Revisiting β-dicyanovinyl substituted calix[4]pyrrole : toward the highly selective detection of hydrazine in solution

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Abstract

The selective detection and quantification of hydrazine, a hazardous pollutant commonly used in industries, was performed by UV-spectroscopy with a repurposed β-dicyanovinyl substituted calix[4]pyrrole as chemosensor. Selectivity was evaluated in acetonitrile towards various nitrogen-containing compounds and a nucleophilic thiol. Moreover, influence of several parameters (time, water content and temperature) on hydrazine detection of the chemosensor was evaluated. This work allows the sensing of hydrazine with a LOD of 1.3 mg/L and a linear response on the 40-1000 µM range. Naked eye detection was also performed.

Introduction

Hydrazine is regarding as a hazardous environmental pollutant and being a carcinogenic chemical has proved to be highly toxic for both humans and animals.[1] The continuous exposure to hydrazine leads to nose, throat, and eye irritation, dizziness, nausea, pulmonary edema, etc., which in turn proves fatal for kidneys, liver and central nervous system in human beings.[2] As a matter of the fact, U.S based Environmental Protection Agency (EPA) has marked hydrazine as potential carcinogen along with a determination of threshold limit of 10 ppb [3] and an Immediately Dangerous to Life and Health (IDLH) value of 50 ppm in air.[4] By virtue of widespread use of hydrazine in industries (pharmaceuticals, polymers, dyes etc.)[5], hydrazine pollution in water and air has become a major concern globally. Indeed, it was reported that upon exposure to about 0.1 ppm hydrazine in air, urine of Japanese workers contains an average of 2.7 ppm of hydrazine.[6] It is also expected to find concentration above 10 ppm in urine in case of an event-level exposure.[7] Thus, there arises a need to efficiently

monitor the level of hydrazine in air, water, human fluids and other related systems in order to avoid global catastrophe. Till now, numerous conventional analytical methods have been employed for the detection of hydrazine viz chromatography, spectrophotometric flow injection, and electroanalytical methods.[8–10] However, Naked eye and UV-visible detection are particularly attractive since it is generally cheap, fast, selective and reliable even if the detection limit is not as low as other methods like molecular fluorescence spectroscopy[11–13]. Analytical methods utilizing optical signatures and supramolecular sensors/receptors have been of great interest to the scientific community in recent year.[14,15] Among diverse supramolecular receptors available, the non-aromatic tetrapyrollic macrocyclic receptor known as calix[4]pyrrole (C4P) has shown a promising role in the recognition of diverse analytes. C4P possess a built-in recognition site able to recognize anions or polar guests, while its functionalization at the level of meso-carbon or pyrrole allow to achieve cation or ion-pair recognition.[16–18]. In this study, we repurposed a β-dicyanovinyl substituted C4P preliminary designed for the recognition of cyanide ion in order to achieve the naked eyed and spectroscopic detection of hydrazine.

Material and Methods

NMR spectra were recorded on a Bruker Ascend 400 spectrometer operating at 400 MHz and 101 MHz for ^{1}H and ^{13}C respectively. Traces of residual solvent were used as internal standard. High resolution mass spectrometry spectra (HRMS) were recorded in positive mode using an Agilent 6540 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC/MS system equipped with an Agilent Jet Stream dual electrospray source. UV-Vis measurements were performed at fixed temperature on a Varian Cary 3500 UV-Vis spectrophotometer equipped with a compact Peltier apparatus. A quartz suprasil 100-QS TO 10 mm pathlength cuvette was used.

