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Simultaneous Determination of Adenosine and Adenosine-5′-triphosphate at Nanogold Modified Indium Tin Oxide Electrode by Osteryoung Square-Wave Voltammetry

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Abstract
Simultaneous determination of adenosine and adenosine-5′-triphosphate has been described using nanogold modified indium tin oxide electrode. Gold nanoparticles catalyze adenosine oxidation which results in increasing separation of oxidation peaks of adenosine and ATP, making it possible to determine adenosine and adenosine-5′-triphosphate simultaneously. The detection limits for adenosine and ATP were found as 0.07 μM and 0.10 μM respectively with sensitivity 22.9 nA μM^{-1} and 20.9 nA μM^{-1}. The proposed method was also used for sensing the compounds in biological samples. Influence of various square-wave parameters and different pH conditions on peak current has also been reported.

Keywords: Gold nanoparticles, Electrocatalysis, Osteryoung square-wave voltammetry, Adenosine, Adenosine-5′-triphosphate

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1. Introduction

Adenine nucleosides and nucleotides are highly critical from the viewpoint of "cellular energy homeostasis". The energy state of a cell is a key factor in controlling its nucleoside/ nucleotide pool [1]. High energy phosphate bonds of adenosine-5′-triphosphate (ATP) make it an important constituent of nature's primary energy cycles, viz. Calvin cycle and Krebs cycle. Adenosine (ADS), a metabolic product of ATP and the sugar analogue of adenine, plays an important role in coronary blood flow control, cardiac arrhythmias, inhibition of adrenergic activity and renal function regulation [2 – 5].

Recently ADS and ATP have been proposed to possess excellent anesthetic property for mammals [6]. Both these compounds being endogenous in nature, have a clear edge over commonly used synthetic and opiate anesthetics which are associated with several undesired cardiovascular and respiratory complications. A balanced ADS/ATP level is critically important in neural transmission also because of its antagonistic effect on nerve excitability and impulse progression [7], which further highlights the importance of ADS/ATP ratio in living systems.

The determination of intracellular level of various nucleosides and nucleotides gives a deep insight into the specific metabolic pathways, which further helps in understanding possible correlations between various pathways [8 – 10]. In view of this, several methods have been developed for the determination of these compounds. Chromatographic methods for simultaneous determination of ADS and ATP have already been reported [11 – 13]. However, chromatographic methods require time consuming complex pretreatment procedure and have high operational cost when compared with electroanalytical procedures. Many references are available describing electrochemical sensing of ADS and ATP individually [14 – 18] while literature describing simultaneous electrochemical determination of ADS and ATP is very limited [19]. High oxidation potential of these compounds [20] limits approach for electroanalytical determination at conventional electrodes. The oxidation potentials of these compounds considerably shift to less positive potentials at nanogold modified electrode owing to electrocatalytic nature of gold nanoparticles. In this paper, simultaneous determination of ADS and ATP at nanogold modified indium tin oxide electrode (NGITO) is described using highly sensitive Osteryoung square-wave voltammetric (OSWV) procedure. The developed method is also illustrated for simultaneous determination of ADS and ATP in biological samples. Interfering effects of common biological metabolites have also been evaluated to optimize the conditions under which modified electrode can be used reliably.

2. Experimental

2.1. Nanogold Modified Electrode

Nanogold modified indium tin oxide electrodes (NGITO) were prepared by 'touch' seed mediated growth method as described in literature [21]. Typically, indium tin oxide sheet was at first modified with Au nano-seed particles which were prepared by $NaBH_4$ reduction. The electrode was then dipped in growth solution having 90 mL of 0.1 M cetyltrimethylammonium bromide, 2.5 mL of 0.01 M $HAuCl_4$, 0.5 mL of 0.1 M ascorbic acid and 0.5 mL of 0.1 M NaOH solutions. Electrode was dried in nitrogen atmosphere before use and was characterized by field emission scanning electron microscopy (FE-SEM). The deposition of spherical nanogold particles (50 – 60 nm) was noticed using low and high resolution FE-SEM images. NGITO used earlier for the determination of uric acid [22] in our laboratory had a very short utility period and a single electrode was useful for a few runs only. To overcome the limitation, densely coated NGITOs have been prepared as described above and their electroanalytical application for determination of ADS and ATP has been illustrated. Electrode surface was equilibrated before each run by keeping it at zero potential in phosphate buffer solution of pH 7.2 under nitrogen atmosphere for 60 seconds.

