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ARTICLE

An organic-inorganic nanohybrid of calix[4]arene based chromogenic chemosensor for simultaneous estimation of ADP and NADH

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The versatility in environmental and biological applications of nanohybrids encouraged us for the preparation of a novel chemosensor based on organic-inorganic nanohybrid (**H1**) employing receptor **1 (R1**), which was synthesized by schiff's base condensation reaction of a calix[4]arene derivative and an aliphatic amine. The techniques such as DLS, TEM were employed for the characterization of organic nanoparticles (**N1**) and **H1**. Further, sensor properties of **H1** were explored towards various biologically important molecules in aqueous media using UV-Visible Spectroscopy. The proposed sensor responded effectively for selective and simultaneous nanomolar determination of adenosine diphosphate (ADP) and reduced nicotiniamide adenine dinucleotide (NADH) and the response was not affected by the presence of each other or any other potentially interfering biomolecule or high concentration of salt. The proposed sensor is also found to show a stable response in an extensive pH range thus widening its practical applicability. **H1** was able to detect a minimum concentration (detection limit) of 6.11×10^{-9} M of ADP and 4.87×10^{-9} M of NADH. The prepared hybrid was subjected to artificially prepared real sample analysis for determination of ADP and NADH in samples prepared artificially by adding known concentration of NADH and ADP in solution and also in mixture of both.

INTRODUCTION

In recent years, sensors for quantitative recognition of various biomolecules (amino acids, DNA etc.) increasingly attract much more interest of researchers due to their imperative physiological functions and numerous applications in medical diagnostics, anti-bioterrorism and food safety.[1] The standard amount of biomolecules is essential for organisms, while the amount too high or too low will lead to certain diseases. For example, adenosine diphosphate (ADP) in the form of ADP-ribose is known to play important role in different cellular processes such as extracellular cell signaling, DNA repair,[2] gene regulation and apoptosis,[3] ADP also plays an imperative function in normal hemostasis and thrombosis, ADP has a crucial role to play in enzymes functioning as bacterial toxins and metabolic regulators,[4] nicotinamide adenine dinucleotide (NAD) play a part in regulation of energy metabolism, DNA repair and transcription,[5] and in maintaining cellular redox homeostasis, the redox couple NAD^+/NADH represents a very important system, being a substrate for a number of dehydrogenase, hydroxylases and oxidoreductase enzymes in natural biological systems.[6] Aberrant level of various biomolecules are known to cause many diseases such as hypoglycemia, hypoxia, ischemia, Parkinson's disease,[7] renal diabetes, cystic fibrosis, diabetes.[8] Change in the level of few of the biomolecules such as NAD/NADH, adenine, thymine and guanine is closely related to many diseases like cancer, AIDS, epilepsy and lupis erythematosus.[9-11] Therefore, it is necessary to develop highly sensitive, prompt, dependable and expedient method for the detection of biomolecules for various biochemical studies and clinical diagnosis. Detection of these substances require complicated separation and purification steps, as well as elaborated sample preparation, large costly instruments and skilled labor.[12] Number of artificially prepared receptors employing various organic units such as benzimidazole, urea, thio-urea, and calixarene moiety have already been reported in literature.[13-14] With-in this context, the development of receptors for multi-analyte detection in real samples is still a challenge for researchers. A few chromogenic and fluorescent sensors for the detection of more than one analyte simultaneously are reported in literature. Such receptors are designed and synthesized either by inserting multi-chromogenic moieties in a single receptor which necessitate tedious synthesis steps or employing several detection techniques such as UV-visible, electrochemical and fluorescence detection method.[15-16] Lately, a shift from

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designing and fabricating a selective receptor to differential receptor has facilitated the recognition of more than one analyte concurrently using one chromophore. Various colorimetric detection techniques employing nanomaterials (nanoparticles, nanorods, quantum dots) have been used in order to get quick, precise, and cost-effective response in samples of biological and environmental origin these days.[17] Nanomaterials, particularly hybrid nanostructures are massively employed these days to meet high-end requirements such as inexpensive, efficient system having high sensitivity and selectivity towards particular ion or molecule. Metallic nanoparticles, particularly, gold nanoparticles (AuNPs) are one of the most explored nanostructures lately owing to its high biocompatibility, conductivity, fluorescent quenching and excellent plasmonic coupling. These exhibit distinctive features such as localized surface Plasmon resonance (LSPR)[18] and a very high molar extinction coefficient. As per Beer-Lambert Law, these show the possibility of reaching a much lower limit of detection (LOD). AuNPs are employed these days for the detection of various biomolecules owing to their comparable sizes. Quantum dots is another class of nanomaterial possessing quantum confined charge carriers, emitting fluorescence with a high quantum yield and possessing remarkable electro-chemiluminescent properties.[19] These are known to conjugate with biomolecules (proteins and DNA) with high sensitivity. Organic nanoparticles (ONPs) are a recently explored class of soft matter which finds application in sensitive and selective chemo-sensing. So, herein, we develop a simple, prompt and responsive colorimetric chemosensor for the estimation of ADP and NADH simultaneously by employing a calix[4]arene derivative and using the interparticle plasmon coupling induced by the aggregation of AuNPs due to binding with a particular analyte.[20-21] Receptor **1** based on calix[4]arene molecule substituted at 1,3-alternate positions on the lower rim is designed in such a manner so as to devise a molecule having tunable cavity size. Such a molecule is bound to have two different cavities competent of accommodating two analytes simultaneously. Because of the presence of the podand arms at the lower rim, the cavity of the calix[4]arene molecule becomes more rigid and is therefore able to selectively and promptly bind a particular analyte of biological and environmental importance. Also, the substituents at the lower rim bear various binding sites which proficiently bind specific analyte. We report development of organic-inorganic hybrid (**H1**) based chemosensor using receptor **1** (R1) for the simultaneous detection of ADP and NADH in an aqueous medium having no interference from any other potentially interferent biomolecules. R**1** was obtained by Schiff's base condensation reaction of 3-(dimethylamino)-1-propylamine and 1, 3-disubstituted *p*-tert butyl calix[4]arene.

