

Nanoparticles and continuous-flow systems combine synergistically for preconcentration

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The exploitation of the singular properties of nanoparticles (NPs) and the well-known characteristics of flow methodologies have led to the development of innovative analytical approaches and applications, opening a new, challenging scenario. The employment of NPs as sorbents in continuous preconcentration systems has become one of the most important applications of NPs and constitutes an excellent example of this powerful combination.

This article overviews issues with respect to on-line procedures and NPs as sorbents, namely, the nature of NPs, the immobilization mode, the location of the preconcentration unit in the flow manifold, and applications to a wide variety of samples.

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1. Introduction

In recent decades, the leading role played by automation towards simplification and miniaturization of analytical process has been amply demonstrated [1]. Among all the steps that are involved in the analytical process, the greatest focus has been on the automation of sample treatment to take advantage of the well-known promises of flow techniques: reproducibility in sample handling; saving both sample and reagent consumption; improvement in sampling throughput; and, reducing risk of sample contamination and waste generation.

Nowadays, automated analytical methods that can achieve low limits of detection (LODs) are required. There are many preparation techniques that are employed in analytical chemistry to improve the sensitivity of the method by increasing analyte concentration and its selectivity by removing interferences from the sample matrix. Among them, solid-phase extraction (SPE) is the most attractive procedure for automation, due to its ease of implementation, its ability to be combined with different detection techniques and its high capability in preconcentration, so that it overcomes low LODs. However, automa-

tion of SPE allows reagent and sample consumption to be minimized, reducing time of analysis and avoiding exposure to potential hazards. In addition, losses due to evaporation are averted since all the steps in on-line SPE are performed in a closed system.

In general, flow techniques are used to condition the extraction column, to introduce a suitable volume of sample through the column where the analyte is retained, to wash the column to remove interfering material, and finally to elute the analytes from the column with a discrete volume of solvent and transport it to the detection system. The compounds of interest can be directly retained on the adsorbent or previously derivatized in a chemical form that can be properly adsorbed.

As in manual mode, the selection of an appropriate SPE sorbent is critical to obtain an efficient extraction procedure, namely a high percentage of the analyte must be trapped by the solid extractant, and then easily and completely eluted from the solid phase. The sorption process must be reversible and SPE sorbents should have large surface areas, exhibit stability toward the sample matrix and the elution solvents, and have good surface contact with the sample solution [2].

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Although automated preconcentration methods are not limited to SPE, these are easier to automate, and not require the use of additional devices (e.g., flow segmenter or phase separator), resulting in a simpler flow manifold.

The use of nanoparticles (NPs) as analytical tools to improve analytical methods or to develop new ones has had a major impact in recent years. The unique properties of NPs are exploited to improve the performance of the analytical method (e.g., its sensibility or selectivity), besides increasing miniaturization in the whole process [3].

To this end, NPs have been used in sample preparation, as sorbents, pseudo-stationary phases or filters, and in detection, as sensors. With respect to detection, NPs have been widely applied in the preparation of electrochemical sensors [4,5] and chemiluminescence (CL) reactions [6–8], and proved to be very efficient, leading to the development of more sensitive and more selective (bio)assays.

Certainly, there has been a lot of research around the exploitation of NPs as sorptive materials due to their

fascinating chemical and physical properties. Large specific surface area, high chemical resistance and high adsorptive capacity are the main characteristics that make NPs very attractive for the development of solid-phase preconcentration. NPs of different nature (carbon, metallic, silica) have been widely applied as sorbents to determine organic and inorganic analytes in a variety of matrix samples [9].

In this article, we review the state-of-the-art in using NPs as sorbent material in continuous preconcentration procedures and the synergistic effect between NPs and continuous-flow (CF) systems. In order to give an overview for those readers who are not familiar with flow techniques or those recently introduced to the subject, we briefly describe different flow configurations based on the position of preconcentration units (PUs) in the system. Then, we give an exhaustive description of different types of PUs, based on the nature of the NPs and the immobilization strategy. Finally, we summarize different PUs and applications in flow methods.

