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Determination of Salicylic Acid by Differential Pulse Voltammetry Using ZnO/Al_2O_3 Nanocomposite Modified Graphite Screen Printed Electrode

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A simple strategy for determination of salicylic acid (SA) based on ZnO/Al_2O_3 nanocomposite modified graphite screen printed electrode (ZnO/Al_2O_3/SPE) is reported. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were employed to evaluate the performance of the sensor. The ZnO/Al_2O_3/SPE demonstrated noticeable electrochemical catalytic activity. The oxidation of salicylic acid at ZnO/Al_2O_3/SPE was accompanied by the considerable decrease in overpotential and dramatic increase in peak current. The response of salicylic acid under the optimized conditions is linear within the ranges of 0.5-80.0 μM. A low detection limit of 0.25 μM was found for salicylic acid target. The function of this modified electrode in the concentration measurement of the real samples such as urine and pharmaceuticals was acceptable and approved its practical application.

Keywords: Salicylic acid, ZnO/Al_2O_3 nanocomposite, Graphite screen printed electrode, Voltammetry, Real sample analysis

1. INTRODUCTION

Salicylic acid (SA) as a class of beta hydroxyl acids, is an important compound in the plants due to its role as an endogenous signal and defense response against pathogens [1]. The function of SA

as a phytohormone has also been known in regulating the physiological and biochemical events in plants such as seed germination, flowering, stomatal closure, membrane permeability, ion absorption, etc. [2, 3]. It is also an active metabolite of acetyl salicylic acid (ASA) which is a common anti-inflammatory medication [4, 5]. Extremely high levels of salicylic acid in human can manifest toxicity which is along with severe headache, fatigue, dizziness, and hearing problems. Therefore, the importance of the fabrication a sensor with high level of accuracy, sensitivity and efficiency for the determination of salicylic acid seems essential.

Various techniques including spectrofluorimetry, gas chromatography, high-performance liquid chromatography (HPLC), spectrophotometry and enzymatic methods [6-10], have been used for the determination of salicylic acid. Nevertheless, limitations including being time-consuming and necessity of sample-dependent treatments like extraction and pre-concentration, together with the high cost of instrumentations and operation impose problems in this regard. As an alternative for the analysis of salicylic acid, the electrochemical approaches have been introduced since they offer advantages of fast response, low detection limit, moderate cost, and simpler operation due to the lack of necessity for sample pretreatment [11].

Screen printed electrodes (SPEs), have become attractive and widely used by electroanalysts due to their ability to provide large-scale production of electrodes possessing features such as high versatility, portability, easy to use, cost-effectiveness, and possibility to produce in miniature size for analysis of microvolume samples, as well [12,13]. A screen printed electrode overcomes the limitations of conventional electrode systems with two or three electrodes, which frequently require re-calibration, polishing process, as well as being ineffectiveness for on-site analysis since this kind of analysis procedure is time consuming [14]. In recent years, the modification of electrode surfaces with nanostructure materials is one of the important developments in various fields of electrochemistry [15-28].

Nanostructured modified electrodes render good electro-catalytic activity, high sensitivity and selectivity, lower detection limit in comparison with unmodified electrodes [29-49]. Among nanoparticles, zinc oxide nanoparticles (ZnO) with a wide band gap of 3.27 eV, large excitation binding energy (60 eV), non-toxicity, near ultraviolet (UV) emission, piezoelectricity as well as high electron communication properties is more favorable for the fabrication of efficient electrochemical sensors. ZnO has been very well known as both an electronic and a structural promoter which can greatly influence the activity of catalysts, while alumina or other refractory oxides simply act as structural promoters. Hence, using ZnO/Al_2O_3 nanocomposite in catalyst formulations has been constantly interesting [50-53].

In the light of the above, developing effective routes for the analysis of salicylic acid can be regarded as a critical need and hence the current work was focused on the use of novel ZnO/Al_2O_3 nanocomposite for modifying a graphite screen printed electrode (SPE) for using in the voltammetric determination of this analyte. The results proved that the resulting modified SPE offers a good electrocatalytic effect toward salicylic acid. The electrode was finally tested for evaluation of its efficiency in the determination of the analyte in real samples.

