International Journal of Advancements in Bio-Informatics and Bio-Technology– IJABB
Volume 2 : Issue 1 [ISSN 2475-2258]
Publication Date : 30 October, 2015

Green and rapid synthesis of biocompatible gold and silver nanoparticles using fruit juices, green tea and coffee

A comparative and systematic study

Aryane Tofanello¶, Érica G. A. Miranda¶, Juliana C. Araújo-Chaves, Carlos E. de Castro, Fernando C. Giacomelli, Alejandro Zúñiga and Iseli L. Nantes*

***Abstract*—A facile, rapid and green synthesis of gold and silver nanoparticles (GNPs and SNPs) using juice of different fruits as reducing and stabilizing agent is reported. Seven different fruits were used in the comparative study with green tea and coffee: lemon, melon, pineapple, banana, orange and tomato. The efficiency of the fruits for the synthesis and stabilization of metallic nanoparticles was compared at pH 3, 7 and 10. The effect of the buffer agent, phosphate and HEPES, was also studied. The early analysis of NP formation was done by observing the color change of the samples. Alkaline pH was favorable for the synthesis of metallic NPs at all the tested conditions. Green tea and coffee were the most efficient agents for the synthesis of GNPs and SNPs. The formation of the GNPs was evident only few seconds after the addition of gold salt solution in the extracts. The most efficient fruits for GNP synthesis were lemon, banana and orange. In the presence of these fruits the formation of GNPs was evident few minutes after the addition of gold salt. HEPES buffer accelerated significantly the rate of GNP formation by fruits. In the presence of HEPES GNP formation using fruits was evident immediately after the addition of gold salt. The fruit-mediated SNP synthesis was relatively slow, spent hours or days according to the fruit type and were not accelerated by HEPES. The GNPs synthetized using fruits were characterized by UV–Vis spectroscopy, scanning electron microscopy (SEM), Fourier transform infrared spectrometry (FTIR) and zeta potential. The GNPs were predominantly spherical and presented different zeta potential values indicating that biomolecules composing fruit juices such as sugars and flavonoids remained capping the NPs.**

***Keywords*—metallic nanoparticles, gold nanoparticles, silver nanoparticles, fruit extracts, HEPES buffer, surface plasmon resonance, zeta potential.**

From Universidade Federal do ABC, Santo André, SP, Brazil. ¶AT and EGAM contributed equally for this paper; *Corresponding author: Iseli L. Nantes:

I. Introduction

Metallic nanoparticles (MNPs) have been considered important area of research due to their unique properties for application in different areas such as: medicine, biotechnology, light harvesting, impairment of bacteria proliferation, images, drug deliver among others (1-5). In recent years, biosynthetic processes for MNPs synthesis have received crescent attention as an alternative for the use of toxic and pollutant reactants and stabilizing (6-8). Therefore, it is crescent the adoption of green chemistry principles by nanotechnology that resulted in a green nanotechnology. Synthesis of nanoparticles using biological entities has great interest due to their unusual optical, photoelectro-chemical and electronic properties (9-14). The synthesis and assembly of nanoparticles would benefit from the development of clean, nontoxic and environmentally acceptable procedure. Currently bioreduction methods based on fungi, microorganisms, plant extracts are being attempted due to the ease of synthesis, environmentally benign nature and greater stability of nanoparticles (6, 15). Compared with traditional chemical syntheses, biomolecule-assisted syntheses of noble metal nanomaterials have a number of advantages (16-18). Once biomolecule-mediated syntheses are carried out at room temperature and under aqueous conditions, energy entry is reduced and the reagents used are nontoxic – factors that minimize environmental damage and enhance human health. The structural diversity of biomolecules results in nanostrucutres with a wide range of sizes and morphologies that determine their physical-chemical properties and consequently their applications. Biomolecules can coat the surface of nanoparticles, making them stable and preventing particle aggregation. Furthermore, biomolecules with different functional groups facilitate the post-functionalization of the surface for many different applications (19). Natural extracts are inherently rich in proteins, flavonoids, polymers such as lignin, hemicellulose and pectins all of them potentially able to contribute for the synthesis and capping of metallic nanoparticles. In the present study, we explored a rapid, simple and viable route for the synthesis of gold nanoparticles using some natural extracts of fruits, coffee and green tea in the presence of two different buffers. In terms of chemical composition, fruit, coffee and green tea consist of water and dry matter.