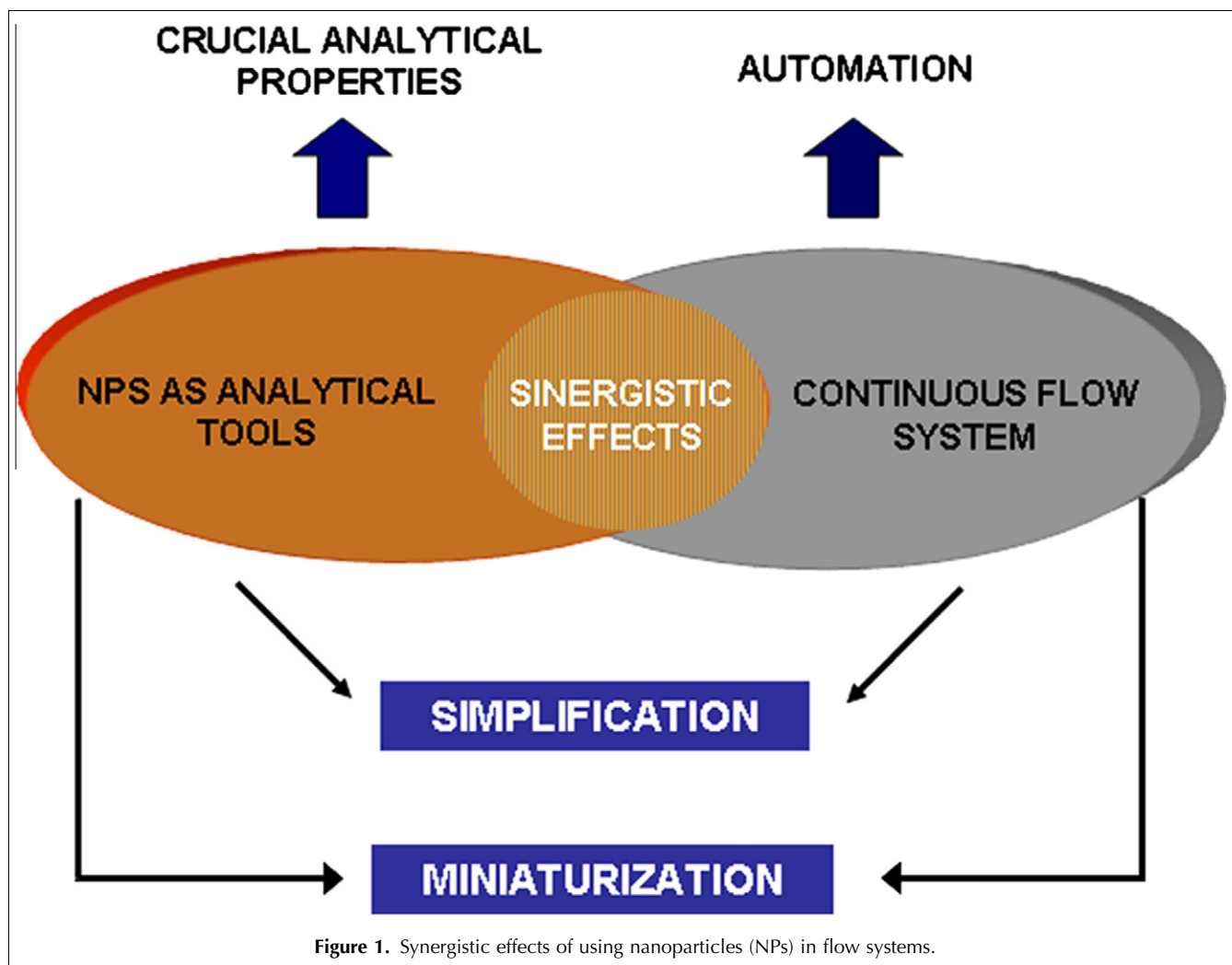


Figure 1. Synergistic effects of using nanoparticles (NPs) in flow systems.

2. Synergistic effect

Since nanotechnology has had such an important role in science in general and analytical chemistry in particular, the amazing features of the nanostructured materials have led to their inclusion in nearly all analytical approaches and applications. Flow methodologies have been no exception and have been employed at different steps of the analytical process.

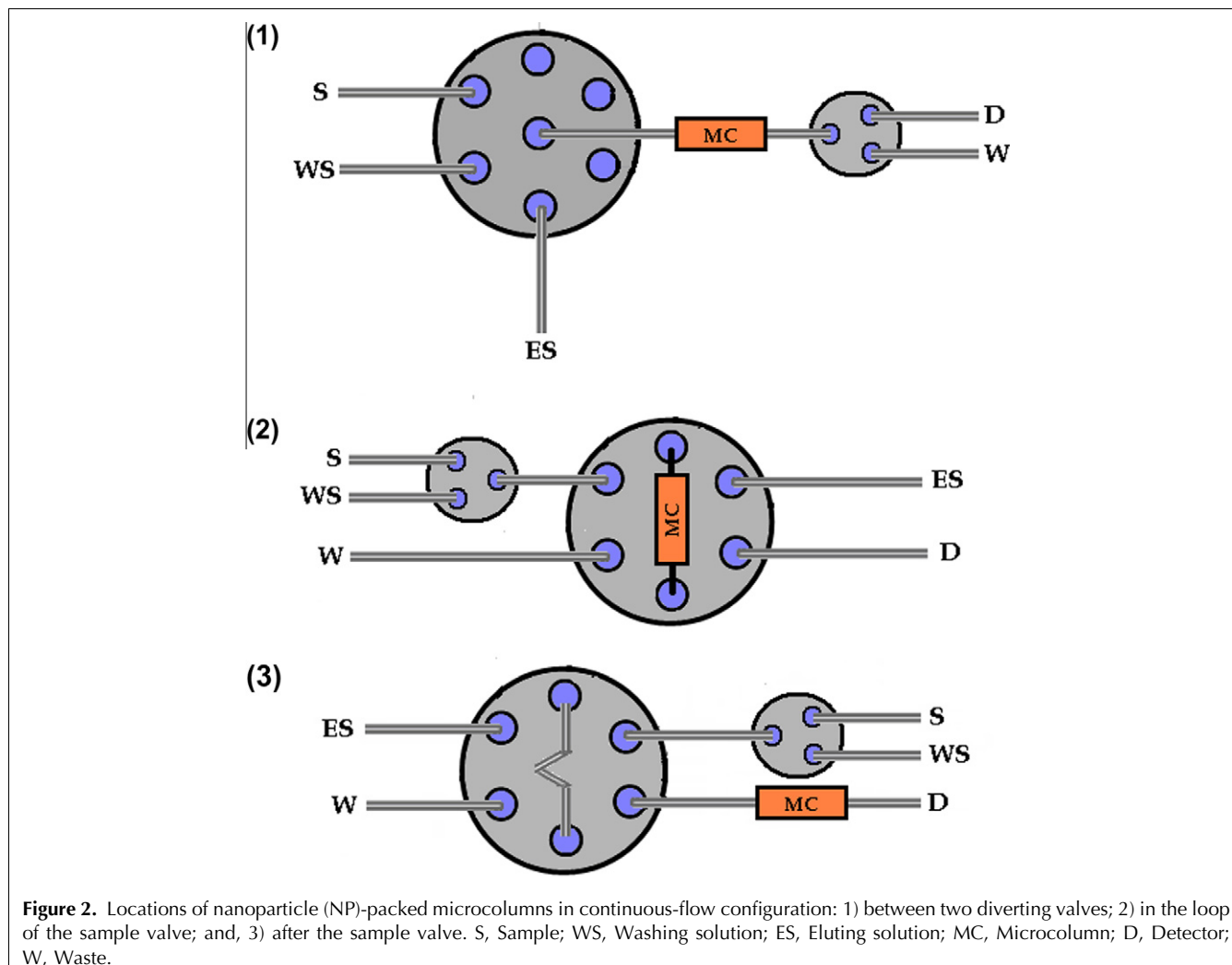
The inherent advantages of the automation of the analytical methods combined with the size and the exceptional properties of NPs have opened up a brand new outlook for simplification and miniaturization of the analytical process (Fig. 1).

