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Contributed Paper

# Gold Nanoparticles Decorated on Carbon Nanotube Modified Screen-printed Electrode for Flow Injection Amperometric Determination of Methyldopa

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## ABSTRACT

Gold nanoparticles (AuNPs) decorated on multi-walled carbon nanotubes (MWCNTs) were used as a modifier on the surface of a screen-printed carbon electrode (SPCE) for sensitive determination of methyldopa, which is widely used as an antihypertensive medicine. The electrochemical oxidation of methyldopa on the modified electrode was carried out in flow injection amperometric (FI–amp) system at the applied potential of +400 mV vs Ag/AgCl. As a result, SPCE modified with 20%w/w of gold nanoparticles on carbon nanotube (AuNPs-CNT/SPCE) provided an excellent sensitivity to methyldopa detection. Morphology of AuNPs-CNT was characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy dispersive spectroscopy (EDS), and x-ray diffraction (XRD), which indicated a proper distribution of AuNPs on CNT and high electrode areas. Several parameters that affect the response of the modified electrode were studied. The plot of peak height versus methyldopa concentration showed a linear range of 0.2-100 μM with a detection limit of 0.1 μM. The proposed flow injection amperometric system was successfully applied for methyldopa determination in real samples.

**Keywords:** carbon nanotubes, flow injection analysis, gold nanoparticle, methyldopa, screen printed electrode

## 1. INTRODUCTION

Methyldopa is a catechol derivative which is catecholamine and widely used as an antihypertensive agent for the treatment of high blood pressure or hypertension. Methyldopa inhibits the dopadecarboxylase and metabolizes L-DOPA to the dopamine

which is a precursor of norepinephrine and epinephrine [1]. Methyldopa is converted to methylnorepinephrine in the adrenergic nerve terminals, and its antihypertensive activity is due to the stimulation of the central adrenergic receptors, which reduces the sympathetic tone and produces a fall in blood pressure [2]. The side effects of methyldopa treatment consist of dizziness, dryness of mouth, flatulence, headache, weakness, sedation, and depression [3]. Several analytical methods have been reported for the quantitative determination of methyldopa including spectrophotometry [4, 5], chromatography [6, 7], potentiometry [8], and electrochemical methods [9, 10]. Some methods require a time-consuming, sample pretreatments, and expensive instruments which are inappropriate for routine analysis. Furthermore, electrochemical methods have more attention due to their simplicity, rapidness, high sensitivity, and without requiring pretreatments.

Flow injection analysis (FIA) technique have been employed in many applications because of less amount of chemical reagent and sample consumption, short analysis time, low cost, good reproducibility, and high sample throughput. Some reports have been applied FIA with a spectrophotometer for determination of methyldopa [11, 12], but there was no report on FIA with an electrochemical method for quantification of methyldopa. Therefore, the incorporation of a flow injection system with amperometry (FI-amp) for methyldopa determination should provide advantages as mentioned above.

Various materials were used as a working electrode in the amperometric sensor, such as gold electrode [13], glassy carbon electrode (GCE) [14, 15], and screen-printed electrode (SPE) [16, 17]. In recent years, SPE has received much attention due to its portable, field-based size, disposable, low cost, and highly reproducible. The electrodes modified with nanomaterials have attracted considerable attention due to their specific properties including wide electrochemical windows and good electrical conductivity, fast electron transfer, and especially large surface area [18, 19]. For example, a simple and sensitive sensor was developed using carbon paste electrode modified with chitosan-coated $Fe_3O_4$ magnetic nanoparticle for the electrochemical determination of morphine [20]. Palladium nanoparticles decorated multi-walled carbon nanotubes modified on glassy carbon electrode was applied for the detection of 5-hydroxytryptophan in biological fluids samples [21].

In this work, we developed the FI-amp system for determination of methyldopa. Sensitivity for methyldopa determination was enhanced by modifying a screen-printed carbon electrode with gold nanoparticles decorated on carbon nanotubes. Some parameters affecting sensitivity such as applied potential, flow rate, and pH of solution were optimized. The results demonstrate that the AuNPs-CNT increased electroactive surface area and promoted electron transfer of methyldopa oxidation. The proposed system is convenient operation, sensitive, selective, and reliable for the determination of methyldopa in real sample.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals and Materials

All chemicals were analytical reagent grade, and deionized water was obtained from a system of Milli-Q, Millipore, Sweden. Methyldopa was purchased from Sigma-Aldrich. Working standard solutions were daily prepared and diluted with 0.1 M acetate buffer pH 5.5. Carbon ink (ACHESON, Singapore) was diluted in

diethylene glycol monobutyl ether before screening on a polyvinyl chloride sheet substrate as described in the previous report [22].

