doi:10.1017/S1431927616009909
Microsc. Microanal. 22 (Suppl 3), 2016

Electron Microscopy of Spirulina (*Arthrospira spp*) Nanoparticles Obtained by Means of Mechanical Milling

H. A. Calderon[1], K. Bustillo[2], E.E. Neri-Torres[3], J. J. Chanona-Pérez[3], N. Torres-Figueredo[4], G. A. Chamorro-Ceballos[5], G. H. Calderón-Domínguez[3].

[1.] Depto. Física, ESFM-IPN, Ciudad de Mexico, Mexico.
[2.] NCEM-LBNL, Berkeley CA, USA.
[3.] Depto. Ing. Bioquímica, ENCB-IPN Ciudad de México, Mexico.
[4.] CICATA Legaria, IPN, Ciudad de México, Mexico and CEADEN, La Habana, Cuba.
[5.] Depto. Toxicología, ENCB-IPN Ciudad de México, Mexico.

Spirulina is a cyanobacteria (Arthospira spp) that grows naturally, it has various applications. Nowadays, it is cultivated for mass production under controlled conditions. It has been extensively studied for its nutraceutical value and it has been used as a food supplement. Mainly, Spirulina is processed by using spray drying and subsequent drying in trays, in order to obtain encapsulated powders with maltodextrin and other wall materials [1]. The objective of this work is to synthesize Spirulina (Arthrospira spp) nanoparticles (SNPs) by using high impact mechanical milling and characterize them through electron microscopy and spectroscopy techniques. Additionally the number mean size (NMS) has been correlated to powder flow properties and specific surface area [2]. The milling products are analyzed after various processing times (1-4 h). The smallest particles are obtained after three hours of milling, with a NMS of 55.6 ± 3.6 nm, with 95% of the particles being smaller than 100 nm.

The SNPs have been imaged by STEM and their chemical composition has been measured by energy dispersive X ray spectroscopy (EDS), an example is given in Figures 1 and 2. Different chemical elements are found such as Ca, Fe, K, Mg, Na and Zn with a rather heterogeneous spatial distribution but highlighting specific zones.

Transmission electron microscopy shows that the SNPs have amorphous regions linked to biomacromolecules of Spirulina. There are also some crystalline regions that can be related to wall materials (maltodextrin) contained in the spray dryer Spirulina powder (SDSP). Their lattice planes are estimated to be 0.29 ± 0.04nm. Figures 3a,c and d show an experimental images in TEM under a low dose to preserve the sample by reducing beam sample interaction. The dose rate in use is 35 $e^{-}/Å^{2}s$. Experimental images are rather dark in such conditions thus an exit wave reconstruction procedure is necessary to determine amplitude and phase images with the help of 40 experimental images at different defocus settings. The software Mac Tempas ® is used for images taken in the TEAM 0.5 instrument. Figures 3b and e show two typical phase images that give both accurate lattice spacings and intensities that can be related to chemical composition of the specific atomic (or molecular) columns in the image.

References:

[1] M. E. Gershwin, A. Belay, Spirulina in Human Nutrition and Health. CRC Press, 2007.
[2] E.E. Neri-Torres *et al*, submitted to Microscopy and Microanalysis (Dec. 2015).
[2] The authors acknowledge funding from IPN, CONACYT (grants 148304, FOINS 75/2012) and COFFA IPN. The use of TEAM 05 at NCEM-LBNL is supported by the Molecular Foundry, which is

supported by the Office of Science, the Office of Basic Energy Sciences, the U.S. Department of Energy under Con- tract No. DE-AC02-05CH11231.

