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Paper Carbon dots based fluorescence sensor for distinction of organic and inorganic sulphur in analytes

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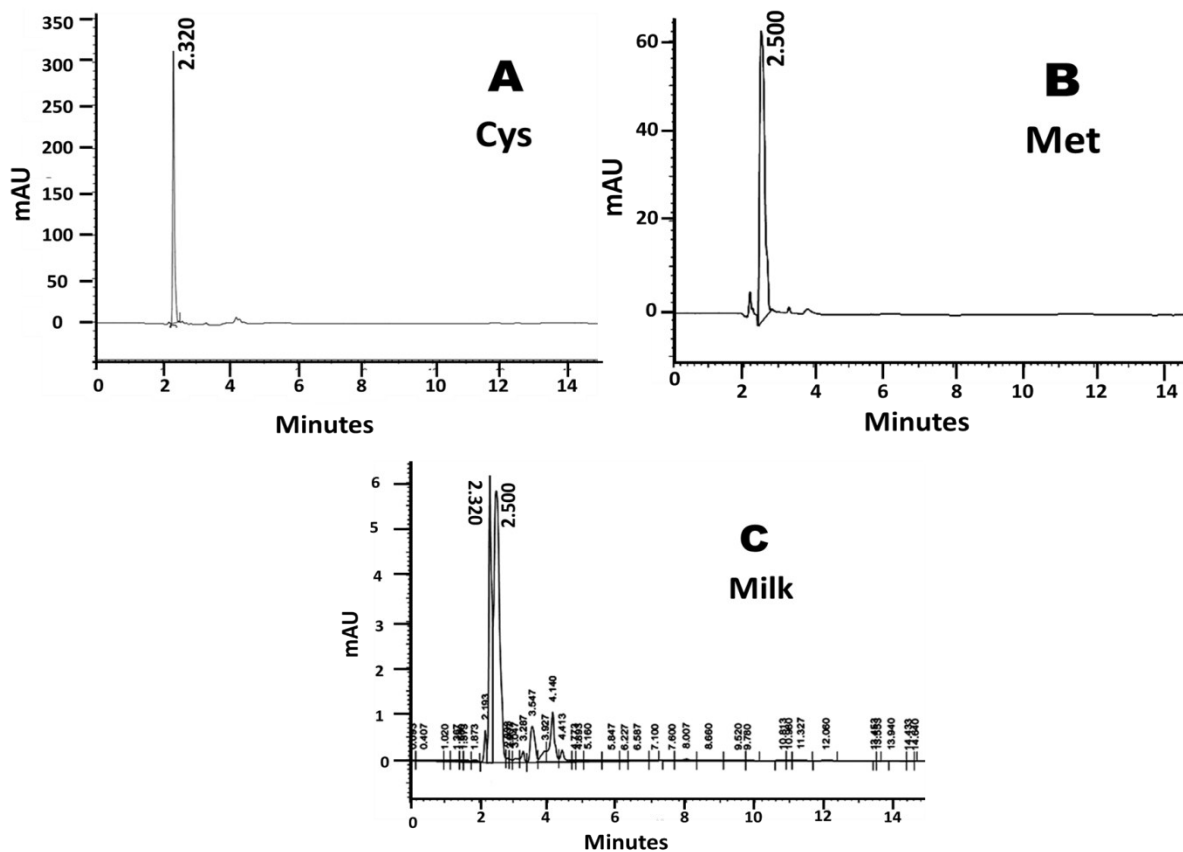
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HPLC Analysis

Procedure

In order to determine the amino acid composition of milk, they must be released from their protein-bound form. In our study raw cow milk was collected from local vendor and was hydrolyzed for amino acids by following the technique reported by Sanchez et al. with slight variations¹. Briefly, 1mL of cow milk was defatted with 0.5 mL of diethyl ether and the supernatant was collected. The collected supernatant was then hydrolyzed with 1N HCl and mixed slowly. The sample was heated for 16hrs at 110°C and filtered through 0.45 µm membrane filter. The filtrate was then subjected to HPLC analysis.