It is worth noting that, currently, the main use of flow systems is automation and simplification of sample-treatment processes. In this regard, preconcentration techniques based on adsorption onto NPs of different types are becoming increasingly important.

To the above advantages of automating SPE, we add the advantages of using NPs as sorbents. As expected,

incorporating an NP-sorbent column into a flow system substantially improves the sensitivity of the method because of a greater preconcentration of the target analyte compared with the use of other common sorbents [10]. Furthermore, the amount of NPs used as SPE material is lower than the conventional sorbents used in SPE cartridges, offering the possibility of designing miniaturized on-line extraction devices.

However, implementation of NP-packed microcolumns in flow-based systems for automation and simplification of sample treatment suffers from some limitations because of possible entanglement and aggregation. The sorption capacity can therefore be affected as a result of significant reduction in the effective surface area. Also, compaction of NPs is observed due to the sorption/desorption cycles resulting in not only deterioration of the adsorption process but also overpressure in the flow system. As in a conventional column-based extraction system, the formation of flow passages through the packed column is possible, leading to poor column efficiency. To incorporate an extraction



microcolumn in a flow system and overcome these shortcomings, different strategies to immobilize NPs can be applied.

3. Continuous-flow configurations

Although flow methodologies are well-known, we briefly describe different configurations that can be adopted to perform an on-line SPE.

Designs of flow manifolds that are employed for extraction and preconcentration of analytes using NPs are the same as those used for other sorbents and vary according to the position of the PUs in the system. The use of each configuration depends on different variables (e.g., the available amount of sample, the amount of solvent needed for elution or the type of detection that is employed).

In short, PUs packed with NPs can be placed at three different positions in the flow stream, as shown in Fig. 2:

- (1) after the selection valve, where no injection step is necessary and sample is directly introduced as a CF stream. The preconcentration device is placed in the CF stream and the sample passes through it with the aid of a pump adjusted to the desired flow rate. After that, the eluent passes through the sorbent, desorbs the analyte retained on the NPs and transports it to the detection system [11,12];
- (2) in the sample valve, instead of the sample loop, so that extraction and preconcentration of the analyte can be achieved directly in the loop of the sample valve and the eluted solution is inserted into the carrier stream. As a rule, the eluent is propelled through the column in the opposite way that the sample is introduced to avoid the continuous impact of the solutions on the sorbent [13–16];
- (3) immediately after the sample valve, where an exact volume of sample, eluent or both can be injected into the carrier stream, and pass through the microcolumn to perform the extraction [17–19].

4. Preconcentration units

To carry out SPE, PUs are packed with a certain quantity of NPs and can have different formats (e.g., syringe barrels, membrane discs, or even a tip of a micropipette), even though cylindrical packed-bed microcolumns are the most frequently used format in CF methods.

PUs can be described according to the nature of the NPs that are employed as sorbents and the strategy that is employed to immobilize them. Immobilization of NPs avoids overpressure problems and losses of adsorbent material, besides allowing reuse of microcolumns a greater number of times, without them losing their adsorption capability.

Each packed-column and immobilize strategy can be applied to different types of NPs, as shown in Table 1. In the following sub-section, we highlight examples of PUs for NPs of different natures, applied in CF systems.

4.1. Carbon nanoparticles (CNPs)

4.1.1. Fullerenes. Buckminster fullerenes are closed-cage carbon molecules where the carbon atoms are arranged in pentagonal and hexagonal rings. Fullerenes have less tendency to aggregate than other CNPs, which makes them promising candidates for use as sorbents in SPE based on CF devices. Usually, 60–80 mg are packed in polytetrafluoroethylene (PTFE) microcolumns ~1.0 cm long and 3 mm inner diameter, in powder form, without any previous immobilization procedure, and are conditioned to perform the preconcentration. Microcolumns are sealed at both ends with cotton wool in order to avoid losses of material.

The potential of fullerenes as sorbent materials for on-line preconcentration or determination of metal ions was studied for the first time by Gallego et al. [10]. A flow-injection analysis (FIA) method was developed and applied to the determination of traces of lead in water. As the retention of the metal on the fullerene surface requires the formation of a neutral chelate, ammonium pyrrolidinedithiocarbamate was used as ligand. The

Table 1. Different combinations for packing preconcentration units (PUs) in flow systems, taking into account the nature of nanoparticles (NPs) and mode of immobilization

		NP nature		
		Carbon ^(a)	Metallic ^(a)	Silica ^(a)
Immobilization mode	Without immobilization	●	●	
	Multilayer format	●		
	Chemically modified		●	●
	Chemically bonded to a support	●	●	●
	Dispersed on inert particles	●		
	Retained on filters	●		
	Retained by magnetic field		●	

^aCircles (●) indicate the actual combination for packing PUs in flow systems.

chelate was formed in the CF system, sorbed on a C₆₀ fullerene minicolumn, eluted and directly introduced into an atomic absorption spectrometer. Results were compared with those obtained by performing the same determination using C₁₈-bonded silica and activated carbon as sorbents. Fullerenes exhibited the highest preconcentration factor (PCF), lowest LOD and best selectivity. Similar flow configurations were used to determine other metals (e.g., copper and cadmium [20,21]) in different matrices (e.g., water and fish liver, respectively).

