Surface-Enhanced Raman Scattering Sensing of Transition Metal Ions in Waters

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ABSTRACT: In this mini-review, we provide a coherent discussion on the sensing schemes exploited in the surface-enhanced Raman scattering (SERS) analysis of transition metal ions in waters. A critical approach was used where illustrative examples are selected to discuss key drawbacks and challenges associated with various experimental configurations and the employed enhancing substrates.

INTRODUCTION

Recent years have witnessed growing ecological and public health concerns associated with transition metal contamination. Although these metals are naturally occurring elements, anthropogenic activities have caused a dramatic increase in human exposure. Furthermore, even when transition metals are essential nutrients for multiple biochemical and physiological functions, acute and chronic exposures can cause major adverse health effects, including the development of neurodegenerative and cancer diseases.

The extent of their toxicity and carcinogenicity is strictly related to the type and speciation of the transition metal, as well as exposure time and dose. Accordingly, their maximum allowable concentrations in the environment significantly vary from element to element. However, guidelines for drinking water quality, recommended by the World Health Organization, the European Union, or the Environmental Protection Agency, typically set maximum contaminant limits between the pm and ppb regimes.

A great effort has been devoted to the exploitation of the unique properties of nanoscale materials for the design of advanced analytical tools fulfilling sensitivity, selectivity, and reproducibility requirements while simultaneously enabling their implementation into cost-effective miniaturized devices for real-time monitoring of these pollutants. A paradigmatic example of a nanoscale analytical technique is surface-enhanced Raman scattering (SERS) spectroscopy. SERS exploits the strong electromagnetic fields generated at the surface of plasmonic nanostructures upon excitation with light (i.e., localized surface plasmon resonances, LSPRs) to magnify the Raman scattering of molecules located in their close proximity by many orders of magnitude. This high sensitivity granted by the plasmonic-mediated intensification combines with the high specificity and experimental flexibility of Raman spectroscopy making SERS an ultrasensitive technique. At the same time, the possibility of engineering integrated portable SERS-based sensors paved the way for the implementation of SERS as a potential tool to perform rapid on-field quantification of analytes in real-life environmental and biological media.

SERS detection of transition metals in waters is almost a 15 years old field of research which has been exponentially expanding in recent years. This growth is built upon the advances in nanofabrication techniques as well as the design of molecular ligands and receptors which are progressively facilitating the transition from basic proof-of-concept studies on simple artificial matrices to on-field quantification of transition metals via portable, multifunctional, optical devices. This minireview is mainly devoted to a critical overview and coherent discussion of the different sensing schemes devised to enable the SERS-based quantification of transition metals in aqueous media. In this context, the general features and ideal requirements of the employed plasmonic substrates are...
discussed. Also, representative examples are selected to illustrate key concepts and applications.

**PLASMONIC PLATFORMS**

Colloidal spherical particles synthesized via wet-chemistry methods are typically low-cost substrates that can be easily produced in large batches. However, isolated round nanoparticles do not provide sufficient enhancements for ultrasensitive detections. Thus, nanofabrication methods have been devised to tackle this issue mostly by engineering: (i) anisotropic individual colloidal nanoparticles;\(^1\) (ii) ordered assemblies of nanoparticles stable in solution;\(^1\) (iii) assemblies of nanoparticles on solid inert surfaces;\(^1\) and (iv) nanostructured 2D and 3D surfaces via either colloidal assembly\(^4\) or nanolithography.\(^1\)

The strong dependence of the LSPLR on the nanoparticle composition, size, and shape facilitates the tunability of its plasmonic response. Single-particle morphologies such as nanorods, nanocubes, or nanostars localize large electromagnetic fields at the corners and tips, making them far more efficient enhancers than their spherical counterparts.\(^1\) Even more outstanding improvements of the enhancing performances can be achieved by promoting the assembling of nanoparticles into superstructures with closely spaced configurations.\(^12,13\) In this scenario, giant electromagnetic (EM) fields are concentrated at the narrow interparticle gaps (hot spots) as a result of the particle plasmon coupling, yielding enhancements of the Raman signal up to \(10^{10} - 10^{11}\) for those molecules positioned at these interstitial volumes. It is also worth noting that further intensifications of the SERS signals can be determined by an increase of the Raman cross-section of the adsorbed molecule at these interstitial volumes. This phenomenon is known as chemical enhancement and its contribution to the overall signal magnification is typically much smaller than the EM one (commonly within the \(10^3 - 10^4\) range).\(^5\)

