Electrochemical Determination of Adrenaline at Ru(III) Schiff Base Complex Modified Carbon Electrodes

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Abstract: The anodic oxidation of adrenaline on ruthenium(III) Schiff base complex modified carbon electrodes was used for determination of adrenaline by flow-injection analysis, cyclic voltammetry, differential pulse voltammetry and hydrodynamic amperometry. The electrocatalytic properties of ruthenium(III) complex at glassy and screen printed carbon electrodes were enhanced by addition of cellulose acetate and multi-walled carbon nanotubes. Flow injection amperometric measurements were performed at 100 mV *vs.* Ag / AgCl in 0.1 M phosphate buffer pH 7.5 at 0.4 mL min⁻¹ flow rate. Novel sensor provided a linear dynamic range up to 50 mg L⁻¹ of adrenaline with detection limit of 53 µg L⁻¹ at physiological pH. Determination of adrenaline in commercial sample was carried out by flow-injection method with excellent recoveries 99.8–101 %.

Keywords: adrenaline, multi-walled carbon nanotubes, Ru(III) complex, Schiff base, flow injection amperometry, cyclic voltammetry, differential pulse voltammetry.

INTRODUCTION

ADRENALINE (AD), also known as epinephrine, is a hormone, neurotransmitter and medicament. Fast and reliable determination of adrenalin with low detection limits has huge importance due to its significance. It plays an important role as a mediator of stress caused by the development of anxiety disorders and depression.[1] Adrenalin is used as the medication for several conditions related to anaphylaxis, bleeding and cardiac arrest.[2] In blood it affects the blood pressure regulation, heart rate and glycogen metabolism.[2]

Many methods are available for determination of adrenaline[3] but electrochemical methods have several advantages such as low cost, simplicity, speed of determination, high sensitivity and low detection limits.[4]

Many electrochemical sensors have been developed for the adrenaline determination, based on carbon electrodes modified with various electron transfer mediators, such as poly(caffeic acid), poly(L-aspartic acid), poly(indoleacetic acid), poly(L-methionine), 2-(4-Oxo-3-phenyl-3,4-dihy-droquinazolinyl)-*N'*-phenyl-hy-drazinecarbothioamid, valine, MnO_2 /Nafion, osmium complex and ruthenium complex (ruthenium oxide/ferrocyanide).[5–15]

Ruthenium complexes are being the subject of great interest due to prominent properties as biologically active compounds,[16–18] efficient catalyst[19] and good electrochemical mediators.[20,21] In this study, we report the development of an efficient and stable sensor based on water insoluble mediator, Sodium bis[*N*-2-oxyphenyl-5-bromosalicylideneiminato-*ONO*]ruthenate(III) complex (hereinafter referred as Na[RuL₂] complex), cellulose acetate and multi-walled carbon nanotubes (MWCNTs) for sensitive and selective adrenaline determination in pharmaceutical samples.

EXPERIMENTAL

Reagents and Solutions

All chemicals were supplied from commercial sources as analytically pure. The synthesis of Na[RuL$_2$] complex was carried out by reported procedure.[22] Multi-walled carbon nanotubes (type: L.MWCNTs-1030) were purchased from Advanced Chemicals. Adrenaline (Epinephrine) was purchased from Sigma Aldrich. An ampoule of 1 mg mL^{-1} adrenaline was obtained from Rotex Medica Trittau, Germany. All measurements were carried out using helium deoxygenated 0.1 M phosphate buffer pH 7.5 prepared with doubly distilled water. Adrenaline stock solutions were prepared in buffer by dissolving appropriate amount of adrenaline and used immediately. Working solutions of adrenaline were prepared prior to measurements by diluting stock solution or sample solution of adrenaline from ampoule with buffer.

Electrode Preparation

Alumina (0.05 µm) was used for glassy carbon electrode polishing until mirror shine was obtained. Screen printed electrodes were prepared on inert porcelain plates (Coors Ceramic GmbH, Chattanooga, TN, USA). Six modified electrodes with Na[RuL$_2$] complex, cellulose acetate, multiwalled carbon nanotubes and Nafion® were prepared. Electrode modification mixture was prepared by mixing the ruthenium complex (4 mg) dissolved in 50 µL of ethanol and depending on the modification: 2 mg of multi-walled carbon nanotubes, 2 mg cellulose acetate and 5 µL 0.05 % Nafion. The mixtures were homogenized for 15 minutes with ultrasound (Ultraschallgenerator PHYWE) before electrode modification. The glassy carbon and screen printed electrodes were modified by adding 5 µL of homogenous suspension and dried for 30 minutes at room temperature.