Fullerenes have also been applied to continuous preconcentration of organometallic compounds of mercury and tin in water samples [22], cobalt in wheat flour [23] and organic compounds [24,25] in wastewater samples, followed by determination off-line.

4.1.2. Carbon nanotubes (CNTs). CNTs were first reported following arc-discharged synthesis of C₆₀ in 1991 [26]. They comprise graphene sheets rolled coaxially into cylinders of nm diameter, and can be classified as single-walled CNTs (SWCNTs) or multi-walled CNTs (MWCNTs), depending on the number of carbon sheets [27]. Their well-known unique properties are due to their large surface area-to-volume ratio and their ability to establish π - π electrostatic interactions, so they have been widely exploited in adsorption processes [28]. Due to better interaction with the analyte, MWCNTs are preferred to SWCNTs in SPE procedures [29], but, in spite of that, many works have successfully used SWCNTs for preconcentration.

As far as flow-solid-phase preconcentration is concerned, several studies have related to the use of CNTs packed in microcolumns. The simplest approximation is carried out by packing a PTFE or poly (methylmethacrylate) (PMMA) cylindrical tube with CNTs in their powder form, and sealing it with cotton or glass wool frits at both ends. Lengths of microcolumns are in the range 1 mm to 1.0 cm, while inner diameters are, in general, of about 3 mm. Usually, a short column length with a relatively larger column diameter is recommended to avoid deterioration or tailing of the peak profiles attributable to the concentration gradient along the microcolumn. This strategy was employed by Du et al., who carried out SPE on-line by using oxidized MWCNTs as sorbents for the isolation of hemoglobin and cytochrome C in biological-sample matrices [30]. The microcolumn was placed in a sequential-injection analysis (SIA) system coupled to spectrophotometric detection. As the surfaces of the MWCNTs were negatively charged, electrostatic interactions occurred between the sorbent and the proteins, which were adsorbed. The retained protein species were eluted in a sequential way by using appropriate solvents of adequate pH.

To surmount the possible agglomeration of packed CNTs that could give rise to entanglement, overpressure

in the system and poor analytical performance, the microcolumn can be loaded in a multilayer format, inserting PTFE chunks as layers between the CNTs and furnished with polyethylene frits. In this way, the microcolumn could be integrated in a fully automated system and be reused without a fast deterioration of the sorbent capacity. This strategy was employed to carry out on-line uptake and preconcentration of water-leachable hexavalent chromium from soils [31] by using an MWCNT microcolumn in an SIA system. Automation of the method allowed determination of the water-soluble Cr (VI) fraction in soils in 6 h against more than 24 h for the standard method.

Although PUs with a cylindrical shape are most commonly used, extraction microcolumns with conical shape are also employed. The conical design increases the enrichment factor by reducing dispersion. Wu et al. [32] developed an FIA method for the simultaneous speciation of inorganic arsenic and antimony in natural water samples by employing two micropipette tips to prepare the SWCNT-extraction microcolumn that was placed in the loop of an injection valve. In addition, the microcolumn was partitioned into four compartments with a portion of glass wool between them to try to avoid the backpressure in the FIA system. Furthermore, the eluting solution was pumped in a direction opposite to that in which the sample solution was introduced. As a result, the microcolumn could be regenerated and it enabled more than 30 preconcentration/elution cycles with no significant effect on the sorbent capacity. For total inorganic arsenic and antimony determination, a quantitative reduction of As(V) and Sb(V) present in the sample were carried out. The FIA system was coupled to a hydride-generation dual-channel atomic fluorescence spectrometer to carry out the detection. To achieve suitable acidity for the generation of volatile As and Sb species, HNO₃ solution was selected as eluent. Satisfactory enrichment factors [i.e. 25.4 for As(III) and 24.6 for Sb(III)] were found with a preconcentration time of 180 s. The conical design of the preconcentration microcolumn using MWCNTs as sorbent was employed in the determination of cobalt [33] and cadmium [34] with on-line detection by flame atomic absorption spectrometry (FAAS) and inductively-coupled plasma optical emission spectrometry (ICP-OES), respectively.

A microcolumn can also be packed with CNTs in the form of paper sheets called "buckypapers" [35]. Here, the CNTs are dispersed in a solvent and filtered through an inert membrane that is removed at the end of the process, leading to packed bundles of CNTs. This interesting strategy was implemented in a flow system by Zheng et al. [36] to preconcentrate organic vapors. SWCNTs were suspended in toluene, filtered through a PTFE membrane and allowed to dry. The CNT layer was then peeled from the membrane, and packed into a metal tube with gas inlet and outlet tubes, where toluene,

methyl ethyl ketone (MEK) and dimethyl methylphosphonate (DMMP) were adsorbed and preconcentrated. The adsorbed trace organic vapors were released from the preconcentration column by a temperature-programmed desorption method and detected downstream with a flexural plate wave vapor sensor. The SWCNT-paper method was better for flow-through applications than the conventional CNT-powder-packed method because the paper occupied only about 25% of the volume of the tube interior, so avoiding overpressure problems.

