

# Simultaneous and sensitive determination of procaine and its metabolite for pharmaceutical quality control and pharmacokinetic research by using a graphite paste electrode

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**Abstract** A simple, rapid, sensitive, and accurate method for simultaneous electrochemical determination of procaine and its metabolite (*p*-aminobenzoic acid, PABA) for pharmaceutical quality control and pharmacokinetic research was developed using a graphite paste electrode. The differential pulse voltammetric results revealed that procaine and *p*-aminobenzoic acid, respectively, showed well-defined anodic oxidation peaks on a carbon paste electrode with a current peak separation of 155 mV at a scan rate of 100 mV s<sup>-1</sup>. This well separation of the current peaks for these two compounds in voltammetry enables us to simultaneously determine them. Good linearity ( $r > 0.998$ ) between oxidation peak current and concentration was obtained in the range of  $5.0 \times 10^{-7}$ – $5.0 \times 10^{-5}$  M for procaine and  $5.0 \times 10^{-7}$ – $2.0 \times 10^{-5}$  M for PABA in pH 4.50 acetate buffer solution. The detection limit for both analytes is  $5 \times 10^{-8}$  M ( $S/N=3:1$ ). The present voltammetric method has been successfully used to determine trace *p*-aminobenzoic acid in procaine hydrochloride injection and procaine in

plasma with a linear relationship of current to its concentration ranging from  $1.0 \times 10^{-6}$  to  $5.0 \times 10^{-5}$  M (correlation coefficient of 0.9981) with a low detection limit of  $5.0 \times 10^{-7}$  M ( $S/N=3:1$ ). This validated method is promising to the study of pharmacokinetics in Sprague–Dawley rat and rabbit plasma after an intravenous administration of procaine hydrochloride injection.

**Keywords** Procaine · *p*-Aminobenzoic acid · Graphite paste electrode · Rat plasma · Pharmacokinetics · Electrochemical analysis

## Introduction

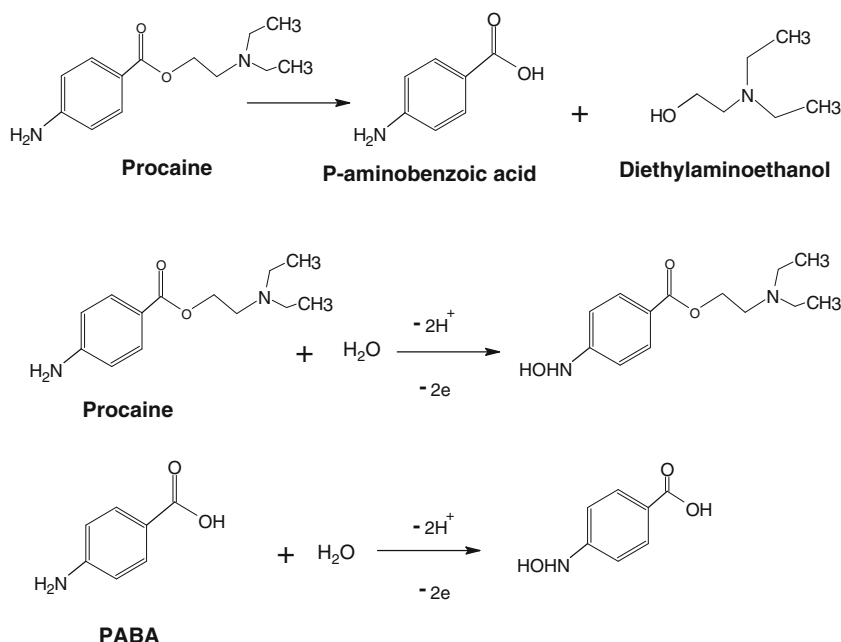
Procaine, 2-diethylaminoethyl 4-aminobenzoate (chemical structure illustrated in Scheme 1) synthesized in 1905, was the first injectable man-made local anesthetic. For its less toxicity and low cost than other hard stuffs such as cocaine and tetracaine, procaine has been widely employed in the treatment of clinical surgery and often used for anesthesia, block therapy, and as a stimulating drug [1–3]. It reversibly blocks impulse conduction along nerve axons and other excitable membranes that utilize sodium channels as the primary means of action potential generation. This action can be clinically used to block pain sensation from specific areas of the body [4]. It is metabolized primarily by plasma butyrylcholinesterase and secondarily by liver esterase. Hydrolysis of procaine produces *p*-aminobenzoic acid (PABA) and diethylaminoethanol (chemical structure illustrated in Scheme 1). *p*-Aminobenzoic acid can interfere with the action of sulfonamide antibiotics [2, 3]. Moreover, the decomposition product, PABA, is the main material which causes an allergic reaction in surgery when procaine

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**Scheme 1** The hydrolysis reaction of procaine and the electrochemical reactions of procaine and PABA on a graphite paste electrode



is administrated as a local anesthetic. It can induce anaphylactic shock if the allergy is strong enough. Therefore, the quality control or/and the purity determination of procaine are imperative [3].