Apparatus

All measurements were performed with Autolab PGSTAT-12 potentiostat / galvanostat with GPES software (Autolab software version 4.9). Modified and unmodified glassy and screen printed electrodes were used as working electrodes. The flow-injection system consisted of a high performance liquid chromatographic pump (Model 510, Waters, Milford, MA, USA), a sample injection valve (U6K, Waters), and a thin-layer electrochemical cell (CC5, BAS Bioanalytical systems Inc., West Lafayette IN, USA). Teflon spacers (MF-1047, MF-1048, BAS) were used to adjust the thickness of the flow-through cell. A conventional three-electrode flow cell BAS 100 (BASi Dual 3mm glassy carbon electrode MF-1000 for thin layer flow cells, BAS CC-5) was used for measurements. Silver/silver chloride electrode (3M KCl, model RE-1, BAS) and the back plate of flow cell were used as the reference and counter electrode, respectively.

For hydrodynamic amperometry, cyclic voltammetry and differential pulse voltammetry, a three electrode cell was used with a platinum wire as the counter electrode, Ag / AgCl (Model 6.1227.000; Metrohm) as the reference electrode, and a modified glassy carbon or screen printed working electrode. The pH values were measured using pH meter (Thermo Orion, model 210+; Orion, Model SA 720) with the appropriate pH electrodes (SenTix 22 plus (A043019007).

Measurement Procedure

All measurements were performed in 0.1 M pH 7.5 phosphate buffer at ambient temperature using Ag/AgCl as reference electrode. Flow-injection analyses were performed at applied potentials of 100 mV at 0.40 mL min^{-1} flow rate with 100 µL injection volume. Hydrodynamic amperometric was performed at applied potentials of 100 mV. Cyclic voltammograms were recorded in -300 – 400 mV potential region using 50–400 mV s^{-1} scan rates at 20 mV step potential and 2 scans *per* measurements. Differential pulse voltammograms were recorded in from −750 to 750 mV potential region using 120 mV step potential, 400 mV modulation amplitude, 0.006 s modulation time and 0.6 s interval time.

RESULTS AND DISCUSSION

Mediating Properties of Ru(III) Schiff Base Complex

Cyclic voltammetry, differential pulse voltammetry and hydrodynamic amperometry were used to demonstrate mediating properties of Na[RuL$_2$] complex for adrenaline oxidation at modified glassy carbon electrode.

Typical cyclic voltammograms of adrenaline at unmodified and Na[RuL$_2$] modified glassy carbon electrode are shown in Figure 1A. Well-defined anodic peak obtained using complex modified glassy carbon electrode that appeared at +220 mV clearly indicates excellent mediating properties of Ru(III) Schiff base complex for oxidation of adrenaline. At bare glassy carbon electrode adrenaline showed broad oxidation wave at wide potential range (−0.2 V to 0.3 V) with almost constant amperometric response (about 5 µA). On contrary, well-defined anodic peak of adrenaline oxidation was found at Na[RuL$_2$] modified GC electrode (I_{pa} = 52.50 µA).

