Available online at www.sciencedirect.com
SCIENCE DIRECT®
Talanta 67 (2005) 625–633
Talanta
www.elsevier.com/locate/talanta

# Determination of benorilate in pharmaceutical formulations and its metabolite in urine at carbon paste electrode modified by silver nanoparticles

Cheng Yin Wang, Xiao Ya Hu *

*Department of Chemistry and Chemical Engineering, Yangzhou University, 180 Siwangting lu, Yangzhou 225002, China*

Received 30 October 2004; received in revised form 11 March 2005; accepted 15 March 2005
Available online 18 April 2005

**Abstract**

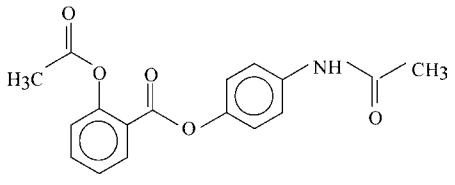
Benorilate was determined by the differential pulse voltammetry (DPV) using a carbon paste electrode modified by silver nanoparticles in $1.25 \times 10^{-3}$ mol l$^{-1}$ $KH_2PO_4$ and $Na_2HPO_4$ buffer solution (pH = 6.88, 25 °C) .The anodic peak potential was +0.970 V (versus SCE). A good linear relationship was realized between the anodic peak currents and benorilate concentrations in the range of $1.0 \times 10^{-7}$ to $2.5 \times 10^{-4}$ mol l$^{-1}$ with the detection limit of $1.0 \times 10^{-8}$ mol l$^{-1}$. The recovery was 95.2–103.6% with the relative standard deviation of 3.6% ($n = 9$). The pharmaceutical preparations, benorilate tablets samples and its metabolite (salicylic acid) in urine were determined with the desirable results.


*Keywords:* Benorilate; Carbon paste electrode; Differential pulse voltammetry; Silver nanoparticles; Salicylic acid

## 1. Introduction

Metal nanoparticles play an important role in modern analytical chemistry due to their usefulness for the preparation of sensors giving rise to improved responses from compounds with respect to those observed at conventional metal surfaces. In particular, gold nanoparticles (Au NPs) have demonstrated to be very appropriate for the development of modified electrodes and to exhibit high catalytic activity towards some electrooxidation reactions [1]. Au NPs modified electrodes have been prepared using carbon paste as electrode substrate [2–5], and were of good long-term stability and high sensitivity [6–8]. Concerning electrodes based on use of silver nanoparticles (Ag NPs), however, there were a limited number of references found in the literature on electrochemical sensors modified by Ag NPs. Very recently, Ag NPs, which are easy to synthesize, have attracted some researchers attention due to their quantum characteristics of small granule diameter and large specific surface area as well as their ability to quickly transfer photoinduced electrons at the surfaces of colloidal particles [9]. Wang et al. reported an amperometric sensor used for determination of thiocyanate with a Ag NPs modified electrode with a surprisingly high electroactivity for the first time in the electrochemistry [10]. Zhu and co-workers have introduced an electrochemical biosensor constructed by nanosized silver particles doped sol–gel film [11]. Li and co-workers investigated effect of Ag NPs on the electron-transfer reactivity and the catalytic activity of myoglobin [12]. There were no antecedents found in the literature on Ag NPs carbon paste sensors, although similar designs using gold nanoparticles have been cited above.

The molecular structure of benorilate (benorylate, benoral, 4′-(acetamido) phenyl-2-acetoxybenzoate) is




* Corresponding author. Tel.: +86 514 7888454; fax: +86 514 7975244.
*E-mail address:* xyhu@yzu.edu.cn (X.Y. Hu).

0039-9140/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2005.03.009

It is the esterification product of paracetamol and acetylsalicylic acid in order to put two different compounds together possessing the same activity [13]. It belongs to a class of medicines called non-steroidal anti-inflammatory drugs [14]. It works by blocking the production of chemicals (prostaglandins) which the body produces in response to injury or certain diseases. These prostaglandins would otherwise go on to cause swelling, pain and inflammation. The drug has also analgesic and antipyretic properties, and helps reduce fever and discomfort in viral illnesses like flu [15]. Benorilate is probably absorbed as the intact molecule which accounts for its good gastric tolerance. After absorption, benorilate is hydrolyzed into its components, salicylate and acetaminophen, which then follows the usual routes of metabolism [16]. Benorilate has shown that it probably has an anti-inflammatory action of its own [17]. Clinical studies have compared benorilate with ibuprofen and have shown its value in rheumatoid arthritis, osteoarthrosis and other musculoskeletal conditions [18]. A long-term study was carried out to determine the possible toxic effects of therapeutic doses of benorilate in patients [19]. The overall tolerance is excellent. Some minor gastric disturbances were reported, but these were less than the gastric disturbances with comparable doses of aspirin. Studies measuring occult blood loss in the stools have shown that this is not a significant problem with benorilate and that the majority of patients lose no more blood than the controls [20].

But there are a limited number of techniques described for the determination of benorilate in pharmaceuticals formulations. The Chinese pharmacopoeia method for conventional assaying benorilate is based on spectrophotometric measurement at 240 nm in the solvent of anhydrous ethanol [21]. The absorption coefficient is $E^{1\%}_{1\,cm} = 730–760$. Chen reported a UV spectrophotometric method for the determination of dissolution of benorilate tablets, 1% sodium laurylsulfate solution was used as a medium, and a paddles method was used with sampling after 45 min [22]. The linear range was 2–14 $\mu g\,ml^{-1}$. Stevens and Gill proposed an HPLC method for the determination of benorilate on an ODS-silica packing material, and three isocratic eluents prepared from isopropanol, formic acid and an aqueous phosphate buffer were used [23]. Marzo et al. developed a HPLC analytical method which detected benorilate and its active metabolites by extraction from plasma or tissue homogenate with diethyl ether and acetate in two steps [24]. Some investigations have been focused on the GC method for determination of benorilate and its metabolites [25,26].

However, many of above methods required several time-consuming manipulation steps, sophisticated instruments and special training. For these reasons, the rapid, simple and accurate method with high sensitivity is expected to be established. There is no report for quantitative determination of benorilate by the electrochemical method. Carbon paste electrode (CPE) has been widely used in determination of drugs, biomolecule, and other organic species because of their easy preparation and wider potential window of −1.4 to +1.3 V (versus SCE) according to experimental conditions [27,28]. Their residual currents are 10 times lower than those of the glassy carbon electrodes or noble metallic electrodes [29].