Many analytical methods have been proposed to determine procaine and PABA in pharmaceutical preparations or biological samples. The British and US pharmacopoeial methods for assaying procaine hydrochloride are based on the solvent extraction of procaine hydrochloride, followed by either spectrophotometric measurement at 280 nm or titration with sodium hydroxide [1]. In Chinese pharmacopoeia, procaine is titrated with sodium hydroxide and PABA in procaine hydrochloride is determined by thin layer chromatography [2]. Additionally, procaine or PABA has also been analyzed using other methods, including colorimetric [5, 6], spectrophotometry [7–10], gas chromatography [11–13], high-performance liquid chromatography (HPLC) [14–18], fluorimetric detection [19–21], infrared spectroscopy [22], Raman spectroscopy [23], chemiluminescence [24, 25], and flow-injection analysis [26]. However, only a few methods were developed for the simultaneous determination of both compounds, mainly based on HPLC [27–31], LC–MS/MS [32], and multiway partial least-squares coupled to residual trilinearization (N-PLS/RTL) [33]. Although the above methods are very sensitive and selective, LC–MS/MS is a relatively expensive technology that is not always readily available in academic research laboratories. The HPLC methods for the independent determination of procaine or PABA from biological matrices are reasonably sensitive, but they are time-consuming and require tedious extraction procedures. The N-PLS/RTL method is complicated [33]. Obviously,

the development of a simple, rapid, reliable, and inexpensive method to simultaneously determine procaine and PABA is challenging.

Both procaine and *p*-aminobenzoic acid have the aniline structure which is electroactive and can be electrochemically oxidized at proper potentials. Recently, electrochemical methods have been applied to determine procaine with high sensitivity. For example, polarography [34–36], coulometric titration [37] and potentiometric titration [38], ion-selective electrode [39], and voltammetric method based on modified electrode [40–42] were employed to detect procaine or PABA. However, the electrochemical simultaneous determination of procaine and PABA has not been reported yet. As we know, the electron transfer reaction of procaine and PABA on noble metal electrodes of gold and platinum is a slow process, and high over-potentials are required to oxidize them. In addition, the strong adsorption of PABA and procaine at the surface of noble metal electrodes will seriously block and foul these electrodes [43]; thus, the determination of these compounds using novel metal electrodes often obtains unsatisfactory results.

Graphite paste electrode (GPE) is a mixture of an electrically conducting graphite powder and a pasting liquid and can be prepared very easily. It has exhibited various excellent properties such as wide potential window, good anti-fouling ability, reproducible surface, low residual current, and low cost [44–47]. Thus, the GPE has been widely used in the determination of drugs, biomolecules, and other organic species.

In this work, we described a rapid and simple electrochemical method for the simultaneous detection of procaine and PABA using a GPE. The electrochemistry of procaine

and PABA on GPE was first studied using voltammetry. Two well-defined oxidation peaks, respectively, for procaine and PABA on this electrode appeared, which enabled us to simultaneously determine these two compounds with high sensitivity and selectivity using the GPE. Results also showed that the present method can be successfully used to detect trace *p*-aminobenzoic acid in procaine hydrochloride injection and procaine in the plasma of rats and rabbits. This simple electrochemical method has been successfully used in pharmaceutical analysis and pharmacokinetics study.

## Experimental

### Chemical and reagents

Procaine hydrochloride and *p*-aminobenzoic acid were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The spectroscopy pure graphite powder was obtained from Sinopharm Chemical Reagent Co. Ltd (China). All reagents were of analytical grade and double-distilled water was used for the preparation of all solutions. Procaine hydrochloride injection ( $40 \text{ mg}^2 \text{ mL}^{-1}$ , Guangzhou Baiyunshan Mingxing Pharmaceutical Co., Ltd., No. 080801) was purchased from a local drug store. Heparin sodium injection (12500 IU/2 mL, No. 0709110) was purchased from Jiangsu Wanbang Biopharma Co., Ltd. (China). Blank, drug-free plasma samples were collected from healthy adult and male Sprague–Dawley (SD) rats and male rabbits.

### Apparatus

Electrochemical measurements were performed on a CHI 660C electrochemical workstation (CH Instruments, USA). A three-electrode system consisting of a graphite paste electrode (3.0 mm in diameter) as the working electrode, a saturated calomel electrode as the reference electrode, and a platinum wire as the auxiliary electrode was used. All potentials in this paper refer to this reference electrode. The electrochemical experiments were conducted in acetate buffer solution (0.05 M, pH 4.50) at room temperature with a potential scan rate of  $100 \text{ mV s}^{-1}$  unless otherwise stated. The pH measurements were carried out with a PHS-3B pH-meter (Shanghai Precision & Scientific Instruments, China) at room temperature.