### 2.2 Preparation of the AuNPs-CNT Modified SPCE

AuNPs-CNT 0.0020 g was dispersed in 1.00 mL ethanol, and then it was placed into an ultrasonic bath for 60 min. Before modification, the SPCE was treated in a chamber of plasma cleaner for 1 min to explore the more active site of the electrode. An aliquot of 10 µL of the suspension was dropped onto the electrode surface, and the solvent was evaporated at room temperature.

### 2.3 Instrument Setup

A µAutolab type II (Eco Chemie, Utrecht, Netherlands) was used for operating the cyclic voltammetry for characterization of the modified electrodes. The developed flow injection amperometric system is shown in Figure 1, which consisted of acetate buffer carrier solution, a peristaltic pump (Ismatec, Switzerland), an injection valve (I), a mixing coil, an electrochemical cell (EC) which has a SPCE electrode as a working electrode (WE), a stainless steel as an auxiliary electrode (AE), and a Ag/AgCl electrode (3M KCl) as a reference electrode (RE), a homemade amperometer [23] as a detector (D), an analog to digital converter unit for data recording

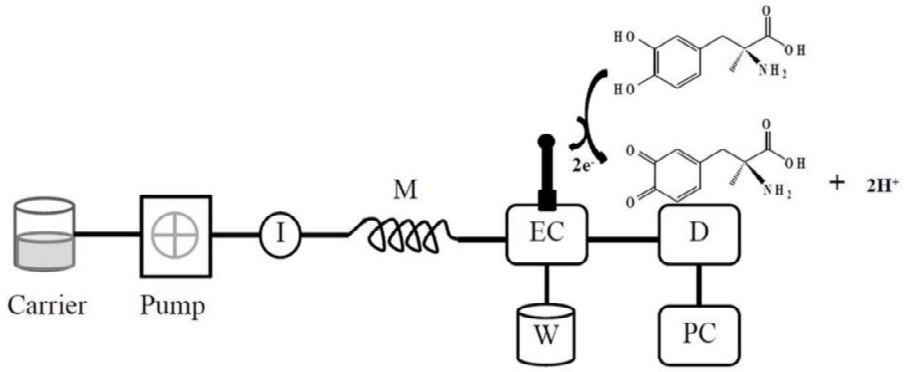


**Figure 1.** FI-amp system for determination of methyldopa.

with the developed software, and a personal computer (PC).

### 2.4 Sample Preparation

Pharmaceutical samples containing methyldopa were acquired from a local drugstore. A tablet of methyldopa was dissolved in 100 mL of DI water by ultrasonication and filtered. Then, the solution was diluted to appropriate concentration with acetate buffer solution. For urine sample preparation, it was kept in the dark place and stored in the refrigerator immediately after collection. 10 mL of urine was filtered out and diluted ten times with acetate buffer for analysis without any further pretreatment. All measurements were carried out by the FI-amp system using the standard addition method and performed in triplicate.

## 3. RESULTS AND DISCUSSION

### 3.1 The Working Electrode

An SPCE of 3 mm diameter was applied as a working electrode in the flow injection amperometric system for

determination of methyldopa because of the large surface area, low cost, disposable, small size, portable, and easy to modify. To obtain the higher sensitivity, noble metal nanoparticles on nanocarbon support and conducting polymer were used to modify on the electrode surface such as gold nanoparticles decorated on carbon nanotube (AuNPs-CNT), bimetallic of PtNPs-AuNPs on CNT, graphene oxide (GO), and reduced graphene oxide with polydopamine (PDArGO). Figure 2 shows the sensitivities of various modified electrodes on methyldopa determination. It was found that AuNPs-CNT/SPCE gave the highest sensitivity due to the synergistic between carbon nanotube and gold nanoparticles which improved electron transfer and increased the surface area of the electrode. Therefore, this modified electrode was chosen as a working electrode for further experiments.