In this work, an electrochemical analysis for benorilate was proposed by differential pulse voltammetry at a CPE modified by Ag NPs (diameter 2–3 nm) for the first time. A sensitive anodic oxidative peak of benorilate was used for quantitative determination. A good linear relationship was realized between the anodic peak currents and benorilate concentrations in the range of $1.0 \times 10^{-7}$ to $2.5 \times 10^{-4}\,mol\,l^{-1}$ with the detection limit of $1.0 \times 10^{-8}\,mol\,l^{-1}$ and good reproducibility. The validation parameters of the method were evaluated. Compared with the UV spectrophotometry, the detection limit of this method decreased two orders of magnitude, and its sensitivity was higher than that of HPLC method [23,24]. The developed method was applied to the analysis of two different commercial pharmaceutical tablet formulations with the desirable results which were coincident with those derived from UV spectrophotometry [21]. This method has the advantages of lower interference, rapid and simple operation and high accuracy.

## 2. Experiment

### 2.1. Reagents

A stock anhydrous ethanol solution of benorilate ($1.0 \times 10^{-3}\,mol\,l^{-1}$) was prepared, and kept in the dark under refrigeration (low 4 °C). All reagents were of analytical grade. All solutions were prepared with double-distilled water. The spectroscopial pure graphite powder was obtained from Shanghai carbon factory (China).

### 2.2. Apparatus

Electrochemical measurements were performed using a 660A CHI (CH Instrument, Ins., USA). A three-electrodes system was used with a CPE modified by Ag NPs as working electrode, 3.0 mm diameter, 0.071 $cm^2$ geometrical area, a platinum plate electrode as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. All tests were carried out under room temperature.

### 2.3. Synthesis of the Ag NPs [30]

Under the rapidly stirring, 20 ml of $5 \times 10^{-3}\,mol\,l^{-1}$ sodium diethyldithiocarbamate (DDTC) was added to 20 ml of $5 \times 10^{-3}\,mol\,l^{-1}$ $AgNO_3$ aqueous solution. After about 2 min, there was a great deal of deposition. Then 2.0 ml chloroform was added into the solution. The deposition was extracted to the organic phase from the above solution, and the mixture was stirred for 5 min. Twenty milliliters of $0.05\,mol\,l^{-1}$ $NaBH_4$ was added drop-wise slowly. Then the solution was stirred for 2 h. The organic phase was separated. When the chloroform was volatilized in the air at normal tem-

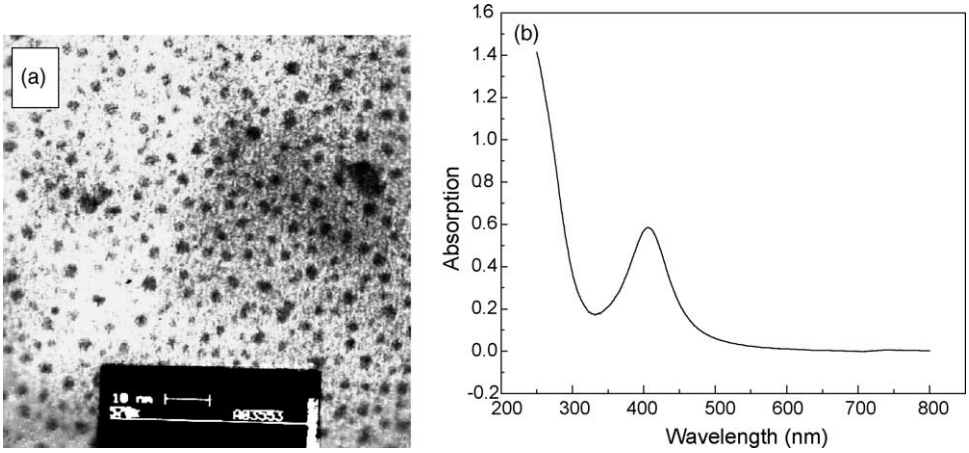



Fig. 1. TEM micrograph of Ag NPs in toluene (a) and UV–vis spectrum of the Ag NPs (b).

perature, the salmon pink Ag NPs were obtained. The 10 mg Ag NPs were dissolved into 20 ml toluene and conserved in the refrigerator below 4 °C. Fig. 1(a) shows that the diameters of Ag NPs were about 2–3 nm by using the Philip Tecnai1 2 transmission electron microscopy (Philip Tecnai1 2, Holland) and Fig. 1(b) shows the characteristic UV–vis absorption of Ag NPs. The extinction band appeared at 414 nm with a full width at half-maximum of 72 nm.

### 2.4. Preparation of the CPE modified by Ag NPs

The carbon paste modified by Ag NPs was prepared by carefully mixing 0.5 g of graphite powders and 3.0 mg Ag NPs (6.0 ml Ag NPs-toluene solution) in a mortar, which the ratio of Ag NPs to graphite powders was 0.6% (m/m). After the solvent had volatilized for 24 h in the mortar, 0.3 g Nujol oil was added and blended. The carbon paste was filled in the glass tube with diameter of 6 mm and length of 12 cm. A graphite carbon electrode was inserted in the glass tube. The tip of graphite carbon was sealed with a copper wire as an electrical conduct. The thickness of the carbon paste was about 6 mm in the glass tube. When the surface of the electrode needed renewed, 2 mm carbon paste was squeezed out with the graphite bar like a piston, and the carbon paste was then scratched and smoothed on the surface of a piece of glass. A good reproducibility of electrode response was achieved by simply renewing the surface of carbon paste repetitively.

### 2.5. Determination of benorilate

The CPE modified by Ag NPs, the platinum plate counter electrode, and the saturated calomel reference electrode (SCE) were immersed in 20.0 ml of $1.25 \times 10^{-3}$ mol l$^{-1}$ $KH_2PO_4$ and $Na_2HPO_4$ buffer solution (pH 6.88). Before this determination, the CPE modified by Ag nanoparticles was pre-activated usually by cycle voltammetry with 100 mV s$^{-1}$ scan rate in the base solution, composing of $KH_2PO_4$ and $Na_2HPO_4$ buffer solution. The cycle voltammetry was carried out from −1.5 to 1.5 V until cycle voltammograms stabilization after several scan segments. A certain amount of benorilate was added, and the solution was stirred by a magnetic stirrer. The stirring was stopped after the electrochemical accumulation for 30 s at 0.0 V. The differential pulse voltammetry was immediately performed from 0 to 1.4 V. In order to establish the optimum conditions for the determination of benorilate by means of DPV technique, various instrumental variables were studied, and the optimum conditions were as follows: scan rate: 10 mV s$^{-1}$, sampling width: 0.05 s, pulse amplitude: 50 mV, and pulse period: 0.2 s. The anodic peak current at 0.970 V was recorded (Fig. 2). The standard addition method was applied for quantitative determination of benorilate concentration. After each determination, the electrode was washed in $1.25 \times 10^{-3}$ mol l$^{-1}$ phosphate buffer solutions for several minutes under stirring, and the scan of DPV was simultaneously carried out until there was no peak wave at 0.970 V as the memory effect on CPE. Otherwise, the renewal step should be repeated again.