II. Materials and Methods

A. *Chemicals*

All chemicals were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). All aqueous suspensions and solutions were prepared with deionized water (mixed bed of ion exchanger, Millipore®), and the pH was measured using a combined glass electrode (Orion Glass pH SURE-FLOWTM). The reference electrode (ROSS™, model 8102) was filled with OrionFilling Solutions (ROSS™). The pHmeter was calibrated using METREPAK pHydrion standard buffer solutions (Brooklyn, NY) Chemicals.

B. *Preparation of fruit extracts, green tea and coffee for NP synthesis*

The fresh fruit were sanitized with a HClO solution (1%) for 15 minutes and then, they were washed with milliQ®

water. For the natural extracts, pulp of fruits were stirred at ULTRA TURRAX® (IKA® T25 Digital – Germany) at 3000 rpm for some minutes (without adding water) to form the extracts. Green tea and coffee were prepared by weighing 1.2 g of material and adding 30 mL of water. Tea and coffee were filtered.

All glasswares were cleaned with an aqua regia solution (1:3 – HNO_3/HCl) for complete removal of potential artificial nucleation sites. GNPs and SNPs were synthetized at pH values of 3, 7 and 10 by using 30.0 mM phosphate buffer (in the presence and in the absence of 30.0 mM HEPES buffer), 10 µL of fruit extract / 1.5 mL of buffer, and 200 µM salt $HAuCl_4$ or $AgNO_3$). The syntheses were carried out at room temperature and the size and nanostructuration of MNPs were dependents of the fruits and buffer (figure 1).

C. *Determination of zeta potential (ζ)*

The measurements of Zeta potential were carried out in a Zetasizer Nano ZS, Malvern Instruments, Ltd. (London, UK), at the temperature of 25°C. For each zeta potential measurements, the samples were previously equilibrated for 2 min at 25°C.

D. *Dinamic Light Scattering (DLS)*

DLS measurements were performed using an ALV/CGS-3 compact goniometer system consisting of a 22 mW HeNe linearly polarized laser operating at a wavelength of 633 nm, an ALV 7004 digital correlator, and a pair of avalanche photodiodes operating in the pseudo-cross-correlation mode. The samples were placed in 10 mm diameter glass cells and maintained at a constant temperature of 25±1°C. The autocorrelation functions reported are based on three independent runs of 60 s counting time.

E. *FTIR analysis*

The FTIR spectra were collected at resolution of 4 cm^{-1} in the transmission mode (4000–400 cm^{-1}) using a Shimadzu FTIR spectrophotometer (FTIR 8400).

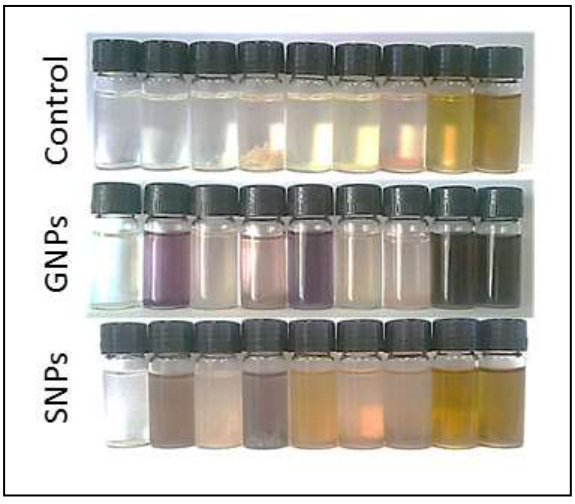

Figure 1. Snapshot of MNPs after 7 days incubation with $HAuCl_4$ or $AgNO_3$ with the following fruit extracts, green tea and coffee at pH 10. The upper row of tubes corresponds to the controls, the intermediate row to GNPs and the lower row, to SNPs. From A to I: control, lemon, melon, banana, orange, pineapple, tomato, green tea and coffee.

