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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.

Key Words: Creatinine; DLS selectivity; Calix[4]arene; Gold nanoparticles; Biosensor

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 behaviours 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 [15-17].

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 error-proof, 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 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

3.1 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, 0.040-0.063mm) was used for column chromatography.

3.2 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}. ^1H 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-210027 FT-IR (200 kV). DLS measurements were performed using Metrohm Nanotrac instrument.

3.3 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}HNMR: δ_H($CDCl_3$,400MH_Z):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).

3.4 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: 3280cm^{-1}(Ar-CH), 3430cm^{-1}(Ar-OH). ^{1}H NMR: δH ($CDCl_3$,500MHZ), 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^{O}C. ESI MS (m/z) 677.1 (M+1), Solubility: Soluble in $CHCl_3$, CH_2Cl_2, CH_3CN and insoluble in H_2O.

3.5 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, hexane9: ethyl acetate 1); Solubility: Soluble in $CHCl_3$, CH_2Cl_2, CH_3CN and insoluble in H_2O. Yield 2.7 g, (97%), m.p. >250 ^{O}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}HNMR: δ_H($CDCl_3$, 400MH_Z):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).

3.6 Synthesis of p-tert butyl-25, 27-bis(dimethoxy dimethyl sulfonyl)- 26,28, dimethoxycalix[4]arene (D)

To 40 ml of acetonitrile having 2.0 g of C, 0.28 g (3.65 mmol) of thiourea was added and the resultant mixture was heated under reflux overnight. Acetonitrile was then removed under reduced pressure. The resulting solid product was mixed with 0.37 g (6.59 mmol) of NaOH and an aliquot of 40 ml of deionized water. The mixture was refluxed for 2 h. The compound was extracted with 1 M HCl and CH_2Cl_2, dried with $MgSO_4$(s), and purified with column chromatography (SiO_2, hexane/ EtOAc 1:3), Solubility: Soluble in $CHCl_3$, CH_2Cl_2, CH_3CN and insoluble in H_2O. Yield 1.7g. 85%, m.p. >250 ^{O}C Elemental analysis for

$C_{56}H_{68}S_2O_6$, Calcd. C; 72.42%, H; 8.27%, O; 11.58, S; 7.73% Found: C; 72.31%, H; 8.15%, O; 11.17%, S; 7.69 %. ^{1}H NMR: δ_H($CDCl_3$,400MH_Z): 1.71(18H, t-butyl, s) 1.11 (18H, t-butyl, s) ,3.67 (6H, -OCH_3, s), 3.01(4H, ArCH_2Ar ,d),1.51(2H,-SH,s),4.30 (4H, ArCH_2Ar, d), 6.42 (4H, Ar-H, s), 6.85 (4H, Ar-H, s). ESI MS (m/z) 829 (M+1).

3.7 Synthesis of p-sulphonato-25, 27 bis (dimethoxy dimethyl sulfanyl) - 26, 28, dimethoxy calix[4]arene (E)

A solution of D (1.7 g, 0.65 mmol) in 5 ml of sulphuric acid (96%) was stirred at 80^{o}C for 5 h. An aliquot was withdrawn from the solution and poured in to water to determine the progress of the reaction. The reaction was completed when water insoluble material was not detected. After cooling, the precipitate was separated by filtration. The precipitate was dissolved in 8 ml of water before addition of 20 ml $BaCO_3$ solution for neutralization. Precipitated $BaSO_4$ was removed by filtration. The product was obtained after evaporation of water in the filtrate. The product was dried in a high vacuum oven. Solubility: Soluble in H_2O, CH_3CN and insoluble in $CHCl_3$, DCM. Yield 1.5 g (90%), m.p. >250 ^{O}C. Elemental analysis for $C_{34}H_{36}S_6O_{18}$, Calcd. C; 44.15%, H; 3.92% Found: C; 44.12%, H; 3.71% ^{1}H NMR : δ_H ($CDCl_3$,400MH_Z):1.88 (18H, t-butyl, s), 1.16 (18H, t-butyl, s) ,4.28 (8H, -OCH_2, t), 3.78 (6H, -OCH_3, s), 3.08 (4H, ArCH_2Ar ,d), 1.51 (2H, -SH, s), 4.30 (4H, ArCH_2Ar, d), 6.42 (4H, Ar-H, s), 6.85 (4H, Ar-H, s),9.31 (4H,-SO_3H,s). ESI MS (m/z) 925 (M+1).