Mediating role of Ru^{III}/Ru^{II} pair for oxidation of adrenaline at glassy carbon electrode was demonstrated also using differential pulse voltammetry. Better defined anodic peak corresponding to adrenaline oxidation is observed at Ru(III) complex modified compared to unmodified glassy electrode (Figure 1B). Furthermore, hydrodynamic amperometry for adrenaline oxidation at +100 mV operating potential showed fast response and
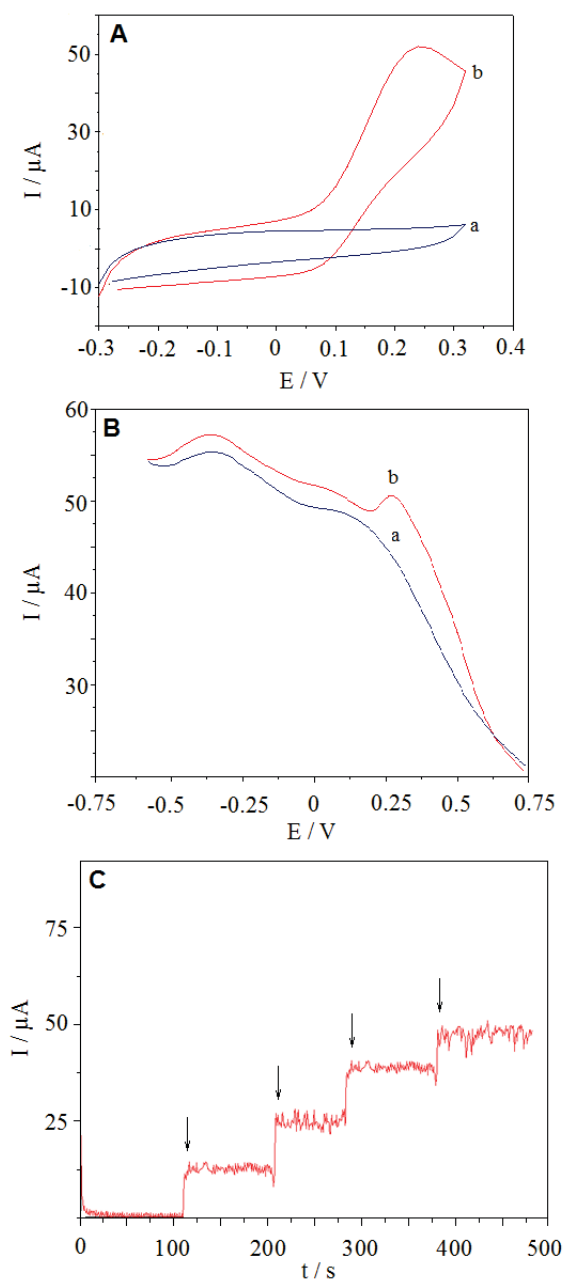
Figure 1. Behavior of adrenaline (200 mg L^{-1}) at (a) unmodified and (b) Na[RuL$_2$] modified GCE electrode in 0.1 M phosphate buffer pH 7.5 measured by different techniques: (A) cyclic voltammetry; (B) differential pulse voltammetry and (C) hydrodynamic amperometry at 100 mV potential (arrows indicate successive additions of adrenaline).

reproducibility of new adrenaline sensor based on Ru(III) Schiff base complex as mediator (Figure 1C).
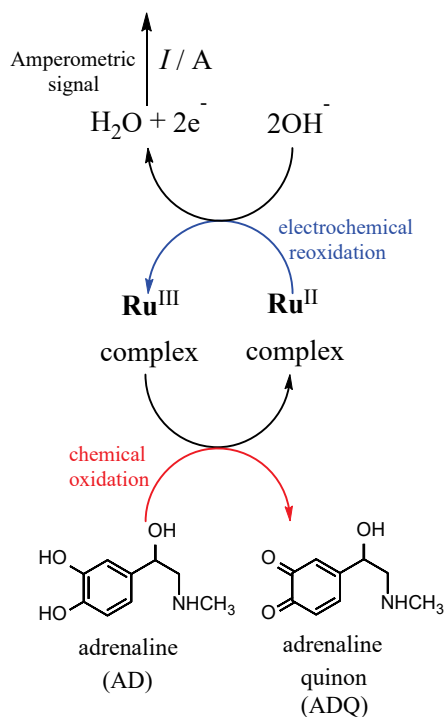
Scheme 1. Adrenaline oxidation at the surface of Na[RuL$_2$] modified electrodes.

At the surface of Na[RuL$_2$] modified glassy carbon electrode adrenaline is oxidized to adrenaline quinone, while Ru(III) is reduce to Ru(II). Generated Ru(II) is than electrochemically oxidized back to Ru(III) generating an oxidation current proportional to the adrenaline concentration (Scheme 1).

Electrode Kinetics of Modified GCE

In order to investigate kinetics of Ru(III) Schiff base complex modified glassy carbon electrode cyclic voltammetry was used. Cyclic voltammograms of adrenaline in 0.1 M phosphate buffer pH 7.5 were collected at 50 to 400 mV s^{-1} scan rates (Figure 2A). Linear relationships between peak currents and scan rate (v) (Figure 2B) and peak current and square root of the scan rate ($v^{1/2}$) (Figure 2C) was found.

