Review

Recent trends in nanomaterial-based microanalytical systems for the speciation of trace elements: A critical review

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HIGHLIGHTS

- First critical review comparing different nanomaterial-based speciation techniques.
- Recent developments and applications of nanomaterials in element speciation assays.
- Important trends in speciation research in environmental science and biomedicine.
- Novel platforms combining nanomaterials and microfluidics for elemental speciation.

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ABSTRACT

Trace element speciation in biomedical and environmental science has gained increasing attention over the past decade as researchers have begun to realize its importance in toxicological studies. Several nanomaterials, including titanium dioxide nanoparticles (nano-TiO2), carbon nanotubes (CNTs), and magnetic nanoparticles (MNPs), have been used as sorbents to separate and preconcentrate trace element species prior to detection through mass spectrometry or optical spectroscopy. Recently, these nanomaterial-based speciation techniques have been integrated with microfluidics to minimize sample and reagent consumption and simplify analyses. This review provides a critical look into the present state and recent applications of nanomaterial-based microanalytical systems in the speciation of trace elements. The adsorption and preconcentration efficiencies, sample volume requirements, and detection limits of these nanomaterial-based speciation techniques are detailed, and their applications in environmental and biological analyses are discussed. Current perspectives and future trends into the increasing use of nanomaterial-based microfluidic techniques for trace element speciation are highlighted.

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1. **Introduction**

The analysis of trace elements in biological and environmental samples is important because they play crucial roles in human life. Several analytical instruments, including atomic absorption spectroscopy (AAS), atomic fluorescence spectroscopy (AFS), inductively coupled plasma optical emission spectroscopy (ICP-OES), and inductively coupled plasma mass spectrometry (ICP-MS), can be used to analyze the total amounts of trace elements in biological, environmental, and food samples. Sample pretreatment (e.g., acid digestion [1]) is usually required to minimize matrix interference, but it can be laborious, costly, and environmentally unfriendly. Accordingly, for many analytical methods, focus has been placed on applying simpler sample pretreatment steps, such as dilution [2], extraction, filtration, solubilization [3], liquid–liquid extraction (LLE) [4–6], co-precipitation [7–9], solid phase extraction (SPE) [10–12], membrane filtration [13–15], and cloud-point extraction (CPE) [16–18]. These pretreatment methods can be used to separate and preconcentrate trace elements while eliminating matrix interference, resulting in high enrichments and recoveries.

Although these sample pretreatment methods have made trace element assays more convenient, they often require large sample or reagent volumes (several tens to hundreds of milliliters) to obtain high enrichment factors (EFs) or preconcentration factors (PFs). In cases where it is difficult or impossible to obtain such large sample volumes, these techniques often provide low EFs, resulting in high detection limits. To overcome these obstacles and improve analytical efficiencies, various materials have been tested to increase the sorption, extraction, and preconcentration of trace elements. Among these, nanomaterials are attracting increasing attention because of their specific binding affinities toward analyte elements; nanomaterials functionalized with various groups (e.g., chelating resins, peptides, proteins, and even other elements) can exhibit highly specific binding affinities. To date, nanomaterials have been used largely and effectively as SPE sorbents for the separation and preconcentration of analyte elements in total content analyses.

Although total content analysis of trace elements is important, their speciation is just as necessary. Trace element species exist in different oxidation states when in the form of free ions and nanoparticles, or as inorganic and organic complexes. Many
species are pH-dependent, are readily reduced or oxidized, or interact strongly with other reactive groups that bind and stabilize them. Some element species have varying toxicities; for instance, hexavalent chromium is much more toxic than trivalent chromium, while inorganic arsenic species are more toxic than their organic counterparts. Differentiating between toxic and less toxic trace element species is, therefore, just as important as their total content determination. Recent trends in trace element research have shifted from total content to speciation analysis [19], as the need to investigate the roles of trace elements and their species in health and disease has become of prime importance. Nevertheless, because of very often complex sample matrices and extremely low concentrations after extraction or fractionation, extremely sensitive and selective detectors are required. Hyphenated techniques based on the coupling of separation techniques with element-selective detection processes are becoming fundamental tools for metal speciation research in both biological and environmental science [20,21]. In addition, when analyte enrichment is required, on-line coupling with SPE can be incorporated to build more useful integrated systems for the determination of trace element species. Improvements to these hyphenated techniques remain necessary to avoid complicated sample pretreatment processes, the use of harmful reagents, and time-consuming analyses.

Approximately 1800 articles on trace element speciation have been published over the past decade, some of which included important developments using nanomaterial- and microfluidic (MF)-based techniques. Similar to their applications in total element analysis, nanomaterials have been used largely and effectively as SPE sorbents to separate and preconcentrate element species for speciation analysis. These nanomaterial-based speciation techniques have still required large sample volumes – a major analytical obstacle; to overcome it, the use of MF techniques decreases the sample volume requirements significantly, from several milliliters to microliters, while simplifying the analytical procedure. Microfluidics are a technology characterized by the engineered manipulation of fluids at the sub-milliliter scale [22]. Microfluidic devices have found multidisciplinary uses because of their requirements for low volumes of samples and reagents. Whereas previous analyses of environmental samples had been limited by their volumetric abundances, the application of microfluidics to trace element speciation has facilitated the analyses of biomedical samples available in only small volumes. To reach practical use, however, MF devices must incorporate and automate on-line sample loading and preparation with analyte separation and detection [23].

The recent integration of nanomaterials and microfluidics in trace element speciation has greatly simplified and enhanced analytical performance. Nevertheless, the development and application of nanomaterial-based integrated MF systems for trace element speciation remain in their nascent stages. This review examines the various nanomaterials used as SPE sorbents for trace element assays and speciation, and the advantages and disadvantages of different assaying and speciation techniques. Recent applications of nanomaterials in trace element speciation are elaborated, with emphasis on advanced approaches for the adsorption, extraction, and preconcentration of trace element species. Applications of microfluidics and nanomaterial-based MF techniques for trace element speciation are also discussed, as are future trends in nanomaterial-based MF techniques and their development in trace element speciation.

2. Fundamentals of nanomaterials

Nanomaterials have been used in trace element assays for many decades because of their ease of fabrication and their ability to simplify analytical processes. Commonly used nanomaterials for total element analysis include titanium dioxide nanoparticles (nano-TiO₂), carbon nanotubes (CNTs), and magnetic nanoparticles (MNPs). Nanomaterials have been used in trace element assays of a wide range of samples. Panigati et al. used nano-TiO₂ to determine Pb and Cd in cosmetics [24]; Li et al. used nano-TiO₂ to determine W(VI) in water and sediments [25]; and Wang et al. used poly(4-vinylaniline) nanoparticles to determine Cr(VI) in wastewater samples [26]. As these papers suggest, research in trace element assays over the past 10 years has been moving from total content to speciation analysis as the need to determine trace element species had become more apparent. This section covers the adsorption characteristics and applications of nanomaterials that have been used for total content and speciation analyses of trace elements.

2.1. Application in trace element assays

Based on the NCBI PubMed database, more than 8000 publications on nanomaterial-based trace element assays have appeared over the past 10 years. Nanomaterials that are commonly used as SPE sorbents in trace element assays include nano-TiO₂, CNTs, and MNPs. Among them, nano-TiO₂ is also used as a photocatalyst in the chemical reduction of various elements (e.g., As, Fe, Hg, Se) [27–31]. Most nanomaterial-based studies of trace elements use adsorption and desorption through SPE to facilitate the determination process. The functionalization of these nanomaterials with chemical groups can further increase the analytical specificity and efficiency toward the analyte elements [12,32]. The magnetic properties of MNPs can also improve both analytical specificity and efficiency, because MNPs are readily separated from nonmagnetic materials under a magnetic or electromagnetic field [33–36]. MNPs functionalized with chemical compounds have the same adsorption mechanisms as their non-functionalized counterparts for the determination of trace elements.

Over the past 10 years, nanomaterials have been used to extract and preconcentrate a wide range of trace elements, including chromium (Cr) [37–44], cobalt (Co) [33,37,45,46], nickel (Ni) [37,46–48], selenium (Se) [49], cadmium (Cd) [45,46,50–52], zinc (Zn) [45,51,52], copper (Cu) [50,52–65], manganese (Mn), cerium (Ce), dysprosium (Dy), europium (Eu), lanthanum (La), ytterbium (Yb), lead (Pb) [50,51,56,63,66], silver (Ag) [50], antimony (Sb), mercury (Hg) [52,56], iron (Fe) [66], and vanadium (V) [32]. Publications on total Cu analysis have been the most common, followed by those for Cr and Cd.