Synthetic procedure for compound 3: To a clear low melting mixture (5g) of L-(+)-TA and DMU in the ratio of 3:7 at 70 ºC, was added freshly distilled pyrrole (5 ml, 72.07 mmol, 1 equiv) and acetone (26.69 ml, 360.34 mmol, 5 equiv). The reaction mixture was stirred at 70 ºC for 4-5 h and was monitored by TLC. After the completion of reaction, the warm reaction mixture was diluted with water (20 ml). The reaction mixture was cooled to room temperature, filtered through sintered glass funnel and the solid material was washed with water (4 x 20 mL). The crude product was purified by silica gel column chromatography using (5% EtOAc:petroleum ether). The first eluted fraction was simple C4P **3** (4.64 g, 60% yield, white solid) and the second eluted fraction was N-confused calix[4]pyrrole (N-C4P) (0.7g, 9% yield, light yellow solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (s, 4H), 5.93 (d, J = 2.4 Hz, 8H), 1.54 (s, 24H).^{13}C NMR (101 MHz, $CDCl_3$) δ 138.67, 102.93, 35.61, 29.09; HRMS (ESI, Q-TOF) m/z: calculated for $C_{28}H_{36}N_4$ $[M+H]^+$ 429.3013, found: 429.3021**.** The spectral data is in accordance with the literature.

Synthetic procedure for compound 4: In a dried two-necked 50 ml round bottomed flask, $POCl_3$ (0.52 ml, 5.599 mmol) was added dropwise at 0º C under inert nitrogen atmosphere to dry DMF (0.43 ml, 5.599 mmol) with vigorous stirring. After some time, dry CH_2Cl_2 (5 ml) was added to this reaction mixture and left for stirring at 0º C for 45 minutes under inert atmosphere to obtain a Vilsmeier reagent. This prepared reagent was later on added dropwise at 0º C under inert atmosphere to a stirred solution of C4P 25 (2.4g, 5.599 mmol)

in dry CH_2Cl_2 (100 ml) prepared in another two-necked 250 ml round bottomed flask. The solution turns dark red and was left for stirring at 0º C for 2 h. After the completion of the reaction, the reaction mixture was thoroughly washed with saturated aqueous Na_2CO_3 and NaCl. The organic contents were extracted in CH_2Cl_2, dried over Na_2SO_4, filtered, and concentrated over vacuum to afford crude red solid. This crude mixture was later on subjected to column chromatography (SiO_2, CH_2Cl_2 followed by 5% CH_2Cl_2:ethyl acetate) to get pure **4** (0.537 g, 21% yield, white solid). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.09 (s, 1H), 7.35 (s, 2H), 6.96 (s,1H), 6.47 (s,1H), 6.0 – 5.83 (m, 7H), 1.73 (s, 6H), 1.53 (d, s, 12H), 1.49 (s, 6H). The spectral data is in accordance with the literature.