RESULTS AND DISCUSSION

Synthesis and characterization of sensor

Synthesis of the R**1** was carried out by reacting 3-(dimethylamino)-1-propylamine with calix[4]arene-based dipodal aldehyde **1** in acetonitrile at room temperature using previously reported literature method[22] (**Scheme 1[SI]**), which in turn was synthesized by reacting *p-tert*-butyl calix[4]arene and 2-(2-bromoethoxy)benzaldehyde in acetonitrile as depicted in scheme 1. The R**1** (**Figure 1**) thus formed was white in color, having a yield of 70% and was characterized using elemental analysis.

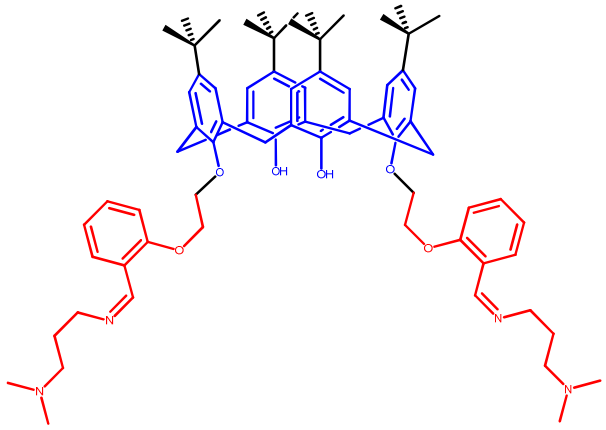

Figure 1: Design of Receptor **1**

Fabrication of Organic nanoparticles (N1) of receptor 1

Organic nanoparticles (ONPs) are fine suspensions of solid particles composed of organic compound in aqueous medium ranging in diameter from 10 nm to 1 μm. Because of their optical properties[23] and competency to generate intense electromagnetic fields, these particles have imperious relevance in various fields such as conducting materials, sensors,[24] medicine,[25] biotechnology and innumerable other applications like catalysis, diagnostics[26] and photo-thermal therapeutics. Of all the procedures known in literature for the fabrication of ONPs, single step re-precipitation technique is usually followed because it is economical and can be easily reproduced. Keeping that in mind, organic nanoparticles (**N1**) of R**1** were obtained by employing single-step re-precipitation method. For this, 1 mL (0.6 μM) of the compound dissolved in pure tetrahydrofuran (THF) was injected manually into deionised water (100 mL) taken in 250 mL beaker, using a micro syringe at a constant rate under sonication for atleast half an hour. The change in photophysical profile of R**1** was studied using UV-Visible and Fluorescence Spectroscopy (Figure 2a and 2b).

Organic ligand dissolved in pure THF shows low absorption peaks in UV-visible spectra. As the amount of water was increased, ONP formation occurred and the solution became slightly cloudy, indicating uniform distribution of nanoparticles in aqueous medium. *p-tert*-butyl calix[4]arene molecule in $CHCl_3$ is known to show appreciable absorption bands at 230 and 280 nm due to n-π* transitions and these absorption bands are expected to shift towards longer wavelengths as polarity of the solvent is increased.[27] The UV-Visible profile of

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N1 shows peaks at 286 nm and 317 nm attributed to n-π* transitions, while emission profile showed peaks at 303 nm and 372 nm. Both the spectral profiles showed marked enhancement in the peak intensity which can now be used to estimate the changes in photophysical profile of the **R1**.

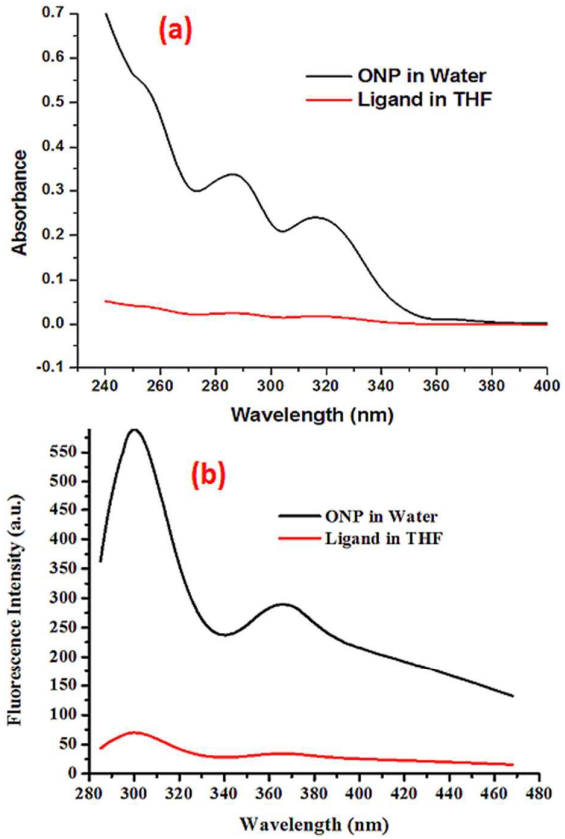

Figure 2: (a) UV-Visible, and (b) Fluorescence Spectra of Ligand in THF and ONPs in water showing noticeable enhancement in peak intensity as ONPs are fabricated.