Nanomaterials display different binding affinities toward different trace elements and toward different trace element species; accordingly, early studies of the speciation of trace elements used nanomaterial-based techniques. Many nanomaterial-based approaches have been applied to studies of trace elements in environmental samples, including river water [38,39,46,59], lake water [49,56,57], tap water [33,49,56,62], mineral water, underground water [33], rainwater, stream water [62], seawater [62], wastewater [38,52], and drinking water [43]. Analyses of foods have included black tea, coffee, tomatoes, cabbages, zucchinis, apples, and chicken [32,48]. There have been relatively fewer studies into the analyses of biological samples, such as urine [50], human hair, and blood [62], due to the smaller quantities of such samples available for analysis.

The analytical emphasis on environmental samples is largely due to the extraction and preconcentration capabilities of current analytical techniques. To obtain higher EFs, large sample volumes (up to 500 mL) are often required; therefore, environmental samples that are readily available in quantities much larger than biological samples usually yield higher EFs and more sensitive analyses. With the possible exceptions of blood and urine, biological samples are difficult to obtain in large volumes, greatly

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W.-C. Tseng et al. / Analytica Chimica Acta 884 (2015) 1–18
limiting the applicability of many trace element-based analytical techniques toward biological samples.

Another way to circumvent the large volume requirements of current nanomaterial-based trace element speciation techniques is to use sensitive detectors. Nanomaterials and MF devices have been involved only in analyte separation and preconcentration; therefore, they require appropriate detection systems. When using a highly sensitive detector, high EFs and PFs are relatively unimportant. Analytical instruments that have been used to determine trace elements include inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma optical emission spectrometry (ICP-OES) [45,67,68]; inductively coupled plasma mass spectrometry (ICP-MS) [3,28,29,31,69–79]; electrothermal atomic absorption spectrometry (ET-AAS) and graphite furnace atomic absorption spectrometry (GF-AAS) [4,12,33,36,38,80–87]; hydride-generation atomic absorption spectrometry (HG-AAS) [88]; flame atomic absorption spectrometry (FAAS) [35,89,90]; and atomic fluorescence spectrometry (AFS) [27,74,91–95].

ICP-MS is favored for its low detection limits, requiring lower EFs and, therefore, lower sample volumes. In addition, the on-line analytical capabilities of ICP-MS allow samples to be analyzed directly after SPE and preconcentration. Although ET-AAS provides similar, if not lower, detection limits to those of ICP-MS, and can lessen matrix interference, it is most commonly used in off-line analytical systems because it is difficult to perform on-line analysis with its instrumental design. ICP-AES, ICP-OES, FAAS, and AFS systems have higher detection limits and require larger sample volumes—two major analytical issues. Unlike the dynamic element range of these aforementioned techniques, HG-AAS is limited to only a few elements, namely As, Se, Sb, and Hg, and is, thus, the least practical of these techniques. Accordingly, ICP-MS and ET-AAS are the most commonly used detection instruments for trace element analysis, followed by ICP-AES, AFS, FAAS, and

Table 1
Analytical characteristics of nanomaterial-based chromium speciation methods.

<table>
<thead>
<tr>
<th>Separation technique</th>
<th>Mechanism of speciation</th>
<th>EF or PF</th>
<th>LOD (µg L⁻¹)</th>
<th>Capacity (mg g⁻¹)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nanomaterial sorbents</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SPE (TDNTs)</strong></td>
<td>Cr(III): TDNTs and reduction of Cr(VI)</td>
<td>67</td>
<td>ICP-MS</td>
<td>0.0075 – 9.0</td>
<td>[75]</td>
</tr>
<tr>
<td><strong>SPE (SWCNTs)</strong></td>
<td>Cr(III): SWCNTs, Cr(VI) in solution</td>
<td>63</td>
<td>ICP-MS</td>
<td>0.01 – 0.024, 0.73 – 0.74</td>
<td>[76]</td>
</tr>
<tr>
<td><strong>On-line POM/MS SPE (CsPOM)</strong></td>
<td>Cr(III): CsPOM, Cr(VI) in solution</td>
<td>–</td>
<td>ICP-MS</td>
<td>0.5 – 0.3</td>
<td>[71]</td>
</tr>
<tr>
<td><strong>On-line SPE and photocatalysis reduction (nano-Au/TiO₂)</strong></td>
<td>Adsorption of Cr(III) and Cr(VI) photoreduction of Cr(VI)</td>
<td>Cr(III): 7.1 Cr(VI); 4.6</td>
<td>FI–ET-AAS</td>
<td>0.08 – 0.13</td>
<td>[82]</td>
</tr>
<tr>
<td><strong>On-line SPE (nano-TiO₂)</strong></td>
<td>Cr(III): TiO₂ and Cr(VI)–TiO₂</td>
<td>–</td>
<td>FI–ET-AAS</td>
<td>0.006 – 0.01</td>
<td>[83]</td>
</tr>
<tr>
<td><strong>MSPE (zincon-Si-MNPs)</strong></td>
<td>Cr(III): zincon-Si-MNPs and Cr(VI): zincon-Si-MNPs at different pH</td>
<td>Cr(III): 100 Cr(VI); 150</td>
<td>ET-AAS</td>
<td>0.016 – 0.011, 9.16 – 18.22</td>
<td>[36]</td>
</tr>
<tr>
<td><strong>SPE (nano-TiO₂)</strong></td>
<td>Cr(III): TiO₂ and reduction of Cr(VI)</td>
<td>Cr(III): 50</td>
<td>ICP-MS</td>
<td>0.22 – 7.04</td>
<td>[116]</td>
</tr>
<tr>
<td><strong>SPE (D₂EHPA-MWCNTs)</strong></td>
<td>Cr(III): D₂EHPA-MWCNTs, Cr(VI) in solution</td>
<td>60</td>
<td>ICP-AES</td>
<td>0.05 – 0.96</td>
<td>[68]</td>
</tr>
<tr>
<td><strong>On-line SPE (MWCNTs)</strong></td>
<td>Cr(III): MWCNTs and reduction of Cr(VI)</td>
<td>Cr(III): 22</td>
<td>FAAS</td>
<td>1.15 – 7.1</td>
<td>[111]</td>
</tr>
<tr>
<td>**MMHSPE (Fe₃O₄@ZrO₂)</td>
<td>Cr(III): Fe₃O₄@ZrO₂ and reduction of Cr(VI)</td>
<td>25</td>
<td>FAAS</td>
<td>0.69 – 24.5</td>
<td>[89]</td>
</tr>
<tr>
<td>**MMHSPE (Fe₂O₃/Al₂O₃)</td>
<td>Cr(III): Fe₂O₃/Al₂O₃ and reduction of Cr(VI)</td>
<td>120.6</td>
<td>FAAS</td>
<td>1.4 – –</td>
<td>[33]</td>
</tr>
<tr>
<td><strong>SPE (APDC-MWCNTs)</strong></td>
<td>Cr(VI): ADPC–MWCNTs and oxidation of Cr(III)</td>
<td>100</td>
<td>FAAS</td>
<td>– 0.90 – 9.5</td>
<td>[38]</td>
</tr>
<tr>
<td><strong>SPE (Ni–Al NO₃⁻–LDH)</strong></td>
<td>Cr(VI): Ni–Al(NO₃⁻⁻) LDH and oxidation of Cr(III)</td>
<td>100</td>
<td>FAAS</td>
<td>– 0.51 – 6.5</td>
<td>[151]</td>
</tr>
</tbody>
</table>