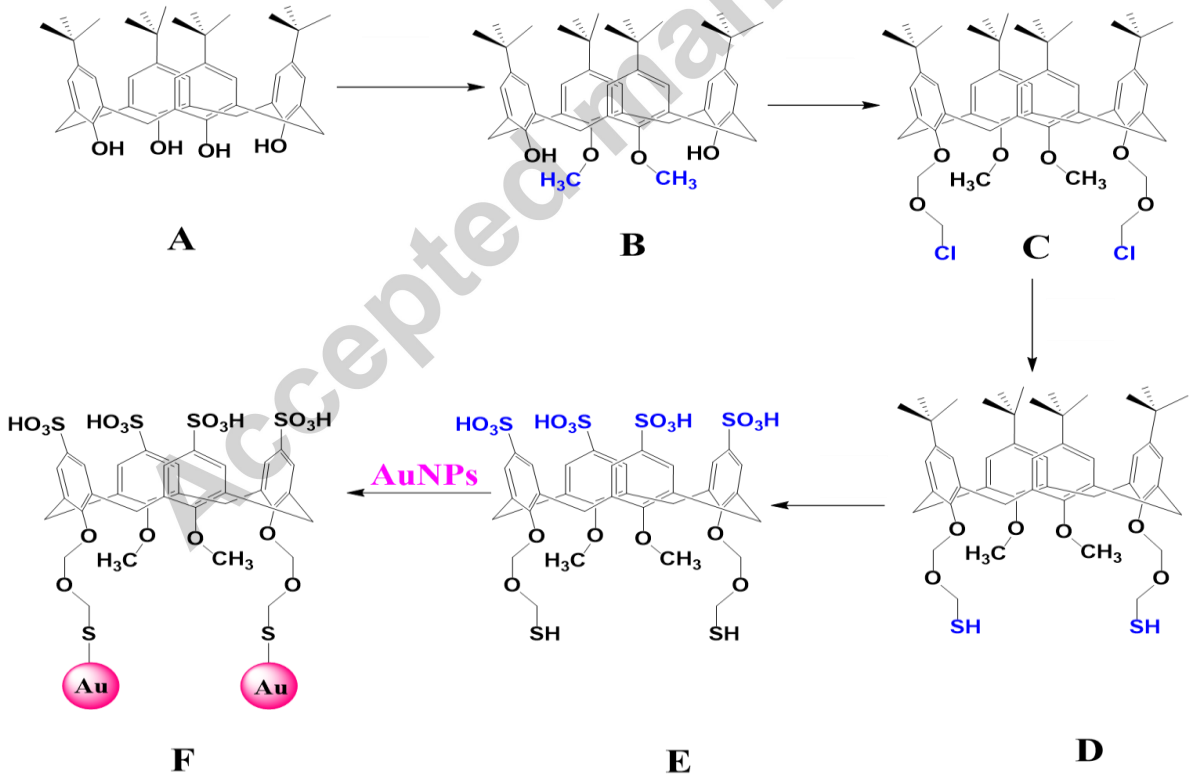
3.8 Microwave synthesis of AuNPs

The procedure was essentially the same as those developed by Menon et al [31] with difference only in the use of microwave for the synthesis .The molar ratio of $HAuCl_4$ to sodium citrate also has been changed accordingly. All glassware was thoroughly cleaned with freshly prepared 3:1 HCl/ HNO_3 (aqua regia) and rinsed thoroughly with Milli-Q water prior to use. The synthesis was carried out in a modified CEM Discover microwave using single

mode and continuous power at 2.45 GHz. The reactions were carried out in sealed reaction vessel containing 3 ml of 0. 4 mM $HAuCl_4$ solution and 2 ml of 13 mM sodium citrate and was heated at 75 ^{0}C at a power up to 300 W for 4 min and obtained 0.24 mM AuNp solution. The solution changed from pale yellow to burgundy to yield Au nanoparticles of 28 nm as per TEM.

3.9 Synthesis of p-sulphonato-25, 27 bis (dimethoxy dimethyl sulfanyl) - 26, 28, dimethoxy calix[4]arene capped gold nanoparticles ($pSDSC_4$ -AuNp) (F)

Initially, 0.24 mM gold nanoparticles were added to 0.2 mM p-sulphonato-25, 27 bis (dimethoxy dimethyl sulfanyl) - 26, 28, dimethoxy calix[4]arene, F ($pSDSC_4$–thio) and kept stirring for 1 h. Then, 25, 27-bis (ethelene amine\ carbonyl methoxy)-26, 28-dihydroxy-p-sulphonato calix[4]arene capped gold nanoparticles ($pSDSC_4$-AuNp) was obtained (Scheme



Scheme 1: Synthesis of the **pSDSC$_4$** [**p**-**s**ulphonato-25,27 bis-dimethoxy – 26,28 (**s**ulfanylmethoxy)methoxy**c**alix[**4**]arene]-AuNPs ligand.

1).

3.10 Preparation of urine samples

For the analytical application of proposed probe, we have analysed urine samples for recognition and determination of creatinine. The developed sensor was successfully used in the determination of creatinine in spiked urine samples. The samples were collected from healthy volunteer for determination of creatinine level by developed method. The urine specimens were centrifuged at 1500 rpm for 5 min before subjecting the supernatant to analysis by developed method. For the extraction of creatinine into aqueous phase, we took 6 ml of ligand solution in water and 4 ml of urine sample in chloroform into separating funnel and shaken for half an hour. The aqueous layer was separated made up to 10 ml with water adjusted the pH 4.0 and measured the absorbance to find out the concentration of creatinine. The results of real sample analysis are given in Table 2.

