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High Performance Liquid Chromatography Coupled with Mass Spectrometry for/and Nanomaterials: An Overview

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On the occasion of the Centenary 1919-2019 from the first Mass Spectrometer

Abstract. The story of mass spectrometry applied to the nanoparticles world is very young but actually it is expected to evolve as one of the most powerful tool for the characterization of the smaller-size nanoparticles in terms of composition, size, shape, surface chemistry, because of its versatility, sensitivity, reliability and the possibility to be coupled with high performance chromatographic separation techniques. A short overview on different fields where liquid chromatography coupled with mass spectrometry, nanomaterials and nanotechnology come together is herein given.

INTRODUCTION

It was 1919 when the young researcher Francis W. Aston, working with Thomson on isotopes, set up the first example of high resolution mass spectrograph. The precision mass measurements led him to the *whole-number rule*, and provided a fundamental contribution to the understanding of the atomic structure of matter. For this, Aston was awarded the Nobel Prize in Chemistry in 1922 [1-3], and after that mass spectrometry took off.

Mass spectrometry is based on the measurement of atomic or molecular mass by ionization of the analyzed compound and detection of the ions after acceleration with an electric field and separation with a magnetic field according to their different m/z value.

Nowadays mass spectrometry finds application in an extremely broad number of fields, from the isotopic investigation to the molecular structure examination of organic compounds, including small molecules as well as biological macromolecules.

First employed at the beginning of 1900 for the separation of plant pigments, chromatography is based on the different partition coefficient of compounds between two phases, called mobile phase and stationary phase, resulting in a different speed of compounds carried by the mobile phase along the stationary phase. The compounds in the mixture are consequently detected at different time (retention time), and can be individually analyzed. After the Nobel Prize in Chemistry in 1952 to Martin and Synge for their fundamental work on chromatographic technique, also chromatography took off.

Thereafter, advances in technology continually improved the technical performance of chromatography, in terms of separation, time of analysis and amount of sample. The advance in technology also allowed to interface the highly performing gas-chromatography (GC) and liquid-chromatography (LC) with mass spectrometry (MS) detectors.

Mass spectrometry coupled with chromatographic separation techniques as GC, high-performance and ultra-performance LC (HPLC and UPLC, respectively) can be considered nowadays a leader and powerful tool for the analysis of complex matrices. In particular, HPLC-MS is commonly used for biological and pharmaceutical applications, i.e. pharmacokinetic, drug development, proteomic, metabolomic, in vitro and in vivo drug metabolism studies [4-7]; it is used for food, beverages and environmental applications, i.e. characterization, fingerprinting,

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analysis of antioxidants, allergens, mycotoxins, phytotoxins, pesticides, authenticity and fraud, process and quality control [8-13]; it is used for chemical applications, i.e. synthetic process and mechanistic investigation [14-20].

The analytical potentialities of the two-dimensional combination of LC with MS are ever-increasing with the continuous advancement of technology, concerning the interface between the liquid phase and the vacuum system of the mass spectrometer, the ionization sources and techniques, the ever-smaller particles for column for separation and suitable high performing pumps.

Widely used for decades in a variety of research fields, MS applied to the nanoparticles (NPs) has a very young story, but for its own nature it is expected to evolve as one of the most versatile, sensitive, reliable analytical tools for the characterization of NPs, in terms of composition, size, shape and surface chemistry. As a consequence, MS also coupled with HPLC offers a precious tool to advance the knowledge of properties and behavior of nanomaterials, to develop new synthetic strategies, to improve performances, and to monitor eventual long-term effects on health and environment, not predictable so far.

In the present paper, a short overview on different fields where LC/MS and nanomaterials comes together is given.

LC/MS FOR NANOMATERIALS CHARACTERIZATION

The increasing attention paid to mass spectrometry for nanomaterials had to be expected, resolution and sensitivity being the most important criterions the different MS techniques are based on. Mass spectrometry was actually destined to become a powerful tool in elucidating the accurate chemical structures and the exact masses of the smaller-size NPs (< 5 nm). So, strong attention and efforts are due to researchers, leaders in the field, in developing MS techniques with increasing resolution and sensitivity for applications on nanomaterials.

