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# Sulfonated nanocellulose for the efficient dispersive micro solid-phase extraction and determination of silver nanoparticles in food products

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ABSTRACT

This paper reports a simple approach to Analytical Nanoscience and Nanotechnology (AN&N) that integrates the nanotool, sulfonated nanocellulose (s-NC), and nanoanalyte, silver nanoparticles (AgNPs), in the same analytical process by using an efficient, environmentally friendly dispersive micro solid-phase extraction (D-μSPE) capillary electrophoresis (CE) method with s-NC as sorbent material. Introducing negatively charged sulfate groups onto the surface of cellulose enhances its surface chemistry and enables the extraction and preconcentration of AgNPs of variable diameter (10, 20 and 60 nm) and shell composition (citrate and polyvinylpyrrolidone coatings) from complex matrices into a cationic surfactant. In this way, AgNPs of diverse nature were successfully extracted onto the s-NC sorbent and then desorbed into an aqueous solution containing thiotic acid (TA) prior to CE without the need for any labor-intensive cleanup. The ensuing eco-friendly D-μSPE method exhibited a linear response to AgNPs with a limit of detection (LOD) of 20 μg/L. Its ability to specifically recognize AgNPs of different sizes was checked in orange juice and mussels, which afforded recoveries of 70.9–108.4%. The repeatability of the method at the limit of quantitation (LOQ) level was 5.6%. Based on the results, sulfonated nanocellulose provides an efficient, cost-effective analytical nanotool for the extraction of AgNPs from food products.



## 1. Introduction

In recent years, the fascinating chemical and physical properties of metallic nanoparticles have furthered their use in biology [1], catalysis [2], food technology [3,4] and even analytical chemistry [5]. AgNPs in particular have attracted much attention by virtue of their unique antimicrobial features [6] in relation to other metallic nanoparticles [7]. The fact that silver in the form of AgNPs possesses marked antibacterial properties – especially those with larger specific surface area due to their smaller size- has led researchers to explore its potential for use in common products such as cosmetics, coatings for medical tools, wound dressings, soaps, paints, laundry additives, water purification devices, food supplements or even packaging materials [8]. For example, the use of AgNPs in food and beverage storage containers has been found to enhance the quality and safety of packaged products (e.g., orange juice [9]) through their bactericidal effects, citrate capped NPs (cit-AgNPs) being the most widely used silver nanoparticles for this purpose. Unfortunately, because size matters for toxic effects, the promise of NPs has a cost. Although the toxicity mechanism of metallic NPs is still unclear, silver NPs were found to be less toxic than ionic silver, but more than micron-sized AgNPs probably related to the easier cellular internalization but also for the released of dissolved silver ions [10]. Similar studies prove a higher toxicity for those citrate AgNPs if compared with polymeric coated AgNPs in accordance to their stability [10,11]. Thus, AgNPs are expected to reach aquatic ecosystems in the near future as a result of their unselective discharge into the environment and possibly have toxic effects on aquatic organisms such as mussels [12]. The anticipated widespread exposure to AgNPs has raised concerns among the public about the safety of their uses [13,14]; furthermore, the scientific community is increasingly calling for innovative and more environmentally friendly methods to extract and determine the nanoparticles in different scenarios.

Regarding non-toxic nanoparticles, NC, which can be obtained from renewable resources, is being increasingly considered an effective choice for microextraction techniques by virtue of its excellent sorbent properties. In fact, NC which is a nanomaterial consisting of nanofibers bearing hydroxyl groups, can be highly useful for analytical applications thanks to the ease with which it can be surface modified. However, the hydrophilicity of cellulose surfaces favors the formation of strong intermolecular and intramolecular hydrogen bonds, and causes cellulose to aggregate and become inefficient as a sorbent material as a result. NC obtained by sulfuric

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acid hydrolysis [15,16] provides stable colloid suspensions containing isolated cellulose whiskers thanks to the negatively charged surface resulting from esterification of hydroxyl groups by sulfate ions [17]. The ability to produce well-individualized nanofiber dispersions in ionic liquids has enabled the obtainment of NC based composites as excellent extraction platforms for heterocyclic amine [18]. Effective NC platforms can also be easily obtained by surface modification with a variety of molecules for efficient extraction of contaminants from both simple [19–22] and complex matrices [23,24]. Self-assembly of danofloxacin with cyclodextrin-modified NC packed in a minicolumn for solid-phase microextraction (SPME) to obtain a suitable encapsulation and thus, the resulting inclusion complex is an excellent example [24].

In line with the current trend in Analytical Nanoscience and Nanotechnology, whereby NPs are simultaneously involved as analytical tools and analytes in analytical process [14,25,26], in this work we developed a simple sustainable method for the extraction of AgNPs using a cationic surfactant in combination with s-NC as dispersed extractant. The ease with which NC can thus be prepared and its excellent sorbent capabilities toward AgNPs in D-μSPE are demonstrated here. The main variables influencing extraction efficiency and elution were optimized, and performance of the s-NC as extracting material was assessed. The proposed system was applied to such complex matrices as those of food products (specifically, orange juice and mussels). Complete preconcentration of the analyte was accomplished by using only 5 mg of functionalized nanomaterial and the separation of the eluted AgNPs was achieved by CE analysis for differing between sizes and shells [27,28].

