Analytica Chimica Acta 889 (2015) 22–34

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Review

Nanoparticle-based assays in automated flow systems: A review

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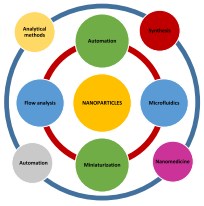
HIGHLIGHTS

- The state of the art of flowing stream systems comprising NPs was reviewed.
- The use of different types of nanoparticles in each flow technique is discussed.
- The most expressive and profitable applications are summarized.
- The main conclusions and future perspectives were compiled in the final section.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 10 January 2015
Received in revised form
20 May 2015
Accepted 22 May 2015
Available online 8 July 2015

Keywords:
Nanoparticle
Automation
Flow analysis
Microfluidics

ABSTRACT

Nanoparticles (NPs) exhibit a number of distinctive and entrancing properties that explain their ever increasing application in analytical chemistry, mainly as chemosensors, signaling tags, catalysts, analytical signal enhancers, reactive species generators, analyte recognition and scavenging/separation entities.

The prospect of associating NPs with automated flow-based analytical is undoubtedly a challenging perspective as it would permit confined, cost-effective and reliable analysis, within a shorter timeframe, while exploiting the features of NPs.

This article aims at examining state-of-the-art on continuous flow analysis and microfluidic approaches involving NPs such as noble metals (gold and silver), magnetic materials, carbon, silica or quantum dots. Emphasis is devoted to NP format, main practical achievements and fields of application. In this context, the functionalization of NPs with distinct chemical species and ligands is debated in what concerns the motivations and strengths of developed approaches. The utilization of NPs to improve detector's performance in electrochemical application is out of the scope of this review.

The works discussed in this review were published in the period of time comprised between the years 2000 and 2013.

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http://dx.doi.org/10.1016/j.aca.2015.05.052
0003-2670/

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1. Introduction

Nanotechnology represents one of the most exciting forefront fields for exploitation in analytical chemistry as by means of a wide variety of nanomaterials with distinctive physicochemical properties it could bring about new concepts and trends and provide innovative tools and devices, for facilitating (bio)analytical measurements [1]. According to IUPAC, NPs are particles whose size is comprised between 1 and 100 nm. The broad applicability of NPs is related with their unique and markedly altered physical, chemical and biological properties compared to their macro scaled counterparts [2]. The composition of nanomaterial may include noble metals like gold [3] and silver [2], other metals and metal oxides [4,5], carbon [6], silica, lipid and solid lipid-based particles like liposomes [7], and polymer-based materials [8].

Each type of NP presents assorted and particular physicochemical properties such as high surface-to-volume ratio, surface charge, adjustable solubility, size, shape, variable aggregation tendencies, etc. By designing and controlling the structure of nanoparticles, researchers can influence the resulting properties and, ultimately, modulate materials according to a certain purpose or function.

Fundamental research elucidating NP structure, physical and chemical properties, and toxicity, has led to the development of a large variety of NPs with functional application in areas as dissimilar as electronics, energy, textiles, catalysis, environment, biotechnology and medicine, bio-imaging, bio-sensing, and drug delivery [9–11].

The use of NPs in analytical processes is presently the most extensively exploited area of nanotechnology. This is greatly related with the peculiar properties of NPs that enable the improvement of well-established analytical methods or the development of novel methodologies for new analytes or matrices. Indeed, the emblematic advantages of NPs create conditions to improve the selectivity, sensitivity, rapidity as well as the portability of analytical systems [12].

Regarding their incorporation into particular processes, NPs can be applied in a variety of analytical formats. A typical use is as quantitation tags, when an analyte triggered NP property change (either electrical, optical, thermal, magnetic, chemical) is quantified. In this context, NPs have been applied on the construction of optical (absorbance, luminescence, SERS, SPR), electrochemical, and mass-sensitive sensors [13,14]. In another possibility, NPs can be functionalized to guarantee enhanced applicability since the introduction of modifications of NPs surface usually led to important transformations in their intrinsic physical and/or chemical properties which can confer them novel and specific functions, broadening their suitability for appliance in medical and biological fields [13,15]. In this context, noble metal NPs, in particular AuNPs, and magnetic NPs have assumed great prominence because they could be functionalized with distinct classes of molecules, while assuring high *in vivo* stabilization, thus providing the NPs with the ability to track and to establish interactions with a given biological target. Taking advantage of the abovementioned properties as well as of the comparable dimensions of nanomaterials and biomolecular structures, multiple applications in the referred fields are envisioned. Indeed, the possible uses of NPs in biomedical applications is extremely vast and goes further beyond nanosensing [3,16] as in the case of the utilization of NPs as controlled release reservoirs for targeted delivery of drugs in the treatment of several diseases [17].

Considering the huge potential of NPs utilization, the implementation of NPs-based assays in automated and miniaturized systems will lead to a powerful assembly, particularly when compared to traditional batch-wise procedures. Effectively, in recent years, automated flow analytical methods have widely incorporated NPs for varied applications. Automation of all stages of reaction enables the implementation of effective in-line sample pre-treatments while guaranteeing reproducibility during solutions insertion and reaction zone implementation and transport towards detection. In addition, this strategy provides conditions to carry out the assays in a closed environment, with inherent advantages in terms of reaction control, reactants consumption and analysis time enabling at the same time the implementation of more complex reaction schemes or multiparametric determinations. Furthermore, the easy assembly of flow manifolds through the use of readily-available and low cost equipment and the straightforward coupling to conventional detection systems create conditions for the easy automation of NP based assays by a broad analyst audience without a high level of expertise. On a distinct but not less interesting perspective, flow-based techniques present adequate characteristics for the synthesis of nanomaterials, since they provide large surface areas and reduced diffusion resulting in fast mass transport due to the downsized dimensions of

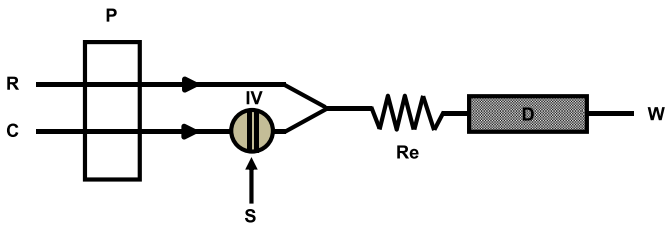

Fig. 1. Schematic illustration of a FIA manifold, where a defined volume of sample (S) is injected by means of a rotary injection valve (IV) into a continuous flowing carrier stream (C) through a pump (P), which is subsequently merged with the reagent (R) stream. After passing a reactor (Re), the ensuing transient generation of product is monitored by a suitable detector (D), and then expelled by the waste (W).

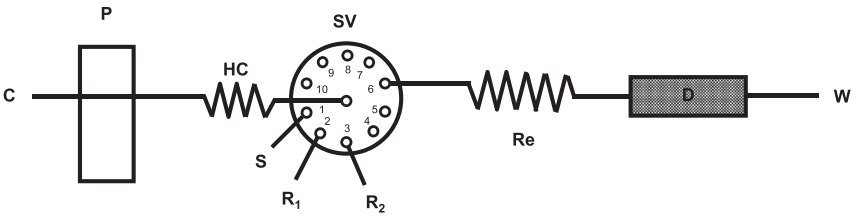

Fig. 2. Schematic illustration of a SIA manifold, where defined volumes of sample (S) and reagents (R1 and R2) are sequentially aspirated to the holding coil (HC) through a selection valve (SV) by means of a bidirectional pump (P). After flow reversal, the stacked zones are sent through a reactor (Re) by the carrier (C) and the ensuing transient generation of product is monitored by a suitable detector (D), and then expelled by the waste (W). It is important to note that different devices could be easily accommodated in the available lateral inlets of the SV (e.g. gas diffusion unit, extraction unit, mixing chamber).

tubing or channels [18], covering a wide range of compositions and tunable sizes. These characteristics facilitate the attainment of high synthesis yields by means of a precise control of the reaction environment while reducing the consumption of expensive or toxic reactants as well as waste production.