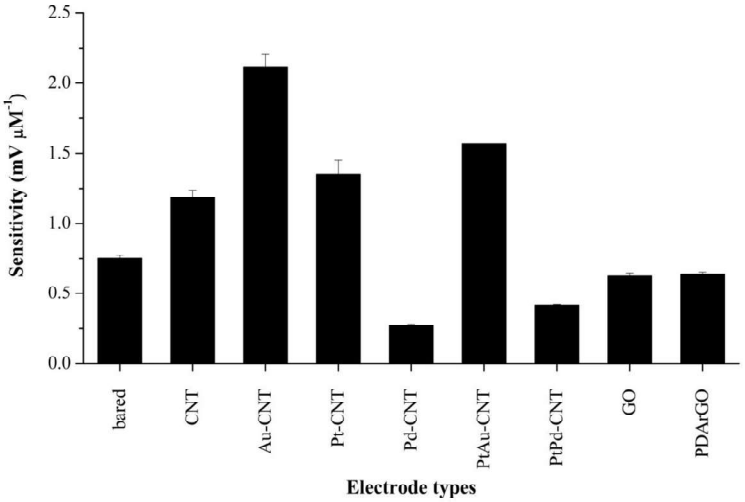


**Figure 2.** The sensitivity on the various modified electrodes for methyldopa detection.

To study the effect of %loading of AuNPs, the parameter was investigated in the range of 10-30%. The highest sensitivity was obtained at 20% of AuNPs on CNT and decreased after %loading more than 20% because there were agglomeration and low dispersion of AuNPs on CNT leading to the decrease in surface area. Thus, 20% loading of AuNPs on CNT was chosen for the next study.

### 3.2 Characterization of the AuNPs-CNT

The morphology of nanostructures was characterized by SEM and TEM techniques. The electrode surface was covered with well dispersed AuNPs-CNT as shown in the SEM image (Figure 3a) and TEM image in Figure 3b confirms that AuNPs is decorated on CNT and diameter of CNT and AuNPs were smaller than 30 and 10 nm, respectively. Moreover, the elemental composition of nanoparticle was corroborated by EDS measurement as shown in Figure 3c indicating that the major compositions are carbon and gold. At last the crystallization and surface compositions of AuNPs-CNT were also investigated by XRD experiment. Figure 3d shows a typical AuNPs-CNT peaks pattern of AuNPs and CNT, which the peaks at 25.9° corresponding to C (002) of characteristic of CNT (carbon) materials. While four characteristic peaks at 38.2°, 44.3°, 64.7° and 77.7° were assigned to the (111), (200), (220)

and (311) crystal planes of face centered cubic (fcc) AuNPs (JCPDS 4-0783), respectively. The results further ascribe to the presence of the AuNPs structures decorated on CNT. According to the Scherrer equation [24, 25], the average particle size of AuNPs was calculated about 6.5 nm, consistent with TEM results.

