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Quantification of lactate in synovia by microchip with contactless conductivity detection

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ABSTRACT

This article describes the determination of lactate in synovia by microchip capillary electrophoresis (MCE) integrated with contactless conductivity detection (CCD). The optimal running buffer consists of 10 mM tris(hydroxymethyl)aminomethane, 1 mM HCl, and 0.1 mM hexadecyltrimethylammonium bromide (pH 9.1). The quantitative measurement of lactate in dilute synovia samples can be finished in less than 40 s. The results indicated that the peak area had a good linear relationship with lactate concentration in the range of 20 to 1000 μM, and the correlation coefficient was 0.9984. The average recovery was from 96.6% to 106.1%, and the interday relative standard deviation was less than 4.0% ($n = 6$). The limit of detection (signal/noise = 3) reached 6.5 μM. To validate the assay results, we compared the current method with the high-performance liquid chromatography method by measuring lactate in synovia samples. The data analysis verified that there was no significant difference between the two methods. Due to significant features such as low cost, integration, and miniaturization, the MCE–CCD method may have great potential in clinical diagnosis.

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The early and accurate diagnosis of arthritic disease is of great clinical importance. In the case of septic arthritis, bacterial infections can cause complete destruction of joints without treatment. In general, the diagnosis of arthritic disease can be carried out by examination of the changes in synovia fluid (SF)[1] because both chemical composition and cellular constitution of SF are clinically relevant to arthritic disease. It is well known that SF lactate is an important marker of arthritis [1]. Lactate widely exists in biological fluids such as plasma and synovia. Lactate is also an important intermediate metabolite related to carbohydrates (e.g., glucose), lipids, and proteins. Glucose molecules are decomposed and then converted into pyruvate via the glycolysis pathway. Only a small amount of pyruvate existing in cytoplasmic matrix can be transformed to lactate by lactate dehydrogenase (LDH) under normal physiological conditions [2]. Synovia lactate is normally 0.5 to 1.7 mmol/L in healthy persons but can rise to more than 10 mmol/L during arthritic disease [3].

Lactate in clinical samples is usually analyzed based on the enzymatic conversion to pyruvate in the presence of LDH. The by-product, nicotinamide adenine dinucleotide (NADH), can be measured optically for indirect quantification of lactate. Moreover, photometric absorbance measurements and fluorimetric methods have been developed [4–6]. However, these methods require quite large volumes of samples and inevitably encounter the problem of potential interferences. Chromatographic methods, such as gas chromatography and high-performance liquid chromatography (HPLC), have also been reported [7]. Doyle and coworkers [8] reported a quantitative method for lactic acid measurement by gas–liquid chromatography in which sample pretreatment of esterification reaction was necessary to render lactate volatile by adding absolute ethyl alcohol and sulfuric acid. Determination by HPLC also requires derivatization to allow ultraviolet (UV) or fluorescence detection because lactate is a poor UV absorber. Simonides and coworkers [9] described a method using HPLC in which the esterification of lactate with the UV-absorbing compound α-*p*-dibromoacetophenone was followed by separation and quantitation of the ester by reversed-phase HPLC. In another report [10], lactate was detected in the UV range by derivatization with α-bromoacetophenone. Biosensors are the other choice for lactate assays [11–13], which are also mostly based on LDH or lactate oxidase (LOD). For instance, an electrochemiluminescence-based disposable biosensor was characterized recently [14]. All of the needed reagents in this lactate recognition system were

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[1] *Abbreviations used:* SF, synovia fluid; LDH, lactate dehydrogenase; HPLC, high-performance liquid chromatography; UV, ultraviolet; LOD, lactate oxidase; ^{1}H MRS, proton magnetic resonance spectroscopy; CE, capillary electrophoresis; CCD, contactless conductivity detection; MCE, microchip capillary electrophoresis; CTAB, hexadecyltrimethylammonium bromide; Tris, tris(hydroxymethyl)aminomethane; ACN, acetonitrile; HCl, hydrochloric acid; PMMA, poly(methyl methacrylate); BGE, background electrolyte; EOF, electroosmotic flow; LOD, limit of detection; RSD, relative standard deviation.

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immobilized by a methocel membrane placed on the working electrode of the screen-printed electrochemical cell. In addition, Romero and coworkers developed an amperometric biosensor for direct blood lactate detection [15]. This approach involved the use of a Nafion membrane between the platinum electrode and the mucin/albumin hydrogel. LOD can be immobilized in the hydrogel matrix without suffering major structural changes and loss of activity. Nafion was used to minimize interferences of other electroactive compounds. Nevertheless, in all cases, a significant loss of sensitivity has been reported regardless of whether Nafion was mixed with the matrix [16] or placed on the sensor surface [17]. Despite biosensors' specificity, they often suffer from some limitations such as long analysis time, low stability, and poor reproducibility. A further technique, proton magnetic resonance spectroscopy (^{1}H MRS), has been tried recently. Cetin and coworkers [18] studied the possibility of in vivo human fetal brain lactate detection by ^{1}H MRS in fetuses with intrauterine growth restriction (IUGR). However, a magnetic resonance instrument may occupy a quite large area and be of high cost when compared with other medical devices.

