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Inverse supercritical fluid extraction as a sample preparation method for the analysis of the nanoparticle content in sunscreen agents

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ABSTRACT

We demonstrate the use of inverse supercritical carbon dioxide (scCO$_2$) extraction as a novel method of sample preparation for the analysis of complex nanoparticle-containing samples, in our case a model sunscreen agent with titanium dioxide nanoparticles. The sample was prepared for analysis in a simplified process using a lab scale supercritical fluid extraction system. The residual material was easily dispersed in an aqueous solution and analyzed by Asymmetrical Flow Field-Flow Fractionation (AF4) hyphenated with UV- and Multi-Angle Light Scattering detection. The obtained results allowed an unambiguous determination of the presence of nanoparticles within the sample, with almost no background from the matrix itself, and showed that the size distribution of the nanoparticles is essentially maintained. These results are especially relevant in view of recently introduced regulatory requirements concerning the labeling of nanoparticle-containing products. The novel sample preparation method is potentially applicable to commercial sunscreens or other emulsion-based cosmetic products and has important ecological advantages over currently used sample preparation techniques involving organic solvents.

1. Introduction

Today, a growing number of consumer products make use of the unique physical and chemical properties of nanomaterials. As the number of such products increases, the ability to thoroughly characterize their properties and functionality becomes critical. In particular, the recent regulatory efforts concerning the labeling of nanoparticle-containing consumer products, e.g., the EU regulations on cosmetics [1] and food [2], call for the development of simple and robust sample preparation protocols enabling a reliable detection and quantification of nanoparticulate ingredients in complex matrices [3–5]. This problem is especially challenging in case of emulsion-based consumer products such as cosmetics, which often

consist of complex multicomponent matrices [6]. Commercially available sunscreen formulations for example usually contain more than 20 ingredients with different functions and physicochemical properties. Moreover, such viscous samples cannot be directly injected into an analytical system, and need to be liquefied prior to analysis. Commonly applied sample preparation protocols include chemical treatments using organic solvents [7–11]. Such complex processes are both time-consuming and have a considerable environmental impact due to the extensive use of organic solvents of which many are ecologically harmful [12–14]. The generalization and simplification of sample preparation workflows, as well as the reduced usage of organic solvents, is therefore likely to have a significant impact on the utility of analyses of nanoparticle-containing samples.

To this end, we herein report the use of inverse supercritical fluid extraction (inverse SFE) [14–18], a more ecological and simpler sample preparation method based on the use of supercritical fluids. For our application we selected supercritical carbon dioxide

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($scCO_2$), as many of the chemical excipients found in large numbers in emulsion-based cosmetic products are of a fatty and non-polar nature and therefore exhibit a high solubility in CO_2. Furthermore, $scCO_2$ is chemically inert [18], nontoxic, nonflammable [19], and it is well-known for its application in SFE processes, where it is commonly used to extract small and/or non-polar molecules from natural materials under very mild conditions [20–23]. Besides the extraction of essential oils from herbs and spices [24,25], the most prominent application of SFE is the removal of caffeine from coffee beans [26,27]. The process has also been employed for the extraction and analysis of antioxidants, preservatives and sunscreen agents in cosmetics [28,29]. In these applications, however, the SFE is used to dissolve and extract the analyte from the matrix. In this work, inverse SFE is used as a sample treatment to simplify the matrix by removing unwanted components, thus keeping the target nanomaterials in the residual sample. Inverse SFE has also been studied for over twenty years. To date, it has primarily been used for the isolation of non-polar pharmaceutical formulations from polar analytes [14,16,17] and not for the pre-treatment of nanoparticle-containing samples. The minimal surface tension, low viscosities and gas-like diffusivities of $scCO_2$ allow for thorough sample penetration whilst maintaining the structure of the residual material [14]. Once the sample treatment is completed, the CO_2 is simply removed by lowering the pressure to below the critical threshold and returning to ambient conditions. The remaining material consists of the polar components (thickening agents) along with the nanoparticles that accordingly, can easily be rewet and subsequent dispersed in a direct manner. To demonstrate the potential utility of such a sample preparation process in the analysis of nanoparticle containing sunscreens, we integrated the $scCO_2$ treatment with Asymmetrical Flow Field-Flow Fractionation (AF4) hyphenated with UV and Multi-Angle Light Scattering (MALS) detection, and tested the method with a model sunscreen sample. The obtained findings were verified by Scanning Transmission Electron Microscopy (STEM) and Energy-Dispersive X-ray (EDX) analysis. Although the method is demonstrated using a model sunscreen matrix, we expect it to be applicable to commercial sunscreens or other emulsion-based cosmetic products, which include fatty additives with a high solubility in $scCO_2$.