2. EXPERIMENTAL

2.1. Apparatus and chemicals

The analyses were carried out using an Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands), using a General Purpose Electrochemical System (GPES) software for controlling the operating conditions. The screen-printed electrode (DropSens, DRP-110, Spain) includes three electrodes featuring graphite counter electrode, a silver pseudo-reference electrode and a graphite working electrode.

Double distilled water was used in the preparation of the test solutions. All reagents in analytical grade were purchased from Merck Co. and used without further treatment. For the preparation of buffer solutions with pH values in the range of 2.0-9.0, orthophosphoric acid and its corresponding salts were used.

2.2. Synthesis of ZnO/Al_2O_3 nanocomposite

The aluminum hydroxide was prepared by dissolving of 3 g of Al $(NO_3)_3.9H_2O$ in 100 ml of distilled water. The pH of solution was set to 8 by ammonia solution and it was kept at 60 ^oC for 18 h. The precipitate was washed by ethanol and aceton three times, respectively. The $Al(OH)_3$ was prepared by aging of precipitate at 75 ^oC for 24 h.

A solution of zinc nitrate (0.3 M) was prepared in 80 ml of distilled water. The pH of solution was set to 9.5 by ammonium solution (25%) and the 0.13 g of aluminum hydroxide was added to the solution and the solution was mixed for 2h at room temperature. The solution was aged at 90 ^oC for 4 h at 250 rpm stirring rate. The precipitate of ZnO/Al_2O_3 nanocomposite was washed by ethanol and distilled water, respectively.

2.3. Modification of the electrode

The typical route for the modification of the bare graphite screen printed electrode involved preparing a stock suspension of the ZnO/Al_2O_3 nanocomposite through admixing a 1 mL/1mg of water and the ZnO/Al_2O_3 nanocomposite through ultrasonication for 60 minutes. Next 5 µl of the suspension was cast on bare electrodes, and then left to dry under ambient temperature.

2.4. The real samples

In the case of the acetyl salicylic acid tablets, 5 tablets were grinded and then 300 mg of the resulting powder was dissolved in 25 mL of distilled water under sonication. Next, various amounts of the solution were cast to a volumetric flask (25 mL) and by using PBS (pH=7.0) and diluted to the mark. These samples were analyzed through the standard addition method.

As with the urine samples, the specimens were instantly transferred to and stored in a refrigerator upon collection. Prior to analyses, 10 mL of each sample was taken and centrifuged at

2000 rpm for 15 minutes, and the supernatant was cleared using a filter (0.45 µm). Next, different amounts of the supernatant was poured into a 25 mL flask and diluted to the mark using PBS (pH=7.0), and eventually spiked with various amounts of salicylic acid.

3. RESULTS AND DISCUSSION

3.1. Electrocatalytic oxidation of salicylic acid at a ZnO/Al_2O_3/SPE

pH has a critical role in determining the electrochemical behavior of salicylic acid. Hence, the effect of this parameter on the electrochemical behavior of salicylic acid was assessed using different solution of the analyte at 0.1 M in PBS with various pH values from 2.0 to 9.0, using the modified ZnO/Al_2O_3/SPE through cyclic voltammetry. The results revealed that the optimal electrochemical behavior could be observed around neutral pH values. Accordingly, pH of 7.0 was determined and used as the optimal value for the rest of the experiments using the ZnO/Al_2O_3/SPEs.

The CVs obtained for 80.0 µM salicylic acid solutions using ZnO/Al_2O_3/SPE (curve a) and bare SPE (curve b) are illustrated in Fig. 1.