### Preparation of graphite paste electrode

Graphite paste electrode was prepared by putting a thoroughly mixed admixture of graphite powder and paraffin oil (3:1 *w/w*) into a plastic tube (inner diameter of

3.0 mm). A copper wire was inserted into the graphite paste from the backside of the plastic tube and connected with a brass screw to provide an external electrical contact. By turning the brass screw forward, the GPE surface can be easily renewed by polishing on a weighing paper. The plastic tube had a line to connect the carbon paste and ferrule of conductor joint. In order to control the efflux velocity of the carbon paste, we put a rotatable screw to the end of the plastic pipe.

### Preparation of stock and standard solutions

A primary stock solution of  $1.0 \times 10^{-2}$  M procaine was prepared by dissolving procaine hydrochloride in a hydrochloric acid aqueous solution (pH=4.50). A primary stock solution of  $1.0 \times 10^{-2}$  M PABA was prepared by dissolving PABA in water and storing in the dark at  $4^\circ\text{C}$  prior to use. A total of 50 mM acetate buffer solution containing 10 mM NaCl was prepared by dissolving the appropriate quantity of acetic acid (HAc), sodium acetate (NaAc), and sodium hydroxide (NaOH) in water and then adjusting the pH with HAc or NaOH. The working standard solutions of procaine and PABA were prepared by diluting the primary stock solution with acetate buffer solution before taking measurements.

### Analytical procedure

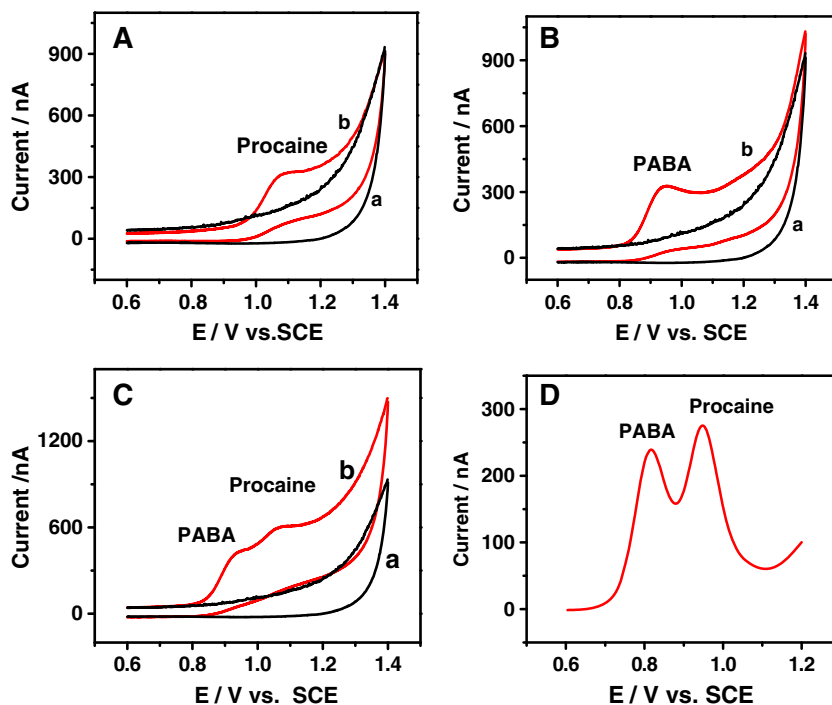
The required volume of standard solution or sample solutions was added to 10 mL acetate buffer solution (pH 4.50). Then, cyclic voltammograms (CVs) were recorded in the potential region from 0.6 to 1.2 V at scan rates from 10 to  $200 \text{ mV s}^{-1}$ . The differential potential voltammograms (DPV) were recorded from 0.6 to 1.2 V with an amplitude of 0.05 V, pulse width of 0.05 s, and pulse period of 0.2 s.

### Application to the pharmacokinetics study in rats and rabbits

The experimental protocol was approved by the Institutional Animal Ethics Committee for the use of experimental animals and all animal studies were carried out according to the Guide for Care and Use of Laboratory Animals.

Plasma standards were prepared using drug-free plasma. The plasma standard solution of procaine was prepared in bland plasma. Then, 100  $\mu\text{L}$  of plasma standard solution were spiked into each of a series of 10-mL volume flasks and made up to volume with 50 mM acetate buffer solution (pH 4.50). Then, this test solution was placed in an electrochemical cell for the determination of procaine using the above DPV method. Calibration curves were obtained by plotting the oxidation peak current versus procaine concentrations in the plasma standard sample.

