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Study on the potential application of salivary inorganic anions in clinical diagnosis by capillary electrophoresis coupled with contactless conductivity detection

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ABSTRACT

A capillary electrophoresis approach with capacitively coupled contactless conductivity detection method has been developed for the determination of inorganic metabolites (thiocyanate, nitrite and nitrate) in human saliva. Field amplified sample injection, as a simple sample stacking technique, was used in conjunction for online preconcentration of above inorganic anions. A selective separation for the target anions from other coexisting constituents present in saliva could be obtained within 14 min in a 10 mmol/L His—90 mmol/L HAc buffer (pH 3.70) at the separation voltage of −18 kV. The limits of detection and limits of quantification of the three analytes were within the range of 3.1–4.9 ng/mL ($S/N = 3$) and 10–16 ng/mL ($S/N = 10$), respectively. The average recovery data were in the range of 81–108% at three different concentrations. This method provides a simple, rapid and direct approach for metabolite analyses of nitric oxide and cyanide based on noninvasive saliva sample, which presents a potential fast screening tool for clinical test.

1. Introduction

Saliva is a readily accessible and informative biofluid, containing a variety of important compounds. Compared with blood sample, saliva collection is safe, convenient and non-invasive, which not only has no pain for patients, but also no risk of hematogenous spread of the disease except for communicable diseases. In contrast with urine and exhaled breath condensate samples, saliva can achieve real-time sampling without any auxiliary equipment. Many researches have shown that the inorganic or organic species in saliva could be used as biomarkers for diagnoses of the physiological and biological state of an individual [1–3]. For example, nitrite (NO_2^-) and nitrate (NO_3^-), as the nitric oxide metabolites from human body fluids, are two useful biomarkers of oxidative and nitrosative stress [4,5]. Thiocyanate (SCN^-), as an end product of detoxification of cyanide, is significantly correlated with daily cigarette consumption. SCN^- is considered as a good biomarker for distinguishing smokers and non-smokers, and its quantitative analysis could be interesting in health screening programmers for the evaluation of smoking behavior [6]. Besides, it is a substrate of peroxidase, and an inhibitor on NO formation [7,8]. Therefore, it is interesting to develop some sensitive and reliable methods to monitor above inorganic anions in human saliva.

Many chromatographic methods have been reported for the determination of NO_2^-, NO_3^- and/or SCN^- in various biological samples including saliva [9–14], and several reviews have summarized the characteristics of different analytical methods including high performance liquid chromatography (HPLC), gas chromatography (GC), and ion chromatography (IC) [15–17]. Although various methods have their own advantages, their main disadvantages are relatively low sensitivity, inability to directly or simultaneously detect NO_2^-/NO_3^- with a need for derivatization or complicated sample pretreatment [15–17]. Capillary electrophoresis (CE) has gained prominence among the available separation techniques due to its high-resolution power, low running cost and environment-

Abbreviations: C^4D, capacitively coupled contactless conductivity detection; CD, conductivity detector; CE, capillary electrophoresis; DAD, diode array detection; FASI, field amplified sample injection; GC, gas chromatography; HAc, acetic acid; His, L-histidine; HPLC, high performance liquid chromatography; IC, ion chromatography; LODs, limits of detection; LOQs, limits of quantification; NO_3^-, nitrate; NO_2^-, Nitrite; RSD, relative standard deviation; SCN^-, thiocyanate; UV, ultraviolet.

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friendly factors [18,19]. Now, several methods have been developed for the determination of above inorganic anions in saliva based on CE coupled with ultraviolet (UV) [20–27], diode array detection (DAD) [28] and conductivity detector (CD) [29]. Capacitively coupled contactless conductivity detection (C^4D) has been considered as an universal detection technique for CE, because of its various advantages including elimination of electrode surface fouling, effective isolation from high separation voltages, simplified detector design and electrode alignment [30,31], particularly compared with conventional CD. In our previous work, CE with C^4D (CE–C^4D) method has been employed for direct determination of four main polyamines in saliva [32]. However, CE–C^4D has not yet been applied for simultaneous determination of the above inorganic metabolic anions in saliva sample.

In this work, a directly analytical method has been developed for simultaneous determination of NO_2^-, NO_3^- and SCN^- based on CE–C^4D approach, and the sensitivity of this method was further improved by field amplified sample injection (FASI) technique [33]. Various parameters affecting electrophoretic separation and detection sensitivity were investigated. This FASI/CE–C^4D method has been applied to analyze human saliva originated from healthy humans, smokers and patients that suffer from asthma.

2. Materials and methods

2.1. Chemicals

The standard compounds ($NaNO_3$, $NaNO_2$ and KSCN) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Other referred chemicals including L-histidine (His), NaCl, NaBr, Na_2SO_4, NaH_2PO_4, KI, formic acid, lactic acid, uric acid, propionic acid and acetic acid (HAc) were purchased from China National Pharmaceutical Group Corporation (Shanghai, China), and the solvents (methanol, ethanol and acetonitrile) were purchased from Shanghai Chemical Reagent Co., LTD (Shanghai, China). All chemicals and reagents were of analytical grade.

