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Short communication

Flow-injection biamperometric direct determination of calcium dobesilate in irreversible couple system

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Abstract

A flow-injection biamperometric method for direct determination of calcium dobesilate had been proposed based on biamperometric detection for irreversible couple. The detection was realized by coupling the oxidation of dobesilate at one platinum wire electrode with the reduction of MnO_4^- at another one with the applied potential difference of 0 V between two platinum wire electrodes. Dobesilate was determined in the range of 4.0×10^{-6} to 1.0×10^{-4} M with the detection limit of 8.0×10^{-7} M ($S/N = 3$). The relative standard derivation of 1.7% was obtained for 24 successive determinations of 4.0×10^{-5} M dobesilate. The proposed method had been shown to be sensitive, simple and rapid. © 2003 Elsevier B.V. All rights reserved.

Keywords: Calcium dobesilate; Flow-injection analysis; Biamperometry; Platinum wire electrode

1. Introduction

Calcium dobesilate (doxium) is an angioprotective agent, which acted on capillary vessel selectively. It not only modulated osmose and crispness of patients of the capillary vessel, also inhibited active substance such as hormonal peptide. As calcium dobesilate was principally used to cure disease of capillary vessel, it was necessary to develop a simple, sensitive and rapid method for the determination of it. Several methods had been recommended for the determination of calcium dobesilate such as spectrophotometry [1], HPLC [2], thin-layer chromatography and UV spectroscopy [3].

Biamperometry for reversible couples system had been used in flow-injection analysis [4–8]. Based on the principle of biamperometric endpoint detection, the method showed high sensitivity, high selectivity and signal to noise (S/N) ratio attributed to a small potential differences applied (usually < 200 mV). Unfortunately, the method was restricted in reversible couple field when both the oxidized and the reduced forms of the same species coexist in the flow detector, and only few reversible couples such as Br_2/Br^- and I_2/I^- had been used successively so far. In our previous work, the flow-injection biamperometry for irreversible couple was introduced [9]. In the method, the biamperometric detection scheme was established by coupling two irreversible and independent couples that their half-wave potential were close to each other as possible. The resulting

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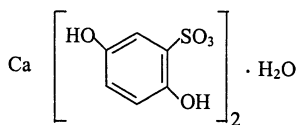
current was limited by the smaller among anodic and cathodic currents that were related to the concentration of each reactant, respectively. When the concentration of one reactant was high enough, the linearity between the signal current and the concentration of the other reactant of interest could be obtained over certain concentration range. The method had the advantages of high selectivity and low noise, and the determination could be performed at a very small potential difference, even 0 V. The flow-injection biampometry had been successfully applied to detect the ascorbic acid [10], hydroxylamine [11], cysteine [12] and hydrazine [13] by coupling the reduction of PtO at one platinum wire electrode with the oxidation of these analytes with low oxidation potential at another one.

As shown in Scheme 1, dobesilate belonged to phenolic compounds. Since the electro-oxidation of dobesilate at the platinum wire electrode was irreversible at very high oxidation potential, another irreversible couple that can be reduced at high potential was needed for establishing a biampometric detection system for the determination of dobesilate. In present work, KMnO_4 was selected to be as another irreversible couple. By coupling the oxidation of dobesilate and the reduction of permanganate, a flow-injection biampometric method with the applied potential difference of 0 V was proposed for the direct determination of dobesilate.

2. Experimental

2.1. Apparatus

A CHI660 electrochemical workstation (CH Instruments, USA) equipped with a personal computer. The dimensions and assembly of the homemade biampometric detector were shown



Scheme 1. Structure of calcium dobesilate.

as Fig. 1. The two platinum wire electrodes (1.1 cm length, \varnothing 0.5 mm) were separated by means of a salt bridge between two electrode rooms. The internal volume of each room was estimated to be 20 μl (1.2-cm length, 0.7 mm i.d.). The platinum wire electrodes were cleaned electrochemically by alternating polarization between +0.5 and -0.5 V in 0.1 M H_2SO_4 solution prior to the measurements. A potential difference (ΔE) was imposed between two platinum wire electrodes of the biampometric detector by connecting both the auxiliary electrode and the reference electrode led to one side of the detector and the working electrode led to the other side.

