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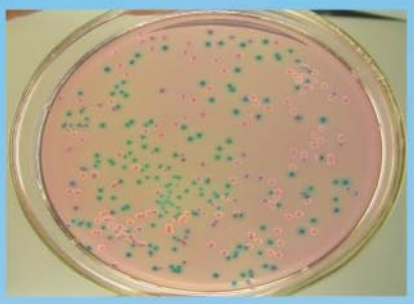



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Interdisciplinary Researches

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

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"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

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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 1×10^{-9} mol·L^{-1} were prepared by dilution of 1,000 g·mL^{-1} standard solution plasma-pure (Leeman Labs, Inc.). Tris (Mallinckrodt Chemical Works, NY, USA) solution 1×10^{-2} mol·L^{-1} was prepared by weighting and subsequent dilution with ultrapure water and adjusted to the desired pH, with aqueous $HClO_4$ (Merck, Darmstadt, Germany) or NaOH (Mallinckrodt Chemical Works, NY, USA). $AgNO_3$ (Sigma-Aldrich, St. Louis, USA) 1×10^{-3} mol·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 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) (1.2×10^{-4}-2.93×10^{2} ng·L^{-1}), 100 μL buffer Tris solution 1×10^{-2} mol·L^{-1} (pH = 6.3) and 500 μL 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 λ_{em} = 348 nm using λ_{exc} = 240 nm.

2.7 Accuracy Study

1 mL of biological samples was spiked with increasing amounts of Ni(II) (1.2×10^{-4}-2.93×10^{2} ng·L^{-1}). 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^{-1} 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, especific 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 μL for subjects with minor exposure and of 2.5 μL 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^{-1} and quantification limit 0.12 pg·L^{-1} were obtained. The calibration sensitivity was 2×10^{14} L·pg^{-1}·cm^{-1} for the new methodology, with a range of linearity of six orders of magnitude between 0.12 and 2.93×10^{5} pg·L^{-1} (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^{-1})
LOD	--	0.036
LOQ	--	0.12
Range of linearity[(1)]	0.998	0.12-184
Range of linearity [(2)]	0.999	120-36,400
Range of linearity [(3)]	0.998	2,440-293,000

[(1)] Slit excitation: 5 nm; slit emission: 3 nm. [(2)] Slit excitation: 3 nm; slit emission: 1.5 nm. [(3)] 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 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.

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

Sample*	Ni(II) added ($ng \cdot L^{-1}$)	Ni(II) found CV ($ng \cdot L^{-1}$)		Recovery (%, n = 6)
	-	13.59	0.01	-
1	59	72.36	0.08	98.52
	77	91.02	0.03	103.16
	-	14.88	0.06	-
2	59	74.01	0.05	100.87
	77	92.12	0.04	101.61
	-	45.52	0.05	-
3	77	123.11	0.04	99.11
	102	146.32	0.09	97.36
	-	77.33	0.10	-
4	77	155.1	0.07	101.00
	102	180.02	0.01	100.89
	-	1,152	0.10	-
5	1,010	2,162	0.02	100.0
	1,230	2,349	0.06	97.13
	-	1,560	0.06	-
6	1,010	2,567	0.02	99.80
	1,230	2,792	0.07	100.10
	-	3,445	0.09	-
7	2,753	6,200	0.03	100.05
	3,850	7,293	0.04	99.94
	-	4,465	0.04	-
8	2,753	7,216	0.08	99.95
	3,850	8,313	0.01	99.95

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