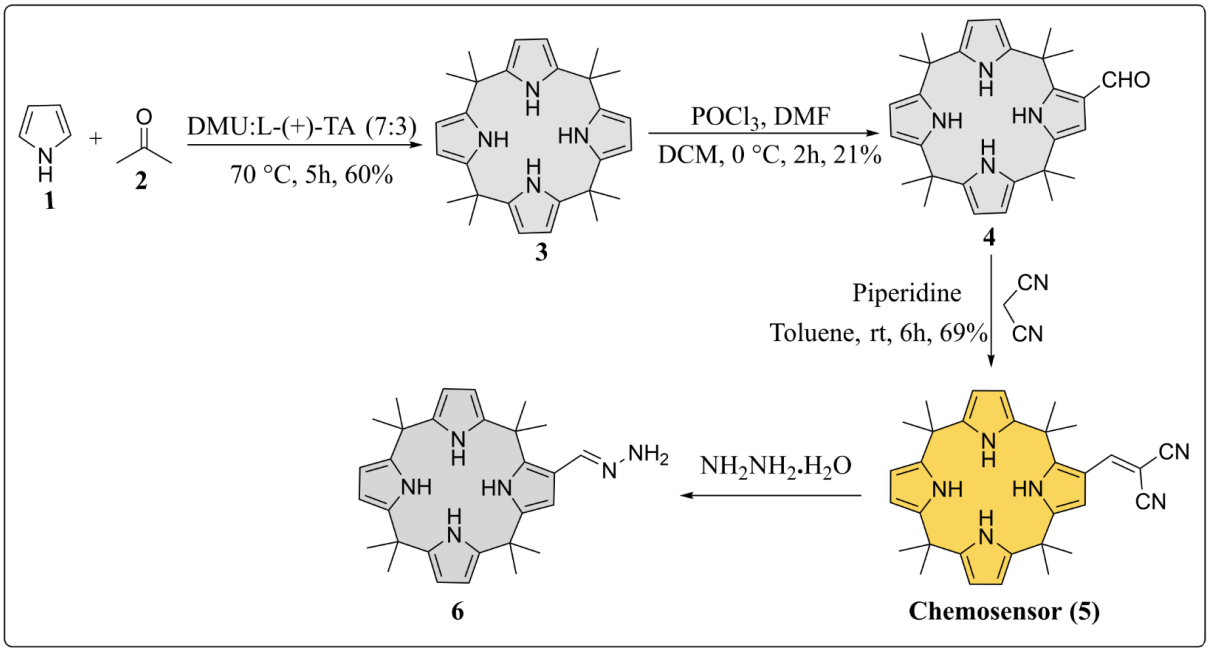
Synthetic procedure for chemosensor 5: In a dried two-necked 50 ml round bottomed flask, compound 26 (0.5 g, 1.1 mmol) and malononitrile (89 µl, 1.60 mmol) were dissolved in 18 ml of toluene at room temperature. To this stirred solution, piperidine (0.15 ml, 1.52 mmol) was added and left for stirring at room temperature for 6 h. After the reaction gets completed, the organic contents were extracted in CH_2Cl_2, dried over anhydrous Na_2SO_4, and concentrated over vacuum to afford crude mixture. This crude mixture was then subjected to column chromatography (SiO_2, pure CH_2Cl_2) to afford pure chemosensor **5** (381 mg, 69% yield) as a yellowish solid. ^{1}H NMR (400 MHz, CDCl3) δ 7.95 (s, 1H), 7.42 (bs, 1H), 7.26 (bs, 1H), 7.20 (bs, 1H), 6.95 (d, J=2.7 Hz, 1H), 6.88 (bs,1H), 5.95-6.02 (m,4H), 5.91 (m,1H), 5.85 (m, 1H), 1.68 (s, 3H), 1.55 (s, 3H), 1.53 (s, 3H), 1.50 (s, 3H). The spectral data is in accordance with the literature.

UV-spectroscopy analysis : For UV spectroscopy analysis, a stock solution of chemosensor **5** was prepared in pure acetonitrile at 1.10^{-3} M and then diluted to 10 µM (10 mL). Stock solutions of amino compounds were also prepared in pure acetonitrile at 1.10^{-1} M. An aliquot of the nucleophilic compounds (100 equivalents) was added to the 10 µM solution of chemosensor **5** and then UV spectra were recorded every 10 minutes during 2 hours. For calibration curves, 7 solutions of compound **5** at a concentration of 10 µM in acetonitrile were incubated in a water bath at 25°C. Every two minutes, to a solution was added a known concentration of hydrazine (i.e. 10 equiv. for solution 2, 20 equiv. for solution 3, 40 equiv for solution 4 etc.). The two minutes interval between hydrazine addition allow the operator to fill the UV cuvette and record UV spectra at known time interval of 1, 2 and 4 hours.

Results and discussion:

1) synthesis and characterization

The synthesis of chemosensor **5** is described in Scheme 1. Firstly, octamethylcalix[4]pyrrole **3** was obtained from the condensation of pyrrole with acetone following a green procedure employing deep eutectic solvent.[19] Then compound **4** and **5** were obtained following previously published procedures.[20,21] ^{1}H NMR spectra in $CDCl_3$ is in accordance with literature (Figures S1-2).