It has been reported that the relationship between the redox peak current and the scan rate may be expressed by the following relationships: [35–37]

$$I_{pa} = kv^x \tag{1}$$

$$\log I_{pa} = \log k + x \log v \tag{2}$$

where I_{pa} is the anodic current density (mA cm^{-2}), v is scan rate (mV s^{-1}), k is a proportionality constant and x is the scan rate exponent. Since the electrode kinetic meets (1) electrochemical redox reaction can be under control of: (*i*)
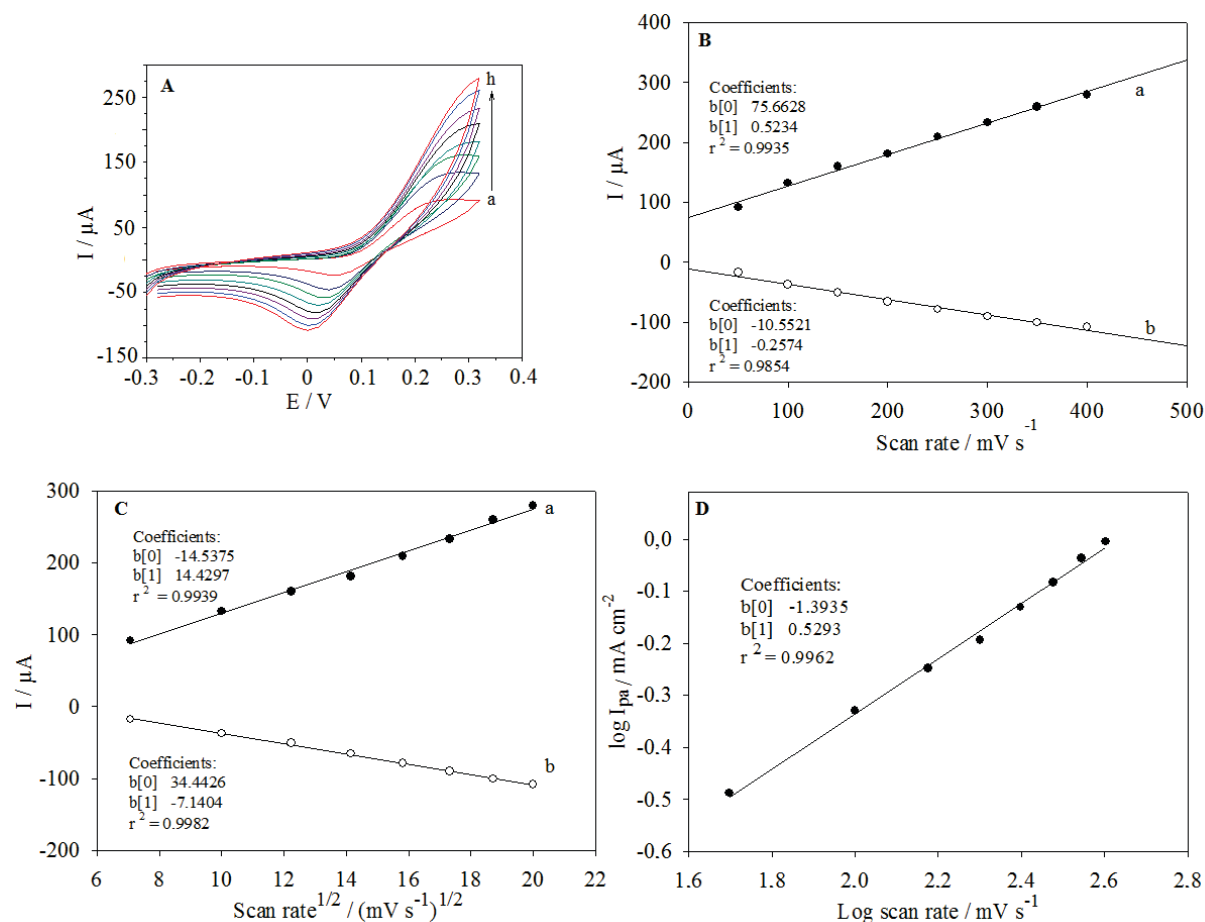
Figure 2. Cyclic voltammograms of adrenaline (200 mg L^{-1}) in 0.1 M phosphate buffer pH 7.5 at Na[RuL$_2$] / GCE using various scan rates (a – 50, h – 400 mV s^{-1}); (B) Plot of the peak current (a – anodic, b – cathodic) *vs* scan rate; (C) Plot of the peak current logarithm (a – anodic, b – cathodic) *vs* scan rate square root; (D) Plot of the peak current logarithm *vs* scan rate logarithm.

electron transfer (x = 1) and (*ii*) reactant diffusion (x = 0.5). Anodic current density logarithm (log I_{pa}) as a function of the scan rate logarithm (log v) showed linear relationship for oxidation of adrenaline at glassy carbon electrode (Figure 2D). The exponent of the scan rate is found to be 0.5293 indicating that the diffusion of adrenaline from the bulk solution to the electrode surface is rate limiting.