This paper aims to review, highlight and discuss the different applications involving the utilization of NPs and nanomaterials in combination with automated and miniaturized flow-based systems and seeks to gather and to provide information that can result in future developments on the field of nanotechnology.

2. Automation of nanoparticle-based methodologies

The methodologies resulting from automation of NP-based processes exhibit an enormous potential due to the combination of the inherent advantageous NPs properties, the precise and reproducible control of NPs/analyte chemical sensing mechanisms and the simplicity, versatility and ease of operation of automated miniaturized approaches such as flow-based techniques and microfluidics.

Flow-based techniques, such as flow injection analysis (FIA) [19], sequential injection analysis (SIA) [20], multicommutation (MCFIA) [21], multipumping (MPFS) [22] and single interface flow analysis (SIFA) [23] are nowadays recognized as valuable automation tools that permit the development of NP-based assays providing high versatility, simplicity, sensitivity, repeatability, portability and low cost. In a similar manner, the association of NPs with miniaturized systems usually called microfluidics [24] has become a dominant trend in this research field with application in a broad variety of fields.

Flow injection analysis (FIA) emerged in 1975 as an alternative for the automation of chemical methods aiming at the development of simple and reproducible automated methodologies [19]. Generally, a FIA system incorporates an injection valve and a propulsion device that in combination enable the injection of an aliquot of sample in a stream of reagent with formation of a product that is measured in a detector physically connected to the FIA manifold (Fig. 1).

The main principles of FIA rely on the control of the dispersion of the reaction zone in the flow system and on the precise timing of the operations. This technique is generally based on simple and low cost manifolds with possibility to be adapted to distinct analytical requests. These features associated to low sample volumes and the carrying out of detection and measurement before the chemical reaction reaches equilibrium, justify the widespread utilization of FIA for the automation of analytical procedures in the last decades. This tendency is also observed when dealing with NP-based assays even in comparison with the more sophisticated flow techniques available. As stated above this can be related with the inherent simplicity of the manifolds and also with the fact that the majority of these assays are based on sensors development that can be easily integrated in basic FIA systems.

The emergence of sequential injection analysis (SIA) in 1990 significantly expanded the compass of chemical analysis by surmount some of the disadvantages that hindered the utilization of FIA in routine assays [20]. The continuous circulation of reagents and the need of physical reconfiguration to perform different determinations were crucial factors affecting FIA applicability. SIA soon showed to exhibit operational characteristics amenable to the implementation of analytical procedures in diverse fields [25]. The core element of SIA systems is the selection valve that works in synchronization with a bi-directional propelling unit (Fig. 2). Thus, the solutions do not need to flow continuously in SIA as happens in FIA, which contributes to a significant reduction not only of reagents and sample consumption but also on effluents production.

In the last years, flow researchers witnessed the implementation of NP based assays in SIA systems mainly with analytical purposes exploring the versatility of SIA that enables the incorporation of additional devices as sensors, reactors or sample pre-treatment devices allowing the realization of several concomitant actions. Thus automation of NP based assays resorting to SIA explores mainly the versatility of the selection valve and the computer controlled mode of operation that besides enabling the implementation of parallel events on the lateral inlets of the valve permits also the rigorous control of the reaction conditions in terms of space and time. These possibilities make this technique very adequate not only for analytical applications but also for the implementation of synthetic processes in which, as it is known, the assay conditions determine the properties of the resulting NPs.

Multicommutation flow analysis (MCFIA) appeared in 1994 with the aim of increasing the versatility of flow-based systems, facilitating automation and decreasing reagent consumption [21]. This analytical strategy embraces some of the advantages of both FIA and SIA overcoming at the same time some of their disadvantages. Multipumping flow systems (MPFS) are characterized by the particularity of incorporating only one device, solenoid micropumps that function at the same time as insertion, selecting, mixing and commuting elements [22]. Moreover, the pulsed flow originated by micropumps causes an improvement of the mixing efficiency that is particularly evident in situations of reduced dispersion, making this strategy adequate for the determination of short-lived species, as occurs in chemiluminescence assays.

The concept of single interface flow analysis (SIFA), which was proposed in 2008 by Silvestre and co-workers, is based on the establishment of a unique interface where all implicated solutions are put in contact for the development of the chemical reactions [23]. This strategy does not demand the utilization of pre-defined volumes of sample and reagent which simplifies significantly the control and operation of the system.

On a distinct basis, microfluidics appeared in the 1990s aiming at the reduction of the costs of analysis by reducing the consumption of expensive reagents and by increasing throughput and

Table 1
Flow-based methodologies based on nanoparticles.

