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Screen-printed Electrochemical Sensor Based on Reduced Graphene doped Poly (L-dopa)/PEDOT:PSS Composites for Epinephrine Detection

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In current work, the highly sensitive screen-printed electrochemical sensor for epinephrine (EP) detection was constructed based on reduced graphene doped poly (L-dopa)/PEDOT:PSS composites by the simple solution coating method and electropolymerization. The constructed sensor exhibited excellent sensor performances and recorded the linear response of EP in the concentration range of 0.10–16.50 μM with detection limit of 0.02 μM. The practical analytical utility provides great promise for sensitive measurements of EP in human serum, and has broad prospects in clinical and pharmaceutical applications.

Keywords: Reduced Graphene, Screen-printed Electrochemical Sensor, Poly (L-dopa), PEDOT:PSS, Epinephrine

1. INTRODUCTION

Epinephrine (EP), commonly known as adrenaline, is a catecholamine， and its chemical molecular structure is shown in Fig. 1. As one of the key neurotransmitters in the human central nervous system, EP plays an enormous role in the transmission of nerve impulses [1]. Under physiological stress or hypoglycemia, the adrenal glands and certain neurons can produce EP [2]. The normal level of EP in the body helps to react during stress and sudden action by controlling physiological activities such as heart rate and blood pressure, but abnormal level of EP is associated with many diseases, such as heart diseases, schizophrenic disorders, Huntington's diseases and Parkinson's diseases [3]. Because the intake of EP provokes the speed and strength of athletes, the world anti-doping agency (WADA) prohibits athletes from taking EP in sports competitions [4, 5].

Furtherly, EP has been used as a drug for treating some diseases, such as severe anaphylaxis, myocardial infarction, sudden cardiac arrest and hypertensive disease [3, 4].

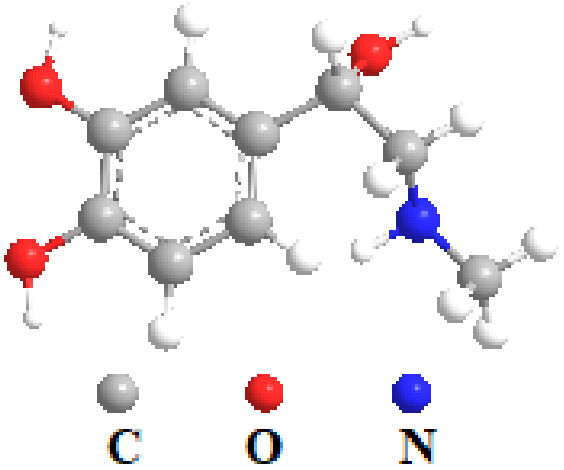


Figure 1. Chemical structure of epinephrine.

In view of the fact that EP is not only very important for various body functions, but also is of great significance to medicine and life science [6], a variety of methods for the determination of EP have been reported, including high performance liquid chromatography, spectrophotometric method, flow injection analysis, chemoluminescence method, and high performance capillary electrophoresis [7 - 10]. As an alternative to those based on optical properties and chromatography, electroanalysis is a simple and highly sensitive method for the quantification of EP [11]. In many cases, electrochemical oxidation of EP at conventional electrodes is hindered by slow electrode kinetics and high overpotential [3]. In order to solute those defects, many scientists have carried out in-depth research to develop new surface modified electrodes [12]. Various materials, such as nanostructured materials, conductive polymers and biomolecules, have been widely studied as modifiers in this regard [1].

One of the graphene derivatives, graphene oxide (GO), has been considered as a promising material for various applications due to its unique two-dimensional structure and special electronic, mechanical and electrochemical properties [13]. GO is less conductive due to the breakage of its sp^2 bonding networks, but the reduction of GO can recover its honeycomb hexagonal lattice and greatly improves its conductivity [14].

As a heavily doped p-type organic semiconductor, PEDOT:PSS consists of PEDOT chains (semiconductor) and PSS (dopant), and the holes in the PEDOT chains are compensated by sulfonate anions on the PSS [15]. PEDOT:PSS is a promising material for the electrochemical sensors because of its soft nature mixed electronic/ionic conductivity and printability [16]. Levodopa ((2S)-2-amino-3-(3, 4-dihydroxyphenyl) propionic acid, L-dopa), has a negatively charged carboxyl group and a positively charged amine group, which is extremely advantageous to be used as electrode modification material [17].

