.1 \text{ } \mu\text{g L}^{-1}$ to $10 \text{ } \mu\text{g L}^{-1}$, and the LOD was $0.1 \text{ } \mu\text{g L}^{-1}$. After the samples were treated by DMISPE, the adsorption recovery rate of AFB1 was greater than 88%. After 5 adsorption-desorption cycles, the adsorption capacity of DMISPE could still maintain 90.3% of its initial value. The application of SERS sensors based on MIPs in the detection of mycotoxins in feed is relatively scarce, which may be related to their low sensitivity, difficulties in synthesis and characterization, and poor site accessibility. Despite these limitations, as a specific recognition material with high stability and low production cost, molecularly imprinted polymers still remain prospective solutions in the detection of mycotoxins in feed.

3.3 Combination of SERS with other technologies

Despite its high sensitivity and rapid detection capabilities, SERS has limitations in mycotoxin analysis. Thus, integrating it with other technologies is necessary to meet on-site, rapid, accurate, and high-throughput detection demands. This section presents typical cases of SERS combined with chromatographic techniques, immunochromatography, and microfluidics, with relevant information summarized in Table 3. By combining with other technologies, SERS technology can compensate for its own limitations to varying degrees. In particular, the integration of SERS technology with lateral flow immunoassay technology not only preserves the high sensitivity inherent to SERS but also offers enhanced advantages in multiplex detection. In addition, it partly compensates for the long detection time. This combination may still be the mainstream direction for the detection of feed mycotoxins in the future.

3.3.1 Combination with chromatography techniques. The matrix in feed samples often has a complex composition. During SERS detection, complex pretreatment is usually required. Therefore, selecting an efficient separation technique will contribute to the rapid and accurate detection and analysis of target molecules in feed samples. Chromatography is a method that separates and analyzes substances based on the differences in their partition coefficients between the stationary phase and the mobile phase. It is commonly used for the separation of components in complex samples. By combining chromatography with SERS, we can achieve the goal of eliminating matrix interference and further improving the performance of SERS detection. Among the commonly used chromatography techniques are gas chromatography, liquid chromatography, and TLC. However, to date, only the

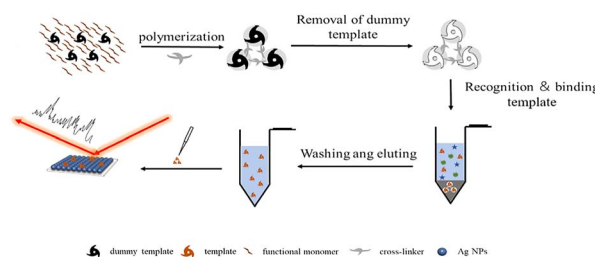


Fig. 6 Principle of SERS sensor detection based on MIPs.⁶⁵



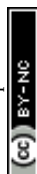
Table 3 Summary of combination of SERS with other technologies

Combined technology	Mycotoxins	Raman signal molecule	SERS substrates	Sample	Limit of detection	Rate of recovery	Analysis time	Ref.
Chromatography	AFs	—	AuNPs	Peanut	1.5×10^{-6} (AFB1), 1.1×10^{-5} (AFB2), 1.2×10^{-6} (AFG1), 6.0×10^{-7} (AFG2) M	87.5% $\pm$ 9.4% (AFB1), 92.7% $\pm$ 8.5% (AFG1), 114.0% $\pm$ 10.8% (AFG2)	5 min	66
Lateral flow immunoassay	FB1; AFB1; ZEN	5,5'-Dithiobis-(2-nitrobenzoic acid)	GO@Au–Au	Peanut, maize, river, and lake water	0.529 (FB1), 0.745 (AFB1), 5.90 (ZEN) pg mL ⁻¹	90.03–113.75% (average)	20 min	70
	AFB1; OTA	4-Mercaptobenzoic acid; 5,5'-dithiobis (2-nitrobenzoic acid)	Au@SiO ₂	Corn, rice, wheat	0.24 (AFB1), 0.37 (OTA) pg mL ⁻¹	91.0% $\pm$ 6.3–104.8% $\pm$ 5.6% (AFB1), 87.0% $\pm$ 4.2–112.0% $\pm$ 3.3% (OTA)	—	71
	AFB1; ZEA; FB1; DON; OTA; T2	4-Mercaptobenzoic acid; 5,5'-dithiobis-2-nitrobenzoic acid	Au@Ag NPs	Maize	0.96 (AFB1), 6.2 (ZEA), 0.26 (FB1), 0.11 (DON), 15.7 (OTA), 8.6 (T2) pg mL ⁻¹	83.2–106.2% (AFB1), 78.9–97.3% (ZEA), 81.1–104.5% (DON), 79.5–102.3% (FB1), 82.7–97.7% (OTA), 81.3–100.5% (T2)	20 min	72
Microfluidic	OTA	—	Au triangle array	Standard solution	—	—	—	76

