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Analytical Developments for the Characterization of Nanomaterials in Consumer Products, Environmental and Medicinal Samples

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Abstract - Nanomaterials (NM) open huge prospects for innovation in different fields such as medicine, electronics, cosmetics and materials. However, their uses raise questions about possible risks to the environment and humans. The development of suitable protocols for the physicochemical characterization (size distribution, shape and chemical composition) of such materials is a fundamental issue for coming years. To meet the needs of various industrials producing or using NM, UT2A has developed new analytical approaches. The first one is focused on the determination of the size distribution of nano-scale particles using Dynamic Light Scattering detector (DLS) and a splitting system (by size and weight) such as Asymmetrical Flow Field Flow Fractionation hyphenated with a Multi Angle Laser Light Scattering detector (A4F-MALLS). The second approach is based on a comprehensive physicochemical characterization made by the combination of A4F-MALLS with an UV detector and an Inductively Coupled Plasma Mass Spectrometer (ICP-MS). The Single Particle-ICPMS has also been used to characterize NM. This study is first focused on the characterization of NM in consumer products such as sunscreens, candies or juices. The results obtained by the different analytical approaches are also discussed. Then the same techniques were used for environmental (colloids and NPs in effluent processes) and pharmaceutical applications. This work has enabled to develop and validate an approach to global physicochemical characterization of nanomaterials in complex matrices.

Keywords**:** Nanomaterials, characterization, consumer products, industrial and environmental applications

1. Introduction

Nowadays, nanomaterials (NMs) and nanoparticles (NPs) are used in many fields of activities. Apart from environmental NPs and natural colloids, the NMs can be classified according to their production in voluntarily and involuntarily produced. Currently, more than 1600 nano-based products are introduced to the market [1] due to their properties, such as antimicrobial agent [2], color additive [3], ultraviolet (UV) filter [4,5], processing additive, agent for clarification, pH control [6] or carrying of flavors and aromas [7]. Some of the most common uses of NPs are in medicine (Au, CeO_2), food (Ag, TiO_2 SiO_2, Fe), cosmetics (TiO_2, ZnO), sport clothes (Ag), electronics, energy and fuel additives (CeO_2), nutraceuticals and pharmaceuticals (silicate and aluminosilicate) and for wastewater treatment or cleaning (Fe,TiO_2), etc. [8].

In spite of the extended use of NPs in diverse consumer products, there is a great concern over the unexpected impact or effects on humans due to exposure [8]. Thus, recently, regulations focused on the allowed and not allowed appearance of NPs in daily products have released [9]. For example, for cosmetics, the Regulation (EC) No 1223/2009 [10] provides with a list of the prohibited and restricted uses of diverse metals and organic compounds. In relation with NMs, a report of the product should be submitted to the commission six months in advance of the release of the product into the market (article 16) and the labeling should include the word 'nano' in brackets (article 19). Similarly, European Food Safety Authority (EFSA) published an opinion essay focused on the potential risks that may arise from nanoscience to food and feed safety [11].

According to these regulations, the key parameter to be determined for the characterization of these NPs lies in particle size for perfect spheres or equivalent spherical diameters (ESD) for non-spherical NMs [10,12]. Other parameters that affect the toxicity and should be taken into account such as size distribution, structure, shape, composition, concentration, surface charge or functionality, porosity, aggregation or agglomeration state, etc.

A wide range of techniques is available for NPs characterization, covering microscopy, spectroscopy and size separation techniques. Traditionally, microscopy-based techniques (Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM)) give information of size and shape of NPs, and also on elemental composition after coupling with Energy Dispersive X-Ray Fluorescence Spectrometry (EDX). Light Scattering methods, including Dynamic Light Scattering (DLS) and Multi-angle Laser Light Scattering (MALLS) are also available. Information about the hydrodynamic diameter (Dh) and the RMS radius (Rg) of NPs is obtained. Of those methods, DLS has been commonly used due to its simple operation and rapid analysis but may result in an overestimation when small and large particles and their aggregates co-exist in a sample [13]. MALLS is often used as detector after size fractionation of particles by different modes of Flow-Field Fractionation techniques (A4F) [14]. A4F techniques can also be hyphenated with ICP-MS to obtain information about NPs composition. More recently, Single-Particle Inductively Coupled Plasma Mass Spectrometry (SP-ICP-MS) has been used to quantify the number, concentration and size of NPs by using the frequency of analytical signals and the relationship between mass and density, respectively [15]. This technique is gaining more and more importance, especially on a routine basis due to its rapidity [16,17].