Analysis of **N1** particle size was confirmed by TEM (Transmission Electron Microscopy) which showed the development of nanoparticles, spherical in shape having a size of 12 nm (Figure 5a). The concentration of **R1** for the fabrication of ONPs was required to be optimized by varying the injected amount of **R1** in 100 ml of distilled water in order to achieve more intense peaks both in UV-visible and Fluorescence spectra. It is quite evidently perceived from the results (Figure 3a & 3b) using both UV-visible spectroscopy and fluorescence spectroscopy that as the amount of **R1** was increased from 0.2 μM to 0.6 μM the intensity of the peaks increased. On further increasing the amount of **R1** the intensity of the peaks decreased considerably which is ascribed to increase in particle size leading to agglomeration. The same has been confirmed using DLS studies (Figure S1). In order to explore the effect of amount of water on the photophysical profile of **R1**, various compositions of THF:water were examined by UV-Visible and fluorimetric profiles of the same (Figure 4a and 4b).

It is quite evident that with the increasing concentration of water, the intensities of the peaks increased considerably and best peaks are obtained with composition of 1:99, THF: water. Therefore providing us with best suited media for formation of optimum sized ONPs. The optimum sized ONP were confirmed using TEM analysis and DLS (Dynamic Light Scattering) and had a size of 12 nm (Figure 5a and 5b).

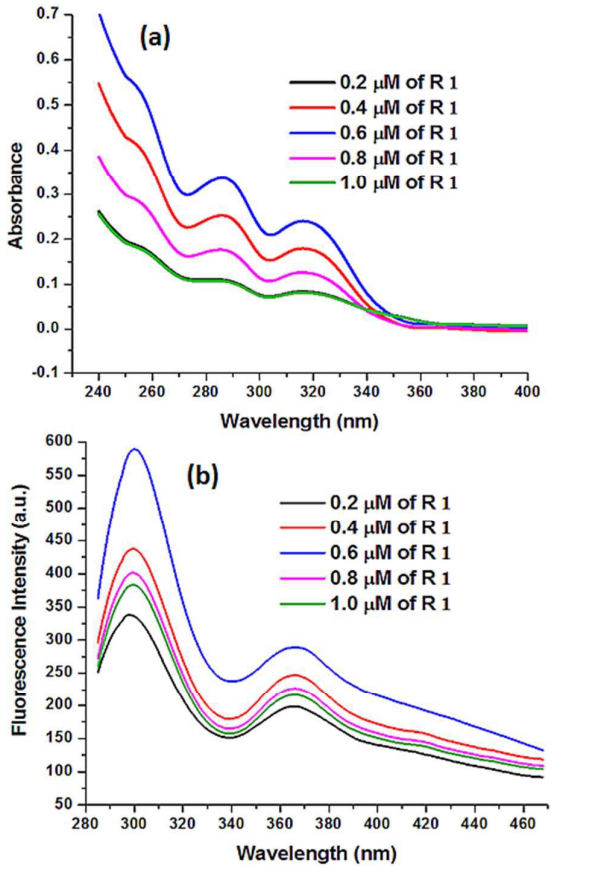

Figure 3: (a) UV-Visible Spectra, (b) Fluorescence Spectra of **N1** with increasing concentrations of **R1.**

Fabrication of organic-inorganic hybrid (H1)

Many different techniques have been developed to generate organic-inorganic hybrids (**H1)** using reduction of gold as gold nanoparticles (AuNPs) on surface of organic nanoparticles.[26,28-30] For this, stock solutions of $HAuCl_4$ (1.0×10^{-3} M), and ascorbic acid (1.0×10^{-3} M) were made using de-ionized water, and subsequent dilutions (100 times) were made from the stock solutions and were then tried in different ratios along with previously prepared **N1** (0.1 μM) to obtain **H1**. It was deduced that $HAuCl_4$, **N1** and ascorbic acid when mixed in the ratio of 9:1:9 leads to the formation of **H1.** Formation of **H1** from **N1** happened with dramatic change in color from colourless to pink (Figure S2). Both UV-Visible and fluorescence spectra (Figure 6a and 6b) confirmed the formation of **H1,** as a clear surface plasma resonance (SPR) band appeared in visible region at around 528 nm due to reduction of Au (III) to Au (0) over organic ligand, while the

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emission spectra demonstrated quenched emission bands of **N1** at 303 nm and 372 nm attributed to deactivation of the surface of **N1** by the immobilization of AuNPs over its surface. This was further confirmed using TEM (Transmission Electron Microscopy) which showed an appearance of black spots on the surface of **N1** having a size of 18-22 nm and also confirmed by DLS studies (Figure S3).