A flow-injection system was constructed with polyethylene tubing (0.8 mm i.d.) to perform the detection. The system included a model IFIS-B automatic sampling system (Ruik Electronic Instrument Limited Co., Xi'an, China), which was equipped with two peristaltic pumps and a six-way injection valve, controlled by a microcomputer.

Agilent 1100 HPLC system (Hewlett Packard, USA). The chromatographic column: ZORBAX SB-C₁₈ 3.5 μm (150 \times 4.6 mm) column; mobile phase: $\text{CH}_3\text{OH}-\text{H}_2\text{O}$ (60:40, v/v); flow rate: 1.0 ml/min; the wavelength of the detector: 295.8 nm.

2.2. Materials

The standard stock solutions of calcium dobesilate were prepared daily by dissolving calcium

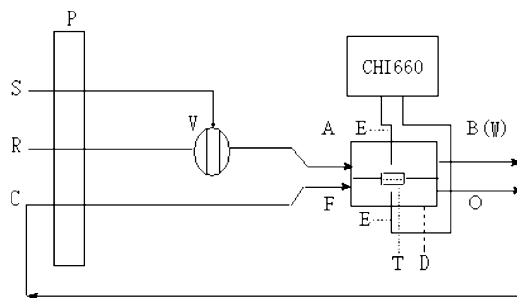


Fig. 1. Schematic diagram of the flow-injection biampometric detection system. (P): peristaltic pump; (V): valve; (S): sample; (R): 0.05 M H_2SO_4 carrier solution; (C): 0.001 M KMnO_4 counter solution; (W): waste; (D): biampometric detector, (A): sample inlet; (B): sample outlet, (E): platinum wire electrode; (F): counter solution inlet; (O): counter solution outlet; (T): salt bridge.

dobesilate (Xi'an Rejoy Pharmaceutical Co., Ltd, Xi'an, China) in water.

0.001 M potassium permanganate solution was prepared by dissolving 0.0158 g potassium permanganate (Xi'an Chemical Plant, Xi'an, China) in 100 ml of 0.05 M H₂SO₄ solution. All chemicals used were of analytical reagent grade. Redistilled water was used throughout.

2.3. Procedure

The biamperometric detection was carried out according to following procedure. By keeping the valve in the washing position, 2.8 ml/min of carrier solution of 0.05 M H₂SO₄ and the same rate of counter solution of 1.0×10^{-3} M KMnO₄ were continuously pumped into the biamperometric detector. When baseline was established on the recorder, 100 μ l of standard or sample solution was injected into the detector. A potential difference (ΔE) of 0 V was kept across the two platinum wire electrodes and the signal current was recorded by the CHI660 workstation. Calibration graphs of signal current versus concentration of dobessilate were plotted, and the content of each sample was determined.

The cyclic voltammetric experiments were performed independently in a cell with three electrodes, a small-sized platinum wire working electrode, a platinum wire auxiliary electrode, and a saturated calomel electrode (SCE).

3. Results and discussion

3.1. Voltammetric investigation of the biamperometric system

In order to check the biamperometric detection system, voltammetric behaviors of dobessilate and MnO₄⁻ at platinum wire electrodes were examined in the potential range from -0.2 to 1.55 V, respectively. Fig. 2, curve a showed a cyclic voltammogram of 1.0×10^{-3} M dobessilate in 0.05 M H₂SO₄ solution at a platinum wire electrode. Before adding dobessilate, an irreversible reduction wave P₁ appeared at about 0.25 V (vs. SCE) on the cathodic scan. The reduction wave

had been extensively studied [11,14] and mainly attributed to the reduction of platinum oxide formed during the anodic pretreatment of platinum wire electrode. After adding dobessilate, besides the reduction wave P₁ kept changeless, a new irreversible oxidation wave P₂ appeared at about 0.78 V (vs. SCE), corresponding to the oxidation of dobessilate. This was due to the oxidation of two hydroxyl groups connecting the phenyl ring in dobessilate.