Nanoparticle	Analyte	Detection/Application	Limit of detection	Sample	Reference
Epoxysilane-modified magnetic (Fe_3O_4/SiO_2) NP	Carcinoembryonic antigen	Electrochemical impedance spectroscopy	0.5 ng mL^{-1}	Human serum	[110]
Epoxysilane-MIPs modified magnetic (Fe_3O_4/SiO_2) NP	Sulfadiazine	Chemiluminescence	1.54×10^{-7} mol L^{-1}	Human urine	[112]
Magnetic graphene nanosheets + QtDs functionalized silica nanospheres	Prostate specific antigen	Electrochemiluminescence	0.72 pg mL^{-1}	Human serum	[101]
Fe_3O_4 NPs (magnetic) Silica NPs	Aflatoxin B1	Fluorescence/Competitive immunoassay	0.2 ng mL^{-1}	Peanuts	[74]
Amine saline modified silica NPs (magnetic) AuNPs	Methyl-3-quinoxaline-2-carboxylic acid residues	PCR/Competitive immunoassay	1.4 amol L^{-1}	Pork muscle	[73]
Polystyrene NPs with COOH groups (magnetic)	Progesterone	Chemiluminescence/Enzymatic immunoassay	8.5 fg L^{-1}	Saliva	[79]
Fe_3O_4 NPs (magnetic)	Glucose	Spectrophotometry	–	n.a.*	[75]
Octadecylsilane functionalized silica NPs (magnetic)	Quercetin	Voltammetry/Separation and preconcentration	1.3×10^{-9} mol L^{-1}	Urine Red wine	[77]
Octadecylsilane functionalized silica NPs (magnetic)	Metals	Electrothermal atomic absorption spectrometry/Separation and preconcentration	3 ng L^{-1}	Natural waters	[80]
Polyacrylic acid functionalized iron based NPs (magnetic)	Heavy metals	Inductively coupled plasma mass spectrometry/Separation and preconcentration	0.04–0.06 μg L^{-1}	Urine (reference material)	[78]
SDS functionalized alumina coated Fe_3O_4 NPs	Metals	Electrothermal atomic absorption spectrometry/Separation and preconcentration	6 ng L^{-1}	Natural waters	[76]
AgNPs	Lisinopril	Chemiluminescence (Luminol – $KMnO_4$)	0.027 mg L^{-1}	Tablets Human urine	[40]
AgNPs	Naproxen	Chemiluminescence (Eu^{3+}-Ce(IV)–$Na_2S_2O_4$)	0.11 ng mL^{-1}	Pharmaceutical samples Human urine	[38]
AuNPs	Estrogens	Chemiluminescence (luminol – H_2O_2)	3.2–49 nmo L^{-1}	Pharmaceutical samples Human urine	[41]
AuNPs	Mefenamic acid	Chemiluminescence (luminol – periodate)	0.16 μg L^{-1}	Pharmaceutical samples	[42]
AuNPs	Timolol maleate	Chemiluminescence (luminol – N-bromosuccinimide)	7.6 μg L^{-1}	Eye drops Human urine	[43]
Polythymine functionalized AgNPs	Hg^{2+}	Fluorescence/Extraction	3 pmol L^{-1}	Tap water	[50]
Au-coated silica	Hg^{2+}	Atomic fluorescence/Extraction	180 pg L^{-1}	Natural and wastewaters	[51]
Carbon dots	Nitrite	Chemiluminescence	5.3×10^{-8} mol L^{-1}	Water Milk	[99]
MWCNTs functionalized with poly(diallyldimethylammonium chloride	Acidic proteins	UV/Vis spectrophotometry/Solid phase extraction	1 μg mL^{-1}	Whole blood	[103,104]
MWCNTs	Basic proteins	UV/Vis spectrophotometry/Solid phase extraction	0.06–0.12 μg mL^{-1}	Whole blood	[102]
MWCNTs self-assembled on quartz wool	Lysozyme	UV/Vis spectrophotometry/Solid phase extraction	0.04 μg mL^{-1}	Egg	[107]
Polyelectrolyte-modified MWCNTs	Cr(VI)	Electrothermal atomic absorption spectrometry	0.016 μg L^{-1}	Water	[105]
Carbon nanofibers MWCNTs Oxidized carbon nanotubes	Chlorotriazine residues and metabolites	Liquid chromatography	0.004–0.03 ng mL^{-1}	Environmental waters	[106]
Graphene + Fe_3O_4 + MIP	L-tryptophane	Chemiluminescence	2.11×10^{-8} mol L^{-1}	Pharmaceutical samples	[100]
CdTe QDs + carbon nanotubes + chitosan + indium tin oxide glass (sensor)	Dopamine	Electrochemiluminescence	3.6 pmol L^{-1}	Cerebro-spinal fluid	[127]
CdTe/ZnSe QDs	Volatile phenols	Fluorescence	2.7×10^{-9} g L^{-1}	Environmental waters	[123]
CdS QDs + paramagnetic microbeads	Influenza viral protein	Differential pulse voltammetry	0.1 μg mL^{-1}	n.a.	[124]
CdS mercaptoacetic capped QDs	As(III) As(V)	Fluorescence/Speciation and determination	As(III)–20 μg L^{-1}/70 μg L^{-1} As(V)–40 μg L^{-1}	Sediment Ground water	[128,129]
CdTe mercaptoacetic capped QDs	Ibandronate	Fluorescence		Pharmaceutical samples	[131]
CdTe mercaptoacetic capped QDs	Ascorbic acid and hydroxytyrosol	Fluorescence		Pharmaceutical samples Food	[132]
CdTe mercaptoacetic capped QDs	Acetylcysteine	Fluorescence		Pharmaceutical samples	[135]
CdTe QDs	Quinolones	Fluorescence		Pharmaceutical samples	[130]
CdTe QDs	Hypoglycaemic drugs	Chemiluminescence		Pharmaceutical samples	[133]
CdTe QDs	Epinephrine	Chemiluminescence		Pharmaceutical samples	[134]
CdTe QDs	Chemical oxygen demand	Chemiluminescence		Wastewaters	[136]
Polyanyline NPs	pH measurement	Raman spectroscopy	–	n.a.*	[142]
Liposomes	Inhibition of PLA_2	UV–Vis spectrophotometry	–	n.a.*	[143]

n.a. –not available.

automation [26]. These miniaturized systems, also called lab-on-a-chip, became an important research tool on several fields from medicine to analytical chemistry [27–29]. This was a consequence not only of the possibility of downscaling conventional procedures but also of the possibility of executing analysis under conditions that are impractical in large scale instruments due to the reduced dimensions and large surface area of the microconduits. In microfluidics, a vast number of possible approaches can be implemented by using branched networks of channels with adequate hydrodynamic resistances enabling the generation of linear profiles, double peaks and sawtooth shape, among other more complicated shapes. All this, associated to the diffusion limited mixing and improved heat transfer/dissipation justify the increasing application of microfluidics in a variety of fields. In this context, the scientific community has assisted also, in the last decades, to an increasing number of applications combining microfluidics and nanomaterials with objectives ranging from synthesis to analytical determinations and nanomedicine [29].

In all these techniques, a significant reduction of the consumption of reagents and increased throughput analysis are attained. Additionally, there is an effective reduction of the characteristic time of the transport processes at the micrometer length scale which becomes comparable to that of the chemical reaction. All this opens promising perspectives regarding the implementation of complete analysis in specific or hostile environments.

In this context, the number of reports where NPs have been used coupled to a flow-based strategy or as a part of a microfluidic device has been increasing, and emphasis has been given by researchers to the synthesis, characterization and toxicity evaluation of these particles.

The following section discusses several developments related to NP-based methods exploiting flow systems and microfluidics devices, where the different assays are grouped by the chemical nature of NPs (Table 1).

2.1. Metal nanoparticles based automated methodologies

Metal nanoparticles of distinct natures are being applied extensively in all fields of chemistry due to their particular optical properties and metal size-dependent nature. These group of NP is indeed characterized by variable properties dependent on the kind of metal employed to produce the nanomaterial. Amongst metal NP particles, noble metal based and magnetic NPs are the most studied and applied types due to their analytical potential mainly regarding sensitivity enhancement.

For instance, in the last decades, the use of noble metal NPs as nanocatalysts has been strengthened and metal NP-enhanced chemiluminescence (CL) measurements have been exploited in analytical chemistry, in order to achieve higher sensitivity [30,31]. One of the most interesting and promising applications of NPs in analytical applications is their use for the capture, concentration, and separation of target analytes from complex matrices [12,32]. This is related with the ability of NPs to be readily dispersible in solutions and with specific properties such as large surface area, high adsorption capacity, and size comparable to many analytes of interest. In particular, NPs based on metal oxides, noble metals (like silver and gold) have been pointed out as promising solid-phase extractants and as contamination scavengers [12,32]. There are already evidences of their adequacy to substitute organic solvents and reactive complexants in the extraction and preconcentration of trace metals and organic pollutants from environmental sources, namely from natural waters [33].

On the following subsections, automated applications of metal based NPs will be discussed, divided by NP chemical nature.

2.1.1. NPs based on noble metals (Au and Ag)

Among the enormous variety of metal NPs, gold (Au) and silver (Ag) NPs have attracted increasing interest due to their distinctive physico-chemical properties, including high electrical and thermal conductivity, surface-enhanced Raman scattering (SERS), long-term stability, catalytic activity and biological compatibility [34]. Additionally, these NPs have specific surface plasmon resonance (SPR) properties, where the surface plasmon band arises from the coherent existence of free electrons in the conduction band due to small particle sizes [35]. The surface plasmon in these NPs can be excited by incident light, leading to a pronounced SPR absorption in the visible or UV parts of the spectrum [36], thus functioning as a signal amplification approach.

