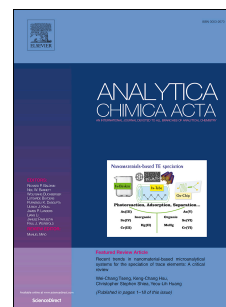


# Accepted Manuscript

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PII: S0003-2670(18)30357-X

DOI: [10.1016/j.aca.2018.02.075](https://doi.org/10.1016/j.aca.2018.02.075)

Reference: ACA 235791

To appear in: *Analytica Chimica Acta*

Received Date: 30 November 2017

Revised Date: 26 February 2018

Accepted Date: 28 February 2018

Please cite this article as: M.V. Taboada-López, S. Iglesias-López, P. Herbello-Hermelo, P. Bermejo-Barrera, A. Moreda-Piñeiro, Ultrasound assisted enzymatic hydrolysis for isolating titanium dioxide nanoparticles from bivalve mollusk before sp-ICP-MS, *Analytica Chimica Acta* (2018), doi: 10.1016/j.aca.2018.02.075.

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# Ultrasound assisted enzymatic hydrolysis for isolating titanium dioxide nanoparticles from bivalve mollusk before sp-ICP-MS

María Vanesa Taboada-López, Sara Iglesias-López, Paloma Herbello-Hermelo, Pilar Bermejo-Barrera, Antonio Moreda-Piñeiro<sup>1</sup>

Trace Element, Spectroscopy and Speciation Group (GETEE), Health Research Institute of Santiago de Compostela (IDIS). Department of Analytical Chemistry, Nutrition and Bromatology. Faculty of Chemistry. Universidade de Santiago de Compostela. Avenida das Ciencias, s/n. 15782 – Santiago de Compostela. Spain.

## Abstract

Applicability of single-particle inductively coupled plasma mass spectrometry (sp-ICP-MS) using dwell times equal to or shorter than 100  $\mu$ s has been tested for assessing titanium dioxide nanoparticles (TiO<sub>2</sub> NPs) in bivalve mollusks. TiO<sub>2</sub> NPs isolation from fresh mollusk tissues was achieved by ultrasound assisted enzymatic hydrolysis procedure using a pancreatin/lipase mixture. Optimum extraction conditions imply ultrasonication (60% amplitude) for 10 min, and 7.5 mL of a solution containing 3.0 g L<sup>-1</sup> of pancreatin and lipase (pH 7.4). The developed method was found to be repeatable (repeatability of 17% for the over-all procedure, TiO<sub>2</sub> NPs concentration of  $5.33 \times 10^7 \pm 8.89 \times 10^6$ , n=11), showing a limit of detection of  $5.28 \times 10^6$  NPs g<sup>-1</sup>, and a limit of detection in size of 24.4-30.4 nm, based on the 3 $\sigma$  criteria, and on the 3 $\sigma$ /5  $\sigma$  criteria, respectively. The analytical recovery within the 90-99% range (use of TiO<sub>2</sub> NPs standards of 50 nm at 7 and 14  $\mu$ g L<sup>-1</sup> as Ti). Several bivalve mollusks (clams, cockles, mussels, razor clams, oysters and variegated scallops) were analyzed for total titanium (ICP-MS after microwave assisted acid digestion), and for TiO<sub>2</sub> NPs by the proposed method. TiO<sub>2</sub> NPs concentrations were within the  $2.36 \times 10^7$ - $1.25 \times 10^8$  NPs g<sup>-1</sup> range, and the most frequent

<sup>1</sup>Corresponding author: E-mail address: antonio.moreda@usc.es

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