2.2. Standard solution and sample preparation

The stock solution of each analyte (1.0 mg/mL) was prepared with ultra-pure water (ultrapure water meter, Shanghai Taihe Instrument Co., Ltd., China), and the standard solution of NO_2^- was stable within 30 days. The main coexisting inorganic anions including Cl^-, Br^-, SO_4^{2-}, $H_2PO_4^-$ and I^- (1.0 mg/mL each) were prepared from their corresponding sodium salts and potassium salts with ultra-pure water, respectively. The organic anions (1.0 mg/mL each) including formic acid, lactic acid, propionic acid and acetic acid were also prepared with ultra-pure water, except uric acid (1.0 mg/mL) prepared with 10 mmol/L NaOH. A fresh working solution was prepared daily by diluting the stock solution with running buffer (10 mmol/L His—90 mmol/L HAc buffer) to the desired concentration. Ten calibration standard solutions were used for regression analysis, and the detailed concentrations were 0.010, 0.020, 0.050, 0.10, 0.20, 0.50, 1.0, 2.0 and 5.0 μg/mL. Before use, all solutions were stored at 4 °C refrigerator.

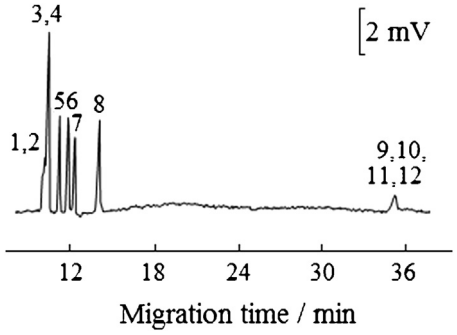

Fig. 1. The electropherogram of the mixture standard solution for both target anions and the main coexisting substances.
Experimental conditions: capillary tube: 23.5 μm id × 360 μm od × 80.0 cm, effective length of 73.0 cm; running buffer: 10 mmol/L His − 90 mmol/L HAc; excitation frequency for C^4D: 450 kHz; peak-to-peak voltage for C^4D: 80 V_{pp}; separation voltage, −18 kV; injection time, 6 s (at −18 kV); peak identifications: (1) F^-, (2) Br^-, (3) Cl^-, (4) I^-, (5) NO_3^-, (6) SO_4^{2-}, (7) SCN^-, (8) NO_2^-, (9) $H_2PO_4^-$, (10) formic acid, (11) propionic acid and (12) uric acid; the concentration of each anion: 10 μg/mL.

Saliva samples of healthy humans and adult smokers were from volunteers in our campus, and those of patients suffering asthma were obtained from Shanghai Putuo District People's Hospital (Shanghai, China). All samples were collected with the permission of each provider, and the purpose and usage of sample collection were clearly informed. All fresh samples were centrifuged for 10 min (10,000 r/min), then filtered by 0.45 um pin type filter, finally stored at −20 °C. Before use, each thawed saliva sample was diluted 75-fold with 5% methanol solution.

2.3. Instrumentation and electrophoretic conditions

The laboratory-built CE–C^4D system employed was described previously [32]. Fused silica capillary (23.5 μm id × 360 μm od × 80.0 cm) was obtained from Polymicro Technologies (Phoenix, AZ, USA) and the effective length was 73.0 cm. Before use, the capillary was conditioned by washing with 0.1 mol/L NaOH, ultrapure water and the running buffer for 15 min, respectively. Electrophoretic separation was completed in a running buffer of 10 mmol/L His—90 mmol/L HAc (pH 3.70) at the separation voltage of −18 kV. All samples were injected electrokinetically at the negative terminal, and the injection time was 6 s (at −18 kV). The excitation frequency was set to 450 kHz and the amplitude to 80 V_{pp} (peak-to-peak voltage) for C^4D. All experiments were performed at room temperature.

3. Results and discussion

3.1. Optimization of electrophoretic conditions

Since saliva still contains a variety of inorganic and organic ions after removal of proteins, the optimization of the buffer composition plays an important role in method development. In order to obtain a good separation of the target analytes and coexisting

Table 1
The regression equations, LODs and LOQs of the target anions ($n = 3$).

Analytes	Regression equations		r^2	Linear range (μg/mL)	LODs (ng/mL)	LOQs (ng/mL)
	Slope	Intercept				
NO_3^-	78.0 ± 1.7	0.2 ± 0.01	0.9997	0.010–5.0	3.1	10
SCN^-	45.8 ± 1.0	0.6 ± 0.05	0.9994	0.016–5.0	4.9	16
NO_2^-	71.9 ± 1.0	0.9 ± 0.02	0.9993	0.011–5.0	3.3	11

*FASI/CE–C^4D conditions were the same as those in Fig. 1.
**In the regression equation, the x value was the concentration of analytes (μg/mL), the y value was the peak area (mV.s).