Scheme 1. Synthesis of β-vinyl substituted calix[4]pyrrole (chemosensor **5**) and its reaction with hydrazine

2) NMR investigation

Prior to UV-spectroscopy studies, the reaction of chemosensor **5** with hydrazine was investigated by ^{1}H NMR. Addition of an excess hydrazine to a solution of chemosensor **5** in CD_3CN lead after a few minutes to a change of color from yellow to almost colorless. Aromatic part of ^{1}H NMR of chemosensor **5** and its reaction product with hydrazine are presented in Figure 1. It can be seen that the reaction produce a single new product, presenting a significant upfield shift of the proton present on the pyrrole bearing the sensing arm ($\Delta\delta$ = 0.69 ppm) and a slight shielding of vinylidene singlet proton at 8.12 ppm ($\Delta\delta$ = 0.008) ppm. Those observations are fully coherent with previously published literature employing the dicyanovinyl group as sensing unit.[22] Moreover, the proposed structure of compound **6** was in agreement with high resolution mass spectrometry experiment (Figure S4).

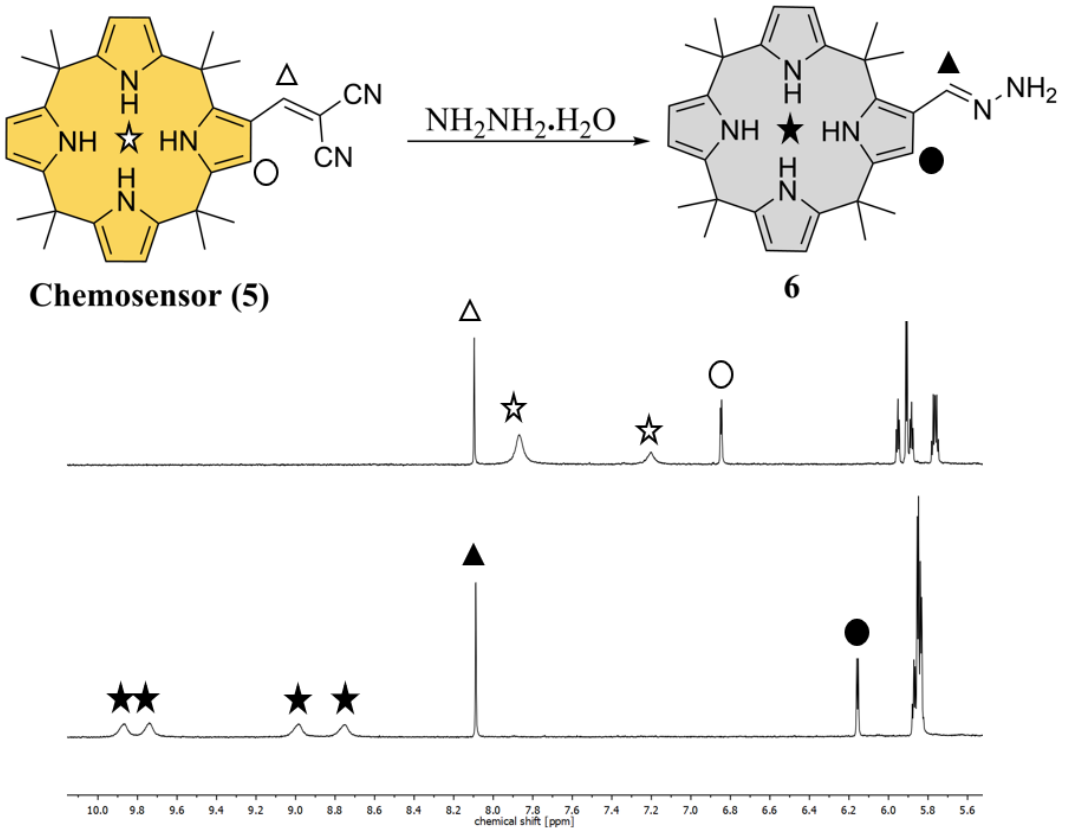


Figure 1 : Partial ^{1}H NMR spectra (400 MHz, CD_3CN, 298K) of chemosensor **5** before (top) and after (bottom) addition of an excess of hydrazine. (full spectra are provided in Figure S5)