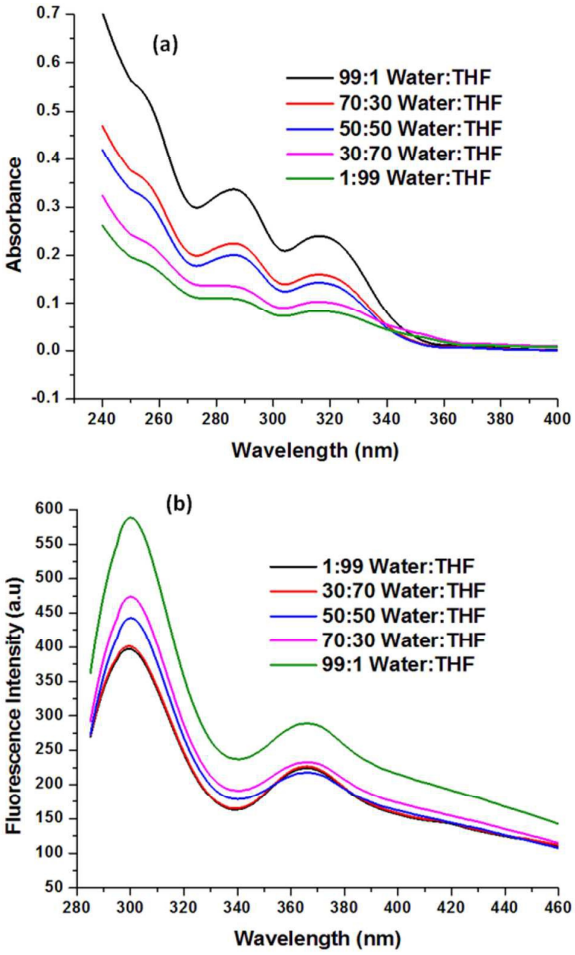

Figure 4: Effect of various composition of solvent (Water:THF) on (a) UV-Visible absorption (b) fluorescence spectra of **N1**.

It is quite evident from Figure 6 that the absorption band has clearly shifted from UV region to visible region, which clearly justified our decision to prepare inorganic-organic nanohybrids from organic nanoparticles for sensor applications.

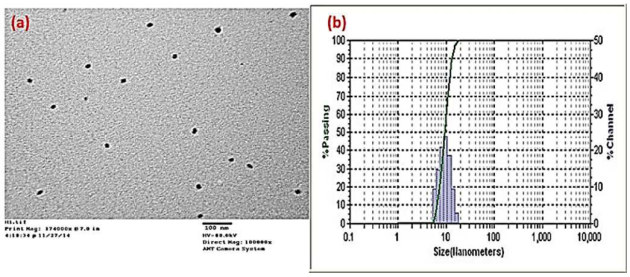

Figure 5: (a) TEM images of ONPs in water (**N1**), and (b) DLS histogram of **N1**

RECOGNITION STUDIES

Binding of biomolecules with organic-inorganic hybrids (H1)

In order to study the binding ability of the developed **R1** in the form of **H1** towards various biomolecules in an aqueous medium, solution of **H1** was taken in a volumetric flask (5 mL) and was subjected to 100 µL (5µM) solution of different biomolecules (NAD, NADH, NADP, AMP, ADP, ATP, Uracil, Adenine, Cytosine and Guanine) and their UV-visible spectra were noted. It was perceived that upon addition of biomolecules to the fixed concentration of **H1** (6µM), negligible change in UV-visible profile with all biomolecules except ADP and NADH (Figure 7).

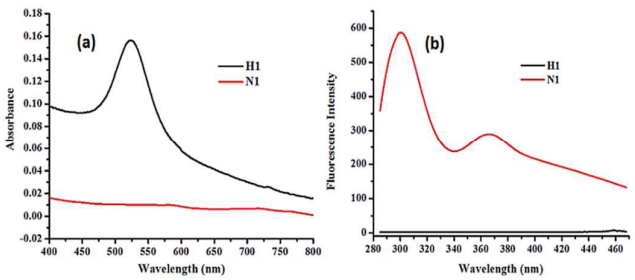

Figure 6: Comparison of (a) UV-Visible, and (b) Fluorescence Spectra of inorganic organic hybrid nanoparticles (**H1**) and organic nanoparticles (**N1**)

Interaction with NADH with **H1** showed a shoulder peak at 648 nm, whereas addition of ADP showed a new peak at 712 nm, along with decrease in absorbance peak at 528 nm. Binding of the receptor with anion leads to stabilisation of the excited state in comparison with the ground state. This leads to bathochromic shift in the absorption spectrum.[31] These spectroscopic changes may be assigned to the formation of a charge transfer complex. The observed bathochromic shift both in case of ADP and NADH may be attributed to intermolecular hydrogen bonding between receptor **1** in the form of **H1**and biomolecules. To authenticate the binding behaviour of **H1** with both ATP and NADH, titrations were performed by adding small aliquots of both separately in 10 mL solution of **H1 (**Figure 8a & 8b**)**.

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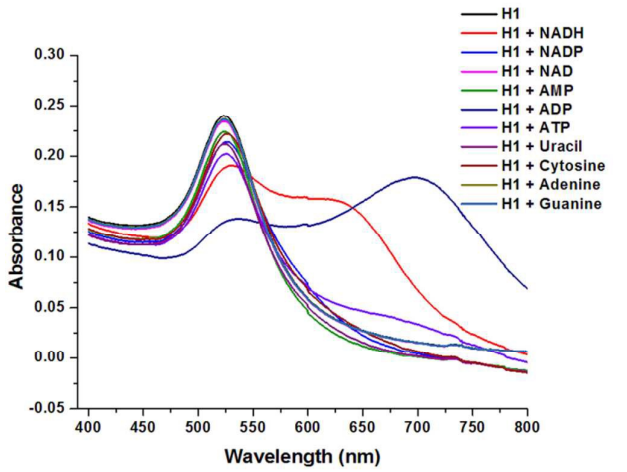
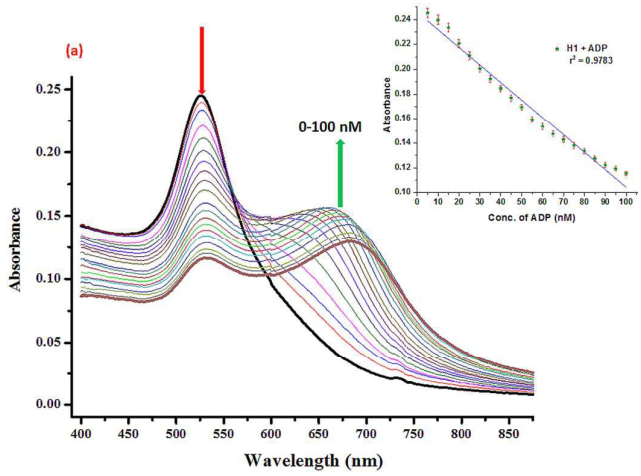