Optimisation of Working Conditions

Since Ru(III) Schiff base complex showed excellent mediating properties of adrenaline oxidation operating conditions for analytical determination of adrenaline were optimized using flow injection analysis by the means of working potential and pH.[23] The most important parameter for the amperometric response of the sensor is the operating potential. Figure 3A shows dependence of the amperometric response of adrenaline oxidation and

background current to applied potential in -300 to 400 mV range. The modified electrode demonstrates low background current in the broad potential range. Optimal working potential was found to be +100 mV since: (*i*) it is adequately low to reduce any interferences, (*ii*) background current is stable and approaches in nanoamper scale to zero, (*iii*) amperometric response is satisfactory and highly reproducible. Dependence of amperometric response and background current to pH at +100 mV operating potential was investigated in 3–9 pH range (Figure 3B). At acidic conditions (pH 3–6) background current and adrenaline amperometric response are very unstable and irreproducible. At neutral and slightly basic conditions (pH 7–9) background current is stabilized around zero and current response of adrenaline oxidation increases. Although the current response is higher at pH 9 measurements were performed at pH 7.5 since there is a
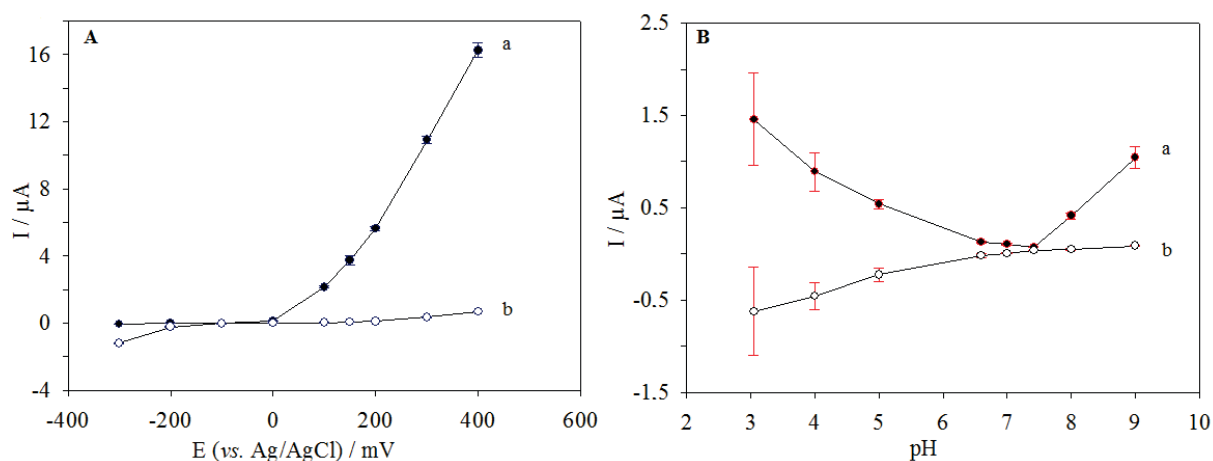
Figure 3. Dependence of flow injection amperometric response of (a) adrenaline (200 mg L^{-1}), (b) background current on (A) applied potential at pH 7.5 and (B) applied pH in 0.1 M phosphate buffer using 0.4 mL min^{-1} flow rate and 100 µL injection volume at 0 mV.

need for imitation of physiological ambient in biological fluids and pharmacological products. Moreover, at pH 9 reproducibility of adrenaline amperometric response is slightly lower.

Performances of the Sensor

Linearity and detection limit were investigated in 0.1 M phosphate buffer at three Ru(III) Schiff base complex modified carbon electrodes: one glassy carbon and two screen printed carbon electrodes with addition of (*i*) cellulose acetate or (*ii*) multi-walled carbon nanotubes (Table 1).

The widest linearity was obtained at screen printed carbon electrode modified with complex and multi-walled carbon nanotubes (Figure 4). Additionally, its lowest detection limit, among used electrodes, indicate that mediating properties of Ru(III) Schiff base complex for adrenaline oxidation can be improved by addition of multi-walled carbon nanotubes. The detection limit is given as triple standard deviation (3σ) of ten successive

Table 1. Linearity and detection limit of adrenaline determination at modified electrodes in 0.1 M phosphate buffer pH 7.5 at 100 mV operating potential and 100 µL injection volume

