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Short communication

Direct amperometric determination of lactate at a carbon fiber bundle microdisk electrode by capillary zone electrophoresis

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Abstract

Capillary zone electrophoresis was employed for the determination of lactate using end-column amperometric detection at a carbon fiber bundle microdisk electrode. The optimum conditions of separation and detection are 3.6×10^{-3} mol/l Na_2HPO_4–1.4×10^{-3} mol/l NaH_2PO (pH 7.2) for the buffer solution, 18 kV for the separation voltage and 1.60 V versus the saturated calomel electrode for the detection potential. The limit of detection is 7.6×10^{-7} mol/l or 1.7 fmol ($S/N=3$) and the linear range is 1.7×10^{-6}–8.2×10^{-4} mol/l for the injection voltage of 6 kV and injection time of 5 s. The RSD is 1.8% for the migration time and 3.3% for the electrophoretic peak current. The method was applied to the determination of lactate in human saliva. The recovery of the method is between 95 and 109%.

Keywords: Carbon fiber bundle microdisk electrode; Lactate

1. Introduction

Lactate is known to be one of the most important metabolites in clinical analysis. The determination of lactate is used in diabetes control, food analysis and sports medicine to help athletes tailor their training [1–3]. It has already been demonstrated that lactate in blood increases after meals [1,4]. The results of saliva lactate reflect the same trend frequently obtained with monitoring of blood lactate [3]. Electrochemical biosensors and selected electrodes are commonly used for the determination of lactate [5–15]. Recently, an approach based on voltammetry has been reported [16]. In these methods, the electrodes normally have to be modified by enzymes or polymers. If these biosensors or electrodes are used with biological samples, electrode fouling by macromolecular components in the medium becomes a problem. Therefore, using a separation technique can overcome this difficulty. Recently, capillary zone electrophoresis (CZE) has rapidly become an important instrumental analysis technique suitable for rapid separation and detection of complex mixtures [17–19]. The primary strength of CZE is its ability to provide extremely high separation efficiencies in over a short time. Lactate does not possess any fluorescing properties amenable for sensitive detection. It is also not easily derivatized. Therefore, it cannot be quantitated by direct fluorescence detection. Xue and Yeung [20] used an indirect fluorescence method for determination of lactate. Amperometric detection in CZE has been demonstrated to be a highly sensitive method for the determination

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of a wide range of electroactive compounds. Voegel and Baldwin have reviewed this detector in CZE in detail [21]. Nevertheless, there are no reports on the determination of lactate by CZE with electrochemical detection. We found that lactate can be oxidized at carbon fiber electrodes. This behavior can be used for direct determination of lactate if CZE is used.

In CZE with electrochemical detection, end-column amperometric detection with a normally sized working electrode is convenient for the alignment of the electrode and capillary [22], because the area of the electrode is much larger than that of the inside cross-section of the capillary. For constructing the normally sized carbon fiber electrodes, carbon fiber bundles with ~30 6-μm carbon fibers were used. This work demonstrates the utility of end-column amperometric detection at a carbon fiber bundle microdisk electrode with CZE for the measurement of lactate. The separation was performed in a 30- or 20-μm I.D. fused-silica capillary. The detection was carried out by potentiostatic control of the electrode potential by means of a three-electrode system. Because of its excellent selectivity, the method has been used to determine lactate in human saliva. Only one peak of lactate appears on the electropherograms.

2. Experimental

2.1. Apparatus

2.1.1. Linear sweep voltammetry

In linear sweep voltammetry, an electrochemical analyzer (Model 800, CH Instruments, Austin, TX, USA) was used in connection with a cell, using potentiostatic control of the electrode potential by means of a three-electrode system. It consisted of a carbon fiber bundle electrode as the working electrode, a Pt wire as the auxiliary electrode and an SCE as the reference electrode. The reference electrode was connected to the analyte via a salt bridge filled with the same supporting electrolyte as the cell.

2.1.2. Capillary zone electrophoresis

A reversible high-voltage power supply (Model 9323-HVPS, Beijing Institute of New Technology, Beijing, PR China) provided a variable voltage of 0–30 kV across the capillary with outlet of the capillary at ground potential. Fused-silica capillaries (375 μm O.D., 20 or 30 μm I.D.) were purchased from Yongnian Optical Conductive Fiber Plant, Yongnian, PR China. They were cut to a length of 40 or 63 cm and placed between two buffer reservoirs. A high voltage was applied at the injection end, while the reservoir containing the electrochemical detection cell was held at ground potential. Separations were carried out at an applied voltage of 18 kV. The arrangement of the electrochemical detection cell was illustrated in Ref. [23] in detail. The electrochemical detection at a constant potential with CZE was performed using the end-column amperometric approach with an electrochemical analyzer (Model CHI800, CH Instruments, Austin, TX, USA). The detection cell and detector were housed in a Faraday cage in order to minimize the interference from external sources of noise. Electrochemical detection was carried out with a three-electrode system. It consisted of a carbon fiber microdisk bundle electrode as the working electrode, a coiled Pt wire as the auxiliary electrode, which also served as the ground for the high potential drop across the capillary mentioned above, and an SCE as the reference electrode.