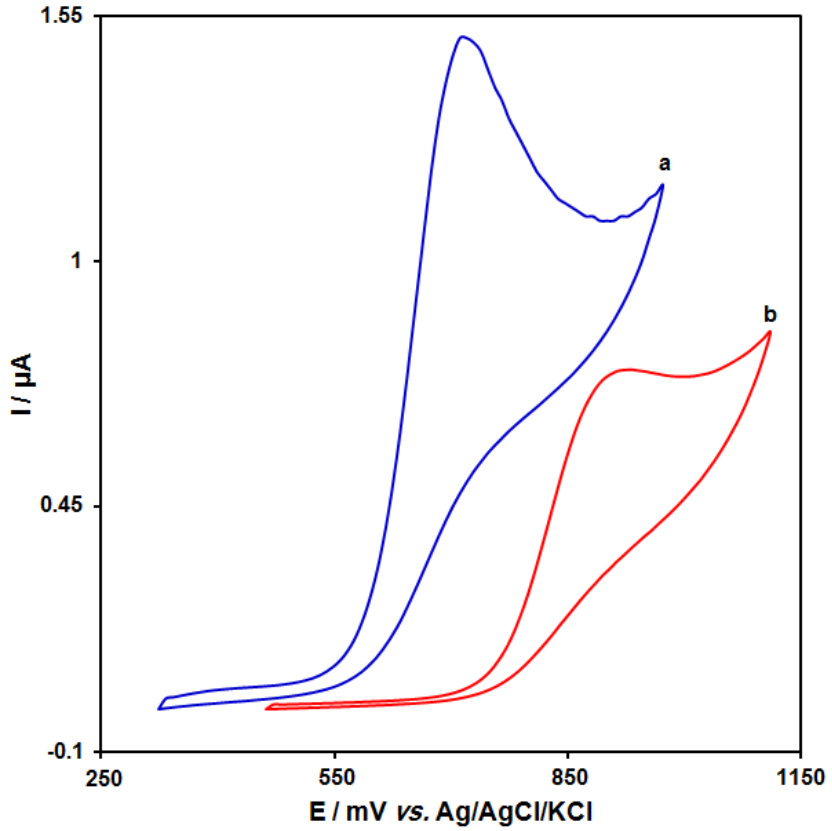

Figure 1. Two cyclic voltammograms of ZnO/Al_2O_3/SPE (a) and bare SPE (b) in 0.1 M PBS (pH 7.0) in the presence of 80.0 µM salicylic acid at the scan rate 50 mVs^{-1}.

In the former case, the anodic peak potential can be observed at around 720 mV as opposed to 930 mV in the case of bare SPE. Also, in the case of the modified electrode the anodic peak current was found to be relatively enhanced. Both of these results indicate that the modification using the ZnO/Al_2O_3 nanocomposites greatly improves the signal corresponding to the oxidation of salicylic acid.

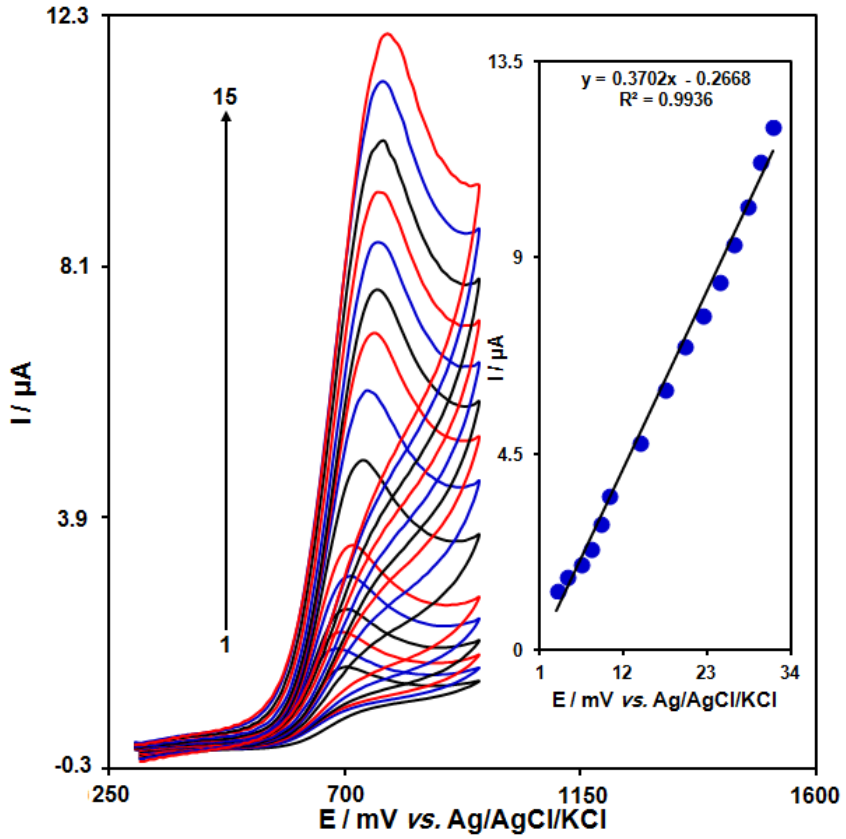

Figure 2. Cyclic voltammograms of ZnO/Al_2O_3/SPE in 0.1 M PBS (pH 7.0) containing 100.0 µM salicylic acid at various scan rates; numbers 1-15 correspond to 10, 20, 40, 60, 80, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mV s^{-1}, respectively. Inset: variation of anodic and cathodic peak current vs. $v^{1/2}$.