3 Results and Discussion:

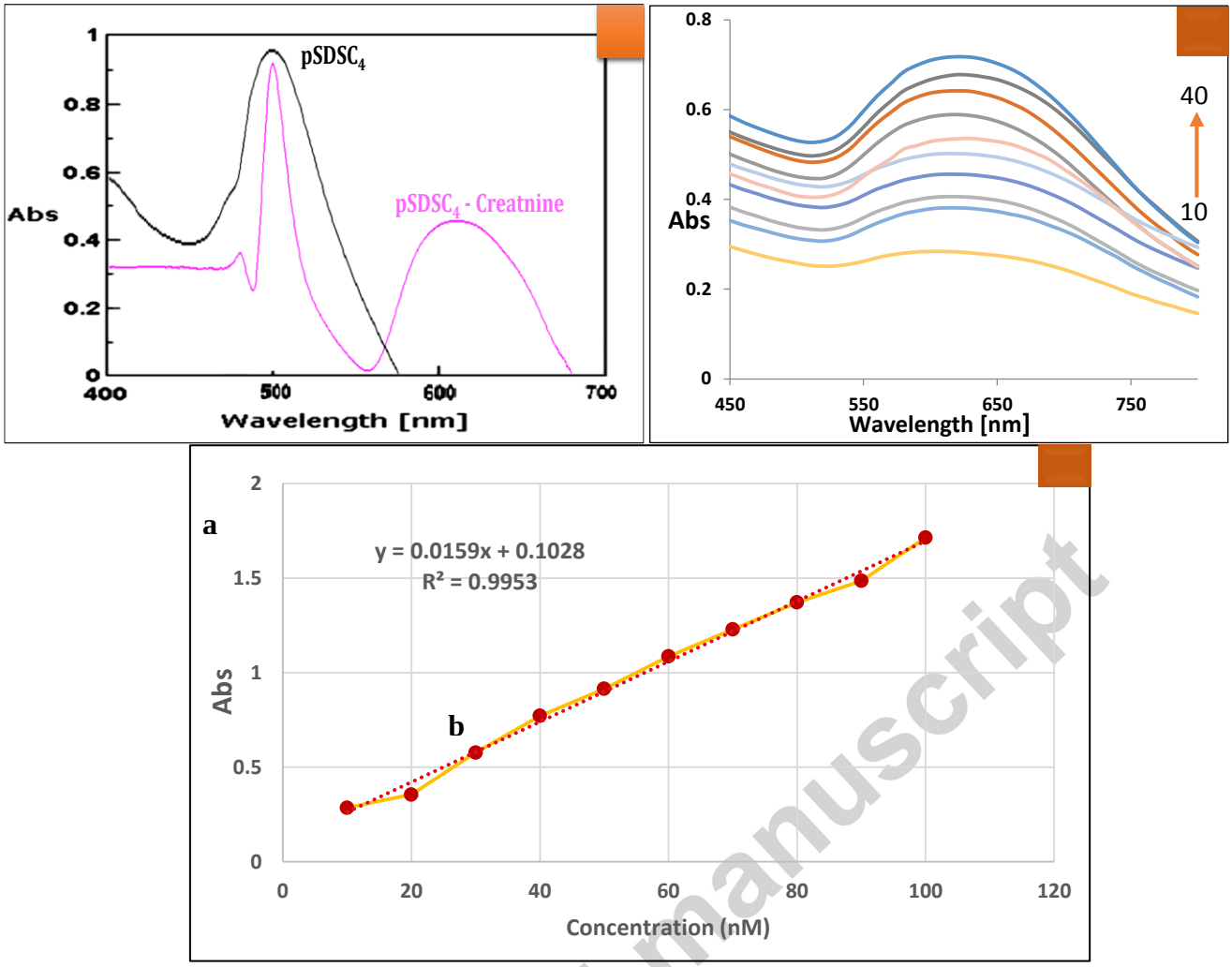


Fig. 1: (**A**) UV–Vis spectra of (a) $pSDSC_4$-AuNPs (b) $pSDSC_4$-AuNPs with 25 nM creatinine concentration (**B**) UV-Visible spectra of $pSDSC_4$-AuNPs 0.24 mM responding to various concentrations of creatinine (10, 20, 22, 25, 28, 30, 32, 35, 38, 40 nM.) (**C**) The linear range for creatinine, using 0.24 mM $pSDSC_4$-AuNPs is found to be 10 nM- 100 nM (R^2=0.9953)

Several calibration samples with different concentrations of creatinine (three measurements for each concentration) were analyzed and UV-visible absorption spectra were collected. UV–visible spectra were recorded in the absence and presence of different concentrations of creatinine. The color of 0.24 mM $pSDSC_4$-AuNPs is wine red and displayed a characteristic absorption band at 522 nm (Fig.1A). However, a color change to dark blue took place due to aggregation and significant change in the spectral profile was noticed upon the gradual addition of different concentrations of creatinine from 25 nM to 10 nM with 0.24 mM $pSDSC_4$-AuNPs at pH 4 adjusted with sodium acetate/acetic acid buffer.

These changes were due to gradual decrease in the surface plasmon resonance at about 522 nm and appearance of a new band at 619 nm (Fig.1B) which are ascribed to the aggregation of AuNPs and exhibit selective binding with creatinine via electrostatic interaction and H-bonding between creatinine and $pSDSC_4$- AuNPs. Prominently, it was found that even the addition of trace creatinine could lead to an obvious color change which could be distinguished with the naked eye. The absorption was used to quantify the concentration of

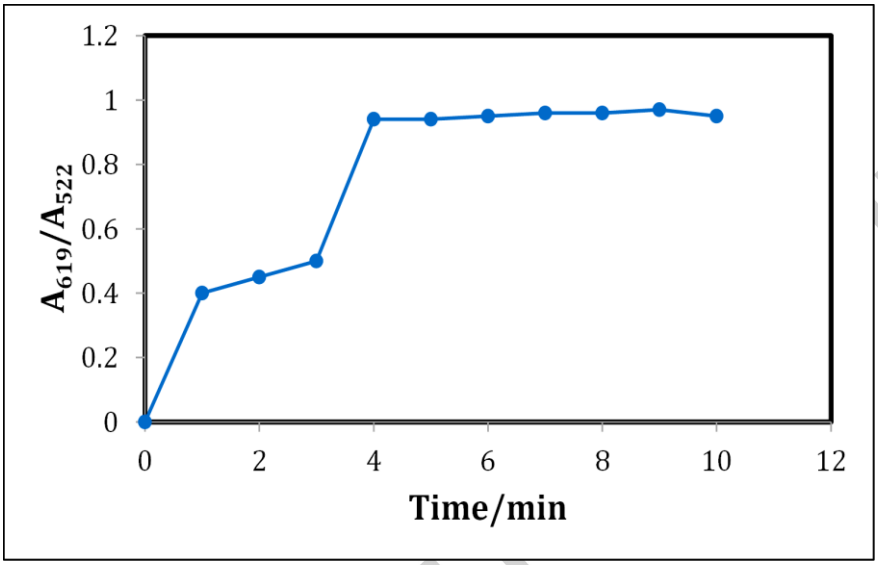


Figure 2: Shows the response behaviour after addition of creatinine molecule in 0.24 mM $pSDSC_4$-AuNPs.

creatinine. It is concluded that the change in solution colour and degree of red shift are associated with the increase in the size of aggregated nanoparticles (Fig. 1B). However after 5 days, colour decreases very slowly due to sedimentation. The linear range for creatinine, using 0.24 mM $pSDSC_4$-AuNPs is found to be 10 nM- 100 nM (R^2=0.9953) (Fig. 1C). The lower detection limit is 2.16 nM calculated as per 3σ IUPAC criteria. As shown in (Fig. 2) the absorption ratio A_{619}/A_{522} remained unchanged after 4 min, indicating the interaction was almost completed under this condition. Therefore, all the absorption measurements were performed after 4 min.

