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Towards routine analysis of TiO_2 (nano-)particle size in consumer products: Evaluation of potential techniques

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ABSTRACT

In this work, the potential of several techniques commonly used in research studies for TiO_2 nanoparticles' (NPs) characterization was evaluated for the implementation in routine analysis. Namely, Dynamic Light Scattering (DLS), single-particle mode Inductively Coupled Plasma Mass Spectrometry (spICP-MS) and Asymmetrical Flow Field-Flow Fractionation coupled to Multi Angle Light Scattering and (single particle) Inductively-Coupled Plasma Mass Spectrometry (AF4-MALS-(sp)ICP-MS) were assessed for this purpose. Electron Microscopy was also used to confirm the validity of results and to obtain information about the shape of the particles. Each instrument was optimized according to routine analysis criteria using reference materials for instrument performance and quality assurance. Then, the methodology was applied to two types of samples of consumer products (sunscreens and sugar-coated chocolate candies), where particle size and concentration obtained by each technique were discussed. Results indicated that TiO_2 particles were found in both samples. AF4-MALS-ICP-MS and spICP-MS were powerful tools to characterize TiO_2 NPs in real samples, with spICP-MS being more adapted to routine analysis. DLS and electron microscopy provided comparable results for the particle size. The studied sunscreen complied with the European regulation (No 1223/2009) in relation with NPs because the particle size found for TiO_2 was in the range 80–110 nm and the reference to "nano" required is present in the label of the product. Sugar-coated chocolate candies may contain NPs according to DLS and AF4-MALS-ICP-MS results, but particles larger than 100 nm were found by spICP-MS.

1. Introduction

In the last few years, the use of nanoparticles (NPs) has increased in different daily products, such as cosmetics or food. Due to their possible toxicological effects on the human body and other living organisms, as well as their potential impact on the environment, legislation has started to be implemented at a European level. Indeed, the European Commission (EC) has already recommended the definition of a nanomaterial (NMs) as a material with 50% or more of the particles in number-based size distribution in the range 1–100 nm [1].

According to the regulations EC No 1223/2009 [2] and No 1169/2011 [3], the presence of NPs in consumer products requires the addition of the word "nano" between brackets in the list of ingredients in cosmetics and food products, respectively. For cosmetics, only TiO_2 and ZnO in nanoform are allowed as inorganic ultraviolet (UV) filters to protect the skin against UV radiation but their presence needs to be mentioned in the label [2,4]. Concerning food products, the current European regulation still remains unclear, particularly for food additives containing NPs. The presence of calcium carbonate (E170), vegetable carbon (E170) and titanium dioxide (E171) is allowed in nanoforms, but the particle size should be included in the specifications of the product [3]. In the case of TiO_2, the European Food Safety Authority (EFSA) recently concluded that its use as a food additive does not raise genotoxic effects and more reliable data are still needed to show its impact on the reproductive functions [5]. Other additives such as iron oxides and hydroxides (E172), silver (E174) and gold (E175), silicon dioxide (E551), calcium silicate (E552), magnesium silicate (E553a) and talc (hydrated magnesium silicate) (E553b) will be evaluated in the next few years. In relation to the properties describing NPs, the EC only focuses on the size (mean diameter) and number-based size distribution. Other properties necessary for evaluation in the future for consumer products will be particle shape, chemical composition, charge, surface area, stability, etc. These parameters can be related to their behavior, interaction with biological systems, fate, effects and

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degradation (by oxidation reactions) [6].

Several works have been published describing sample preparation before the analysis of TiO_2 NPs in cosmetics [7–20] and foods [9,21–25]. For cosmetics, direct analysis of the sample or its dispersion in ethanol before deposition onto a support [7,14,15], resin embedding [10] or only dispersion in water/ethanol [12,14] were used. In most studies, a degreasing step is applied using organic solvent (hexane, methanol and chloroform) before or after dispersion in water/methanol with the aid of a probe or bath sonication [8,9,11,19,20]. This step is often necessary when NPs' size separation is performed before the detection. It is also possible to only disperse the cream sample in water and NPs are analyzed after dilution [11,17,18]. Different studies are also available for the analysis of TiO_2 NPs in commercial food products such as cakes, candies, chewing gum, wheat flour, sugar glass, coffee cream, confectionary products, cookies, semolina and pasta [9,21–25]. Sample preparation procedures involve several steps in order to release NPs from the sample, such as matrix destruction, extraction and/or purification. For example, the addition of H_2O_2 [21] or H_2O_2/HNO_3 [22] and heating enable the destruction of the organic matrix of the food sample and the separation of TiO_2 NPs from the larger particles. In other samples, such as chewing gum, extraction of TiO_2 NPs from the matrix consists of water extraction, centrifugation and washing steps [23]. Enzymatic digestion has been applied to seed samples by adding α-amylase for carbohydrate degradation after protein destruction with a Tris buffer [25].