The dependence of oxidation current of salicylic acid on potential scan rates was investigated (Fig. 2). According to the results, increment in the potential scan rate resulted in increasing the peak current [54-58]. Besides, it was revealed that the oxidation process is under the control of diffusion as concluded from the linear relationship between the anodic peak current (I_p) and the square root of the potential scan rate ($v^{1/2}$) over a wide range from 10 to 1000 mV s^{-1}.

3.2. Chronoamperometry studies

The studies were performed by adjusting the potential of the working electrode at 0.8 V for all sample solutions (SA in PBS (pH=7.0)) and the results are given in Fig. 3. It is known that the

electrochemical current observes under the mass transport limited conditions; in the case of an electroactive material like SA can be described using Cottrell's equation [59].

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2} \quad (1)$$

Where D and C_b represent the diffusion coefficient ($cm^2\ s^{-1}$) and the bulk concentration (mol cm^{-3}), respectively.

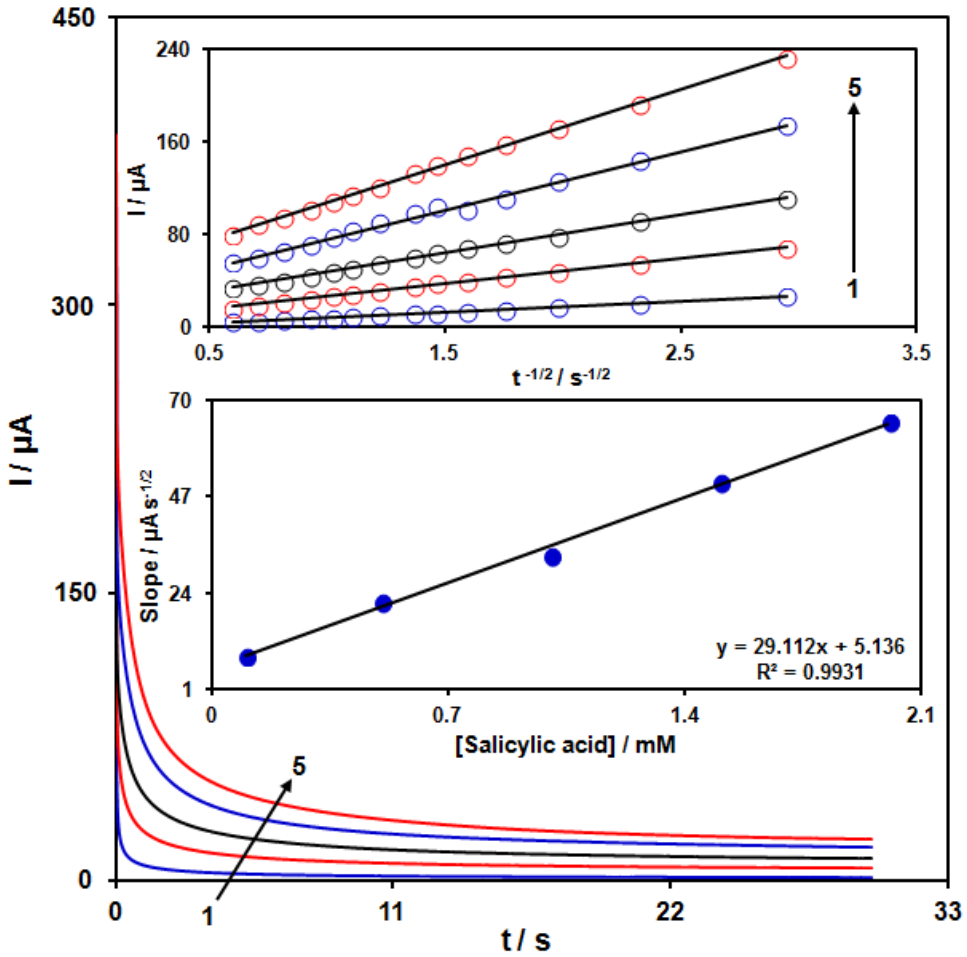

Figure 3. The resulted chronoamperograms at ZnO/Al_2O_3/SPE in 0.1 M PBS (pH 7.0) for various concentration of salicylic acid. The numbers 1–5 related to 0.1, 0.5, 1.0, 1.5 and 2.0 mM of salicylic acid. Insets: (A) Plots of I vs. $t^{-1/2}$ resulted from chronoamperograms 1–5. (B) Plot of the slope of the straight lines against salicylic acid concentration.

The plots of I vs. $t^{-1/2}$ obtained from experimental data demonstrated the best fits for various concentration of salicylic acid (Fig. 3A). Then, the slopes of the lines obtained from the plots in fig. 3A were plotted against the salicylic acid concentration (Fig. 3B). The resulting slope and Cottrell equation were employed for the determination of mean value of D and determined to be 7.2×10^{-5} cm^2/s.

3.3. Calibration plot and limit of detection

In order to determine the salicylic acid in solution, the peak current of its oxidation at the surface of the modified electrode can be used.