Figure 7: UV-Visible profile of **H1** in the presence of 5µM of various biomolecules

It is quite evident from the calibration curves that **H1** responds linearly for ADP and NADH with a linear dynamic range of 0-100 nM for ADP and 0-80 nM for NADH, having a regression coefficient of 0.9896 and 0.9783, respectively. **H1** was able to detect a minimum concentration (detection limit) of 6.11×10^{-9} M of ADP and 4.87×10^{-9} M of NADH. The lower detection limit was calculated by using standard IUPAC 3σ method.[32]



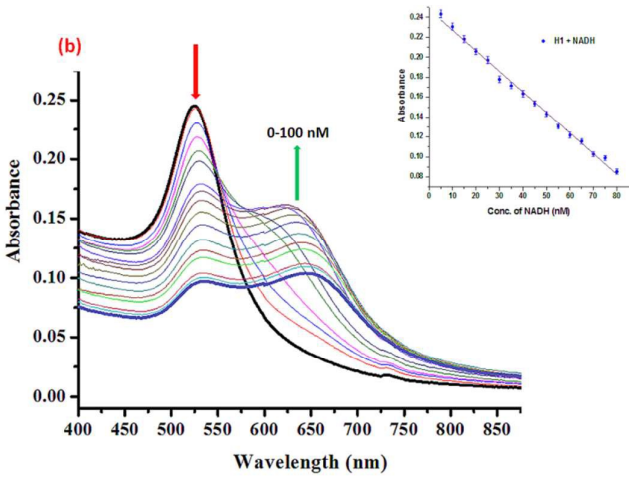


Figure 8: UV-visible Spectra of **H1** in the presence of increasing concentrations (a) (0-100 nM) of ADP, and (b) (0-80 nM) of NADH (Inset A: Calibration plot for ADP, Inset B: calibration plot for NADH)

Further, in order to project it as a sensor for simultaneous determination of ADP and NADH, simultaneous binding assay was performed (Figure S4 & S5). The experiment was performed by adding one component in excess as interferent and doing the titration with the second component as analyte of interest and calibration curves were plotted for both NADH and ADP, in presence of each other and compared with the calibration curve of NADH and ADP in absence of interferent. It is quite evident from the calibration plots (Figure S4 & S5) that calibration curve remains the same i.e. neither slope nor intensity changes on addition of one component as interferent. The differential behaviour of **H1** with NADH and ADP respectively may be ascribed to distinct binding sites in the molecule for both NADH and ADP. For a sensor to have practical application in real sample analysis beside selectivity, it should be able to work in complex environment like in the presence of various potential interferent, varying pH and salt concentration. In order to achieve this, interference studies were carried out and UV-vis spectra were noted. In 10 mL volumetric flasks, **H1** containing 0.1 µM of ADP and NADH respectively, were subjected to various other biomolecules (NADP, NAD, AMP, ATP, Uracil, Adenine, Guanine, and Cytosine) and their UV-vis profiles were investigated (Figure 9).

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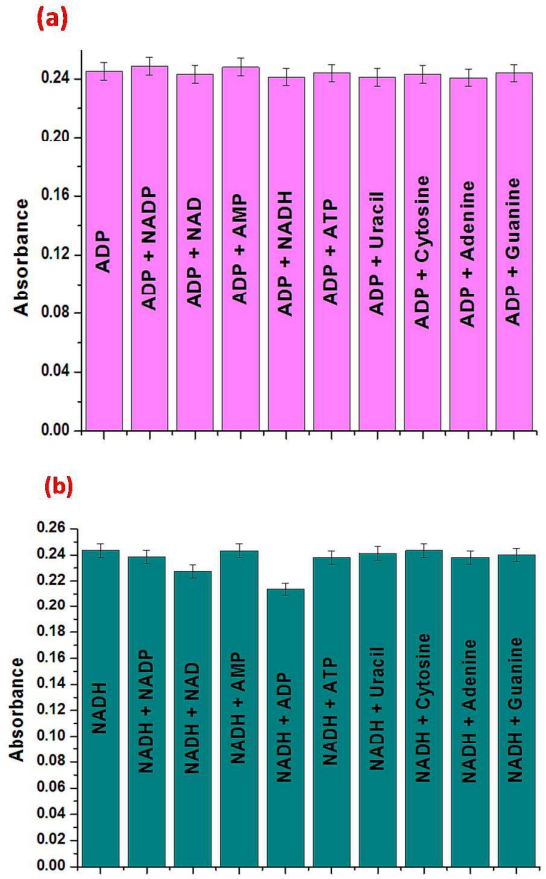

Figure 9: UV-vis absorption spectra showing negligible interference due to different biomolecules in the recognition behaviour of H1 towards (a) ADP, and (b) NADH respectively.