As shown in Fig. 2 by curve a, the oxidation potential of dobessilate apart from the reduction potential of platinum oxide was about 0.53 V. Provided that the biamperometric detection scheme was constructed from these two couples, a large potential difference (ΔE) between two platinum wire electrodes had to be applied to obtain current response. However, the increasing of the applied ΔE would undoubtedly lead to the decrease of selectivity and S/N level. In order to decrease the applied ΔE , another irreversible couple with opposite electrode process and close $E_{1/2}$ to dobessilate was needed. Among various alternatives, permanganate was selected in present work. As shown in Fig. 2 by curve b, the adding of 1.0×10^{-3} M KMnO₄ led to two reduction waves P₃ and P₄. The wave P₃ was the reduction of platinum oxide and the wave P₄ at about 0.84 V (vs. SCE) corresponds to the reduction of MnO₄⁻ to Mn(II) [15], respectively.

From Fig. 2, the curves a and b, the oxidation wave P₂ and the reduction wave P₄ were separately in anodic and cathodic polarized curves and were from two independent and irreversible couples. These two waves overlapped. By coupling the oxidation of dobessilate and the reduction of MnO₄⁻, establishing the biamperometric detection scheme was very ideal. In this case, the determination could be carried out when the applied ΔE between two platinum wire electrodes was 0 V. Moreover, because a homogeneous redox reaction of dobessilate with MnO₄⁻ could occur if mix them together, they were separated into two electrode rooms by means of salt bridge when performing the flow-injection determination. In doing so, the influences from the homogeneous reaction were avoided.

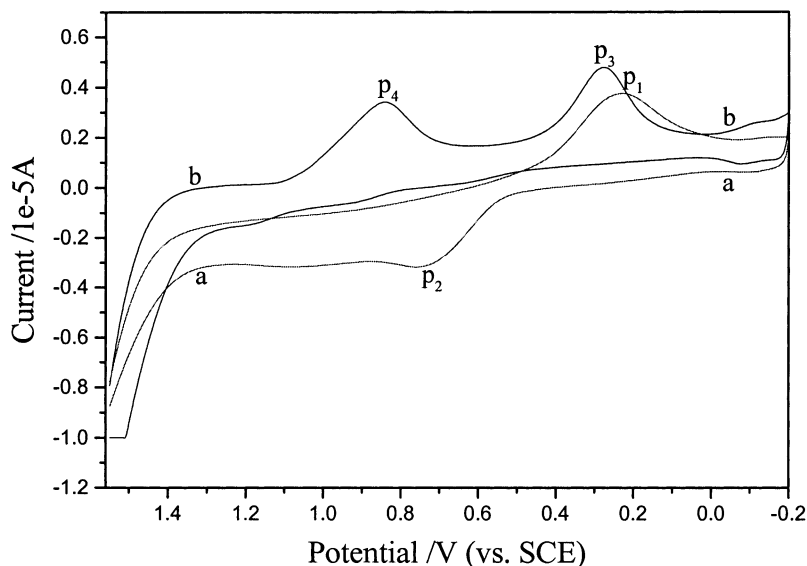


Fig. 2. Cyclic voltammetric curves of dobesilate/permanganate biamperometric system at platinum wire electrode. Curve a: the presence of 1.0×10^{-3} M dobesilate; curve b: the presence of 1.0×10^{-3} M permanganate. Initial potential -0.20 V, reversal potential 1.55 V, scan rate $v = 100$ mV/s.

The biamperometric detection scheme could be further proved with performing the following experiments. When 2.0×10^{-5} M dobesilate solution was injected into the sample room and 0.05 M H_2SO_4 solution was injected into the counter room, no signal current was obtained (Fig. 3, curve a). When 0.05 M H_2SO_4 solution without dobesilate was injected into the sample room and 1.0×10^{-3} M KMnO_4 solution was injected into the counter room, in like manner, no signal current was obtained (Fig. 3, curve c) too. However, when both dobesilate and KMnO_4 solutions were separately injected into the sample room and the counter room, good signal currents were obtained (Fig. 3, curve b). The results indicated the contribution of both dobesilate and MnO_4^- to the biamperometric detection scheme.