combination of TLC and SERS has been validated for use in feed analysis. Qu *et al.*⁶⁶ successfully separated four different Afs using TLC. Subsequently, Au colloid was used as the SERS-active substrate, and a small portable Raman spectrometer was employed to quantitatively determine the mycotoxins. The established TLC-SERS system demonstrated extremely high selectivity and was able to successfully identify aflatoxins in complex peanut samples. Although the application of each chromatography technique has certain limitations, through increased research on the combined application of chromatography and SERS techniques in the future, the advantages of both can be fully exploited to achieve highly sensitive and high-resolution analysis of samples.

3.3.2 Combination with lateral flow immunoassay technology. The on-site detection of feed often requires rapid and multiplex testing. Immunochromatography assay (ICA), as a point-of-care testing (POCT) technology, offers advantages such as convenience, timely feedback, simplicity, and low cost.⁶⁷ However, although AuNPs are the most widely used signal tags in the ICA system, the weak colorimetric signal output and lack of quantification ability pose serious obstacles to the sensitive multi-target detection by ICA.^{68,69} However, as a technique with trace-level detection capabilities, SERS, when combined with ICA, enables the rapid, quantitative, and multiplex detection of on-site feed. However, antibody-based specific recognition elements are still employed in the detection process, where antibodies or antigens of target toxins are immobilized on test lines to enable sandwich or competitive assay formats. Multiplex detection using SERS sensors based on immunochromatography generally involves two strategies. One strategy utilizes SERS probes modified with a single Raman signal molecule for a single test line, and achieves multiplexing by increasing the number of test lines. For example, Zheng *et al.*⁷⁰ coated capture antigens of AFB1, FB1, and ZEN onto

three separate T lines of a nitrocellulose membrane, conjugated their respective antibodies to SERS nanotags, and employed a competitive assay for detection (Fig. 7A). The resulting LODs for FB1, AFB1, and ZEN were 0.529 pg mL⁻¹, 0.745 pg mL⁻¹, and 5.90 pg mL⁻¹, respectively, with the entire assay completed within 20 minutes. The other approach employs SERS nanotags modified with two types of Raman signal molecules for detection on a single test line, and enables multiplexing by increasing the number of test lines. Chen *et al.*⁷¹ co-immobilized two capture antigens, AFB1-BSA and OTA-BSA, onto a single test line (T-line), while modifying anti-AFB1, anti-OTA antibodies, and Raman signal molecules onto Au@SiO₂ nanoparticles to form SERS nanotags with silica shells (Au^{4-MBA}@SiO₂ and Au^{DTNB}@SiO₂) (Fig. 7B). This strategy enabled simultaneous detection of the two mycotoxins, achieving LODs of 0.24 pg mL⁻¹ for AFB1 and 0.37 pg mL⁻¹ for OTA, with the entire assay completed in approximately 15 minutes. Whereas Zhang *et al.*⁷² developed a SERS-LFIA sensor with dual Raman signal molecules and triple test lines for simultaneous detection of six major mycotoxins. They designed three combinations of capture antigens: AFB1-BSA & ZEA-BSA, DON-BSA & FB1-BSA, and OTA-BSA & T2-BSA, which were respectively coated onto three T-lines of a nitrocellulose membrane (NC). Anti-AFB1, anti-FB1, and anti-OTA antibodies were individually modified onto DTNB-Au@Ag NPs, while anti-ZEA, anti-DON, and anti-T2 antibodies were separately conjugated to MBA-Au@Ag NPs to form nanoprobe (Fig. 7C). Under this sensor configuration, the LODs were 0.96 pg mL⁻¹ for AFB1, 6.2 pg mL⁻¹ for ZEN, 0.26 ng mL⁻¹ for FB1, and 0.11 ng mL⁻¹, 15.7 pg mL⁻¹, and 8.6 pg mL⁻¹ for DON, OTA, and T2, respectively. By integrating with immunochromatographic assay (ICA) technology, SERS sensors demonstrate enhanced sensitivity, cost-effectiveness, and multiplex detection capabilities, with assay times reduced to under 20 minutes. These