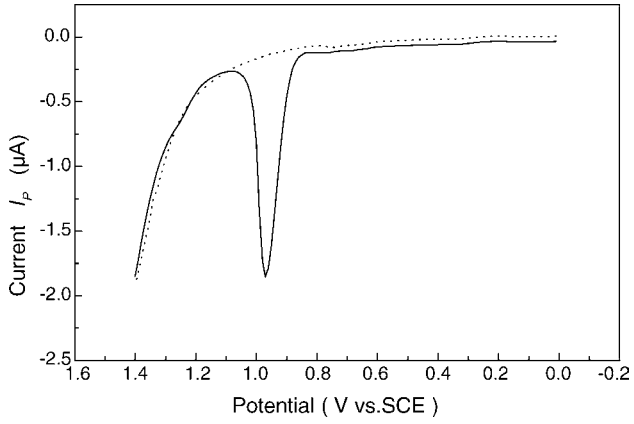



Fig. 2. Differential pulse voltammograms of the Ag NPs modified CPE in $1.25 \times 10^{-3}$ mol l$^{-1}$ $KH_2PO_4$ and $Na_2HPO_4$ buffer solution (pH 6.88), accumulation potential under stirring: 0.0 V, accumulation time: 30 s, quiet time: 20 s, scan rate: 0.01 V s$^{-1}$, pulse height: 0.050 V, sampling width: 0.05 s, pulse period: 0.2 s, sensitivity: $1.0 \times 10^{-6}$ A V$^{-1}$, solid line: in $2.0 \times 10^{-6}$ mol l$^{-1}$ benorilate, short dot line: in absence of benorilate.

## 3. Results and discussion

### 3.1. Optimization of analysis condition

Some supporting electrolytes were examined, such as HCl, $H_2SO_4$, HAc–NaAc, $NH_4Cl$–$NH_3{\cdot}H_2O$, KCl, NaOH, $HClO_4$, and phosphate buffer solution. It was found that pH value of the base solution, but not the type of ions in particular supporting electrolyte, played a critical role. Fig. 3 shows that in acidic media, HAc–NaAc solution (pH 3.39), hydrolysis of benorilate took partially place. So the anodic peak current of benorilate decreased and the peak of acetaminophen was observed (about +0.18 V). In basic media, $Na_2HPO_4$–NaOH solution (pH 10.1), the anodic peak current of benorilate decreased similarly and the peak of acetaminophen the peak of acetaminophen (about +0.18 V) were observed distinctly. It was also due to the hydrolysis. The extent of hydrolysis depends strongly on pH of the medium; hence the hydrolysis influences strongly the concentration of benorilate in the supporting electrolyte. However, benorilate was of stability to hydrolysis in $KH_2PO_4$–$Na_2HPO_4$ buffer solution (pH 6.88) chosen as the base solution during this test. When benorilate concentration was lower than $1.0 \times 10^{-6}$ mol l$^{-1}$, the peak current increased with the increase of accumulation time. But this increase was ceased when the accumulation time was over 30 s, indicating that an adsorption of benorilate at electrode surface reached saturation. So the optimal time of accumulation was chosen as 30 s in following experiments.

### 3.2. Electrochemical behaviors of benorilate

The coulometry was carried out to determine number of electrons involved in the oxidation of benorilate. The benorilate solution ($2.5 \times 10^{-6}$ mol l$^{-1}$) was electrolyzed exhaustively at +1.038 V until the electrolytic current was close to zero, and the quantity of electrical charge $Q_1$ was recorded. Likewise, the background solution was electrolyzed under the same conditions, and the quantity of electrical charge $Q_2$ was obtained. The charge difference $Q = (Q_1 - Q_2)$ represents the quantities of electrical charge consumed by benorilate:

$$Q = nFCV \quad (1)$$

$C$ and $V$ here are the concentration and vo1ume of the benorilate solution respectively. $n$ and $F$ have their conventional meanings. From Eq. (1), the electron number $n$ of the electrochemical reaction of benorilate could be calculated to be 1.91. So $n$ was 2 approximately. The experimental results indicated that there was a good linear relationship between the peak potentials and log $v$ with the slope of 0.0663 V which equals $0.059/\beta n$ according to Nernst equation. Here $\beta$ was the coefficient of electrons transfer. Then $\beta n$ was found to be 0.88. On the other hand, as for the irreversible reaction on the electrode surface, there existed such an equation between the half-peak width of the differential pulse voltammogram and electron-transfer number ($n$) as: $W_{1/2} = 62.5/\beta n$ (mV) [31]. $W_{1/2}$ was 72.1 mV from Fig. 4, so $\beta n$ was found to be 0.87. It was coincident with above results. The pH dependence studies were also preformed. The pH value affected the peak potential of benorilate. The peak potentials shifted towards positive direction with decrease of pH value. A good linear relationship was obtained between the peak potential and pH value in the range of pH 5.9–8.1 with the slope of −0.0619 from Nernst equation $E_p = K - (0.059\,m/\beta n)$ pH. Here $m$ was the number of $H^+$ transfer. $m$ could be calculated to 0.92. So $m$ was 1 approximately.

In order to study some aspects of electrochemical behaviors of benorilate, the cyclic voltammetry was carried out in $2.5 \times 10^{-2}$ mol l$^{-1}$ $KH_2PO_4$ and $Na_2HPO_4$ buffer solution (pH 6.88). It was found that the cyclic voltammograms were very similar with those of phenacetin (acetophenetidin)

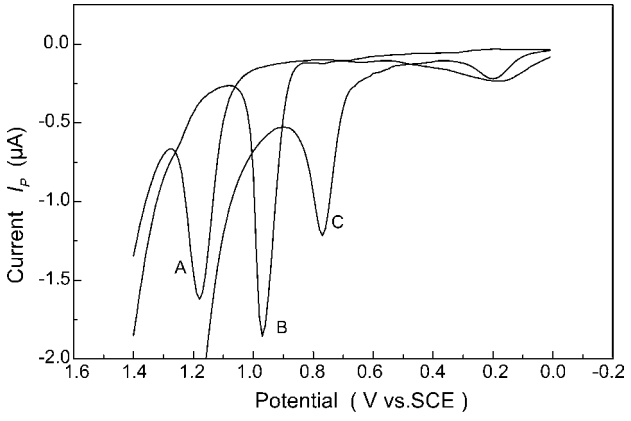



Fig. 3. Differential pulse voltammograms of the Ag NPs modified CPE in different base solutions ($2.0 \times 10^{-6}$ mol l$^{-1}$ benorilate had been prepared for 1 h). (A) 0.01 mol l$^{-1}$ HAc + $1 \times 10^{-3}$ mol l$^{-1}$ NaAc (pH 3.39), (B) $1.25 \times 10^{-3}$ mol l$^{-1}$ $KH_2PO_4$ and $Na_2HPO_4$ buffer solution (pH 3.32), (C) $1.5 \times 10^{-3}$ mol l$^{-1}$ $Na_2HPO_4$ + $2 \times 10^{-3}$ mol l$^{-1}$ NaOH (pH 10.1). The other experimental conditions were: accumulation potential under stirring: 0.0 V, accumulation time: 30 s, quiet time: 20 s, scan rate: 0.01 V s$^{-1}$, pulse height: 0.050 V, sampling width: 0.05 s, pulse period: 0.2 s, sensitivity: $1.0 \times 10^{-6}$ A V$^{-1}$.