While sensing platforms relying on colloidal nanoparticles, including the anisotropic ones, profit from the relative simplicity of their fabrication, their direct applicability as on-site ultrasensitive and quantitative analytical tools can be challenging. In fact, nanocolloidal stability in complex media with high ionic strength and rich in organic matter is a cause of concern that can severely impact the signal reproducibility. This limitation can be circumvented by organizing the nanostructures into fixed configurations onto solid supports. Such plasmonic films can be generated both from colloidal suspensions and by physical methods. In both cases, the production of aggregated films by casting, or silver islands by physical vapor deposition, is simple and inexpensive. However, these techniques commonly face major difficulties at precisely controlling the nanoparticle organization which, in turn, limits the degree of reproducibility. On the other hand, refined methods such as the controlled fabrication of colloidal supercrystals\(^14\) or the physical evaporation on patterned substrates\(^15\) make possible the production of uniform and highly reproducible plasmonic substrates with well-defined nanostructured features but commonly to the increment of production costs.

Figure 1 illustrates representative examples of different classes of plasmonic substrates employed in the fabrication of SERS sensors for transition metal ions detection. Round colloidal gold nanoparticles of \(\sim 50 \text{ nm diameter} (\text{Figure 1A})\) display a characteristic LSPLR centered around \(540 \text{ cm}^{-1}\) (Figure 1C, green curve).\(^16\) while gold nanostars of \(\sim 80 \text{ nm diameter} (\text{Figure 1B})\) support a more intense plasmon resonance centered at 785 nm which is further slightly red-shifted upon coating with a 3 nm silica layer as a result of the change in refractive index of surrounding medium (Figure 1C, black and red curves, respectively).\(^15\) Spherical nanoparticles (\(\sim 50 \text{ nm diameter}, \text{gold}\)) have been also used upon assembly onto polystyrene microbeads (3 \(\mu\)m) via a layer-by-layer protocol (Figure 1D).\(^16\) The resulting hybrid plasmonic microstructures are colloidal stable microcarriers of highly localized 3D collections of closely spaced nanoparticles, thereby yielding intense and averaged SERS signals at the single-particle level. When the polymeric core is replaced with silica-coated \(\text{Fe}_3\text{O}_4\) microspheres, the hybrid construct is further equipped with magnetic properties that enable the straightforward separation of the SERS platform from the media by simply applying a magnet.\(^18\) Finally, plasmonic ordered arrays have also been exploited as SERS substrates for their outstanding signal reproducibility, structural uniformity, and excellent SERS enhancing properties.\(^19,20\) For instance, Song et al. fabricated silver nanorods array prepared by oblique angle deposition technique (Figure 1E).\(^19\) With this approach, the authors, rather than organizing colloidal particles onto a solid surface, physically evaporated and deposited silver on top of a rotating surface (a 200 nm thick, smooth Ag film)
easy operation, and convenient modifi-
cations (e.g., high sensitivity and reproducibility, robustness, cost,
time-consuming process unless using state-of-the-art techniques
mapping of large areas with high spectral resolution can be a
plasmonic platform. Nonetheless, it is worth stressing that SERS
at smaller and highly electromagnetically active locations of the
ups that favor the illumination of fewer molecules concentrated
bility but decreased sensitivity as compared to experimental set
will provide highly averaged signals with improved reproduc-
cability. It is also important to highlight that the analytical performances of the
substrate are also dependent on the measuring setup. For
instance, acquiring SERS spectra via simultaneous interrogation of large portions of substrates by using, for instance, macrolenses
will provide highly averaged signals with improved reproduc-
bility but decreased sensitivity as compared to experimental set
ups that favor the illumination of fewer molecules concentrated at
smaller and highly electromagnetically active locations of the
plasmonic platform. Nonetheless, it is worth stressing that SERS
mapping of large areas with high spectral resolution can be a
time-consuming process unless using state-of-the-art techniques
such as SERS holography.22