Electrode	Linearity / mg L^{-1}	LOD / mg L^{-1}
Na[RuL$_2$] / GCE	0.5 – 25	0.64
SP / Na[RuL$_2$] / cellulose acetate / CE	5 – 50	0.24
SP / Na[RuL$_2$] / MWCNTs / CE	0.5 – 50	0.05
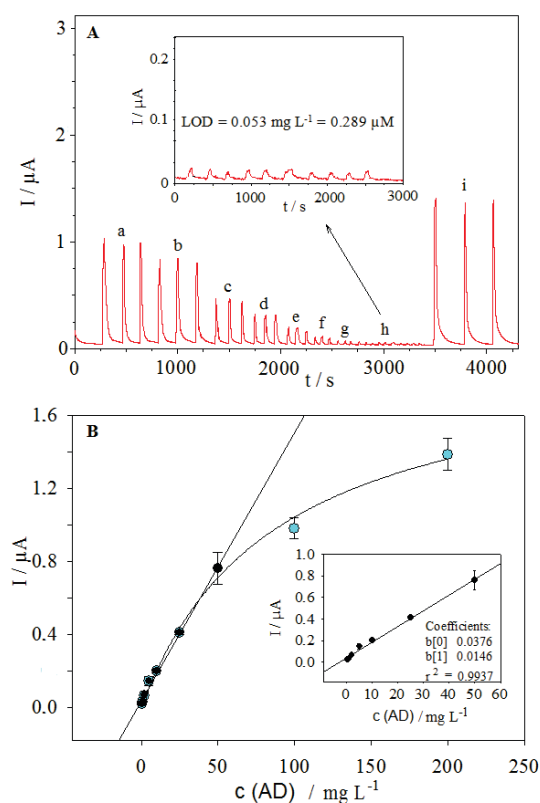
Figure 4. (A) Amperometric flow injection response for various concentrations of adrenaline (a – 100, b – 50, c – 25, d – 10, e – 5, f – 2, g – 1, h – 0.5, j – 200 mg L^{-1}) at SP / Na[RuL$_2$] / MWCNTs / CE in 0.1 M phosphate buffer pH 7.5; working conditions: potential 100 mV, flow rate 0.4 mL min^{-1} and an injection volume of 100 µL. (B) Calibration curve based on (A).

Table 2. Comparison of the proposed sensor for adrenaline determination with others described in the literature

Method	Linearity / μM	LOD / μM	Ref	
A	10 – 110	2.54	[3]	
CV	2 – 300	0.60	[9]	
CV	10 – 20 0.5 – 1.0	0.10	[25]	
CV	20 – 4000	7.00	[27]	
CV	4.5 – 10 10 – 140	0.76	[29]	
CV	0.1 – 8.0 10 – 100	0.04	[30]	
CV	10 – 200	3.40	[31]	
DPV	0.1 – 500	0.04	[26]	
DPV	1 – 600	0.20	[28]	
DPV	10 – 400 400 – 6000	2.80	[32]	
DPV	0.1 – 10	0.052	[34]	
FIA	0.02 – 100	0.007	[33]	
FIA	50 – 350	15.00	[24]	
FIA	3 – 136	3.49		1
FIA	27 – 273	1.35	This work	2
FIA	3 – 273	0.289		3

FIA - Flow injection amperometry, DPV - Differential pulse voltammetry, CV – Cyclic voltammetry, A – Amperometry, 1 – Na[RuL₂] / GCE, 2 – SP / Na[RuL₂] / cellulose acetate / CE, 3 – SP / Na[RuL₂] / MWCNTs / CE.

measurements of 0.5 mg L^{-1} adrenaline concentration. The repeatability was found as 1.7 %.

The comparison of sensors performances with other described in the literature is summarized in Table 2. Proposed method for adrenaline determination has comparable or even better performances than those reported in literature. Most importantly, developed sensor for adrenaline determination based on Ru(III) complex of *N*-(2-hydroxyphenyl)-5-bromosalicylideneimine as electron transfer mediator has better mediating properties and wider linearity range than previously developed sensors based on ruthenium complexes.[33,34]

Pharmaceutical Adrenaline Sample Analysis

Flow injection amperometric analysis at screen printed carbon electrodes modified with Ru(III) Schiff base complex and (*i*) cellulose acetate or (*ii*) multi-walled carbon nanotubes were used for determination of adrenaline in pharmaceutical sample used in medical emergencies (Figure 5).

Quantitative determinations were carried out by injecting diluted sample solutions into the FIA system. Analysis results are given in Table 3. Excellent recovery values along with good repeatability prove high accuracy and precision of newly developed sensor based on Ru(III) Schiff base complex as mediator for adrenaline determination in pharmaceutical samples.