As already referred in this section, AgNPs and AuNPs, in particular, are being extensively applied in CL reactions to promote an enhancement of the inherent sensitivity of the determinations while expanding the scope of application of this kind of detection technique [30,31,37]. The advantages of this association can be further highlighted through combination with FIA due to the simple configuration and unidirectional operation mode of the systems that allow the effective mixture of the analyte with the CL reagents immediately before the detector resulting in methodologies with high sensitivity, low background noise and good reproducibility [38]. Taking advantage of these features, AgNPs have been used to enhance the CL of systems as luminol–$AgNO_3$ [39], luminol–$KMnO_4$ [40] or Eu^{3+}-Ce(IV)–$Na_2S_2O_4$ [38]. Generally, in this strategy, the analytes exert a concentration-dependent enhancing or inhibiting effect on the CL promoted by the mentioned systems, enabling their accurate determination in real samples. Several analytes were already determined by means of the implementation of this strategy on FIA systems namely amino acids and other organic compounds [39] and drugs like lisinopril and naproxen in pharmaceutical formulations and biological fluids [38,40].

Similarly, the CL enhancing effect of AuNPs on the luminol–H_2O_2 [41], periodate-pyrogallol/luminol [42] and luminol–N-bromosuccinimide [43] systems was evidenced resorting to FIA methodologies based either on the inhibiting effect of estrogens [41] and mefenamic acid [42] or on the enhancing effect of timolol maleate [43]. This enabled the determination of the analytes in biological fluids and pharmaceutical formulations. Even though it is known that the effect of gold nanocatalists on CL analytical signal is dependent on the synthetic route adopted for the preparation of gold colloids, all the optimized methodologies proved to be simple and sensitive with very good repeatability.

The incorporation of metallic NPs, mainly AgNPs and AuNPs, in sensors is also a very effective and widely used strategy to explore their optical properties with analytical purposes. Following this tendency, there are some reports of FIA and microfluidic methods involving the utilization of bio and immunosensors based on AuNPs [44–48]. In terms of analytical performance, further enhancements in sensitivity were attained through the association of three of the abovementioned sensors with CL detection resulting in highly

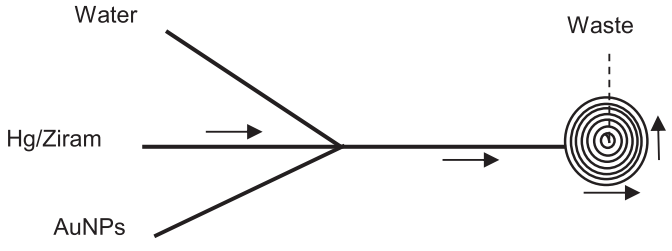

Fig. 3. Schematic representation of a microfluidic sensor for the analysis of environmental contaminants (adapted from Ref. [47]).

sensitive methods for the determination of glucose in human serum [44], DNA hybridization [45] and 2,4-dinitrotoluene [48]. In particular, the method developed for the determination of 2,4-dinitrotoluene was based on a CL enzyme immunoassay in which signal amplification was attained by the use of AuNPs codified with both horseradish peroxidase and an anti-mouse antibody. Aiming at the development of a portable device, AuNPs and gold nanoclusters were combined with rhodamine and bovine serum albumin for the development of sensors for in field detection of environmental pollutants like Hg or pesticide ziram (Fig. 3) [47].

As stated before, AgNPs and AuNPs can also find application in solid phase extraction processes [32,49]. In terms of FIA approaches, these applications rely on the use of polythymine functionalized AgNPs [50] and AuNPs coated silica [51] for the quantification of mercury in tap, natural and wastewaters. In the assay for the determination of Hg^{2+} resorting to polythymine-AgNPs as extraction material, only the detection step was performed in a FIA system. The steps of NP functionalization, analyte extraction and elution were performed off-line not taking advantage of the controlled conditions offered by the system which could be profitable during extraction and elution steps. Distinctly, the microcolumn packed with AuNPs coated silica was incorporated in a FIA system coupled to an atomic fluorescence spectrometer reaching a higher automation degree with advantages in terms of repeatability, robustness and sensitivity [51]. The simplicity of the manifold impelled the development of a portable version for in-situ monitoring.

In the field of microfluidics, AuNPs have been widely applied to enhance the analytical performance of microchip electrophoresis [52–55]. This strategy can be implemented through distinct approaches involving the coating of the walls of the microfluidic channels with NPs or with mixtures of NPs with other materials like for instance PDDA [52], polyvinyl pyrrolidone or polyethylene oxide [53]. With this, the efficiency of the separation can be enhanced and the sensitivity and selectivity of the methodologies are both significantly increased.

On the bio-analytical field, NPs are being extensively used in methodologies based on DNA and nucleic acid determinations. The miniaturization of these methodologies resorting to microfluidic devices further enhances their applicability due to the dramatic decrease of bio-material consumption and to the rigorous control of the assays conditions which are of utmost importance while performing these assays. For instance, surface modified AuNPs were incorporated in a microchip system with the specific purpose of providing a novel and efficient cell lysis method prior to bacteria detection by polymerase chain reaction (PCR) [56] while AuNPs were used for DNA labeling during the development of a microstructured electrode array on a chip for the determination of DNA [55]. This method is based on the electrical detection of NP-labeled DNA through a metal enhancement step that leads to the deposition of conductive material on the NPs. The procedure is still very dependent on operator intervention and the automation of the incubation steps would greatly increase the analytical performance of the chip. On a distinct approach, a microfluidic DNA platform was designed and applied to the colorimetric detection of DNA from *Mycobacterium tuberculosis* through non-crosslinking hybridization of DNA-functionalised AuNPs [57]. Another example is the use of AgNPs on a microfluidic strategy for the detection of clinically relevant nucleic acid sequences of *Chlamydia trachomatis* [58]. On this application, AgNPs provided a suitable metal surface for enhancement of the analytical signal through surface-enhanced resonance Raman scattering (SERRS) during the analysis of nucleic acid sequences of *C. trachomatis* [58]. Microfluidic devices have already proved to be exceedingly suitable for the implementation of methods based on the study of adsorbed molecules on metal surfaces like SERRS. This is related to the possibility of confining the active particles in novel ways, enabling a more in-depth understanding of the effect, and to the exquisite control of the reaction spatially enabled by the circulation of both analyte and active particles under conditions of laminar flow. In this context, surface-enhanced Raman scattering (SERS) has also been quantitatively exploited in microfluidic devices resorting to AgNPs for the determination of phenothiazine, promethazine, mitoxantrone, nicotine [59,60], paraquat [61] and drugs of abuse [62]. Liquid/liquid two-phase segmented flow was implemented to prevent the adhesion of AgNPs aggregates to the channels of the microfluidic device [59,60]. In the case of paraquat AgNPs and NaCl were inserted in the microfluidic channel and subsequently encapsulated by a continuous oil phase forming microdroplets that exhibit the ability to adsorb the analyte. Aiming at the development of optical detectors, AgNPs and Au–AgNPs were also studied regarding their resonant light scattering (RLS) [63].

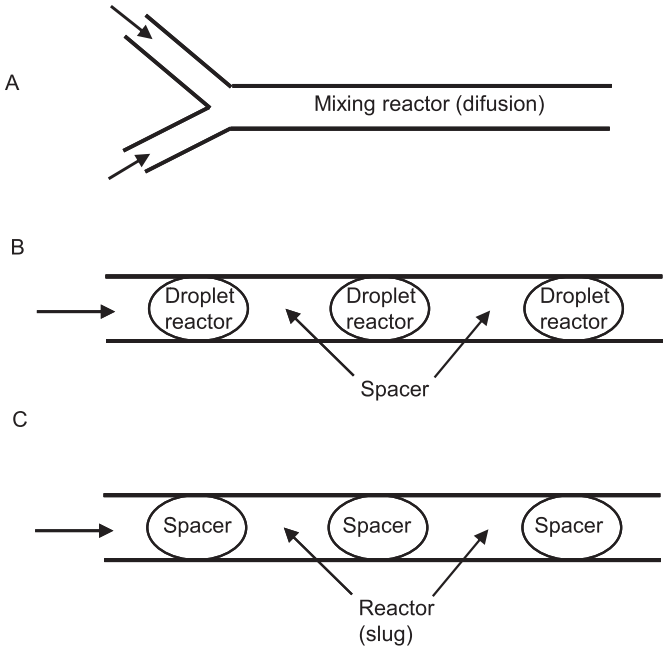

Fig. 4. Main categories of microfluidic reactors for the synthesis of nanomaterials (adapted from Ref. [29]).