**Other speciation methods**

<table>
<thead>
<tr>
<th>Mechanism of speciation</th>
<th>EF or PF</th>
<th>LOD (µg L⁻¹)</th>
<th>Capacity (mg g⁻¹)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chromatography (resin)</strong></td>
<td>IEC</td>
<td>–</td>
<td>HPLC–ICP-MS</td>
<td>0.04 – 0.02</td>
</tr>
<tr>
<td><strong>SPE (iron phosphate)</strong></td>
<td>Cr(III) and reduction of Cr(VI)</td>
<td>8.7</td>
<td>ET-AAS</td>
<td>0.02 – 8.12</td>
</tr>
<tr>
<td><strong>Cloud point extraction (Triton X-100)</strong></td>
<td>Cr(III): 1-phenyl-3-methyl-4-benzoyl-pyrrozal-5-one and reduction of Cr(VI)</td>
<td>20</td>
<td>ICP-AES</td>
<td>0.81 – –</td>
</tr>
<tr>
<td><strong>SPE (Bacillus subtilis-loaded Diaion SP-850 resin)</strong></td>
<td>Cr(III) and reduction of Cr(VI)</td>
<td>50</td>
<td>FAAS</td>
<td>0.50 – 6.95</td>
</tr>
<tr>
<td><strong>Chromatography (C18)</strong></td>
<td>Reversed-phase chromatography</td>
<td>–</td>
<td>HPLC–UV</td>
<td>1.9</td>
</tr>
<tr>
<td><strong>SPE (C18)</strong></td>
<td>Cr(VI): DPC and oxidation of Cr(III)</td>
<td>12</td>
<td>UV–vis</td>
<td>2.4</td>
</tr>
</tbody>
</table>

**SPE:** solid phase extraction; **MSPE:** magnetic solid phase extraction; **MMHSPE:** magnetic mixed hemimicelles solid phase extraction; **MF:** microfluidic; **TiO₂:** titanium dioxide; **TDNTs:** titanium dioxide nanotubes; **APDC:** ammonium pyrrolidinedithiocarbamate; **MWCNTs:** multiwalled carbon nanotubes; **D₂EHPA:** di-(2-ethyl hexyl)-phosphoric acid; **SWCNTs:** single-walled carbon nanotubes; **zincon-Si-MNPs:** zincon-immobilized silica-coated magnetic Fe₃O₄ nanoparticles; **POM:** polyanionmetalate cluster; **Ni–Al LDH:** nickel–aluminum layered double hydroxide; **HPLC:** high-performance liquid chromatography; **EF/PF:** enrichment factor/preconcentration factor; **LOD:** limit of detection.
HG-AAS. Nanomaterial-based separation and preconcentration systems are, therefore, generally coupled to highly sensitive ICP-MS and ET-AAS systems. Because current trace element analytical techniques for practical analyses of biological samples favor on-line systems with low sample volume requirements and low detection limits, combining a MF-based system with a highly sensitive detector is critical. As discussed above, ICP-MS is the most favored detection instrument for on-line coupling of nanomaterial- and MF-based trace element speciation systems.

2.2. Analytical characteristics of various nanomaterials for trace element speciation

Over the past decade, only several dozen papers have appeared related to trace element speciation, most of which have been published quite recently, indicating a trend in research toward trace element speciation. Various nanomaterials, pretreatment methods, and analytical instruments have been used in the speciation of different trace elements, all of which are covered in this review.

Nanomaterial-based speciation techniques have been developed for Cr [35,36,38,68,71,75,76,82,83,89,90,96,97], Se [74,79, 90–92,98,99], As [31,67,69,81,87,93,100–102], Sb [67,87,88,90,93], Hg [91–95,101–103], Te [77], and V [84]. Although still in its early stages of development, nanomaterial-based speciation is facilitated by the ready fabrication, high selectivity, and cost-effectiveness of nanomaterials. The speciation of Cr has been the subject of the majority of nanomaterial-based speciation studies. Table 1 lists the various nanomaterials that have been used for Cr speciation, including titanium dioxide nanotubes (TDNTs) [75], single-walled carbon nanotubes (SWCNTs) [76], cesium polyoxometalate clusters (CsPOMs) [71], nano-Au/TiO2 [82], nano-TiO2 [83], zincin-immobilized silica-coated magnetic Fe3O4 nanoparticles (zincin-Si-MNPs) [36], di(2-ethylhexyl) phosphoric acid on multiwalled carbon nanotubes (D2EHPA-MWCNTs) [68], multiwalled carbon nanotubes (MWCNTs), Fe3O4@ZrO2 nanoparticles [89], FeO4/Al2O3 nanoparticles [39], ammonium pyrrolidinedithiocarbamate on multiwalled carbon nanoparticles (APDC-MWCNTs) [38], and nickel–aluminum nitrate layered double hydroxides (Ni–Al NO3–LDH). This section covers the various nanomaterial-based trace element speciation methods that have been developed, including resin-packed columns for ion exchange chromatography (IEC) [103,104], ion phosphates for SPE [105], Triton X-100 for CPE [106], and CNT for reverse phase chromatography [107,108]. Regardless of the nanomaterials and techniques selected for Cr speciation (Table 1), these analyses generally involve the use of sorbents for separation and preconcentration of Cr(III) rather than Cr(VI). The use of nanomaterials as adsorbents has yielded EFs higher than those obtained without them, because nanomaterials generally provide stronger and more specific binding affinities toward trace elements than do their bulk counterparts.

According to Table 1, the detection limits for Cr in the hyphenated ICP-MS systems are similar. Except for the on-line CsPOM/MF/ICP-MS study by Hsu et al., the detection limits in ICP-MS-based speciation techniques have ranged from 0.3 to 0.5 μg L−1 [71]. The speciation of Cr reported by Hsu et al. was based on on-line CsPOM SPE, where no preconcentration was used. To accommodate low sample volumes (only 20 μL), they obtained a detection limit higher than those of other ICP-MS-based Cr speciation techniques. ET-AAS-based methods for Cr speciation have provided detection limits of 0.02–0.1 μg L−1; ICP-OES-based methods, 0.1–0.8 μg L−1; FAAS-based methods, 0.5–1.4 μg L−1. Although UV-vis spectroscopy-based methods for Cr have provided detection limits of 1.0–2.4 μg L−1, such detection limits are too high and not suitable for nanomaterial-based speciation.

The differences in detection limits among these techniques are greatly influenced by the nature of the analytical instrument. Therefore, ICP-MS-based techniques for trace element assays are the most powerful, requiring small sample volumes and on-line nanomaterial-based pretreatment techniques. On the other hand, the maximum adsorption capacity for target ions is a concern when using SPE-based separation systems. Adsorption isotherms are widely for calculating adsorption capacities. In general, the adsorption capacity of a sorbent has been obtained using the batch technique, where a certain quantity of a nanomaterial is added to a solution containing analytes and reacted for a certain time. The equation

$$q_e = \frac{(C_0 - C_f)V}{m}$$

is used to calculate the adsorption capacity, where $C_0$ is the initial solution concentration, $C_f$ is the solution concentration after sorption, $V$ is the sample volume (L), and $m$ is the mass of the adsorbent (g) [109]. Table 1 also lists the adsorption capacities of various adsorbents. In general, nanomaterials have large surface areas and, thus, high adsorption capacities. Nevertheless, the density of functionalized surface groups is sometimes more important than the total surface area, especially for carbon-based nanomaterials [110]. Another advantage of functionalized sorbents is that the resistance to interference, or the analyte selectivity, can be increased by adding functional groups, but such approaches are beyond the scope of this review.