3.1 Particle Size analysis:

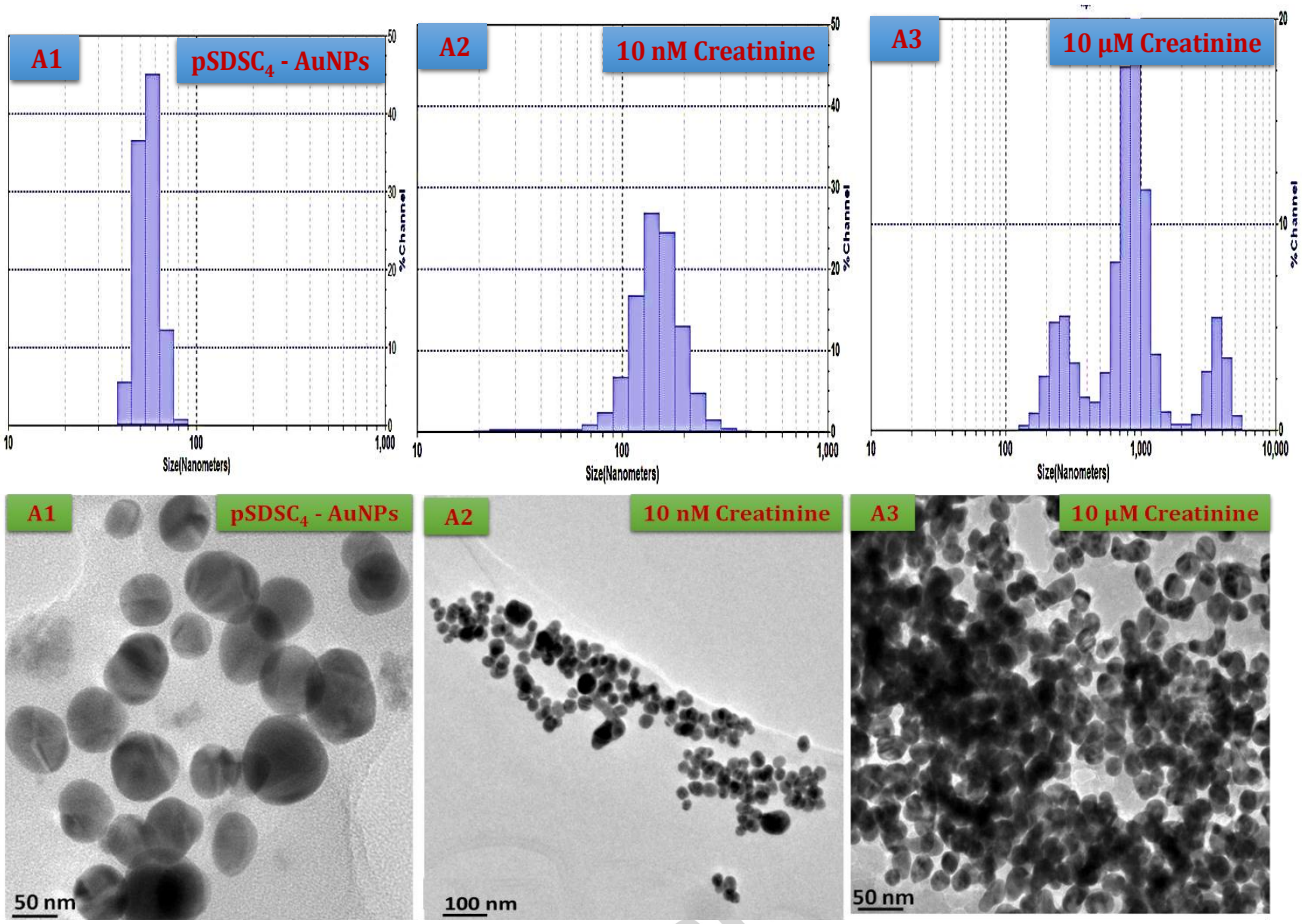


Figure 3: Size distribution of $pSDSC_4$ conjugated AuNPs measured by DLS **(A1)** in the absence of creatinine **(A2)** in the presence of 10 nM creatinine and **(A3)** 10 µM creatinine, and In TEM analysis **(A1)** in the absence of creatinine **(A2)** in the presence of 10 nM creatinine and **(A3)** 10 µM creatinine.

To evaluate the aggregation of $pSDSC_4$-AuNPs-creatinine, we examined $pSDSC_4$ attached gold nanoparticles using DLS technique. Dynamic light scattering (DLS) technique illustrates that before surface modification, the nanoparticles had an average hydrodynamic diameter of ~42 nm which maintain their size and stability. After ligand exchange, the $pSDSC_4$ coating on AuNPs average hydrodynamic diameter increased to ~48 nm (Fig.3 A1). In contrast, upon addition of different concentration of creatinine (10 nM, 10 µM), gold nanoparticles agglomerated and size increased to (~139 nm,~967 nm) respectively (Fig.3 A2-A3). Thus, $pSDSC_4$ conjugated AuNPs based DLS assay shows that, at a lower concentration of creatinine only smaller aggregates are formed and at higher concentration of creatinine molecule shows bigger aggregation. To appraise the aggregation of $pSDSC_4$-AuNPs-creatinine further the TEM mesurements of the $pSDSC_4$-AuNPs assambly was carriedout.

The TEM micrograph for the typical sample resultant from $pSDSC_4$ - AuNPs were discrete completely with a mean diameter of ~48 nm (Fig 3 A1). But after addition of different concentration (10nM, 10µM) of creatinine into $pSDSC_4$-AuNPs, TEM measurements indicates aggregate formation of $pSDSC_4$-AuNPs due to electrostatic interaction of $pSDSC_4$ with creatinine (Fig.3 A2-A3).

3.2 pH study:

It was perceived that $pSDSC_4$ - AuNPs exhibited stability within the range of pH of 2 to 4 for weeks to months with no sign of further aggregation. Furthermore, $pSDSC_4$-AuNPs assembly displays a high stability at room temperature with no sign of aggregation. The optimization o pH for the detection of creatinine was found to be pH-4 a shown in Fig.4 and hence all studies were carried out at pH 4 using sodium acetate-acetic acid buffer.