2.1.3. Electrode preparation

For linear sweep voltammetry carbon fiber bundle electrodes were used. Approximately 30 carbon fibers 6 μm in diameter and soaked with acetone, were inserted into a fused-silica capillary (~200 μm I.D., 375 μm O.D., 1.5 cm length). Next the carbon fiber bundle was immersed into ethyl α-cyanoacrylate adhesive and the adhesive was passed through the entire carbon fiber bundle in the fused-silica capillary. After drying, the fused-silica capillary with the carbon fiber bundle was inserted into a glass capillary (~0.5 mm I.D., 1 mm O.D. and 2.5 cm length) with a small amount of mercury. The carbon fiber bundle was connected to a copper wire (0.4 mm diameter, 5 cm length) via the mercury junction by pushing a copper wire down. The fused-silica capillary with the carbon fiber bundle and the copper wire were bonded to both ends of the glass capillary using epoxy. Finally, the carbon fiber bundle protruding from the fused-silica capillary was cut to 4 cm.

For capillary zone electrophoresis, the process of manufacture of the carbon fiber bundle electrode was similar to that described above for linear sweep voltammetry, except that only 10 carbon fibers were used instead of 30. Finally, the carbon fiber bundle protruding from the fused-silica capillary was trimmed. Both kinds of carbon fiber electrodes have been illustrated in the literature [24]. Before use all carbon fiber microdisk bundle electrodes were cleaned in alcohol and washed with double distilled water for 5 min with a supersonic wave cleaner.

2.2. Reagents

All reagents were of analytical grade, purchased from standard suppliers, and used without further purification. A stock lactate solution was prepared by dissolving an appropriate amount of sodium lactate (chemically pure quality, Shanghai Chemical Reagents, Shanghai China) in water and kept refrigerated at 4 °C. Dilute solutions were obtained by serial dilution of the stock solution with water. Other reagents were of analytical grade. All solutions were prepared with double distilled water.

2.3. Procedure

For linear sweep cyclic voltammetry, the carbon fiber bundle electrode was directly inserted into the experimental solution containing lactate and a linear sweep cyclic voltammogram was recorded. In CZE, the carbon fiber microdisk bundle electrode was fixed onto a microscope slide, which was placed over a laboratory-made XYZ micro-manipulator and glued in place. The position of the carbon fiber microdisk bundle electrode was adjusted (under a microscope) against the end of the capillary so that the electrode and the capillary were in contact. This arrangement allowed one to easily remove and realign both the capillary and the electrode. The other end of the capillary was inserted into a plastic syringe tip (the metal needle was previously removed) and glued in place with a small amount of epoxy glue. Before each run, the capillaries were flushed with double distilled water, 0.1 mol/l NaOH, double distilled water and the corresponding separation electrolyte, respectively, by means of a syringe. In addition, the electrolyte solution at the electrochemical cell was also replaced before each run. During the experiments the separation voltage was applied across the capillary and the detection potential was applied at the working electrode. After the electroosmotic current reached a constant value (after 20 min), the electromigration injection was carried out and the electropherogram was recorded. All potentials were measured versus SCE.

3. Results and discussion

3.1. Linear sweep voltammogram of lactate

The voltammetric characteristic of sodium lactate at the carbon fiber bundle electrode has not been reported so far. Fig. 1, curve 2, shows its typical linear sweep voltammogram in Na_2HPO_4–NaH_2PO_4 of pH 7.2. No oxidation peaks of lactate appear on the voltammogram. Its anodic current is higher than that of the buffer shown in Fig. 1, curve 1, especially for the potentials more positive than 1.4 V. This means that lactate can be oxidized at the carbon fiber bundle electrode in Na_2HPO_4–NaH_2PO_4. Since a constant potential is applied at the working electrode

