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A simple and rapid creatinine sensing via DLS selectivity, using calix[4]arene thiol functionalized gold nanoparticles

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ABSTRACT

A new, simple, ultra-sensitive and selective approach has been reported for the “on spot” colorimetric detection of creatinine based on calix[4]arene functionalized gold nanoparticles (AuNPs) with excellent discrimination in the presence of other biomolecules. The lower detection limit of the method is 2.16 nM. The gold nanoparticles and p-tert-butylcalix[4]arene were synthesized by microwave assisted method. Specifically, in our study, we used dynamic light scattering (DLS) which is a powerful method for the determination of small changes in particle size, improved selectivity and sensitivity of the creatinine detection system over colorimetric method. The nanoassembly is characterized by Transmission electron microscopy (TEM), DLS, UV–vis and ESI–MS spectroscopy, which demonstrates the binding affinity due its ability of hydrogen bonding and electrostatic interaction between –NH group of creatinine and pSDSC$_4$. It exhibits fast response time (< 60 s) to creatinine and has long shelf-life (> 5 weeks). The developed pSDSC$_4$–AuNPs based creatinine biosensor will be established as simple, reliable and accurate tool for the determination of creatinine in human urine samples.

1. Introduction

Creatinine is one of the components of human blood, which is the final product of creatine metabolism in mammals which undergoes in skeletal muscles to release energy. Creatinine is extracted from the body by renal excretion at a relatively constant rate. Kidney problems, thyroid malfunction and muscular disorders cause an increase in creatinine concentration in blood serum, therefore measuring creatinine concentration in blood or serum or urine leads to the diagnosis of those disorders. The normal clinical range for creatinine is 44–106 mmol L^{-1}, however it can be less or more according to age and gender. In kidney malfunction, creatinine concentration can exceed 1000 mmol L^{-1}. Values more than 140 mmol L^{-1} indicates the need for more clinical assays while more than 530 mmol L^{-1} is a sign of having kidney disease. Patients suffering from kidney disease need a device to control creatinine concentration in blood daily. Thus it is necessary to develop chemical sensor which can selectively detect creatinine and determine its concentration in real samples [1–7].

Research on molecular recognition of amino compounds, such as biogenic amines, amino acids, peptides, proteins and carbohydrate like essential substrates in biological processes, by synthetic receptors is a challenging issue of great relevance from both supramolecular chemistry and analytical application point of view [8]. Colorimetric biosensors derived from gold nanoparticles (AuNPs) have attracted particular interest in diagnostics and environmental monitoring. The functional mechanism for such biosensors derive from the aggregating behaviors of AuNPs [9–14]. The functionalization of metal substrate with organic ligand has become a standard practice in surface science and nanomaterial chemistry. Conceivably, the most ubiquitous example is the chemisorption of thiol on Au, a simple yet versatile approach for preparing absorbing surface with chemical or physical properties or biological recognition elements. The stability is usually achieved by the use of thiolate ligands which form a protective shell around the particles to which they are attached through strong Au–S interaction [15–19].

There are various classical methods described in the literature for creatinine determination which includes electrochemical technique [22], nafion coated copper plating electrode [23] spectrophotometric methods [24], colorimetry [25] and potentiometric sensors [26]. Although these approaches are powerful and errorproof, some of them are laborious, complex, time consuming and very often they do not offer the necessary sensitivity and specificity towards the target. Calix[4]arene are ideal frame work for