3.2. Effect of the imposed potential difference

In biamperometry for irreversible couples system, the potential difference ΔE imposed between two electrodes was dependent on the potential difference $\Delta E_{1/2}$ of the half-wave potential from two irreversible couples. When the $\Delta E_{1/2}$ was larger, the appropriate ΔE imposed between two

electrodes would improve analytical sensitivity and selectivity. However, the increase of the ΔE was accompanied by the decrease of both selectivity and S/N ratio, which was not expected in practical determinations. In present work, when the ΔE was 0 V, the true potentials of two electrodes were between 0.78 and 0.84 V and were kept the same value, as shown in Fig. 2. In this case, the true potentials of two electrodes had been just in the limiting current domains of both reactants, the obtained signal current was maximal. Therefore, the ΔE of 0 V was chosen.

3.3. Selection of carrier solution

The carrier solution was selected based on the effect of pH value of carrier solution on peak height and peak width of the signal current. Effects of the acidity of the carrier solution on the determination were examined at ΔE of 0 V using 2.0×10^{-5} M dobesilate standard solution. In operation, 0.05 M of H_2SO_4 carrier solution normally used, shown in Fig. 1, was replaced with Britton–Robinson buffer (H_3PO_4 – HOAc – H_3BO_3 – NaOH) solutions of different pH values in the range 1.0 – 10.0 . Fig. 4 showed the effect of

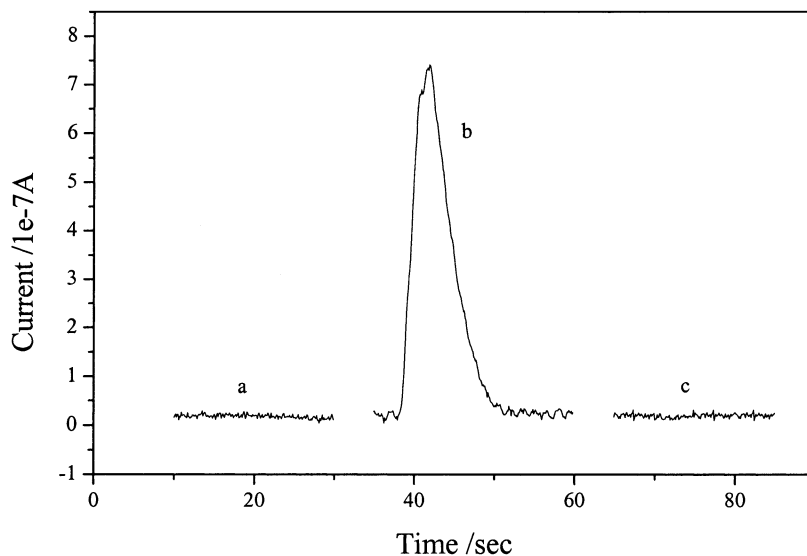


Fig. 3. Flow-injection signals obtained for 2.0×10^{-5} M dobesilate in the absence (a) and the presence (b) of permanganate in the counter room of the detector, and the signal obtained in the presence of permanganate in the counter room of the detector but in the absence of dobesilate (c) in the sample room of the detector. $\Delta E = 0$ V, flow rate $r = 2.8$ ml/min.

pH value on S/N ratio. The signal current decreased with the increasing of pH value of the carrier solution, at the same time, the background current increased gradually. Using the carrier solution with low pH value was, therefore, favorable to achieve high sensitivity and low background. In present work, 0.05 M H_2SO_4 solution was selected as the carrier solution.

3.4. Selection of $KMnO_4$ concentration

In order to obtain the linearity between signal current and dobesilate concentration, $KMnO_4$ concentration should be kept enough high since the current flowing through the biamperometric detector was limited by the smaller one among the anodic current of dobesilate and the cathodic current of MnO_4^- . A 1.0×10^{-3} M $KMnO_4$ solution was selected because dobesilate concentration in present work was generally less than 1.0×10^{-4} M. In view of that $KMnO_4$ concentration was much higher than that of dobesilate, the consumption of $KMnO_4$ owing to the reduction was regardless. No obvious influence had been observed when using 100 ml of 1.0×10^{-3} M $KMnO_4$ solution for no less than 400 samplings.