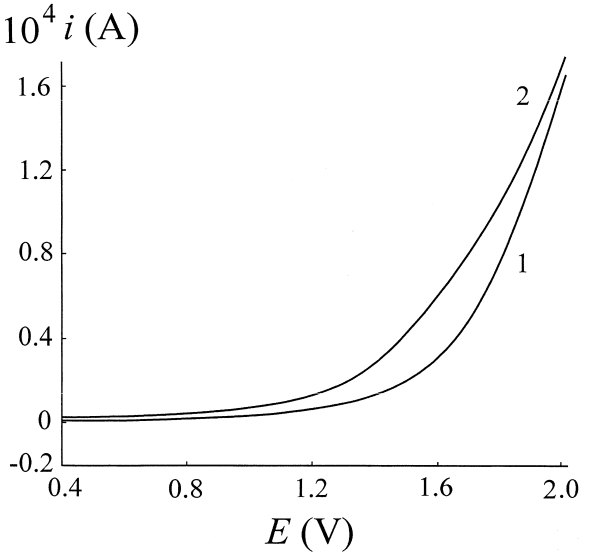

Fig. 1. Typical linear sweep voltammogram of lactate at the carbon fiber bundle electrode in Na_2HPO_4–NaH_2PO (pH 7.2). Scan rate: 100 mV s^{-1}. (1) 3.6×10^{-3} mol/l Na_2HPO_4–1.4×10^{-3} mol/l NaH_2PO_4; (2) (1)+8.2×10^{-3} mol/l lactate.

in CZE with electrochemical detection, a constant background current is obtained. When lactate is present in the solution, an electrophoretic peak corresponding to the oxidation of lactate over the constant background current will be recorded, if the potential of the electrode is positive enough. Thus lactate can be directly measured electrochemically by CZE with a constant potential scheme. This idea has been used for the determination of metal ions [25,26], myoglobin [27] and transferrin [28].

3.2. Conditions of CZE

The electrophoretic behavior of lactate in five Na_2HPO_4–NaH_2PO_4 solutions at different pH around pH 7.2 was investigated. The peak current, i_p, the migration time, t_m, the width at the half-peak, $W_{1/2}$, on the electropherograms and the number of theoretical plates, n, at different pH are listed in Table 1. It can be found that n and i_p have maximum values in 3.6×10^{-3} mol/l Na_2HPO_4–1.4×10^{-3} mol/l NaH_2PO of pH 7.2. Therefore, this buffer was selected in subsequent experiments.

From the relationship between i_p and the detection potential, E_d, it was found that when E_d is lower than 1.4 V, i_p is almost a constant and when E_d is more positive than 1.4 V, i_p increases with increasing E_d. E_d of 1.60 V was chosen in our experiments because of less noise.

The separation voltage, V_s, exerts an influence on t_m and n [29]. It was found that $1/t_m$ is proportional to V_s, which agrees with the prediction of the theory. i_p has maximum value at 18 kV. Therefore, 18 kV for V_s was chosen.

3.3. Reproducibility, limit of detection and linear range

The response for a series of six injections of 1.34×10^{-4} mol/l lactate resulted in a RSD of 1.8% for t_m and 3.3% for i_p, respectively. The limit of detection was 7.6×10^{-7} mol/l or 1.7 fmol for the injected volume calculated (according to the signal-to-noise ratio of 3), which was estimated from the electropherogram obtained for 1.7×10^{-6} mol/l lactate. A linear relationship holds between the peak current detected and concentration in the range of 1.7×10^{-6}–8.2×10^{-4} mol/l for the injection voltage of 6 kV and injection time of 5 s. Least-squares treatment of these data yielded a slope 1.97 pA μmol^{-1} 1 with a correlation coefficient of 0.996.

3.4. Determination of lactate in human saliva

The concentration of lactate in human saliva can be readily and directly determined by using this CZE-electrochemical detection system without any pre-treatment. Fresh human saliva samples from four subjects (two females and two males) working in the laboratory were collected in the fasting state 2 h before eating and at 30 min after eating.

After 0.99 ml electrophoretic buffer was added into 0.01-ml fresh saliva sample, the sample solution was directly injected into the CZE/end-column

Table 1
Values of t_m, i_p, $W_{1/2}$ and n in Na_2HPO_4–NaH_2PO_4 at different pH

Buffer	pH	t_m (s)	i_p (nA)	$W_{1/2}$ (s)	n (10^{-4})
3.1×10^{-3} mol/l Na_2HPO_4 –2.0×10^{-3} mol/l NaH_2PO_4	7.0	273	223	3.1	4.3
3.6×10^{-3} mol/l Na_2HPO_4 –1.4×10^{-3} mol/l NaH_2PO_4	7.2	327	355	3.1	6.2
4.1×10^{-3} mol/l Na_2HPO_4 –9.0×10^{-4} mol/l NaH_2PO_4	7.4	284	183	3.2	4.4
4.4×10^{-3} mol/l Na_2HPO_4 –6.5×10^{-4} mol/l NaH_2PO_4	7.6	279	174	3.2	4.2
4.8×10^{-3} mol/l Na_2HPO_4 –2.5×10^{-4} mol/l NaH_2PO_4	7.8	278	98.3	3.2	4.2

8.44×10^{-5} mol/l lactate; capillary, 63 cm length, 30 μm I.D.; injection, 6 kV for 10 s; separation voltage, 18 kV; detection potential, 1.60 V.