Previous comparisons have been made between nanomaterial-based analytical methods and other methods for trace element speciation. Wu et al. [83] compared the analytical performance of a nano-TiO2 system with techniques using similar separation and preconcentration approaches when combined with ET-AAS detection. They found that the sensitivity of their protocol was comparable with those of the other procedures. Tuzen and Soylok [38] compared the results of different MWCNT-based Cr speciation studies. The optimal conditions were comparable for the procedure in the literature with quantitative recovery values and lower detection limits for chromium speciation. They found that MWCNT-based speciation procedures were superior to other Cr speciation methods in terms of selectivity, detection limit, applicable pH range, capacity, degree of enrichment, and consumption of organic solvents. Chen et al. [76] compared the analytical performances of SWCNTs with other adsorbents for Cr speciation. They found that the analytical efficiencies of SWCNT-based speciation methods were better than or similar to those of other methods. Yu et al. [111] compared data from a MWCNT-based system with those from other SPE-based studies coupled to FAAS, where the detection limits, RSDs, and sample consumption of the compared techniques were similar. Wu et al. [89] compared the performance of a Fe3O4@ZrO2 MNP-based method with other Cr speciation methods, with the former having the superior LOD. Another study compared the performance of an on-line CsPOM-MF/ICP-MS system with other methods for Cr speciation [71]; the former required similar or lower sample volumes relative to those of methods involving chromatographic separation. The separation procedure of the on-line CsPOM-MF/ICP-MS system is simpler and faster than chromatographic separation. Compared with off-line non-chromatographic separation methods, the on-line CsPOM-MF/ICP-MS system not only markedly decreased run times and sample consumption but also minimized external contamination. These major advantages over non-chromatographic methods indicate a trend toward the use of nanomaterial-based MF techniques. In general, nanomaterial-based methods have higher sensitivities, lower sample volume requirements, and faster analysis times.
3. Nanomaterials for trace element speciation in the past decade

The number of publications on the use of nanomaterials in trace element speciation has increased in the past decade, but it remains a relatively new field, with only several dozen papers available as of 2015. There is enough information in those papers, however, to adequately assess the advantages and disadvantages and the analytical performance of nanomaterial-based speciation techniques. The nanomaterials used most commonly for trace element speciation have been nano-TiO2, CNTs, and MNPs, similar to those used in nanomaterial-based analyses of the total amounts of trace elements. Section 3.1 discusses the trends in nanomaterial-based speciation techniques, as well as the pros and cons of these techniques. Although some studies have focused on using nanomaterials for the speciation of free ions and their nanoparticles [112], due to the rapid growth in the commercial use of (e.g., Ag) nanoparticles, this review does not discuss nanoparticle speciation because only a few papers have appeared on this topic.

3.1. Titanium dioxide nanoparticles (nano-TiO2)

Nano-TiO2 has been used for the speciation of Se [27,29,74,92,98,113–115], Cr [75,82,83,116], As [31,67,81], Sb [67,88], and Hg [91]. The most commonly studied trace element in speciation research is Se, followed by Cr, As, Sb, and Hg. Nano-TiO2 has been involved in the photocatalysis, chemical reduction, and SPE of trace element species [27,28,31,67,74,75,81–83,88,92,98]. The analytical performances of these trace element speciation techniques are elaborated below. The use of TiO2 for speciation bypasses many pretreatment steps and reagents, while inducing the redox reactions required to detect element species (Fig. 1).

Table 2 lists previous studies that have used nano-TiO2 for Se, Cr, As, Sb, and Hg speciation. In most of these studies, Se speciation was performed using nano-TiO2, because Se(VI) can be reduced to Se(IV) through UV-induced redox reactions. Nano-TiO2 in the tubing is stimulated by UV light to donate an electron to reduce Se(VI) to Se(IV), without hydride generation or the use of tetrahydroborate-based techniques. Fig. 2 displays an on-line hyphenated HPLC speciation system using nano-TiO2 for the photocatalytic reduction of Se(VI) to Se(IV) via UV-induced redox reactions. Similar on-line systems for Se speciation have been reported, including HPLC–(UV–UV/UV) PCRD–ECVG)–AFS, HPLC–UV/nano-TiO2–ICP-MS, LC/SPADVI/ICP-MS, and HPLC–AFS [27,28,92]. Se(VI) can be reduced to Se(IV) via hydride generation using tetrahydroborates, but a drawback of on-line hydride generation is the introduction of large amounts of hydride vapor into the detection instrument, increasing matrix interference. The use of nano-TiO2 avoids the production of these chemicals and, therefore, a greener alternative for Se reduction. Thus, these on-line UV/nano-TiO2 systems do not deliver hydride vapor and large amounts of hydrogen into the ICP-MS.

To detect low concentrations of trace elements when using on-line column-based UV/nano-TiO2 systems for trace element speciation, it is necessary to improve the EF or PF by increasing the sample injection volume. As revealed in Table 2, the sample volume used in an on-line method is generally 100 μL. Nevertheless, to accommodate the pressure tolerance of the column, the maximum sample volume in a column-based system is approximately 500 μL; thus, the detection limit is affected by the sample injection volume. To bypass this limitation, the use of sensitive detection instruments (e.g., ICP-MS) provides detection limits that are 10–100 times lower than those of AFS. To date, ICP-MS has been the analytical instrument of choice for hyphenated on-line UV/nano-TiO2 systems for trace element speciation.

Table 2 lists the nano-TiO2 and functionalized nano-TiO2 nanoparticles that have been used as SPE sorbents for Se, Cr, As, and Sb speciation. In many of these studies, the pH was adjusted for optimal separation and preconcentration of specific element species. Because these speciation studies were performed at different values of pH, the nano-TiO2 samples in the separation columns had varying adsorption behaviors (Fig. 3). Adjusting the pH can be used to quantitatively identify different elements and their species. Huang et al. [67] used a dimercaptosuccinic acid-modified TiO2 (DMSA-TiO2) micro-column for As and Sb speciation. They found that both trivalent and pentavalent inorganic As and Sb species were quantitatively adsorbed on DMSA-TiO2 within a pH range of 4–7. Only As(III) and Sb(III) were retained quantitatively on the micro-column within a pH range of 10–11, while As(V) and Sb(V) passed through the micro-column without retention.

The adsorption capacities and enrichment efficiencies of nano-TiO2 toward trace elements are often taken into consideration when performing SPE. The adsorption capacities of packed column-based techniques are also of concern, because analyte enrichment will not increase beyond the adsorption capacity. To achieve a high PF, the sample volume must be increased. The use of

