Analytica Chimica Acta 1007 (2018) 40–49

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

# Citrate-capped silver nanoparticles as a probe for sensitive and selective colorimetric and spectrophotometric sensing of creatinine in human urine

Melisew Tadele Alula [a, 1], Leshern Karamchand [b, 1], Nicolette R. Hendricks [b], Jonathan M. Blackburn [b, *]

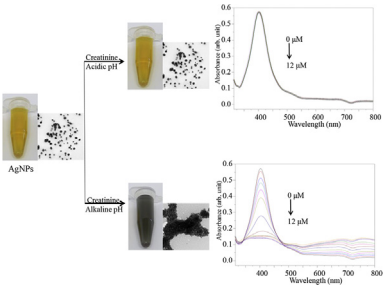
[a] College of Sciences, Department of Chemical and Forensic Sciences, Botswana International University of Science and Technology, Plot 10071, Private Bag 16, Palapye, Botswana

[b] Institute of Infectious Disease and Molecular Medicine, Department of Integrative Biomedical Sciences, University of Cape Town, Cape Town 7925, South Africa

## HIGHLIGHTS

- A colorimetric, LSPR citrate-capped AgNPs (cc-AgNPs) sensor capable of selective quantitation of creatinine was developed.
- Creatinine induced concentration-dependent aggregation of cc-AgNPs at pH 12.
- Inorganic ions and organic molecules present in urine do not interfere with cc-AgNPs-assay of creatinine.
- The $A_{670}/A_{403}$ extinction ratio of cc-AgNPs increased linearly in aqueous creatinine concentration range of 0 −4.2 μM.
- A nanomolar urinary creatinine limit of detection (66 nM) was achieved with the cc-AgNPs assay.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 30 October 2017
Accepted 6 December 2017
Available online 28 December 2017

*Keywords:*
Citrate-capped silver nanoparticles
Creatinine
Aggregation
Localized surface plasmon resonance (LSPR)
UV-Visible spectrophotometry

## ABSTRACT

Urinary creatinine concentration is a critical physiological parameter that enables reliable assessment of patient renal function and diagnosis of a broad spectrum of diseases. In this study, a simple and inexpensive sensor comprising monodisperse, citrate-capped silver nanoparticles (cc-AgNPs) was developed, which enabled rapid, sensitive and selective quantitation of creatinine directly in unprocessed urine. The mechanism of this sensor entails the creatinine-mediated aggregation of the cc-AgNPs (within 1 min) under alkaline conditions (pH 12). This is attributed to the tautomerization of creatinine to its amino anionic species at alkaline pH, which cross-link the cc-AgNPs *via* hydrogen bond networks with the negatively charged citrate caps. Creatinine elicited visibly-discernable color changes of the cc-AgNPs colloids in a concentration-dependent manner up to 10 μM. UV-visible spectroscopic analyses of the cc-AgNPs revealed that creatinine elicited a concentration-dependent decrease in intensity of the localized surface plasmon resonance (LSPR) band centered around 403 nm, with a concomitant increase in intensity of the red-shifted LSPR band at 670 nm. This observation denotes a creatinine-mediated

* Corresponding author.
*E-mail address:* jonathan.blackburn@uct.ac.za (J.M. Blackburn).
[1] Joint-first authors.

https://doi.org/10.1016/j.aca.2017.12.016

increase in cc-AgNP particle size *via* aggregation, as confirmed by transmission electron microscopy analysis. The cc-AgNP sensor exhibited a linear correlation between the $A_{670}/A_{403}$ extinction ratio and creatinine concentration range of 0–4.2 μM in aqueous solutions ($R^2 = 0.996$), and a low detection limit of 53.4 nM. Hence, the simplicity, short assay time, and high sensitivity and selectivity of our cc-AgNP sensor affirms its utility as a creatinine monitoring assay for low-resource, point-of-care settings.



## 1. Introduction

Creatinine is a metabolite of creatine and end-product of nitrogen metabolism, which is filtered by the kidneys and excreted from the human body in urine. The concentration of creatinine in urine and serum reflects the glomerular filtration rate, which is a clinically important physiological parameter used to diagnose kidney disease and monitor renal function [1,2]. Various analytical methods, such as, chromatography [3–5], electrochemistry [6–8], capillary electrophoresis [9,10], liquid chromatography–isotope dilution mass spectrometry (LC–IDMS) [11], mass spectrometry [12,13], and surface-enhanced Raman spectroscopy (SERS) [14,15] have been employed in the quantitation of creatinine in biological fluids. However, these methods have multiple drawbacks, including laborious sample preparation steps, complicated and environmentally toxic reagents, the need for expensive, sophisticated instruments, as well as highly-trained technicians to operate them. Hence, conventional laboratory methods are unsuited to, or impractical for the point-of-care and real-time batch quantitation of creatinine in human specimens, particularly in low resource settings. Alternatively, Jaffe's reaction, which is a colorimetric method based on the complex formation between creatinine and picric acid in alkaline medium, is well documented [16]. However, interference from other metabolites and drugs impairs the specificity of the reagent, which severely limits its clinical application. Moreover, picric acid is highly corrosive and explosive, and therefore unsafe to use in routine clinical analysis. Hence, there is a pressing need for a fast, simple, inexpensive, highly sensitive and specific, point-of-care method to detect, and quantitate creatinine in biological fluids.