2. Materials and methods

2.1. Chemicals

2.1.1. Titanium dioxide nanoparticle samples

A titanium dioxide (TiO_2)—nanoparticle dispersion, AERODISP® W 740 X (40% w/w, EVONIK Industries, Hanau, Germany) was diluted with ultrapure water (MilliQ, Billerica, USA). This was followed by addition of 0.2% (v/v) NovaChem (Postnova Analytics GmbH, Landsberg, Germany) to yield a final particle concentration of 0.2 mg/mL. NovaChem is a mixture of non-ionic and ionic detergents that helps to prevent particle agglomeration. Prior to analysis, the sample was placed in an ultrasonic bath (Sonorex Digital 10 P, Bandelin, Berlin, Germany) and sonicated at maximum power (320 W, 35 kHz) for 30 min to further reduce eventual particle agglomerates.

2.1.2. Model sunscreens

The novel sample preparation method was tested on two complex sunscreen model samples, one with and one without nanoparticles. The creams were produced separately, although both consisted of the following excipients: Avicel® PC611 (FMC Biopolymer, Brussels, Belgium), glycerin (Thai Oleochemicals Ltd., Bangkok, Thailand), KELTROL® T (Bronson & Jacobs Pty Ltd., Villawood, NSW, Australia), potassium sorbate (APAC Chemical Corp.,

Arcadia, CA, USA) and ultrapure water (MilliQ, Billerica, USA) in the water phase and Antaron™ V216 (ISP Ltd., Tadworth, UK), Arlacel™ 165 (JEEN, Fairfield, NJ, USA), capric/caprilic triglycerine (HENKEL KGaA, Düsseldorf, Germany), cyclomethiocone (Momentive Amer Ind., Waterford, NY, USA), Emulsiphos® (Symrise, Holzminden, Germany), isostearyl isostearate (UNIQEMA Corp., New Castle, DE, USA), octyl palmitate (Eigenmann & Veronelli, Milano, Italy), stearyl alcohol (Temix International, Milano, Italy), TEGO® Care 450 (EVONIK Industries, Essen, Germany), Finsolv® TN (Innospec, Englewood, CO, USA) and tocopheryl acetate (BASF SE, Ludwigshafen, Germany) in the oil phase. Both phases were mixed independently for 15 min using a L4R Mixer (Silverson Machines Inc., East Longmeadow, MA, USA) at 6000 rpm before they were homogenized together for another 15 min using again the L4R at 6000 rpm. In the last step, Dow Corning® 1503 (Dow Corning Corporation, Midland, MI, USA), Euxyl® PE 9010 (Schülke & Mayr GmbH, Norderstedt, Germany) and 12.5% w/w of a AERODISP® W 740 X TiO_2 nanoparticle dispersion (40% w/w, EVONIK Industries, Essen, Germany) were added to one cream, resulting in a TiO_2 particle concentration of 5.0% w/w, a concentration typically found in commercial sunscreens [9,30]. In the blank cream, the AERODISP® nanoparticles were replaced with corresponding amounts of ultrapure water (MilliQ, Billerica, USA). Both creams were homogenized again for 5 min at 4000 rpm, before they were filled into tubes and stored at room temperature.

2.2. Sample treatment

2.2.1. Extraction equipment

Extraction was performed using a lab scale supercritical fluid extraction system (Lab SFE 100 mL, Separex, Champigneulles, France). The system was equipped with a high-pressure CO_2 pump, a pressure/flow regulating system, and a horizontally mounted 100 mL extraction vessel housed in a thermostated oven.

2.2.2. Supercritical CO_2 sample treatment

The model sunscreen (Fig. 1A) was placed on a Teflon cartridge surrounded by a stainless steel holder (Fig. 1B). The Teflon part contained a small recess resulting in a cavity with dimensions of $60 \times 10 \times 0.2$ mm. To ensure that a reproducible sample volume was assayed, excess sunscreen was removed each time using a spatula. The Teflon cartridge was then removed from its holder (Fig. 1C) and placed in the extraction vessel (100 mL, Separex, Champigneulles, France). The sample was then subjected to a constant $scCO_2$ flow of 100 g/min for 30 min at 40 °C and 131 bars. The optimum parameters were selected by performing a series of measurements with varying processing times, temperatures and pressures. Less aggressive conditions (such as shorter processing times, lower temperatures and lower pressures) resulted in reduced extraction efficiencies of the fatty components, leading to reduced solubility in water, whilst harsher conditions led to more extensive particle aggregation and reduced reproducibility. The treated sample (Fig. 1D) was then removed from the cartridge (Fig. 1E) and dissolved in ultrapure water (MilliQ, Billerica, USA), to which 0.2% (v/v) NovaChem (Postnova Analytics GmbH, Landsberg am Lech, Germany) was added until a concentration of less than 0.2 mg TiO_2 (related to a recovery of 100%) per mL of solvent (Fig. 1F). Sample dilution is necessary to prevent overloading effects, which cause peak shifts and further advanced particle aggregation. Extractions for both creams (with and without nanoparticles) were performed in triplicate.