As a matter of fact, the application of SERS to in situ
monitoring of transition metals demands the implementation of the
plasmonic substrates into all-in-one portable devices. In
addition to a compact Raman spectrometer for optical detection,
these systems must integrate additional functionalities that
would enable, besides optical detection, sample processing such as
filtration, preconcentration, and separation when the complexity of the sample matrix would require it (e.g.,
wastewaters, freshwaters). In fact, while the intrinsic sensitivity and
selectivity of a SERS method are primarily defined by the
enhancing ability of the plasmonic substrate and the molecular properties of the surface receptors, respectively, competitive
adsorptions of matrix interferences may significantly deteriorate the
sensing performances. This issue is particularly relevant
when the target analyte is present at very low concentrations. To
this end, recent years have witnessed multidisciplinary
approaches to integrate SERS sensing with microfluidics, paper-based substrates, magnetic functionalities, and capillary-
based chips to facilitate automated sample handling and
processing.23 For instance, magnetic-based extraction and
preconcentration of the target analyte from complex media
enable high throughput sample processing with the additional
improvement of the limit of detection (LOD) by several orders of
magnitude.24 On the other hand, separation tools such as
capillary techniques are beneficial for the selective quantification of multiple target analytes.25 However, it has to be stressed that
excessively extensive and laborious sample processing makes far
less attractive the use of nanotechnology in decentralized
analysis.

DIRECT SENSING

The simplest approach to discriminate and quantify the presence
of a target species in a mixture by SERS is via the acquisition of the
intrinsic SERS signal of the analyte and measurement of its
intensity (direct SERS sensing). Evidently, this approach is not
suitable for vibrationless species such atomic ions and, thus, is
limited to the detection of transition metals involved in
inorganic, metalorganic, or organometallic compounds. For
instance, arsenic in waters commonly exists as a combination of
relatively harmless organoarsenic compounds and the toxic
inorganic species such as arsenite (As(III)) and arsenate (As(V)). The relative toxicity of the arsenic species is tightly
related to the oxidation state of the metal cation (where As(III)
is much more toxic than As(V)). Thus, chemical speciation of
the different forms is key for environmental analysis. However, the high chemical reactivity of As(III) poses important
challenges for laboratory methods to perform a reliable
speciation analysis due to the favorable oxidation of arsenite
species during the steps of sample collection, storage, and
transportation. For instance, Yang et al. described a simple
qualitative approach for the direct SERS speciation of arsenic
compounds based on the physical separation achieved by
exploiting the coffee ring effect. A 2 μL portion of an arsenic
solution in phosphate buffer was placed on a silver film prepared
by electrostatic deposition of ~45 nm silver nanoparticles onto
an amino-modified glass slide (Figure 2A). As evaporation
occurs, arsenic species migrate to different extents toward the
outer edge promoting, therefore, their partial separation and
preconcentration in preferential area of the Ag film (a surfactant,
SDS, was also added to the sample to extend the movement of the
solvent onto the film). SERS spectra collected at a different
di was placed on a silver

Figure 2. Direct SERS speciation of arsenic forms. (A) Outline of the sensing scheme for physical separation and SERS speciation of four arsenic forms:
arsenite (As(III)), arsenate (As(V)), monomethylarsonic acid (MMA(V)), and dimethylarsinic acid (DMA(V)). (B) SERS spectra of separate
solutions of arsenite and arsenate on Ag film (black curves, signal obtained at the center of the droplet; red curves, signal obtained at the edge of the
dried sample). The spectra were acquired by focusing a 785 nm laser with a x20 optical lens (100 μm spot size) (Adapted with permission from ref 26.
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vibrational fingerprints of each molecular species. Figure 2B shows the characteristic SERS fingerprints of As(III) and As(V) species in the 350–900 cm⁻¹ spectral range of both the droplet and the dried sample (black and red curves, respectively). Besides the speculation of the arsenite and arsenate forms, this approach also allowed the discrimination of the structurally analogous organoarsenic species monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA).