As far as we know, the results obtained by different techniques have never been compared considering the same sample preparation procedure. Among the different available techniques, DLS, and SP-ICP-MS were used in this work due to the complement information provided by each one. AF4-MALLS(-ICP-MS) can be considered as a reference technique for NPs characterization even if this method requires a long time of analysis and is subjected to matrix effects. DLS and SP-ICP-MS were also selected because of their simplicity and availability in laboratories making them more adapted to routine analysis. After optimization and evaluation of the analytical performances of each technique, the presence of NPs was studied in various consumer products and environmental and medicinal samples.

2. Experimental

To characterize nanomaterials, three analytical techniques were employed. First, a Dynamic Light Scattering (DLS) detector (Vasco2, Cordouan Technologies) was used. This technique is a common tool to determine size, size distribution and polidispersity of nanomaterials. An Eclipse 2 Field Flow Fractionation (A4F) splitting system was coupled with a Dawn Heleos II Multi Angle Laser Light Scattering (MALLS, Wyatt Technology) detector to determine size and size distribution. An Inductively Coupled Plasma Mass Spectrometer detector (ICPMS, Agilent 7900, Agilent Technologies) was also used on-line to determine simultaneously the composition of nanoparticles. A refractive index (RI) detector (T-Rex, Wyatt Technology) was also use to quantify particle concentration in medicinal samples. Another ICPMS (Nexion 300, Perkin Elmer) was used in Single Particle mode (SP-ICPMS) to determine in a faster way the size and composition of nanomaterials in consumer products.

Different kinds of samples were considered in this study. Beverage products (alcoholic drinks, coffee, sport drinks, hot chocolate, cappuccino, wine, juices) were diluted in ultrapure water previous to their analyses. Chocolate candies, chewing-gum and decorative pearls were dissolved in ultrapure water and sonicated in a ultrasonic bath for 30 min. Nanomaterials in cosmetics were first extracted with hexane and centrifuged (5 min, 3000 rpm). Hexane was then removed and residues were dispersed in water and sonicated 30 min in an ultrasonic bath. For environmental (waste water, soils) and medicinal samples (nanopharmaceuticals) any samples preparation was performed. Three gold nanoparticles standards from NIST (RM 8011 10 nm, RM 8012 30 nm and RM 8013 60 nm) were used to evaluate the suitability of analytical approaches. Each Au standards was diluted according to the technique employed.

3. Method Validation

Prior to the analysis of real samples, a validation step was realized to evaluate the suitability of the different equipment with standards. For each analytical technique, gold standards were analyzed and results are reported in table 2. The limit of detection by size and concentration and the other parameters such as repeatability and reproducibility were determined simultaneously and are also presented in this table.

Table 1: Characterization of gold stanrds nanoparticles by DLS, SP-ICPMS and FFF-MALLS-ICPMS.

Sample	Theoretical diameter (nm)	Experimental diameter (nm)		
		DLS	SP-ICPMS	A4F-MALLS-ICPMS
RM 8011 (10 nm)	9.9 ± 0.1 (SEM) 8.9 ± 0.1 (TEM) 13.5 ± 0.9 (DLS)	11.1 ± 0.2	< LOD	13 ± 2
RM 8012 (30 nm)	26.9 ± 0.1 (SEM) 27.6 ± 2.1 (TEM) 28.6 ± 0.9 (DLS)	29 ± 3	27 ± 1	28.9 ± 0.6
RM 8013 (60 nm)	54.9 ±0.4 (SEM) 56.0 ± 0.5 (TEM) 56.6 ± 1.4 (DLS)	59.0 ± 0.2	64 ± 2	58.4 ± 0.3
Parameter		**DLS**	**SP-ICPMS**	**A4F-MALLS-ICPMS**
Limit Of Detection (nm)		1	18 nm	10
Limit Of Detection (concentration)		0.025 – 1 mg/L	1- 200 ng/L	50-600 µg/L