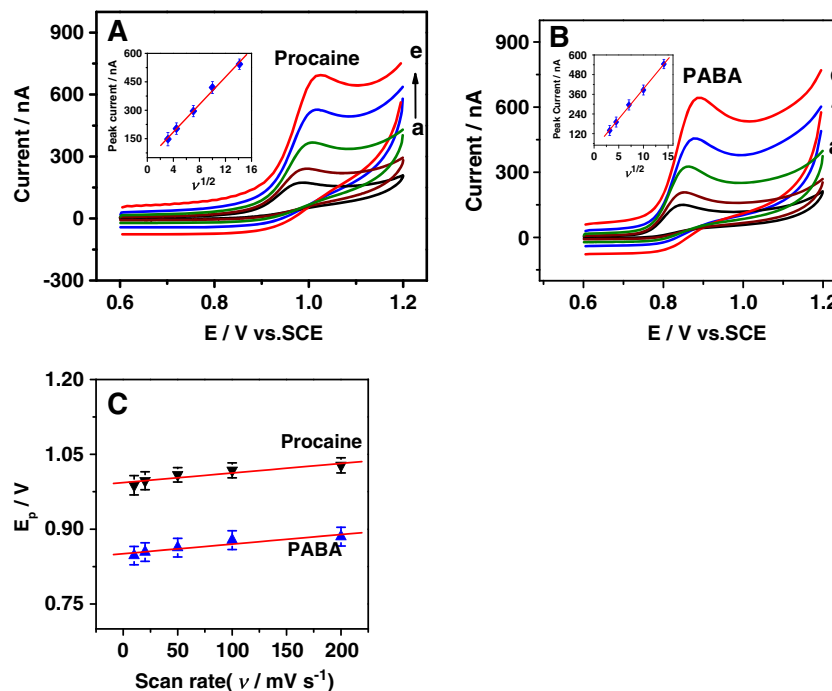
**Fig. 1** Cyclic voltammograms of 10  $\mu\text{M}$  procaine (a), 10  $\mu\text{M}$  PABA (b), and the mixture solution (c) of 10  $\mu\text{M}$  procaine and 10  $\mu\text{M}$  PABA at a GPE in pH 4.50 acetate buffer solution. d The DPV of 10  $\mu\text{M}$  procaine and 10  $\mu\text{M}$  PABA at the GPE in pH 4.50 acetate buffer solution. Scan rate, 100  $\text{mV s}^{-1}$

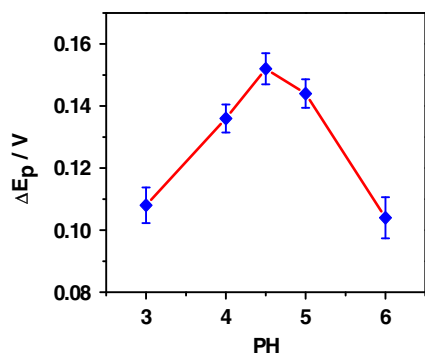


A pharmacokinetics study was performed in male Sprague–Dawley rats ( $n=4$ , weight range 200–220 g) and male rabbits ( $n=4$ , weight range 2,400–2,600 g) obtained from the Laboratory Animal Center of Fujian Medical University. SD rats and rabbits were housed in standard cages and allowed free movement in an environmentally controlled breeding room (temperature  $23\pm 2^\circ\text{C}$ ) for 4 days before the experiments, and they were fed with standard

laboratory food and water ad libitum during the whole experiments. Procaine was intravenously administered to each rat and rabbit at a dose of 40 and 16  $\text{mg kg}^{-1}$ , respectively. Blood samples were collected from rat tail vessels under light and marginal veins of rabbit ear post-dosing and transferred immediately into microfuge tubes containing heparin as anti-coagulant. Plasma samples were obtained by immediately centrifuging the blood samples at

**Fig. 2** CVs of procaine (a) and PABA (b) at a different scan rate in pH 4.50 acetate buffer. Scan rate (from a to e): 10, 20, 50, 100, and 200  $\text{mV s}^{-1}$ . Insets of a and b show the dependence of peak currents on the scan rate. c The influence of scan rate on the oxidation peak potential





**Fig. 3** The current peak separation ( $\Delta E_{pa}$ ) of procaine and PABA as a function of solution pH

10,000 rpm for 5 min and these were stored frozen at  $-20^\circ\text{C}$  until analysis. All plasma samples were analyzed within 2 days.