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molecular recognition because of their ability of being modified at both the upper and lower rims. The recognition and development of selective complexes with biological compounds are particularly fascinating applications of the functionalized calixarenes. Calixarenes, with their unique three-dimensional exterior, are one of the superlative known host molecules along with cyclodextrins, cucurbiturils, cryptands and crown ethers. Calix[4] arene based chemosensors have engrossed a great deal of consideration due to their ability to visually sense analytes with high sensitivity as well as fast response time [20,21]. Our approach for the synthesis of receptor molecule was by attaching thiol unit to calix[4]arene at the lower rim which can offer a suitable binding site and form enough strong electrostatic interaction with creatinine molecule for recognition. Our research group has been working on the development of sensors for cations, anions and biomolecules by supra-nano assemblies. Recently, we reported potassium ion recognition by benzo-15-crown-5-gold nanoparticles [27], lysine, arginine and histidine recognition by functionalized calix[4]arene thiol Au nanoparticles [28], detection of sulfide using calix[4]arene modified gold nanoparticles [29], calixarene capped ZnS quantum dots for detection and determination of menadione [30], a non-enzymatic glucose biosensor based on calix[4]arene functionalized boronic acid gold nanoprobe [31] and calix[4]arene thiol functionalized silver nanoprobe for recognition of ferric ion [32]. These results motivated us to develop chemosensor for recognition and determination of creatinine from urine samples [33–35].

Herein, we designed a new promising approach using AuNPs based colorimetric sensing system (ANCSS) by using functionalized p-sulphonatocalix[4]arene-Au assembly for creatinine detection. This assembly was characterised by different spectroscopic methods for the confirmation of recognition of creatinine.

2. Experimental

2.1. Chemicals and reagents

All chemicals used were of analytical grade or of the highest purity available. All solutions were prepared with double-distilled and deionized water. $HAuCl_4$, sodium citrate, creatinine, ascorbic acid, mannose, vitamin K2, cholesterol, citric acid, urea, glucose and fructose (99% purity) were purchased from Aldrich. Silica gel (Merck, 0.040–0.063 mm) was used for column chromatography.

2.2. Apparatus

Melting points were taken on Opti-Melt (Automated melting point system). GmbH Vario Micro cube elementar analyzer was used for elemental analysis. The FT-IR spectra were recorded as KBr pellet on Bruker TENSOR-27 in the range of 4000–400 cm^{-1}. ^{1}H NMR spectra was scanned on 400 MHz FT-NMR Bruker Avance-400 in the range of 0.5–15 ppm using internal standard tetramethylsilane (TMS) and $CDCl_3$ as a solvent. ESI Mass spectra were taken on a Shimadzu GCMS-QP 2000A. Working standard solutions were prepared daily in deionised water. UV–vis absorption spectra were acquired on a Jasco V-570 UV–vis spectrophotometer. Transmission Electron Micrograph (TEM) was recorded by JEOL, JEM-210027FT-IR (200 kV). DLS measurements were performed using Metrohm Nanotrac instrument.

2.3. Microwave assisted synthesis of p-tert-butylcalix[4]arene (A)

The procedure was essentially the same as those developed by Menon et al. [29] with minor modification. A mixture of p-tert-butyl phenol (4.0 g, 0.33 mM), sodium hydroxide (NaOH) (1 g) and formaldehyde (1.8 ml, 0.18 mM) solution was taken in an open vessel and was irradiated with 50 W power in a microwave synthesizer Discover (CEM) by stirring for 3 min. Cooling for 10 min, resulted yellow solid mass. Next, 4 ml of toluene and 30 ml of diphenyl ether was added in this yellow solid, again irradiated with microwave power of 100 W for 5 min with stirring and obtained a dark brown solution. Further, this solution was added in to 75 ml of ethyl acetate and kept for 2 h. Finally, white precipitate was obtained which was filtered and washed with ethyl acetate and dried. **Yield**, 3.5 g (96%). **Elemental analysis** for $C_{44}H_{56}O_4$, Calcd. C;81.44%, H;8.70%, O; 9.80%, Found:C; 81.11%, H; 8.261%, O; 9.90% **^{1}H NMR:** δ_H($CDCl_3$, 400 MHz):1.28(36H, s, tBu), 3.81(8H, s, $ArCH_2Ar$), 7.12(8H, s, Ar–H), 9.61(4H, s, Ar–OH). **ESI MS** (*m*/*z*)648 (*M*+1).