Ascorbic Acid as Interference

Since Ru(III) Schiff base complex, as electron transfer mediator, showed prominent properties for adrenaline determination in pharmaceutical samples effect of ascorbic
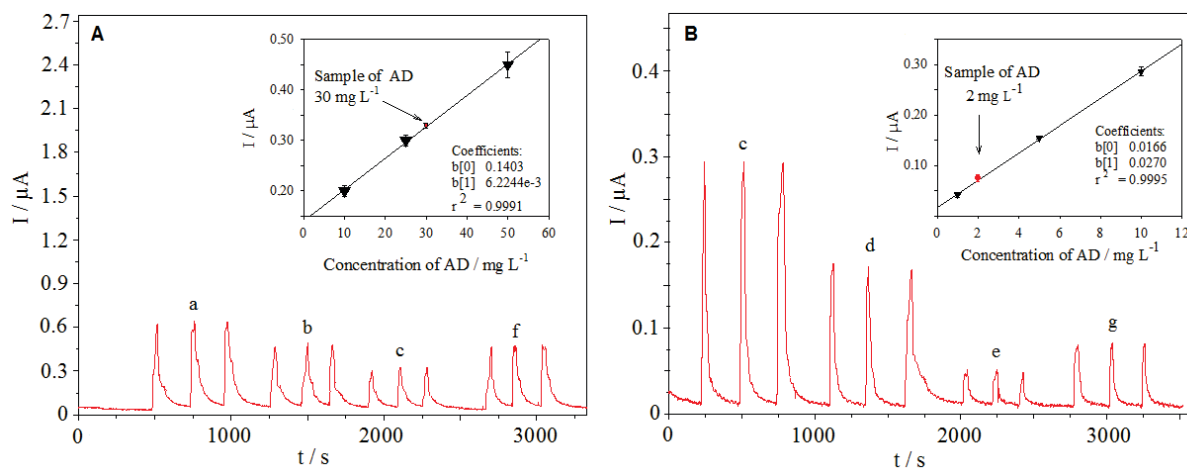
Figure 5. Flow injection amperometric response of various concentrations of adrenaline (a – 50, b – 25, c – 10, d – 5, e – 1, f – 30 and g – 2 mg L^{-1}) in 0.1 M phosphate buffer pH 7.5 at 100 mV operating potential with 0.4 mL min^{-1} flow rate and 100 μL injection volume at SPCE modified with Na[RuL₂] and (A) cellulose acetate; (B) MWCNTs.

Table 3. Determination of adrenaline in a pharmaceutical sample using FIA method at differently modified SPCEs

Electrode	γ (adrenaline) / mg mL^{-1}		Recovery / %
	Labelled	Found	
1	1.00	1.010	101
2	1.00	0.998	99.8

1 – SP / Na[RuL$_2$] /cellulose acetate / CE.

2 – SP / Na[RuL$_2$] / MWCNTs/CE.

acid (AA) on adrenaline determination was investigated. Ascorbic acid is most widely spread redox active specie that coexists with adrenaline in biological fluids.[38–40] Since ascorbic acid is oxidized at low potentials and present in notable concentration, possibility of adrenaline determination in presence of ascorbic acid can be used as a rough measure whether the sensor can be used for analysis of biological samples. In order to investigate selectivity of newly developed sensors electrochemical behavior of adrenaline (50 mg L^{-1}) in presence of equal

concentration of ascorbic acid was investigated using flow injection amperometry (Figure 6). Ru(III) Schiff base complex modified screen printed carbon (un)modified with multi-walled carbon nanotubes and/or Nafion® were used for these purposes.

Amperometric measurements provided several conclusions: (*i*) addition of multi-walled carbon nanotubes increases adrenalin signal response almost twice but does not eliminate oxidation of ascorbate (Figure 6A and 6B); (*ii*) in absence of Nafion® amperometric signal of adrenalin is increased owing to oxidation of ascorbate (Figure 6A and 6B); (*iii*) Nafion® effectively blocks ascorbate ions while amperometric response is proportional to adrenalin concentration (Figure 6C and 6D).