**Results and discussion**

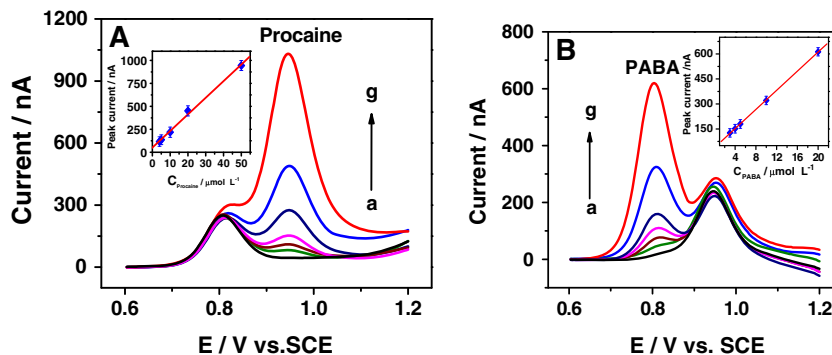
The electrochemical behavior of procaine and *p*-aminobenzoic acid at the GPE

The GPE shows an excellent electrochemical activity towards the oxidation of procaine and PABA. The cyclic voltammogram of procaine on a GPE in 0.05 M acetate buffer solution (pH 4.50) is shown in Fig. 1a. The electrochemical oxidation started at ca. 0.90 V and reached a current peak at  $E_{pa}=1.075$  V (curve b) in the positive potential scan. There appeared only a small oxidation peak in the negative potential scan, indicating that the electrochemistry of procaine on GPE is irreversible. Similarly, the electrocatalytic oxidation of PABA on the GPE in 0.05 M acetate buffer solution (pH 4.50) showed the irreversibly electrochemical behavior. The CV showed the electro-oxidation current profiles in both the positive and negative

potential scans (Fig. 1b) with the peak potential at  $E_{pa}=0.945$  V. The large difference in the oxidation peak of these two compounds revealed that the electrochemistry of procaine and PABA on GPE can be well distinguished. As shown in Fig. 1c, two electrooxidation peaks at 1.065 and 0.940 V respective to procaine and PABA were resolved on GPE in a 0.05 M acetate buffer (pH 4.50) containing a mixture of 10  $\mu\text{M}$  procaine and 10  $\mu\text{M}$  PABA. These two oxidation peaks could be resolved more clearly when differential pulse voltammetric technique was used. As shown in Fig. 1d, the oxidation peaks appeared at 0.800 and 0.955 V, respectively, for procaine and PABA. The large peak separation of the anodic peaks for these two compounds allows us to simultaneously determine them in mixture solutions.

The electrochemical behavior of individual procaine and PABA on GPE at different scan rates was also studied. As shown in Fig. 2, the anodic peak current of procaine (Fig. 2a) and PABA (Fig. 2b) increased and the anodic peak potentials shifted to positive values with increasing the scan rate. Plots of the irreversible anodic peak currents ( $I_p$ ) of procaine and PABA versus the scan rate ( $v^{1/2}$ ) yielded straight lines in the range from 10 to 500  $\text{mV s}^{-1}$  ( $I_{pa, \text{procaine}} (\text{nA})=36.345 v^{1/2}+39.507, r=0.9978$ ;  $I_{pa, \text{PABA}} (\text{nA})=27.967 v^{1/2}+36.493, r=0.9992$ ) (insets in Fig. 2a,b). These results imply that the electrode processes of procaine and PABA are irreversibly diffusion-controlled.

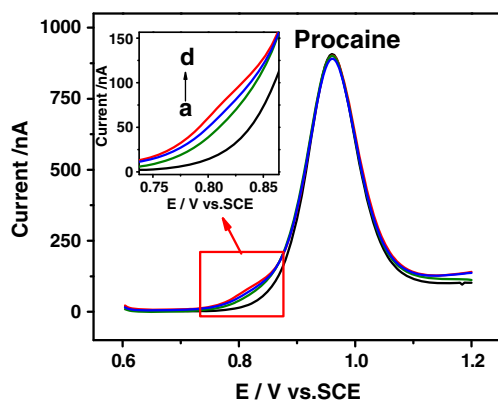
For an irreversible electrode process, its oxidation peak potential ( $E_{pa}$ ) is a function of the scan rate. It shifts in the positive direction by  $30/an$  mV at  $25^\circ\text{C}$  for each tenfold increase in scan rate  $v$  [48]. This shift of  $30/an$  mV was also observed in the case of procaine and PABA (Fig. 2c). Generally, the electron transfer coefficient ( $\alpha$ ) is about 0.5 in a totally irreversible electrode process [48]. Therefore, the number of electrons ( $n$ ) involved in the oxidation of procaine and PABA can be estimated to be ca. 2, which is in agreement with the previous reports [40, 42].