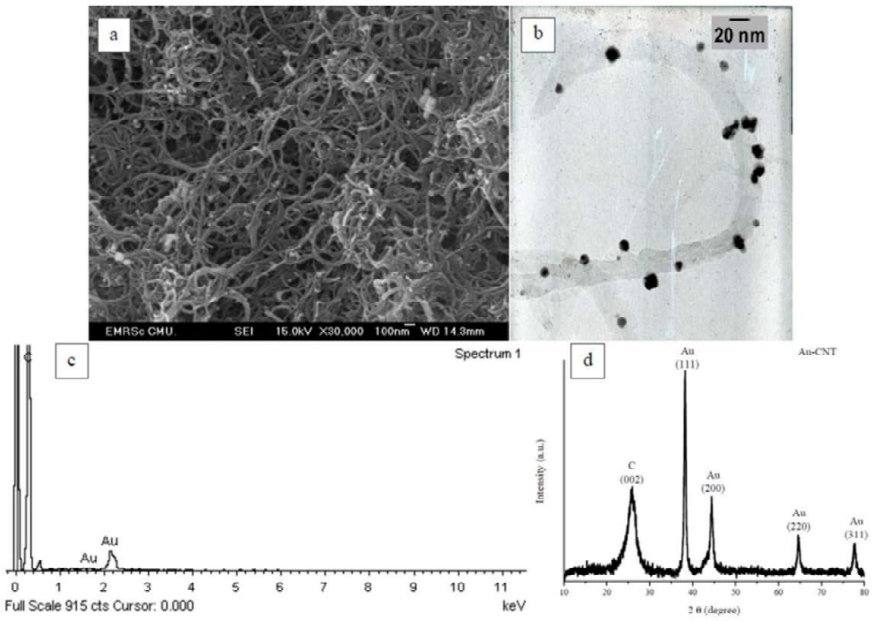


**Figure 3.** a) SEM image, b) TEM image, c) EDS spectrum, and d) XRD pattern of AuNPs-CNT.

### 3.3 Electrochemical Characterization of the Modified Electrode

The modified electrodes were characterized using cyclic voltammetry to study the feature of the electrodes. Figure 4 shows the cyclic voltammograms of different modified electrodes in the potential range of -1.0 to +1.0 V in 0.1 M acetate buffer solution pH 5.5 at a scan rate of 50 mV $s^{-1}$. SPCE provided poor redox peaks in Figure 4a, which might be the low conductivity of the bared electrode. After modification with CNT in Figure 4b, the electrode exhibited higher peak current indicating that CNT can improve the properties of the electrode. Furthermore, well-defined peaks and the increased peak current were obtained at SPCE modified with AuNPs-CNT as shown in Figure 4c, suggesting the larger surface area and faster electron transfer rate due to the good conductivity of carbon nanotube and gold nanoparticles.

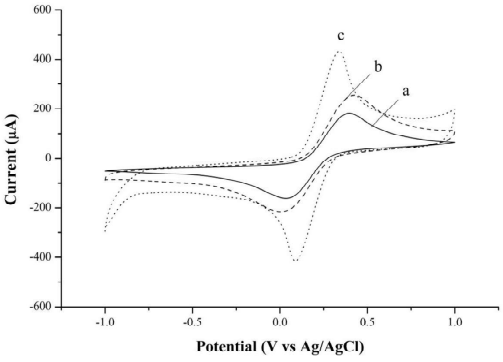


**Figure 4.** Cyclic voltammograms of a) SPCE, b) CNT/SPCE, and c) AuNPs-CNT/SPCE in 10 mM $[Fe(CN)_6]^{3-/4-}$ solution.

Electrochemical impedance spectroscopy (EIS) is an effective method to investigate the interfacial properties of surface-modified electrode. The electron-transfer resistance ($R_{et}$) is the most directive and sensitive parameter that responds on the change of the electrode interface, as represented by the diameter of the semicircle in the Nyquist plot. The result in Figure 5a presented that the bare SPCE exhibited large $R_{et}$ while SPCE modified with CNT showed decreasing of $R_{et}$ as displayed in Figure 5b due to an excellent conductivity of CNT providing more conductive of the electrode. Then a further decrease of $R_{et}$ was observed after AuNPs-CNT was modified on the electrode in Figure 5c, this may be ascribed to that AuNPs-CNT has an excellent potential to be an electrical conductive material which is also able to accelerate the electron transfer reaction. Moreover, it was in good agreement with the CV results.

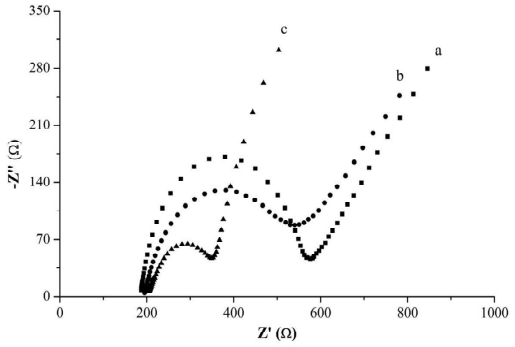


**Figure 5.** Nyquist plots of the different electrodes. a) SPCE, b) CNT/SPCE, and c) AuNPs-CNT/SPCE in 10 mM $[Fe(CN)_6]^{3-/4-}$ solution.

### 3.4 Optimization of the FI-Amp Sensor

An SPCE modified with AuNPs decorated on CNT was used as a working electrode in the FIA system. To obtain better sensitivity, some parameters of FIA that affected the methyldopa determination were optimized by considering the sensitivity of methyldopa in the range of 10-50 μM. First, the applied potential was studied in the positive potential range of +300 to +500 mV vs Ag/AgCl. The result indicated that the sensitivity increased with increasing of applied potential and no significant difference at +400 mV hereafter. Therefore, the applied potential of +400 mV should be selected as the optimum potential in order to avoid interference from substances that could be electrochemically oxidized at higher positive potential [26].