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**Fig. 1.** Schematic representation of trace element analytical and speciation systems using various nanomaterials and detection instruments.
<table>
<thead>
<tr>
<th>Trace elements and species</th>
<th>Role of nano-TiO₂</th>
<th>Sample volume</th>
<th>EF or PF</th>
<th>Detection system</th>
<th>Detection limit (µg L⁻¹)</th>
<th>Precision (RSD%)</th>
<th>Analyzed samples</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se speciation</td>
<td></td>
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<tr>
<td>• SeCys Se(VI), SeMet Se(VI)</td>
<td>On-line photolysis and reduction</td>
<td>100 µL</td>
<td>–</td>
<td>AFS</td>
<td>SeCys: 2.1 Se (IV): 2.9</td>
<td>SeCys: 3.4</td>
<td>Water-soluble extracts of garlic shoots [27]</td>
<td></td>
</tr>
<tr>
<td>• Se(VI), Se(VI)</td>
<td>HPLC–UV–UV/TiO₂, PCRD–EVCYG–AFS</td>
<td>400 µL</td>
<td>–</td>
<td>ICP-MS</td>
<td>Se(VI): 0.06 Se (VI): 0.03</td>
<td>Se(VI): 3.4 Se (VI): 6.5</td>
<td>Agricultural irrigation water, groundwater Extracts of supplements urine [28]</td>
<td></td>
</tr>
<tr>
<td>• Se(VI), Se(VI), SeMet</td>
<td>On-line sequential photocatalyst-assisted digestion and vaporization LC/SPADVD/ICP-MS</td>
<td>100 µL</td>
<td>–</td>
<td>AFS</td>
<td>Se(VI): 0.0039 Se(VI): 0.0072 SeMet: 0.0083</td>
<td>Se(VI): 3.7 Se (VI): 2.7</td>
<td>Wine mineral water, mineral water, wastewater [92]</td>
<td></td>
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<tr>
<td>• Se(VI), Se(VI)</td>
<td>Photochemical vapor generation low temperature: only Se(VI) using boiling water bath: total Se photo-CVG-AFS/ICP-MS</td>
<td>2.4 mL</td>
<td>–</td>
<td>AFS or ICP-MS</td>
<td>Se(VI): 2.38 Se (VI): 3.39</td>
<td>Se(VI): 0.02 (SeV): 0.1</td>
<td>Tap water, green tea, fruit juice, energy drink</td>
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<tr>
<td>• Se(VI), Se(VI)</td>
<td>SPE, Se(VI) and Se(VI) adsorbed on n-TiO₂ at different pH</td>
<td>100 mL</td>
<td>50</td>
<td>GFAAS</td>
<td>Se(VI): 0.0047 Se(VI): 0.0063</td>
<td>Se(VI): 0.7 Se (VI): 0.9</td>
<td>Lake water, seawater, sediment Lake water [113]</td>
<td></td>
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<tr>
<td>• Se(VI), Se(VI), SeMet SeCys</td>
<td>On-line dual column SPE column 1: nano-Al₂O₃ column 2: DMASA-modified TiO₂</td>
<td>4 mL</td>
<td>Se(VI): 5 Se(VI): 2</td>
<td>ICP-MS</td>
<td>Se(VI): 0.05 Se (VI): 0.12</td>
<td>Se(VI): 7.7 Se (VI): 8.8</td>
<td>Tap water, drinking water Tea leaves, tea infusion [83]</td>
<td></td>
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<tr>
<td>• Se(VI), Se(VI)</td>
<td>SPE, Se(VI) and Se(VI) adsorbed on n-TiO₂</td>
<td>100 mL</td>
<td>50</td>
<td>GFAAS</td>
<td>Se(VI): 0.0047 Se(VI): 0.0063</td>
<td>Se(VI): 0.7 Se (VI): 0.9</td>
<td>Lake water, seawater, sediment Lake water [113]</td>
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<tr>
<td>• Se(VI), Se(VI)</td>
<td>SPE, Se(VI) adsorbed on nano-TiO₂</td>
<td>2.4 mL</td>
<td>–</td>
<td>ICP-MS</td>
<td>Se(VI): 0.02 (SeV): 0.02</td>
<td>Se(VI): 7.8 Se (VI): 7.0</td>
<td>Lake water, river water [115]</td>
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<td>Cr speciation</td>
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<tr>
<td>• Cr(III), Cr(VI)</td>
<td>SPE Cr(III): TiO₂ and reduction of Cr (VI)</td>
<td>50 mL</td>
<td>50</td>
<td>ICP-AES</td>
<td>Cr(III): 0.22 Cr (VI): 3.5</td>
<td>Tap water, lake water [116]</td>
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<tr>
<td>• Cr(III), Cr(VI)</td>
<td>On-line SPE and photo-reduction adsorption of Cr(III) and Cr(VI) photo-reduction of Cr(VI)</td>
<td>2 mL</td>
<td>Cr(III): 7.1 Cr (VI): 4.6</td>
<td>FI-ET-AAS</td>
<td>Cr(III): 0.08 Cr (VI): 0.13</td>
<td>Cr(III): 4.0 Cr (VI): 6.2</td>
<td>Urine [82]</td>
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<tr>
<td>• Cr(III), Cr(VI)</td>
<td>On-line SPE adsorption of Cr(III) and Cr(VI)</td>
<td>100 µL</td>
<td>–</td>
<td>FI-ET-AAS</td>
<td>Cr(III): 0.006 Cr (VI): 0.01</td>
<td>Cr(III): 2.0 Cr (VI): 3.2</td>
<td>Tap water, drinking water Tea leaves, tea infusion [83]</td>
<td></td>
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<tr>
<td>• Cr(III), Cr(VI)</td>
<td>SPE</td>
<td>20 mL</td>
<td>67</td>
<td>ICP-MS</td>
<td>Cr(III): 0.0075</td>
<td>Cr(III): 3.8</td>
<td>Tap water, lake water [81]</td>
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<tr>
<td>As speciation</td>
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<tr>
<td>• As(III), AsB, MMA, As(V), DMA</td>
<td>On-line photocatalytic oxidation LC–UV/nano-TiO₂ film reactor-HG–ICP-MS</td>
<td>10 µL</td>
<td>–</td>
<td>ICP-MS</td>
<td>As(III): 0.46 AsB: 0.24 MMA: 0.34 As (V): 0.40 DMA: 0.49 As(III): 0.0024</td>
<td>As(III): 5.3 AsB: 5.3 MMA: 3.4 As (V): 4.7 DMA: 3.7 As(III): 4.8</td>
<td>Microbialysisate of blood or target organs [31]</td>
<td></td>
</tr>
<tr>
<td>• As(III), As(V)</td>
<td>SPE adsorption of As(III) at pH 10 adsorption of As(III) and As(V) pH 6</td>
<td>50 mL</td>
<td>50</td>
<td>ET-AAS</td>
<td>As(III): 0.11 As (V): 0.10</td>
<td>As(III): 5.5 As (V): 3.5</td>
<td>Tap water, lake water [81]</td>
<td></td>
</tr>
<tr>
<td>• As(III), As(V)</td>
<td>Off-line SPE (DMASA-TiO₂) adsorption of As(III) at pH 10 adsorption of As(III) and As(V) at pH 6</td>
<td>40 mL</td>
<td>50</td>
<td>ICP-AES</td>
<td>As(III): 0.53 As (V): 0.49</td>
<td>As(III): 3.9 As (V): 1.5</td>
<td>Lake water, well water, pond water [67]</td>
<td></td>
</tr>
<tr>
<td>• As(III), As(V)</td>
<td>Off-line SPE (DMASA-TiO₂) adsorption of As(III) at pH 10 adsorption of As(III) and As(V) at pH 6</td>
<td>8 mL</td>
<td>10</td>
<td>ICP-AES</td>
<td>As(III): 0.53 As (V): 0.49</td>
<td>As(III): 3.9 As (V): 1.5</td>
<td>Lake water, well water, pond water [67]</td>
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<tr>
<td>Sb speciation</td>
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<tr>
<td>• Sb(III), Sb(V)</td>
<td>Off-line SPE (DMASA-TiO₂) adsorption of Sb(III) at pH 10 adsorption of Sb(III) and Sb(V) at pH 6</td>
<td>40 mL</td>
<td>50</td>
<td>ICP-AES</td>
<td>Sb(III): 0.15 Sb (V): 0.13</td>
<td>Sb(III): 6.7 Sb (V): 5.9</td>
<td>Lake water, well water, pond water [67]</td>
<td></td>
</tr>
<tr>
<td>• Sb(III), Sb(V)</td>
<td>Off-line SPE (DMASA-TiO₂) adsorption of Sb(III) at pH 10 adsorption of Sb(III) and Sb(V) at pH 6</td>
<td>8 mL</td>
<td>10</td>
<td>ICP-AES</td>
<td>Sb(III): 0.77 Sb (V): 0.71</td>
<td>Sb(III): 5.2 Sb (V): 4.6</td>
<td>Lake water, well water, pond water [67]</td>
<td></td>
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</table>
Table 2 (Continued)

<table>
<thead>
<tr>
<th>Trace elements and species</th>
<th>Role of nano-TiO₂</th>
<th>Sample volume</th>
<th>EF or PF</th>
<th>Detection system</th>
<th>Detection limit (µg L⁻¹)</th>
<th>Precision (RSD%)</th>
<th>Analyzed samples</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>adsorption of Sb(III) and Sb(V) at pH 6</td>
<td>500 mL</td>
<td>50</td>
<td>HGAAS</td>
<td>Sb(II): 0.05 Sb (V): 0.06</td>
<td>–</td>
<td>Lake water, well water, pond water</td>
<td>[88]</td>
</tr>
<tr>
<td>• Sb(III), Sb(V)</td>
<td></td>
<td></td>
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<tr>
<td>Hg speciation</td>
<td>SPE adsorption of Sb(III) and Sb(V) reduction of Sb(V)</td>
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<tr>
<td>• Hg(II), MMC, EMC, PMC</td>
<td>On-line UV/TiO₂ photocatalysis HPLC-(UV/TiO₂ PCRD)-AFS</td>
<td>–</td>
<td>–</td>
<td>AFS</td>
<td>Hg(II): 0.01 MMC: 0.02 EMC: 0.03 PMC: 0.07</td>
<td>All Hg species &lt;6</td>
<td>Lake sediment, seafood</td>
<td>[91]</td>
</tr>
</tbody>
</table>

EF: enrichment factor; PF: preconcentration factor; SeCys: seleno-α-cysteine; SeMet: seleno-α-methionine; PCRD: photocatalysis reduction device; ECVG: electrochemical vapor generation; DMSA: dimercapto succinic acid; IC-CD: ion chromatography-conductivity detection; SPE: solid phase extraction; TDNTs: titanium dioxide nanotubes; MMA: monomethylarsenic acid; DMA: dimethylarsinic acid; AsB: arsenobetaine; MMC: methylmercury chloride; EMC: ethylmercury chloride; PMC: phenylmercury chloride; PCRD: photocatalysis reaction device.

nano-TiO₂ as a sorbent in on-line SPE of trace element species regularly requires sample volumes of 0.1–8 mL to obtain EFs of 1–10 times; conversely, off-line SPE requires sample volumes of 20–500 mL to achieve EFs of 30–67 times. Because of such large sample volume requirements, the applicability of this technique is greatly limited to environmental water samples or other samples that are available in large volumes (Table 2). Therefore, the application of nano-TiO₂ in trace element speciation will require lowering the sample volume for preconcentration, thereby extending the applicability of current SPE techniques to samples available in small volumes.