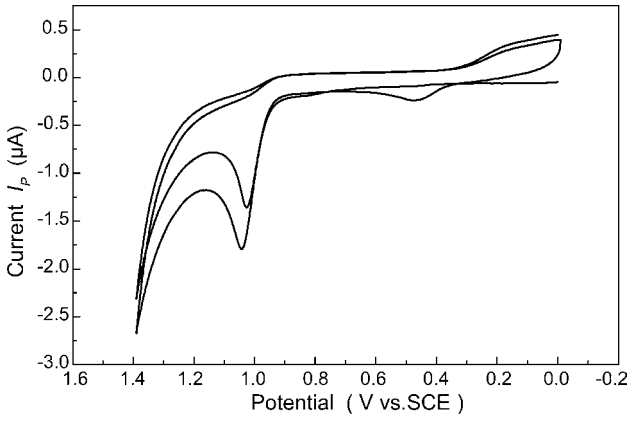



Fig. 4. Cycle voltammograms of $2.0 \times 10^{-6}$ mol l$^{-1}$ benorilate on Ag NPs modified CPE in $2.5 \times 10^{-2}$ mol l$^{-1}$ $KH_2PO_4$ and $Na_2HPO_4$ buffer solution (pH 6.88), accumulation potential under stirring: 0.0 V, accumulation time: 30 s, quiet time: 20 s, scan rate: 0.1 V s$^{-1}$, sensitivity: $1.0 \times 10^{-6}$ A V$^{-1}$.

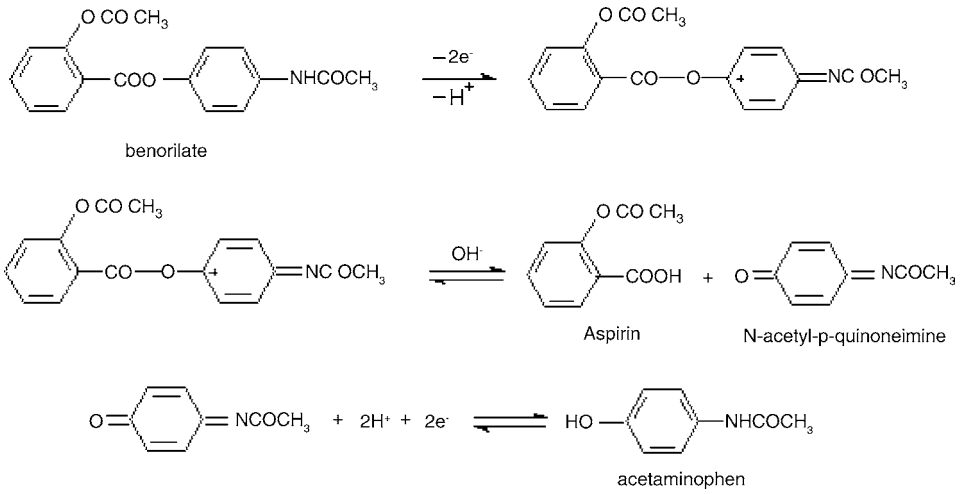



Fig. 5. Schematic reaction mechanism at the electrode.

which was reported by Kissinger and co-workers [32]. The mechanism was illustrated by the cycle voltammograms of benorilate shown in Fig. 4. Firstly, the benorilate was oxidised to formation of an *N*-acetyl-*p*-quinoneimine but the oxygen remains substituted and acquires a positive charge (the peak at 1.038 V in Fig. 4). It was likely that hydroxide ion was principally responsible for attacking rapidly the charged intermediate, liberating the aspirin and *N*-acetyl-*p*-quinoneimine [32]. Following the initial oxidation, a corresponding reduction scan was not observed. Instead, a redox couple attributable to the reaction of *N*-acetyl-*p*-quinoneimine to be reduced back to acetaminophen and the subsequent reoxidation of acetaminophen were observed obviously on second and subsequent scans (the reduction peak at 0.161 V and the oxidation peak at 0.477 V in Fig. 4). Experimental results indicated that the peak currents of cyclic voltammetry at 1.059 V in $2.0 \times 10^{-5}$ mol l$^{-1}$ benorilate solution were proportional to the scan rates ($\nu$) implying that the electrode process was a typical surface adsorption process. When benorilate concentration exceeded $5.0 \times 10^{-5}$ mol l$^{-1}$, the peak currents were proportional to the $\nu^{1/2}$ indicating that the electrode process was controlled by the diffusion process.

From above discussion and reference, the electrochemical reaction mechanism of benorilate could be concluded that the redox of a benorilate molecule was a process of one $H^+$ and two electrons [32]. The possible reaction by EC mechanism was shown in Fig. 5.

### 3.3. The role of the Ag NPs

The anodic peak currents of benorilate and the stability of electrode were considerably related to the ratio of Ag NPs to graphite powders in the modified carbon paste. The Ag NPs contents of 0, 0.3, 0.6, 1.2% (m/m) corresponded with only graphite powders, 0.5 g of graphite powders with 1.5 mg Ag NPs (3.0 ml Ag NPs-toluene solution), 0.5 g of graphite powders with 3.0 mg Ag NPs (6.0 ml Ag NPs-toluene solution), 0.5 g of graphite powders with 6.0 mg Ag NPs (12 ml Ag NPs-toluene solution), respectively. Fig. 6 shows that the anodic peak current of benorilate at +1.040 V was recorded for the conventional CPE, and at +0.970 V was recorded for the CPE by modified Ag NPs. The peak potential obviously shifted negatively about 70 mV. The peak potential shifts towards more negative potentials by increasing the content of Ag NPs. The reason is that the increase of the content of Ag NPs can enhance the catalysis of the electrode, whereas the non-metallic properties of the silver sub-nanoparticles can lower the electron conductivity of electrode by increasing the content of Ag NPs [33].