To improve the analyte–CNT interaction and the sorption capability, CNTs can be chemically bonded to a support. Suarez et al. proposed chemical immobilization of CNTs onto the surface of controlled porous glass [37]. The carboxylated SWCNTs were aligned perpendicular to the glass surface as consequence of the coupling reaction between the carboxylic groups and the amino-modified glass surface. The new material presented the sorption capability of SWCNTs and an ionic surface that could interact with analytes via electrostatic interactions leading to an improvement of the preconcentration procedure. Furthermore, the microcolumn packing was easier and the use of high flow rates allowed. The effectiveness of the sorbent was proved in an FIA system with the at-line determination of non-steroidal anti-inflammatory drugs (NSAIDs) from urine samples by capillary electrophoresis (CE).

Another attempt to improve the analyte–CNT interaction involved dispersing the NPs in inert particles. This was the proposal of Du et al. [38], by assembling MWCNTs on a silica surface using the polyelectrolyte-assisted layer-by-layer assembly technique. The modification of silica with a layer of hydrophilic polymer minimizes the non-specific adsorption of proteins by silica surface. So, the mechanical properties and the large surface area of the silica gel beads (75–150 mm/100–200 mesh, narrow pore) are combined with the favorable protein retention of MWCNTs. MWCNT/SiO₂ was used as novel SPE sorbent for the separation and the preconcentration of cytochrome-c from bovine serum albumin (BSA). The MWCNT/SiO₂-packed microcolumn was integrated into an SIA system leading to better separation efficiency and higher enrichment factor compared with adsorption in an SiO₂-packed microcolumn. The retained proteins were eluted with NaCl from the microcolumn and quantified by visible spectrophotometry at 410 nm.

A promising approach to overcome the limitations of column-based sorption systems is to convert microporous or macroporous membranes into efficient adsorbers by attaching functional groups to their inner surface [39,40]. Preparation of a thin layer of MWCNTs onto a hydrophilic MF-Millipore membrane was proposed [41]. At first, CNTs were dispersed into polydiallyldimethylammonium chloride (PDDA) by ultrasound in order to form a stable suspension of the PDDA–MWCNT

composite. Subsequently, the suspension was filtered through an MF-Millipore membrane onto which a thin layer of PDDA–MWCNT composite was uniformly immobilized. A piece of the membrane obtained (4.0 × 1.0 × 0.01 cm) was supported by a stainless-steel wire mesh in a PTFE film and then placed between two porous PTFE films with flow-through channels. A polymeric substrate fixed the individual parts into the body to form an extraction module, which was incorporated into an SIA system to perform on-line separation and preconcentration of acidic proteins. Compared with the packed microcolumn mode (column dimensions 5.0 mm × 2.8 mm) [30], a significant reduction in flow resistance and a 146-fold improvement was achieved. The minimization of the diffusive mass transfer and a lower pressure drop, resulted in better sorption/separation efficiency. However, sorptive disks are prone to analyte breakthrough as a consequence of limited uptake capacity.

A different proposal was made by Boonjob et al. [42] in replacing the loop of the injection valve by a microchamber in an SIA system. The cylindrical chamber was designed to contain a suitable quantity of CNTs or carbon nanofibers (CNFs) as sorbents and, a magnetic bar in order to disperse the CNPs and avoid entanglement. Discrete volumes of sample and eluent were delivered sequentially by the top of the chamber through a selection valve. The bottom outlet was equipped with a polyethylene frit to prevent loss of sorbent material, while the solutions were allowed to flow freely. In this way, preconcentration of triazine herbicides in environmental waters and soil extracts was accomplished. The SIA system was coupled on-line to a high-pressure liquid chromatography (HPLC) instrument to carry out the determination.

4.1.3. Carbon nanofibers (CNFs). CNFs also have great potential as SPE sorbents for separation, preconcentration and determination of trace or ultra-trace inorganic and organic compounds in samples of different matrices. They possess the same chemical and mechanical characteristics as CNTs but differ from them in the absence of a hollow cavity and in the size of the diameters, generally higher than those corresponding to CNTs [43]. In addition, CNFs could be functionalized with a simple chemical treatment improving their adsorption capability by forming polar groups on their surfaces [44].

CNFs were applied as sorbent material for the on-line preconcentration of trace rare-earth elements from human-hair samples [45] and inorganic arsenic species in groundwater and lake water [46]. In both cases, a similar flow configuration was used. The microcolumn was packed with oxidized CNFs (ox-CNFs) in their powder form, sealed at both ends with glass wool and located in a CF system coupled on-line to ICP-MS. The adsorption capacity of functionalized CNFs was compared with

activated carbon and MWCNTs under the same conditions, resulting in better results for CNFs.

4.2. Metal-oxide nanoparticles

The chemistry of the surface of some oxides (e.g., TiO₂, Al₂O₃, ZrO₂, CeO₂ and MnO) has been extensively investigated. One of their outstanding properties is that they possess unsaturated atoms on the surface that can react with other atoms leading to a high sorption capacity [47,48].

Nanometer (nm)-sized metal oxides have a high surface reactivity, which allows strong chemisorption of a large range of compounds from different sample matrices [49]. However, due to their small size, coagulation and loss of activity are often problems, and the application in flow methods could be limited. Furthermore, these nm-sized metal oxides are not selective enough. So, in order to prevent agglomeration of particles and to increase their selectivity towards analytes, physical or chemical modifications of the sorbent surface with a specific chelate reagent is usually required.

This strategy has been used for speciation of inorganic and organic selenium compounds in environmental and biological samples [50] where nm-TiO₂ was chemically modified with dimercaptosuccinic acid (DMSA) to carry out the preconcentration of organic selenium [selenocystine (SeCys2) and selenomethionine (Se-Met)]. For this purpose, an FIA method with a dual-column preconcentration/separation step, coupled on-line to ICP-MS was employed.