In the proposed work, we report a novel sensing strategy for EP based on reduced graphene (rGO) doped poly (L-dopa)/PEDOT:PSS composites. The rGO doped poly (L-dopa)/PEDOT:PSS

composites were prepared on the screen-printed graphite electrodes (SPCE) via drop-coating and electrochemical methods. Further, the proposed work is simple and cost effective, and can successfully detect trace EP in human serum.

2. EXPERMENT

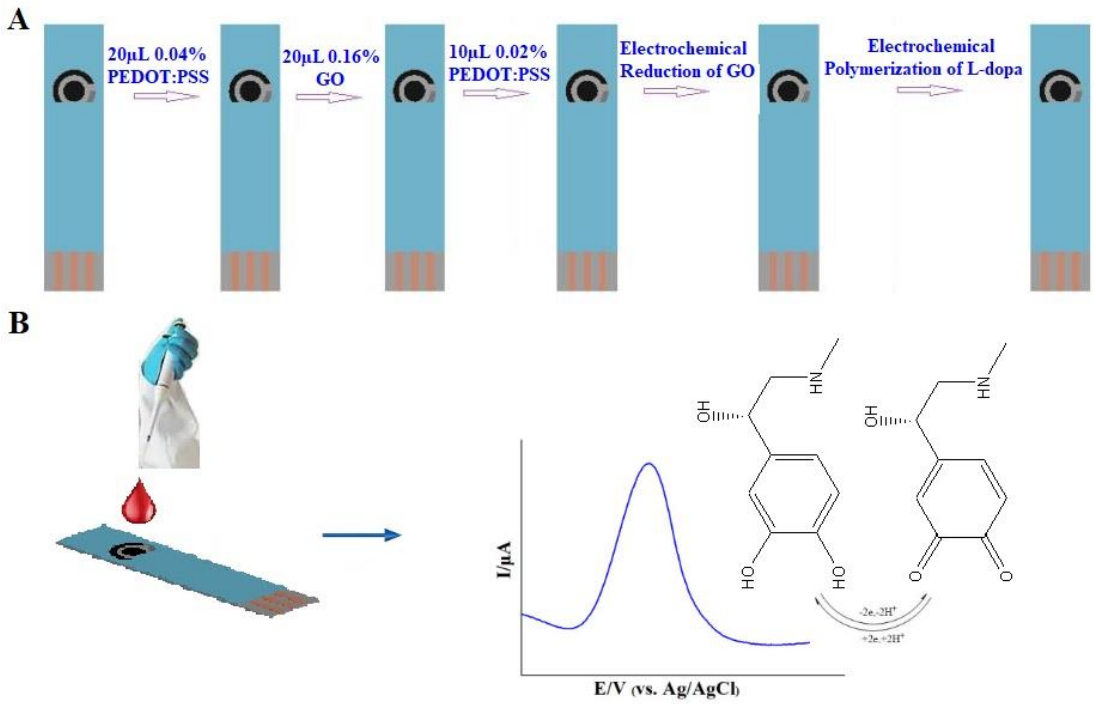
2.1. Apparatus, materials and chemicals

A CHI 830 electrochemical workstation (Chenhua ,Shanghai) equipped with data processing software was used for electrochemical measurements. Commercial screen-printed graphite electrodes (SPCEs) were purchased from DropSens (ref. 110). The PHS-3C pH meter (INESA, Shanghai) was used for pH measurements.

Graphene oxide (GO), H_3BO_3, K_2HPO_4, CH_3COOH, H_3PO_4 and KH_2PO_4 were provided by Macklin Reagent (Shanghai, China). $K_3[Fe(CN)_6]$, $K_4[Fe(CN)_6]$, NaOH, KNO_3 and KCl were provided by Sinopharm Chemical Reagent (Shanghai, China). PEDOT:PSS (1.5 wt% in water) and Levodopa (L-dopa) were obtaibed from Aladdin Reagent (Shanghai, China). Double distilled water was self-made in the laboratory, and was used as solvent throughout the experiment. Unless stated otherwise, all other analytical grade chemicals were also purchased from Macklin Reagent (Shanghai, China) and no further purification was carried out.