Results indicates that experimental diameters determined are in accordance with theoretical values. However for RM8011, the theoretical value (10 nm) is lower than the LOD estimated by SP-ICPMS (18 nm). As shown in table1, DLS is the most sensitive technique to determine the size of small nanoparticles (LOD of 1 nm). However this technique is the less sensitive to determine the composition of nanoparticles. To reach this goal, SP-ICPMS is the more sensitive technique. This step was necessary to evaluate the suitability of the operating conditions to characterize nanomaterials.

4. Characterization of Nanomaterials in Consumer Products

In next paragraphs, examples of raw data obtained by each technique are presented to show information that can be determined. Figures reported are representative of what was obtained for all samples. Size and size distribution determined by DLS for beverage are reported in Fig. 1.

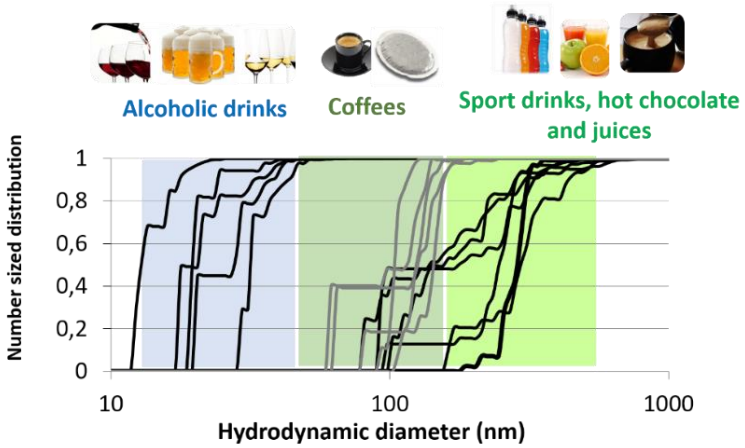

Fig. 1: Determination of size distrution of particles in beverage by DLS.

Beverage can be divided in three categories according to the size distribution. Alcoholic drinks are composed with smaller particles (from 10 to 100 nm), coffees with particles between 70 and 300 nm and other drinks with bigger particles from 100 nm to 1µm. These results indicates that DLS is a very suitable tool to determine size and size distribution of nanoparticles and nanomaterials in drinks. However, for this kind of application, polysaccharides, micelles, biopolymers or metallic nanoparticles can be used and DLS does not allowed determining the composition of nanomaterials.

To determine simultaneously size and composition of nanoparticles the A4F-MALLS-ICPMS system was used and Fig. 2 presents an example of a fractogramm obtained for the characterization of metallic nanoparticles in a sunscreen.

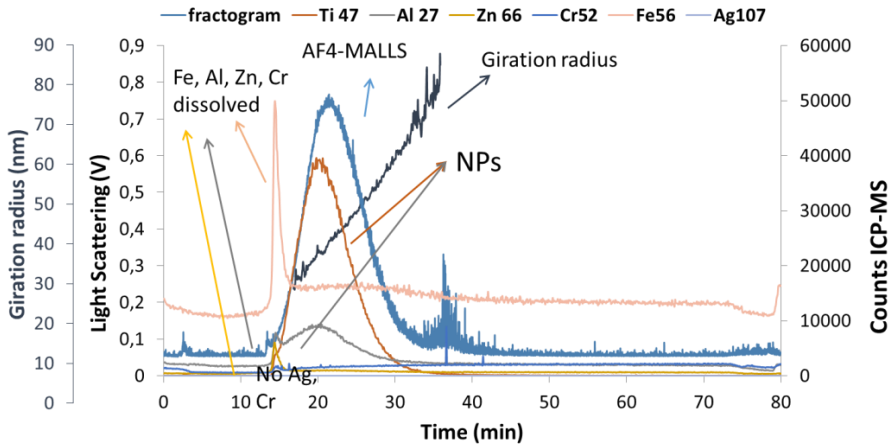

Fig. 2: Characterization of metallic nanoparticles in sunscreens by A4F-MALLS-ICPMS.