Then the appropriate flow rate was investigated by varying at 1.0, 1.3, 1.5, 1.8, and 2.0 mL $min^{-1}$. The best sensitivity was obtained at 1.5 mL $min^{-1}$. For a reason, it might be well diffused of methyldopa in the microtube at slower flow rate leading to more dilution (low concentration) and get low signal, and at faster flow rate also obtained low sensitivity as the zone of methyldopa fastly passed the working electrode, and methyldopa cannot oxidize effectively at a short time.

The injected sample volume is one of the effective ways to achieve higher sensitivity. Therefore, the sample volume was optimized in the range of 50 to 300 μL. The sensitivity increased with increasing of sample volume and quite stable at 200 μL onward. Thus, the injected sample volume of 200 μL was chosen because it offered good sensitivity and used a small amount of sample and reagent solutions.

Methyldopa as a catechol derivative can be oxidized at positive potential, which also depends on pH of the solution [27]. The electrocatalytic oxidation of methyldopa is a proton involved reaction, so the effect of pH of 0.1 M acetate buffer solution on the response of methyldopa was investigated over the pH range of 4.0-7.0. The sensitivities also changed with pH with the largest sensitivity appearing at pH 5.5 which is in

agreement with those previously obtained for detection of methyldopa [28] and this value was used throughout the experiments.

### 3.5 Analytical Performance of the Sensor

Under the optimum conditions, the electrooxidation of methyldopa was investigated to obtain the calibration graph. Figure 6a shows the FIAgram of methyldopa oxidation at AuNPs-CNT/SPCE in the dynamic range of 0.2-500 μM. The peak heights were linearly proportional to the concentrations of methyldopa over the range of 0.2-100 μM as presented in Figure 6b and a linear regression equation was y (mV) = 1.8635 + 4.0045x (μM) with a correlation coefficient of 0.9987 where the detection limit was 0.1 μM (based on S/N = 3).

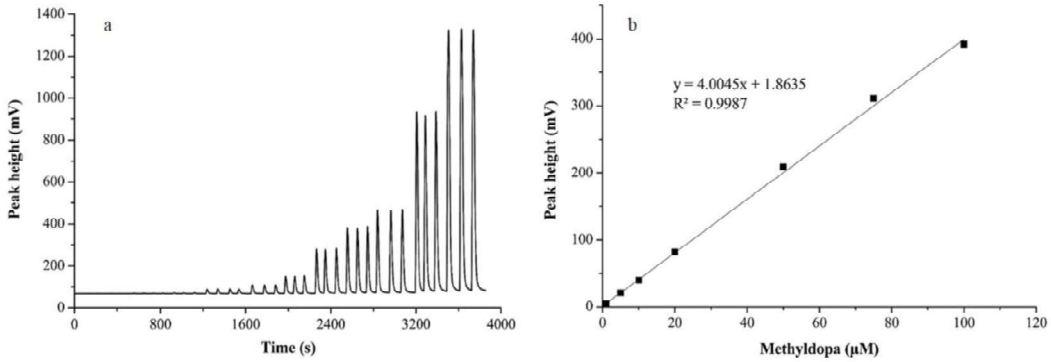


**Figure 6.** FIAgram a) of signal response to methyldopa concentration at 0.2. 0.5, 1, 5, 10, 20, 50, 75, 100, 250, and 500 μM and b) the calibration graph of 0.2-100 μM.

Table 1 shows the figures of merit of the proposed sensor comparing with the other modified electrodes. It can be found that the FI-amperometric sensor offered a comparable detection limit for methyldopa determination to other reported methods. Moreover, this work also provided more advantages such as simple and convenient operation, high sample throughput (36 $h^{-1}$), cost-effective, and fast analysis.