Taking advantage of the intrinsic small volume of microreactors as well as of their large surface area and mixing efficiency, microfluidic technologies have been extensively exploited for the synthesis of several types of NPs to produce nanomaterials in large quantities but with well-defined properties recurring to distinct strategies (Fig. 4).

Considering the existence of previous reviews on this topic [18], herein, only recent works on this field and others that were not previously highlighted will be discussed. Distinct methodologies for the synthesis of AuNPs were implemented in microfluidic systems in the last years [64–66]. NPs were synthesized resorting to pulsed mixing [64], segmented flow [66], and laminar syringe driven flow [65]. The segmented flow approach was also applied to the synthesis of AgNPs in a spiral microreactor [67]. The exquisite control of the reaction conditions provided by the microfluidic operation conducted to nanoparticles with narrow particle size distribution. There are also a few applications of microfluidic synthesis of combined AuNPs such as AuNPs-chitosan functionalized microbeads [68], multicolor Au/AgNPs [69], Au–SiO_2 nanoshells recurring to the droplet approach [68] or to the generation of a concentration-gradient droplet array [69].

Considering the potential health effects of NPs as well as the adequacy of microfluidics for nano-based assays, a couple of devices for the evaluation of the toxicity of Au and AgNPs, based on their effect on cell cultures, were developed. The continuous monitoring of collagen production by human fibroblast cells, as an indicator of cellular stress, was performed through the cultivation of cells on a chip surface and subsequent administration of the NPs to study cellular responses [70]. The cytotoxicity of AgNPs was also assessed through microfluidic image cytometry resorting to HeLa cell [71]. The device incorporated a concentration gradient generator and straight channels for cell culture so that after the generation of a concentration gradient of NPs, the cell culture was exposed to distinct concentrations under continuous flow conditions. Both approaches can significantly increase data acquisition in the field of nano-toxicity while evidencing the advantages of microfluidics for the study of NPs' effects and accumulation on cells.

2.1.2. Magnetic NPs

The magnetic properties of some metallic NPs are receiving increased attention because these NPs can be controlled in terms of motion and location by means of externally applied magnetic fields. This allows tracking and visualization of the local environment through the use of imaging techniques and enables the development of detection probes through magnetic tagging of molecules [72]. These particular properties make magnetic NPs suitable to be employed in automated systems as well as to be associated to other NPs conferring magnetic properties to the resulting nanomaterials. The automated applications based on the association of magnetic NPs to other NPs will be discussed on the subsections corresponding to the associated NPs.

Regarding the use of magnetic NPs in flow-based systems, it is quite evident that the particular basic configuration of a SIA system makes this technique very suitable for assays based on these NPs since it is simple to adapt a permanent magnet to the system, independently from its final configuration. Thus, magnetic NPs can be inserted into the system from one of the lateral inlets and trapped in particular spots depending on the adopted analytical strategy and detection technique. In this context, magnetic NPs have been exploited in SIA methodologies as immobilization supports in immuno [73,74] and bioassays [75] and as adsorbents for the extraction and preconcentration of specific analytes [76–78]. In the immunoassays, magnetic NPs based on iron oxide [74], amine saline modified silica [73] or polystyrene with COOH groups on the surface [79] were used for antibody's immobilization [79] or for the attachment of antigens to form immunosensing probes [73,74]. The immobilization of antibodies on magnetic nanoparticles is a common practice in immunoassays as it enables a strong interaction between antigen and antibody while enabling the reutilization of the antibody after regeneration. The peculiar properties of SIA further enhance the applicability of the immobilized antibody through its entrapment by the action of a magnet. This strategy was applied to the determination of progesterone in human saliva by means of horseradish peroxidase catalyzed CL detection performed after the immunocompetitive assay [79]. The sensitivity of the determination was further enhanced through the implementation of a supported liquid membrane assay, easily implemented on the SIA system through coupling of the membrane unit to one of the inlets of the selection valve. The utilization of magnetic NPs as supports for the interaction with antigens in immunoassays has also been coupled to the immobilization of the antibody on a distinct kind of NP such as AuNPs [73] or silica NPs doped with rhodamine B [74] for the determination of methyl-3-quinoxaline-2-carboxylic acid residues (as detectable biomarker of olaquindox) in pork muscle samples and aflatoxin B1 in peanuts, respectively. The strategy adopted for the determination of the biomarker takes full advantage of the versatility and bidirectional flow of SIA and aimed the substitution of conventional CL mixtures through the coupling to a PCR apparatus for the quantification of the acid residues. During the analysis of aflatoxin B_1, the aspiration of the NP antigen probe and immobilized antibody was performed through lateral inlets of the selection valve without clotting or pressure events. The flow reversal mode of operation facilitated the implementation of the bead-based approach on a lateral inlet of the valve. In the field of bioassays, glucose oxidase and lactate dehydrogenase were immobilized in Fe_3O_4 NPs for automated glucose monitoring in bioprocesses confirming the adequacy of SIA for enzyme based processes [75]. The possibility of controlling rigorously the reaction conditions offered by SIA was a key factor for the successful implementation of the strategy.

Regarding the utilization of magnetic NPs as sorbent materials, there are applications in both SIA and lab-on-a-valve (LOV) manifolds involving the entrapment of silica [76,80] or iron [76,78] based magnetic NPs into micro-columns through the action of magnetic external forces. The nano-adsorbent materials were prepared by modification of the surface of the magnetic NPs with octadecylsilane [77,80], sodium dodecyl sulfate (SDS) [76] or polyacrilic acid [78] to create adequate conditions for the separation and preconcentration of quercetin [77] and metals [76,78,80]. The versatility of the designed systems enabled the hyphenation with electrothermal atomic absorption spectrometry [76,80] or inductively coupled plasma mass spectrometry [78] for the determination of distinct metals. On other hand, quercetin was determined by voltammetry after solid-phase extraction on a LOV manifold confirming the adequacy of this flow-based approach for nanomaterials manipulation [77].

Microfluidics have also proved to be adequate for magnetic NP based assays mainly for bioassays centered on enzymes and biosensors in which NPs were used to enhance the analytical performance of the methodologies [81–87]. Magnetic NPs have also been applied in a microfluidic device as part of a planar half effect sensor for the detection of biomolecules [88]. The potential of the combination of magnetic NPs and microfluidics is well evidenced on a microchip developed for the extraction of DNA from bacterial cell lysates through DNA–DNA hybridization. The silicon-based microchip incorporates a heater to cause cell lysis and allows the implementation of a liquid phase hybridization approach resorting to avidin-coated magnetic NPs for genome extraction [89].