In the last two decades, the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS (freely outlined in Fig. 1) turned out useful as a technique for fully characterizing nanoscale materials and elucidating structure-property relationships.

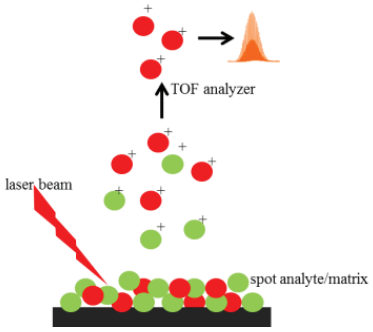

FIGURE 1. Scheme of MALDI-TOF mass spectrometry: the analyte co-crystallised with the matrix on a target plate, after desorption and ionization by pulsed laser light, is analysed by time-of-flight MS mode.

At the end of ’90s, a very accurate analysis of shape, size and passivation of thiol-passivated Au nanocrystals in the 1.5-3.5 nm size-range was obtained by laser desorption ionization (LDI) MS [21], capable of resolving small quantities of species differing in size by less than one lattice spacing. Combined with other independent measurements, MS provided evidences for the discrete nature and the stability of those passivated elemental gold (Au) nanocrystal materials, with applications as advanced optical, electronic, and catalytic materials.

The accurate determination of size and size distribution of a series of *n*-hexadecylamine(HDA)-passivated ZnS nanocrystals, 2.5-3.7 nm size-range, was also obtained by MALDI-TOF MS, using dithranol or anthracene as the matrix, irradiation with a pulsed nitrogen laser for desorption and ionization of nanocrystals, and the TOF mode for ions detection. The particles size distribution obtained by MS analysis was found systematically correlating with their absolute size and size dispersity obtained by optical and Transmission Electron Microscopy (TEM) analysis. For the first time the described MS method had found application to inorganic nanomaterials in the field of semiconductors [22].

More recently, MALDI-TOF MS has been used to characterize colloidal platinum nanoparticles (PtNPs) synthesized in the 1-4 nm size range, by using polyvinylpyrrolidone (PVP) as capping agent to stabilize the PtNPs in solution [23].

Experiments were carried out on both PVP-capped and uncapped NPs, and a relationship between the size of the NPs and the number of polymer chains involved in the capped forms was evidenced. In particular, a single polymer chain was found surrounding the smaller 1.85 nm NPs. Also in this case, the particles sizes determined by MS data were found in good agreement with sizes determined by TEM and X-Ray Diffraction (XRD), as shown in Table 1.

This kind of studies provides a better understanding of the molecular-level behavior of these materials as catalysts. The method was suggested to be extended to colloidal NPs of other metals as Rh, Pd, Ru, also using other capping agents as dendrimers, with application on biomaterials.

Noteworthy, a detailed characterization of the NPs in the whole (i.e., core and ligands) is important to improve new synthesis routes and final properties of nanomaterials as well as to study the impact on human health.

Surprisingly, sensitivity of MALDI-TOF technique was found lowering with increasing mass up to lose ions with larger masses, the same behavior occurring with both heavy and non-heavy metal NPs. A typical example is the characterization of carbon nanoparticles (CNP) [24].

The interest in CNP is growing and growing, as witnessed by the increasing number of reports on new synthetic routes and applications. Recently, a series of interesting results on the complete characterization of individual CNP species in synthesized CNP complex mixtures has been reported, by using LC to separate CNP and MS to determine the sizes and the masses of the different CNP fractions.