3) **Study of selectivity**

Recognition behavior of chemosensor **5** was firstly studied by UV-spectroscopy in pure acetonitrile towards various nitrogen containing compounds (hydrazine, ammonia, propylamine, diethylamine, triethylamine, pyrrole, aniline) and a nucleophile (2-mercaptoethanol). Chemosensor **5** UV spectrum present a maximum absorption at 373 nm which is similar to previously published data[20] recorded in ACN-DMSO (3%) mixture and possess a molar extinction coefficient of 19303 $L.mol^{-1}.cm^{-1}$ (Figure S6). This value should allow the observation of reasonably low concentration of analytes.[23]

As expected following NMR experiments, addition of 100 equivalents of hydrazine to a solution of chemosensor **5** at 10 µM in ACN lead to a decrease in absorbance at 373 nm (Figure 2) with the emergence of an isobestic point at 298 nm. It is important to note that the reaction between the dicyanovinyl group of chemosensor **5** and hydrazine is time-dependant. To take into account the dependency to time of the UV response, UV spectra were acquired every 10 minutes during 2 hours for each nitrogen containing compounds (Figures S7-13). To our delight, addition of 100 equivalents of others amino compounds does not lead to a significant modification of the absorption spectra of chemosensor **5** after 60 or 120 minutes (Figure 3), indicating that chemosensor **5** is highly selective towards hydrazine. Kinetic studies indicate that the reaction between hydrazine and calixpyrrole **5** is pseudo-first order (Figure S14)

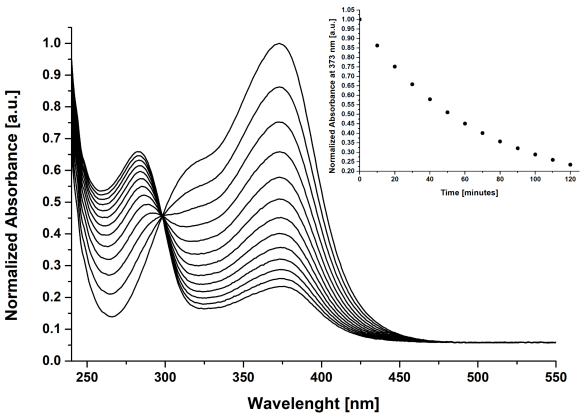


Figure 2: UV absorption of chemosensor **5** (1.10-5M, 25°C) upon addition of 100 equivalents of hydrazine in acetonitrile. Spectra acquired every 10 min. Inset : variation of absorbance at 373 nm

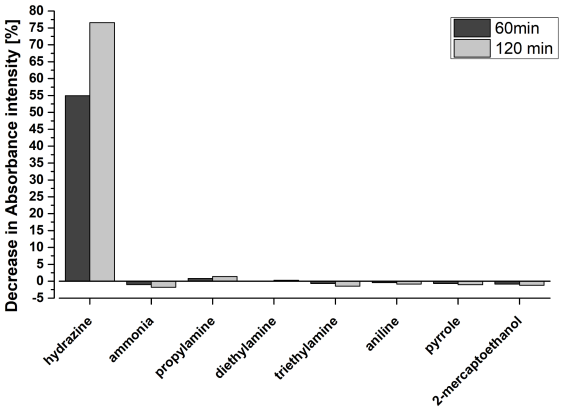


Figure 3: Variation of UV absorption at 373 nm of chemosensor **5** (1.10^{-5}M, 25°C) upon addition of 100 equivalents of different competing nucleophiles in pure acetonitrile.

This great selectivity was also demonstrated at higher concentration (Figure 4). Addition of 100 equivalents hydrazine to a 1 mM solution of chemosensor **5** lead to a complete bleaching after less than 90 seconds that can be perceived by naked eye.