2.2. Material and Instrumentation

The electrochemical experiments were carried out using BAS (Bioanalytical Systems, West Lafayette, IN, USA) CV – 50W voltammetric workstation having a single-compartment three-electrode glass cell comprising NGITO as the working electrode, a platinum wire as counter electrode and Ag/AgCl (3 M NaCl) electrode as reference (BAS; Model MF-2052 RB-5B) electrode. All potentials reported are referenced to the Ag/AgCl electrode at an ambient temperature of $25 \pm 2\,^{\circ}C$.

Adenosine was purchased from Merck, Germany while adenosine-5′-triphosphate was purchased from Sisco Research Laboratory, Mumbai, India. Unimolar phosphate buffer solutions, PBS, of pH 3 – 10 were prepared by the reported procedure [23]. All other reagents used were of analytical grade.

2.3. Biological Samples

Azadirachta Indica (*Neem*) leaves were properly crushed with mortar and pestle and were boiled in 200 mL of double distilled water for 20 min [24]. A mixture of 4.0 mL of the filtered extract and 4.0 mL of 0.1 M PBS was then used for recording Osteryoung square-wave voltammogram.

Wheat grains (150 gm) were kept in wet conditions for sprouting. Sprouted grains were properly crushed with mixer and grinder in 150 mL of double distilled water and the extract was filtered. The filtered extract (4.0 mL) was mixed with 4.0 mL of 0.1 M PBS and then analyzed by Osteryoung square-wave voltammetry (OSWV).

Urine samples were collected from laboratory personnel. The collected samples were diluted 100 times with 0.1 M PBS solution to minimize matrix complexity. Diluted urine samples were then spiked with different amount of ADS and ATP solutions followed by recording their Osteryoung square-wave voltammogram.

2.4. Procedure

Stock solutions of ADS and ATP (4 mM) were prepared in double distilled water. Required amount of their stock solution was added to 4.0 mL of phosphate buffer solution of pH 7.2 and the total volume was made to 8.0 mL with double distilled water so that the effective concentration of analyte ranges between 0.10 μM to 10.00 μM maintaining effective concentration of PBS in each test solution at 0.05 M. The electrochemical measurements were then carried out. Osteryoung square-wave voltammetric experiments were performed with the following parameters of the excitation signal: Initial E: 0 mV, Final E: 1200 mV, Square-wave amplitude (E_{sw}): ±28 mV, Potential step (E): 4 mV, Frequency (f): 5 Hz.

3. Results and Discussion

Initially linear sweep voltammetric studies of adenosine and adenosine-5′-phosphate were carried out. However, it was noticed that both these compounds do not exhibit a peak in the entire pH range. Hence, it was considered necessary to use more sensitive technique viz., Osteryoung square-wave voltammetry (OSWV) in which both the compounds exhibit well defined oxidation peak.

3.1. Electrooxidation of Adenosine (ADS)

At bare indium tin oxide electrode (ITO) at pH 7.2, signal for electrooxidation of ADS is observed at ca. 1160 mV in Osteryoung square-wave voltammogram. At bulk gold electrode, however, peak potential of ADS was ca. 970 mV. In contrast, well-defined oxidation peak was observed for ADS centered ca.650 mV at NGITO and thus shows electrocatalytic nature of the nanogold modified electrode. A comparison of the voltammograms observed for ADS at different electrodes is presented in Fig. 1A. The peak potential of ADS at modified electrode was dependent on pH and shifted to less positive potential with increase in pH. The E_p (in mV) *versus* pH plot suggests that protons are involved in the electrode reaction. The dependence of E_p on pH can be expressed by the relation