3.2. Carbon nanotubes (CNTs)

Table 3 lists the studies using CNTs for the speciation of various trace elements, including Cr [38,68,76,111], As [69,87,93], Sb [87,93], Ti [85,86], and V [84]. CNTs of various shapes and sizes have been used, including carbon nanofibers (CNFs), SWCNTs, and MWCNTs. CNTs have also been functionalized to create D₂EHPA-MWCNTs, ammonium pyrrolinedithiocarbamate-MWCNTs (APDC-MWCNTs), APDC-CNFs, APDC-SWCNTs, and L-tyrosine-CNTs (L-tyr-CNTs), which are specific to certain analytes.

![Fig. 2. Schematic representation of a Se speciation system using UV/nano-TiO₂ photocatalytic reduction. Reproduced with permission [29].](image-url)
3.3. Magnetic nanoparticles (MNPs)

Table 4 reveals that there have been only a few publications related to trace element speciation using MNPs. This field remains nascent, with the application of MNPs as sorbents for speciation only having emerged over the last five years, with Cr speciation [35,36,89] being the most common, followed by Te [77] and Hg [95]. The reported studies have involved the use of zincon-Si-MNPs [36], Fe₃O₄@ZrO₂ [89], Fe₃O₄/Al₂O₃ [35], γ-mercaptopropyltrimethoxysilane-MNPs (γ-MPTMS-MNPs) [77], and Fe₃O₄ MNPs [95].

The separation of trace element species using MNPs is greatly influenced by the solution pH. Some trace element species have been combined with chemical compounds and adsorbed onto MNP surfaces at different values of pH. Because of the magnetic properties of MNPs, analytes adsorbed on MNPs can be effectively separated from sample matrices under an electromagnetic field. Such separation is known as magnetic solid phase extraction (MSPE) [36,77,89]. Fig. 5 displays the speciation of Cr and Te using functionalized MNPs at different values of pH. As revealed in Fig. 5(a), Cr(III) was retained on the absorbent (zincon-Si-MNPs) quantitatively at pH 9.1, while both Cr(III) and Cr(VI) were retained at pH 6.5, allowing measurement of the total Cr content [36]. Fig. 5(b) reveals that γ-MPTMS-MNPs adsorbed Te(IV) at all values of pH, while Te(VI) did not adsorb at any pH [77]; the different binding affinities enabled separation of the Te species. MSPE can be used to effectively separate trace element species adsorbed on MNPs from other species, but the binding specificity of MNPs for certain species must be high to prevent nonspecific binding and inaccurate results.

Another MNP-based SPE method, magnetic mixed hemimicelle solid phase extraction (MMHSE) [35], involves the coating of Al₂O₃ on Fe₃O₄ nanoparticles to create Fe₃O₄/Al₂O₃ nanoparticles, followed by coating with Triton X-114. The resultant mixed hemimicelles can be used to adsorb different trace elements at different values of pH for speciation studies. In addition to the aforementioned methods, it is possible to modify hemimicelles by coating 1–(2-pyridylazo)-2-naphthol (PAN) to create TX-114-PAN-coated Fe₃O₄/Al₂O₃ nanoparticles. Fe₃O₄ MNPs have been used to perform post-column oxidation in techniques based on HPLC [95]; in that study, organomercury was converted to Hg(II) in the presence of H₂O₂ in an on-line HPLC system using a Fe₃O₄-packed column.

Similar to nano-TiO₂ and CNTs, MNPs are commonly used to separate and preconcentrate trace elements through SPE, with large sample volumes required to attain high EFs when analyzing trace elements near the instrument detection limit. Table 5 lists the studies that have used MNPs in SPE for trace element speciation; the sample volumes and EFs have ranged from 10–250 and 25–320 mL, respectively. Although these techniques have low detection limits, because they have employed ICP-MS as the detection instrument, they were suitable only for analyses of environmental water samples because of the need for high sample volumes. Improvements to these techniques will be required if they are to be used to analyze samples available in small volumes (e.g., biomedical samples) with high EFs.

In addition to nano-TiO₂, CNTs, and MNPs, several other nanomaterials have been developed, including aluminum-based nanomaterials [e.g., nanometer-sized alumina (nano-Al₂O₃), nickel–aluminum layered double hydroxide (Ni–Al LDH), nano-γ-alumina], gold nanoparticles (GNPs), cadmium sulfide quantum dots (CdS-MAA QDs), nano-structured lead dioxide (nano-PbO₂), and cerium oxide nanoparticles (CeO₂-NPs). Similar to the requirements for many nanomaterial-based speciations, these nanomaterials are most commonly used in the SPE of analytes in environmental water samples or other samples with large volumes. Again, improvements in these methods will be needed to

![Fig. 3](image-url) Adsorption of (a) Se, (b) Cr, and (c) As and Sb species on nano-TiO₂ or functionalized nano-TiO₂ at various values of pH. Reproduced with permission [67,115,116].

(IV) is combined with CDTA, V(IV)-CDTA cannot bind to MWCNTs, regardless of pH – a phenomenon that can be exploited for separation of the two species.

CNT-based techniques have problems similar to those of nano-TiO₂-based techniques in that they both require large sample volumes to achieve high EFs for improved analytical performance. This feature limits the applicability of TiO₂/CNT-based methods to the determination of environmental samples, because it can be highly impractical to obtain large volumes of biomedical samples – a major problem for both trace element determination and speciation.
increase the applicability of these techniques toward different samples, especially for biological samples.

4. Novel microanalytical platforms combining nanomaterials and microfluidics

Over the past 20 years, miniaturization has become an important trend in both chemical and environmental monitoring, because it can allow chemical reactions to be performed efficiently in small volumes, consume lower amounts of samples and reagents, and increase analytical speed. Microfluidics is the science of manipulating and controlling fluids on the microscale or submicroscale. Microfluidic platforms and microchips can be used to construct a large number of miniaturized devices with various forms and functions [117]; they have attracted much interest in bioanalytical research [118–121]. Recently, several MF platforms have demonstrated great potential as suitable tools for biological and chemical analyses [122–125]. Applications of MF techniques for the analyses of trace elements remain, however, nascent. In general, complex sample pretreatment procedures and highly sensitive detection methods are often required for trace element quantification. Because the use of such processes and tools is often tedious and inconvenient, simplification and miniaturization of trace element analytical systems is a growing trend. This section discusses the trend toward miniaturization of trace element analytical systems, as well as applications of nanomaterial-based MF devices in trace element speciation.

4.1. Fabrication of MF platforms

First-generation MF chips were commonly prepared using wet and dry etching methods. These chips were made of silicon or glass, conferring resistance to organic solvents, stable electro-osmotic flows, and ease of metal deposition as a result of their inertness [126]. Nevertheless, the fabrication of silicon- and glass-based chips is expensive and time-consuming. To accommodate a
broad range of biological applications, other materials have been introduced for the preparation of MF chips, including poly(methyl methacrylate) (PMMA), polydimethylsiloxane (PDMS), polystyrene (PS), and polycarbonate (PC). Among them, PDMS and PMMA have become the most widely used polymers in MF devices; they provide ease of fabrication, high transmittance, and low costs. Because most of the MF devices applied in trace element assays have been fabricated using PDMS and PMMA, here we focus on fabrications involving these two polymeric materials. In addition, we also discuss the various methods that have been used in the fabrication of polymeric MF devices, including hot embossing, injection molding, soft lithography, and laser micromachining.

The miniaturization of trace element analytical platforms can lower analytical costs and improve analytical efficiency. Replica molding is one of the most commonly used techniques for the

![Graph](image)

Fig. 4. Adsorption of (a) Cr, (b) As and Sb, (c) Ti, and (d) V species on CNTs or functionalized CNTs at various values of pH. Reproduced with permission [84,85,93,111].