HPLC analysis confirms the presence of cysteine and methionine in our milk sample. The retention time of cysteine and methionine were found to be 2.320 and 2.500 respectively in the standards. Also in our milk sample two peaks at 2.320 and 2.500 were observed along with many other peaks at different retention time. These two peaks confirmed the presence of sulphur containing amino acids i.e. cysteine and methionine which in turn helped in the increase in PL intensity of PCD-Au³⁺ system upon addition of Milk sample.

References:

1. C. L. Sánchez, J. Cubero, J. Sánchez, L. Franco, A. B. Rodríguez, M. Rivero, C. Barriga *Food Anal. Methods*, 2012, **5**, 312–318

PL response of PCD-Au³⁺ system towards other structurally similar organic and inorganic analytes

To study the exclusive response of PCD-Au³⁺ system towards sulphur containing organic and inorganic analytes, the influence of non-sulphur containing organic and inorganic analytes were tested. For this study L-Aspartic acid (Asp), L-Phenylalanine (Phe), L-Tyrosine (Tyr), NaCl and Na₂CO₃ were considered as test organic and inorganic test analytes. As observed earlier, addition of HAuCl₄ to PCDs resulted in 75.52% intensity drop-off.

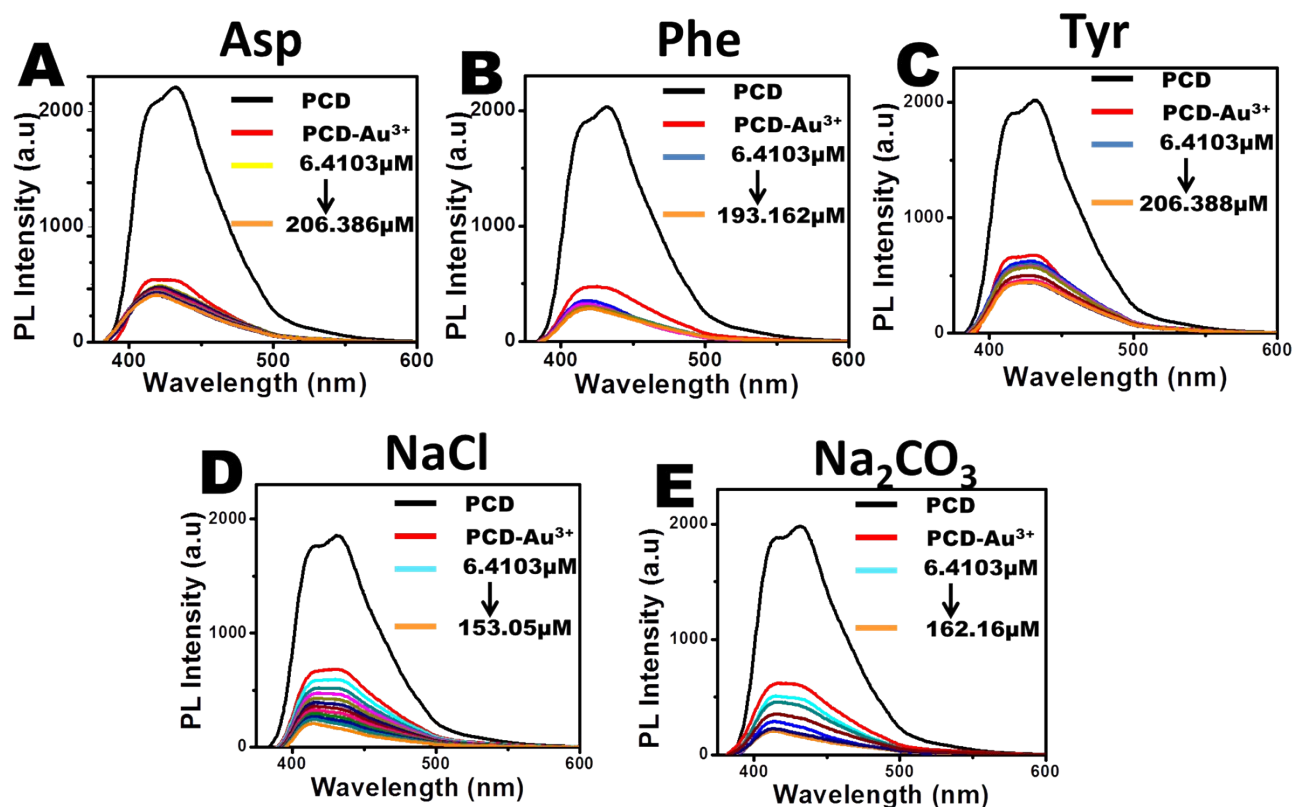


Figure S2: (A) Stacked PL emission spectra of PCDs, PCD-Au³⁺ and PCD-Au³⁺-Asp at a constant wavelength (340 nm), (B) Stacked PL emission spectra of PCDs, PCD-Au³⁺ and PCD-Au³⁺-Phe at a constant wavelength (340 nm), (C) Stacked PL emission spectra of PCDs, PCD-Au³⁺ and PCD-Au³⁺-Tyr at a constant wavelength (340 nm), (D) Stacked PL emission spectra of PCDs, PCD-Au³⁺ and PCD-Au³⁺-NaCl at a constant wavelength (340 nm) and (E) Stacked PL emission spectra of PCDs, PCD-Au³⁺ and PCD-Au³⁺-Na₂CO₃ at a constant wavelength (340 nm).

