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A direct and rapid method to determine cyanide in urine by capillary electrophoresis

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ABSTRACT

Cyanides are poisonous chemicals that widely exist in nature and industrial processes as well as accidental fires. Rapid and accurate determination of cyanide exposure would facilitate forensic investigation, medical diagnosis, and chronic cyanide monitoring. Here, a rapid and direct method was developed for the determination of cyanide ions in urinary samples. This technique was based on an integrated capillary electrophoresis system coupled with laser-induced fluorescence (LIF) detection. Cyanide ions were derivatized with naphthalene-2,3-dicarboxaldehyde (NDA) and a primary amine (glycine) for LIF detection. Three separate reagents, NDA, glycine, and cyanide sample, were mixed online, which secured uniform conditions between samples for cyanide derivatization and reduced the risk of precipitation formation of mixtures. Conditions were optimized; the derivatization was completed in 2–4 min, and the separation was observed in 25 s. The limit of detection (LOD) was 4.0 nM at 3-fold signal-to-noise ratio for standard cyanide in buffer. The cyanide levels in urine samples from smokers and non-smokers were determined by using the method of standard addition, which demonstrated significant difference of cyanide levels in urinary samples from the two groups of people. The developed method was rapid and accurate, and is anticipated to be applicable to cyanide detection in waste water with appropriate modification.

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1. Introduction

Cyanides in the form of hydrogen cyanide (HCN) and its alkali metal salts (such as NaCN and KCN etc.) are extremely toxic chemicals which widely exist in nature and industrial processes [1,2]. Deliberate or accidental cyanide poisoning is rare but may be fatal to involved humans or animals [3]. The most common accidental cyanide exposure is the inhalation of smoke from residential and industrial fires that produce cyanides through nitrogen-containing combustibles [4]. The natural sources of cyanides mainly attribute to the nitrogen metabolic products of bacteria, fungi and algae and to the degradation of cyanogenic glycosides in some plants including bitter almonds, cassava roots, and whole sorghum [5]. Tobacco smoking also produces cyanide thus increasing cyanide levels in blood and urine of smokers but rarely intoxicating. On the other hand, cyanides have important industrial applications including pesticides, electroplating, mineral refinery, and polymer synthesis [1,6], in which cyanides are possibly released into environments. Therefore, cyanide exposure is difficult to avoid, and thus quick identification of potential cyanide intoxication is valuable for forensic lab testing, determining chronic low-level cyanide exposure, and chemical warfare monitoring.

Various methods have been developed and reviewed for cyanide detection [5,7–9]. These methods may be based on spectrophotometry, potentiometry, or fluorometry [7]. Involved instrumentation can be HPLC (high-performance liquid chromatography), CE (capillary electrophoresis), GC (gas chromatography), UV/Vis absorbance, or sensors. Although most of these techniques meet application requirements, there are operational limitations such as complexity and multiple steps of sample pretreatment. Therefore, simple and rapid methods are highly desired to facilitate the quick clues of cyanide exposure so that involved patients could be timely and appropriately treated. The indicators of cyanide intoxication can be biological markers (thiocyanate and 2-aminothiazoline-4-carboxylic acid (ATCA)) or the direct measurement of cyanide ions (CN^-) [8]. The requirement of the detection sensitivity depends on the sources of biological fluids. For instance, cyanide ion concentrations are in micromolar ranges in blood while in nanomolar in urine due to the fast metabolic processes that convert cyanide mainly to thiocyanate (~80%) and other species [8]. The typical half-life of CN^- is approximately 20–60 min

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[8], which further suggests that appropriate cyanide conservation methods or quick measurements be performed.

Tobacco smokers expose themselves to cyanide and would have elevated levels of cyanide in blood and urine. Rapid and accurate determination of the cyanide levels would help to investigate the effect of smoking between smokers and non-smokers. Here we report a direct electrophoretic method for rapid determination of cyanide in urine. In this method, urine samples were mixed online with fluorogenic reagents, NDA (naphthalene-2,3-dicarboxaldehyde) and glycine, to produce fluorescent derivatives. The mixture was then injected for electrophoretic separations. The separation was completed in 25 s and successive injections could be performed. Cyanide concentrations were rapidly measured by using the method of standard addition.

2. Experimental

2.1. Materials and reagents

Sodium tetraborate and all amino acids were purchased from Sigma (St. Louis, MO, USA). Potassium cyanide, acetonitrile (ACN), dimethylsulfoxide (DMSO), dimethylformamide (DMF), ethylenediaminetetraacetic acid (EDTA), creatinine, creatine, urea, and sodium hydroxide were purchased from Fisher Scientific (Chicago, IL, USA). NDA was ordered from Invitrogen (Eugene, OR, USA). Glycine amide [5-(aminoacetamido)fluorescein] (FL-Gly) was purchased from Setareh Biotech (Eugene, OR, USA). Fused silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA).