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

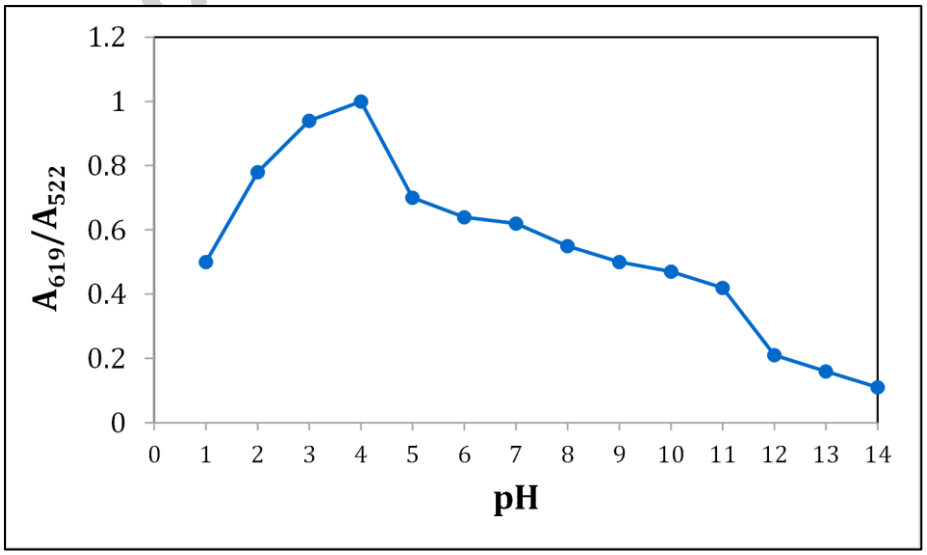


Figure 4: Shows the plot after addition of creatinine 30 nM into $pSDSC_4$-AuNPs at different pH conditions.

3.3 Mechanisam of interaction:

Table 1: Stability of pSDSC_4-AuNPs assembly at different pH conditions

Supramoleular organization such as calix[4]arene are ideal framework for molecular recognition because of their conformational and structural flexibility and ability of being modified at both the upper and lower rims. Introduction of moieties containing O, N and S on the calixarene brings the selectivity toward transition metal ions[20]. Appropriate functionalization can improve the solubility and extend its range of applications in the ion and molecular recognition properties of 1,3-disubstituted derivative of calix[4]arene. Thus,

when we investigated spectrophtotometrically, our observation indicated that the bathochromic shift is perceived during interaction of the $pSDSC_4$-AuNPs with creatinine which is due to existence manifold binding site of calixarene complexing with creatinine, as creatinine has three different tautomeric forms with similar energy that can bind to other molecules through hydrogen bond donor–acceptor arrays[36]. However, due to the negative groups of $pSDSC_4$ which adsorb on the surface of AuNPs the SO^{3-} groups provide the

hydrogen binding site for the creatinine. The $pSDSC_4$-AuNPs were easily aggregated upon addition of creatinine via hydrogen bond formation between - NH group of creatinine and– SO^{3-} groups of $pSDSC_4$ assisted by electrostatic interaction, resulting color change from red to violet that is used as a simple and selective method for determination of creatinine (Fig. 5)[36]. The proposed mechanism of recognition of creatinine via hydrogen bonding and electrostatic interaction by $pSDSC_4$- AuNPs is shown in Fig.5.

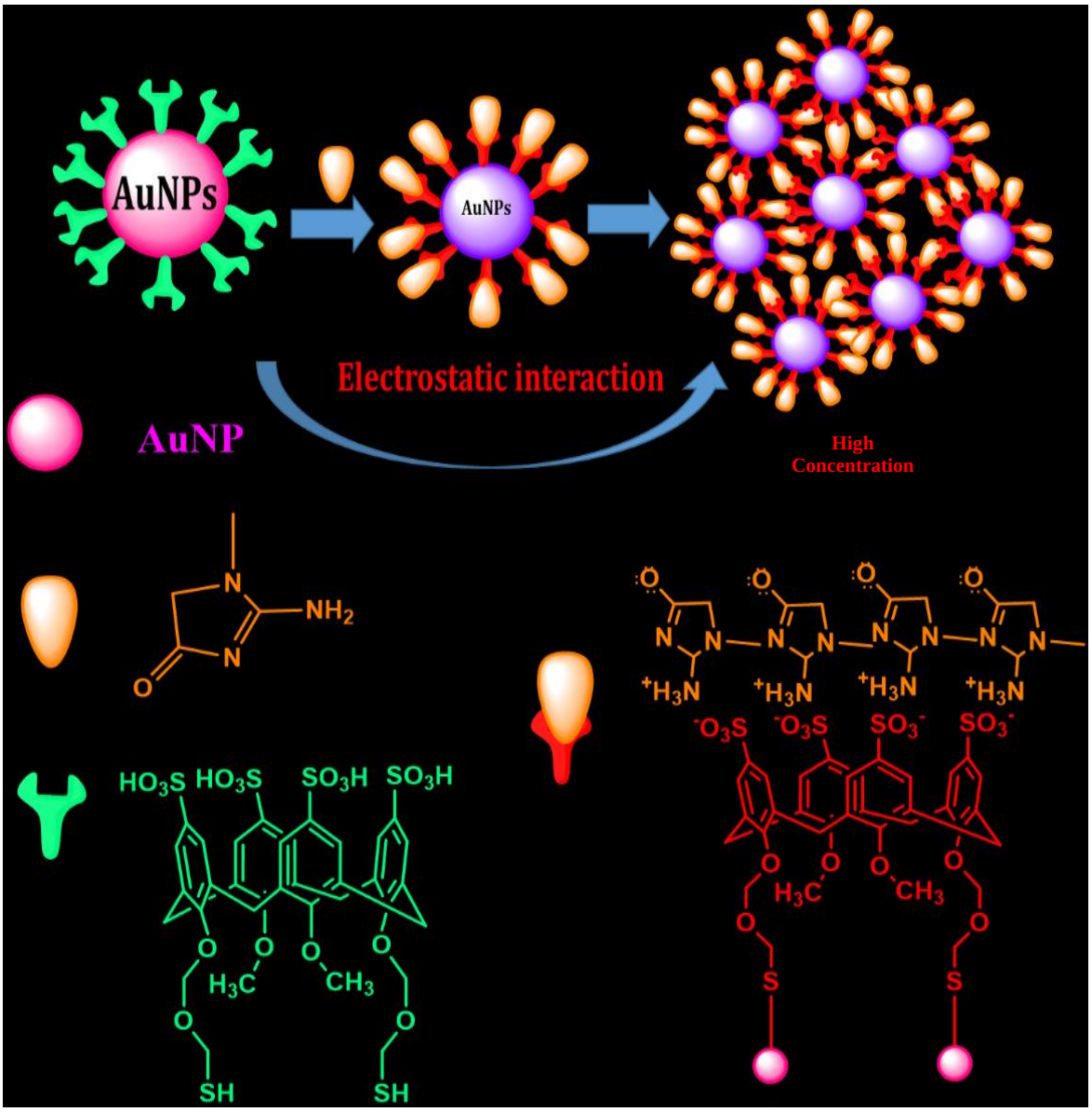


Figure 5 Schematics of the creatinine molecule sensing with $pSDSC_4$ modified AuNPs via nanoaggregation due to hydrogen bonding and electrostatic interaction with creatinine and $pSDSC_4$.