**Fig. 4** a DPVs of procaine at a GPE in the presence of 10  $\mu\text{M}$  PABA in pH 4.50 acetate buffer. Procaine concentrations (from a to g): 0.5, 1, 3, 5, 10, 20, and 50  $\mu\text{M}$ . b DPVs of PABA at a GPE in the presence of 10  $\mu\text{M}$

procaine in pH 4.50 acetate buffer. PABA concentrations (from a to h): 0.5, 1, 2, 3, 4, 10, and 20  $\mu\text{M}$ . Scan rate, 100  $\text{mV s}^{-1}$ . Insets show the plot of peak current of procaine as a function of procaine concentration





**Fig. 5** The DPVs of the procaine hydrochloride injection sample and the spiked sample, with the *inset* showing the local enlargements: *a* the procaine hydrochloride injection sample, *b* the sample spiked with  $1.14 \text{ mg L}^{-1}$  PABA (the less amount as the admissible concentration), *c* the sample spiked with  $1.638 \text{ mg L}^{-1}$  PABA (the admissible concentration), *d* the sample spiked with  $2.28 \text{ mg L}^{-1}$  PABA (the more amount as the admissible concentration). Scan rate,  $100 \text{ mV s}^{-1}$

In addition, the influence of the solution pH ranging from 3.0 to 6.0 on the electrochemical behavior of procaine and PABA was also studied since procaine is not stable in strong acid or alkaline solution [2]. The anodic peak potentials for the oxidation of procaine and PABA shifted to negative values with the increase of pH (not shown). The linear segment was found with slope values of  $-55.2 \text{ mV pH}^{-1}$  and  $-56.8 \text{ mV pH}^{-1}$  for procaine and PABA in the pH ranges of 3.0–6.0, respectively, which is in accordance to the Nernst equation. It can, therefore, be concluded that an equal number of electrons and protons is involved in the electrochemical reactions.

From the foregoing results and discussion, the mechanism for electrochemical oxidation of procaine and PABA at the GPE can be proposed as in Scheme 1, which is in agreement with the previous reports [40, 42].

#### Simultaneous DPV determination of procaine and PABA at GPE

##### *Influence of solution pH*

The influence of solution pH (3.0–6.0) on the separation of the oxidation peak potentials ( $\Delta E_{\text{pa}}$ ) of

procaine and PABA on GPE in DPV was studied. As shown in Fig. 3, the anodic peak difference  $\Delta E_{\text{pa}}$  increased slightly with the increase of solution pH and reached a maximum at pH 4.50. At a solution pH higher than 4.50,  $\Delta E_{\text{pa}}$ , on the contrary, decreased with increasing the solution pH. Thus, the buffer solution pH of 4.50 should give a maximum current peak separation for these two compounds and will be used in the following measurements.

##### *Simultaneous determination of procaine and PABA*

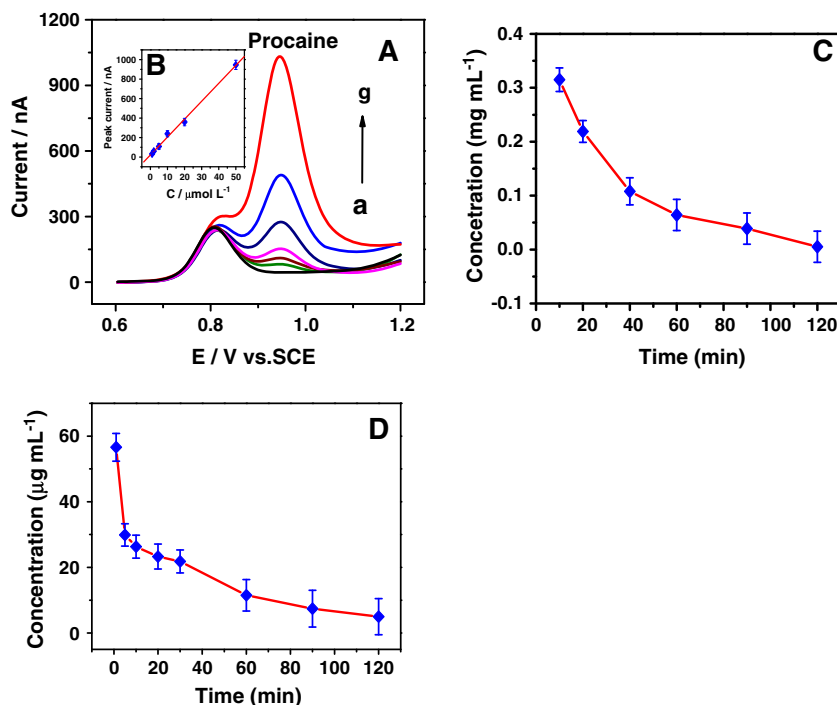
Under the optimum conditions mentioned above, the simultaneous determination of procaine and PABA using the GPE can be achieved. In the first case, the electrochemical response of procaine as a function of its concentration was studied by keeping the concentration of PABA constant. As shown in Fig. 4a, the electrochemical response of procaine increased with the increase of procaine concentration. The peak current at ca. 0.95 V increased linearly with the increase of procaine concentration (Fig. 4a, inset), which established the fundamental for the quantitative determination of procaine from the mixture solutions. In addition, it was also found that the concentration of procaine did not have a significant influence on the peak current and peak potential of PABA. Similarly, the oxidation peak current of PABA increased linearly with the increase of PABA concentration when the concentration of procaine was kept constant (Fig. 4b).