The underlying reason for the catalytic oxidation kinetics exhibited by Ag NPs will be the subject of further study. An aspect deserving of closer scrutiny is the possibility that nanometer-sized silver particles can become now ideal can-

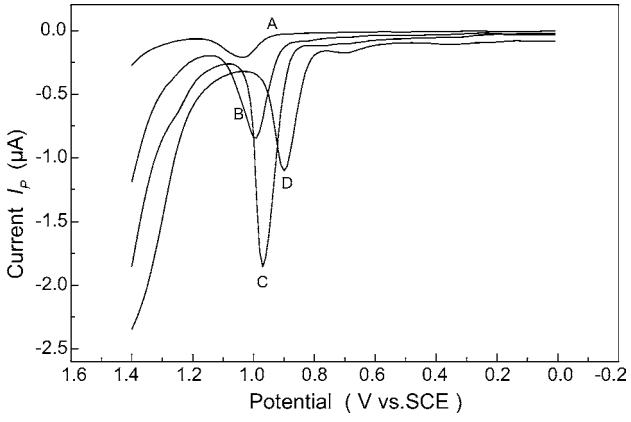



Fig. 6. Differential pulse voltammograms of $2.0 \times 10^{-6}$ mol l$^{-1}$ benorilate with various contents of Ag NPs on the CPE in phosphate buffer, the contents of Ag NPs: (A) 0%, (B) 0.3%, (C) 0.6%, (D) 1.2% (m/m). The other experimental conditions were: accumulation potential under stirring, 0.0 V; accumulation time, 30 s; quiet time, 20 s; scan rate, 0.01 V s$^{-1}$; pulse height, 0.050 V; sampling width, 0.05 s; pulse period, 0.2 s; sensitivity, $1.0 \times 10^{-6}$ A V$^{-1}$.

didates for being used as catalyst by providing huge surface areas. Zheng and Liu investigated their quantum characteristics of small granule diameter and large specific surface area as well as their ability to quickly transfer electrons at the surfaces of colloidal particles [34,35]. Li et al. reported Ag NPs might be a competent material for making such shuttles, and can greatly enhance the electron-transfer reactivity of myoglobin and its catalytic ability toward hydrogen peroxide [12]. Our experimental results have revealed that a fast electron-transfer reactivity of benorilate can be obtained with the help of Ag NPs, and a well-defined oxidation peak can be observed at the modified electrode. The catalytic ability and sensitivity of benorilate have also been improved by the Ag NPs. The optimum content of Ag NPs was 0.6% for the determination of benorilate.

### 3.4. Calibration curve and detection limit

The anodic peak currents were proportional to benorilate concentrations in the range of $1.0 \times 10^{-7}$ to $2.5 \times 10^{-4}$ mol l$^{-1}$ under the optimum experimental conditions. The linear equation was $I_p\,(\mu A) = 0.013 + 0.850 \times 10^6 C\,(\text{mol l}^{-1})$ with the correlation coefficient $r = 0.998$. The calibration curve deviated from the linear relationship when the benorilate concentration was more than $2.5 \times 10^{-4}$ mol l$^{-1}$. In this study, the detection limit of benorilate was $1.0 \times 10^{-8}$ mol l$^{-1}$ in terms of the role of signal-to-noise ratio of 3:1 (S/N = 3). A further lower detection limit could be obtained by prolonging the time of accumulation.

### 3.5. Reproducibility

The benorilate of $2.0 \times 10^{-6}$ mol l$^{-1}$ was determined repeatedly with the same CPE for nine times. The average current was 1.72 μA with the R.S.D. of 2.2%. Then the precision of peak current values with the CPE renewed after each determination was also measured. The average peak current of $2.0 \times 10^{-6}$ mol l$^{-1}$ benorilate was 1.69 μA with the R.S.D. of 3.1% ($n = 9$). With the same electrode, the measurement results were in a good agreement in 3 months indicating that the reproducibility of the electrode was remarkable.

### 3.6. Interference

When $5.0 \times 10^{-6}$ mol l$^{-1}$ benorilate was determined under the optimum experimental conditions, no interferences were observed in the presence of $1.0 \times 10^{-3}$ mol l$^{-1}$ of NaCl, $KNO_3$, $(NH_4)SO_4$, $ZnSO_4$, $Al(NO_3)_3$, $Mg(NO_3)_2$, $Ca(NO_3)_2$, glucose, oxalic acid, urea, tartaric acid, caffeine, $5.0 \times 10^{-4}$ mol l$^{-1}$ of cysteine, cystine, DL-tyrosine, glutamic acid, citrate, malic acid, $CuSO_4$, $2.0 \times 10^{-4}$ mol l$^{-1}$ of albumin fraction and $Fe(NO_3)_3$ respectively. Some conventional medicaments, such as $1.0 \times 10^{-4}$ mol l$^{-1}$ of Vitamin $B_1$, Vitamin $B_2$, Vitamin $B_6$, atropine, penicillin, and theine, did not interfere with the determination. For the following solid pharmaceutical formulations analysis, tablet excipient, such as starch, was added in the system of the determination. It was not found that the results were changed in the presence of the excipient. Some impurities, such as salicylic acid, acetaminophen and aspirin from benorilate had some responses at the electrode in the ranges of scan potential. Fig. 7 shows that the peak of salicylic acid is very near one of benorilate (benorilate: 0.970 V, salicylic acid: 0.880 V), but does not interfere with the measurement of benorilate when the concentration of salicylic acid is lower than $1.5 \times 10^{-5}$ mol l$^{-1}$. In spite of their peaks of oxidation currents approach, the method could be applicable to our samples due to the fact that the amount of salicylic acid, depending on the specific benorilate formulation, was limited to 0.1%, as specified by the pharmacopoeia in solid pharmaceutical formulations [21]. Fig. 8 shows that acetaminophen does not interfere with the oxidation peak currents of benorilate when the concentration of acetaminophen is lower than $1.0 \times 10^{-4}$ mol l$^{-1}$. The determination of benorilate in the presence of *p*-aminophenol did not also be interfered because obvious separation of anodic peak potentials. But we had found the $1.0 \times 10^{-5}$ mol l$^{-1}$ acetylsalicylic acid (aspirin) interfered with the determination (Fig. 9). The peak current of benorilate was decreased with increasing the concentration of acetylsalicylic acid. In the presence of aspirin and excessive acetaminophen, the peak currents of benorilate can decrease owing to Le Chatelier's Principle.