Nanosized alumina (n-Al₂O₃) chemically modified with 3-(8-quinolinyloxy)-4-hydroxybenzoic acid (QAHBA) was used to carry out the simultaneous on-line preconcentration and ICP-MS determination of Ag, Pd, Au, Ga, In and Nb in geological and environmental samples [51]. A total of 50 mg of QAHBA-modified n-Al₂O₃ was filled into a PTFE microcolumn and plugged with a small portion of glass wool at both ends. In this study, the chemical stability of n-Al₂O₃ modified with QAHBA and its efficient analytical performance in an on-line preconcentration system was proved.

A different proposal to avoid coagulation of metal-oxide NPs in on-line extraction columns is use of sol-gel methods [52] where a colloidal solution acts as precursor for a continuous polymer network. Using this strategy, nano Au/TiO₂ tubing was prepared by coating support substrates with a TiO₂ solution [53]. A colloidal solution of gold NPs (AuNPs) was injected into Tygon tubing, followed by injection of a TiO₂-colloid solution. Finally, the Tygon tubing was incubated and used to preconcentrate cobalt and nickel in water samples. The extraction procedure was coupled on-line to an HPLC instrument with ultraviolet (UV) detection prior to derivatization of the eluted solution with 8-hydroxyquinoline.

The same strategy to overcome particle agglomeration was applied to test the adsorptive capability of TiO₂ immobilized on silica gel towards metal ions. Microcolumns packed with immobilized TiO₂ located in a CF configuration were employed for preconcentration of trace lead [54] and gold [55] from water samples prior to its determination by graphite-furnace atomic absorption spectrometry (GF-AAS) and FAAS, respectively. The sorbent showed enough stability and the microcolumns could be reused, after regeneration, for at least 20 adsorption-elution cycles. In both methods, tap and lake water samples were previously filtered through a 0.45-mm membrane filter and adjusted to a suitable pH value for analysis. No further pre-treatment was required.

Although some modification is recommended, the use of metal-oxide NPs in continuous systems without any previous treatment is also possible. Yin et al. developed an FIA method to carry out simultaneous on-line preconcentration of trace metals by using n-Al₂O₃, and subsequent on-line determination by ICP-MS [56]. Here, a PTFE microcolumn was filled with n-Al₂O₃ and capped with glass wool at both ends, without immobilization of the material. The FIA manifold is constructed with the microcolumn placed in the loop of a proportional injector. The sample was drawn through the column for 60 s to carry out the preconcentration. After that, the eluent was propelled for 60 s through the column in the opposite direction. By changing the direction of the flow, the continuous impact on the sorbent and therefore the entanglement of particles was averted, making it possible to reuse the column 50 times, without affecting recoveries.

4.2.1. Magnetic nanoparticles (MNPs). MNPs have not only the advantages of metallic NPs as sorbents, but also unique magnetic properties. Due to these special characteristics, they can adsorb, preconcentrate and separate the target analyte, and then they can be easily separated from the sample matrix by applying an external magnetic field. These NPs are extensively used in biotechnology and biomedicine for a wide range of applications [57–59]. In batch procedure, a solution that contains the analytes and MNPs is stirred for a certain time. Subsequently, a magnet is placed in the external part of the vessel and NPs with the adsorbed analytes are collected while the solution is discarded. Afterwards, the analytes are desorbed with a suitable solvent while the MNPs are regenerated and can be reused.

This procedure can be laborious and time consuming, so the development of automated procedures is an excellent option, although SPE using MNPs is not usually used in combination with flow methods, probably because of its tendency to form large aggregates. Huang et al. overcame this limitation and reported magnetic immobilization of amine-functionalized

magnetite (AF-Fe₃O₄) onto the inner walls of a knotted reactor, employing a tubular magnet [60], with no need for any frit or plugs. The AF-Fe₃O₄-packed knotted reactor was placed in the loop of an injection valve to perform the on-line SPE of trace amounts of chromium from tap water, and was coupled to ICP-MS to carry out the determination. The samples were acidified to pH 4.0 with a diluted solution of HNO₃, prior to their introduction into the FIA system. The magnetic knotted reactor can be used for at least 75 cycles of preconcentration without a significant loss of extraction capability.

Another attempt to integrate MNPs in flow methods was by Lee et al. [61], who developed an SPE lab-on-valve (LOV) system for the trace analysis of Mn²⁺, Co²⁺, Cu²⁺, Zn²⁺ and Pb²⁺ in environmental and biological samples. The nanoadsorbent was prepared by surface modification of iron-based MNPs with polyacrylic acid (PAA), and immobilized in PTFE tubing by external magnetic force. The nanoadsorbents could thus withstand the gravity force and elution force during sample loading, buffer conditioning, and analyte elution. Also, the unequal distribution of immobilized MNP-PAA in the microcolumn was minimized, reducing the effect of eddy diffusion and leading to a more symmetric peak shape. The system is useful for trace analysis of multiple heavy metals in urine and near-shore sea water that are introduced into the LOV system with no pre-treatment.

Recently, Wang et al. presented an SIA-LOV system where the MNPs were trapped inside a microchannel using a permanent magnet [62]. The MNPs were functionalized with sodium dodecyl sulfate and employed as sorbent for extraction of cobalt. Extraction, elution and detection procedures were performed sequentially in the SIA-LOV system followed by electrothermal atomic absorption spectrometry (ET-AAS).

Another interesting approach is to employ MNPs in conjunction with molecularly-imprinted polymers (MIPs) to carry out preconcentration of analytes and, at the same time, improve the selectivity of the detection method, avoiding possible interferences. This could be accomplished by coating MIPs on Fe₃O₄ or Fe₂O₃ MNPs. In this way, the new polymer material, MMIPs, had the advantages of MIPs and was magnetically susceptible and, therefore, easily separated by an external magnetic field after adsorption and recognition. Their high adsorption capability and selectivity made them excellent sorbent material to achieve enrichment, separation and sensors of the target molecule [63].