CONCLUSIONS

Combining the unique catalytic properties of Ru(III) Schiff base complexes of *N*-(2-hydroxyphenyl)-5-bromosalicyli-deneimine, along with favorable electrochemical pro-
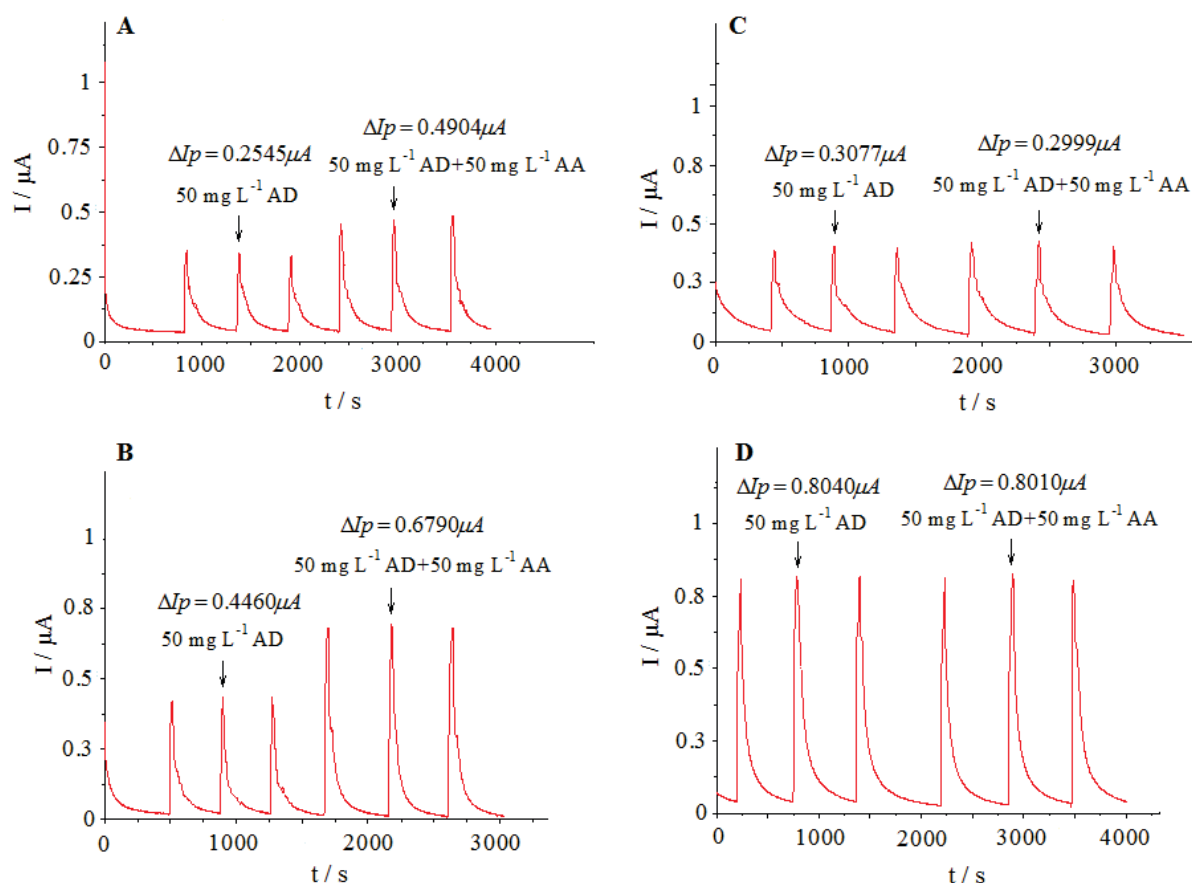
Figure 6. Flow injection amperometric response of adrenaline (AD) and ascorbic acid (AA) at equal concentrations (50 mg L^{-1}) at Na[RuL$_2$] modified SPCEs without (A,C) and with (B,D) MWCNTs and/or Nafion® (C,D). The operating potential 100 mV *vs.* Ag / AgCl in 0.1 M pH 7.5 phosphate buffer at 0.4 mL min^{-1} flow rate and 100 μL injection volume.

perties of multi-walled carbon nanotubes and cellulose acetate provide very stable and selective sensor for determination of adrenaline in pharmaceutical samples. Under the optimal conditions (0.1 M phosphate buffer pH 7.5, +100 mV working potential and 0.4 mL min^{-1} flow rate) developed sensor showed good stability (more than 2 weeks), sensitivity (LOD = 0.289 µM), repeatability (1.7 %), wide linear range (3–273 µM) and excellent recovery (101 % and 99.8 %) for determination of adrenaline. Moreover, ascorbic acid as interference for adrenalin determination is effectively eliminated by addition of Nafion® to modification mixture. Promising results encourage further study of this sensor for determination of adrenaline in biological samples and investigation of Ru(III) Schiff base complexes as very efficient mediators for sensing of redox active (bio)organic molecules.

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