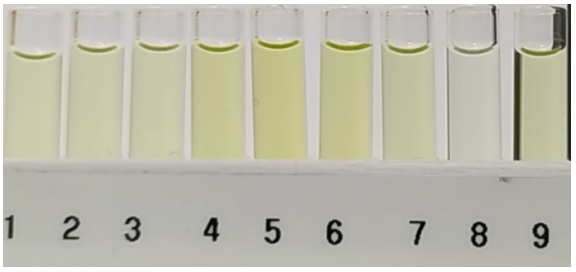


Figure 4: Color change of chemosensor **5** (1 mM) in the presence of 100 equivalent of nucleophiles. 2-mercaptoethanol (1), pyrrole (2), aniline (3), triethylamine (4), diethylamine (5), propylamine (6), ammonia (7), hydrazine (8), chemosensor **5** alone (9). Picture was taken 90s after hydrazine addition.

4) Study of the influence of water

Influence of water on the detection capacity of chemosensor **5** was evaluated by following the absorbance at the maximum absorbance of a solution of calixpyrrole at 10 µM in the presence of 100 equivalents of hydrazine with different percentage of water at 25°C. Addition of water lead to a slight red shift of the maximum absorption reaching 376 nm for 40% of water. It can be observed on Figure 5 that the response decrease with the increasing amount of water. This can be explained by the lowest nucleophilicity of hydrazine in water compared to acetonitrile.[24] Nevertheless, detection capacity of chemosensor **5** can be easily restored by enhancing the reaction rate by increasing the temperature to 50°C (Figure 5). This finding would allow the use of chemosensor **5** for the detection of environmental samples on aqueous media.

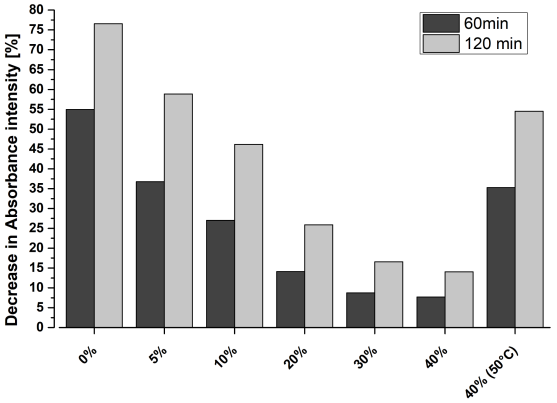


Figure 5: Variation of UV absorption at maximum absorption of chemosensor **5** (1.10^{-5}M, 25°C or 50°C) upon addition of 100 equivalents of hydrazine in containing increasing water content.

5) Analytical performance towards hydrazine

In order to evaluate the analytical performance of chemosensor **5**, calibration curves were constructed by monitoring absorbance at 373 nm with increasing amount of hydrazine (0 to 100 equiv.). To take into account the time dependency of the reaction between calixpyrrole and hydrazine, 7 individual solutions of compound **5** at a concentration of 10 µM were prepared and incubated in a water bath at 25°C. Then an increasing amount of hydrazine was added to each solution with a time interval of 2 minutes in order to allow the operator to fill the UV cuvette and record UV spectra. The series of solutions was analyzed after 1, 2 and 4 hours. Illustrative calibration curves are depicted in Figure 6.

One can observe that good correlation coefficient can be obtained after 1 and 2 hours. After 4h, high hydrazine concentrations (i.e. 80 and 100 equiv.) start to reach a plateau that derive from linearity and therefore do not allow to quantify accurately hydrazine. However, the first 5 points of the calibration curve remain aligned with good correlation coefficient (Figure 6 D). In our hand, the best result was achieved after 2 hours of incubation (Figure 6 B) with a correlation coefficient of 0.997. Limit of detection (LOD) and limit of quantification (LOQ) were deduced from this calibration curve according to the following equations[25]:

LOD = 3.3 x σ / slope
LOQ = 10 x σ / slope
with σ = the standard deviation of y-intercept of the regression line
LOD was evaluated at 4.10^{-5} M (1.3 mg/L) and LOQ at $1.2.10^{-4}$M (3.9 mg/L).