TABLE 1. Particle masses found from MALDI-TOF mass spectra and particle sizes, *d*, calculated from MALDI-TOF MS, TEM, and XRD measurements [23]. Reprinted with permission from [23]. Copyright 2009, American Chemical Society.

m/z (amu)	fwhm (amu)	total mass (amu)	Pt core mass (amu)	d, MALDI-TOF MS (nm)	d, TEM (nm)
73 000	58 500	73 000	42 000	1.84	1.85 ± 0.34
93 600	88 700	187 000	125 000	2.65	2.60 ± 0.56
189 000	286 000	189 000	127 000	2.66	2.60 ± 0.56
107 000	89 200	214 000	152 000	2.82	2.94 ± 0.61
216 000	259 000	216 000	154 000	2.84	2.94 ± 0.61
117 000	94 300	351 000	258 000	3.37	3.69 ± 0.62
245 000	206 000	368 000	275 000	3.44	3.69 ± 0.62

A first report concerns the development of a reverse phase RP-HPLC method coupled with fluorescence detection (FD) for an efficient analytical separation of high purity CNP fractions to be individually characterized by UV-vis adsorption, photoluminescence (PL) spectroscopy, MALDI-TOF MS and TEM [25]. The spectral properties, size, and surface-attached functionality of each individual CNP species were provided. The RP-HPLC method also resulted suitable to tune the synthesis conditions for desired specific CNP species.

A RP-HPLC separation coupled with MALDI-TOF analysis was also developed for the separation and the characterization of *N,N*-dimethylformamide (DMF)-stabilized Pd nanoparticles (DMF-PdNPs), the results revealing a complex mixture of ultrasmall Pd_xNPs stabilized with different numbers of DMF ligands [26].

Advanced results were obtained by using the fast and accurate ultra-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry (UPLC-ESI-Q-TOF-MS/MS) for the separation and the structural elucidation of fluorescent CNP [27]. Six different kinds of chemical formulas were found. CNPs were found as supramolecular clusters in the aqueous phase, the individual monomers linked together through non-covalent bonding forces, of major significance in bio-imaging where chemical structure strongly influences the PL performance of CNP. Further, the sensitivity of ESI-Q-TOF-MS/MS towards CNP was found much better than MALDI-TOF regard to the capture of ions in higher mass range. Similar results on nitrogen and sulfur codoped CNP were also reported [28].

NPs were also recently proposed as new matrix for the analysis of polymers by MALDI MS. As an example, a matrix containing an iron oxide NP core capped with citric acid was successfully used for the analysis of polymers of different hydrophobicity, simplifying matrix selection and sample preparation processes [29].

Just to resume this short paragraph, MALDI-TOF mass spectrometry can be considered as an emerging technique to characterize different types of nanomaterials, particularly useful for the smaller-sized nanoparticles (< 5

nm). Coupled with other techniques, it may be used to determine the size and the mass of the NP core but also the mass of the capping agent. Further, a solvent-free MALDI-TOF method has been proposed, with the advantage of application to insoluble solid samples properly treated [30].

Another mass spectrometry technique, coupled with HPLC, has been used in a recent first report on the investigation of the effect of particulate and dissolved forms of gold on microalgae cells, in order to study the effect of gold species on aquatic microorganisms in the environment. Inductively coupled plasma (ICP) MS also coupled with LC (HPLC-ICP MS) was applied to measure total gold content and AuNPs size after an exposure of algae to gold species at environmentally relevant concentration [31]. It is a high sensitive analytical technique combining a plasma torch, for the ionization of metal and non-metal inorganic compounds, with MS for ions separation and detection. A quantitative uptake was observed for Au(III), whereas a size dependent uptake was found for AuNPs, increasing for increasing particle size, even if no effect on algal growth was observed, at least during the one-day incubation period studied.

NANOTECHNOLOGY AND NANOMATERIALS FOR LC/MS DETECTION

Nanotechnology is also being exploited in analytical chemistry and most of the scientific reports encourage more investigation on developing methods where nanomaterials and nanotechnology could be somehow involved.

As an example, nanotechnology plays an important role in the development of sensors and biosensors, based on nanomaterials synthetized and functionalized according to different methods, with application in medicine, i.e. enzymatic biosensors, DNA sensors, immunosensors and cell sensors [32], in environment and food, i.e. highly selective sensors for caffeine in beverages and drug formulations [33-34], and used as catalysts, immobilization platforms, optical or electro-active labels, in order to enhance the sensing performance [35-37].