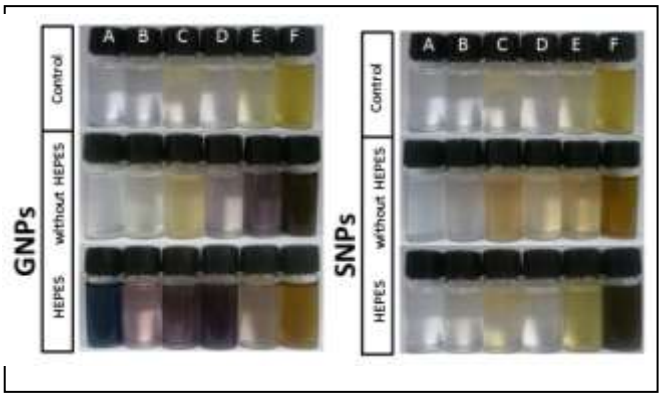

Figure 2. Snapshot of GNPs and SNPs after 1 h incubation $HAuCl_4$ and $AgNO_3$ with the following fruit extracts: A- control of 30 mM phosphate in the absence and presence of 30 mM HEPES, B- melon, C- banana, D- lemon, E-orange, F- green tea. The upper row of tubes corresponds to the synthesis in the absence of HEPES and the lower row to the synthesis in the presence of HEPES.

F. *Scanning Electron Microscopy(SEM)*

The samples (deposited onto a carbon ribbon) were carried out using a scanning electron microscopy (FEG-SEM) field emission by scanning of ultra-high vacuum FESEM JMS-6701F, JEOL (Tokyo, Japan) operated at an 5 kV acceleration voltage and 60,000 and 370,000 X magnification.

III. Results and Discussion

A facile, rapid and green synthesis of gold and silver nanoparticles (GNPs and SNPs) using juice of different fruits as reducing and stabilizing agent was systematically studied. Seven different fruits were used in the comparative study with green tea and coffee: lemon, melon, pineapple, banana, orange, tangerine and tomato.

A. *Effect of pH and buffer*

Gold and silver NPs were synthesized at pH values of 3, 7 and 10 in the presence of 30 mM phosphate buffer using lemon, melon, pineapple, banana, orange, tangerine and tomato in comparison with green tea and coffee. The best efficiency and stability were achieved at pH 10. Figure 1 shows the effect samples of MNPs synthesized using fruits, green tea and coffee after 7 days incubation. The color of the colloidal suspensions are compared with solutions of the reducing agents: fruits, green tea and coffee.

In the following, it was verified the effect of the addition of HEPES buffer in the medium. HEPES is well-known as a reducing and stabilizing agent for the synthesis of multibranched gold nanoparticles featured as nanostars and sea urchin-like which form deep blue color suspensions. HEPES accelerated significantly the synthesis of GNPs (Fig. 1) and did not have significant effect on the synthesis of SNPs (not shown). One exception for the accelerating effect of HEPES was observed for the synthesis using orange as reducing agent. HEPES impaired the formation of GNPs by orange that is evident by comparing the upper and lower tubes assigned as *E*.