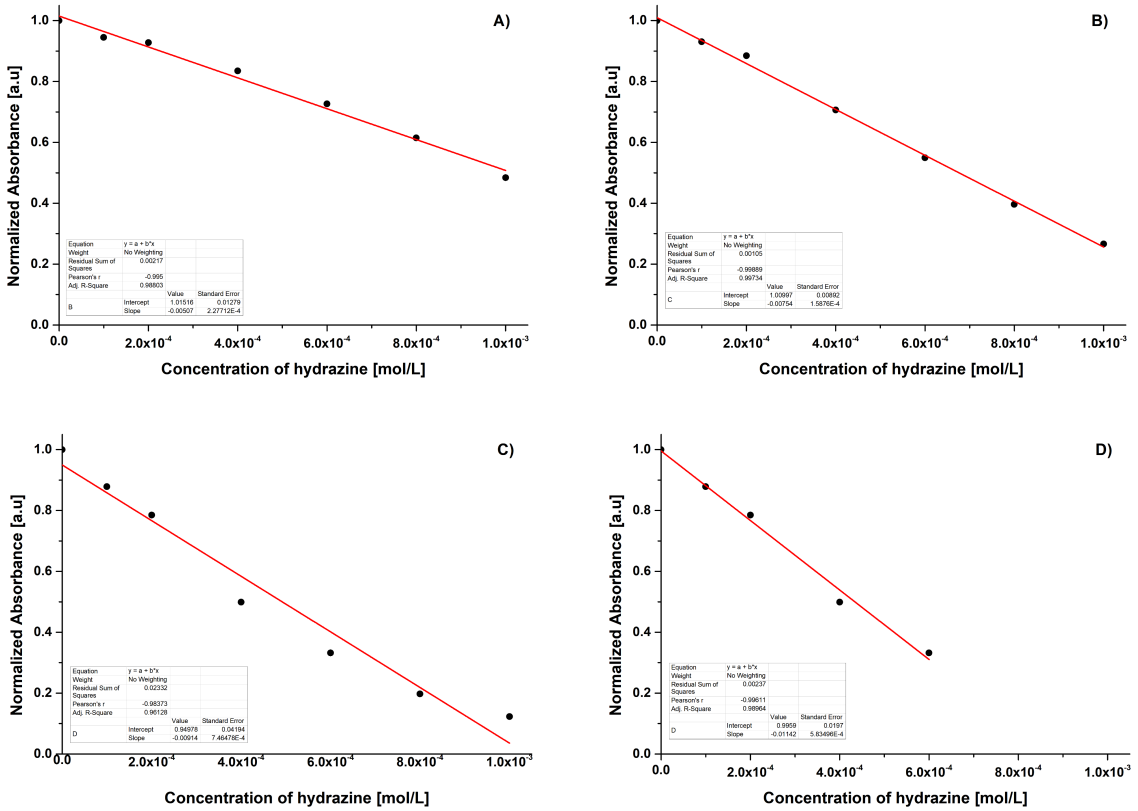


Figure 6 : Calibration curves for hydrazine monitoring in the range 10-1000 µM obtained after A) 1h, B) 2h, C) 4h and D) 4h with only 5 calibration points.

Conclusion

To conclude, we have successfully repurposed a β-dicyanovinyl substituted calix[4]pyrrole chemodosimetric sensor initially designed for the sensing of cyanide ion in order to achieve the spectroscopic detection of hydrazine. Chemosensor **5** prove to be highly selective towards hydrazine among other competing aliphatic or aromatic amino compounds (ammonia, propylamine, diethylamine, triethylamine, pyrrole, aniline) and a nucleophile (2-mercaptoethanol). Sensing capacities were evaluated by UV-spectroscopy in pure acetonitrile and in acetonitrile-water mixtures with up to 40% water. Calibration curve obtained after an incubation time of 2 hours at 25°C prove to be linear 40-1000 µm range and it allows to determinate a limit of detection at 4.10^{-5}M (1.3 mg/L) and a limit of quantification at $1.2.10^{-4}$M (3.9 mg/L). Finally, chemosensor **5** was employed for the naked eye detection of hydrazine, which is the only compounds studied that induce a total bleaching of the yellow solution.

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Conflicts of interest

There are no conflicts to declare.

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