2.2. Fabrication of Sensors

For the fabrication of sensor, the surface of SPCE was cleaned by ultrasonic, and absolute ethanol and double distilled water were successively used as ultrasonic cleaning solution [16, 18]. Then, 20 μL of a PEDOT:PSS solution (0.40mg/ml) was dropped directly onto the cleaned SPCE surface and dried under the room temperature conditions to form a PEDOT:PSS film. Subsequently, the surface of PEDOT:PSS/SPCE was carefully drop-coated with 20 μL of a freshly made dispersion of GO solution (1.60mg/mL) and dried under the room temperature conditions. In order to improve the stability of the electrode, 10 μL of a PEDOT:PSS solution (0.20mg/ml) was then placed directly onto the GO/PEDOT:PSS/SPCE surface. For electrochemical reduction of GO, the GO/PEDOT:PSS/SPCE was immersed in 0.1M phosphate buffer solution (pH 6.0) and Cyclic voltammetry (CV) was carried out between -1.40 to +0.50 V for 10 cycles at 0.02 V/s [19, 20]. Finally, the rGO/PEDOT:PSS/SPCE was immersed in a 0.10 M KNO_3 (pH 6.0) containing 1.50 mM L-dopa solution and the electropolymerization of L-dopa was prepared by CV in -0.40 to +0.60 V for 20 cycles at 0.025 V/s [17, 21]. So far, the fabrication of rGO doped poly (L-dopa)/PEDOT:PSS composites modified SPCE (rGO/L-dopa/PEDOT:PSS/SPCE) was completed, as represented in Scheme 1.



Scheme 1. Schematic representation of rGO doped poly(L-dopa) /PEDOT:PSS composites modified SPCE (poly(L-dopa)/rGO/PEDOT:PSS/SPCE) for epinephrine detection.

2.3. Electrochemical measurements

CV was carried out for exploring the electrochemical performance in 0.1 M KCl solution containing 0.2 mM $[Fe(CN)_6]^{3-/4-}$ (1:1). For epinephrine, CV was carried out from -0.2 to +0.6 V, and linear sweep voltammetry (LSV) was performed from 0 to +0.4 V. Britton-Robinson buffer solution (BRBS) was used as a buffer solution for epinephrine detection.

The human serum sample was obtained from Henan university of science and technology first affiliated hospital, and stored in the fresh-keeping refrigerator for standby. When in use, human serum was diluted 10 times with BRBS (pH 4.5). The concentrations of EP in human serum were measured by standard addition method.

3. RESULTS and DISCUSSION

3.1 Electrochemical characterization of Various Modified Sensors

The electrochemical behaviors of various modified SPCEs were investigated using ferricyanide/ferrocyanide as redox probes by CV. As can be seen from Fig. 2, the well-defined redox couples appeared at bare SPCE and modified SPCEs. After the SPCE was modified with PEDOT:PSS, the redox peak current increased significantly, indicating that the excellent electro-conduction of PEDOT:PSS improved the electrochemical performance of bare SPCE. When the GO was doped into PEDOT:PSS, the redox peak current decreased by about 13%, which was mainly due to the poor

conductivity of GO [14]. Later, GO was reduced to rGO by electrochemical reduction, and the current signals increased to close to PEDOT:PSS/SPCE, which suggested that the reduction of GO results in the recovery of its electrical conductivity [14].

When the rGO/PEDOT:PSS/SPCE was electropolymerized with L-DOPA, the signal currents were increased about 2%. This showed that poly(L-DOPA) had a little effect on improving the electrochemical catalytic activity of the sensor.

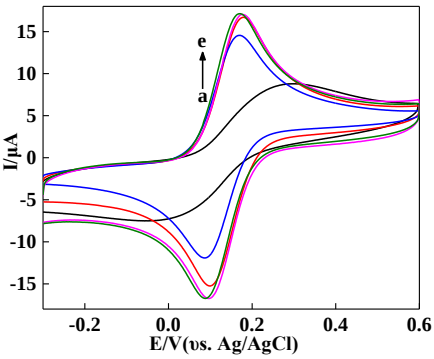


Figure 2. CVs obtained at bare SPCE (a), GO/PEDOT:PSS/SPCE (b), PEDOT:PSS/SPCE(c), rGO/PEDOT:PSS/SPCE (d) and poly(L-dopa)/rGO/PEDOT:PSS/SPCE (e) in 0.20 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) containing 0.10 M KCl, scan rate: 0.05 V/s.