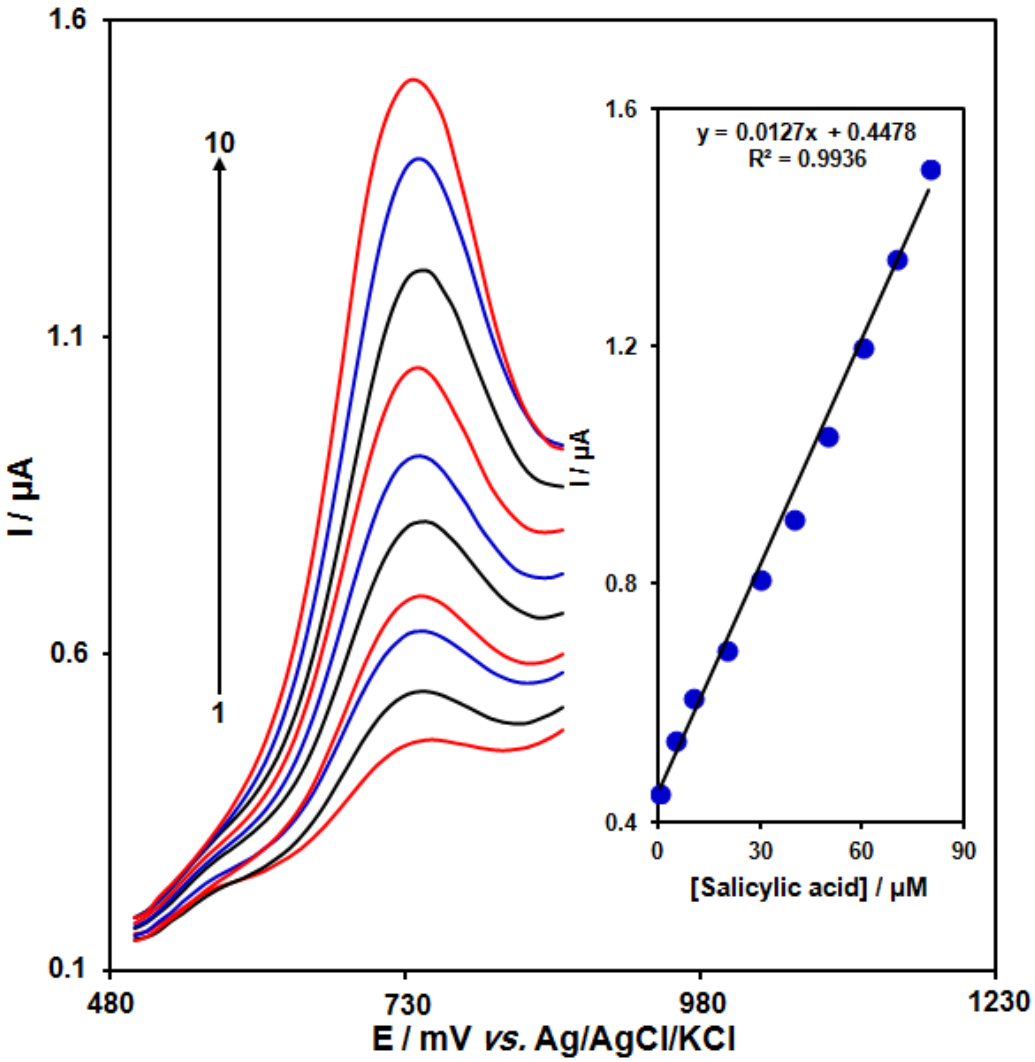

Figure 4. DPVs of ZnO/Al_2O_3/SPE in 0.1 M (pH 7.0) containing different concentrations of salicylic acid. Numbers 1–10 related to 0.5, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0 and 80.0 µM of salicylic acid. Insets: (A) plots of the electrocatalytic peak current as a function of salicylic acid concentration within the range of 0.5-80.0 µM.

Therefore, for different concentrations of salicylic acid, differential pulse voltammetry (DPV) experiments were performed. It was demonstrated, at the surface of a modified electrode the oxidation peak currents of salicylic acid were proportional to the salicylic acid concentration within the range from 0.5 to 80.0 µM (Fig. 4). The detection limit (3σ) of salicylic acid was determined to be 2.5×10^{-7} M. These values are comparable with values reported by other research groups for electro- oxidation salicylic acid at the surface of chemically modified electrodes (see Table 1).

Table 1. Comparison of the efficiency of some methods used in detection of salicylic acid.

Electrode	Modifier	LOD	LDR	Ref.
Carbon paste electrode	Nickel titanate ($NiTiO_3$) nanoceramics	68.0 nM	3.0-1000.0 μM	[1]
Pt electrode	Co/Al hydrotalcite-like compound	0.2 μM	0.1-500.0 μM	[2]
Glassy carbon electrode	Au@Fe_3O_4-chitosan	0.1 μM	1.0-1200.0 μM	[60]
Pt disk electrode	Platinum nanoparticles	6.4 μM	0.2-500.0 μM	[61]
Glassy carbon electrode	Molecularly imprinted polymer	0.0035 nM	0.005-1000.0 μM	[62]
Glassy carbon electrode	Multiwalled carbon nanotubes-chitosan	0.1 μM	0.67-48.82 μM	[63]
Glassy carbon electrode	Molecularly imprinted polymer	0.2 μM	0.6-100.0 μM	[64]