Recently, colorimetric sensors based on silver and gold nanoparticles (AgNPs and AuNPs) have received considerable attention because of their size, shape, composition and distance dependent optical properties, and high extinction coefficients [17]. Specifically, the high extinction cross section of AgNPs and AuNPs in the visible region renders them suitable for colorimetric assays, which are readily detectable by the unaided human eye.

The color difference between dispersed and aggregated noble metal nanoparticles is attributed to differences in their localized surface plasmon resonance (LSPR) properties, which forms the basis of colorimetric detection. The aggregation of nanoparticles, which is usually induced by their selective interaction with specific analytes, results in coupling of the plasmonic nanostructures. This, in turn, elicits a color change. Hence, the degree of color change of the colloid is a function of the extent of nanoparticle aggregation - a process governed by the type and concentration of the analyte, which affects the electrostatic and/or steric stability of the plasmonic nanostructures *via* hydrogen bonding or electrostatic interactions. The color changes of the nanoparticles are further reflected in the corresponding changes of the absorption maximum of their UV-visible extinction spectra, which appear as a red shift, and broadening of the LSPR band. These spectral changes are readily detectable and quantifiable with modular, USB-powered UV-visible spectrophotometers, which permit on-site environmental monitoring, as well as point-of-care biomedical diagnostics [18,19]. Silver nanoparticles are preferred to gold nanoparticles of the same size, due to their higher extinction coefficients and superior plasmonic properties, lower material costs, ease and simplicity of synthesis with tunable size and shape, and practicability [20]. The aforementioned properties of AgNPs are significant advantages that favor their use as platforms in the development of point-of-care sensors.

Indeed, several colorimetric sensors based on noble metal nanostructures have recently been developed for the detection of analytes, including, metal ions, small molecules, biomolecules, and microorganisms [21–24]. Similarly, various colorimetric methods based on AgNPs/AuNPs have been reported for the detection of creatinine in different biological fluids [25–29]. For example, Yi He et al. reported the detection of creatinine in spiked human urine using citrate-capped AuNPs [25]. Jianjun Du et al. reported the detection of creatinine using uric acid and $Hg^{2+}$ modified AuNPs [26]. Sulfonic acid-functionalized silica gel was employed to selectively extract creatinine from urine samples, which was subsequently detected using AuNPs [27]. Colorimetric sensors based on picric acid [28] and 2, 2′-thiodiacetic acid (TDA)-capped [29] AgNPs were developed to detect creatinine in blood, cerebrospinal fluid and urine. Although these studies achieved significant advances in developing plasmonic based sensors to detect creatinine, they still fall short of the requirements of being simple, rapid, and inexpensive due to: (i) the complicated procedures employed to functionalize the nanoparticles [26,28,29]; (ii) the laborious process required to extract creatinine from the specimen [27]; and (iii) high cost [25,27]. To address these problems, we report here a simple, rapid, and inexpensive, colorimetric sensor, based on citrate-capped AgNPs (cc-AgNPs), to detect and quantitate creatinine and demonstrate its highly specific and sensitive detection of creatinine in aqueous solutions and human urine.

## 2. Experimental

### 2.1. Chemicals

All reagents were of analytical grade and used as received without additional purification. All analyte solutions were prepared with Millipore ultrapure double deionized water (18 MΩ). Silver nitrate was obtained from South Africa Precious Metals (Pty) Ltd. Glucose, sodium chloride, sodium hydroxide, sodium citrate tribasic dehydrate, ascorbic acid, glycine, and urea were obtained from Sigma-Aldrich. Uric acid, creatinine (≥98%), $Fe_2(SO_4)_3.xH_2O$, $FeSO_4.7H_2O$, and $Zn(NO_3)_2.6H_2O$ were purchased from Alfa Aesar. Potassium chloride, and $CaCl_2.2H_2O$ were obtained from MERCK. Glutamic acid was purchased from Fluka.

### 2.2. Instrumentation

UV-visible spectra were recorded using a Cary 50 UV-visible spectrophotometer. The cc-AgNPs were deposited on silicon nitride mesh substrates, and transmission electron microscopy