3.5. Selection of the length of wire electrodes

As could be expected, the surface area of the working electrodes directly influenced the signal current in magnitude. In this work, two working wire electrodes were made of platinum wire with the same diameter (0.5 mm). The effect of the length of the working electrodes on the measurement was examined. When 4.0 and 8.0 mm in length wire electrodes were used instead of 1.1 cm electrodes, the current signal decreased by 20 and 16%, respectively. Further measurements were done with the longer electrodes in spite of slightly larger tailing.

3.6. Optimization of FIA operation

Because there was no homogeneous reaction in the proposed method, considerations for some conditions like reaction time, and temperature were needless. Some parameters of the FIA operation such as flow rate and injection volume and other had been optimized.

The flow rate and injection volume were optimized by using 4.0×10^{-5} M dobesilate standard solution. The choice of the flow rate must also take

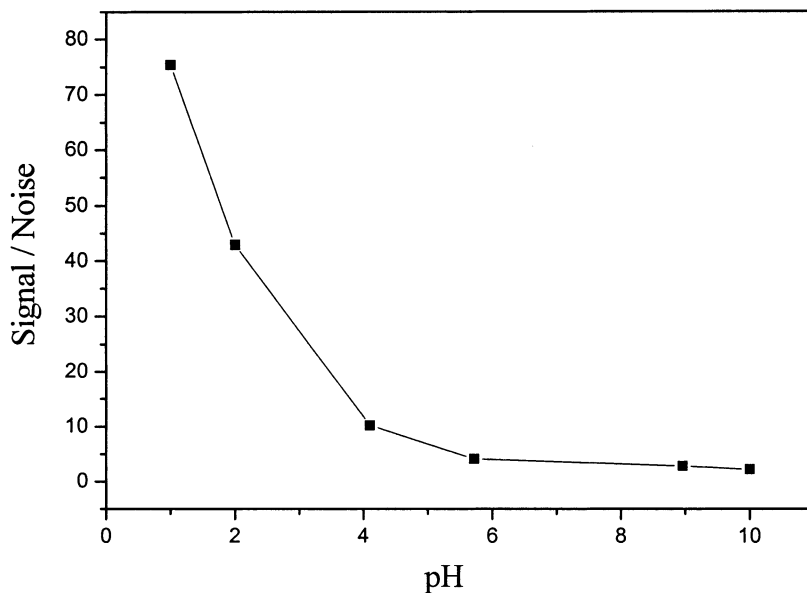


Fig. 4. Effect of pH value of carrier solution on signal/noise (S/N) ratio. $C_{\text{dobesilate}} = 2 \times 10^{-5}$ M, $\Delta E = 0$ V, flow rate $r = 2.8$ ml/min.

into account the change in peak height with flow rate as well as peak width and the rate of the return to the baseline, which influenced the sampling throughput. In this system, the peak height was found decreasing with the flow rate increasing from 0.5 to 6 ml/min. At low flow rate, however, the peak broadens. A compromised value of 2.8 ml/min was selected. The injection volume was found to have a strong effect on peak height and peak width when changing from 10 to 100 μl . In general, the increasing of injection volume led to a higher peak height and a boarder peak width. After 100 μl , the peak height reached a plateau. So an injection volume of 100 μl was chosen. The distance between detector and valve had no obvious effect on detector response and a value of 24 cm was used.

3.7. Stability of the detector

It was known that the anodic oxidation of some aromatic compounds on the solid electrodes led to the loss of electrode response and precision since the formation of polymeric films on electrode surfaces. However, no loss of electrode response was observed for detection at low concentration of

aromatic compounds [16]. Due to dobesilate concentration used in present work was less than 10^{-4} M, and the platinum wire electrodes were cleaned electrochemically prior to the measurements, the contamination of the pair of the electrode was not found. Under the selected conditions of present work, the detector showed satisfactory long-term stability. The RSD obtained for 24 successive determinations was 1.7 and 2.1% for 4.0×10^{-5} and 8.0×10^{-6} M dobesilate, respectively.