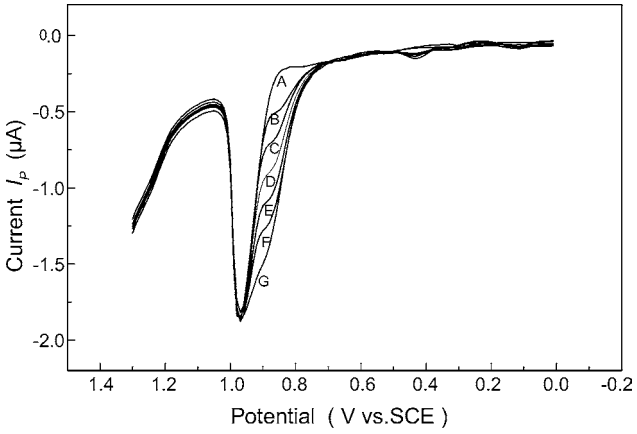



Fig. 7. Differential pulse voltammetry of $2.0 \times 10^{-6}$ mol l$^{-1}$ benorilate in the presence of salicylic acid, (A) benorilate, salicylic acid concentration: (B) $1.5 \times 10^{-6}$ mol l$^{-1}$, (C) $5.0 \times 10^{-6}$ mol l$^{-1}$, (D) $7.5 \times 10^{-6}$ mol l$^{-1}$, (E) $1.0 \times 10^{-5}$ mol l$^{-1}$, (F) $1.25 \times 10^{-5}$ mol l$^{-1}$, (G) $1.5 \times 10^{-5}$ mol l$^{-1}$. The other experimental conditions were: accumulation potential under stirring, 0.0 V; accumulation time, 30 s; quiet time, 20 s; scan rate, 0.01 V s$^{-1}$; pulse height, 0.050 V; sampling width, 0.05 s; pulse period, 0.2 s.

### 3.7. Recovery

The recovery tests of benorilate ranging from $5.0 \times 10^{-7}$ to $2.5 \times 10^{-4}$ mol l$^{-1}$ were performed. The results are listed in Table 1. The recoveries varied on the range from 95.2 to 103.6% and the R.S.D. was 3.6%.

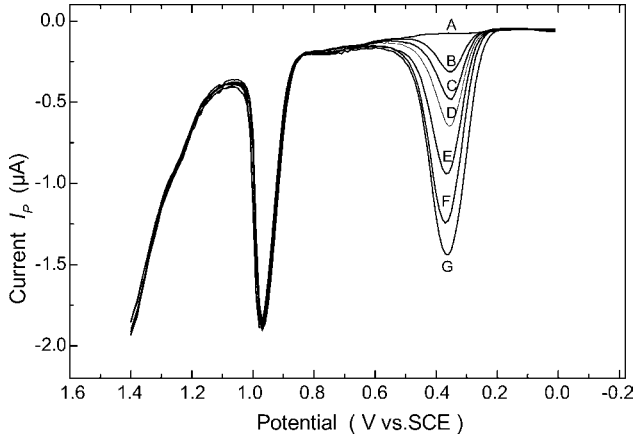



Fig. 8. Differential pulse voltammetry of $2.0 \times 10^{-6}$ mol l$^{-1}$ benorilate in the presence of acetaminophen, (A) benorilate, paracetamol concentration: (B) $2.0 \times 10^{-6}$ mol l$^{-1}$, (C) $4.0 \times 10^{-6}$ mol l$^{-1}$, (D) $6.0 \times 10^{-6}$ mol l$^{-1}$, (E) $1.0 \times 10^{-5}$ mol l$^{-1}$, (F) $1.5 \times 10^{-5}$ mol l$^{-1}$, (G) $2.0 \times 10^{-5}$ mol l$^{-1}$. The peak at 0.380 V was caused by paracetamol. The other experimental conditions were: accumulation potential under stirring, 0.0 V; accumulation time, 30 s; quiet time, 20 s; scan rate, 0.01 V s$^{-1}$; pulse height, 0.050 V; sampling width, 0.05 s; pulse period, 0.2 s.

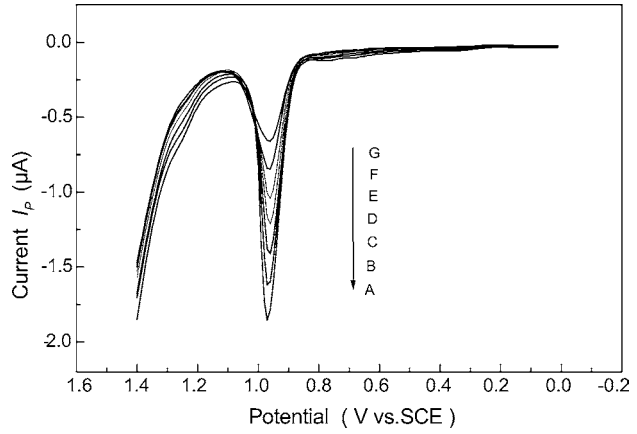



Fig. 9. Differential pulse voltammetry of $2.0 \times 10^{-6}$ mol l$^{-1}$ benorilate in the presence of acetylsalicylic acid, (A) benorilate, acetylsalicylic acid concentration: (B) $1.0 \times 10^{-5}$ mol l$^{-1}$, (C) $2.0 \times 10^{-5}$ mol l$^{-1}$, (D) $3.0 \times 10^{-5}$ mol l$^{-1}$, (E) $4.0 \times 10^{-5}$ mol l$^{-1}$, (F) $5.0 \times 10^{-5}$ mol l$^{-1}$, (G) $6.0 \times 10^{-5}$ mol l$^{-1}$. The other experimental conditions were: accumulation potential under stirring, 0.0 V; accumulation time, 30 s; quiet time, 20 s; scan rate, 0.01 V s$^{-1}$; pulse height, 0.050 V; sampling width, 0.05 s; pulse period, 0.2 s.

### 3.8. Determination of the pharmaceutical preparation samples

The developed DPV method for the benorilate determination was applied to two different commercial preparations (benorilate tablets: HEFEN Pharmaceutical Co. Ltd., China, Jingnuopiling®, batch No. 0301001055 and 0303021075, labeled amount of 0.5 g per tablet. DIAO groups Co. Ltd., China, Bolelai®, batch No. 030715, labeled amount of 0.4 g per tablet). A known number of tablets were grounded to fine powders, and an accurate mass of powders about 500.0 mg was transferred to a 50 ml calibrated flasks. It was extracted for 5 min with 20.0 ml of cold ethanol. No change of the peak potentials in the presence of the excipient was observed. Table 2 gives the results of DPV analysis of the commercial preparations. The Chinese pharmacopoeia methods (UV spectrophotometry) was employed as a comparison to evaluate the validity of the developed method [21]. There was no significant difference between methods and a good agreement was achieved. The results obtained from this study show that the proposed methods would be recommended for the determination of benorilate in tablets. The developed method could be easily used in quality control laboratory for the analysis of benorilate in solid pharmaceutical formulations.