### INDIRECT SENSING VIA SELECTIVE NANOPIECE ASSEMBLING

Designing efficient direct sensing strategies for analytes in complex media presents major problems that are often impossible to overcome. First of all, even if the target displays a good affinity for the plasmonic substrate, the presence of additional compounds in the sample matrix that compete with the analyte for surface adsorption can drastically reduce both the sensitivity and selectivity of the method. Thus, prepurification steps are typically required to isolate the target analyte from complex environments. However, this prerequisite essentially precludes the viability of SERS as a rapid technique for remote sensing. Furthermore, as previously mentioned, direct SERS has the intrinsic inability of detecting “silent” species such as free metal cations.

Far more suited for an environmental sensing scenario are indirect SERS approaches relying on the use of molecular receptors bound to the plasmonic substrate that are capable of selectively interact with high affinity with the target transition metal via covalent and/or noncovalent specific interactions or even selectively undergo metal ion catalytic reactions. In indirect methods, the measurable readout signal is provided by an external molecular source that yields resonant Raman scattering when irradiated with the 785 nm laser (i.e., resonant Raman label). In the example illustrated in Figure 3, two batches of ca. 45 nm gold nanoparticles were derivatized with either nitritolatriacetic acid (NTA) or histidine (His) functionalities via EDC/Sulfo-NHS coupling reaction, to provide anchoring sites for nickel ions binding (L-carnosine was used as a source of histidine residues). Subsequently, the infusion of a near-infrared dye (NIR-797 isothiocyanate) onto the metallic surface of the NTA-gold nanoparticles equipped the system with a Raman label that yields resonant Raman scattering when irradiated with the 785 nm laser (i.e., resonant Raman label). This phenomenon entails an additional enhancement to the SERS signal that enables the acquisition of intense spectra even for very low NIR-797 surface coverages, thereby minimizing the risk of colloidal destabilization of the individual particles. The two sets of NTA- and His-gold nanoparticles were combined, and upon addition of Ni(II) solutions in deionized water, rapid aggregation takes place (less than 3 min). This results from the interparticle linking due to the formation of an octahedral metal complex coordinating the NTA moiety and the closely packed histidines present on the nanoparticles (Figure 3A). Interparticle plasmon coupling resulting from Ni(II) binding is both highlighted by a change of the extinction profile (Figure 3B) and an increase of the SERS intensity, resulting from the concentration of high EM fields at the interparticle junctions (Figure 3C), which can be quantitatively correlated with the metal ion content. Specifically, colorimetric sensing provided a wider linear response for Ni(II) concentration range using ca. 45 nm diameter gold or silver nanoparticles. The SERS spectra were acquired by focusing a 785 nm laser with a ×20 long-working distance objective (Adapted with permission from ref 27. Copyright 2012 Wiley-VCH).
to their analogous gold counterparts, thus improving the overall sensitivity of the SERS-based method. This is exemplified in Figure 3D where the SERS intensity of the NIR-797 band at 523 cm⁻¹, for two colloidal systems comprising spherical gold or silver nanoparticles of identical size, is plotted against the Ni(II) concentration. However, the sensing system displayed a similar affinity for Cu(II) ions, which prevents its applicability as Ni(II) SERS sensors in natural waters where the copper content is typically much higher than the nickel. On the other hand, selectivity can be primarily improved by designing receptors with higher specificity for the target analyte. Further improvements can be also achieved by adjusting the sample conditions (e.g., pH) as well as upon addition of water-soluble masking agents that can selectively sequester the interfering ion from the medium (e.g., EDTA).