3.8. Interferences study

The effects of inorganic ions and organic compounds commonly existed in pharmaceuticals and biological samples on the determination of 4×10^{-5} mol/l dobesilate was studied. Since the applied potential was 0 V, the detector was free from interferences induced by direct oxidation or reduction of the additives. The tolerance limit was defined as the concentration ratio of additive/dobesilate causing less than $\pm 5\%$ relative error. The tolerance limit of additives to 4×10^{-5} mol/l dobesilate was ≥ 200 -fold of Ni^{2+} , Al^{3+} , Co^{2+} , Cu^{2+} , Bi^{3+} , Cr^{3+} , Cd^{2+} , La^{3+} , Na^{+} , K^{+} , SO_4^{2-} , CO_3^{2-} , PO_4^{3-} , Cl^{-} , NO_3^{-} , 100-fold of

Table 1
Determination of calcium dobesilate in capsules

Sample	Proposed method (mg/capsule)	RSD (%) ($n = 5$)	HPLC method (mg/capsule)	RSD (%) ($n = 5$)	Error (%)
1	494.2		491.2		0.6
2	488.9		492.9		-0.8
3	484.4	1.2	489.8	0.6	-1.1
4	497.9		496.7		0.2
5	497.9		496.4		0.3

Label amount 500 mg/capsule.

Ca^{2+} , Fe^{3+} , glucose, lactose, starch, sucrose, mannitol, ephedrina, L-valine, L-threonine, L-cystine, L-serine, L-glutamic acid, L-arginine, L-histidine, and 25-fold of Zn^{2+} , Fe^{2+} , Mg^{2+} , Ag^{+} .

3.9. Calibration curves and precision

Because dobesilate concentration was far lower than permanganate concentration in present work, the obtained signal current was decided by the dobesilate concentration. Under the optimized conditions, the peak height varied rectilinearly with dobesilate concentration in the range of 4.0×10^{-6} to 1.0×10^{-4} M. The linear regression equation was

$$i \text{ (nA)} = 46.0 + 6.1 \times 10^6 C \quad (r = 0.9988, n = 9)$$

The precision of the method shown by RSD of 0.8% for nine replicate determination of 4.0×10^{-5} M dobesilate was good. The detection limit estimated as the blank signal plus three times was 8.0×10^{-7} M.

Table 2
Results of recovery tests in urine samples

Sample	Added (10^{-5} M)	Found (10^{-5} M)	Recovery (%)	Average (%)
1	4.00	4.27	106.8	
2	4.00	3.96	99.0	
3	4.00	4.04	101.0	101.9
4	4.00	4.16	104.0	
5	4.00	3.94	98.5	

3.10. Determination of calcium dobesilate capsules

The sample solutions were prepared by dissolving 0.1216 g powder in water from the well-proportioned mixture of ten calcium dobesilate capsules with label amount of 500 mg. The determination of calcium dobesilate was conducted by the proposed method and the HPLC method [2], respectively. As showed in Table 1, the results obtained by the two methods were in good agreement. The proposed method suited the rapid and direct determination of calcium dobesilate in pharmaceutical preparations.

3.11. Recovery test

The investigations showed that calcium dobesilate did not have any significant decomposition in human plasma [17], and the urinary elimination of calcium dobesilate in the first 24 h reached 75% after intravenous medication and 50% after oral medication [2]. Accordingly, in present work, recovery tests were carried out by adding certain amount of calcium dobesilate standards into the five diluted urine samples of healthy specimen. Each 1 ml of fresh sample was taken and diluted to

10 ml with water prior to the injection. The recoveries obtained were listed in Table 2. As can be seen, the recovery for the determination of calcium dobesilate added to urine samples was good. It showed the proposed method was available for the determination of calcium dobesilate in urine samples too.

4. Conclusions

Present work developed a rapid, simple and economical method for determination of calcium dobesilate by flow-injection biamperometry. Under the optimized conditions, 150 samples could be analyzed every hour with good resolution. Since there was no homogeneous reaction in the proposed method, the KMnO_4 was cyclically used. And the flow-injection system was very simple. The easily controlled measuring conditions and low noise level, owing to the applied potential difference of 0 V, made the method suitable to in situ or online monitoring. Because the applied potential difference was 0 V, the determination was free from most interferences induced by direct oxidation or reduction at electrodes.

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