### Table 4

<table>
<thead>
<tr>
<th>Trace elements and species</th>
<th>Role of MNPs</th>
<th>Sample volume</th>
<th>EF or PF</th>
<th>Detection system</th>
<th>Detection limit (pg L⁻¹)</th>
<th>Precision (RSD%)</th>
<th>Analyzed samples</th>
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<tr>
<td>Cr(III), Cr(VI)</td>
<td>MSPE (zincon-Si-MNPs) Cr (III) and Cr(VI); zincon-Si-MNPs at different pH</td>
<td>100 mL</td>
<td>Cr(III): 100 Cr (VI): 150</td>
<td>ET-AAS</td>
<td>Cr(III): 0.0156 Cr(VI): 0.0111</td>
<td>Cr(III): 6.0 Cr (VI): 6.2</td>
<td>Tap water, lake water</td>
<td>[36]</td>
</tr>
<tr>
<td>Cr(III), Cr(VI)</td>
<td>MSPE (Fe₃O₄@ZrO₂) Cr (III): Fe₃O₄@ZrO₂ and reduction of Cr(VI)</td>
<td>10–75 mL</td>
<td>Cr(III): 25</td>
<td>FAAS</td>
<td>Cr(III): 0.69 Cr(VI): 2.1</td>
<td>Cr(III): 2.1</td>
<td>River water, lake water, sediment, human serum, human urine</td>
<td>[89]</td>
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<tr>
<td>Cr(III), Cr(VI)</td>
<td>MMHSPE (Fe₃O₄/Al₂O₃) Cr (III): Fe₃O₄/Al₂O₃ and reduction of Cr(VI)</td>
<td>250 mL</td>
<td>Cr(III): 120.6</td>
<td>FAAS</td>
<td>Cr(III): 1.4</td>
<td>–</td>
<td>River water, wastewater, tap water, mineral water</td>
<td>[35]</td>
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<tr>
<td>Te species</td>
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<td>Te(VI), Te(VI)</td>
<td>MSPE (γ-MPTMS-MNPs) Te(VI)/γ-MPTMS-MNPs reduction of Te(VI) to Te (IV)</td>
<td>160 mL</td>
<td>Te(VI): 320</td>
<td>ICP-MS</td>
<td>Te(VI): 0.079 ng L⁻¹</td>
<td>Te(VI): 7.0</td>
<td>Seawater</td>
<td>[77]</td>
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<td>Hg species</td>
<td></td>
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<tr>
<td>Hg(II), MeHg, EtHg, PhHg</td>
<td>Post-column oxidation (Fe₃O₄, MNPs), on-line conversion of organomercury to Hg(II)</td>
<td>20 μL</td>
<td>–</td>
<td>AFS</td>
<td>Hg(II): 0.7 MeHg: 1.1 EtHg: 0.8 PhHg: 0.9</td>
<td>Hg(II): 3.9 MeHg: 1.6 EtHg: 4.8 PhHg: 2.1</td>
<td>River water, pool water</td>
<td>[95]</td>
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MNPs: magnetic nanoparticles; MSPEL: magnetic solid phase extraction; MMHSPE: magnetic mixed hemimicelles solid-phase extraction; γ-MPTMS: γ-mercaptopropyltrimethoxysilane; MeHg: methylmercury; EtHg: ethylmercury; PhHg: phenylmercury.
fabrication of PDMS-based MF analytical platforms for trace elements. A master mold is generally created first for casting of the microstructures onto PDMS devices [126,130,131]. After a mixture of PDMS base and curing agent is cured on the master, the PDMS replica is peeled away from the master mold, and the channels are sealed to a flat substrate (e.g., PDMS, PMMA, glass). This technique can produce casts with high fidelities (<0.1 μm). In addition, fabrication of PDMS is simple, cheap, and rapid, making it ideal for MF analytical platforms. Notably, the dimethylsiloxane groups create a highly hydrophobic surface, resulting in poor wettability with aqueous solutions. Because this phenomenon prevents interference from polar analytes and reagents, surface

Fig. 5. Speciation of (a) Cr using zincon-Si-MNPs and (b) Te using γ-MPTMS-MNPs at various values of pH. Reproduced with permission [36,77].
modification is often required to functionalize PDMS for specific interactions with polar analytes. Recently, chemical modification and physical masking have been used to increase the surface hydrophilicity of PDMS.

PMMA is another polymeric material used widely in the fabrication of MF devices for trace element assays. In contrast to the fabrication of PDMS-based MF devices, the etching methods (particularly laser micromachining) used for the fabrication of PMMA-based MF devices are rapid. Although UV and infrared lasers have been used for laser micromachining of polymers, CO₂ laser etching has become the most frequently used method for PMMA chip fabrication [132–134]. During CO₂ laser irradiation of polymer surfaces, the laser energy is strongly absorbed, resulting in the formation of channels and cavities through polymer melting and decomposition at the irradiation sites. Because laser ablation results in rugged channels and cavities, MF devices fabricated using laser etching methods cannot be applied for optical detection.

In short, the fabrication of MF devices from polymeric materials is cost-effective and simple. In contrast to laser micromachining, replica molding is suitable for fabrication of PDMS-based devices. Laser micromachining is a rapid fabrication method, but its formation of rugged ablation surfaces limits its applications in optical detection systems. Nevertheless, the former method requires more advanced and expensive technologies.

On the other hand, several sample handling processes (e.g., purification, enrichment, pretreatment) have been integrated successfully onto MF devices for various bioapplications, including gene and protein analysis, cell culture analysis, and biochemical and pathogen detection. Current MF techniques encounter difficulties, however, when performing complete on-chip sample handling for trace element assays, except for samples having simple compositions, because of the lack of advancements in the miniaturization of the pretreatment techniques. A major problem is interference caused by complicated sample matrices. Moreover, although some novel adsorbents have been developed for adsorption of certain elements, the lack of effective or specific trace element extractants remains a major limitation. As a result, off-line sample pretreatment is often required. MF devices that integrate sample handling and trace element assays are quite rare.

4.2. Microfluidics for trace element assays

Microfluidic devices for trace element assays can be broadly classified as operating through derivatization- or adsorption-based methods. Trace elements can be derivatized through reactions with fluorescent molecules prior to detection of the derivatized complexes. Several studies have led to the development of MF-based analytical systems for the determination of the total content of trace elements.

Zhang et al. published a series of papers describing the use of fluorescence-based MF devices for the detection of trace metal ions; their goal was to develop inexpensive and real-time monitoring methods for the detection of trace metal ions including Cd²⁺ [135], Hg²⁺ [136], and Pb²⁺ [137]. Dalavoy et al. [138] developed an MF-based Pb²⁺ sensor employing, as a recognition element, a Pb²⁺-specific DNazyme that cleaves its complementary DNA substrate strand in the presence of Pb²⁺; in their study, the DNazyme was immobilized on the microchannels of a PMMA MF device. Xue et al. [139] reported a rapid and simple enrichment system on an MF chip integrated with on-line complexation and fluorescence detection; under optimized conditions, the micro-SPE system effectively enriched Ca²⁺, Mg²⁺, Zn²⁺, and Pb²⁺ ions by 520-, 565-, 578-, and 487-fold, respectively. Fan et al. [140] reported a portable, power-free MF device for the rapid and sensitive detection of Pb²⁺; here, 11-mercaptoundecanoic acid (MUA)-functionalized gold nanoparticles (MUA-AuNPs) were used as probes to chelate Pb²⁺, leading to aggregation of the MUA-AuNPs.

In general, the direct determination of trace metal ions in complex matrices is limited mainly by matrix interference. Preconcentration and/or separation are often necessary to improve the sensitivity and selectivity of determination. Therefore, MF devices that incorporate on-chip extraction (e.g., LLE and SPE) will facilitate the realization of practical lab-on-a-chip devices. SPE has been the most common extraction method applied with MF devices. Hylton and Mitra [141] reported the continuous on-line extraction of As(V) through MF membrane extraction using a conventional hollow-fiber membrane. Here, on-line membrane extraction of As(V) through chelation in a liquid membrane support was followed by conventional colorimetric detection using a UV–vis spectrophotometer. The extraction and concentration of As were accomplished through supported liquid membrane extraction. An EF of close to 30 was obtained with a detection limit of 27 µg L⁻¹. Date et al. reported the trace-level analysis of Hg (II) in aqueous samples using a method based on SPE followed by an MF immunoassay [142]. Their MF device featured a main channel for introducing the sample solution and washing buffer and a sub-channel for manipulating the beads. The detection area was aligned with the optical source (LED) and photodiodes. Using this MF platform, their ultrasensitive Hg(II) immunoassay yielded results within 10 min with a detection limit of 0.13 µg L⁻¹.