Also in the field of microfluidic, several authors developed efforts to automatize the synthesis of magnetic NPs and functionalized magnetic NPs aiming at a reduction of the synthesis time and a deeper control of the particles properties. A good example of these efforts is the droplet microreactor developed for the synthesis of biogenic magnetic NPs resorting to bacteria with the ability to assemble metal ions [90]. The flow of two metal solutions generated droplets that could encapsulate cells of recombinant *Escherichia coli* expressing metal binding proteins and metal ions. The NPs formed inside the bacteria cells were detected by means of an external magnet that caused the movement of the cells on its direction. The particles were isolated from the cells and extensively characterized and an improvement of the size homogeneity was attained. Also on this field, Koziej and co-workers exploited for the first time the association of a microwave to a microfluidic device to dielectrically heat non-aqueous droplets for the preparation of tungsten oxide NPs with drastic reduction of the synthesis time [91].

Besides synthesis, microfluidics has been also used as a tool for the characterization of NPs and for the study of the behavior of particular nanomaterials. Some fundamental studies on the behavior of magnetic NPs in microfluidic devices illustrate the importance and potential of those materials [92–95]. Generally, the

information gathered from these works and in some cases the developed models constitute important tools for the future development of lab-on-a-chip systems with analytical and synthetic potential.

2.2. Carbon-NP based automated methodologies

Carbon-based nanomaterials have also become greatly important due to their excellent photoluminescence properties, mainly when dealing with carbon NPs (CNPs) [6]. Moreover, these nanomaterials, especially those based on carbon nanotubes and nanofibers, are promising sorption materials with applicability in solid-phase extraction of various species due to their mechanical, chemical and electrochemical properties [96,97].

CNPs have found application in FIA methodologies, mainly in CL based ones due to their adequacy for this detection technique and to the conditions offered by these systems for this kind of detection [61]. In this context, three approaches were reported including carbon nanomaterials either as CL inducing compounds [98,99] or as sensors for the recognition and adsorption of L-tryptophane in pharmaceutical formulations, prior to CL detection [100]. The first strategy was implemented in a FIA system for the determination of nitrite in water and milk samples based on the carbon dots induced CL in the presence of peroxynitrous acid that was generated in-line through the mixing of acidified H_2O_2 [99]. Similarly, carbon nanospheres were used to enhance the weak CL of the reaction between hydrogen peroxide and hydrogen carbonate, aiming at the determination of the first on tap and snow water in a simple flow manifold incorporating an injection valve and two peristaltic pumps for the insertion of sample, reagents and NPs, respectively [98]. On a distinct basis, the abovementioned sensor for L-tryptophane was based on the association of graphene with Fe_3O_4 NPs and L-tryptophan MIP and it was placed in the sample line, before the injection valve while the CL reagent, $KMnO_4$–$SnCl_2$–CHOH was mixed in-line by confluence [100].

The association of graphene nanosheets (bounded to an antibody) with silica nanospheres functionalized with CdTe-QDs used as labels enabled the development of an electrochemiluminescence immunosensor for prostate specific antigen and evidenced the advantages of combining the special features of distinct nanomaterials [101]. Even though this approach is of high analytical interest, its impact in terms of flow strategy is very low since the fabrication process is totally performed offline and only afterwards the immunosensor is inserted into a homemade detection cell with reduced internal volume. The detection cell however exhibits particular properties that improve the analytical performance of the methodology with possibilities of finding applications in similar approaches.

Generally, the use of carbon nanomaterials in SIA methodologies is limited to a major application: as sorbents for the isolation and separation of analytes from complex matrices [102–106]. In this context, the sorbent properties of multiwalled carcon nanotubes (MWCNTs) were exploited in an automated format by Du and co-workers for the isolation of both acidic [103,104] and basic [102] proteins. The developed methodologies involved either the packing of the MWCNTs in micro-columns [102,103] or the preparation of a thin layer of MWCNTs onto a hydrophilic membrane [104]. Both devices exhibited properties that enabled their incorporation in SIA systems through lateral inlets of the selection valve and connection to the detector by means of three way connectors. This configuration enabled the on-line solid phase extraction of the proteins from biological matrices without significant flow impedance events. It was concluded that the thin layer approach is more effective than the conventional column strategy due to the reduction of the flow resistance and to the creation of a convective flow pattern through the device. The approach for acidic proteins involved the prior functionalization of MWCNTs with poly(diallyldimethylam monium) chloride (PDDA) combining the advantages of both materials and thus providing stronger interactions that enhance the yield of extraction [104]. Similarly, the same authors resorted to quartz wool [107] and L-histidine [108] as ligands for the functionalization of MWCNTs aiming at the separation of lysozyme in egg white and immunoglobulin G in serum, respectively. The automated micro-columns SPE approaches were identical to the one described above. PDDA modified MWCNTs were also used for the adsorption of Cr(VI) in a SIA system with electrothermal atomic absorption detection [105]. As described before, the functionalized nanotubes were also packed in a microcolumn that was connected to the detector.

With the objective of circumvent the issues of undue pressure drop and nanoparticle bundling usually associated to the utilization of CNPs as sorptive materials, Boonjob and co-workers [106] developed a stirred-flow microchamber for the efficient handling and reutilization of these materials. The microchamber was integrated in a hyphenated SIA–FIA system and the SPE approach was used as a front-end to liquid chromatography. The developed methodology was validated through the quantification of target herbicides in water and soils. This methodology is a good example of the potentialities of automated micro SPE due to the important reported improvements.

2.3. Mesoporous silica based automated methodologies

Mesoporous silica materials are being extensively applied on distinct fields due to their high surface area (>900 nm g^{-1}), tunable pore size (2–20 nm) and uniform pore morphology. These particularities associated to novel synthesis procedures that conduct to more biocompatible NPs, make these nanomaterials very adequate mainly for biomedical applications [109].

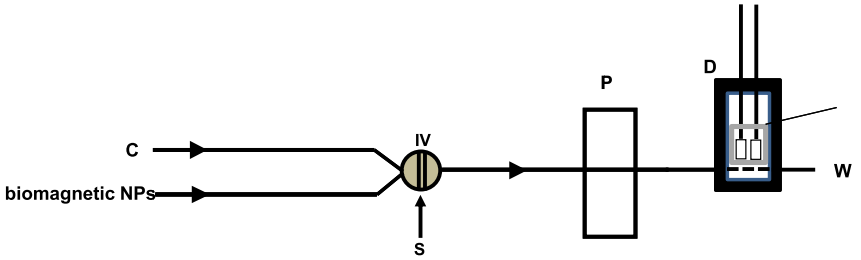

Fig. 5. Schematic illustration of the FIA manifold for implementation of a magneto-controlled immunosensing strategy (adapted from Ref. [110]). Biomagnetic NPs (with immobilized anti-carcinoembryonic antibody) were injected by means of a rotary injection valve (IV) into the detection cell (D) and attached to the surface of the electrode. After that, the sample (S) was injected into the detection cell and the immunoreaction occurred. Finally, the electrode was regenerated by detaching the permanent magnet and by flowing the carrier (C) through the pump (P), being expelled by the waste (W).

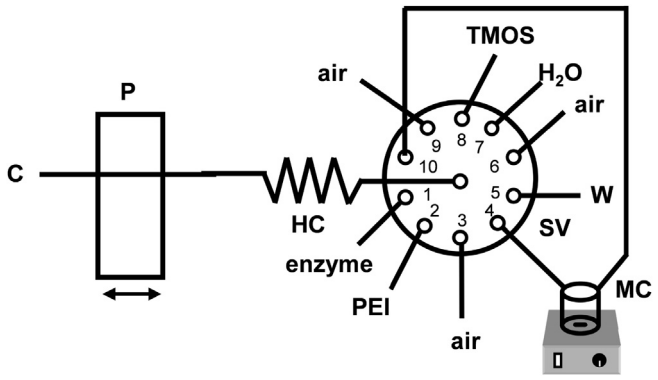

Fig. 6. Schematic illustration of the SIA manifold for simultaneous implementation of synthesis of biosilica nanoparticles and enzyme encapsulation (adapted from Ref. [113]). A mono-segmented flow approach was used, which implied that the sequence zones were isolated between two air bubbles before entering in the mixing chamber. Polyethylenimine (PEI) and enzyme were sequentially aspirated to the holding coil (HC) through the selection valve (SV) by means of a bidirectional pump (P). After flow reversal, the stacked zones were sent to the mixing chamber (MC) (port 4). After that an aliquot of water and the tetramethylorthosilicate (TMOS) was aspirated to the HC and sent, by flow reversal, to the MC, enabling the synthesis of biosilica nanostructures. Finally, the system was cleaned with the carrier solution (C) through the waste (W), to be ready for a new synthesis procedure.