$$E_p \text{ (in mV)} = -65.10\ \text{pH} + 1081.2 \quad (1)$$

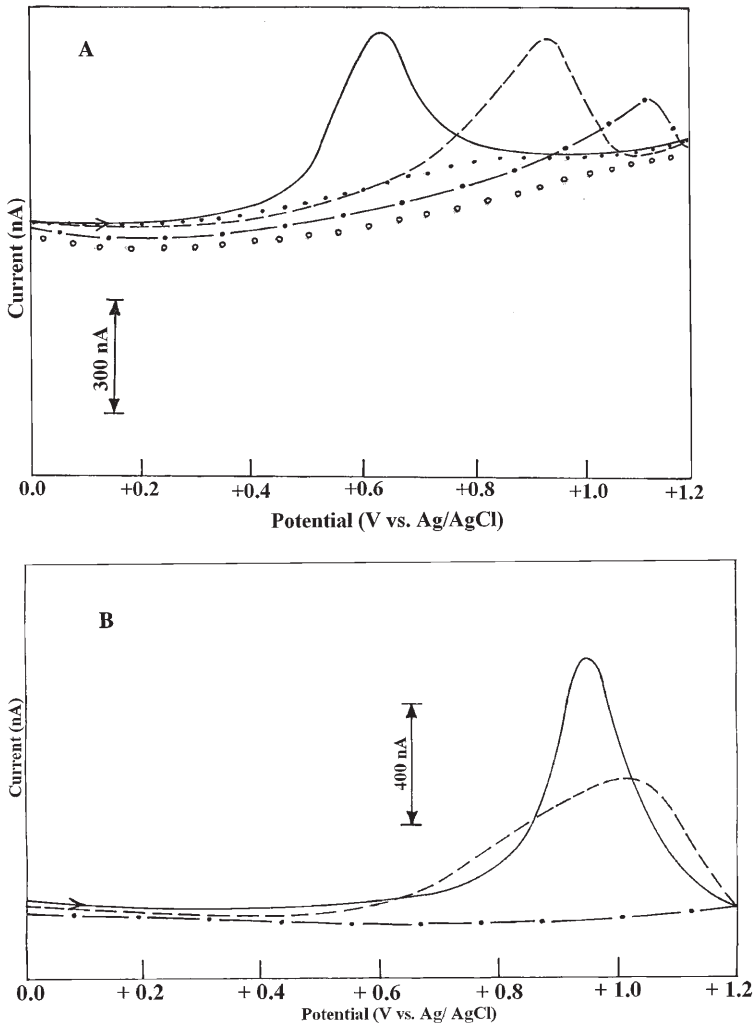

Fig. 1. A) Typical OSWV for 6.0 μM adenosine recorded at NGITO (—), bare ITO (–·–) and gold (– – –) electrodes with superimposed Osteryoung square-wave voltammograms of 0.05 M blank phosphate buffer solution recorded at gold and bare ITO (◦◦◦◦◦) electrodes and at NGITO (·····) electrode. B) Typical OSWV for 6.0 μM adenosine 5′-triphosphate recorded at NGITO(—), bare ITO (–·–) and gold (– – –) electrodes.

with correlation coefficient 0.990 (Fig. 2A).The $\partial E_p/\partial$pH value for ADS indicates that equal number of electrons and protons are involved in the reaction [25]. The peak current (i_p) of the oxidation peak increased with the increase in concentrations of ADS. The i_p (after subtracting background current) vs. concentration plot was linear in the concentration range 0.10 μM to 10.00 μM (Fig. 3A). The correlation coefficient for linear relationship between i_p (in nA) and concentration (in μM) of ADS was found to be 0.983 with a sensitivity of 22.9 nA μM^{-1}.

The E_p of ADS oxidation peak was also dependent on applied frequency and shifted towards more positive potentials with increasing frequency (f). The E_p versus log f plot (Fig. 4A) was linear having a correlation coefficient of 0.972 for ADS. The effect of pulse amplitude on i_p was also studied and the peak current was found to be linearly increasing with increase in pulse amplitude.

3.2. Electrooxidation of Adenosine-5′-triphosphate (ATP)

ATP does not electrooxidize in the potential range 0 to 1200 mV at bare ITO electrode, while at gold electrode; it exhibits a broad peak with E_p ca.1020 mV at pH 7.2. However, a sharp oxidation peak for electrooxidation of ATP was noticed at NGITO having $E_p \approx 980$ mV (Fig. 1B). The appearance of sharp oxidation peak indicates excellent electrocatalytic nature of the gold nanoparticles. The effect of pH on the E_p of ATP was studied in the pH range 3 – 10. It was observed that with increase in pH, the E_p shifted to less positive potential. The linear dependence of E_p on pH can be expressed by the relation

$$E_p \text{ (in mV)} = -55.88 \text{ pH} + 1391.3 \quad (2)$$

with correlation coefficient 0.966 (Fig. 2B). The $\partial E_p/\partial$pH of 55.9 mV clearly indicate [25] that number of electrons and protons involved in electrooxidation of ATP are same. The