### INDIRECT SENSING VIA CONFORMATIONAL CHANGES OF THE MOLECULAR RECEPTOR

The use of DNA aptamers for the SERS quantification of Hg(II) has been extensively reported in the literature⁶,³¹,³² as it combines the detection of an analyte of high interest with the availability of extremely selective surface receptors (i.e., thymine-rich aptamers) which are readily amenable to chemical synthesis and a la carte modifications. Most notably, the implementation of DNA aptamers onto solid substrates for the quantification of Hg(II) via a “turn-on/turn-off” response undeniably represents the most widespread application of SERS-based sensing of transition metals in real water systems. ³¹ In this approach, one extremity of the aptamer is typically conjugated with a resonant dye which relative proximity to the plasmonic surface is determined by the Hg(II)-mediated conformational change of the DNA chain. Therefore, DNA structural reorganization to different forms (e.g., single strand, duplex, hairpin) exposes the Raman label to higher electromagnetic fields (turn-on SERS), when the label is closer to the metal surface, or to lower enhancements (turn-off SERS), when the relative distance between the two components is increased. The work by Shi et al.³¹ affords an illustrative example of this approach. A thymine rich single-stranded DNA labeled with carboxyfluorescein (FAM) is covalently attached to a silver nanoparticle film on a silicon wafer via sulfur bond (Figure 4A). For sufficiently high oligonucleotide surface coverages, the single strands preferably adopt a tilted geometry with the Raman label spaced away from the metal surface (i.e., weak SERS background signal). Upon Hg(II) addition, the formation of a hairpin structure forces the close approximation of the label to the substrate, which is revealed by an increase of the SERS intensity. A second oligo receptor was also introduced onto the Ag film, consisting of a DNA duplex obtained by hybridization of the surface-bound Pb(II)-specific DNAzyme strand labeled with 6-carboxy-X-rhodamine (ROX) with the corresponding substrate strand (17DS). The rigid duplex structure, that places the ROX label far from the silver film, is disrupted in the presence of lead ions via cleavage of the substrate strand 17DS by the Pb(II) activated DNAzyme strand (Figure 4B). The acquired flexibility of the DNAzyme strand allows the ROX moiety to approach the plasmonic film. The catalytic reaction was completed after ~1 h, which was set as the experimental incubation period for metal ion quantification. Notably, the Ag film was also decorated with 4-aminothiophenol (4-ATP) molecules, which provides an easily distinguishable SERS signal with an intensity comparable to those of ROX and FAM labels. Figure 3C shows the SERS spectra obtained in the presence of equimolar buffer solution of Hg(II) and Pb(II) at increasing concentration, where three bands at 1079 (4-ATP), 1322 (FAM), and 1503 cm⁻¹ (ROX) can be discerned. For quantification purposes, rather than plotting the absolute intensities of the FAM and ROX marker bands (I₁₃₂₂ and I₁₅₀₃) vs the metal ion concentration, ratiometric values were calculated using the 4-ATP marker band as the internal standard (I₁₃₂₂/I₁₀₇₉ and I₁₅₀₃/I₁₀₇₉). Such an approach has been shown to significantly reduce the signal fluctuation associated with interferences in the local environment as well as instrumental and procedural errors.³¹ Ratiometric analysis allows a broader linear dynamic range (from 1 nM to 10 μM for Hg(II) and from 100 pM to 10 μM for Pb(II)) and lowers the limits of detection (LODs) (~1 nM for Hg(II) and 0.1 nM for Pb(II)) as compared to noncalibrated SERS data (in buffer solutions).³¹ Finally, the authors demonstrated the viability of the sensing tool to real-life applications by detecting Pb(II) and Hg(II) spiked into industrial wastewater, previously filtered to remove large-sized particles, using a portable hand-held Raman instrument. Unfortunately, the design of specific oligonucleotide sequences for selective capturing of transition metal ions is currently restrained to very few species, which significantly limits their broader use in environmental sensing.
**INDIRECT SENSING VIA ELECTRONIC CHANGES IN THE MOLECULAR RECEPTOR**

The formation of T−Hg(II)−T base pairs not only induces structural reorganization and reorientation of oligonucleotides over the metallic surface but also can be detected by monitoring the spectral alterations of the intrinsic DNA vibrational profiles which, in turn, can be quantitatively correlated with the Hg(II) content. Thus, DNA strands can act as chemoreceptors by simultaneously performing the role of selective molecular ligands and SERS transducers. Most notably, a key advantage of this approach is the use of the chemoreceptor itself as the internal reference, thereby enabling a straightforward implementation of ratiometric SERS sensing with improved sensitivity and reliability.

