



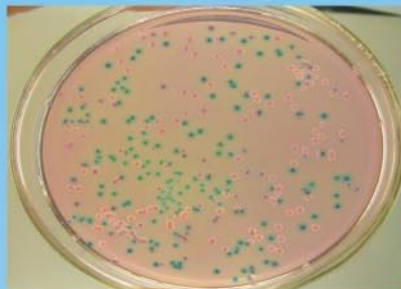
David Publishing Company  
www.davidpublishing.com

ISSN 1934-7391 (Print) ISSN 1934-7405 (Online)

# JLS

## *Journal of Life Sciences*

Volume 6, Number 1, January 2012



From Knowledge to Wisdom

### **Publication Information**

*Journal of Life Sciences* is published monthly in hard copy (ISSN 1934-7391) and online (ISSN 1934-7405) by David Publishing Company located at 9460 TELSTAR AVE SUITE 5, EL MONTE, CA 91731, USA.

### **Aims and Scope**

*Journal of Life Sciences*, a monthly professional academic journal, covers all sorts of researches on molecular biology, microbiology, botany, zoology, genetics, bioengineering, ecology, cytology, biochemistry, and biophysics, as well as other issues related to life sciences.

### **Editorial Board Members**

Dr. Stefan Hershberger (USA), Dr. Suiyun Chen (China), Dr. Farzana Perveen (Pakistan), Dr. Francisco Torrens (Spain), Dr. Filipa João (Portugal), Dr. Masahiro Yoshida (Japan), Dr. Reyhan Erdogan (Turkey), Dr. Grzegorz Żurek (Poland), Dr. Ali Izadpanah (Canada), Dr. Barbara Wiewióra (Poland), Dr. Valery Lyubimov (Russia), Dr. Amanda de Moraes Narcizo (Brasil), Dr. Marinus Frederik Willem te Pas (The Netherlands), Dr. Anthony Luke Byrne (Australia), Dr. Xingjun Li (China), Dr. Stefania Staibano (Italy), Dr. Wenle Xia (USA), Hamed Khalilvandi-Behroozyar (Iran).

Manuscripts and correspondence are invited for publication. You can submit your papers via Web Submission, or E-mail to [life-sciences@davidpublishing.com](mailto:life-sciences@davidpublishing.com) or [life-sciences@hotmail.com](mailto:life-sciences@hotmail.com). Submission guidelines and Web Submission system are available at <http://www.davidpublishing.com>.

### **Editorial Office**

9460 TELSTAR AVE SUITE 5, EL MONTE, CA 91731, USA

Tel: 1-323-9847526, Fax: 1-323-9847374

E-mail: [life-sciences@davidpublishing.com](mailto:life-sciences@davidpublishing.com), [life-sciences@hotmail.com](mailto:life-sciences@hotmail.com)

Copyright©2012 by David Publishing Company and individual contributors. All rights reserved. David Publishing Company holds the exclusive copyright of all the contents of this journal. In accordance with the international convention, no part of this journal may be reproduced or transmitted by any media or publishing organs (including various websites) without the written permission of the copyright holder. Otherwise, any conduct would be considered as the violation of the copyright. The contents of this journal are available for any citation. However, all the citations should be clearly indicated with the title of this journal, serial number and the name of the author.

### **Abstracted / Indexed in**

Database of EBSCO, Massachusetts, USA

Chemical Abstracts Service (CAS), USA

Cambridge Scientific Abstracts (CSA), USA

Chinese Database of CEPS, American Federal Computer Library center (OCLC), USA

Ulrich's Periodicals Directory, USA

Chinese Scientific Journals Database, VIP Corporation, Chongqing, China

### **Subscription Information**

Price (per year): Print \$520, Online \$360, Print and Online \$680.