Lu et al. [64] developed a novel FIA-CL method for determination of sulfadiazine (SDZ) employing MMIPs packed into a colorless glass tube. The MMIPs column was connected to the CL flow system as recognition element for the sensor. The determination consisted of

the adsorption of SDZ from the sample matrix, washing the column to remove some possible interferences, CL detection and column cleaning. The procedure is simple and fast and the sensitivity of the CL method greatly improved.

4.3. Silica nanoparticles

Silica particles of nm size (n-SiO₂) are a promising material as extractant because of their large specific surface area, high adsorption capacity, and thermal and chemical stability. Despite that, some compounds are poorly adsorbed on its surface [65] and chemical or physical modification of the n-SiO₂ surface with certain functional groups containing some donor atoms (e.g., oxygen, nitrogen, sulfur and phosphorus) is necessary. Two main strategies are used to improve the sorption of n-SiO₂: chemical modification of the surface with organic compounds [66,67]; or, metal coating [68]. Both were applied to extraction and preconcentration of analytes in flow methods.

The chemical-functionalization strategy was used for on-line SPE of Cu(II), Fe(III) and Pb(II) [69] {e.g., n-SiO₂ modified with 4-(2-aminoethylamino)-N-(2-(2-aminoethyl amino) ethyl) butanamide (n-SiO₂-AAEB) was used as sorbent}. The adsorption efficiency of the nm sorbent was compared with silica gel chemically modified with the same compound (SG-AAEB), and distinct chemical activity and selectivity towards metals was found. By comparison, n-SiO₂-AAEB showed a high selectivity to Pb(II) and had larger adsorption capacity. The method was applied to the determination of these metals in a certified reference material (GBW 08301, river sediment), kaolin ore and nature water samples.

Gold-coated n-SiO₂ (Au/SiO₂) was used as adsorbent for on-line preconcentration and determination of dissolved mercury in natural waters and wastewaters by atomic fluorescence spectrometry (AFS) [70]. The affinity of AuNPs for mercury and the stability of the Au/SiO₂ adsorbent to heat cycles, which are necessary to release Hg⁰ from the microcolumn, provides high efficiency in the preconcentration of Hg species. The microcolumn was incorporated into a fully automated FIA system directly coupled to AFS. The sample was injected and passed through the column where mercury species were adsorbed onto the Au/SiO₂ material. A heating wire was coiled around the column. The temperature inside the column was kept at 100°C until all remaining Hg solution has been completely evaporated. After that, the temperature was increased to 550°C and Hg was released and transported by an argon stream to the AFS. In this way, there is no need for species conversion or use of solvents for desorption, and the method could easily be applied with a portable FIA system. Furthermore, the microcolumn could be used in more than 180 analyses, without losing precision or sensitivity.

Table 2. Applications of preconcentration units (PUs) used in continuous-flow systems for the preconcentration of inorganic and organic analytes in diverse matrixes

	Preconcentration units					Analyte	Sample	Ref.
	NPs	Immobilization	Amount (mg)	Vs (mL)	Ve (μL)			
Carbon	Fullerenes	No	50	6.0-15.0	200	Pb (II)	Water	[10]
	Fullerenes	No	80	5.0	150	Metalocenes, organoleads	Water	[13]
	Fullerenes	No	80	6.0	250	Cu (II)	Water	[20]
	Fullerenes	No	80	10.0	200	Cd-methallothioneins	Fish liver	[21]
	Fullerenes	No	160	50	250	Hg, Sn organometallic compounds	Water and coastal sediments	[22]
	Fullerenes	No	80	25	500	Co (II)	Wheat flour	[23]
	Fullerenes	No	60	15	150	BTEX	Sea water, wastewater	[24]
	Fullerenes	No	80	25	150	Nitrosamines	Wastewater	[25]
	MWCNTs	No	30	20.0	1000	Pb (II)	Garlic, Ginkgo Biloba	[15]
	MWCNTs	No	80	104	1000	Sulfonamides	Egg, pork meat	[16]
	MWCNTs	No	10 ^g	2.0	75	TI	Tap water	[17]
	MWCNTs	No	25 ^g	1.0	50	V(IV), V(V)	Natural water	[18]
	MWCNTs	No	6.0	10.0	200	Tetracycline residues	Ground and surface water	[19]
	MWCNTs	No	8.0-10	2.0	200	Hemoglobin, cytochrome c	Human blood	[30]
	MWCNTs	No	7.0	3.0	800	Cr(VI)	Soil	[31]
	SWCNTs	No	40	13.5	2000	As (III), Sb (III)	Sea, river and lake water	[32]
	Tyr-MWCNTs	No	10	10	50	Co (II)	Natural water	[33]
	ox-MWCNTs	No	4.0	5.0	40	Cd (II)	Environmental samples	[34]
	SWCNTs	No	37	60	TD ^f	Toluene, MEK, DMMP	Air	[36]
	SWCNTs	CBS ^a	15	5.0	200	NSAIDs	Urine samples	[37]
	MWCNTs	DIP ^b	30	1.0	100	Cytochrome C	Bovine serum albumin	[38]
	MWCNTs	RF ^c	8.0-10	2.0	200	Bovine serum albumin	Human blood	[41]
	CNF	No	20	150	1000	Mn (II), Co (II), Ni (II)	Water samples	[12]
	CNF	No	80	10.0	470	Chlorotriazine residues	Water, agricultural soils	[42]
	Ox-CNF	No	20	200	2000	Trace rare elements	Human hair	[45]
	Ox-CNF	No	30	100	3000	As (III), As (V)	Groundwater, lake water	[46]
Metallic oxides	TiO ₂	No	100	50	2000	Cr (III), Cr (VI)	Tap and lake water	[11]
	Al ₂ O ₃	No	50	4.0	800	Se (IV), Se (VI)	Yeast, tea leaves	[50]
	DMSA-TiO ₂	CM ^d	50	4.0	800	SeCys2, Se-Met	Yeast, tea leaves	[50]
	QAHBA-Al ₂ O ₃	CM ^d	50	6.0	600	Ag, Pd, Au, Ga, In, Nb	Lake water	[51]
	TiO ₂ -SiO ₂	CBS ^a	50	50	1000	Pb (II)	Tap and lake water	[54]
	TiO ₂ -SiO ₂	CBS ^a	50	50	1000	Au (III)	Tap, lake water	[55]
	Al ₂ O ₃	No	50	2.0	400	V, Cr, Mn, Co, Ni, Cu, Zn, Cd.	Natural water	[56]
	AF-Fe ₃ O ₄	RMF ^e	18	2.4	467	Cr (III)	Tap water	[60]
	PAA-Fe ₃ O ₄	RMF ^e	5	0.02	200	Mn (II), Co (II), Zn (II), Pb (II)	Environmental and biological aqueous samples	[61]
	Fe ₃ O ₄ / Al ₂ O ₃	RMF ^e	100	2.0	100	Co (II)	Tap and groundwater	[62]
Silica	n-SiO ₂ -AAEB	CM ^d	35	300	4000	Cu (II), Fe (III), Pb (II)	Kaolin ore, tap and river water	[69]
	Au/SiO ₂	CBS ^a	1000	7.0	TD ^f	Total Hg	Estuary and river water	[70]