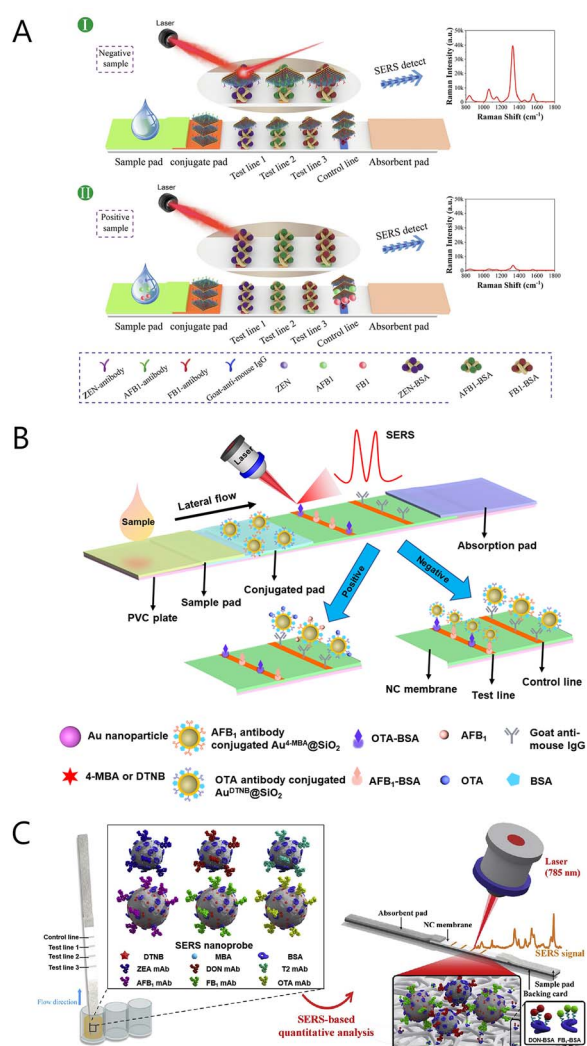


Fig. 7 Schematic diagram of the combination of SERS technology and lateral immunochromatography technology. (A) Achieves multiplex detection using a single Raman signal molecule.⁷⁰ (B) and (C) Realize multiplex detection with two Raman signal molecules.^{71,72}

attributes render them highly promising for on-site, high-throughput screening of feed contaminants.

3.3.3 Combination with microfluidic technology. Microfluidic systems, also known as “labs-on-a-chip”, operate on the principle of integrating all necessary procedures onto a chip. By manipulating fluids in micro-scale channels, they enable low-dose, high-efficiency, and precise analysis of samples.^{73,74} Integrating SERS technology with microfluidic technology can provide an efficient, cost-effective, and portable tool for the detection field. Therefore, the primary challenge we face is to effectively integrate SERS-active substrates into microfluidic systems. Currently, there are three methods for integrating substrates into microfluidic platforms: injecting colloidal nanoparticles into microfluidic channels; constructing solid nanostructures within microfluidic channels; and *in situ* fabrication of nanostructures in microfluidic channels. These three integration methods are commonly referred to as “external injection”, “built-in”, and “*in situ* fabrication” respectively.⁷⁵

Currently, in the detection of mycotoxins, only the construction of solid nanostructures in microfluidic channels has been achieved for the detection of OTA. By designing 2D gold triangle nanostructures and modifying them with OTA adapter sequences, embedding the gold triangle array into microfluidic chips to achieve OTA recognition.⁷⁶ The SERS-microfluidic system has yielded numerous research results in other fields and has been analyzed and validated.⁷⁷ For example, Andreou *et al.*⁷⁸ mixed Ag nanoparticles with the analyte and injected it into the microfluidic channel. The mixing process was controlled by a vacuum microfluidic device to generate a favorable SERS signal, thereby successfully detecting ampicillin in milk. Yan *et al.*⁷⁹ proposed a two-step photochemical reduction method. By using a focused laser beam to synthesize Ag nanoparticle aggregates in the microfluidic channel as the SERS substrate, they successfully detected various molecules including crystal violet, rhodamine 6G, and methylene blue, achieving *in situ* single-molecule spectroscopy detection technology on the microfluidic system. As an advanced detection and analysis platform, the SERS-microfluidic system demonstrates great potential in trace detection and real-time monitoring, which has important guiding significance for the future detection of mycotoxins in feed.