David Publishing Company

9460 TELSTAR AVE SUITE 5, EL MONTE, CA 91731, USA

Tel: 1-323-9847526, Fax: 1-323-9847374

E-mail: [order@davidpublishing.com](mailto:order@davidpublishing.com)



David Publishing Company  
[www.davidpublishing.com](http://www.davidpublishing.com)

# JLS

## **Journal of Life Sciences**

Volume 6, Number 1, January 2012 (Serial Number 45)

### **Contents**

#### **Molecular Biology and Medical Biochemistry**

- 1 **Genotypic Assessment by RAPD Markers and Ultrastructural Characteristics of a NaCl-Tolerant Potato Cell Line**

*Filipa Queirós, José M. Almeida, Domingos P.F. Almeida and Fernanda Fidalgo*

- 9 **The Taxonomic Status of *Gymnura bimaculata* and *G. japonica*: Evidence from Mitochondrial DNA Sequences**

*Anglv Shen, Chunyan Ma, Yong Ni, Zhaoli Xu and Lingbo Ma*

- 14 **Parameters Analysis of Gastric Motility Signals in Time Domain and Frequency Domain**

*Zhangyong Li, Likun Xu and Zhui Xu*

- 20 **Validation of Metformin Hydrochloride in Human Plasma by HPLC-Photo Diode Array (PDA) for Application of Bioequivalence Study**

*Yahdiana Harahap, Krisnasari Dianpratami, Mahi Wulandari and Rina Rahmawati*

- 28 **Rotation Thromboelastography for Assessment of Hypercoagulation and Thrombosis in Patients with Cardiovascular Diseases**

*Antoaneta Dimitrova-Karamfilova, Yuliana Patokova, Tania Solarova, Irina Petrova and Gencho Natchev*

- 36 **New Silver Nanosensor for Nickel Traces. Part II: Urinary Nickel Determination Associated to Smoking Addiction**

*María Carolina Talio, Marta O. Luconi and Liliana P. Fernández*

#### **Physiology**

- 41 **Studies on the Antioxidant Potential of Extracts from Unripe Fruit of *Carica papaya***

*Omotade Oloyede, Daniel Roos and Joao Rocha*

- 48 **Effect of Absciscic Acid on NaCl Stressed Callus Proliferation and Plant Regeneration in Rice**  
*Ikram-ul-Haq, Ghulam Yasin, Mumtaz Hussain and Ali Mohammad Dahri*
- 55 **Physiological Response of *Hydrilla verticillata* (L.f.) Royle Exposed to Cadmium Stress**  
*Sibanarayan Mohapatra and Surjendu Kumar Dey*
- 61 **The Potentials of Locally Available Fruits Rich in Iron to Mitigate Iron Deficiency Anemia in Least Developing Countries (LSD)**  
*Abdulkadir A. Egal and Wilna H. Oldewage-Theron*
- 68 **Feeding Habits of the Red Porgy *Pagrus pagrus* (Linnaeus, 1758) from Benghazi Coasts, Libya**  
*Mohammad El-Mor*
- 74 **Nesting Activity and Conservation Status of the Hawksbill Turtle (*Eretmochelys imbricata*) in Persian Gulf**  
*Seyyed Mohammad Bagher Nabavi, Ruhollah Zare and Mahdieh Eftekhari Vaghefi*

### **Interdisciplinary Researches**

- 80 **Economic Feasibility of Simultaneous Production of Pine Sawlogs and Meat Goats on Small-Sized Farms in Alabama**  
*Brandi Broughton, James O. Bukenya and Ermson Nyakatawa*
- 91 **Constructing a Model of Digestion in a Primary School Using a Theatrical Performance**  
*Maria J. Gil-Quílez, Begoña Martínez-Peña, Milagros De la Gándara, Marta Ambite and Marian Laborda*
- 99 **Creationism and Evolution Views of Brazilian Teachers and Teachers-to-Be**  
*Ana Maria de Andrade Caldeira, Elaine S. Nicolini Nabuco de Araujo and Graça S. Carvalho*
- 110 **Research on Behavior of Governing Gene/Epigene Networks as a Problem of Cellular Automata Identification**  
*Rustem Tchuraev*
- 114 **Nymphaeaceae Salisb. and Trapaceae Dumort. Families in the Collection of O.V. Fomina Botanical Garden**  
*Tatyana Mazur, Nikolai Didukh and Anna Didukh*