Table 1
The recoveries of benorilate test using present method

| Added (mol l$^{-1}$) | Found (mol l$^{-1}$) | Recovery (%) |
|---|---|---|
| $5.00 \times 10^{-7}$ | $4.77 \times 10^{-7}$ | 95.4 |
| $1.00 \times 10^{-6}$ | $1.03 \times 10^{-6}$ | 103.0 |
| $2.50 \times 10^{-6}$ | $2.59 \times 10^{-6}$ | 103.6 |
| $5.00 \times 10^{-6}$ | $4.82 \times 10^{-6}$ | 96.4 |
| $1.00 \times 10^{-5}$ | $9.73 \times 10^{-5}$ | 97.3 |
| $5.00 \times 10^{-5}$ | $5.12 \times 10^{-5}$ | 102.4 |
| $7.50 \times 10^{-5}$ | $7.19 \times 10^{-5}$ | 95.9 |
| $1.00 \times 10^{-4}$ | $9.55 \times 10^{-5}$ | 95.5 |
| $2.50 \times 10^{-4}$ | $2.38 \times 10^{-4}$ | 95.2 |

### 3.9. Determination of the metabolite (salicylic acid) in urine

After resorption of benorilate in the gastrointestinal tract, the preparation is entirely hydrolyzed to acetylic salicylic acid and paracetamol [36,37]. The electrochemical oxidation of salicylic acid has been studied on a glassy carbon electrode using cyclic voltammetry and differential pulse voltammetric method in Britton–Robinson buffer solution, pH 2.37 in solid pharmaceutical formulations [38]. We determined salicylic acid by differential pulse voltammetry on CPE modified by Ag NPs, with the experimental conditions similar to Fig. 2. The anodic peak potential of salicylic acid was +0.880 V (vs. SCE). The anodic peak currents were proportional to salicylic acid concentrations in the range of $7.5 \times 10^{-7}$ to $2.5 \times 10^{-4}$ mol l$^{-1}$ un-

Table 2
Determination results of benorilate in tablets

| Batch number | The method in pharmacopoeia ($n = 3$) (g/tablet) | Present method ($n = 5$) (g/tablet) | Present method ($n = 5$) R.S.D. (%) |
|---|---|---|---|
| Sample 1 (0301001055) | 0.498 | 0.493 | 2.5 |
| Sample 2 (0303021075) | 0.497 | 0.494 | 2.8 |
| Sample 3 (030715) | 0.397 | 0.393 | 3.1 |

Table 3
The recoveries of salicylic acid in urine using present method

| Salicylic acid added ($\times 10^{-6}$ mol l$^{-1}$) | Salicylic acid average found ($\times 10^{-6}$ mol l$^{-1}$) ($n = 5$) | R.S.D. ($n = 5$) (%) | Average of recoveries (%) |
|---|---|---|---|
| 0.952 | 0.929 | 4.1 | 97.5 |
| 4.76 | 4.57 | 3.7 | 96.0 |
| 9.52 | 9.75 | 3.2 | 102.4 |
| 14.3 | 13.65 | 3.3 | 95.4 |
| 19.0 | 19.48 | 2.2 | 104.2 |

der the optimum experimental conditions. The linear equation was $I_p$ (μA) = 0.2703 + 5.062 × $10^4 C$ (mol l$^{-1}$) with the correlation coefficient $r = 0.997$. The detection limit was $5.0 \times 10^{-7}$ mol l$^{-1}$ (S/N = 3).

When $1.0 \times 10^{-6}$ mol l$^{-1}$ salicylic acid in urine was determined, no interferences were observed in the presence of $1.0 \times 10^{-3}$ mol l$^{-1}$ of NaCl, $KNO_3$, $(NH_4)SO_4$, $ZnSO_4$, $Al(NO_3)_3$, $Mg(NO_3)_2$, $Ca(NO_3)_2$, glucose, oxalic acid, urea, tartaric acid, caffeine, $5.0 \times 10^{-4}$ mol l$^{-1}$ of cysteine, cystine, DL-tyrosine, glutamic acid, citrate, malic acid, $CuSO_4$, $2.0 \times 10^{-4}$ mol l$^{-1}$ of albumin fraction and $Fe(NO_3)_3$. Some conventional medicaments, such as $1.0 \times 10^{-4}$ mol l$^{-1}$ of Vitamin $B_1$, Vitamin $B_2$, Vitamin $B_6$, atropine, penicillin, and theine, did not interfere with the determination. The content of uric acid, ascorbic acid, adrenaline and dopamine in urine of a healthy human did not interfere with the determination of salicylic acid. Paracetamol was another main metabolite of benorilate. Its anodic peak potential was +0.380 V, and its currents were interfered by uric acid in urine because the anodic peak potential of uric acid was +0.520 V.

An aliquot of original urine was collected. Two certain amounts of salicylic acid and paracetamol were added into 50.0 ml of urine (both the concentrations were $2.0 \times 10^{-6}$ mol l$^{-1}$), then 10.0 ml of above urine sample was transferred into an electrolytic cell. Then 10.0 ml $2.5 \times 10^{-3}$ mol l$^{-1}$ $KH_2PO_4$–$Na_2HPO_4$ buffer solution was added to the electrolytic cell. The solution was stirred by a magnetic stirrer. The accumulation was carried out at 0.0 V for 30 s. Then the stirring stopped with quiet time 20 s. The differential pulse voltammetry was immediately performed from 0.0 to 1.4 V (Fig. 10), and other experimental parameters were similar to Fig. 2. The salicylic acid concentration in the urine was determined by the multiple standard additions of DPV. Table 3 shows the average recoveries of salicylic acid in urine was 95.4–104.2% with the desirable results.

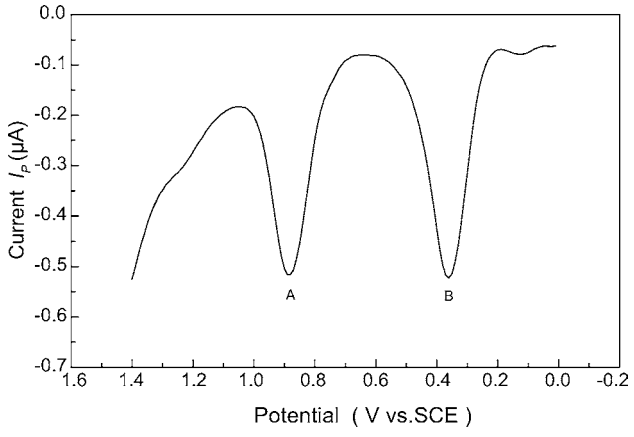



Fig. 10. Differential pulse voltammetry of salicylic acid in urine, supporting electrolyte: $1.25 \times 10^{-3}$ mol l$^{-1}$ $KH_2PO_4$ and $Na_2HPO_4$ buffer solution (pH 6.88), (A) salicylic acid concentration: $2.0 \times 10^{-6}$ mol l$^{-1}$, (B) paracetamol ($2.0 \times 10^{-6}$ mol l$^{-1}$) and the uric acid in urine. The other experimental conditions were: accumulation potential under stirring, 0.0 V; accumulation time, 30 s; quiet time, 20 s; scan rate, 0.01 V s$^{-1}$; pulse height, 0.050 V; sampling width, 0.05 s; pulse period, 0.2 s.