A wide range of techniques is available for NPs' characterization, including microscopy, spectroscopy and size separation techniques. Traditionally, microscopy-based techniques (Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM)) provide information about the size and shape of NPs, and also on the elemental composition after coupling with Energy Dispersive X-Ray Fluorescence Spectrometry (EDXRF). Light Scattering methods, including Dynamic Light Scattering (DLS) based on the diffusion rate of particles and Multi Angle Light Scattering (MALS) based on the angular dependence of light scattering are also available. Information about the hydrodynamic diameter (D_h) and the gyration diameter (D_g) of NPs can be obtained. Among these techniques, DLS is commonly used due to its simple operation and rapid analysis, but an overestimation of particle size may result when small and large particles and their aggregates co-exist in a sample [26]. MALS is often used as a detector after size fractionation of particles by Flow Field-Flow Fractionation techniques (FFF) [27]. These techniques can also be hyphenated to ICP-MS to obtain information about the NPs composition. More recently, Single-Particle Inductively Coupled Plasma Mass Spectrometry (spICP-MS) has been used to quantify the particle concentration and size, assuming spherical particles, by means of the frequency of analytical signals and the relationship between mass and density, respectively [28]. This technique is gaining more and more importance, especially on a routine basis due to its specificity and rapidity [29,30].

In research studies of NPs analysis, the procedures are very time consuming, instruments are often expensive and data treatment is intensive. However, in future years, qualitative characterization of NPs will be part of quality control processes both at industry and institution levels in order to provide a rapid decision of the presence/absence of NPs. Therefore, the current research methodologies need to be adapted to fast and relatively cheap studies considering the perspective towards routine analysis. In fact, the analysis of NPs will require fast screening tests. Thus, the future methodologies will encompass fast, robust, reliable, automated, properly validated procedures, easy-to-use, integrated analytical techniques, etc., as in other routine analysis [31].

In this work, several potential techniques have been assessed on a routine basis for the analysis of TiO_2 NPs. These techniques were commonly used in several research papers. However, an evaluation of their characteristics and requirements have not been yet performed in terms of their implementation in routine analytical laboratories. Some steps such as, the instrument performance, the utilization of relevant tests to ensure the regular working of the instrument, the use of reference materials to check accuracy of the whole analytical process and the study of the repeatability were considered in this work. In order to perform this evaluation and to select the potential techniques more suitable for routine analysis of NPs, the presence of TiO_2 NPs was studied in two consumer products (sunscreen and sugar-coated chocolate candies) using several techniques. DLS and spICP-MS were selected due to their expected easy implementation making them adapted for this objective. AF4-MALS-ICP-MS was selected since it is considered as a reference technique for NPs characterization even if the measurement takes a long time. Microscopy techniques (SEM and TEM) were also used as complementary techniques to confirm the results.

2. Experimental

2.1. Instrumentation

Dynamic Light Scattering (DLS) analyses were performed using a Cordouan Technology VASCO-2 particle size analyzer (Cordouan Technology, Pessac, France) in combination with the software nanoQ v2.

The NexION 300X ICP-MS fitted with a Meinhard nebulizer and a cyclonic spray chamber was used for the analysis of TiO_2 NPs' nanodispersions (without previous separation) according to two modes of operation: a standard one (without reaction gas supply into the cell), and with the collision cell filled with He (Perkin-Elmer, Shelton, CT, USA) for the determination of total Ti content. It was also employed operating in the single particle mode using the Syngistix™ Nano Application Module 1.0 (Perkin-Elmer, Shelton, CT, USA) for nanodispersions analysis.

The Asymmetric Flow Field-Flow Fractionation (AF4) system employed for NPs size separation consists of an Eclipse® (Wyatt Technology, Santa Barbara, CA, USA) using a long channel (275 mm length) and a regenerated cellulose (RC) membrane with a 10 kDa cut-off (Wyatt Technology). The detection chain contains a UV–Vis detector operating at 240 nm (VWD, 1200 series, Agilent Technologies) and a Multi Angle Light Scattering (MALS) detector of 18 angles operating at 663.9 nm (DAWN HELEOS II, Wyatt Technology). Data from the different detectors were collected and treated with the Astra 6.0.2.9 software (Wyatt Technology). The chemical composition of particles eluted from AF4-MALS (particle fractionation) was obtained by coupling AF4-MALS to the Agilent 7500cx ICP-MS (Agilent Technology Ltd., Japan) fitted with a Micromist nebulizer and a Scott spray chamber. The ICP-MS was used according to two modes of operation: a standard one (without reaction gas supply into the cell), and with the cell filled with He. Additionally, the spICP-MS Agilent 7900 ICP-MS (Agilent Technology Ltd., Japan) was used for the analysis of particles eluted from AF4-MALS particle fractionation. This instrument was fitted with a Micromist nebulizer, a Scott spray chamber and with the Single Nanoparticle Application Module for ICP-MS Mass Hunter software (G5714A). These analyses were performed using the collision cell filled with He.

Micrographs were taken with a LVEM5 (Delong Instrument, Brno, Czech Republic), a low-voltage table-sized electron microscope operating both in transmission mode (TEM) and scanning mode (SEM) without requirement of dark room or cooling water. This instrument used a Schottky field emission gun at a nominal acceleration voltage of 5 kV and the SEM mode was obtained using back scattered electrons detectors (BSE detection). Image J software was employed for microscopy data treatment [32].

A hot block digestion system (SCP Sciences, Courtaboeuf, France) and an ultrasonic bath model 5510 (Branson, Switzerland) were used for sample preparation prior to total element determination and extraction of TiO_2 NPs (500 W, 20% power), respectively. Separation of the solid sample from the liquid fraction after defatting with hexane was performed with a model Rotofix 32A centrifuge (Hettich, Marne la