As shown on Fig.2, the size (gyration radius) of particles in sunscreen is between 30 and 80 nm. The mean size is of 86 ± 5 nm. Most, these particles are constituted by titanium and aluminium (respectively in orange and grey). TiO_2 NPs are often coated with a surface layer of aluminium to shield against the harmful effects of hydroxyl radicals (•OH), superoxide anion radicals (O_2^{-}•) and other reactive oxygen species (e.g. H_2O_2) generated when TiO_2 nanoparticles are exposed to UV radiation [13]. Moreover, results corroborate the presence of titanium nanoparticles in the sunscreens as mentioned into bracket on the packaging. These results illustrate the complementarity between granulometric and elemental detectors. The same sample was also analysed by SP-ICPMS and results are presented on Fig. 3.

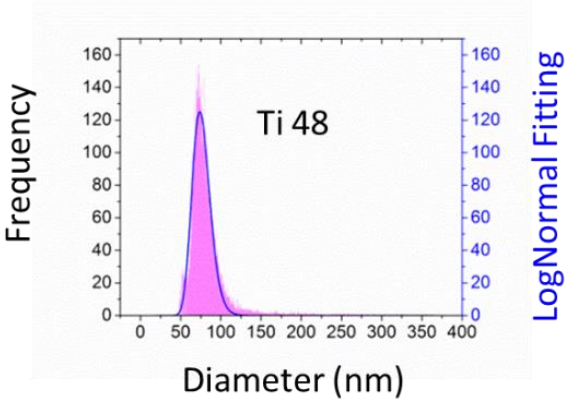

Fig. 3: Analysis of TiO2 nanoparticles in sunscreen by SP-ICPMS.

The mean size of TiO2 nanoparticles determined by SP-ICPMS is of 80 ± 10 nm and results are in accordance with ones obtained by A4F-MALLS-ICPMS. By this way, various consumer products were analysed by these three techniques and results are ported in table2.

NMs are present in all samples investigated. However by DLS it is not possible to identify the origin and/or the kind of NMs. This task can then be realized using elemental detectors such as ICPMS or SP-ICPMS. In most of cases size or size distributions determined by the three techniques for foodstuff and cosmetics products are in accordance. This table demonstrates the suitability of the analytical approaches developed to characterize NMs in consumer products.

Table 2: Summary table of the characterization of NMs in consumer products.

Sample		Diameter (nm)	Composition	Technique
Beverage	**Alcoholic drinks**	20 - 40	No information	DLS
	Coffee	100 - 140	No information	DLS
	Sport drinks	170 - 310	No information	DLS
	Cappuccino	133 ± 20	TiO_2	A4F-MALLS-ICPMS
	Hot chocolate	110 - 330	Organic particles	A4F-MALLS-ICPMS
	Juices	35 - 135	TiO_2 & C particles	A4F-MALLS-ICPMS
	White wine	129 ± 15	Cu NPs	A4F-MALLS-ICPMS
Foodstuff	**Chocolate candies**	124 ± 8 147 ± 3 125 ± 4	No information TiO_2 TiO_2	DLS A4F-MALLS-ICPMS SP-ICPMS
	Chewing-gum	112 ± 4 216 ± 6 126 ± 1	No information TiO_2 TiO_2	DLS A4F-MALLS-ICPMS SP-ICPMS
	Decorative pearls	35 - 225 30 - 150 50 - 200	No information Ag Ag	DLS A4F-MALLS-ICPMS SP-ICPMS
Cosmetic products	**Sunscreen**	109 ± 9 86 ± 5 80 ± 10	No information TiO_2 and ZnO TiO_2 and ZnO	DLS A4F-MALLS-ICPMS SP-ICPMS
	Toothpaste	70 - 125 50 - 100	No information TiO_2	DLS SP-ICPMS
	Lip balm	45 - 175 50 - 90 40 - 100	No information TiO_2 TiO_2	DLS A4F-MALLS-ICPMS SP-ICPMS