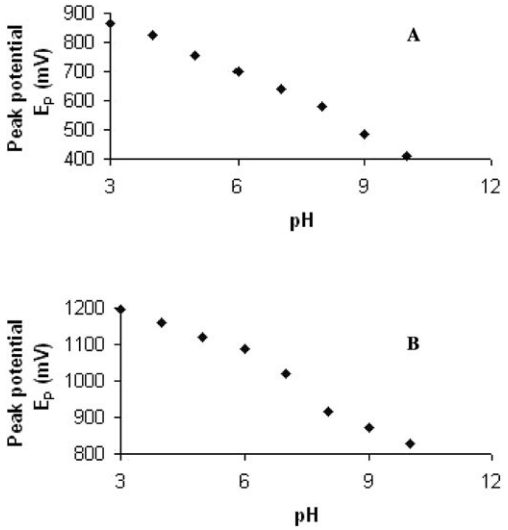

Fig. 2. A) Observed dependence of E_p on pH for 6.0 μM adenosine in the pH range 3 – 10. B) Dependence of E_p on pH for 6.0 μM adenosine 5′-triphosphate in the pH range 3 – 10.

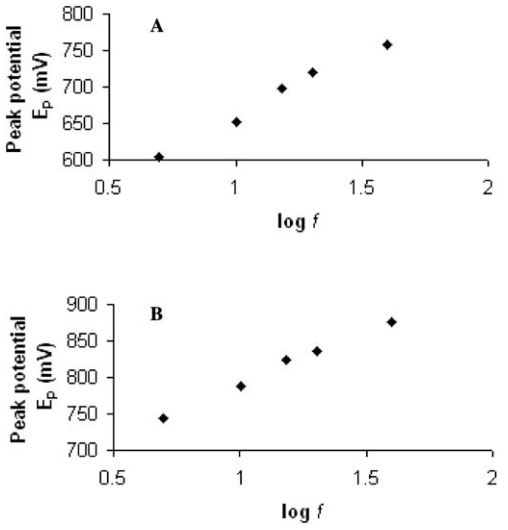

Fig. 4. A) E_p versus log f plot observed for adenosine and b) adenosine-5′-triphosphate for Osteryoung square-wave frequency (f) in the range 5 – 40 Hz. B) E_p versus log f plot observed for adenosine-5′-triphosphate for Osteryoung square-wave frequency (f) in the range 5 – 40 Hz.

peak current was found to increase with increase in concentration of ATP. The i_p (after subtracting background current) vs. concentration plot was linear in the concentration range 0.10 μM to 10.00 μM (Fig. 3B) having correlation coefficient 0.992 with a sensitivity of 20.9 nA μM^{-1}.

The effect of OSWV frequency was also studied on the peak current of ATP. It was found that plot of E_p versus log f was linear in the frequency range 5 – 40 Hz with correlation coefficient 0.991 (Fig. 4B). The variation of peak current with increase in pulse amplitude was also linear. The values of the peak current functions ($i_p\, v^{-1/2}$) remained practically constant with increase in sweep rate in the range 5 mV s^{-1} to 80 mV s^{-1}. This behavior suggests that the nature of electrode reaction is diffusion controlled.

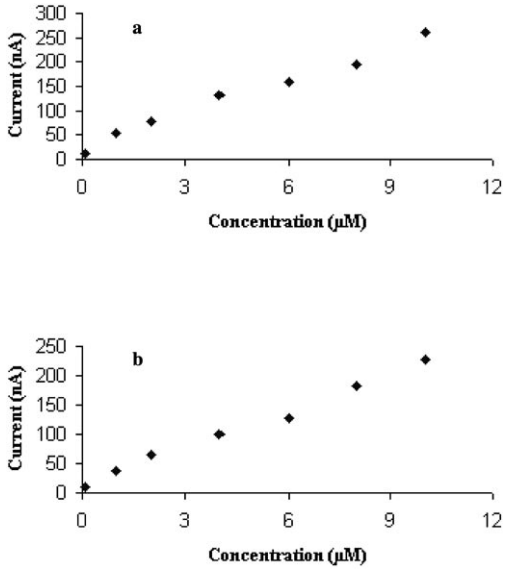

Fig. 3. Dependence of peak current on 0.10 – 10.00 μM concentration of a) adenosine and b) adenosine-5′-triphosphate at pH 7.2.