Nonetheless, to maximize the sensing performance, chemoreceptors are typically selected among small molecules: (i) with high Raman cross-section (i.e., molecules bearing one or more benzene rings with delocalized π-bonding in close contact to the plasmonic surface), yielding strong SERS signals with well-defined, narrow spectral features; (ii) that firmly bind the metallic surface (mostly via thiolated bonds); and (iii) undergo larger electronic and/or structural modifications upon binding of the target pollutant (which is reflected in larger alterations of the spectral profile). The methodological protocol for selecting and characterizing a chemoreceptor preferably involves several steps, which are exemplified by our work summarized in Figure 5A−D. In this study, we exploited a terpyridine (TPY-DTC) dithiocarbamate derivative as a chemoreceptor for the simultaneous ultratrace SERS detection of Cu(II) and Co(II) in buffer solutions. First, the electronic spectra of the pipazinyl TPY precursor (pTPY, Figure 5A) in solution were acquired via UV−vis spectroscopy prior and after the addition of transition metal ions. This affords a first indication of the selectivity of the chemoreceptor toward specific metals, which will be later assessed also by SERS. Moreover, it provides a clue of the extent of electronic perturbation that transition metal binding imposes (which broadly correlates with the alteration of Raman polarizability and, in turn, the degree of spectral alterations in SERS). Among different metal ions, Cu(II) and Co(II) have shown to produce major distinguishable alterations of the TPY absorption spectrum (Figure 5B).

Figure 5. Indirect SERS chemosensing of Co(II) and Cu(II). (A) Molecular structures of 4-(N-piperazinyl)terpyridine (pTPY): free and coordinated with a metal ion M(II). (B) Normalized electronic absorption spectra of pTPY and its equimolar complexes with Co(II) and Cu(II) in 50/50 CH₂Cl₂/Methanol. (C) Theoretical and experimental Raman spectra of the solid samples. The spectra of Cu(II)/pTPY are not shown. (D) SERS spectra of TPY-DTC at different concentrations on Ag colloids. (E) Normalized SERS spectra of TPY-DTC in the ring breathing region before (green line) and after incubation with Co(II) (30 ppb, pink line) and Cu(II) (6.4 ppm, blue line). SERS spectra were acquired on dried colloidal silver nanoparticles using a 785 nm laser with a 50× objective (Adapted with permission from ref 34. Copyright 2013 Royal Society of Chemistry).
ability at higher surface coverages. Once identified the appropriate surface density of the chemoreceptor, SERS spectra are acquired at increasing concentration of metal ions in a buffer solution to experimentally identify the TPY vibrational features that undergo major alterations upon metal ion binding and, supported by the DFT calculations, identify the marker bands for ratiometric quantification of the target analytes (Figure 5E).

Specifically, in this study, we used as spectral markers the ratios between the peak intensities at 1033 and 1040 cm\(^{-1}\) (for Co(II) and Cu(II) detection, respectively), and the peak intensity at 1019 cm\(^{-1}\) as the internal standard (IS), permitting a certain degree of multiplex capabilities for the simultaneous detection of the two transition metal cations. The SERS response of the chemoreceptor must be then tested in the presence of potential interfering analytes, such as transition metal ions dissolved at relatively high concentrations in buffer solutions, and, finally, in real sample matrices.

As for arsenic species, speciation of different Hg forms is essential to determine their bioavailability, mobility, and, thus, their environmental and toxic impact. Most notably, methylmercury MeHg(I) is the most potent mercuric toxin. As it is produced by aquatic microorganisms, MeHg(I) rapidly enters the aquatic food chain where it is subjected to biomagnification of concentration can be easily controlled by adjusting the concentration of the beads in the sample. On the other hand, the MeHg(I) quantification is restricted to a linear range of from 0 to 70 nM, with a LOD of 7 nM. Interestingly, simultaneous quantification of the two species highlighted the dynamic interconversion between Hg(II) and MeHg(I) in water.