3.4 Selectivity:

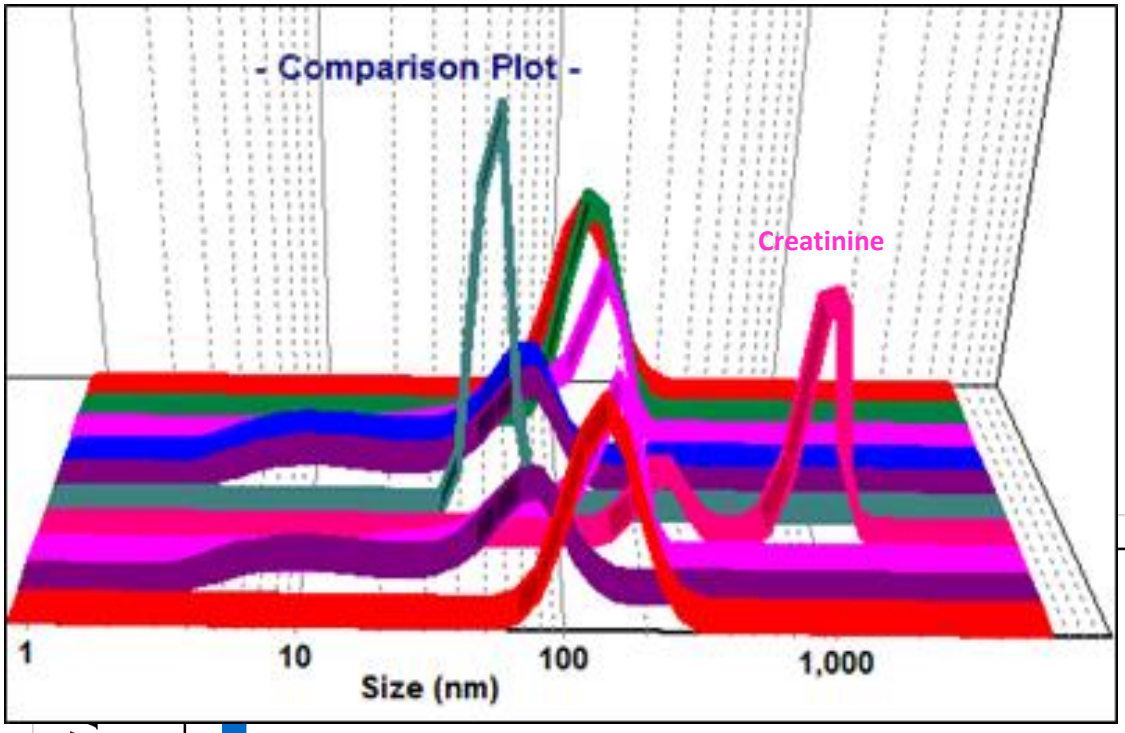


Figure 7 Size distribution of pSDSC4-AuNPs solutions after adding 10 μM of different biomolecules, from top to bottom urea, ascorbic acid, mannose, creatine, vitamine K2, cholesterol, creatinine, citric acid, glucose and fructose.

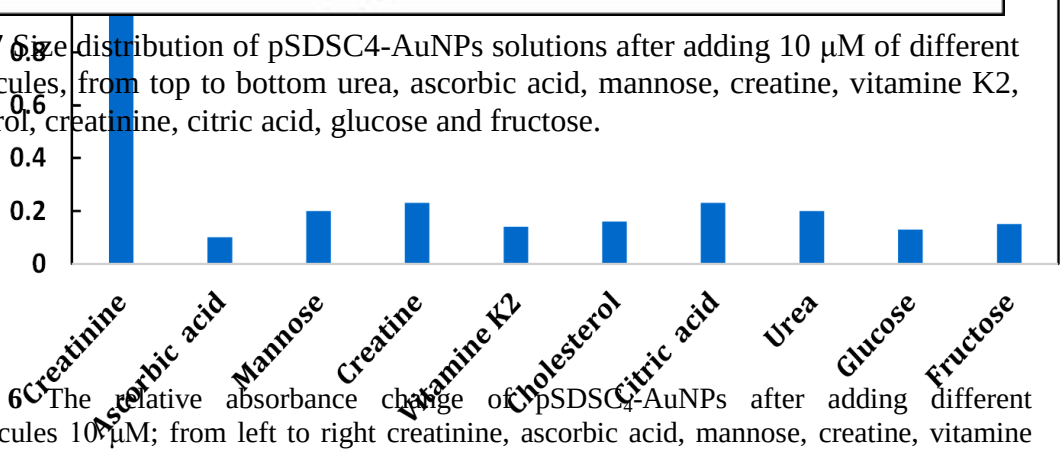


Figure 6 The relative absorbance change of pSDSC$_4$-AuNPs after adding different biomolecules 10 μM; from left to right creatinine, ascorbic acid, mannose, creatine, vitamine K2, cholesterol, citric acid, urea, glucose and fructose.

The selectivity of this assay toward creatinine was evaluated by absorption spectroscopy as well as dynamic light scattering (DLS) techniques with other closely related biomolecules such as, ascorbic acid, mannose, creatine, vitamine K2, cholesterol, citric acid, urea, glucose and fructose (10 μM) in the presence of 0.24 mM pSDSC$_4$-AuNPs. The experimental results were shown in (Fig. 6 & Fig. 7). It was clear that only creatinine showed a remarkable higher absorption ratio (A_{619}/A_{522}), confirming the excellent selectivity of the proposed assay to creatinine. As shown in Fig. 7, the size of pSDSC$_4$-AuNPs is the largest after treating with creatinine (10 μM), which indicates that pSDSC$_4$-AuNPs have a good

selectivity towards creatinine over the above mentioned biomolecules. The DLS analysis shows more sensitivity and selectivity at lower concentration than at high concentration.