**Table 1.** Comparison of the characteristics of some reported electrochemical methods for the determination of methyldopa.

| Modified electrode | Technique | Linear range (μM) | LOD (μM) | Sample throughput ($h^{-1}$) | Ref. |
|---|---|---|---|---|---|
| CA/BMI·N(Tf)$_2$/CPE | SWV | 34.8-370.3 | 5.5 | Normal | [28] |
| ILs/MWCNTPE | SWV | 0.4-400 | 0.1 | Normal | [29] |
| $NiFe_2O_4$/MWCNTs/GCE | DPV | 0.5-900 | 0.08 | Normal | [27] |
| TGA-capped CdSe@$Ag_2Se$/GCE | DPV | 0.09-60 | 0.04 | Normal | [30] |
| AuNPs-CNT/SPCE | FI-amperometry | 0.2-100 | 0.1 | High | This work |

CA = cellulose acetate; BMI·N(Tf)$_2$ = 1-butyl-3-methylimidazolium bis(trifluoromethyl-sulfonyl)imide; CPE = carbon paste electrode; SWV = square wave voltammetry; ILs = ionic liquids; MWCNTPE = multiwall carbon nanotubes paste electrode; MWCNTs = multiwall carbon nanotubes; DPV = differential pulse voltammetry; CMWCNTs = carboxylated multiwall carbon nanotubes; ASV = adsorptive stripping voltammetry; TGA = thioglycolic acid.

In addition, the reproducibility of the sensor was investigated by measuring the signal of 10 μM methyldopa. Eleven modified electrodes were prepared using the same procedure, and the results showed an acceptable relative standard deviation (RSD) of 3.6%, demonstrating that the sensor has good reproducibility. Although the SPCE is a disposable electrode, we interested to study the stability of the modified SPCE for seven days when the electrode was stored at 4 °C. 86% of its initial signal response was maintained, indicating that AuNPs-CNT modified on SPCE has excellent stability. In order to evaluate selectivity of the developed method, the effect of possible interfering substances which are common species presented in pharmaceutical and biological samples were investigated and the peak heights of 10 μM methyldopa without and with interference species were compared. The results showed that 500-fold of $Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$, $F^-$, $Cl^-$, $NH_4^+$, $SO_4^{2-}$, $ClO_4^-$, glucose, sucrose, fructose, lactose, urea, ethanol, and tartaric acid, 300-fold of citric acid, 10-fold of uric acid, 4-fold of ascorbic acid and saturated starch solution did not affect the current response because relative error percentages of the signal were less than 5%, showing a good selectivity of the sensor.

### 3.6 Sample Analysis

To evaluate the applicability of the proposed sensor, pharmaceutical and urine samples were analyzed using the FI-amp system. The standard addition method was used to determine methyldopa in samples and investigated the percentage of recovery. Different concentrations of methyldopa were spiked into samples, and the recoveries were obtained in a range of 101.6-105.0% as presented in Table 2, indicating that the developed sensor is accurate, effective, and it could be applied for the determination of methyldopa in real samples.

**Table 2.** Determination of methyldopa in drug and urine samples using the proposed sensor.

| Sample | Add (μM) | Expected (μM) | Founded (μM) | % Recovery |
|---|---|---|---|---|
| Tablet | - | 5.0 | 5.24 | 104.8 |
| | 1.0 | 6.0 | 6.17 | 102.8 |
| | 10.0 | 15.0 | 15.48 | 103.2 |
| Urine | 1.0 | 1.0 | 1.05 | 105.0 |
| | 10.0 | 10.0 | 10.16 | 101.6 |

## 4. CONCLUSION

In this study, the gold nanoparticles decorated on carbon nanotube modified screen-printed carbon electrode was used in the flow injection amperometric sensor for determination of methyldopa. The modified electrode shows an effective synergistic effect in the electrochemical oxidation of methyldopa leads to an improvement in the sensitivity of the detection dramatically. The proposed sensor displays an advantage of simple preparation and operation, good reproducibility and stability, and high selectivity and sensitivity. Moreover, the sensor was successfully applied for the determination of methyldopa in tablet and urine samples with satisfactory results.

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## REFERENCES

[1] Shahrokhian S. and Rastgar S., *Electrochim. Acta*, 2011; **58**: 125-133.

[2] Perez-Mella B. and Alvarez-Lueje A., *Electroanalysis*, 2013; **25**: 2193-2199.

[3] Spratto G.R. and Woods A.L., *Delmar Nurse's Drug Handbook 2011: Special 20 Year Anniversary*, Delmar Cengage Learning, 2011.