Graphite screen printed electrode	ZnO/Al_2O_3 nanocomposite	0.25 μM	0.5-80.0 μM	This work

3.4. Real samples

The proposed modified electrode was also applied for the determination of salicylic acid in urine samples and acetyl salicylic acid tablets, as mentioned before. The results as expressed in Table 2 were found to be satisfactory. According to the experimental results, desirable recovery was found for salicylic acid. The mean relative standard deviation (R.S.D.) was used to approve the reproducibility of the method.

4. CONCLUSIONS

The present study introduces the fabrication and application of ZnO/Al_2O_3/SPE for the determination of salicylic acid. The salicylic acid oxidation was catalyzed at pH 7.0 and its peak potential was shifted to a less positive potential at the ZnO/Al_2O_3/SPE. The ZnO/Al_2O_3/SPEs proved to have low detection limit, linear and fast response behavior over a wide concentration range, as well as considerable stability. The applicability of the proposed sensor with satisfactory results, proved its efficiency as a promising analytical tool for the analysis of salicylic acid.

Table 2. The performance of ZnO/Al_2O_3/SPE in the determination of salicylic acid in Acetyl salicylic acid tablet and urine samples (n=5). All concentrations are in µM.

Sample	Spiked	Found	Recovery (%)	R.S.D. (%)
Acetyl salicylic acid tablet	0	12.5	-	2.7
	2.5	15.5	103.3	3.2
	5.0	17.3	98.9	1.7
	7.5	20.2	101.0	2.4
	10.0	22.3	99.1	2.1
Urine samples	0	-	-	-
	5.0	5.1	102.0	3.3
	15.0	14.9	99.3	1.8
	25.0	25.3	101.2	2.8
	35.0	34.1	97.4	2.6

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