4 Problems still existing in the practical application of SERS

4.1 Sample processing

As a complex matrix, feed contains numerous interfering factors that compromise detection results, leading to reduced sensitivity or false positives in recognition elements. To improve the LODs, rigorous sample pretreatment steps are often necessary to ensure the accuracy and sensitivity of SERS. Traditional pretreatment methods mainly include solid-phase extraction (SPE) and liquid-liquid extraction (LLE). Although SPE demonstrates remarkable purification efficiency, its cumbersome operation steps hinder integration with on-site SERS detection systems. Meanwhile, extraction solvents used in LLE, such as acetonitrile, chloroform, and dichloromethane, exhibit significant efficiency in mycotoxin extraction but pose risks to human health and the environment due to their toxicity. Therefore, exploring simplified SPE protocols and developing eco-friendly extraction reagents are of great significance for promoting the practicality and safety of on-site rapid detection technologies.

4.2 Portable devices

In the past, trace-detection instruments such as liquid chromatographs (LC), gas chromatographs (GC), and large-scale Raman spectrometers were bulky and required operation by professional technicians. This situation severely restricted the development of on-site rapid detection technologies. The emergence of portable Raman spectrometers has significantly compensated for the defect that Raman spectrometers cannot be carried on-site, demonstrating the great potential of SERS technology in on-site mycotoxin detection. However, the

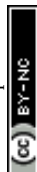


Table 4 Maximum residue limits (MRLs) in feed as stipulated by China and the European Union

	AFB1	OTA	ZEN	DON	T-2	FBs
MRLs (GB 13078-2017) ^a	10 $\mu\text{g kg}^{-1}$	100 $\mu\text{g kg}^{-1}$	500 $\mu\text{g kg}^{-1}$	1000 $\mu\text{g kg}^{-1}$	500 $\mu\text{g kg}^{-1}$	60 000 $\mu\text{g kg}^{-1}$
MRLs (EC NO 1881/2006) ^b	20 $\mu\text{g kg}^{-1}$	5 $\mu\text{g kg}^{-1}$	100 $\mu\text{g kg}^{-1}$	8000 $\mu\text{g kg}^{-1}$	10 $\mu\text{g kg}^{-1}$	1000 $\mu\text{g kg}^{-1}$

^a The MRLs are stipulated in the Chinese Feed Hygiene Standards. The minimum limits vary according to different types of feeds, and the values listed in this table are all the minimum limits for feed raw materials. ^b The MRLs are mainly based on Regulation (EC) No. 1881/2006 and relevant amendments. The minimum limits vary depending on the types of livestock and poultry, and all the values listed in this table are the minimum limits for different livestock and poultry.

accuracy and sensitivity of portable instruments remain lower compared to laboratory instruments, and there is still much room for improvement in hardware. Additionally, how to combine portable devices with algorithms for processing spectral information and cloud-based spectral databases to achieve on-site qualitative and quantitative visual analysis of toxins is also a major challenge we will face in the future.

4.3 Analysis time

On-site feed detection often requires high-throughput and rapid operations. Prolonged detection times for feed raw materials can exacerbate the risk of mold contamination, thereby compromising the overall quality and yield of the feed and leading to significant economic losses. However, most SERS-based sensors require a certain incubation time with the sample before detection can be performed. Chen *et al.*⁸⁰ employed pre-etched silver nanoclusters for SERS detection of AFB1 in peanut oil, which required an incubation period of 10 hours with the sample prior to detection. Hahm *et al.*⁸¹ synthesized SiO₂@Ag core-shell nanoparticles for the detection of alternariol (AOH), which necessitated an incubation time of 12 hours with the sample before detection. Clearly, prolonged testing is detrimental to the on-site determination of mycotoxins. Consequently, SERS technology still offers considerable potential for reducing analysis time.