[4] Chamsaz M., Safavi A. and Fadaee J., *Anal. Chim. Acta*, 2007; **603**: 140-146.

[5] Gadkariem E.A., Ibrahim K.E.E., Kamil N.A.A., Haga M.E.M. and El-Obeid H.A., *Saudi Pharm. J.*, 2009; **17**: 289-293.

[6] Zecevic M., Zivanovic L., Agatonovic-Kustrin S. and Minic D., *J. Pharm. Biomed. Anal.*, 2001; **24**: 1019-1025.

[7] Muzzi C., Bertocci E., Terzuoli L., Porcelli B., Ciari I., Pagani R. and Guerranti R., *Biomed. Pharmacother.*, 2008; **62**: 253-258.

[8] Athanasiou-Malaki E.M. and Koupparis M.A., *Anal. Chim. Acta*, 1984; **161**: 349-353.

[9] Ramirez C., del Valle M.A., Isaacs M. and Armijo F., *Electrochim. Acta*, 2016; **199**: 227-233.

[10] Fouladgar M. and Ahmadzadeh S., *Appl. Surf. Sci.*, 2016; **379**: 150-155.

[11] Tubino M., Junior A.R. and Vila M.M.D.C., *J. Flow Injection Anal.*, 2004; **21**: 132-136.

[12] Ribeiro P.R.S., Neto J.A., Pezza L. and Pezza H.R., *Talanta*, 2005; **67**: 240-244.

[13] Chawla S., Rawal R. and Pundir C.S., *J. Biotechnol.*, 2011; **156**: 39-45.

[14] Jain R., Dhanjai and Sinha A., *Appl. Surf. Sci.*, 2016; **369**: 151-158.

[15] Rahman M.M., Ahmed J. and Asiri A.M., *Sens. Actuators B*, 2017; **242**: 167-175.

[16] Raj M., Gupta P., Goyal R.N. and Shim Y.B., *Sens. Actuators B*, 2017; **239**: 993-1002.

[17] Alonso-Lomillo M.A., Dominguez-Renedo O., Saldana-Botin A. and Arcos-Martinez M.J., *Talanta*, 2017; **174**: 733-737.

[18] Anuar N.S., Basirun W.J., Ladan M., Shalauddin M. and Mehmood M.S., *Sens. Actuators B*, 2018; **266**: 375-383.

[19] Nawaz M.A.H., Majdinasab M., Latif U., Nasir M., Gokce G., Anwar M.W. and Hayat A., *J. Pharm. Biomed. Anal.*, 2018; **159**: 398-405.

[20] Dehdashtian S., Gholivand M.B., Shamsipur M. and Kariminia S., *Mater. Sci. Eng. C*, 2016; **58**: 53-59.

[21] Kumar N., Rosy R. and Goyal R.N., *Sens. Actuators B*, 2017; **239**: 1060-1068.

[22] Reanpang P., Themsirimongkon S., Saipanya S., Chailapakul O. and Jakmunee J., *Talanta*, 2015; **144**: 868-874.

[23] Thunkhamrak C., Reanpang P., Ounnunkad K. and Jakmunee J., *Talanta*, 2017; **171**: 53-60.

[24] Themsirimongkon S., Sarakonsri T., Lapanantnoppakhun S., Jakmunee J. and Saipanya S., *Int. J. Hydrogen Energ.*, 2018; (Article in press).

[25] Zhang Q., Fan X., Wang H., Chen S. and Quan X., *RSC Adv.*, 2016; **6**: 41114-41121.

[26] Vahedi J., Karimi-Maleh H., Baghayeri M., Sanati A.L., Khalilzadeh M.A. and Bahrami M., *Ionics*, 2013; **19**: 1907-1914.

[27] Ensafi A.A., Saeid B., Rezaei B. and Allafchian A.R., *Ionics*, 2015; **21**: 1435-1444.

[28] Moccelini S.K., Franzoi A.C., Vieira I.C., Dupont J. and Scheeren C.W., *Biosens. Bioelectron.*, 2011; **26**: 3549-3554.

[29] Fouladgar M. and Karimi-Maleh H., *Ionics*, 2012; **19**: 1163-1170.

[30] Asadpour-Zeynali K. and Mollarasouli F., *Sens. Actuators B*, 2016; **237**: 387-399.