B. Surface plasmon resonance bands

The MNPs synthetized using fruits were characterized by UV–Vis spectroscopy for the analysis of the surface plasmon resonance bands (SPR). The SPR of GNPs synthesized using melon and banana in the presence of HEPES (Fig. 3A, olive and dark yellow lines) peaked at 539 nm while the SPR bands of GNPs synthesized using orange and lemon (Fig. 3 A, orange and green lines) are broader and 30 nm redshifted. The GNPs synthesized using orange and lemon in the absence of HEPES (Fig. 3B, orange and green lines, respectively) presented narrower and blue shifted SPR bands. However, the SPR band of GNP obtained using banana in the absence of HEPES (Fig. 3B, dark yellow line) was less intense and redshifted relative to that observed for the GNPs produced in the presence of HEPES (Fig. 3 A, dark yellow line). SNPs produced using banana and melon in the presence of HEPES presented SRP bands peaking at 400 and 408 nm, respectively (Fig. 3, dark yellow and olive lines, respectively). In the absence of HEPES a similar result was obtained for banana (Fig. 3D, dark yellow line) while no SPR was detected for the sample using melon. This result shows that HEPES is crucial for SNP synthesis using melon. The SRP bands of SNPs synthesized using orange in the presence of HEPES were a composite of a narrow band peaking at 411 nm overlapped by a broad SPR band peaking around 565 nm (Fig. 3C, orange line). Similar result was obtained using lemon however with a lower yield as evidenced by a lower intensity SPR band (Fig. 3, green line).

In the absence of HEPES, the SNPs obtained using orange and lemon presented also narrow bands suggesting that HEPES favored the formation of a population of larger SNPs that are responsible for the absorption at the higher wavelengths. These redshifted bandas were observed after 4 days of incubation and are consistent with the grayish color of the samples (Inset of Fig. 3).

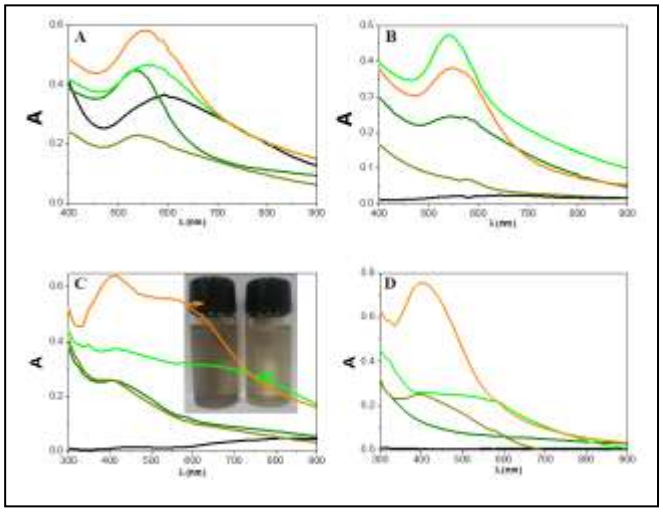

Figure 3. UV-visible spectra of GNPs and SNPs synthesized using fruit extracts. A and B corresponds to SPR of the GNPs synthesized in the presence (A) and in the absence (B) of 30 mM HEPES. C and D corresponds to SPR of the SNPs synthesized in the presence (C) and in the absence (D) of 30 mM HEPES. Black line - control (buffer), olive line - melon, dark yellow line - banana, green line -lemon, orange line - orange.

C. Contribution of fruit phenolic compounds and the particle charge

The samples of GNPs were analyzed by Fourier transform infrared spectrometry (FTIR) and the most significant contribution was identified in the region of 3000-2400 cm^{-1} that can be assigned to phenolic compounds and aldehydes (20, 21). The FTIR spectra of GNPs synthesized usin fruits did not present contributions in the region of amide I and II region (not shown). Therefore, phenolic compounds and aldehydes probably are the best contributors for stabilization of GNPs. The commom phenolic compounds that are found in fruits are: callitephin (melon) sinapinic acid (pineapple), quercertin-3-glucoside

The MNPs were also characterized by the ζ potential (Table I). The MNPs synthesized in the presence of HEPES are all negatively charged at the range of -23 to -33 mV, except those obtained using green tea. Contrary to the effect on the NPs obtained using green tea, the absence of HEPES buffer promoted significant increase of ζ potential for all the other GNPs. It is important to note that HEPES did not influence significantly the zeta potential of MNPs synthesized using melon (Table I).