From Fig. 4, it is clear that the electrooxidation peaks of procaine and PABA at the GPE are well separated from each other, although they coexist in acetate buffer solutions (pH 4.50). It is, therefore, possible to individually or simultaneously determine procaine and PABA from mixture samples using the GPE without insignificant cross-interferences. As shown in the insets of Fig. 4a,b, the oxidation peak currents were linearly proportional to procaine and PABA (shown insets of Fig. 4a,b) concentrations in the ranges of 0.5–50.0 and 0.5–20.0  $\mu\text{M}$ , respectively (correlation coefficients 0.9999 and 0.9980 for procaine and PABA, respectively). The detection limits for both analytes were 0.05  $\mu\text{M}$ .

**Table 1** Determination of procaine hydrochloride in injection sample ( $n=6$ , repetitive measurement number)

Analyst	Labeled ( $\text{mg}^2 \text{ mL}^{-1}$ )	Added ( $\text{mg}^2 \text{ mL}^{-1}$ )	Found ( $\text{mg}^2 \text{ mL}^{-1}$ )	RSD (%)	Recovery (%)
Procaine	39.86	0	39.83	2.1	
	39.86	5.000	44.89	2.6	100.6
	39.86	10.00	49.71	3.1	98.5
	39.86	15.00	54.58	2.3	98.1
	39.86	20.00	60.18	3.5	101.6

**Fig. 6** *A*, DPVs of procaine concentration in plasma: *a* 1, *b* 2, *c* 5, *d* 10, *e* 20, and *f* 50  $\mu\text{M}$ . *A*, the relationship between procaine concentration in plasma and peak current. *C*, concentration–time curve in male SD rats following the intravenous administration of procaine at a dose of 40  $\text{mg kg}^{-1}$  ( $n=4$ ). *D*, concentration–time curve in male rabbits following the intravenous administration of procaine at a dose of 16  $\text{mg kg}^{-1}$  ( $n=4$ )



*Interferences*

To evaluate the interferences of foreign species on the determination of procaine and PABA, possible interferences such as lysine (200  $\mu\text{M}$ ), cysteine (200  $\mu\text{M}$ ), ascorbic acid (200  $\mu\text{M}$ ), citric acid (400  $\mu\text{M}$ ), tartaric acid (400  $\mu\text{M}$ ), uric acid (150  $\mu\text{M}$ ), epinephrine (200  $\mu\text{M}$ ), dopamine (200  $\mu\text{M}$ ), glucose (400  $\mu\text{M}$ ), cyclodextrin (400  $\mu\text{M}$ ), lactose (400  $\mu\text{M}$ ), and saturated starch (400  $\mu\text{M}$ ) were individually added into the standard solution containing 10  $\mu\text{M}$  procaine and PABA. The results indicated that no interference (signal change >5%) on the determination of procaine and PABA was observed.

Application of the present method

*Determination of pharmaceutical product samples*

Figure 5 shows the differential pulse voltammograms of procaine hydrochloride injection sample (Fig. 5a), the sample spiked with 0.1143  $\text{mg L}^{-1}$  PABA (the least amount as the admissible limit of pharmacopoeia; Fig. 5, curve b), the sample spiked with 0.1163  $\text{mg L}^{-1}$  PABA (which is the admissible limit of pharmacopoeia; Fig. 5, curve c), and the sample spiked with 0.2286  $\text{mg L}^{-1}$  PABA (the most amount as the admissible limit of pharmacopoeia; Fig. 5, curve d). From the differential pulse voltammograms, no signal from PABA in the real sample was observed (Fig. 5, curve a),

**Table 2** Pharmacokinetic parameters of procaine in plasma after the intragastric administration of 40- and 16- $\text{mg kg}^{-1}$  dose to SD rats ( $n=4$ ) and male rabbits ( $n=4$ ), respectively