3.5 Mass spectra:

The binding of creatinine with pSDSC$_4$-AuNPs was further confirmed by ESI-MS spectroscopy. Fig. 8 shows the ESI-MS spectra of the mixtures of pSDSC$_4$-AuNPs and creatinine with pSDSC$_4$-AuNPs in aqueous solution. The spectra of pSDSC$_4$-AuNPs showed peak at m/z = 1118 (Fig.8A). When the excess of creatinine was mixed with pSDSC$_4$-AuNPs in aqueous solution, pSDSC$_4$-AuNPs - creatinine peak at m/z =1235.8 was clearly detected (Fig.8B), which indeed suggest the formation of a 1:1 complex as the main species.

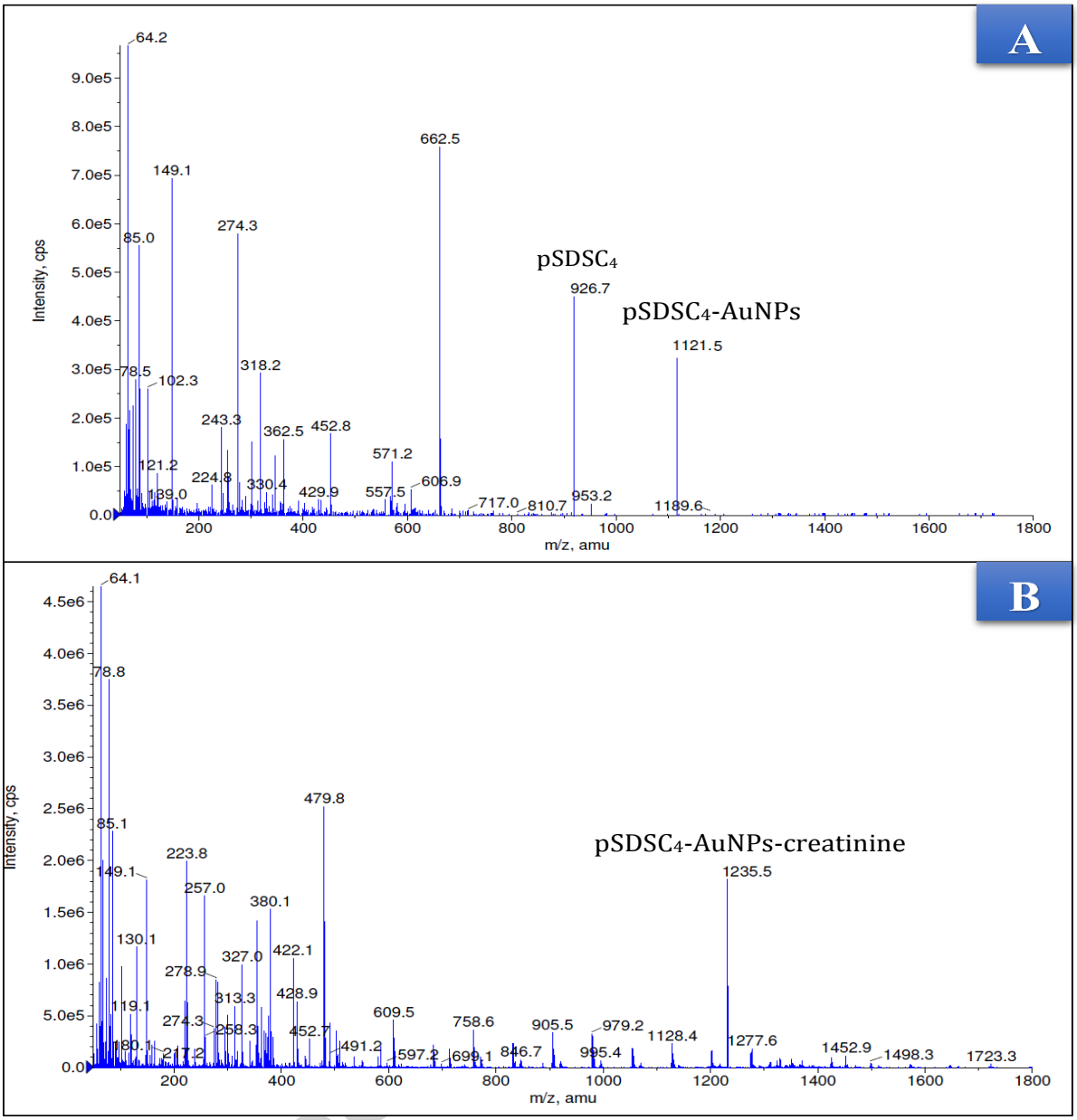


Figure 8: ESI-MS spectra of the mixtures of **(A)** pSDSC$_4$-AuNPs **(B)** pSDSC$_4$-AuNPs – creatinine.

3.6 Real Sample Analysis:

To check the viability of the pSDSC$_4$-AuNPs based creatinine biosensor in practical analysis, it was employed to measure creatinine in human urine samples. Fresh human urine samples were collected from local hospital and analyzed by the developed pSDSC$_4$-AuNPs based creatinine method. In our work, the spiked urine samples were extracted into the ligand as disscused under experimental section and analyzed by the present method. The results are

Sample	Added Creatinine (nM)	Found Creatinine (nM)	Recovery (%)
Urine sample 1	0	6.5	---------
Urine sample 2	10	15.9	96.36 ± 3
Urine sample 3	20	26.1	98.49 ± 2
Urine sample 4	30	40.1	99.45 ± 5
Urine sample 5	100	106.4	99.90 ± 2

Table 2 Determination and recovery of creatinine in human urine samples using the pSDSC$_4$-AuNPs creatinine probe.

shown in Table 2, with quantitative recovery up to 99.9%. The analysis and recovery test results of calix capped gold nanoparticles exhibit that the creatinine biosensor offers an excellent, accurate, fast and precise method for determination of creatinine in human urine samples. This novel method for the recognition and determination of creatinine is compared with some of the previously reported methods (Table 3) and it is evidently seen that this novel developed method for the recognition and determination of creatinine is far better in terms of linearity range and limit of detection.