## 4. Conclusion

This paper described a simple and sensitive method for the detection of benorilate in solid pharmaceutical formulations and its metabolite (salicylic acid) in urine by differential pulse voltammetry with a CPE modified by Ag NPs. Significant advantages were achieved by the simple fabrication of this electrode, rapid determination, excellent sensitivity and selectivity. The reliability and stability of the CPE modified by Ag NPs offered a good possibility for extending the technique in pharmaceutical analysis.

## Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 20175023, 20375034), and Natural Science Foundation of Jiangsu Provincial Education Department (No. 02KJB150013).

## References

[1] K. Yahikozawa, K. Nishimura, M. Kumazawa, N. Tateishi, Y. Takasu, K. Yasuda, Y. Matsuda, Electrochim. Acta 37 (1992) 453.
[2] H.X. Ju, S. Liu, B. Ge, F. Lisdat, F.W. Scheller, Electroanalysis 14 (2002) 141.
[3] M.B. González-Garćıa, C. Fernández-Sánchez, A. Costa-Garćıa, Biosens. Bioelectron. 15 (2000) 315.
[4] H.X. Ju, S.Q. Liu, B. Ge, F. Lisdat, F.W. Scheller, Electroanalysis 14 (2002) 141.
[5] S. Liu, J. Yu, H. Ju, J. Electroanal. Chem. 540 (2003) 61.
[6] Y. Xiao, H.X. Ju, H.Y. Chen, Anal. Biochem. 278 (2000) 22.
[7] L. Agüí, J. Manso, P. Yáñez-Sedeño, J.M. Pingarrón, Talanta 64 (2004) 1041.

[8] K. Kerman, Y. Morita, Y. Takamura, M. Ozsoz, E. Tamiya, Anal. Chim. Acta 510 (2004) 169–174.
[9] T. Liu, J. Zhong, X. Gan, C. Fan, G. Li, N. Matsuda, Chem. Phys. Chem. 4 (2003) 1364.
[10] G.F. Wang, M.G. Li, Y.C. Gao, B. Fang, Sensors 4 (2004) 147.
[11] J.Z. Xu, Y. Zhang, G.X. Li, J.J. Zhu, Mater. Sci. Eng. C 24 (2004) 833.
[12] X. Gan, T. Liu, J. Zhong, X.J. Liu, G.X. Li, Chem. BioChem. 5 (2004) 1686.
[13] S. Similä, S. Keinänen, K. Kouvalainen, Eur. J. Pediatr. 121 (1975) 15.
[14] O.R. Day, G.G. Graham, K.M. Williams, G.D. Champion, J.D. Jager, Pharmacol. Ther. 33 (1987) 383.
[15] H. Müller-Fassbender, M. Schattenkirchner, Scand. J. Rheumatol. Suppl. 13 (1975) 21.
[16] F.M. Williams, U. Moore, R.A. Seymour, E.M. Mutch, E. Nicholson, P. Wright, H. Wynne, P.G. Blain, M.D. Rawlins, J. Clin. Pharmacol. 28 (1989) 703.
[17] M. Franke, G. Manz, J.P. Glynn, Scand. J. Rheumatol. Suppl. 13 (1975) 13.
[18] L.G. Darlington, E.N. Coomes, Rheumatol. Rehabil. 14 (1975) 76.
[19] W.Z. Reiter, Z. Rheumatol. 34 (1975) 270.
[20] V. Wright, Scand. J. Rheumatol. Suppl. 13 (1975) 5.
[21] Pharmacopoeia Committee of the Chinese Ministry of Public Health, Chinese Pharmacopoeia, second part, Chemical Industry Press, Beijing, 2000, p. 58.
[22] Z.D. Chen, K.R. Du, J.Z. Zeng, D. Zhang, F. Deng, Chinese J. Pharm. Anal. 22 (2002) 55.
[23] H.M. Stevens, R. Gill, J. Chromatogr. 370 (1986) 39.
[24] A. Marzo, G. Quadro, E. Treffner, M. Ripamonti, G. Meroni, C. Lucarelli, Arzneimittel-forsch. 40 (1990) 813.
[25] A. Cailleux, P. Cailleux, P. Allain, Therapie 34 (1979) 73.
[26] R.E. Ardrey, A.C. Moffat, J. Chromatogr. A 220 (1981) 195.
[27] C.Y. Wang, X.Y. Hu, G.D. Jin, Z.Z. Leng, J. Pharm. Biomed. Anal. 30 (2002) 131.
[28] C.Y. Wang, X.Y. Hu, Z.Z. Leng, G.J. Yang, G.D. Jin, Anal. Lett. 34 (2001) 2747.
[29] S.J. Dong, G.L. Che, Y.W. Xie, Chemically Modified Electrodes, Chinese Science Press, Beijing, 1995, p. 39.
[30] M. Brust, M. Walker, D. Bethell, D.J. Schiffrin, R. Whyman, J. Chem. Soc., Chem. Commun. 7 (1994) 801.
[31] E. Laviron, J. Electroanal. Chem. 101 (1979) 19.
[32] D.J. Miner, J.R. Rice, R.M. Riggh, P.T. Kissinger, Anal. Chem. 53 (1981) 2258.
[33] F. Remacle, R.D. Levine, Chem. Phys. Chem. 2 (2001) 20.
[34] J. Zheng, G. Chumanov, T.M. Cotton, Chem. Phys. Lett. 349 (2001) 367.
[35] T. Liu, J. Zhong, X. Gan, C. Fan, G. Li, N. Matsuda, Chem. Phys. Chem. 4 (2003) 1364.
[36] E. Liss, A. Robertson, Arzneimittel-forsch. 11 (1975) 1972.
[37] P.W. Lücker, M. Birkel, B. Hey, I. Loose, A. Schaefer, Meth. Find. Exp. Clin. Pharmacol. 25 (2003) 631.
[38] A.A.J. Torriero, J.M. Luco, L. Sereno, J. Raba, Talanta 62 (2004) 247.