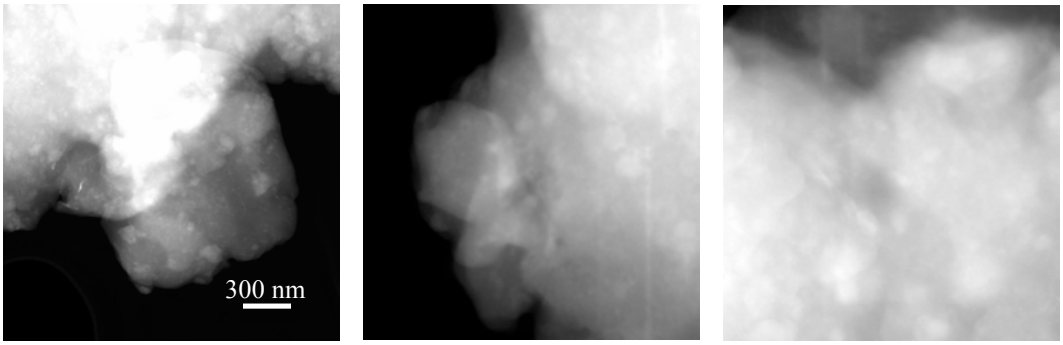

Figure 1. STEM images of Spirulina nanoparticles

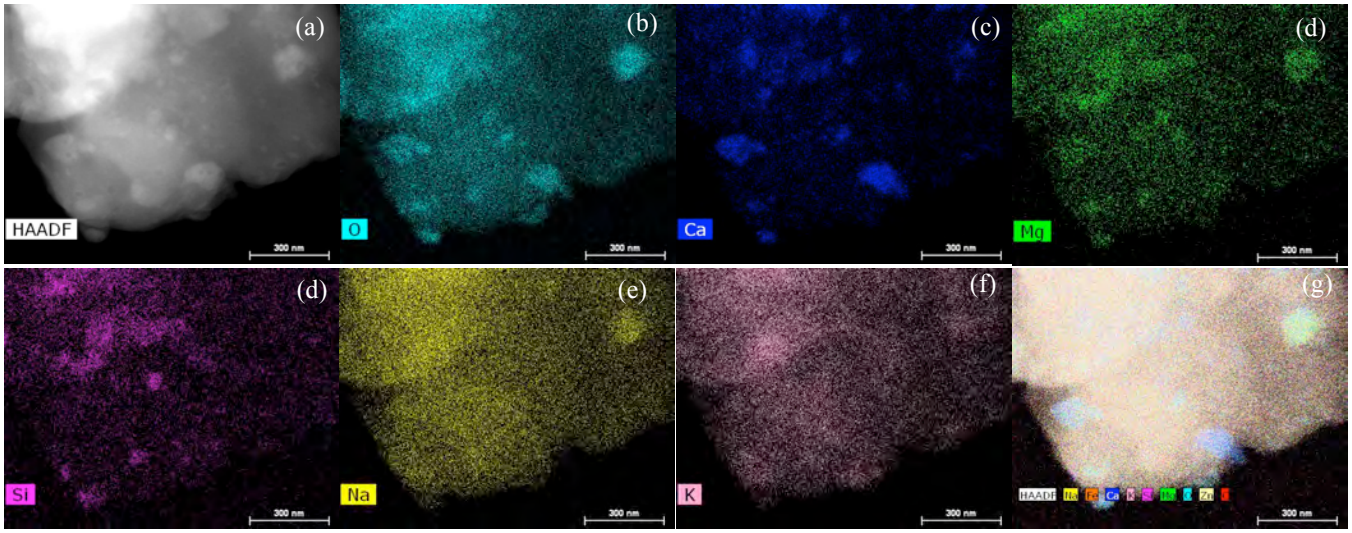

Figure 2. EDS mapping of as milled Spirulina. Some elements concentrate in specific regions. Each compositional mapping indicates the reference element. The STEM image in (A) shows the investigated area.

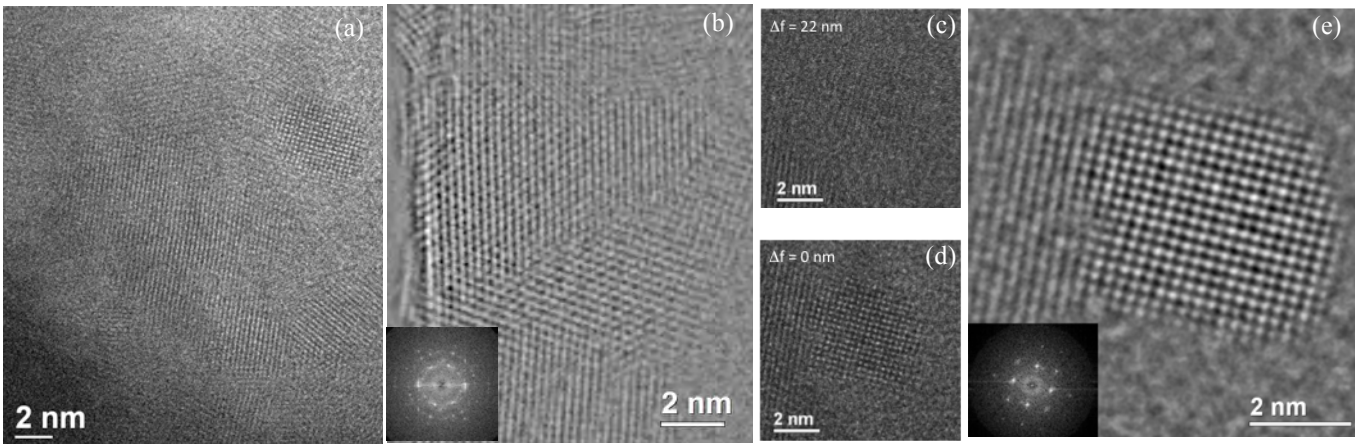

Figure 3. TEM of Maltodextrin wall material. (a) Experimental image at 35 $e^{-}/Å^{2}s$, (b) Phase image of polycrystalline area. (c-d) Experimental images (different defocus settings) for calculation of (e) phase image of a single ordered nanoparticle.

https://doi.org/10.1017/S1431927616009909 Published online by Cambridge University Press