Capillary electrophoresis (CE)-based MF analytical systems have also been applied for MF-based trace element assays. Harrison et al. proposed a system combining CE with an MF device [143], using electrophoretics to achieve sample separation and solvent pumping [144,145]. Several other studies of CE-based microfluidics for trace element speciation have been published [146–149]. Most MF-based electrophoresis systems generally feature (1) a sample and buffer injection zone, (2) an electrophoresis separation channel, and (3) a system for analyte detection. Different analytes will have different migration speeds when placed in an electric field. Therefore, the length of the separation channel is an important factor influencing the efficiency and sensitivity of the separation. Furthermore, matrix effects and the co–migration of analyte ions can also affect the analytical efficiency.
of CE for multi-elemental analyses. Although several methods using CE-MF for analysis of trace metal ions are currently available, we focus herein on the applications of nanomaterials combined with microfluidics for trace element speciation.

The shift toward MF-based trace element speciation, which drastically lowers the required sample and reagent volumes, makes it easier to analyze samples having small volumes (e.g., biomedical samples) in a practical manner, as opposed to non–MF-based techniques that require large sample volumes. Nevertheless, current MF devices lack their own detection systems, requiring on-line hyphenation to other systems (e.g., chromatography, mass spectrometry) for determining the trace element species.

Some recent reports have described combining nanomaterials with microfluidics for trace element speciation (see Section 3). The nanomaterials were integrated in the MF systems to perform their useful roles in preconcentration, catalysis, and separation of analyte species. In general, the parameters influencing separation or preconcentration must be optimized (e.g., adsorption medium pH; adsorbent mass; adsorption/elution flow rate; sample and eluent volumes). To minimize the consumption of nanomaterials in trace element assays, immobilization of nanomaterials within MF devices remains a challenge.

Shih et al. [150] developed a selective and sensitive hyphenated system employing an MF-based vapor generation (VG) system coupled with HPLC separation and ICP-MS detection for the determination of trace inorganic selenium species. The VG system used PMMA substrates of high optical quality to fabricate an MF-based photocatalyst-assisted reduction device (MF-based PCARD). Moreover, to minimize the consumption of the photocatalyst during analytical procedures, an MF-based PCARD coated with nano-TiO2 was employed to avoid consecutive loading; a dynamic coating method was used to simplify the coating procedure and improve the stability of the coating materials. Both Se(IV) and Se(VI) were efficiently vaporized within 15 s; their detection limits were 0.043 and 0.042 μg L⁻¹, respectively. A series of validation experiments confirmed that this method could be applied satisfactorily to the determination of inorganic Se species in environmental water samples.

Hsu et al. developed an on-line polyoxometalate cluster (POM)/MF separation system coupled with ICP-MS detection for the determination of Cr(III) and Cr(VI) in aqueous samples [71]. They fabricated MF chips using a CO₂-laser etching method (Fig. 6). The immobilized POMs were prepared by coating an inert microporous support membrane with a POM solution, after which the POM-immobilized membranes were packed into a reaction chamber. The linearity (>0.9987), precision (<3.69%; n = 7), and accuracy (95.93–108.60%) of this analytical procedure were quite high under optimized conditions.

Chen et al. reported novel methods for (on-chip) magnetic solid phase extraction (MSPE) combined with HPLC/ICP-MS for selenium speciation in selenium-enriched yeast cells [79]. They fabricated an integrated MF chip comprising reaction, mixing, and extraction units for on-chip MSPE: sulfonated polystyrene-coated magnetic nanoparticles (Fe₃O₄@PSS MNPs) were prepared as the adsorption material for MSPE of selenoamino acids and selenopeptides (Fig. 6). Selenium species in various samples were successfully determined using their proposed MF analytic system. The analytical performance of the on-chip MSPE-HPLC-ICP-MS was evaluated under individual optimal conditions. The limits of detection for five target selenium species were 0.025–0.090 and 0.057–0.149 mg L⁻¹ when using MSPE-HPLC-ICP-MS and on-chip MSPE-HPLC-ICP-MS, respectively. The MSPE-HPLC-ICP-MS method is sensitive, rapid, easy-to-operate, and economical.

Shih et al. and Hu et al. combined nanomaterials with MF devices for selenium speciation using TiO₂ and MNPs, respectively [150,67]. In these cases, however, the said nanomaterials were used for photocatalysis and preconcentration, rather than element separation for speciation. Hsu et al. used a CsPOM-packed MF device for chromium speciation. Although this method allowed the speciation of chromium under environmentally friendly conditions, the CsPOM synthesis and device assembly steps were time-consuming, thereby limiting the practicality of the technique. Current methods for trace element analysis often use nanomaterials for the analyses of multiple elements at once or of only a single species. The mechanisms behind these methods generally involve electrostatic adsorption of the analyte elements for the separation of their different species. As described in Section 3, however, many current nanomaterial-based speciation methods can separate only two element species at most. As a result, there are many difficulties encountered when using a single

Fig. 6. Overview of recently developed microfluidic-based trace element speciation systems combined with nanomaterial-based separation and preconcentration.
type of nanomaterial to analyze the different species of multiple elements. Functionalization of nanomaterials is usually necessary for the analysis of different species of multiple elements; this approach is becoming a future trend in nanomaterial-based speciation research.

Most microfluidics-based trace element analytical systems have been developed for analyses of environmental samples, which are readily available so that large amounts of sample can be used to obtain EFs after preconcentration. In contrast, biological samples are often available in limited amounts, making it difficult to obtain EFs as high as those of environmental samples. In addition, the compositions of biological samples are much more complex than those of environmental samples. The use of sensitive detection instruments (e.g., ET-AAS, AFS, ICP-MS) requires pretreatment, such as sample dilution, to lower the analyte concentrations. Improvements in MF-based techniques will be needed to overcome these obstacles in trace element speciation analysis.

Despite advances in the applications of nanomaterials in microfluidics for on-line trace element speciation, there remain limitations that must be addressed if we are to develop techniques that are practical, simple, and green. One limitation is that some off-line sample pretreatment steps (e.g., extraction, filtration, separation) are still required prior to MF-based analysis. A well-designed on-chip sample pretreatment procedure would certainly simplify trace element analysis systems. The adsorption capabilities of the nanomaterials are also critical parameters. Although immobilization of nanomaterials on microchips minimizes the required adsorbent volumes, a longer channel length is required to obtain a sufficient reaction surface area, inevitably increasing the analysis time. Studies of trace element speciation techniques in which effective and sensitive extraction techniques are major factors remain relatively rare.

5. Conclusions

This critical review focuses on the use of nanomaterials to facilitate trace element assays. Many nanomaterial-based trace element analytical systems—based on, for example, nano-TiO₂, CNTs, and MNP—have been developed recently for various applications. These nanomaterials have a range of binding affinities and a range of optimal values of pH for the adsorption of trace element species. Generally, a high EF will improve analytical performance, but will require large sample volumes to overcome the extraction and preconcentration capabilities of current analytical techniques. Therefore, environmental samples, which are generally more readily available in larger quantities than are biological samples, usually yield higher EFs and more sensitive analyses. Improvements in these techniques will be needed to ensure high EFs when analyzing samples available in small volumes (e.g., biomedical samples). On-line systems are preferred for practical analyses of biological, food, and environmental samples using nanomaterial-based techniques, because of their low sample volume requirements and low detection limits. Integration of trace element analytical systems with MF devices can also overcome the issue of sample volume—these miniaturized devices require only several microliters of sample for analysis. Simplification and miniaturization of trace element analytical systems is an important trend, because current sample preparation processes are often tedious and the instrumentation can be expensive and inconvenient. The current trend toward trace element speciation reveals a need to improve existing techniques, where a major obstacle is found in the pretreatment procedures often required when analyzing complex samples. A future trend in the nanomaterial-based MF determination of trace elements will presumably be the simplification and integration of various sample pretreatment and detection processes into single MF devices.

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