### INDIRECT SENSING VIA CHEMICAL REACTIONS OF THE MOLECULAR RECEPTOR

In a very recent work, Zhao et al.\(^{20}\) adopted 4-mercaptophenylboronic acid (4-MBA) as a SERS chemoreporter attached to ordered Au nanorod arrays (Figure 1P) as plasmonic substrates for ultratrace detection of Hg(II). In this case, an electrophilic substitution reaction takes place, in which the transition metal ion displaces the \(-\text{B(OH)}_2\) functional group, leading to the chemical transformation to 4-mercapto phenyl mercury chloride (Figure 6A). Removal of the boronic moiety and the extensive perturbation of the polarizability of the phenyl ring determine a dramatic reshaping of the SERS spectra (Figure 6B), which includes the disappearance of the \(-\text{B(OH)}_2\) functional group, modes at 627 and 1020 cm\(^{-1}\), the striking blue-shifts of the 1061 cm\(^{-1}\) ring breathing and 1558 cm\(^{-1}\) \(\nu(C-C)\) bands, and the appearance of two new intense features at 467 and 1085 cm\(^{-1}\), ascribed to the stretching vibration of newly formed C-Hg bond and the ring deformation. This ensemble of dramatic spectral alterations equips the sensing platform with high discriminative power, which is key to maximize accurate and quantitative SERS performances. Calibration curves showed a linear response over 5 orders of magnitude, with a LOD of 0.1 nM in deionized water, with insignificant interferences from other metal ions and pH conditions. Simulated on-site detection was carried out on the ground and lake waters spiked with Hg(II) (10 nM) and using a hand-held Raman spectrometer (Figure 6C), evidencing a consistent, repeatable response of the sensing platform in real-earth aquatic media. Intriguingly, preliminary studies indicated the potential of 4-mercaptophenylboronic chemoreceptor for discriminating different mercury species (mercury nitrate, methylmercury chloride, and ethylmercury chloride; Figure 6D).

### INDIRECT SENSING BY NANOPIRLE BINDING OF NONAFFINITY METAL SELECTIVE DYES

An untapped wealth of potential SERS chemoreceptors can be found in classical qualitative analytical chemistry, which extensively describes multiple classes of organic reagents with...
the ability to selectively discriminate, and even speciate, most metal ions. Nonetheless, their use in SERS has been significantly hampered by the inability of equipping the organic receptor with a mercapto group, as required for firmly anchoring the plasmonic surface while preserving its selectivity for the target transition metal. For instance, several anthraquinone and flavonoids derivatives have shown a diversity of excellent metal chelation capabilities with, often, emissive properties in the visible region. However, coordination to the target species mainly occurs through the same functional groups that enable their adsorption on silver and gold nanostructures, therefore commonly prevent their application as SERS chemoreceptors. To tackle this issue, we have recently illustrated that the structural and functional plasticity of DNA duplexes for noncovalently interacting with small aromatic molecules via intercalative binding can be exploited to retain the metal chelation capabilities of these classes of reagents onto the plasmonic surfaces.\(^{37}\) Specifically, we demonstrated the validity of this approach using alizarin red S in combination with short DNA duplexes for the simultaneous Fe(III) and Al(III) quantification in spiked tap water.