Aqueous solutions were prepared in deionized (DI) water, and the NDA stock solution was in DMF. The stock solution of KCN (100 mM) was prepared in DI water. Cyanide standard solutions were prepared by diluting the stock KCN solution. Buffer solutions with pH 9.2 were prepared in DI water by using sodium tetraborate ($Na_2B_4H_7{\cdot}10H_2O$). Amine solutions (glycine, alanine, glutamine, and glutamate) were prepared in DI water, and were diluted to appropriate concentrations with DI water or borate buffer.

2.2. Instrumentation

The microfluidic detection system has been described elsewhere [10]. Briefly, a 442-nm laser (Laserglow Technologies, Toronto, ON, Canada) beam was focused on the separation capillary through a 40x oil-immersion objective (Carl Zeiss Microscopy, Thornwood, NY, USA), and the fluorescence was collected by using the same objective and then transported to a photomultiplier tube (Hamamatsu Photonics, Japan) after emission filtration (482 ± 17 nm). Sample injection and separation was performed by using a high-voltage power supply (Model CZE1000R) purchased from Spellman High Voltage Electronics Corporation (Hauppauge, NY, USA). A sample plug was electrokinetically injected via the flow gated injection method, and then separated. This procedure was controlled by a LabVIEW program. A 4-syringe pump (Chemyx Inc., Stafford, TX, USA) was used to supply sample and derivatization reagents through individual gas-tight Hamilton syringes (Reno, Nevada, USA). The flow gate and other interfaces were fabricated with poly(dimethylsiloxane) (PDMS) as described in the reference [10].

A 17-cm long capillary with the effective length of 10 cm, ID 10 μm, and OD 360 μm was used to perform separation and detection. The flow-gated injection was run at -5 kV × 0.5 s with the high voltage applied to the outlet side of the capillary, and the inlet side was grounded. Separations were performed under -23 kV, and the separation buffer was 20-mM tetraborate with pH 9.2.

2.3. Derivatization of cyanide

The derivatization was performed through offline or online mixing of three solutions: NDA in DMF/water at 50/50 by volume, glycine in buffer with EDTA (5.0 mM), and KCN in buffer or in urine at room temperature. The mixing ratio of NDA/glycine/samples was 1:1:1 or 1:1:2 by volume as indicated in Figure captions and/or the text. The concentrations of NDA, Gly and CN^- indicate original concentrations in three separate solutions before mixing. The urinary samples were donated by three male regular smokers and three male non-smokers in their ages of 20-30 years old. No personal information was recorded except that they were smokers or non-smokers. Urine samples were collected fresh on the experimental day and diluted by adding NaOH solution (final 15 mM in urine) to conserve cyanides. The samples with standard additions were prepared via KCN standard solutions; and the final volume of each sample consisted of 95% urine plus NaOH (15 mM) and standard KCN (various concentrations).

2.4. Standard addition and data analysis

Standard cyanide (KCN) was prepared in DI water, and was diluted to appropriate concentrations. The concentrations added to the urine samples were 0, 100, 200, 300, 400, and 500 nM before mixing with the derivatization reagents. The cyanide possibly existing in water and/or DMF was subtracted by using the background signal obtained via replacing the urine sample branch with NaCl solution with an equivalent conductivity to the urine sample. After separations, the cyanide-derivative peaks were integrated and the peak areas were recorded and averaged (>5 repeated injections). The calibration curves were constructed and the cyanide levels in urine were calculated as the absolute values of x-axis intercepts.

3. Results and discussion

3.1. Fluorogenic derivatization of cyanide ions

NDA was first developed to efficiently derivatize primary amines at the existence of cyanide ions in 1986 [11]. Recent years have seen wide application of NDA-derivatization of amino acid neurotransmitters for sensitive laser-induced fluorescence (LIF) detection [12–14]. To measure amines in a sample, excessive NDA and CN^- (typically in the mM range) were often used to derivatize amines to the greatest extent. On the other hand, this derivatization strategy has also been employed to measure cyanide ions by using high concentrations of NDA and an amine compound such as glycine and taurine [15–19]. Similarly, excessive NDA and amine were used to facilitate the derivatization of cyanide ions thus quantitatively determining the levels of cyanide in waste water, blood, and urine.

However, NDA and the primary amine reacted to produce imines. The resulted water-insoluble species caused the solution faintly yellow, brown, and finally black as shown in Fig. 1a. NDA at 5.0 mM and glycine or taurine at 20 mM were mixed at 1:1 by volume at pH 9.2; the mixture changed color rapidly (~ 2 min). Experiments also showed that higher concentrations of amines and/or NDA accelerated the color change, which indicated that the reaction rate was amine- and/or NDA concentration dependent. Jackson et al. observed the similar phenomenon when they mixed NDA and taurine [1], and the formation of NDA-ditaurine species was proposed considering the NDA molecule had two aldehyde groups which could react with primary amines. When the reaction mixture was analyzed with CE, capillary clogging might occur due to the precipitation and should be avoided. To confirm the reaction between NDA and primary amines, FL-Gly and NDA were