^aChemically bonded to a support.^bDispersed on inert particles.^cRetained on filters.^dChemically modified.^eRetained by magnetic field.^fTemperature desorption.^gQuantity equivalent to 10 μL of water in a tip.

Table 3. Representative examples to point out the main advantages of the nanoparticle (NP)-flow system combination

Sorbent	Flow configuration/Preconcentration Unit	Sample/Analyte	Advantages on NP-flow system combination	Ref.
Fullerene	On-line AES /Packed microcolumn	Fish liver/Cadmium	Direct introduction of all eluent volume	[21]
SiO ₂ -CNTs	At-line CE /Packed microcolumn	River water/NSAIDs	Higher flow rate preconcentration without overpressure	[37]
Metallic oxide	Magnetic ICP-MS/ Knotted reactor	Tap water/ Chromium	Reusability in simple matrices. Use of low amounts of sorbents to have a large area	[60]
Metallic oxide	Lab on chip, ETAAS/Packed microcolumn	Environmental and biological samples/ Mn, Co, Cu, Zn, Pb	Possibility to renew in each analysis the sorbent phase. Analysis of complex samples without carryover. Use of low amounts of sorbents	[61]

5. Applications

The potential of NPs as SPE sorbents in flow-preconcentration systems for the enrichment of both inorganic and organic compounds has been greatly exploited in recent years and the number of articles that deal with this task has greatly increased.

Table 2 shows the description of diverse PUs that were employed in flow methods, in terms of nature of NPs, immobilization method, amount of NPs used to pack the microcolumn, sample volume and elution volume, and analytes that were determined in different sample matrices.

As can be seen, CNPs were widely used in flow-preconcentration systems. Among them, fullerenes are basically used in PUs without immobilization for preconcentration of trace metals, organocompounds and, to a lesser extent, organic compounds. CNTs are the most commonly-used sorbents in flow systems and different immobilization strategies have been applied to improve their sorption capabilities. Both organic and inorganic compounds have been successfully preconcentrated using CNTs, even in complex matrixes.

However, PUs with metallic and silica NPs as sorbents have been mainly applied to preconcentrate trace metals in water samples.

Compared with traditional sorbents (e.g., C₁₈, activated carbon, SiO₂ or resins), NPs generally have enhanced adsorption capacity, due to their large surface area and greater interstitial volume. These unique characteristics of NPs make them more effective for preconcentration, presenting larger enhancement factors and higher selectivity than others sorbents [10,23,24,55,69].

In some cases, LODs for the target analyte are also lower than LODs for different common sorbents using the same detection method [15,32,38,54].

The importance of using NPs in microcolumns located in flow systems is to miniaturize the system further, achieving analytical parameters similar to or better than

for most commonly used sorbents. Indeed, it is possible to employ very small quantities of adsorbent and achieve good PCFs [17,19,30,34,41].

6. Final remarks

As different types of NPs have been discovered, besides being characterized, different possible applications have been sought by researchers. One of the most outstanding applications is SPE, which, coupled with the intrinsic properties of flow methodologies, have led to further simplification of analytical procedures.

PUs packed with carbon, metal-oxide or silica NPs located in different parts of the flow system were coupled to diverse detection techniques to perform successfully preconcentration and determination of both inorganic and organic analytes from a wide variety of sample matrices. Furthermore, different strategies of immobilization have been applied in order to improve selectivity and to prevent overpressure problems in the flow system.

Table 3 summarizes four selected strategies in order to point out the importance of the use of the flow system in combination with NPs. As can be seen, four main advantages can be distinguished namely:

- (1) optimization of volume eluent;
- (2) use of high flow-rate preconcentration;
- (3) use of low amount of sorbent material; and,
- (4) removal of the sorbent material in each analysis for the analysis of complex samples.

Undoubtedly, we can say that potential applications in this field are not exhausted. Derivatization of NPs of different types opens a much wider field to develop new nano-sorbents, which can definitely increase selectivity towards target analytes.

Furthermore, MNPs have been exploited very little in continuous systems, despite their inclusion in flow configurations greatly facilitating the adsorption-desorption process. However, possible extension of the use of NPs to

different automated pretreatment techniques would be of interest, since most applications are limited to SPE.

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