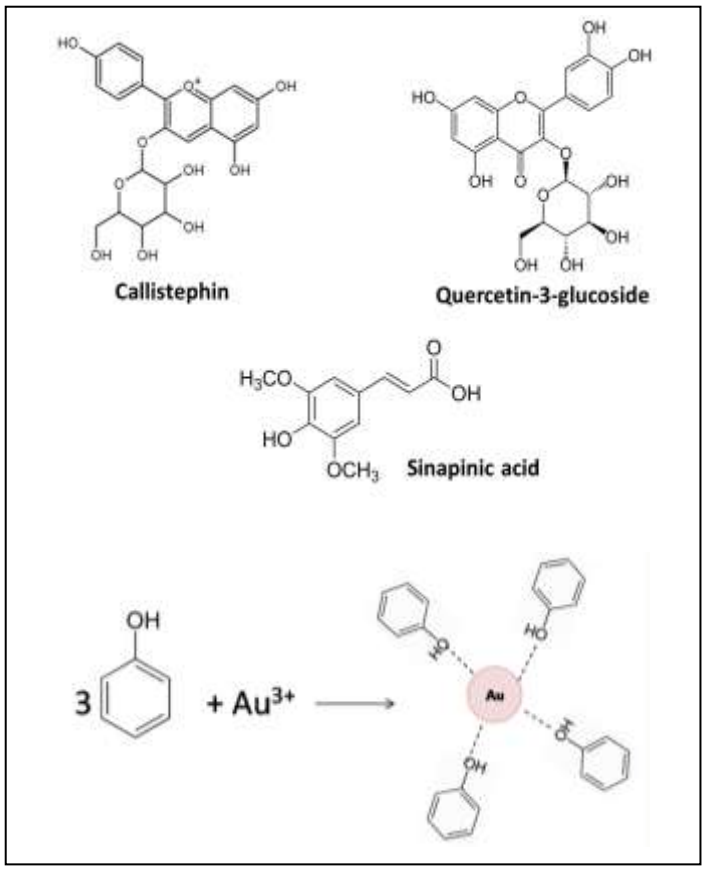

Figure 4. Structure and properties of phenolic compounds for GNP synthesis and stabilization. The upper panel shows the structure of callistephin, sinapinic acid and quercetin-3-glucoside. The lower panel shows a general model of phenolic compounds interaction with GNP surface.

TABLE I. ζ POTENTIAL OF MNPS

Reducing agent	ζ Potential (mV)	
	GNP	*SNP*
Buffer[a]	-35.3 +/- 1 (-)[a]	-
Melon	-33.2 +/- 1 (-35.4 +/- 1)[a]	- 24.2 +/- 1 (- 26.6 +/- 2)[a]
Lemon	-26.5 +/- 1 (-17.1 +/- 1)[a]	- 23.7 +/- 1 (- 15.7 +/- 1)[a]
Banana	- 33.0 +/- 1 (- 26.9 +/- 1)[a]	- 32.5 +/- 2 (- 29.8 +/- 1)[a]
Orange	- 30.5 +/-2 (-26.9 +/- 1)[a]	- 25.5 +/- 2 (- 29.2 +/- 2)[a]
Green Tea	- 12.8 +/- 1 (- 34.2 +/- 1)[a]	-17.2 +/- 2 (- 22.1 +/- 2)[a]

a. Values in parenthesis were determined for MNPs synthesized with sodium phosphate buffer without HEPES.