## 2. Experimental

### 2.1. Reagents and materials

Avicel PH-101 microcrystalline cellulose (50 μm particle size), sulfuric acid (95–98%), sodium dodecyl sulfate (SDS, 98%), 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS, ≥98%) and AgNPs (dispersions of 10, 20 and 60 nm particles) were supplied by Sigma-Aldrich (Madrid, Spain). Hexadecyltrimethylammonium chloride (CTAC, assay 25% in water) and TA (≥98%) were purchased from Fluka (Buchs, Switzerland). All reagents were used as received (i.e., without further purification).

Ultrapure water purified by passage through a Millipore system was used throughout. Orange juice and fresh mussels used as real samples were purchased from a local supermarket.

### 2.2. Instrumentation

UV–vis spectra were obtained by using a tungsten/halogen lamp, and the monochromator and photonic detector of a PTI QuantaMaster™ Spectrofluorometer (Photon Technology International). Felix 32 software was used to acquire and process all absorption data. All optical measurements were made at room temperature, using micro quartz cuvettes of 10 mm light path.

NP images were obtained by using an Agilent AFM 5500 microscope equipped with NCL-W Point probe-Silicon SPM-cantilevers. For AFM images, a 10 μL drop of 0.1% nanocellulose was allowed to stand on the mica for 1 min, rinsed off with water and dried. If needed, the mica was brought into contact with a solution of AgNPs for 1 min, rinsed off with water and dried. The mica surfaces were then attached to an AFM specimen disk and analyzed in the tapping mode.

Infrared spectra were recorded at room temperature on a Tensor 27 FT-MIR spectrophotometer equipped with a Hyperion 2000 microscope to characterize NC surfaces. All samples were prepared as KBr pellets and analyzed over the wavenumber range 4000–400 $cm^{-1}$.

Thermogravimetric measurements were made on a Q 50 TGA instrument. The temperature was raised from 30 to 700 °C at 10 °C/min. These tests were carried out under a nitrogen atmosphere (20 mL/min) in order to prevent thermoxidative degradation.

A P/ACE MDQ Capillary Electrophoresis System from Beckman (Palo Alto, CA, USA) equipped with a diode array detector (DAD) was also used.

Samples were dried by using Hetosicc Model 1481N S1L freeze-dryer under vacuum (~0.1 bar) for 72 h.

### 2.3. Nanomaterial preparation

#### 2.3.1. Preparation of sulfonated nanocellulose from microcrystalline cellulose

s-NC was prepared according to Hun Kim et al. [29], using microcrystalline cellulose (MCC) as precursor. For this purpose, a volume of 50 mL of sulfuric acid was added dropwise to a well-stirred slurry containing 10 g of MCC in 100 mL of deionized water at 0 °C, followed by warming to 45 °C for 2 h and then cooling to room temperature with 500 mL of water to obtain a white dispersion. The dispersion was then centrifuged at 3000 rpm for 15 min and the solid residue purified by repeated resuspension of the solid in distilled water with ultrasound-assisted mixing and centrifuging, and, finally, drying at 60 °C for 24 h (yield 70%). The material, which was designated s-NC, was characterized by using different techniques such as thermogravimetric analysis and infrared spectroscopy.

### 2.4. Protocols

#### 2.4.1. Sample extraction

An amount of 5 mg of s-NC was placed in an Eppendorf tube. Then, 5 mL of the standard/sample solution containing cit-AgNPs or polyvinylpyrrolidone coated NPs (PVP–AgNPs) was mixed with 50 μL of 200 mM CTAC under vigorous stirring on a vortex for 10 s, followed by sonication for 10 min. AgNPs were immediately attached to sulfonated nanocellulose as confirmed by centrifugation at a speed where CTAC-coated AgNPs remained well dispersed. Then, the supernatant was discarded and the residue washed once with 300 μL of distilled water, sonicated and centrifuged. The analyte was easily eluted by using 200 μL of 75 mM TA for CE analysis with monitoring of the typical localized surface plasmon resonance (LSPR) wavelength of AgNPs.

#### 2.4.2. Capillary electrophoresis

Capillary electrophoresis analyses were performed by using a fused silica capillary (Beckman Coulter) of 75 μm of inner diameter, 70.2 cm total length and 40 cm effective separation length. Photometric measurements were made at 400, 414 and 432 nm for 10, 20 and 60 nm sized NPs, respectively. The initial background electrolyte (BGE) was a mixture of 40 mM SDS and 10 mM CAPS adjusted to pH 10 with NaOH. Samples were injected by applying 0.5 psi for 50 s, each run taking 15 min. The applied voltage was 15 kV and the working temperature was set to 25 °C. All solutions were passed through a 0.45 μm membrane filter before use. Initially, the capillary was sequentially conditioned with 1 M HCl (5 min), 0.1 M NaOH (5 min) and ultrapure water (10 min). Between runs, the capillary was sequentially washed with 1 M HCl (1 min), 0.1 M NaOH (5 min), ultrapure water (7 min) and background electrolyte (7 min). At the end of the day, the capillary was washed with 0.1 M NaOH (5 min) and ultrapure water (5 min) and afterwards the capillary was stored empty overnight. A precision study of the