# New Silver Nanosensor for Nickel Traces. Part II: Urinary Nickel Determination Associated to Smoking Addiction

María Carolina Talio<sup>1</sup>, Marta O. Luconi<sup>2</sup> and Liliana P. Fernández<sup>1,2</sup>

1. Chemical Institute of San Luis (INQUISAL-CONICET), Chacabuco y Pedernera, San Luis 5700, Argentine

2. Area of Analytical Chemistry, Faculty of Chemistry, Biochemistry and Pharmacy, Nacional University of San Luis, San Luis 5700, Argentine

Received: June 14, 2011 / Accepted: July 22, 2011 / Published: January 30, 2012.

*"In memoriam" of Dr. Adriana Masi, prominent researcher, dear colleague and friend, who passed away prematurely, as a consequence of public insecurity, killed by a shot in the head at the door of her house.*

**Abstract:** A new fluorescence silver nanosensor assisted by surfactant has been recently synthesized and applied to ultra trace nickel determination. The methodology was validated by the standard addition method and satisfactorily applied to nickel determination in urine without previous treatment, coming from subjects with different smoking addiction levels and second hand smokers. Within-day precision was better than 0.011 CV. The reproducibility (between-days precision) was also evaluated over 3 days by performing six determinations each day with a CV of 0.025. The proposed methodology represents a promising approach in the area of biological monitoring due to its low operation cost, simplicity of instrumentation, high sampling speed and non-polluting solvents. Obtained results of urinary nickel concentration were successfully correlated with the tobacco addiction.

**Key words:** Fluorescence nanosensor, micellar silver nanoparticles, urinary nickel, smoker and non-smoker subjects, second hand smoke exposure.

## 1. Introduction

The smoking habit represents the main preventable cause of human disease and death. Passive smoking or exposure to second hand smoke (SHS) is an associated problem to tobacco addiction and regulations are being introduced to protect non-smokers in working and public places; however, 55% of young people are exposed in their own homes [1].

Tobacco contains numerous harmful substances, among these are toxic metals, which can be inhaled through both active and passive smoking. Although nickel is an essential metal to human life, nickel compounds are human carcinogens by inhalation, and there exists ample evidence for the carcinogenicity of

Ni(II) in humans [2].

Plasma and urine nickel concentrations have shown to be useful biomarkers of nickel inhalation exposure [3]. The development of new methodologies and modern analytical techniques has allowed the use of other matrices that are less or non-invasive [4].

Because of the low concentration level of nickel in biological fluids, a preconcentration step must be introduced in analytical protocols when atomic spectroscopies are used [5], which involves contamination risks associated to sample manipulation. In a previous work a methodology was developed for urinary Ni(II) quantification [6]; a disadvantage of this methodology is that the membranes must be conditioned with a dye and dried for retain by filtration the analyte present in sample. This preparative step is time-consuming; added to this, each membrane can be

---

**Correspondence author:** Liliana P. Fernández, chemical doctor, research fields: luminescent methods and supramolecular systems. E-mail: lfernand@unsl.edu.ar.

used only once for a sample or standard, resulting in an additional cost per analysis.

In this work, a fluorescence sensitive nanosensor is presented as an advantageous alternative to traditional instrumental methods. AgNPs are synthesized in SDS (Sodium Dodecyl Sulfonate) medium (SDS-AgNPs) and the obtained nanomaterials are applied to trace nickel quantification.