5. Characterization of Environmental Samples

Interest in NPs has also increased during the last decades due to their potential role as a carrier for toxicants in natural waters or due to their inherent toxicity. The coupling of ICPMS with A4F-MALLS has been applied to fractionate synthetic nanoparticles and natural colloids (e.g. humic or fulvic acids and bound metal ions) respectively in waste waters (Fig.4A.) and soils (Fig.4B).

The study of the waste water process (Fig.4A) was focused on the evaluation on the efficiency of the treatment unit to trap and eliminate metallic NPs. Two water samples (HB and HT respectively before and after treatment) were considered and the A4F-MALLS-ICPMS analyses were focused on the size characterization of particles (Rg in black) and composition by monitoring of aluminium (blue) and Silver (red). Results indicate the presence of Al and Ag NPs of around 130 nm in untreated wastewater. However, in treated wastewater any particle were determined and Ag and Al ICPMS signals are lower than the LOD. These results prove the efficiency of such process to eliminate metallic NPs in waste waters.

The characterization of soils from wine culture (Fig.4B) indicates strong interaction of aluminium (green) with natural colloids between 50 and 400 nm in soil D1 and 50 to 150 nm in soil DT. Results indicates that interactions are different according to origin of the soil and the metallic element investigated. All these experiments illustrate the efficiency of such analytical approaches to understand the behaviour of NPs and colloids in the environment.

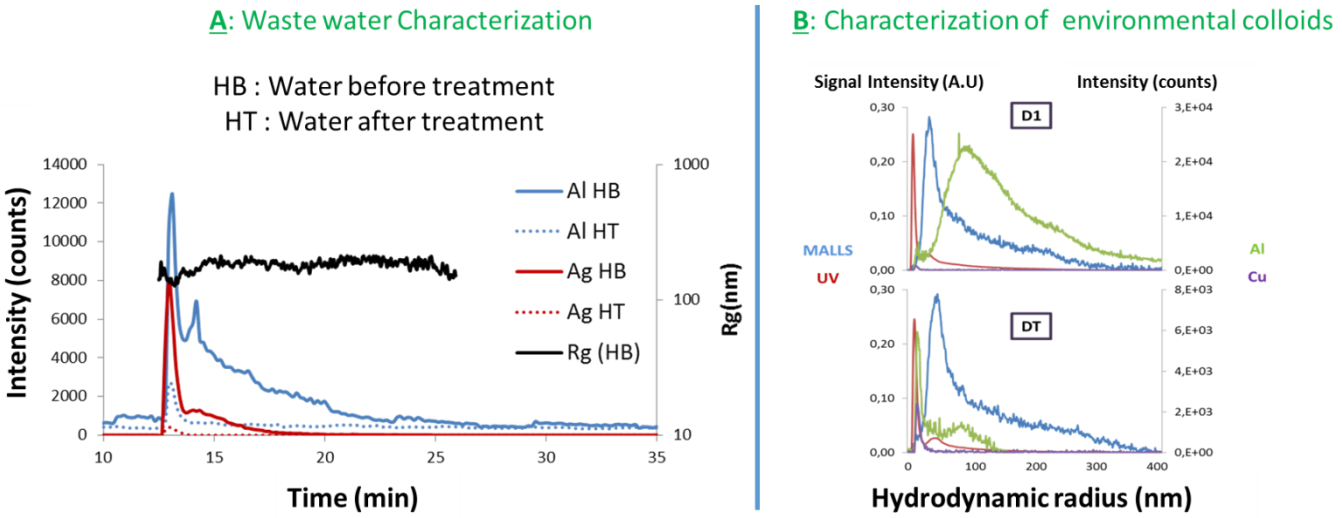

Fig. 4: Characterization of metallic NPs in wastewater (A) and interactions of metallic pollutants with natural colloids (B) by A4F-MALLS-ICPMS.