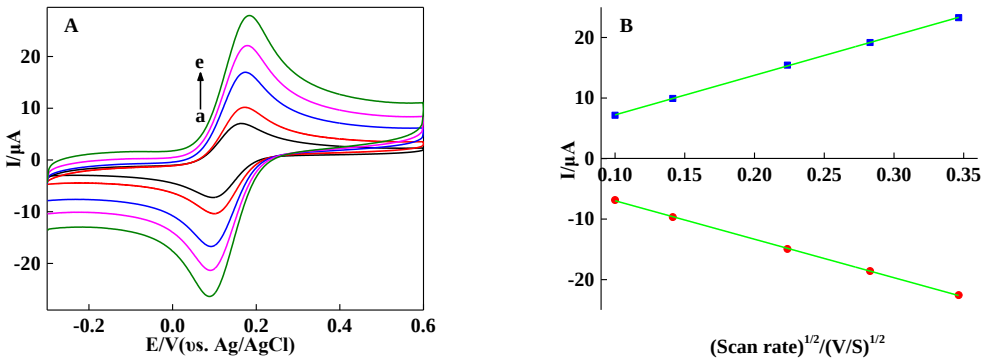


Figure 3. A: CVs at different scan rates (a-e: 0.01-0.12 V/s) at poly(L-dopa)/rGO/PEDOT:PSS/SPCE in 0.20 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) containing 0.10 M KCl. B: Plots of peak current against the square root of the scan rate.

Next, the effect of scan rate upon the redox behaviors of ferricyanide/ferrocyanide at poly(L-dopa)/rGO/PEDOT:PSS/SPCE was explored. As shown in Fig. 3, the peak currents were linearly correlated with $(\text{scan rate})^{1/2}$ (I_{pa} (μA) = 65.431 $v^{1/2}$ + 0.671, R^2 = 0.9998; I_{pc} (μA) = -63.578 $v^{1/2}$ - 0.611, R^2= 0.9997.). This indicates that the electrochemical reaction of ferricyanide/ferrocyanide at poly(L-dopa)/rGO/PEDOT:PSS/SPCE is a diffusion- controlled process [16, 17].

3.2 Analytical Performance of Various Modified Sensors

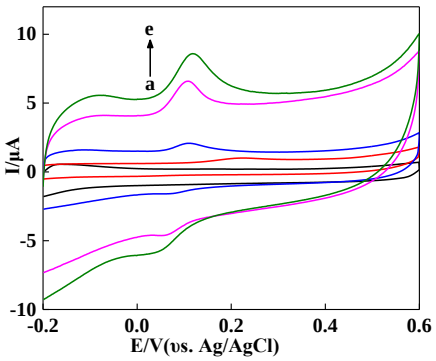


Figure 4. CVs obtained at bare SPCE (a), PEDOT:PSS/SPCE (b), GO/PEDOT:PSS/SPCE (c), rGO/PEDOT:PSS/SPCE (d) and poly(L-dopa)/rGO/PEDOT:PSS/SPCE (e) in BRBS (pH 4.5) containing 0.80 µM EP at the scan rate of 0.05V/s.