## **2. Experimental**

### *2.1 Reagents*

Urine samples were tested using Urine Strip-Wiener Lab (Rosario, Argentina). Ni(II) stock solutions  $1 \times 10^{-9} \text{ mol}\cdot\text{L}^{-1}$  were prepared by dilution of  $1,000 \text{ g}\cdot\text{mL}^{-1}$  standard solution plasma-pure (Leeman Labs, Inc.). Tris (Mallinckrodt Chemical Works, NY, USA) solution  $1 \times 10^{-2} \text{ mol}\cdot\text{L}^{-1}$  was prepared by weighting and subsequent dilution with ultrapure water and adjusted to the desired pH, with aqueous  $\text{HClO}_4$  (Merck, Darmstadt, Germany) or  $\text{NaOH}$  (Mallinckrodt Chemical Works, NY, USA).  $\text{AgNO}_3$  (Sigma-Aldrich, St. Louis, USA)  $1 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$  was prepared by dilution of 17 mg in 100 ml ultrapure water. Citric acid (Hopkin and Williams, England), hexadecyl trimethyl ammonium bromide (J.T. Baker, Mallinckrodt Baker, Inc., NJ, USA) and sodium dodecylsulfate (J.T. Baker, Mallinckrodt Baker, Inc., NJ, USA) were used without further purification. All used reagents were of analytical grade.

### *2.2 Apparatus*

Fluorescence measurements were made using a Shimadzu RF-5301 PC spectrofluorometer equipped with a 150 W xenon lamp and 1.00 cm quartz cells. A combined glass electrode and a pH meter (Orion Expandable Ion Analyzer, Orion Research, Cambridge, MA, USA) model EA 940 were used for pH adjustments. A centrifuge was used in biological sample processing and AgNPs purification. All used glass materials were previously washed with a 10% v/v  $\text{HNO}_3$  water solution and then with ultrapure water.

### *2.3 Biological Sample Collection*

First morning urine samples were collected from eight nickel occupationally unexposed subjects. In order to assure the obtaining of representative samples, subjects previously received detailed information about the collection protocol [6]. Biological samples were collected in nickel-free polystyrene test tubes between 8:00 and 10:00 a.m. to reduce possible circadian contributions. Studied subjects were asked to respond to a written questionnaire in order to obtain information about smoking habits (frequency, length of addiction), age, sex, occupation, etc.. Written informed consents were obtained from all participants.

Samples were centrifuged 10 min at 1,000 g and processed immediately after arriving at the laboratory. No stabilizing agents were added to avoid the incorporation of analytes as impurity [7]. The obtained samples (approximately 10 mL each) were centrifuged for 10 min. Supernants (approximately 5 mL) were reserved for nickel quantification.

### *2.4 Physical Characterization and Semi-Quantitative Determination of Clinical Parameters in Biological Samples*

Biological samples were physically characterized (colour, odor and appearance, presence of sediment, blood and mucus) in order to establish variables that could affect the obtained results. Urine samples were tested using commercial reagent strips.

### *2.5 Dilution Test*

1 mL of each biological sample was taken and dilutions were carried out to obtain dilution factors of 1/2, 1/4, 1/8, 1/16 and 1/20.

### *2.6 Proposed methodology*

Appropriate aliquots of urine/standard solution Ni(II) ( $1.2 \times 10^{-4}$ - $2.93 \times 10^2 \text{ ng}\cdot\text{L}^{-1}$ ), 100  $\mu\text{L}$  buffer Tris solution  $1 \times 10^{-2} \text{ mol}\cdot\text{L}^{-1}$  (pH = 6.3) and 500  $\mu\text{L}$  of synthesized SDS-AgNPs, were placed in a 10 mL graduated centrifuge tube. The whole mixture was

diluted to 3 mL with ultrapure water. Fluorescent emission was measured at  $\lambda_{em} = 348$  nm using  $\lambda_{exc} = 240$  nm.

### 2.7 Accuracy Study

1 mL of biological samples was spiked with increasing amounts of Ni(II) ( $1.2 \times 10^{-4}$ - $2.93 \times 10^2$  ng·L<sup>-1</sup>). Nickel contents were determined by the proposed methodology.