3.3. Simultaneous Determination of ADS and ATP

The simultaneous determination of ADS and ATP at NGITO was carried out by fixing the concentration of one compound and varying the concentration of other. In the first experiment, the concentration of ATP was fixed at 6.00 μM and concentration of ADS was varied. Typical Osteryoung square-wave voltammograms observed in this case are presented in Fig. 5A. It was observed that the i_p of ATP (fixed component) remained almost unchanged while the i_p of ADS increases with an increase in concentration. Similarly in the second experiment, the concentration of ADS was kept constant at 6.00 μM and the concentration of ATP was changed. The peak current of ATP was found to increase with increase in concentration and typical voltammograms observed are presented in Fig. 5B. It is clear from these observations that simultaneous determination of ADS and ATP is possible at NGITO. Currents observed in both the cases for varied component were essentially same as observed during the individual compound study and obeyed the calibration plot.

3.4. Effect of Interferents

Uric acid, ascorbic acid, xanthine and hypoxanthine are common biological compounds present in noticeable amount in living systems and normally interfere due to close oxidation potentials. Thus, it was considered necessary to evaluate the influence of these electroactive compounds

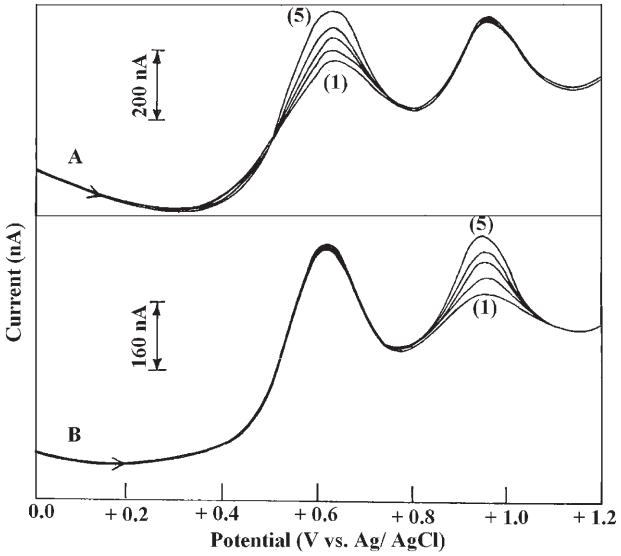

Fig. 5. A) Typical set of OSWV depicting change in peak current values of adenosine at fixed adenosine-5′-triphosphate (6.0 μM). The curves were recorded for 1)1.0, 2) 2.0, 3) 4.0, 4) 6.0, and 5) 8.0 μM of adenosine. B) Square-wave voltammograms depicting change in peak current values of adenosine-5′-triphosphate at fixed adenosine (6.0 μM) concentration. The curves were recorded for 1) 1.0, 2) 2.0, 3) 4.0, 4) 6.0, and 5) 8.0 μM of adenosine-5′-triphosphate.

as interferents in the determination of ADS and ATP. A systematic study of their influence on voltammetric response was carried out by recording OSWV for mixtures containing fixed quantity (1.0 μM) of the analyte (ADS or ATP) and varying concentrations of each interferent in the range 1.0 – 8.0 μM. The results obtained are presented in Table 1. Oxidation peak potential of ADS and ATP were found to remain practically constant and the change in current response in the presence of interferents was within ± 15 nA as compared to their absence (Table 1). If concentration of interferents exceeds their reported value in Table 1, i_p varies significantly. Thus ADS and ATP can be safely determined in biological systems despite coexistence of other electroactive compounds, provided their relative concentration does not exceed the limit shown in Table 1.

3.5. Application in Biological Samples

The utility of the proposed method was examined to determine ADS and ATP in biological samples of whole biotic community; i.e., plants as well as animals. *Azadirachta Indica* leaves and sprouted wheat grains samples from plant origin, and urine samples of human body, were analyzed.