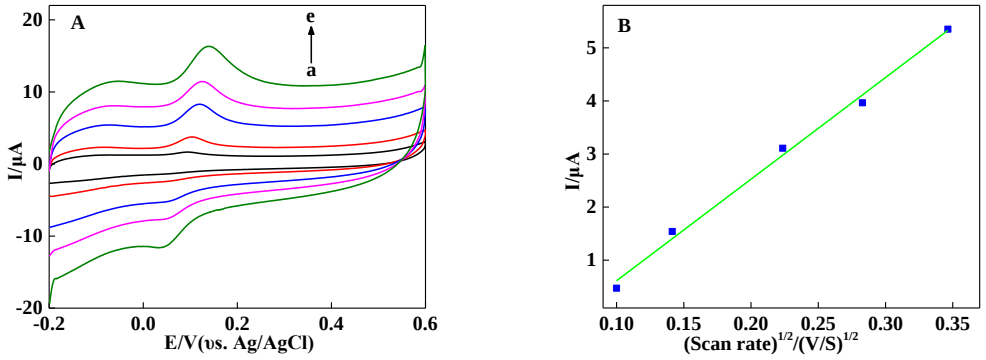


Figure 5. A: CVs at different scan rates (a-e: 0.01-0.12 V/s) at poly(L-dopa)/rGO/PEDOT:PSS/SPCE in BRBS (pH 4.5) containing 0.80 µM EP. B: Plots of oxidation peak current against the square root of the scan rate.

The determination of EP was performed at bare/ modified SPCEs using CV technique. Fig. 4 represented the CV of EP (0.80 µ M) at bare SPCE (a), PEDOT:PSS/SPCE (b), GO/PEDOT:PSS/SPCE (c), rGO/PEDOT:PSS/SPCE (d) and poly(L-DOPA)/rGO/PEDOT:PSS/SPCE (e). A definite oxidation peak was observed at bare/modified SPCEs, which can be attributed to the oxidation of EP to its corresponding quinone (adrenaline quinone) [22, 23]. When the GO was doped into PEDOT:PSS, the redox peak current increased by about 154%, which was mainly due to the fine biocompatibility of GO [14]. Similarly, the incorporation of poly(L-DOPA) also greatly improved the biocompatibility of the electrode, resulting in a 45% improvement in the oxidation peak current signal compared with rGO/PEDOT:PSS/SPCE .

CVs of EP at poly(L-DOPA)/rGO/PEDOT:PSS/SPCE were studied in the range from 0.01 to 0.12 V/s (Fig. 5A). The illustration of Fig. 5B shows the linear relationship between anodic peak current and (scan rate)$^{1/2}$ (I_{pa} (µ A) = 19.144$v^{1/2}$ - 1.302, R^2 = 0.9931), which implies a diffusion-

controlled process [24].

3.3 Effect of supporting electrolyte and pH

The electrochemical behaviour of EP depends on the pH value of the test solution. To achieve the optimization condition, the electrochemical behaviours of EP were studied in BRBS in different pH values (4.5 - 6.5) at poly(L-dopa)/rGO/PEDOT:PSS/SPCE by CV (Fig. 6). It can be seen that the anodic peak current (I_{pa}) decreased sequentially from pH 4.5 to pH 6.5, and the maximum value of I_{pa} was surveyed at pH 4.5. Hence, the BRBS with pH value of 4.5 was selected in the following experiments in order to obtain higher sensitivity.

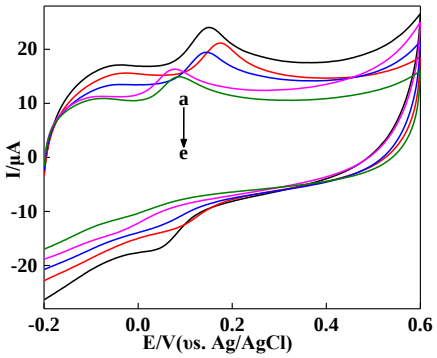


Figure 6. CVs of 0.80 μM EP in different pH values of BRBS (a→e: pH values of 4.5 - 6.5) at poly(L-dopa)/rGO/PEDOT:PSS/SPCE, scan rate: 0.05 V/s.

3.4 Calibration curve, detection limit, and method validation

From the CV studies, it is inferred that the oxidation peak current response of EP is much larger than the reduction peak current response of EP. Considering the above facts, the oxidation process of EP was chosen for sensing. Under the optimized experimental conditions, the LSV curves pertaining to the increasing concentrations of EP were shown in Fig. 7. As indicated in Fig. 7A, there was no response in the bare BRBS with pH 4.5. However, when the EP concentration increased to 0.02 μM, a weak LSV response appeared. According to these facts, it could be determined that the detection limit of EP was 0.02 μ M. The peak current was found to increase with the increase of EP concentration, a linear relationship between the peak current and the EP concentration was observed in the range of 0.10 to 16.50 μM. The calibration plot of I (μA) vs. Concentration (μM) (Fig. 7B) was described by the following equation: I (μA) = 1.969 c + 1.375; R^2 = 0.996.