### 2.8 Precision Study

Repeatability (within-day precision) of the method was evaluated preparing urine replicate samples ( $n = 6$ ) containing 5.81 ng·L<sup>-1</sup> of nickel, and analyte contents were determined by the proposed methodology.

## 3. Results and discussion

Urine represents easily accessible body fluid using non-invasive sampling procedures and it can reflect levels of biomarkers [4].

### 3.1 Studied Subjects

Subjects with different degrees of tobacco addiction (Table 1) were selected to evaluate U-Ni (urinary nickel) contents.

### 3.2 Physical and Chemical Characterization of Biological Samples

Once in the laboratory, biological samples were observed and characterized for physical appearance (colour, odor and appearance, presence of sediment,

blood and mucus) in order to establish variables that could interfere in the determinations. All processed samples can be considered within the normal physical parameters. Samples were immediately centrifuged for 10 min at 1,000 g, and supernants were reserved for nickel examination.

The clinical parameters (pH, urobilinogen, glucose, ketones, bilirubin, proteins, nitrite, blood, specific gravity and leucocytesin) of the urine samples, as determined by commercial urine strips, can be considered within normal values.

### 3.3 Dilution Test

In order to establish the appropriate volume of each sample for performing Ni(II) determination, several sample volumes were assayed. The adequate dilution for each sample was that whose signal fell into the linearity range of the developed methodology. Dilution test was of 25  $\mu$ L for subjects with minor exposure and of 2.5  $\mu$ L for the most exposed subjects. Dilution factors were adopted for the following studies.

### 3.4 Analytical Performance

At optimal experimental conditions, a detection limit of 0.036 pg·L<sup>-1</sup> and quantification limit 0.12 pg·L<sup>-1</sup> were obtained. The calibration sensitivity was  $2 \times 10^{14}$  L·pg<sup>-1</sup>·cm<sup>-1</sup> for the new methodology, with a range of linearity of six orders of magnitude between 0.12 and  $2.93 \times 10^5$  pg·L<sup>-1</sup> (Table 2).

The accuracy of methodology was tested using the standard addition method. The reproducibility of the method was evaluated by performing 6 replicate

**Table 1** Addiction levels for studied subjects.

Subjects	Daily smoked cigarettes	Exposure time (years)
1	0	0
2	0	0
3	SHS*	20
4	SHS*	35
5	5	8
6	20	25
7	40	40
8	TChH**	40

\* Second hand smoker; \*\* Tobacco chewing habit.

**Table 2** Quality parameters for nickel determination using SDS-AgNPs sensor.

Parameters	Regression	Ni(II) (pg·L <sup>-1</sup> )
LOD	--	0.036
LOQ	--	0.12
Range of linearity <sup>(1)</sup>	0.998	0.12-184
Range of linearity <sup>(2)</sup>	0.999	120-36,400
Range of linearity <sup>(3)</sup>	0.998	2,440-293,000

<sup>(1)</sup> Slit excitation: 5 nm; slit emission: 3 nm. <sup>(2)</sup> Slit excitation: 3 nm; slit emission: 1.5 nm. <sup>(3)</sup> Slit excitation: 1.5 nm; slit emission: 1.5 nm.

experiments. Ni(II) contents in each type of sample based on the average of replicate measurements are presented in Table 3. Although urine fluorescent emission constitutes a severe interference in fluorescence measures, the high sensitivity of developed methodology permitted to realize urinary Ni(II) determinations using a very small volume of biological sample (0.0025-0.025 mL depending on exposure tobacco level), minimizing the spectral interference. The results showed that the proposed method was suitable for Ni(II) determination in urine biological samples, and for all the range of studied concentrations.

The precision was better than 0.011 CV for U-Ni. The reproducibility (between-days precision) was also evaluated over 3 days by performing six determinations each day and was 0.025 CV. These results showed that the biological samples were stable during this period of time.