As can be clearly seen from the UV-vis spectra, none of the biomolecules pose any interference to the recognition behaviour of the receptor in the form of **H1.** The effect of varying pH on the recognition behaviour of **H1** and UV-vis spectrum is recorded (Figure S6). It is quite apparent that UV-visible profile of **H1** remains unaltered in wide pH range of 2 to 12. Hence, the proposed sensor is stable in this pH range and can be applied for use in samples of biological and environmental utility without losing its sensitivity or selectivity. In order to investigate the salt effect on recognition behaviour of **H1**, tetrabutylammonium perchlorate salt is added in increasing amount (1-100 equiv.) to **H1** taken in 5 mL volumetric flask and UV-Visible spectra were recorded (Figure S7). It was perceived that even the presence of 100 equivalents of salt does not alter the response of **H1**. Hence, it can be said that prepared nanohybrid complex (**H1**) can be used for real sample analysis of NADH and ADP in samples of biological and environmental importance.

Spiked sample analysis

The prepared hybrid was scrutinized using spiked samples (A1-A4) of NADH. A1 and A2 were made by dissolving ADP and NADH, respectively. A3 and A4 were made by dissolving both NADH and ADP in same solution for knowing the workability of **H1** in simultaneous estimation of NADH and ADP. The results thus obtained are given in Table 1:

Table1: Analysis of artificially made samples of NADH and ADP using **H1**

S.No	Sample Name	Added Conc. (nM)	Recovered Conc. (nM)	Percentage Recovery
1	A1(ADP)	4.5	4.46 ± 0.03	99.1%
2	A2(NADH)	3.7	3.64 ± 0.04	98.38%
3	A3(ADP + NADH)	12.9 6.8	12.73 ±0.06, 6.63 ± 0.04	98.68% 97.5%
4	A4 (ADP + NADH)	7.6 14.8	7.49 ± 0.03 14.48 ± 0.07	98.55% 97.84%

It is quite evident from Table 1 that **H1** can be used for real sample analysis of various spiked samples of analytical importance for NADH and ADP with accuracy of more than 95%. The hybrid can also be successfully employed for simultaneous estimation NADH and ADP, without any lose in its selectivity or sensitivity.

CONCLUSIONS

Receptor **R1** was synthesized by Schiff's base condensation reaction of a calix[4]arene-based dipodal aldehyde and 3-(dimethylamino)-1-propylamine. The receptor was then subjected to organic nanoparticles (**N1**) and further to organic-inorganic nanohybrids (**H1**). Sensor activities of **H1** were investigated by subjecting it to various biomolecules in aqueous media using UV-visible spectroscopy. It was perceived that the receptor worked well for the simultaneous determination of ADP and NADH even in presence of each other as interferent molecules. Proposed sensor is stable in a wide pH range, hence could be used for simultaneous determination of above mentioned biomolecules in aqueous medium for various environmental and biologically important samples.

EXPERIMENTAL

Materials and Methods

All reagents *viz*. *p*-hydroxybenzaldehyde, 1, 2-dibromoethane, *p*-tert butyl phenol, formaldehyde (37%), N, N-dimethylpropane-1, 3-diamine, K_2CO_3, NaOH (analytical grade) were purchased from Loba Chemie and used without further purification. Solvents such as diphenyl ether, ethyl acetate, acetic acid were procured from SD Fine and were used as such, whereas acetonitrile (HPLC Grade) was obtained from Fisher Scientific which was further dried by distillation method before use. All biomolecules *viz.*ADP, NADP, NADH, NAD, ATP, AMP, cytosine, Adenine, Guanine and uracil were obtained from Sigma Aldrich. De-ionized bi-distilled water was used for

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preparation of organic-inorganic nanohybrids and solutions of all biomolecules.

UV-Visible absorption spectra were recorded on Spectroscan 30 spectrophotometer from Biotech Engineering Management Co. Ltd. (UK). The fluorescence measurements were performed on RF-5301 PC spectrofluorophotometer from Shimadzu. The particle sizes and size distribution of the ONPs and AuNPs were determined on Metrohm Microtac Ultra Nanotrac Particle Size Analyzer (dynamic light scattering). Transmission electron micrographs (TEM) were noted on Hitachi (H-7500) instrument working at 120 kV having a resolution of 0.36 nm (point to point) with 40-120 kV operating voltage. For sample preparation, a carbon-coated copper grid (400-mesh) was used. Elemental analysis was carried out on Fisons instrument (Model EA 1108 CHNO).

Synthesis of receptor 1

Calix[4]arene-based dipodal receptor (I) was synthesized as depicted in Scheme 1. The calix[4]arene-based dipodal aldehyde 1 was prepared by the reported method[22] from *p*-tert butyl calix[4]arene and 2-(2-bromoethoxy) benzaldehyde (Scheme 1). Receptor **1** was having a yield of 70%, prepared by the schiff's base condensation reaction of 3-(dimethylamino)-1-propylamine and dipodal aldehyde 1. The receptor **1** thus obtained was white in color having a yield of 70% and was then characterized by elemental analysis. Expected percentage: C = 77.66, H = 8.69, N = 5.03, O = 8.62; Obtained percentage: C = 77.89, H = 8.46, N = 5.16, O = 8.54.

