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ScienceDirect

Procedia Chemistry 20 (2016) 52 – 55

Procedia Chemistry

11th Asian Conference on Chemical Sensors, ACCS 2015

Determination of citric acid in urine by enzymatic flow injection system based on a novel microfluidic chip

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Abstract

A flow injection analysis (FIA) system with enzymatic detection has been proposed for determination of citric acid in urine using a novel microfluidic chip. A microfluidic device based on PDMS channels is designed and fabricated. To determinate the citric acid concentration in real samples and explore interference factors, three models are established based on different standard solutions of citrate, which are prepared with deionized water, artificial urine and real sample without citrate, respectively. The correlation coefficients of these models are 0.9989, 0.9955 and 0.9976 within the range from 0.1mM to 6mM. Meanwhile, a strong correlation is obtained between the three models (Each R^2>0.986) with regression analysis. At last, 20 urine samples are tested and an analysis of the correlation between the results measured by the proposed method and by the capillary electrophoresis method is made. Results indicate that this method looks promising in urine citrate analysis because of its high sensitivity, high selectivity and low consumption of solvent.

Peer-review under responsibility of Universiti Malaysia Perlis

Keywords: Flow injection analysis; Enzymatic detection; Citric acid; Microfluidic device

1. Introduction

Due to unhealthy dieting habits and environmental factors, urolithiasis has become one of the most widespread diseases of urinary system, which is characterized by the formation of stones in the kidneys

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Peer-review under responsibility of Universiti Malaysia Perlis
doi:10.1016/j.proche.2016.07.008

and urinary organs [1]. Many studies[2-4] indicate that citrate present in urine forms soluble complexes with calcium leading in decrease of supersaturation of calcium oxalate and calcium phosphate, thus the presence of citrate in urine contributes to the inhibitory potential against urine stone. Hence, the urinary citrate concentration could be a significant indicator of urolithiasis.

The most commonly used enzymatic method for measure citrate levels is based on the following reactions:

$$Citrate \overset{CL}{\rightleftharpoons} oxaloacetate + acetate \quad (1)$$

$$Oxaloacetate + NADH + H^{+} \overset{MDH}{\rightleftharpoons} L\text{-}malate + NAD^{+} \quad (2)$$

As shown in the Eq.1 and Eq.2, citrate converts to oxaloacetate and acetate by catalytic activity of citrate lyase (CL). In the presence of L-malate dehydrogenase (MDH), oxaloacetate pyruvate is reduced into L-malate by NADH. According to Eq.2, the amount of oxidized NADH level is correlated with the level of citrate in the sample. Citrate concentration is calculated by the decrease in absorbance of NADH at 340 nm.

The traditional enzymatic method for the determination of citrate uses tube and cell without mixing function as a somewhat isolated component, which means the waste of reagent and time. We propose a flow injection analysis (FIA) system with a novel microfluidic chip, which includes microchannels, mixing cell and testing cell, for the determination of citric acid in urine. Using this system, we build three models with different standard solutions prepared in deionized water, artificial urine and real sample without citrate, respectively, and assess their performance characteristics by testing 20 urine samples.

2. Apparatus and Reagents

2.1. FIA System

In order to achieve absorption photometry, a USB fiber micro spectrometer, USB2000+ (Ocean Optics, USA), is chosen to build the measure system. Just as shown in Fig.1, buffer solution, sample and enzyme solution are introduced into the microfluidic chip, which is designed and fabricated based on PDMS with the size of 20mm x 30mm x 8mm, and then discarded after being detected by the micro spectrometer. The structure of microfluidic chip, which is bonding with a piece of quartz glass slice, is presented in Fig.2, and it contains three sections: channels, mixing cell, which provides time and space for mixing and pre-reaction, and testing cell.