Analytical Biochemistry 544 (2018) 1–6

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Selective recognition of creatinine – Development of a colorimetric sensor

Unni Sivasankaran[a], Theresa Chiramal Jos[b], Krishnapillai Girish Kumar[a,*]

[a] Department of Applied Chemistry, Cochin University of Science and Technology, Kochi-22, Kerala, India
[b] Department of Chemistry, Vimala College, Thrissur - 9, Kerala, India

ARTICLE INFO

Keywords:
Creatinine
Copper nanoparticle
Colorimetry
Renal biomarker
Sensor

ABSTRACT

The present report describes a simple and cost effective protocol for colourimetric determination of creatinine (CR), L-cysteine stabilized copper nanoparticles (L-cys-CuNPs) exhibited selective and sensitive interaction with CR. Utilizing this interaction, a colourimetric sensor has been developed based on the reduction in LSPR intensity as monitored by a UV–visible spectrophotometer. The developed sensor exhibited a linear dynamic range of 5.33×10^{-6} to 3.33×10^{-7} M. Proposed sensor is simple and cost - effective compared to methods based on noble metal nanoparticles and the sensitivity to determine CR was as low as 4.54×10^{-10} M. The sensor was successfully applied for quantification of CR in artificial serum and urine samples. Sensor developed in this work has a high potential for rapid and on-site determination of CR in physiological and clinical samples.

Introduction

Kidneys are one of the important chemical factories in our body, which regulates blood pressure, remove waste products of metabolism and maintain the water balance and composition of electrolytes in blood [1]. Generally functioning of kidneys is studied by observing the levels of blood urea nitrogen, creatinine clearance and also by glomerular filtration rate, the flow rate of filtered fluid through the kidney [2]. Hence, research on breakdown products such as urea and creatinine (CR) (2-amino-1-methyl-5H-imidazole-4-one) can explore the biological effects and functions of kidneys [3]. Additionally, concentrations of the above species can also reveal the muscular and thyroid functions [4].

CR is the end product of creatine metabolism [3]. It is produced by body and is filtered from bloodstream by kidneys in relatively constant amounts every day [3]. Normal physiological concentration in blood is 0.9–1.3 mg/dL in men and 0.6–1.1 mg/dL in women, but it can exceed from these levels in certain pathological conditions [5]. In contrast to urea, the concentration of CR in body fluids is not influenced by protein intake, so the level of CR serves as a more reliable indicator of renal function [6]. Hence, quantification of CR is an important area of research in science, especially in the fields of clinical biochemistry and medicine [7,8].

Colourimetry [9], electrochemical methods [10], chromatography [11] etc. are often used for analysis of creatinine. With the exceptions of colourimetry, instruments required for other two methods are costly, requires elaborate sample preparation and skilled persons to operate them, so these are not suitable for routine analysis [12]. Generally, colourimetric methods are easy to perform and can easily detect the presence of analyte. Most common colorimetric determination methods used for CR are Jaffé's reaction [13] and the enzymic colorimetric method [14]. But these methods are limited with respect to selectivity or time-consuming complicated procedure (enzyme linked). In view of above disadvantages, a simple and cost effective colourimetric technique was developed for the determination of CR in physiological solutions. Moreover, the proposed method may serve as an ideal test for clinicopathological investigations associated with renal function.

As part of our research on optical sensors [15–19], a new colourimetric sensor has been developed by utilizing the advantages of copper nanoparticles (CuNPs). Compared to noble metal nanoparticles such as gold and silver, CuNPs is considered to be cost effective and are more catalytic in nature because of the extremely small size and high surface to volume ratio [20]. Herein, L-cysteine is chosen as stabilizing agent for the synthesis of probe. To the best of our knowledge, no colourimetric sensor based on L-cysteine stabilized CuNPs has been reported for the determination of CR. The developed assay was successfully applied for the determination of CR in artificial blood serum and urine samples.

Experimental

Reagents

Creatinine and Hydrazine hydrate were obtained from Spectrochem Pvt. Ltd., Mumbai, India. L-cysteine and Uric acid were purchased from Alpha Aesar, Heysham, England. Dopamine was supplied by Himedia

* Corresponding author.
E-mail address: giri@cusat.ac.in (K. Girish Kumar).

https://doi.org/10.1016/j.ab.2017.12.017
Received 9 September 2017; Received in revised form 7 December 2017; Accepted 12 December 2017
Available online 15 December 2017
0003-2697/