Method	Linear range	Limit of detection	Ref.
Jaffé-based procedure	0–6 mM	0.72 mM	37
Portable miniature fiber optic spectrometer	0 to 40 mgL^{-1}	3.3 mgL^{-1}	38
Multichannel kinetic spectrophotometry	0–250 mg/L	0.76 mg/L	39
Capillary zone electrophoresis	0.2–32 mM	0.05 mM	40
Electroanalytical method	1 to 80 µM	380 nM	41
Conductometric biosensor	1×10^{-1} and 9×10^{-6} M	2×10^{-6} M	42
Citrate-stabilized Gold nanoparticles	15-40 mg/L	13.7 mg/L	43
Citrate-stabilized Gold nanoparticles	0.1 to 20 mM	80 µM	44
glutathione (GSH)-protected gold nanoparticles	10-1000 µM	1.21 µM	45
2,2′-thiodiacetic acid	0.01 to 1.0 µM	3.0 nM	46

functionalized silver nanoparticles			
Present method	10 nM- 100 nM	2.16 nM	-----

Table 3: Comparison of $pSDSC_4$-AuNPs chemosensor method with various reported methods for determination of creatinine.

4 Conclusion

In this study, we have proposed a novel approach for stable and highly sensitive creatinine biosensor which has been successfully made-up using $pSDSC_4$-AuNPs. Plasmon absorption peak was red-shifted from 522 nm to 619nm and good correlation was obtained between the wavelength shift and concentration pointing out the feasibility of the method for the quantitative measurement of creatinine with lower detection limit of 2.16 nM. It is easy to operate and most importantly, it exhibits fast response time to creatinine and has long shelf-life (>5 weeks). The developed $pSDSC_4$-AuNPs based creatinine biosensor has been proved to be a simple, reliable and accurate tool for the determination of creatinine in human urine samples.

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Graphical abstract

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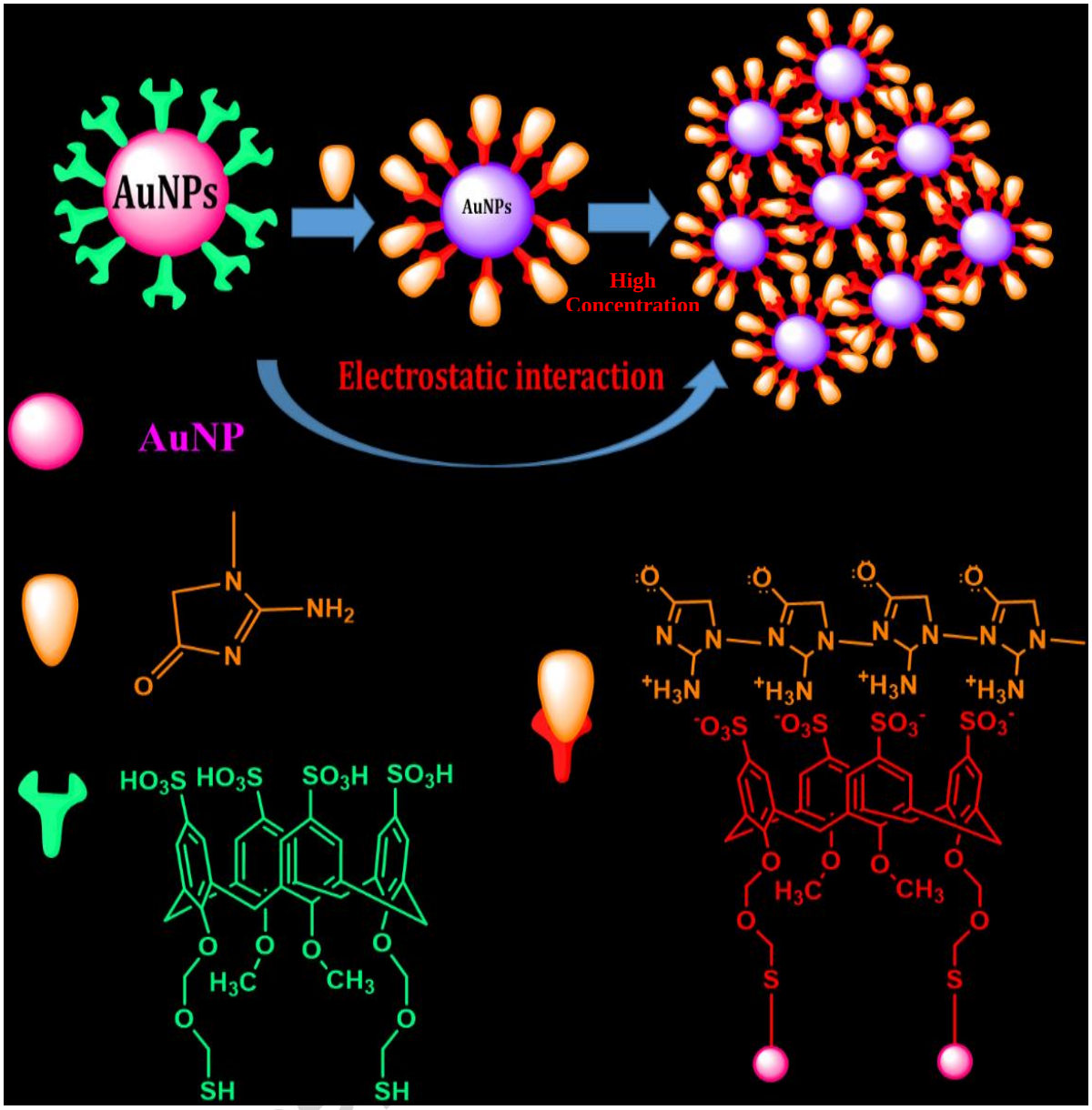

Research Highlights

- A well-designed colorimetric sensor has been constructed for creatinine,
- The strategy has been used for visual detection of creatinine in urine
- Calix[4]arene functionalized gold nanoparticles (AuNPs) based on assembly
- The proposed sensing platform is characterized by rapidness and high throughput
- Developed sensor was characterized by using TEM, DLS, UV-Vis, FT-IR and ESI-MS