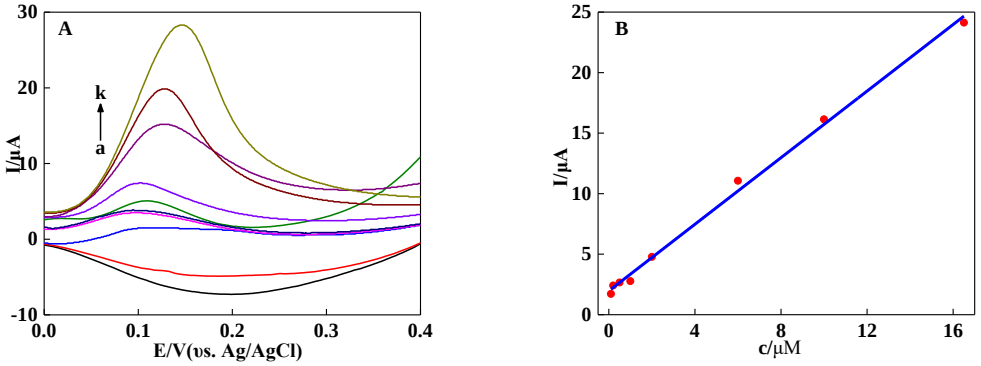


Figure 7. A: LSVs at poly(L-DOPA)/rGO/PEDOT:PSS/SPCE in BRBS (pH 4.5) for simultaneous determination of EP (a→k: 0-16.5 μM) ; B: Plots of oxidation peak current density against EP concentrations (0.1 -16.5 μM)

As shown in Table 1, the comparison of analytical performance between previous reports and this work was mentioned, and the fabricated sensor was superior to earlier reports in terms of the detection limit for EP. Due to the synergistic effect of poly(L-dopa)/PEDOT:PSS polymers and rGO, the poly(L-dopa)/rGO/PEDOT:PSS composites with fine biocompatibility and good conductivity were beneficial to enhance the electrocatalytic activity for EP.

Table 1. List of various electrodes reported in literature for EP detection

Electrode	Linear range (μM)	LOD (μM)	Ref.
$ZNFe_2O_4$/CPE	5–100	0.70	[25]
PBCB/Fe_2O_3/GCE	0.05 − 15	0.31	[26]
Nano-Au/PPyox/GCE	0.30 − 21	0.03	[27]
Pt@SnO2/GCE	13.75–110	0.35	[28]
Caffeic acid/GCE	2- 80	0.60	[29]
MWCNT–Oppy/GCE	0.10-8	0.04	[30]
CNT/GCE	1–50	0.10	[31]
Al-MCM-41/CPE	1–30	0.03	[32]
MnO_2/Nafion/GCE	0.50–100	0.10	[33]
GME/GCE	0.40–13	0.09	[34]
poly(L-DOPA)/rGO/ PEDOT:PSS/SPCE	0.10-16.50	0.02	This work

3.5 Analysis of real samples

In order to evaluate the practical application of the sensor, human serum sample was taken as real sample. The results were summarized in Table 2. For the sake of evaluating the recovery of the

sensor, 4μM, 8μM and 10μM EP were added into the serum samples respectively. The recoveries ranged from 98.1% to 103.2%, which indicated that the prepared sensor was very suitable for EP detection in real samples.

Table 2. Analysis results of EP in human serum (n=5).

Method	Added/(μM)	Determined/(μM)	Recovery/%	RSD/%
LSV	4	4.13	103.2	3.7
	8	8.19	102.4	1.6
	10	9.81	98.1	2.1

4. CONCLUSION

In the study, a novel electrochemical sensor was successfully prepared by electropolymerization of a poly(L-dopa) film on rGO/PEDOT:PSS/SPCE surface. With the fine biocompatibility of poly(L-dopa) and good conductivity of rGO/PEDOT:PSS composites, a simple and facile electrochemical sensor for the sensitive determination of EP was achieved. The detection limit of EP was as low as 0.02 μM, and the linear concentration range was 0.10–16.50 μM. The prepared low-cost electrochemical sensor may be applied to measure EP in clinical samples and drug industry.

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