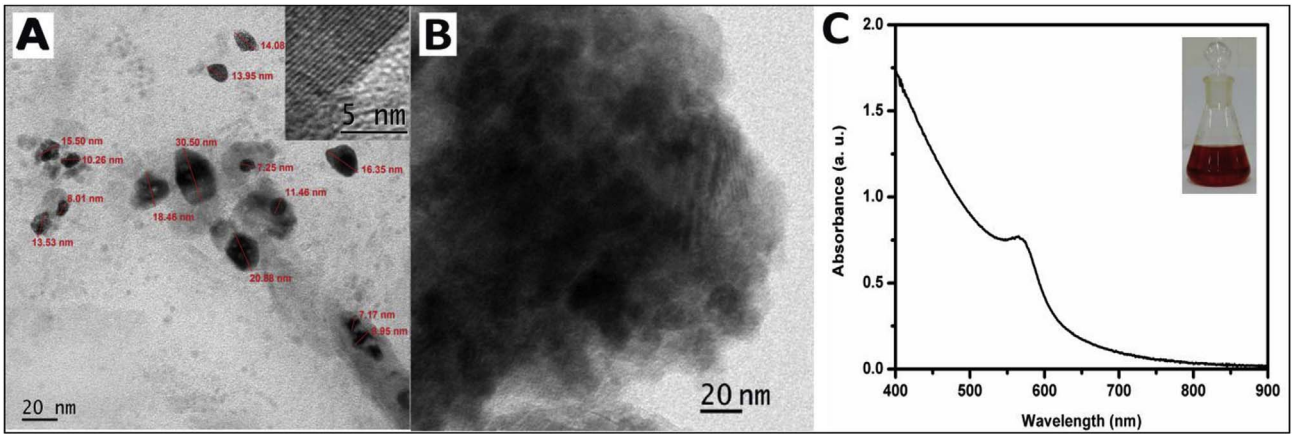

Fig. 1. TEM images of (A) L-cys-CuNPs, inset: lattice spacing of one particle, (B) L-cys-CuNPs in presence of CR, (C) UV–visible absorption spectrum of L-cys-CuNPs, inset: as-synthesized L-cys-CuNPs in visible light.

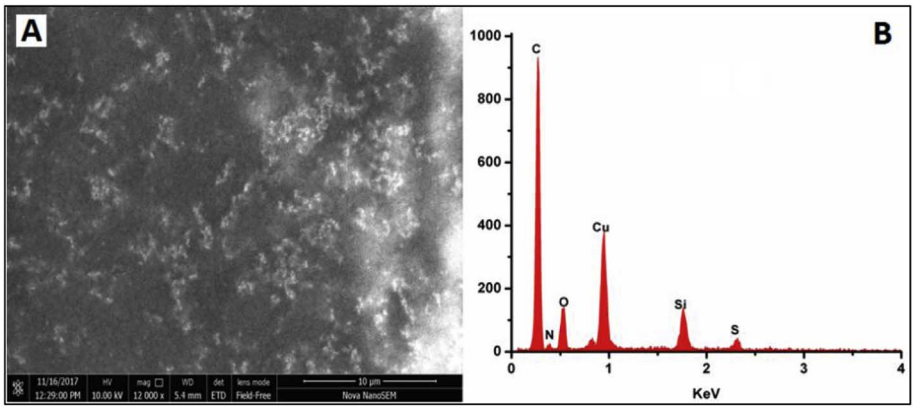

Fig. 2. (A) SEM images of L-cys-CuNPs coated on a glass plate, (B) corresponding EDX spectrum of the area shown in SEM image.

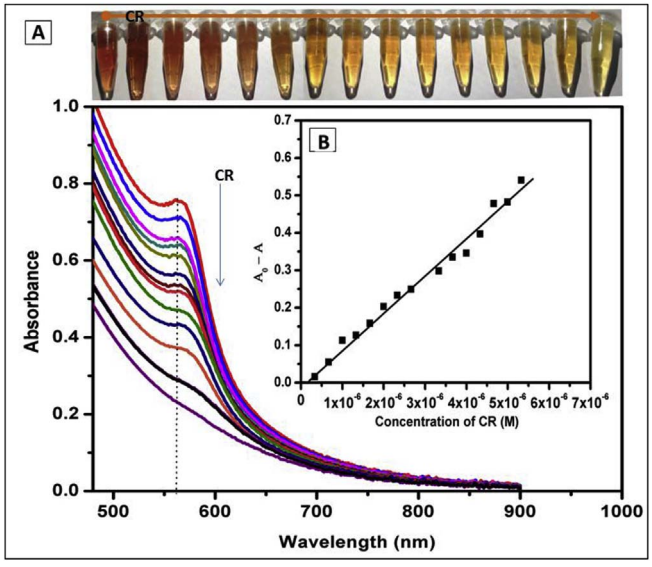

Fig. 3. (A) Effect of concentration of creatinine on the absorption spectrum of L-cys-CuNPs and the observed colour change (left to right) is depicted in inset. (B) Corresponding linear dynamic range of creatinine. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

laboratories Pvt. Ltd, India. Copper chloride and Urea were procured from S.D. Fine Chemicals, India. Ascorbic acid, Sodium chloride, Sodium phosphate and Glucose were supplied by Merck Life Science Pvt. Ltd, India. All chemicals were of analytical grade and used without further purification. Solutions were prepared in Millipore water and all experiments were carried out at room temperature.

Instruments

UV- visible spectra were recorded using Thermoscientific, Evolution 201, China. TEM images were captured using JEM-2100 HRTEM. Particle size was measured using Zetasizer Nano ZS series, Malvern instruments. AFM studies were performed on Composite desktop AFM/ STM-Nanosurf AG, Switzerland.

Synthesis of L-cysteine stabilized CuNPs

L-cysteine stabilized CuNPs were synthesized as per the reported procedure [21]. 3.0 mL of 3 mM solution of $CuCl_2$ was diluted to 80.0 mL using Millipore water and mixed with 2.0 mL of 0.01 M L-cysteine solution. Then, 10.0 mL of 0.1 M hydrazine hydrate was dropped slowly in to the mixed solution. Obtained solution was sealed and reacted for 90 min. Finally a dark red colloid was obtained, which was stored at 4 °C until use. The synthesized CuNPs is stable and gave reproducible results up to 4 months when it was stored in refrigerator.

Analytical procedure

Experimental procedure

L-cys-CuNPs solution was taken in a quartz cuvette and aliquots of CR (3.33×10^{-7} to 5.33×10^{-6} M) were added and the total volume was made up to 3 mL by adding appropriate amount of water. After 7 min incubation at room temperature, the UV–visible spectra were recorded at λ_{max} 563 nm. Difference in absorbance was noted as $\Delta A = A_0 - A$, where A_0 and A are the absorbance of L-cys-CuNPs in the