The extract of *Azadirachta Indica* leaves exhibited three different peaks in the OSWV. The peak present at ca. 980 mV was due to the presence of ATP (Fig. 6). The spiking of ATP resulted in enhancement of signal present only at 980 mV while all other peaks remain unaffected. The sprouted wheat grain extract exhibited four sharp peaks in the OSWV. The peak at 980 mV corresponded to ATP, which was also confirmed by the spiking experiment. To find

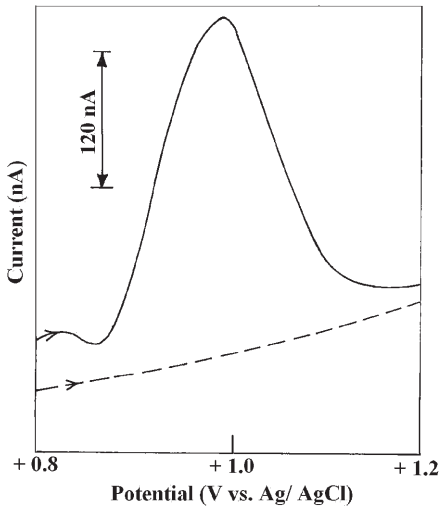

Fig. 6. Square-wave voltammogram observed for the extract of *Azadirachta Indica* leaves (—) corresponding to ATP oxidation and OSWV response for blank PBS (– – –) at NGITO.

Table 1. Influence of common biological electroactive compounds on i_p value of ADS and ATP.

Interferents	Concentration (μM)	Current (nA)	Signal change (nA) [a]
ADS			
(No interferent)	1.0	54.1	–
Ascorbic acid	2.0	55.9	+1.8
Hypoxanthine	8.0	39.7	−14.4
Xanthine	8.0	55.5	+1.4
Uric acid	6.0	66.5	+12.4
ATP			
(No interferent)	1.0	18.0	–
Ascorbic acid	8.0	17.0	−1.0
Hypoxanthine	1.0	9.0	−9.0
Xanthine	8.0	19.0	+1.0
Uric acid	4.0	20.0	+2.0

[a] average of at least three runs.

Table 2. Recovery data observed for ADS and ATP in dilute urine sample.

Spiked (μmol L^{-1})	Detected [a] (μmol L^{-1})	Recovery (%)
ADS		
2.00	2.09	104.5
4.00	3.81	95.3
6.00	6.18	103.0
ATP		
2.00	1.95	97.5
4.00	3.83	95.8
6.00	5.92	98.6

[a] average of at least three runs.

out the peak of ADS, spiking of ADS was done and in both the cases none of the observed peaks show change in i_p. Thus, it is concluded that *A. Indica* leaves and sprouted wheat grain extract do not have free ADS in them.

The recovery tests of ADS and ATP were also carried out by their spiking in highly diluted urine samples. The urine samples were spiked with different amount of ADS and ATP subsequently followed by recording their Osteryoung square-wave voltammograms. The results obtained are listed in Table 2. Recoveries have been found to lie within ±5% of actual spiked value with a relative standard deviation of 1.41% for ATP and 4.9% for ADS.

3.6. Stability of NGITO

Stability and reproducibility of an electrode are the two important features of an electrode performance. Both the features of NGITO were determined to check the utility of the electrodes for the electroanalytical determination of purine nucleosides and nucleotides. To check the reproducibility of the electrode, the variation in current response was observed for five successive sweeps in a solution of fixed concentration. It was observed that the current observed was within 97 – 102% of the i_p observed for the first sweep. Long term stability of NGITO was determined by daily monitoring current response variation for 6.0 μM ADS for one week. The electrode was kept in dry conditions after use and it was found that the electrode retained 95 – 98% current for seven consecutive days after which the peak current values started decreasing with an increase in peak potential. Thus, the electrode is suitable for use for a week. The response time of the electrode was very fast and all measurements were carried out easily and quickly.