#### 4. Conclusions

In the present work we have described the bioanalytical

**Table 3 Urinary nickel determination of subjects ordered by increasing tobacco addiction level of recovery study.**

Sample *	Ni(II) added (ng·L <sup>-1</sup> )	Ni(II) found CV (ng·L <sup>-1</sup> )	Recovery (%; n = 6)
1	-	13.59	0.01
	59	72.36	0.08
	77	91.02	0.03
2	-	14.88	0.06
	59	74.01	0.05
	77	92.12	0.04
3	-	45.52	0.05
	77	123.11	0.04
	102	146.32	0.09
4	-	77.33	0.10
	77	155.1	0.07
	102	180.02	0.01
5	-	1,152	0.10
	1,010	2,162	0.02
	1,230	2,349	0.06
6	-	1,560	0.06
	1,010	2,567	0.02
	1,230	2,792	0.07
7	-	3,445	0.09
	2,753	6,200	0.03
	3,850	7,293	0.04
8	-	4,465	0.04
	2,753	7,216	0.08
	3,850	8,313	0.01

application of a surfactant assisted fluorescent nanosensor for ultra trace nickel quantification, using the enhancement of AgNPs fluorescent signal in presence of Ni(II). The method was successfully applied to the determination of trace amounts of nickel in urine without previous treatment, with good tolerance to regular foreign constituents. The proposed methodology may constitute a promising approach in the area of biological monitoring with low operation costs, simplicity of instrumentation, high sampling speed and non-polluting solvents. Results of urinary nickel were successfully correlated with the tobacco addiction. Considering that high levels of this carcinogenic metal in the studied urine samples from smokers may contribute to pathologic effects, efforts should be made by the control agencies and health agents to discourage the consumption of cigarettes and the tobacco chewing habit.

#### Acknowledgments

The authors wish to thank Instituto de Química San Luis-Consejo Nacional de Investigaciones Científicas y Tecnológicas (INQUISAL-CONICET), FONCYT (Fondo Nacional de Ciencia y Tecnología), National University of San Luis (Proyect 22/Q828) for the financial support.

#### References

- [1] R. Pitarque, A. Bolzan, M.E. Gatella, Tabaquismo en adolescentes escolarizados de la ciudad de Olavarría, Buenos Aires: Prevalencia y factores asociados, Arch. Argent. Pediatr. 105 (2007) 115-121. (in Spanish)
- [2] K. Salnikow, K.S. Kasprzak, Ascorbate depletion: A critical step in nickel carcinogenesis?, Environ. Health Persp. 113 (2005) 577-584.
- [3] F.W. Sunderman Jr., S.M. Hopfer, K.R. Sween, A.H. Marcus, B.M. Most, J. Creason, Nickel absorption and kinetics in human volunteers, Proceedings of the Society for Experimental Biology and Medicine 191 (1989) 5-11.
- [4] M. Esteban, A. Castaño, Non-invasive matrices in human biomonitoring: A review, Environ. Int. 35 (2009) 438-449.
- [5] J.L. Manzoori, G. Karim-Nezhad, Development of a cloud point extraction and preconcentration method



**New Silver Nanosensor for Nickel Traces. Part II: Urinary Nickel  
Determination Associated to Smoking Addiction**

- for Cd and Ni prior to flame atomic absorption spectrometric determination, *Anal. Chim. Acta.* 521 (2004) 173-177.
- [6] M.C. Talio, M.O. Luconi, A.N. Masi, L.P. Fernández, Solid surface spectroscopic methodology for ultra-trace urinary nickel monitoring in smokers and non-smokers' subjects, *J. Pharm. Biomed. Anal.* 52 (2010) 694-700.
- [7] H.G. Seiler, A. Sigel, H. Sigel, *Handbook on Metals in Clinical and Analytical Chemistry*, MerceL Dekker, Ink., 1994, pp. 40-41.
- [8] T. Itakura, K. Torigoe, K. Esumi, Preparation and characterization of ultrafine metal particles in ethanol by UV irradiation using a photoinitiator, *Langmuir* 11 (1995) 4129-4134.