D. *Particle size*

The MNPs were also characterized by the size with determination of the hydrodynamic radius and polydispersivity (Table II, RH and μ2/Γ2). The MNPs produced using fruits as well green tea are polydisperse. In the presence of HEPES, the smaller MNP sizes were obtained using orange and green tea and the larger sizes were obtained using melon, banana and lemon. The synthesis of MNPs carried out in the absence of HEPES resulted in NPs significantly smaller, except for the GNPs synthesized using green tea. The GNPs obtained in the presencer of HEPES did not present polydispersivity significantly different that observed in the absence of this buffer (Table II). However, for SNPs, HEPES buffer increased the polydispersivity. The large sizes with polydispersivity can also result from aggregation.

TABLE II. DLS OF MNPS

Reducing agent	Dynamic Light Scattering (DLS)			
	GNPs		*SNPs*	
	R_H (nm)*	μ_2/Γ^{2*}	R_H (nm)*	μ_2/Γ^{2*}
Buffer[a]	-	-	-	-
Melon	100 (36)[a]	0.52 (0.51)[a]	172 (-)[a]	0.70 (-)[a]
Banana	176 (43)[a]	0.31 (0.43)[a]	238 (67)[a]	0.78 (0.21)[a]
Lemon	114 (59)[a]	0.49 (0.39)[a]	384 (169)[a]	1.23 (0.40)[a]
Orange	24 (25)[a]	0.50 (0.47)[a]	81 (48)[a]	0.31 (0.40)[a]
Green tea	48 (243)[a]	0.44 (0.45)[a]	54 (21)[a]	0.33 (0.52)[a]

a. Values in parenthesis were determined for MNPs synthesized without HEPES.

Samples of GNPs synthesized using melon and banana were analyzed by scanning electronic microscopy (SEM). Consistent with the optical properties, the GNPs produced using melon are spherical and presented aggregation (Fig. 4A). Interestingly, the samples GNPs synthesized using banana presented gold microcrystals (Fig. 4 B)

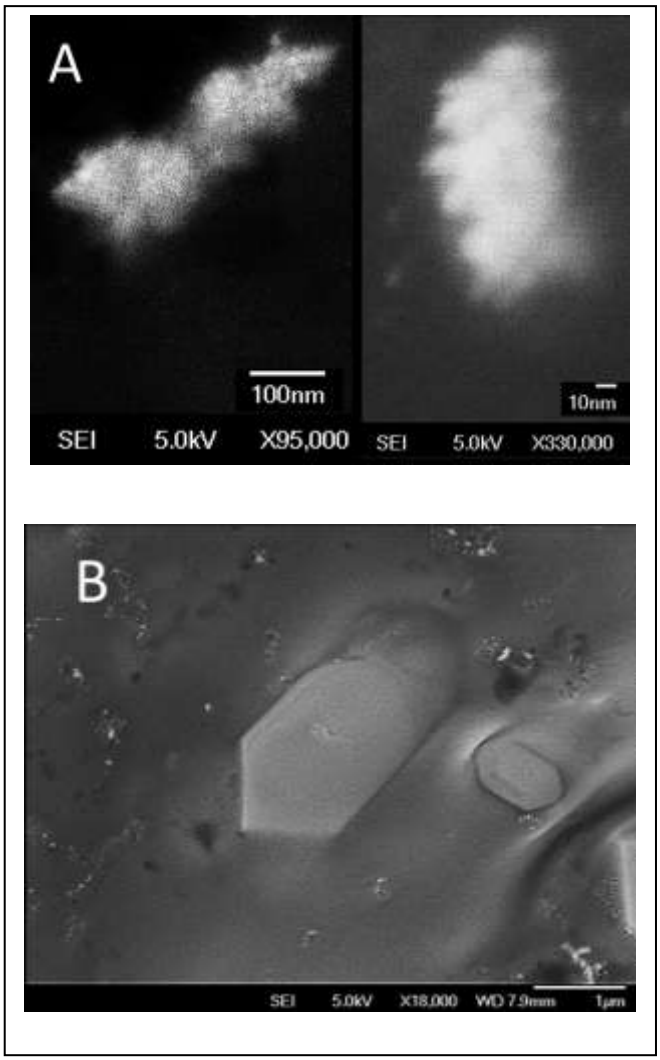

Figure 5. Field emission scanning electron microscopy of GNP sinthesized using melon extract (A) and of gold microcrystals sinthesized using banana extract.