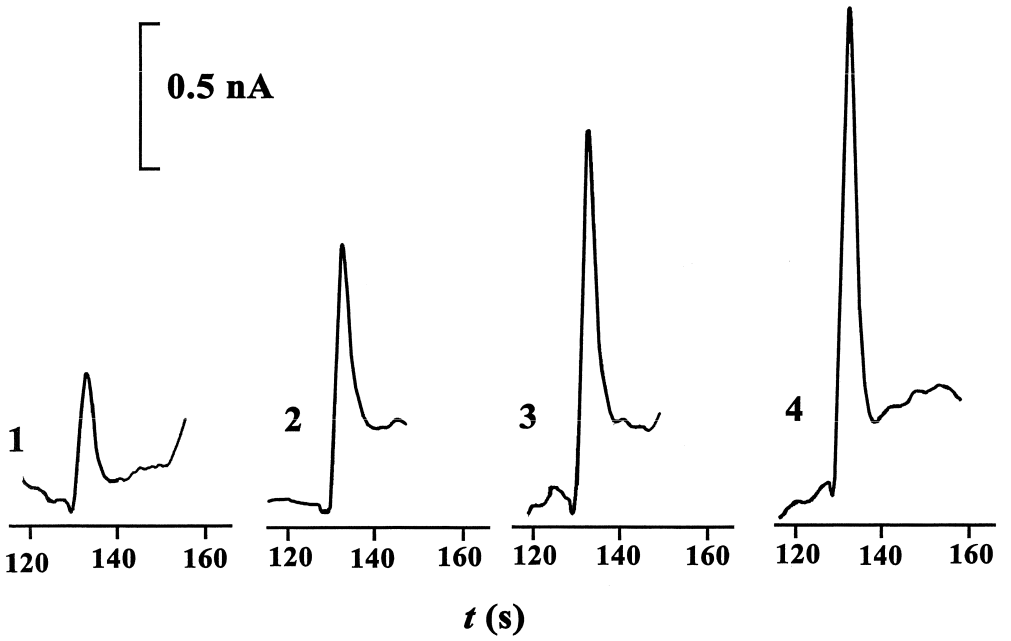

Fig. 2. Electropherograms of lactate in a sample solution of human saliva. Added concentration of lactate (mol/l): 1, 0; 2, 5.00×10^{-6}; 3, 1.00×10^{-5}; 4, 1.50×10^{-5}. 3.6×10^{-3} mol/l Na_2HPO_4–1.4×10^{-3} mol/l NaH_2PO_4. Capillary, 40 cm length, 20 μm I.D. Other conditions as in Table 1.

amperometric system by electromigration.. The typical electropherograms of a human saliva sample without and with the standard solution of lactate are shown in Fig. 2. The results obtained for the saliva samples using the standard addition method are listed in Table 2. The lactate level in saliva varies widely between subjects and the level after a meal increased for all the subjects. The analysis of samples spiked with known quantities of lactate is summarized in Table 2. Of the spiked lactate, 95–109% is recovered. From the electropherograms of the human saliva samples, it can be seen that only one peak of lactate appears. This indicates that the main advantage of the method is the excellent selectivity. In addition, the samples can be directly injected, and no pretreatment of the samples is needed. However, it can be noted that the number of theoretical plates of the peaks becomes poor for these samples (~8000). This is because the other compounds in the saliva samples changed the characteristics of the separation capillary.

4. Conclusions

Lactate can be oxidized at the carbon fiber bundle electrode in Na_2HPO_4–NaH_2PO_4. No oxidation peaks of lactate appear on the voltammogram and the

Table 2
Results of determination of lactate in samples of human saliva (conditions as in Fig. 2)

Sample	Concentration of saliva with fasting (10^{-4} mol/l)	Recovery (%)	Concentration of saliva after meal (10^{-4} mol/l)	Recovery (%)
Female 1	5.0	100	5.5	95
Female 2	3.1	97	6.6	104
Male 1	2.1	95	4.0	98
Male 2	5.0	100	12.2	109

background current is higher at more positive detection potential. In such a case, voltammetry usually cannot be used for its direct determination. However, it still can be determined directly by using CZE with electrochemical detection. This is because a constant potential is applied at the working electrode in CZE with electrochemical detection. The current detected corresponds to the oxidation of lactate over the constant background current. Thus, an electrophoretic peak of lactate over the constant background current will be recorded. The example described here reflects the advantages of CZE using electrochemical detection with a constant potential.

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