Synthesis of organic nanoparticles (N1) of receptor 1

Organic nanoparticles of receptor **1** (**N1**) were primed using reprecipitation method.1 mL of stock solution of **1** (in tetrahydrofuran) was injected with a micro syringe at a steady rate into 100 mL de-ionized water under vigorous stirring. The solution thus obtained was sonicated for half an hour at constant temperature to ensure the formation of **N1**. Size distribution of **N1** formed were scrutinized using particle size analyzer Dynamic Light Scattering (DLS) technique.

Synthesis of organic-inorganic hybrids (H1)

Organic-inorganic nanohybrids (**H1**) were prepared by mixing **N1** in a particular ratio with reduction product of $HAuCl_4$ using ascorbic acid. For this, 250 mL aqueous solutions of $HAuCl_4$ (1 mM) and ascorbic acid (1 mM) were prepared using de-ionized water from stock solutions of $HAuCl_4$ (10.0×10^{-3} M) and ascorbic acid (10.0×10^{-3} M). The solutions were equilibrated at ambient temperature for 2 hours and were then mixed along with **N1** to obtain **H1**. Appearance of pink color in the solution confirmed the formation of **H1.** There are various factors which control the size distribution of the hybrid nanoparticles in the solution such as ratio of the three components i.e. gold, ascorbic acid and the nanoparticles, the order in which the reagents are added and physical conditions such as temperature. The methodology we have followed here is prompt and simple yielding reproducible results and mono-dispersed nanoparticles.

Recognition studies

The UV-Visible Spectroscopy technique was used for the recognition studies of prepared **H1**. A solution of **H1** taken in 5 mL volumetric flask and100 μL various biomolecules (5μM) (NADH, NADP, NAD, AMP, ATP, ADP, Uracil, Cytosine, Adenine and Guanine) were added into it, volumetric flasks were shaken well and equilibrated for half an hour at temperature 25 ± 1 °C before recording the spectrum. To confirm the binding, titrations were carried out with NADH and ADP for which small aliquots of both the biomolecules were added into 10 mL **H1** taken separate volumetric flasks. In order to evaluate interference due to the presence of other biomolecules, interference studies were carried out. For this, UV-vis spectra were recorded for solutions of **H1** containing NADH and ADP, respectively without and with different biomolecules. In order to propose the receptor for simultaneous determination of ADP and NADH without having any interference due to each other when present in excess amount, the experiment was performed in which one analyte was present in excess in a given volume of **H1** and titration was performed with the another analyte. The results showed no discernible interference of one analyte in the detection of other. In addition, effect of high concentration of salt was studied by adding different concentrations of tetrabutyl ammonium perchlorate salts (0-100 equiv.) Experiments showing the response time were also carried out by recording the spectra of **H1** at various concentrations with respect to time. The pH titration was also performed, so as to investigate the consequence that varying pH may bring to the performance of **H1**.

Spiked sample analysis

To extend the use of **H1** as sensor and for real sample analysis, the solutions of known concentrations (A1-A4) of NADH and ATP were made and scrutinized using the prepared **H1.**

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REFERENCES

1. S. Song, Y. Qin, Y. He, Q. Huang, C. Fan and H.-Y. Chen, *Chem. Soc. Rev.*, 2010, **39**, 4234-4243.
2. C. M. Nicolae, E. R. Aho, K. N. Choe, D. Constantin, H.-J. Hu, D. Lee, K. Myung and G.-L. Moldovan, *Nucleic Acids Res.*, doi:10.1093/nar/gkv147.
3. S. Kunzelmann and M. R. Webb, *ACS Chem. Biol.,* 2010, **5(4)**, 415-425.