Regarding automated applications, silica NPs have been associated to several types of other NPs aiming at mainly the development of sensing devices. This is illustrated in the first FIA assay based on NPs, implemented in 2007, resorting to mesoporous silica associated to magnetic NPs for the immobilization of an anti-carcinoembryonic antibody [110]. The implementation of the assay in the flow system (Fig. 5) provided also adequate conditions for the automation of the steps of antigen–antibody interaction and sensor regeneration. Despite the high degree of automation attained in the optimized conditions, the precision of the assay was affected by the well-known problem of non-uniform packing of the NPs in flow conditions evidenced by relatively large variations between different reactors.

The association of silica based materials to magnetic NPs results usually in nanosensors with broad applicability in biomedicine [111] and has been exploited in flow analysis mainly with sensing purposes sometimes with peculiar variations: i) association of the epoxysilane modified magnetic NPs to molecularly imprinted polymers (MIPs) for the detection of specific analytes [112] and ii) association of magnetic graphene nanosheets with silica nanospheres and QDs for the development of an immunosensor [101].

Distinctly, the already mentioned adequacy of automated methods for the synthesis of NPs was evidenced in a recent work based on the development of a SIA generic tool for the synthesis of biosilica NP with concomitant enzyme immobilization (Fig. 6) [113]. This was the first successful attempt to synthesize nanoparticles inside a SIA system and involved also the innovative encapsulation of laccase on the nanostructures. Automation resorting to SIA enabled the precise and exact control of the operations within the system which guarantees repeatable hydrolysis and nucleation and consequently similar particle growth. The particular mode of operation of SIA allowed also the implementation of a mono-segmented approach with the objective of decreasing the dispersion of aspirated solutions while reducing the possibility of formation of solid structures inside the flow systems.

Several types of silica-based nanomaterials have also been synthesized resorting to microfluidics [114–116]. The versatility of microfluidics enabled the integration of a planar baffled micromixer, with three mixing units, in a microreactor chip for the synthesis of silica NPs at high and low flow rates [114]. The microreactor proved to be highly efficient enabling the synthesis of variable size NPs by means of flow rate variation. Fluorescent silica NPs were synthesized inside microfluidic droplets with high reaction rates and enhanced particle size uniformity [116]. These features are once again related to the precise control of the reaction conditions in terms of reagents and time offered by microfluidics. A one step approach for the preparation of silica NPs with non-uniform surface properties was proposed as an alternative to the multi-step, time consuming procedure [115]. A coaxial microfluidic device was used for the implementation of a drop flow procedure that enabled the precise control of particles' morphology, dispersion and size. Similarly, silica nanoporous structures were synthesized in microfluidic channels to be applied to the separation of biomolecules by gel electrophoresis [54].

Mesoporous silica has been also used to improve the performance of microchip-based immunoassays that usually lack sensitivity due to the low capacity of the microfluidic system. In situ deposition and functionalization of SiO_2 nanoparticles on the surface of microchannels transformed the microfluidic surfasse making it more stable enhancing protein capturing also due to the increased surface-area-to-volume ratio [117].

2.4. QDs based automated methodologies

Quantum dots (QDs) are the most studied and applied NPs and are characterized by a nanocrystalline structure usually composed of a semiconductor material [118]. QDs present unique optochemical properties, arising from their size-dependent and tunable photoluminescence, and long-term photostability, and are particularly suitable for application in chemical analysis since their surface chemistry could be easily adjusted to sensing a specific analyte. In this regard, QDs are advantageous and interesting alternatives to the commonly used molecular probes in the biological and biomedical fields [119]. A variety of QDs has been synthesized and cadmium telluride (CdTe) [120], cadmium sulfide (CdS) [120] and zinc selenide (ZnSe) [121]are amongst the most popular exploited materials.

In the field of automated assays, the application of QDs is mostly based on the quenching of their fluorescence by selected analytes or on their use as sensitizers of chemiluminescence determinations. Since the first developments on the analytical features of QDs, the association with FIA showed to be a valuable tool as it enabled the accurate profiteering of the optical and physical chemistry of these peculiar NPs in the analytical perspective. The applications on this field resort to CdTe [122], CdTe/ZnSe [123], CdS [124], ZnS [125] capped with thioglycoloic acid [122], tiopronin [123] or to a Mn surface imprinting polymer [126] coupled to CL [122], electrochemiluminescence [127], fluorescence [123] and pulse voltammetry [124]. The developed approaches enabled the determination of nitrophenol in tap water [125], volatile phenols in environmental samples mediated by analyte induced CL quenching [123], dopamine in cerebro-spinal fluid [127] and influenza viral protein through connection of the dots with paramagnetic microbeads [124]. In the case of dopamine detection, CdTe QDs were associated with carbon nanotubes and chitosan on an indium tin oxide glass for the construction of an electrochemiluminescence electrode applied in a specially designed nano-liter size flow cell [127].

Distinctly, the properties of CdS mercaptoacetic capped QDs were exploited on methodologies for the speciation and determination of arsenic, based on quenching of QDs fluorescence [128,129]. The applicability and sensitivity of the determinations were greatly enhanced by their implementation on a SIA system that enabled the incorporation of gas diffusion units [128,129].

More recently, several QDs-based assays were automated resorting to MCFIA strategies aiming at the development of sensitive analytical methodologies for the determination of quinolones [130], ibandronate [131], ascorbic acid and hydroxytyrosol [132] in pharmaceutical formulations and food. All the approaches were based on the quenching effect of the analytes on the fluorescence of CdTe QDs [130] and mercaptopropionic acid modified CdTe QDs. The multicommutation systems operated resorting to three solenoid valves for the insertion of samples, QDs and carrier solutions driven by a peristaltic pump.

Distinctly, CdTe QDs were applied as sensitizers of the week chemiluminescence emission produced by oxidation of sulphite by Ce(IV) in acidic medium [133]. The reaction was implemented in a MPFS with four micropumps and was applied to the determination of hypoglycaemic drugs that interact with QDs hindering their sensitizer effect. Authors of the same group investigated CdTe QDs as generators of highly reactive oxygen species (ROS) [134] by coupling a photocatalytic unit. The methodology was based on the inhibitory effect of epinephrine on the oxidation of luminol by the produced ROS and, as before, it was applied to the control of pharmaceutical formulations. Due to the short lifetime of ROS, the methodology was implemented in a MPFS that guaranteed proper reaction development and appropriate dispersion before detection, with improvements in terms of efficiency and sensitivity. A simple MPFS was also used for the study of the promoting effect of acetylcysteine on the fluorescence of CdTe QDs modified with mercaptopropionic acid [135]. The developed methodology was successfully applied to the determination of acetylcysteine in pharmaceutical formulations.