Table 1
Stability of pSDSC4–AuNPs assembly at different pH conditions.

pH(Abs)	2(0.78)	4(1.0)	6(0.62)	8(0.51)	10(0.48)
Stability of pSDSC$_4$–AuNp	6 Months	3 Months	2 Weeks	7 h	5 h

2.4. Synthesis of p-tert-butyl -26, 28-dimethoxy calix[4]arene (B)

A mixture of p-tert-butylcalix[4]arene **A** (3.5 g. 0.80 mM), K_2CO_3(1.9 g, 14.0 mM) and 1-iodomethane (4 ml, 14.0 mM) in dry acetone (150 ml) was stirred for 24 h. The actual reaction time was considered by performing thin layer chromatography (TLC) at regular interval of time by using mixture of ethylacetate:hexane (8:2). The solvent was then evaporated under vacuum and the residue taken up with CH_2Cl_2.The organic phase was washed with 0.1 M HCl up to neutrality and dried over anhydrous Na_2SO_4. After complete evaporation of the solvent, the resulting crude product was purified by column chromatography (silica gel, hexane: ethyl acetate 1); 2.9 g, **Yield** (81%). **Elemental analysis** for $C_{46}H_{60}O_4$: C,81.61; H,8.93% Found: C,81.38; H,8.52%. **FT-IR** (KBr) υ: 3280 cm^{-1}(Ar–CH), 3430 cm^{-1}(Ar–OH). **^{1}H NMR**: δH (CDCl3,500 MHZ), 1.20 (18H, t-butyl, s) 0.96(18H, t-butyl, s), 4.28 (4H, $-OCH_3$, t), 3.83 (4H, $ArCH_2Ar$,d), 4.30(4H, $ArCH_2Ar$, d), 6.42 (4H, Ar–H, s), 6.85(4H, Ar–H, s), 9.19 (2H, OH, s), m.p. 223–228 °**C. ESI MS** (*m*/*z*) 677.1 (*M*+1), **Solubility:** Soluble in $CHCl_3$, CH_2Cl_2, CH_3CN and insoluble in H_2O.

2.5. Synthesis of p-tert-butyl-25, 27-bis (chloromethoxymethane) 26, 28, dimethoxy calix[4]arene (C)

A mixture of p-tert-butyl-26, 28-dimethoxy calix[4]arene **B** (2.9 g, 0.005 mol), K_2CO_3 (1.9 g, 1.0 mol) and chloro(chloromethoxy)methane (4 ml, 1.0 mol) in dry acetonitrile (150 ml) was stirred for 24 h. The actual reaction time was considered by performing TLC at regular interval of time by using mixture of ethylacetate:hexane (8:2). The solvent was then evaporated under vacuum and the residue taken up with CH_2Cl_2.The organic phase was washed with 0.1 M HCl up to neutrality and dried over anhydrous Na_2SO_4. After complete evaporation of the solvent, the resulting crude product was purified by column chromatography (silica gel, hexane 9: ethyl acetate 1); **Solubility:** Soluble in $CHCl_3$, CH_2Cl_2, CH_3CN and insoluble in H_2**O. Yield** 2.7 g, (97%), **m. p.** > 250 °C. **Elemental analysis for $C_{50}H_{66}O_6Cl_2$,** Calcd. C; 72.01%, H; 7.98%, O; 11.51% **Found:** C; 71.98%, H; 7.88%, O; 11.47%. **^{1}H NMR:** δ_H**($CDCl_3$, 400 MHz):**1.20 (18H, t-butyl, s), 0.96 (18H, t-butyl, s), 4.28 (4H, OCH_2, t), 3.18 (4H, $ArCH_2Ar$, d), 4.30(4H, $ArCH_2Ar$, d),6.42 (4H, Ar–H, s), 6.85 (4H, Ar–H, s), 3.82 (6H, OCH_3, s), **ESI–MS** (*m*/*z*) 832 (*M*+1).