4.4 Multiplex detection

During feed production and storage, suitable environmental humidity and temperature conditions facilitate the growth of various molds, leading to complex contamination scenarios where multiple mycotoxins often co-occur. For example, AFB1, ZEN, and OTA commonly exist in composite pollution, and the toxic effects of such multiplex contamination are typically more potent than single toxins, posing greater threats to livestock health and food safety. Therefore, on-site multiplex detection of mycotoxins is not only a key means to trace contamination sources and assess risk levels but also a necessary link in feed quality and safety control. Notably, while significant progress has been made in the development of mycotoxin sensors for feed, with some single-toxin detection technologies achieving sensitivities as low as the fg mL⁻¹ level, which is far below the safety thresholds set by China and the EU (Table 4), this research trend of excessive pursuit of ultra-high sensitivity for single toxins is disconnected from the reality of multi-toxin coexistence in actual feed contamination scenarios. Shifting

the research focus from ultrasensitive single-toxin detection to the construction of simultaneous multiplex mycotoxin detection systems can thus reduce sample pretreatment losses, enhance result reliability through cross-validation, and ultimately provide more comprehensive and efficient technical support for feed safety supervision.

5 Summary and outlook

Feed safety is of utmost importance to the entire livestock industry. As a novel detection technique, SERS has advantages such as rapid detection, non-destructiveness, and immunity to water interference, showing great potential in the field of feed detection. This paper reviews different SERS substrates for the detection of mycotoxins in feed, demonstrating that SERS substrates are diverse and extremely flexible in use. They can be selected according to the needs of the detection. Moreover, by combining with different techniques, the shortcomings of SERS can be compensated, further improving its detection performance and enabling rapid and non-destructive detection of mycotoxins in various feed samples. Currently, demands for on-site rapid mycotoxin detection still center on low cost, rapidity, high sensitivity, and multiplexity. Lateral flow chromatography integrated with SERS sensors largely meets these requirements among existing technologies. However, further optimization of this approach is warranted.

(1) Employ aptamers as recognition elements. Aptamers are employed as recognition elements due to their comparable binding performance to antibodies, while their nucleic acid nature endows them with superior stability, facilitating large-scale preparation and storage. In terms of preparation processes, aptamers are obtained *via in vitro* SELEX technology and chemical synthesis, eliminating the need for animal immunization and thus avoiding batch instability caused by individual animal variations. This production model not only shortens the Research and Development cycle but also reduces preparation costs, enabling aptamers to show higher technical feasibility and economic rationality in both basic laboratory research and on-site detection applications.

(2) Adoption of low-cost nanomaterials and optimized synthesis processes. Current SERS substrates are predominantly made of noble metals like Au and Ag, especially enhanced substrates with specialized structures (*e.g.*, tips and gaps). These not only rely on expensive raw materials but also involve complex fabrication processes often using toxic reagents, restricting the large-scale application of SERS



technology. To address this bottleneck, innovations in both alternative material development and synthesis process optimization are urgently needed. For materials, metal oxides or composite metal materials can be explored to replace traditional Au/Ag. In terms of processes, precise and controllable methods should be adopted to regulate experimental parameters, ensuring uniform nanoparticle size and reducing material waste caused by batch inconsistency. Additionally, promoting green synthesis technologies to avoid toxic reagents can lower production costs and environmental risks. Through dual innovations in materials and processes, SERS technology is expected to transition from laboratory research to industrialized applications.

(3) Development of an integrated device combining a microfluidic chip with a portable Raman spectrometer. This device integrates three core functional modules: sample pretreatment, lateral flow chromatography separation, and SERS detection. The microfluidic chip performs sample extraction, purification, and enrichment; lateral flow chromatography achieves separation and concentration of target toxins; and the SERS detection module provides highly sensitive quantitative analysis. Through their collaborative operation, the device simplifies the traditional complex laboratory detection process into a one-stop “sample-in-result-out” operation, significantly reducing detection time and operational threshold to meet the on-site rapid detection needs in feed production, storage, and distribution.

From laboratory Research and Development to field applications, SERS technology still faces numerous challenges in the detection of mycotoxins in feed. Sustained breakthroughs in cost control, anti-interference design for complex matrices, and integration of portable devices are essential to propel SERS technology as the core solution for on-site rapid testing of feed safety in the future.

Conflicts of interest

There are no conflicts to declare.

Data availability

This manuscript does not involve any experimental work.

Acknowledgements

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