Several reports on the determination of lactic acid in clinical samples by the capillary electrophoresis (CE) method have also been presented [19,20]. Lactate was determined by fluorescence detection using derivative reagents, which is generally a time-consuming and labor-intensive process. Such analytical procedures can be simplified when using conductivity detection. In particular, contactless conductivity detection (CCD) is a widely used detection technique that has several advantages, including antifouling, stability of the sensing electrode, high efficiency, short analysis time, and simple instrumentation. CCD was first introduced to CE by Zemann [21]. The CE–CCD method has also been applied for the detection of lactate, creatinine, and carnitine [20,22,23] in clinical samples.

Over the past decades, the miniaturization of analytical techniques, specifically CE, has become a dominant trend in the analytical scope. Microchip capillary electrophoresis (MCE) has drawn tremendous interest for a variety of applications as a chemical and biochemical analysis tool. Great progress in the area of MCE applications of bioanalysis is due to micro-scale analytical devices being able to achieve high-speed electrophoretic separations within seconds, requiring only picoliter or nanoliter sample volumes while maintaining the advantages of conventional CE at the same time. In our previous work, we successfully achieved the innovations of MCE devices and the applications of the MCE method in pharmaceutical analysis [24–28]. MCE–CCD was first introduced by Guijt and coworkers, and the applicability of the system was demonstrated with the separation of two short peptides [29]. During recent years, the application of MCE–CCD in the field of clinical analysis has been explored, mainly due to its high efficiency for fast disease screening and diagnosis. In particular, analyses of inorganic cations, organic ions, and other species by this method have already been presented [30–32].

Synovia lactate is a very important clinical indicator, especially for patients with arthritis. The measurement of synovia lactate concentrations can offer a useful quantitative parameter for the diagnosis of arthritis. In this article, we demonstrate for the first time the use of MCE–CCD as a new and feasible analytical method for the determination of lactate.

Materials and methods

Reagents

All chemicals were of analytical reagent grade. Hexadecyltrimethylammonium bromide (CTAB), tris(hydroxymethyl)aminomethane (Tris), and reference standard of L-lactic acid were obtained from Aladdin Reagent (Shanghai, China). Acetonitrile (ACN) was used for deproteinization of the testing samples. Hydrochloric acid (HCl) and other reagents were obtained from Guangzhou Chemical Reagent (Guangzhou, China). Deionized water (18 MΩ cm) was used throughout this work. The pH value of the buffer was determined with a pH meter (PB-10, Satorius, Germany). Certified synovial fluid reference materials were obtained from the Hospital of Stomatology, Sun Yat-sen University (Guangzhou, China).

Instrumentation

The MCE–CCD system is a homemade instrument that is mainly composed of a high-voltage power supply, a contactless conductivity detector, and a poly(methyl methacrylate) (PMMA) microchip. The construction of this system is shown in Fig. 1. The high-voltage power supply was made of piezoelectric ceramics, providing a potential of constant DC of 0.5 kV for injection and a negative voltage of 0.1 to 5.0 kV for electrophoretic separation. Previously published results have shown that excitation voltage and frequency beyond 60 $V_{p\text{-}p}$ and 60 kHz, respectively, applied to the CCD in conventional CE offer better sensitivity [28]. In this work, the separation voltage was negative 2.0 kV. The excitation voltage and frequency were 90 $V_{p\text{-}p}$ and 60 kHz, respectively. Injection of standard and sample was carried out for 10 s. Before experiments, the microchip was rinsed with 0.1 mol/L NaOH solution (5 min), deionized water (5 min), and running buffer (10 min) to prevent peak distortions.

Preparation of running buffer and standard solution

The running buffer, consisting of 10 mM Tris, 1 mM HCl, and 0.1 mM CTAB (pH 9.1), was prepared daily. Standard solutions

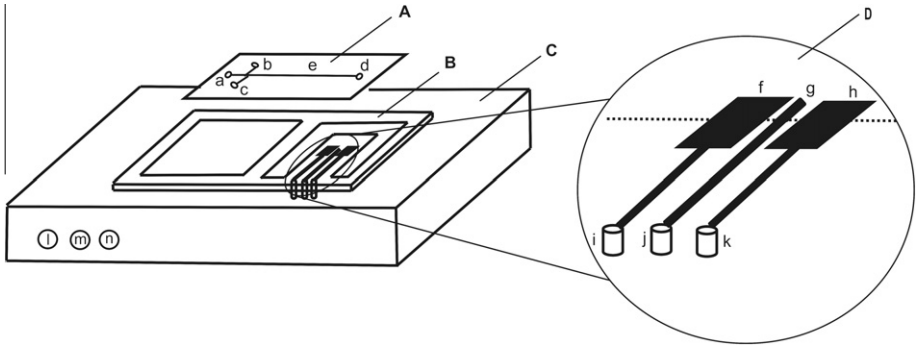

Fig.1. Schematic illustration for microchip system: (A) PMMA microchip; (B) contactless electrode plate; (C) contactless conductivity detector; (D) enlarged illustration of electrodes: (a) buffer reservoir; (b) sample reservoir; (c) sample waste reservoir; (d) buffer waste reservoir; (e) separation channel; (f) input electrode; (g) shielding (earth) electrode; (h) output electrode; (i) input electrode terminal; (j) shielding electrode terminal; (k) output electrode terminal; (l) frequency adjustor; (m) excitation amplitude adjustor; (n) baseline adjustor).

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