**CONCLUSIONS AND OUTLOOK**

In summary, in this mini-review, we aimed at providing a coherent discussion on the sensing schemes exploited in the SERS analysis of transition metals in waters, using illustrative examples to describe the rationale of each approach, their most relevant aspects as well as the diverse classes of plasmonic nanomaterials utilized. Although the studies reported in the literature display a marked methodological diversity, we have broadly classified their sensing schemes into four main classes: (i) direct sensing, (ii) indirect sensing via nanoparticle assembling, (iii) indirect sensing via conformational changes of the molecular receptor in combination with Raman labels, and (iv) indirect chemosensing (either via coordination or chemical reaction of target ions with SERS active receptors). While the advantages and disadvantages of each approach are very case-specific, some general considerations can be drawn. First of all, direct sensing approaches commonly require, in addition to an intrinsic affinity of the target species for the metallic surface, the separation of the analyte from complex media. This largely prevents the applicability of such methods for remote SERS sensing of transition metal species in natural waters. To overcome this limitation, multiple indirect strategies have been developed to enable the selective capture of the target within its original matrix. The simplest indirect sensing design exploits the metal ion-induced assembly of Raman-labeled colloidal nanoparticles equipped with selective surface receptors for the target species. However, the complexity itself of natural matrices can put under significant stress the colloidal stability of the nanoparticles, thereby reducing the reliability of the sensor, especially at low target concentrations. This issue can be overcome by using nanostructured solid surfaces in combination with “SERS silent” molecular receptor such as DNA aptamers or “SERS active” chemosensors. Remarkably, the conformational reorganization of DNA aptamers upon binding of metal ions have been widely exploited in a variety of studies thanks to their high selectivity and reliable performance. Unfortunately, the current availability of aptamer receptors is limited to very few target metal ions (Hg(II), Pb(II), and Ag(I)). Alternatively, molecular receptors that act simultaneously as SERS transducers have recently gained a great deal of attention to increase the number of possible targets, while potentially enabling speculation analysis. Furthermore, chemosensors intrinsically provide an internal standard for ratiometric SERS analysis.

Despite the extensive work carried out at the academic level, the successful design of SERS sensors for remote detection that combines ultrasensitivity and robust and reproducible response in complex and variable matrices with cost-competitiveness remains an unmet challenge. To address this issue, extensive efforts in applied research have been currently devoted to the development of low-cost, manageable, and portable Raman instruments for rapid data acquisition which can be easily integrated into multiperational platforms equipped with automated sample handling and processing functionalities. Concurrently, continuous advances are being pursued in the development of highly efficient plasmonic materials with a reliable and quantitative response that can be fabricated at a large scale and low manufacturing cost using standardized procedures. On the other hand, expanding the range of surface elements, with a specific focus on the rational design of highly selective chemoreceptors, is key to improve the sensing performances in terms of selectivity, sensitivity, multiplexity and resilience against interference.

Finally, SERS technology appears specifically suited for the development of sensing devices for the remote and fast quantification of multiple contaminants with ultralow permissible limits that cannot be otherwise detected by more inexpensive methods. In fact, regardless of the potential improvements, we expect that SERS technique will not be able to compete on price with traditional colorimetric and fluorimetric methods for detection of transition metal ions when ultrasensitivity is not required.

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**Notes**

The authors declare no competing financial interest.

**Biographies**

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Prof. Ramon A. Alvarez-Puebla is an expert in surface science, nanoscience, and spectroscopy with emphasis on the manufacturing and characterization of plasmonic materials and their integration into advanced detection devices especially with applications in nanobiomedicine. He has a degree in chemistry (Universidad de Navarra, 2000) and a doctorate in surface science (Universidad Publica de Navarra, 2003, summa cum laude). He completed his postdoctorate at the University of Windsor (Windsor, ON, Canada) and General Motors Corporation (Warren, MI, USA) in nanofabrication and surface spectroscopy (SERS, SEFS, and SEIRA) with Prof. Ricardo Arora (UWINDSOR) and Prof. Gholam Abbas Nazi (GMC). During 2006–2007, he worked as a Research Officer and Principal Investigator at the National Institute for Nanotechnology of the National Research Council of Canada (NINT-NRC, Edmonton, AB, Canada). In 2008 he returned to Spain as an associate Professor at the Universidade de Vigo. Since 2012, he has been the ICREA Professor at the Universitat Rovira i Virgili in Tarragona, where he leads the Plasmonics and Ultradetection Group (Zeptonic).

**ACKNOWLEDGMENTS**

The authors acknowledge the Spanish Ministerio de Economía y Competitividad (CTQ2017-88648R and RYC-2016-20331), the Generalitat de Cataluña (2017GRS883), and the Universitat Rovira i Virgili (FR 2019-B2).

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