Parameter	SD rats Estimate (mean $\pm$ S.D.)	Rabbit Estimate (mean $\pm$ S.D.)
$t_{1/2}$ (min)	21.08 $\pm$ 3.62	38.21 $\pm$ 4.27
$C_{\text{max}}$ ( $\mu\text{g mL}^{-1}$ )	455.8 $\pm$ 15.3	34.48 $\pm$ 3.54
$AUC_{0-\infty}$ ( $\mu\text{g mL}^{-1}$ )	13,867.5 $\pm$ 1,167.6	2,042.4 $\pm$ 215.3
$AUMC_{0-\infty}$	421,890.1 $\pm$ 26,872.8	120,996.5 $\pm$ 10,132.2
MRT	31.21 $\pm$ 4.12	59.26 $\pm$ 7.15

$t_{1/2}$  apparent elimination half-life,  $C_{\text{max}}$  maximum plasma concentration,  $AUC_{0-\infty}$  areas under the curves,  $AUMC_{0-t}$  the area under the first moment of the plasma concentration–time curve,  $MRT$  mean residence time

demonstrating that PABA content in the tested procaine hydrochloride injection is below the admissible limit. Figure 5, curves b to d, shows that a trace amount of PABA as low as  $0.1143 \text{ mg L}^{-1}$  could be distinguished from a large amount of procaine and confirms that the amount of PABA in the procaine hydrochloride injection tested meets the pharmacopoeia requirements.

Standard addition recovery experiments were performed by adding known amounts of the standard to the procaine hydrochloride injection sample. The results are listed in Table 1. It showed that the recoveries was ranging from 98.1% to 101.6% and the R.S.D. was less than 3.6% in six parallel experiments. Therefore, the present method can be used for the quantitative detection of procaine and simultaneous inspection of trace PABA in real pharmaceutical samples.

#### Study of the pharmacokinetics of procaine

To demonstrate the utility of the present method in *in vivo* conditions, the pharmacokinetics of procaine in the plasma of SD rats and rabbits were studied. Figure 6(a) shows the oxidation peak current of procaine spiked in blank plasma as measured by DPV. The calibration curve for procaine in plasma was obtained based on the results in Fig. 6(a). It showed a linear relationship between the peak current and the spiked procaine concentration ranging from 1.0 to  $50.0 \text{ } \mu\text{M}$  ( $r=0.9981$ ; Fig. 6(b)). In addition, it was found that the current peak potential of procaine in plasma shifted to positive values with the increase of procaine concentration. This phenomenon may be caused by the fact that the major transport form of procaine in vessel is a plasma protein-conjugated procaine which will be electrochemically oxidized at more positive potentials than procaine.

The interferences of foreign species were evaluated on the determination of procaine in plasma; possible interferents such as ascorbic acid ( $200 \text{ } \mu\text{M}$ ), dopamine ( $200 \text{ } \mu\text{M}$ ), uric acid ( $150 \text{ } \mu\text{M}$ ), and acetaminophen ( $150 \text{ } \mu\text{M}$ ) were individually added into the standard solution containing  $10 \text{ } \mu\text{M}$  procaine. The results indicated no interference (signal change  $>5\%$ ) on the determination of procaine in plasma.

Nevertheless, from the calibration curve (Fig. 6(b)), the procaine concentration in SD rats plasma after intravenous bolus injection administration could be determined. The concentration of procaine at different time points was evaluated by means of linear regression analysis in blank plasma. The concentration–time curve in rats following the intravenous administration of procaine at a dose of  $40 \text{ mg kg}^{-1}$  ( $n=4$ ) is shown in Fig. 6(c). The procaine concentration in rat plasma decreased gradually after an injection in the present study. The pharmacokinetics of procaine in rabbit plasma was also studied. As shown in

Fig. 6(d), the concentration of procaine slumped at the beginning after the administration of procaine at a dose of  $16 \text{ mg kg}^{-1}$  ( $n=4$ ) and then decreased gradually. The pharmacokinetic parameters were estimated by the Kinetics version 4.4.1 software (Thermo Electron Corporation, USA). Selected pharmacokinetic parameters are shown in Table 2, which are consistent with the studies using liquid chromatography–mass spectrometry [18].

#### Conclusion

In summary, we demonstrated a sensitive and rapid electrochemical assay method for the simultaneous determination of procaine and PABA from their mixtures. Electrochemical measurements indicated that well-separated current peaks of procaine and PABA on the GPE could be obtained although their electrochemical reactions were irreversible. Their peak currents showed linear responses to concentration over wide ranges. The simultaneous determination of procaine and PABA with minor cross-interferences was achieved from their mixture solutions. This method is very sensitive, and trace amounts of PABA impurity lower than the Chinese pharmacopoeia method in procaine hydrochloride injection could be distinguished. It has also been successfully applied to determine procaine in pharmaceutical product samples and study the plasma pharmacokinetics. The present method is rapid, easy, and of low cost and could be a promising approach to the clinical pharmacokinetics research as well as for intensive medicines monitoring.

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