Nanotechnology found recent application in LC, where the use of nanoLC column and the reduction of the flow rate of the mobile phase to the nanoliters per minute scale (<500 nL/min) allowed to introduce the eluted solution from nanoLC column directly into the source of electron ionization (EI) mass spectrometer (EI-MS), without any interface. Firstly proposed by Cappiello et al. [38], such a miniaturization of LC systems made faster the nebulization and vaporization process, avoiding the use of the nebulizer gas. Further, the ion source contamination and the thermal degradation of analytes more sensitive to temperature resulted reduced. A scheme representing the interface and mechanism of ionization of this nano-LC/MS system is shown in Fig. 2.

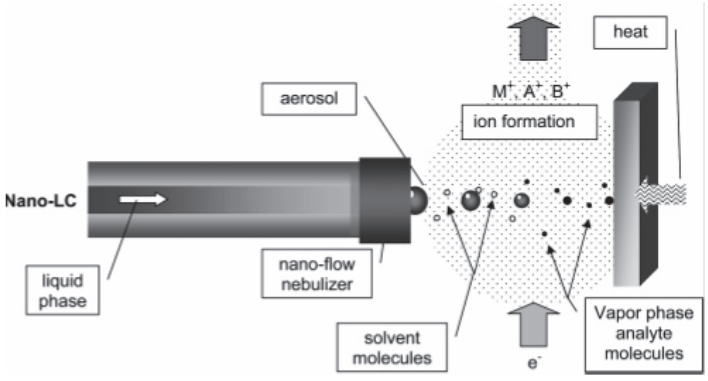

FIGURE 2. Interface and mechanism of ionization of the nanoLC/EI-MS system [39].
Reprinted with permission from [39]. Copyright 2007, American Chemical Society

Since its prototypal stage, nanoLC/EI-MS resulted particularly effective in the analysis of small molecules, polar or thermolabile compounds, or volatile compounds in their native form without needing derivatization step, filling the gap dividing GC/MS and LC/MS in the analysis of small molecules.

Good limits of detection (LOD) and quantification (LOQ), linearity, and repeatability, were obtained for a mixture of small molecules [39], and several applications were reported in literature, as the determination of organo-chlorine pesticides in water [40], pyrolysis compounds in biomass [41], endocrine disruptor in seawater [42], not-

esterified fatty acids in plasma [43]. In particular, fatty acids are usually analyzed by GC after derivatization to make them less polar and more volatile, but the free fatty acids profile in organisms could be affected by the derivatization process. The benefits of nanoLC/EI-MS over GC techniques have been reported on the free fatty acids elucidation in marine organisms, as shown in Fig. 3 [44].

The dramatic increase of industrial manufacture and applications of NPs is calling concern and attention to the impacts of NPs on biological systems and environments [45]. Due to their specific surface area and activity, NPs tend to adsorb many substances in complex matrix, including bio-molecules in biofluids to form the so called biological corona. It was found that size, shape, surface coating and core composition of NPs and the surrounding environment strongly affect the formation of protein corona [46-48].

The analysis of the adsorption of lipids, abundantly present in biological fluids in which they play important biological roles, on three different kinds of NPs have been carried out by nanoflow LC coupled with tandem mass spectrometry (MS/MS), as recently reported [49]. Three different types of NPs were used, as TiO_2 and cellulose nanofibrils (CNF), because added to food products to adjust nutrition contents, color and taste, and polystirene (PS) because more hydrophobic than CNF. As these NPs likely interact with lipids in serum or food matrices, human serum and heavy cream were chosen as matrices. The formation of lipid corona on NPs during incubation with serum and heavy cream was demonstrated, and the lipid profiling from lipid corona on NPs was obtained by nanoflow LC and collision-induced dissociation (CID) mode of tandem mass spectrometry, a technique providing an effective fragmentation route to obtain structural information of lipids. The lipid adsorption profile on NPs was found affected by both NPs and matrix nature.