View Article Online
DOI: 10.1039/C5RA18003A

4. F. Huang, G. Hao, F. Wu and G. Feng, *Analyst*, 2015, **140**, 5873-5876.
5. S. T. Nam, J. H. Hwang, D. H. Kim, M. J. Park, I. H. Lee, H. J. Nam, J. K. Kang, S. K. Kim, J. S. Hwang, H. K. Chung, M. Shong, C.-H. Lee and H. Kim, *BMB Rep*., 2014, **47(9)**, 494-499.
6. F. Sun, C. Dai, J. Xie and X. Hu, *PLoS ONE*, 2012, doi: 10.1371/journal.pone.0034525
7. P. Hu, S. Yang and G. Feng, *Org. Biomol. Chem*., 2014, **12**, 3701-3706.
8. L. H. Feng, Y. Wang, F. Liang, X. J. Wang and L. W. Zhang, *Sens. Actuat. B.*, 2011, **156**, 499–503.
9. P. Cekan and S. T. Sigurdsson, *J. Am. Chem. Soc.*, 2009, **131**, 18054–18056.
10. F. Xiao, F. Zhao, J. Li, L. Liu and B. Zeng, *Electrochim. Acta.*, 2008, **53**, 7781–7788.
11. F. Q. Yang, J. Guan and S. P. Li, *Talanta,* 2007, **73**, 269–273.
12. W. W. Chen, Y. M. Guo, W. S. Zheng, Y. L. Xianyu, W. Zhuo and X. Y. Jiang, *Chin. J. Anal. Chem*., 2014, **42,** 307–314.
13. P. Molina, A. Tarraga and F. Oton, *Org. Biomol. Chem*., 2012, **10**, 1711–1724.
14. P. A. Gale, N. Busschaert, C. J. E. Haynes, L. E. Karagiannidis and I. L. Kirby, *Chem. Soc. Rev.,* 2014, **43**, 205–241.
15. H. Komatsu, D, Citterio, Y. Fujiwara, K. Minamihashi, Y. Araki, M. Hagiwara and K. Suziki, *Org. Lett*., 2005, **7**, 2857-2859.
16. M. Schmittel and H. W. Lin, *Angew. Chem., Int. Ed*., 2007, **46**, 893-896.
17. K. Saha, S. S. Agasti, C. Kim, X. N. Li and V.M. Rotello, *Chem. Rev.,* 2012, **112**, 2739–2779.
18. X. Huang, and M. A. El-Sayed, *J. Adv. Res.,* 2010, **1**, 13-28.
19. Y. Shan, J.-J. Xu and H.-Y. Chen, *Chem. Commun*., 2010, **46**, 4187.
20. H. N. Kim, W. X. Ren, J. S. Kim and J. Yoon, *Chem. Soc. Rev.,* 2012, **41**, 3210–3244.
21. M. Saleem and K. H. Lee, *RSC Adv.*, 2015, **5**, 72150-72287.
22. N. Singh, M. Kumar and G. Hundal, *Tetrahedron*, 2004, **60**, 5393–5405
23. (a) A. Singh, S. Kaur, A. Kaur, T. Aree, N. Kaur, N. Singh and M. S. Bakshi, *ACS Sustainable Chem. Eng*., 2014, **2(4),** 982-990. (b) C. Sanchez, P. Belleville, M. Popall and L. Nicole, *Chem. Soc. Rev.,* 2011, **40**, 696-753.
24. S. Jongjinakool, K. Palasak, N. Bousod and S. Teepoo, Energy Procedia, 2014, 56, 10-18.
25. E. C. Dreaden, A. M. Alkilany, X. Huang, C. J. Murphy and M. A. El-Sayed, *Chem. Soc. Rev*., 2012, **41**, 2740-2779.
26. M. Yu, C. Zhou, J. Liu, J. D. Hankins and J. Zheng, *J. Am. Chem. Soc.,* 2011, **133(29)**, 11014-11017.
27. N. Sharma, S. Rana, H. G. Shivkumar and R. K. Sharma, *Radiat. Protect. Env.,* 2013, **36(2),** 78-84.
28. R. Kaur, J. Singh, A. Saini, N. Singh and N.Kaur, *RSC Adv*., 2014, **4**, 48004-48011.
29. R. Kaur, A. Kaur, G. Singh, M. Kumar and N. Kaur, *Anal. Methods,* 2014, **6,** 5620-5626.
30. A. Saini, J. Singh, R.Kaur, N. Singh and N. Kaur, *Sensor Actuat. B-Chem*., 2015, **209**, 524–529.
31. (a) A. S. F. Farinha, A. C. Tome and J. A. S. Cavaleiro, *Tetrahedron*, 2010, **66**, 7595-7599. (b) J. Yoo, M.-S. Kim, S.-J. Hong, J. L. Sessler and C.-H. Lee, *J. Org. Chem*., 2009, **74**, 1065-1069. (c) P. Thiampanya, N. Muangsin and B. Pulpoka, *Org. Lett.,* 2012, **14 (16),** 4050-4053.
32. F. Allergrini and A. C. Olivieri, *Anal. Chem*., 2014, **86 (15),** 7858-7866.

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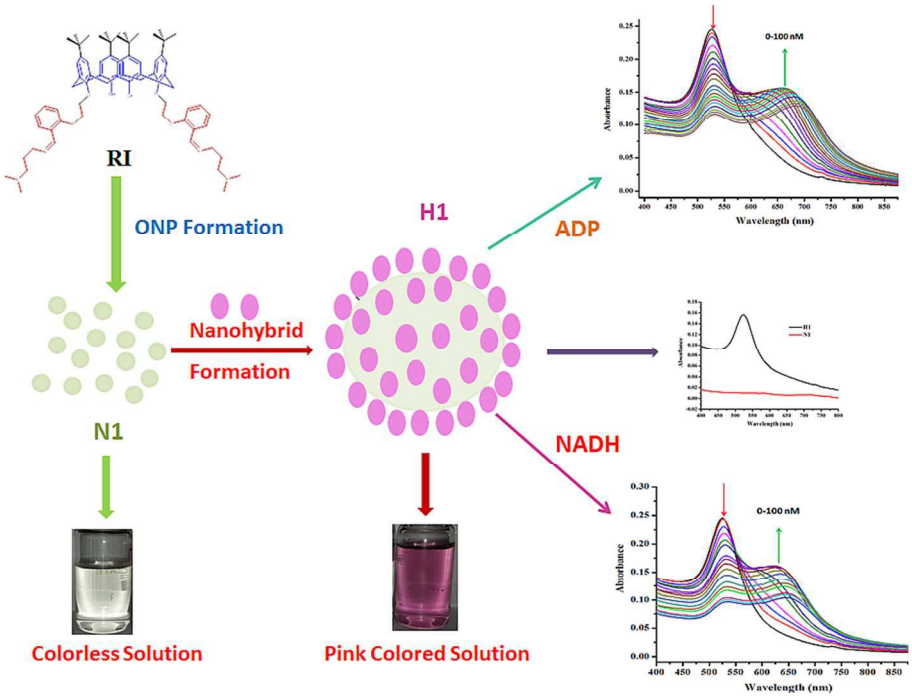

Nanohybrids of calix[4]arene based receptor has been employed for selective and simultaneous estimation of ADP and NADH.