E. *Conclusions*

The most efficient fruit extracts for the synthesis of MNPs were: melon, banana, orange and lemon. The yield, the zeta potential and the size of MNPs synthesized with these fruits were influenced by the presence of HEPES. In the case of SNPs HEPES buffer was crucial for the success of SNP synthesis using melon and favored a population of larger particles obtained using lemon and orange as reducing and stabilizing agents. The MNPs obtained with fruits did not exhibit a significant contribution of proteins as capping agents but exhibit the contribution of aldehydes and phenolic compounds covering the particle surfaces. Banana was the unique fruit that was able to generate gold microcrystals and the components responsible for this structure remain to be investigated.

Acknowledgment

The authors thank to the financial support of Brazilian agencies: FAPESP (2012/07456-7), CNPq and CAPES (5583/2014-04). The present study had also financial support of UFABC (NBB – Núcleo de Bioquímica e Biotecnologia). The authors thank also to Central

Experimental Multiusuários of UFABC for the access to facilities and to David da Mata Lopes for the technical assistance. J.C.A-C is post-doctoral fellow of FAPESP (2012/08322-4).

References

[1] C. Lorenz, N. V. Goetz, M. Scheringer, M. Wormuth, and K. Hungerbühler, "Potential exposure of German consumers to engineered nanoparticles in cosmetics and personal care products", Nanotoxicology, vol. 5, pp. 12-29, March 2011.

[2] J.W. Wiechers, and N. Musee, "Engineered Inorganic Nanoparticles and Cosmetics: Facts, Issues, Knowledge Gaps and Challenges", J. Biomed. Nanotechnol., vol. 6, pp. 408-431, October 2010.

[3] J. Conde, G. Doria, and P. Baptista, "Noble metal nanoparticles applications in cancer", J Drug Deliv, vol -, pp. 1-12, August 2012.

[4] X. H. Huang, S. Neretina, and M. A. El-Sayed, "Gold Nanorods: From Synthesis and Properties to Biological and Biomedical Applications," Advanced Materials, vol. 21, pp. 4880-4910, December 2009.

[5] J. Z. Zhang, "Biomedical Applications of Shape-Controlled Plasmonic Nanostructures: A Case Study of Hollow Gold Nanospheres for Photothermal Ablation Therapy of Cancer," Journal of Physical Chemistry Letters, vol. 1, pp. 686-695, January 2010.

[6] A. R. Tao, S. Habas, and P. D. Yang, "Shape control of colloidal metal nanocrystals" Small, vol. 4, pp. 310-325, March 2008.

[7] Y. Xia, Y. Xiong, B. Lim, S. E. Skrabalak, "Shape-Controlled Synthesis of Metal Nanocrystals: Simple Chemistry Meets Complex Physics?," Angew Chem Int Ed., vol. 48, pp. 60-103, January 2009

[8] K. N. Thakkar, S. S. Mhatre, and R. Y. Parikh, "Biological synthesis of metallic nanoparticles", Nanomedicine, vol. 6, pp. 257-262, April 2010.

[9] S. P. J. Namal Senanayake, "Green tea extract: Chemistry, antioxidant properties and food applications - A review", J Func Foods, vol 5, pp. 1529-1541, September 2013.

[10] S. K. Nune, N. Chanda, R. Shukla, K. Katti, R. R. Kulkarni, S. Thilakavathi, S. Mekapothula, R. Kannan, and K. V. Katti, "Green nanotechnology from tea: phytochemicals in tea as building blocks for production of biocompatible gold Nanoparticles", J Mater Chem., vol 19, pp. 2912-2920, June 2009.

[11] N.J. Liang, and D.D. Kitts, "Antioxidant Property of Coffee Components: Assessment of Methods that Define Mechanisms of Action", Molecules, vol 19, pp. 19180-19208, November 2014.