6. Characterization of Medicinal Samples

NMs are more and more employed in medicine and more precisely in pharmaceuticals for drug delivery. DLS was used to estimate the size and the polidispersity of such nanopharmaceuticals (blank NPs and NPs containing the active ingredient API) elaborated in the H2020 Project NANOPILOT. Results are reported in table 3.

Table 3: Granulometric characterization of nanopharmaceuticals.

Sample	Size (nm)	Polidispersity index
Blank NPs	57 ± 1	0.33 ± 0.01
NPs + API	75 ± 6	0.39 ± 0.04

Results indicates that full NPs are bigger than blank one and that the polidispersity is similar for both samples. The same samples were analysed by A4F-MALLS to estimate the encapsulation efficiency of the API (Fig. 5).

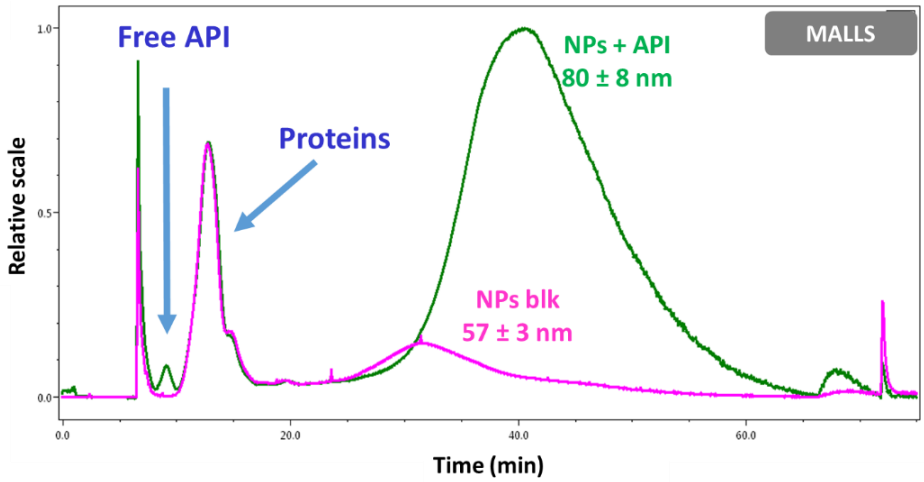

Fig. 5: Characterization of blank (pink) and full (green) nanopharmaceuticals by A4F-MALLS.

Fractogramms indicates that size determined by A4F-MALLS are in accordance with ones obtained by DLS. Moreover, the main components of the formulation (Free API, proteins, NPs) can be identified and quantified by this technique). The encapsulation efficiency (amount of API trapped in NPs) was estimated to 16 %. This results are useful to evaluate the influence of various production parameters and its optimization. Studies are still in progress to validate this analytical methodology.

7. Conclusions

This work shows the complementarity of several techniques for the characterization of NPs in consumer products. DLS appears as the most common tool for the size determination of NMs and is used routinely for this kind of application. However, DLS is not suitable to determine simultaneously the composition of NPs. Even if the A4F-MALLS-ICPMS is the most specific tool and allows the quantification of metallic NPs with various chemical nature in a single run analysis, this technique is time consuming and complex to use. On a contrary, the SP-ICPMS is a very promising tool to obtain both granulometric and composition information in a faster and easier way. SP-ICPMS will be used routinely in quality control lab in future years to perform NMs characterization.

The same analytical approaches have also been used to characterize nanopharmaceuticals. In this context, A4F-MALLS is a relevant tool for evaluating manufacturing yields by identifying and quantifying the different constituents of the formulation. The work also enabled the development of analytical methodologies for raw materials and finished products meeting GLP requirements.

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