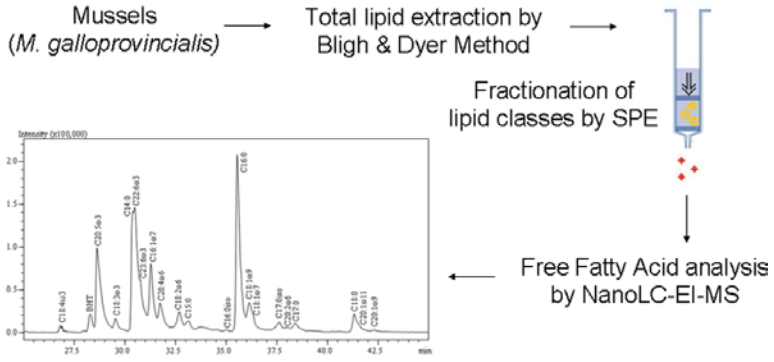

FIGURE 3. Free fatty acids profiling in marine organisms by nanoLC/EI-MS [44].
Reprinted with permission from [44]. Copyright 2016, American Chemical Society.

The analysis of bisphenol A desorption from titania NPs has been recently reported [50]. Bisphenol A (BPA), used as monomer for various consumer products, is a pervasive environmental toxicant and it is a known endocrine disrupting chemical. NPs are an emerging class of contaminants, whose co-presence in the same environment could lead to the binding of BPA on NPs surface. This could cause a not accurate determination of BPA. The co-presence of NPs with contaminants, as TiO_2 and BPA, could also synergistically affect the contaminant bioaccessibility, uptake and toxicity into living organisms [51]. The adsorption/degradation of BPA in water into/by TiO_2 NPs was studied by direct infusion into the electrospray ionization (ESI) source of the mass spectrometer, without previous separation by LC. Free BPA, adsorbed BPA and NPs were introduced into the ESI source, the method resulting simple, fast and cost-effective for the determination of BPA with enhanced accuracy, for application to real sample analysis also in the presence of NPs.

Nanomaterials also find application in analytical chemistry as useful tool for low levels analyte determination that requires pre-concentration techniques, high separation performances and selective and sensitive detection. Solid-phase extraction (SPE) is the pre-concentration mode most widely used for the analysis of low levels of organics in aqueous matrices and new sorbent materials to be used in lower amounts are being explored. In this context, carbon nanotubes (CNTs) have found application as a new kind of sorbent and first used for environmental purpose as the removal of dioxins [52] or trihalomethanes [53], just to name a few. An efficient sorbent capacity is shown also in organic media, as evidenced in the one-step carbon nanotubes-based solid-phase extraction followed by GC/MS analysis reported for the determination of pesticides in virgin olive oil samples [54]. These properties

added to the little quantities of CNTs with respect to the conventional SPE cartridges, make this nanomaterial highly useful in the miniaturization of pre-concentration procedures.

The selective solid-phase extraction by packed multi-walled carbon nanotubes (MWCNTs) followed by LC-MS analysis was described for the determination of two groups of pharmaceuticals highly consumed worldwide, i.e. non-steroidal anti-inflammatory drugs (NSAIDs) and β-blockers, in river and wastewaters [55]. Although not highly persistent, these complex mixtures of pharmaceuticals may exert synergistic toxic effects on biota even at the low environmental concentration levels of μg L^{-1} to ng L^{-1}. The small amount of 20 mg of MWCNTs was used with success to pre-concentrate twelve pharmaceuticals, with high extraction efficiency for most drugs that were successively separated by LC and quantified by the selected reaction monitoring (SRM) mode of MS.

CONCLUSION

The goal of this paper was to provide an overview on the suitability, potentialities and different types of application of different techniques of mass spectrometry coupled with high performing chromatographic separation techniques, for the characterization of nanomaterials. Mass spectrometry application without previous separation by high performance liquid chromatography were also cited for the sake of completeness. Some selected papers herein proposed evidenced a good agreement between data collected with these techniques, quite new for the nanoparticles world, and more conventional techniques as TEM and XRD measurements.

The recent increasing number of papers in which LC/MS are linked with nanomaterials evidences the relevance of the topic we have given a quick look to, and suggests that increasing applications can be predicted, with particular attention to the field in which nanomaterials meet biological systems.

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