[12] C. Lin, L. A. Mueller, J. Mc Carthy, D. Crouzillat, V. Pétiard, and S. D. Tanksley., "Coffee and tomato share common gene repertoires as revealed by deep sequencing of seed and cherry transcripts", Theor Appl Genet., vol 112(1), pp. 114-130, November 2005.

[13] S. P. Tan, C. Stathopoulos, S. Parks, and P. Roach, "An Optimised Aqueous Extract of Phenolic Compounds from Bitter Melon with High Antioxidant Capacity, Antioxidants", Antioxidants, vol 3, pp. 814-829, December 2014.

[14] S. Ranganna, V. S. Govindarajan, K. V. R. Ramana, and J. F. Kefford., "Citrus fruits - Varieties, chemistry, technology, and quality evaluation. Part II. Chemistry, technology, and quality evaluation. A. Chemistry", C R C Critical Reviews in Food Science and Nutrition, vol 18(4), pp. 313-386, 1983.

[15] A. G. Ingale, and A. N. Chaudhari, "Biogenic Synthesis of Nanoparticles and Potential Applications: An EcoFriendly Approach", J. Nanomed. & Nanotechnol, vol. 4, pp. 1-8, February 2013.

[16] [9] M.B. Dickerson, K.H. Sandhage, and R.R. Naik, "Protein- and Peptide-Directed Syntheses of Inorganic Materials", Chem. Rev., vol. 108, pp. 4935-4978, October 2008.

[17] S. Mann, "Biomineralization : principles and concepts in bioinorganic materials chemistry", Oxford chemistry masters, Oxford; New York: Oxford University Press. xii, 210 pages, November 2001.

[18] W.J. Crookes-Goodson, J.M. Slocik, and R.R. Naik, "Bio-directed synthesis and assembly of nanomaterials", Chem. Soc. Rev., vol 37(11), pp. 2403-2412, September 2008.

[19] P.T. Anastas, et al., "The role of catalysis in the design, development, and implementation of green chemistry", Catal. Today, vol 55(1-2), pp. 11-22, January 2000.

[20] J. Y. Song, H.-K. Jang, and B. S. Kim, "Biological synthesis of gold nanoparticles using Magnolia kobus and Diopyros kaki leaf extracts," Process Biochem., vol. 44, pp. 1133-1138, June, 2009.

[21] E. H. Ismail, M. M. H. Khalil, F. A. Al Seif, and F. El-Magdoub, "Byosinthesis of gold nanoparticles using extract of grape (Vitis Vinifera) leaves and seeds," Progress Nanotecnol. Nanomat., vol. 3, pp. 1-12, January, 2014

About Authors:

Aryane Tofanello is PhD student under the guidance of Professor Iseli L. Nantes. Her research interest focuses on the synthesis of gold nanoparticles with thiol-containing peptides.

Erica G. A. Miranda is PhD student under the guidance of Professor Iseli L. Nantes. Her research interest focuses on the photochemical synthesis of gold and silver nanoparticles using dyes.

Juliana C. Araujo-Chaves is Postdoc in UFABC under the supervision of Professor Iseli L. Nantes. Her research interest focuses on porphyrins, membranes, and the synthesis of GNP for application in PDT.

Carlos E. de Castro is Master´s student working under the supervision of Professor Fernando Carlos Giacomelli. His investigations are focused on polymer colloids.

Fernando C. Giacomelli is Associate Professor in the Universidade Federal do ABC - UFABC (Brazil). His main research area is focused on the characterization of complex polymer assemblies.

Alejandro Zúñiga received his PhD in Materials Science and Engineering from the University of California, Davis. His research interests include nanostructured, amorphous materials and electron microscopy.

Iseli L. Nantes is full professor of Bioenergetics in Universidade Federal do ABC, Brazil. She coordinates projects focused on hemeproteins and porphyrins associated to membranes and nanoparticles.