4. Conclusions

In the present study, an electroanalytical approach for simultaneous determination of ADS and ATP is reported with applications in biological systems also. The results presented clearly indicate that gold nanoparticles catalyze the oxidation of ADS and ATP by increasing the peak currents and shifting the E_p to less positive potentials in OSWV. The OSWV method is applied for simultaneous determination of ADS and ATP at NGITO. The E_p of ATP which was not detectable at bare ITO shifted to less positive potentials about 50 mV in comparison to gold electrode whereas the oxidation potential of ADS at bare ITO (1160 mV) is reduced to 650 mV at NGITO giving well defined sharp peak. The oxidation peak of ADS can be attributed to the $6e^-$, $6H^+$oxidation of adenosine to diimine, as reported in literature [20]. The mechanism of electro-oxidation of ATP is not reported in literature to the best of our knowledge, however, it appears reasonable to conclude that the oxidation will involve $6e^-$, $6H^+$ as the primary unit in both the compounds is same. Both the compounds can be determined simultaneously in biological samples. It is expected that the method developed will be useful for monitoring the ratio of ADS/ATP in living systems, a deliberately controlled dose of which is believed to replace conventional anesthetics.

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6. References

[1] T. M. Casey, K. G. Dufall, P. G. Arthur, *Eur. J. Biochem.* **1999**, *261*, 740.
[2] R. M. Berne, *Circ Res.* **1980**, *68*, 807.
[3] K. Kroll, G. V. Martin, *Am. J. Physiol.* **1993**, *269*, H503.
[4] A. Deussen, C. W. Flesche, T. Lauer, M. Sonntag, J. Schrader, *Pfluegers Arch.* **1996**, *432*, 451.
[5] H. Osswald, *Trends Pharmacol. Sci.* **1984**, *5*, 94.
[6] A. F. Fukunaga, *United State Patent 6642209*, Pub. Date 04-11-03.
[7] N. Dale, *J. Physiol.* **1998**, *511.1*, 265.
[8] A. Atlante, P. Calissano, A. Bobba, S. Giannattasio, E. Marra, S. Passarella, *FEBS Lett.* **2001**, *497*, 1.
[9] V. Micheli, C. Ricci, S. Sestini, M. Rocchigiani, M. Pescaglini, G. Pompucci, *Adv. Exp. Med. Biol.* **1991**, *309B*, 323.
[10] D. G. Nicholls, S. Budd, *Physiol. Rev.* **2000**, *80*, 315.
[11] K. Mohri, K. Takeuchi, K. Shinozuka, R. A. Bjur, D. P. Westfall, *Anal. Biochem.* **1993**, *210*, 262.
[12] M. Katayama, Y. Matsuda, K. Shimokawa, S. Tanabe, S. Kaneko, I. Hara, H. Sato, *J. Chrom. B* **2001**, *760*, 159.
[13] O. Korchazhkina, G. Wright, C. Exley, *J. Inorg. Biochem.* **1999**, *76*, 121.
[14] S. Xie, G. Shafer, C. G. Wilson, H. B. Martin, *Diamond Related Mater.* **2006**, *15*, 225.
[15] J. R. Aranzaes, C. Belin, D. Astruc, *Angew. Chem. (Int. Ed.)* **2006**, *45*, 132.
[16] H. Radecka, I. Szymanska, M. Pietraszkiewicz, O. Pietraszkiewicz, H. Aoki, Y. Umezawa, *Chem. Analityczna* **2005**, *50*, 85.
[17] K. A. El-Nour, A. Brajter-Toth, *Analyst* **2003**, *128*, 1056.

[18] S. Hason, S. Simonaho, R. Silvennoinen, V. Vetterl, *Electrochim. Acta* **2003**, *48*, 651.
[19] R. Marczak, V. T. Hoang, K. Noworyta, M. E. Zandler, W. Kutner, F. D'Souza, *J. Mater. Chem.* **2002**, *12*, 2123.
[20] R. N. Goyal, A. Sangal, *J. Electroanal. Chem.* **2002**, *521*, 72.
[21] A. Ali Umar, M. Oyama, *Cryst. Growth Des.* **2005**, *540*, 299.
[22] R. N. Goyal, M. Oyama, A. Sangal, S. P. Singh, *Ind. J. Chem., Sec. A: Inorg., Bio-inorg., Phys., Theor. Anal. Chem.* **2005**, *44A*, 945.
[23] G. D. Christian, W. C. Purdy, *J. Electroanal. Chem.* **1962**, *3*, 363.
[24] J. W. Patterson, P. L. Brezonik, H. D. Putnam, *Environ. Sci. Technol.* **1970**, *4*, 569.
[25] P. Wang, X. Wang, G. Zhu, *New J. Chem.* **2000**, *24*, 481.