CdTe QDs can however find other peculiar applications as demonstrated in a SIFA methodology developed for the determination of chemical oxygen demand of wastewaters. In this approach, CdTe QDs were used to promote the photocatalysis of organic compounds due to the production of strong oxidizing species [136]. The manifold incorporated four solenoid micropumps connected in pairs by Y-shape connectors from where sample, QDs and luminol were propelled to a photo-irradiation unit, positioned in the centre of the system besides the chemiluminescence detector. Considering the short-lived species generated during sample irradiation the adopted configuration was adequate and provided appropriate sensitivity.

In the field of microfluidics, QDs have been extensively applied to enhance the analytical performance of enzyme and biosensor based methodologies [87,137–139]. The mentioned adequacy of QDs for application on the biological and biomedical fields was also demonstrated on microfluidic approaches designed for the extraction of DNA from cell lysates through a multiplexed nucleic acid hybridization assay [140] and for the delivery engineered NPs into the cytoplasm of living cells [141] The nucleic acid hybridization assays was based on the CdSe/ZnS QDs mediated fluorescence resonance energy transfer (FRET) and relied on the modification of the microfluidic channels with biotin to assemble the biorecognition interface [140]. The microfluidic intracellular device specifically designed to deliver engineered NPs into living cells was tested with biologically compatible QDs that can be used to illuminate cytosolic proteins for long-term microscopy studies [141]. In this application, QDs diffuse into the cytosol during the lifetime of transient membrane disruptions that result from cell deformation after passing through a constriction in a microfluidic channel.

2.5. Other NPs

It is possible to find other punctual reports involving the application of NP in flow-based assays such as polyaniline NPs [142] and liposomes [143] to perform pH automated measurements and evaluation of the inhibitory effect of non-steroidal anti-inflammatory drugs (NSAIDs) on the activity of phospholipase A_2 (PLA_2), respectively. In these strategies, the SIA systems were hyphenated with Raman spectroscopy [142] and fluorescence [143] and mixing chambers [143] to increase the analytical performance of the methodologies.

On a distinct perspective, a microfluidic surface trap for the capturing of pH sensitive polymeric nanoparticles via UV light irradiation was developed [144]. The surface and the NPs were both modified with a polymer sensitive to pH variations.

In terms of synthesis, it is important to highlight the preparation of lipid-based NPs in microfluidic devices resorting to the directed self-assembly strategy [145,146]. Limited size NPs were obtained by spontaneous assembly through the millisecond mixing of aqueous and ethanol streams at high flow rates [145]. Microfluidic hydrodynamic focusing was used to attain the convective–diffusive mixing of two NP precursor solutions with the objective of forming lipid vesicles with encapsulated hydrogel precursors [146].

Similarly, a high pressure interdigital multiamination micromixer was selected for the continuous preparation of NPs of methacrylic polymers [147]. In this approach, mixing occurs mainly by diffusion mass transfer, with help of hydrodynamic focusing. The conditions of the synthesis were once again strictly controlled and the authors managed to understand the mechanism of particle's tunability. More recently, a microfluidic device was designed to both synthesize polymeric NPs and encapsulate paclitaxel, an anticancer drug [148]. The device consisted of perpendicular crossed channels that enabled the hydrodynamic mixing of the intervenient solutions and proved to be highly adequate for the controlled synthesis of polymeric nanoparticles with properties to be used in drug delivery.

On the bio-medical field, an interesting microfluidic system, based on a flow chamber, for the quantitative assessment of the accumulation of liposomes (targeted to inflamed endothelium) in cells under physiologically-relevant laminar flow was developed [149].

3. Conclusions and future trends

In this review, a comprehensive understanding of NP-based assays in automated systems from the first generation of flow-based injection techniques to microfluidic technology is provided.

The emphasis is placed on the categorization of NP-based assays in different flow-based strategies highlighting both the analytical potential of the resultant methods, the chemical nature of the employed NPs and the applied analytical format. Under a broad perspective it should be pointed out that, independently from the type of technique, automation provides advantageous features that are mostly related with the exquisite and versatile control of all stages of the assays, from reproducible insertion of sample and reagent solutions, reaction zone formation and transport towards detection in non-equilibrium conditions, including sample pretreatment and multi-analyte determination. Additionally, downsizing of assay conditions guarantees significant reduction of reactants consumption and minimization of waste generation.

Taking a deep insight into FIA NP-based assays, the high number of applications found in literature could be explained by the main features of this flow technique especially manifold simplicity and compatibility with a variety of detectors. In these systems the incorporation of NP-based bio and immunosensors as well as of NP-based solid phase extraction units is greatly facilitated by the systems' continuous functioning mode.

Regarding SIA methodologies, the versatility of the selection valve has been particularly useful for instance for the implementation of bioassays based on magnetic NPs, which can be inserted from one of the valve lateral inlets and be easily controlled by an externally applied magnet field. The flow reversal operation mode has also enabled the easy incorporation of separation and pre-concentration devices based mainly on carbon nanomaterials (MWCNTs) with advantages in terms of analyte loading and elution.

Flow techniques relying on the multicommutation principle showed to be particularly suitable for the implementation of QDs-based assays exploiting their photoluminescent properties, either in terms of intrinsic fluorescence enhancement or quenching or through their use as reaction catalysts in CL measurements. The versatility of solutions handling could be particularly useful in the carrying out of complex reactional schemes.

Analytical assays involving solutions manipulation by microfluidics technology are undoubtedly the most commonly used in combination with NPs research, covering all types of NPs in a variety of formats. This is typically a corollary of the improved assays conditions resulting from associating miniaturization with the possibility of conducting assays in environments comparable to those of biochemical reactions along with low consumption of solutions and energy. Indeed, since the distance that the analytes (such as biomolecules) have to travel inside the micro-channels to reach the sensor surface is markedly shortened, the detection time required for bioassays can be also greatly reduced. Moreover, kinetic binding rates between the biomolecules and the sensor can be determined with enhanced accuracy. These features also justify the particular attention devoted to the development of nanosensing systems, mainly bio and immunosensors, as well as to the application of microfluidic devices on nanomedicine. Microfluidic platforms exhibit also adequate properties and are particularly interesting for the implementation of procedures for the synthesis of NPs, with different chemical composition and variable, customized and controlled size and shape, as the reaction conditions are improved in comparison with traditional “beaker methods”.

In what concerns the contribution of new NPs or nanostructures, a great attention has been paid in recent years to nanopores. Several research groups have been implicated in the development of new technologies for separation purposes by means of new nanoscale transport phenomena, implementation of molecular–level surface interactions or the establishment of nanoporous membranes. These membranes are designed and structurally or functionally modified to explore fundamental transport studies, advanced separation and molecular separation strategies based on molecular size, chemistry and charge and for separation combined with detection by resorting to microfluidic systems.

It is highly probable that future developments on this field will explore mainly the versatility, simplicity and large surface area of microfluidic platforms for the development of novel procedures for the synthesis of NPs with controlled properties. It is also expected that major achievements will be attained in a near future as a result of the application of microfluidic chips in nanomedicine due to their biocompatibility.

Acknowledgments

This work received financial support from the European Union (FEDER funds through COMPETE) and National Funds (FCT, Fundação para a Ciência e Tecnologia) through project UID/QUI/50006/2013. The work also received financial support from the European Union (FEDER funds) under the framework of QREN through Project NORTE-07-0162-FEDER-000124. Marieta L.C. Passos thanks the Fundação para a Ciência e a Tecnologia, Pos-doc grant (SFRH/BPD/72378/2010) in the ambit of “POPH – QREN – Tipologia 4.1 – Formação Avançada” co-sponsored by FSE and national funds of MEC.

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