To this solution Asp was added up to a concentration of 206.386 μ M and the PL response was monitored using a spectrofluorometer. Figure 1(A) shows the stacked PL emission spectra of PCDs, PCD-Au³⁺ and PCD-Au³⁺-Asp at a constant wavelength (340 nm). It was found that upon addition of Asp, the PL intensity further decreased by 85.3% of the original PCD intensity. The same procedure was repeated with Phe. In this case 66.216% intensity drop-off was noticed upon addition of HAuCl₄ to PCDs. Phe was added to a final concentration of 193.162 μ M and the final intensity was found to decrease up to 77.8% of the original PL intensity of the PCDs. Figure 1(B) shows the stacked PL emission spectra of PCDs, PCD-Au³⁺ and PCD-Au³⁺-Phe at a constant wavelength (340 nm) whereas Figure 1 (C) shows the stacked PL emission spectra of PCDs, PCD-Au³⁺ and PCD-Au³⁺-Tyr at a constant wavelength (340 nm). In this case addition of HAuCl₄ to PCDs resulted in 73.102% intensity drop-off. To this solution Tyr was added up to a concentration of 206.386 μ M and the PL response was monitored using a Spectrofluorometer which showed further intensity drop by 79.583%. Figure 1 (D) shows the stacked PL emission spectra of PCDs, PCD-Au³⁺ and PCD-Au³⁺-NaCl at a constant wavelength (340 nm). In this case addition of HAuCl₄ to PCDs resulted in 67.377% intensity drop-off. To this solution NaCl was added up to a concentration of 153.05 μ M and the PL response was monitored using a

Spectrofluorometer which showed further intensity drop by 84.274%. Also in case of Na_2CO_3 , addition of HAuCl_4 to PCDs resulted in 63.471% intensity drop-off. To this solution Na_2CO_3 was added up to a concentration of $162.16 \mu\text{M}$ and the PL response was monitored using a Spectrofluorometer. Figure 1(E) shows the stacked PL emission spectra of PCDs, PCD-Au^{3+} and $\text{PCD-Au}^{3+}-\text{Na}_2\text{CO}_3$ at a constant wavelength (340 nm). It was found that upon addition of Na_2CO_3 , the PL intensity further decreased by 85.043%. Thus, it is to be observed that as discussed in the main manuscript, there is observed enhancement of PL intensity for organic sulphur-bearing analytes whereas for other structurally similar organic analytes like Asp, Phe and Tyr there is further quenching of PL intensity thereby demonstrating the selectivity of the system. Similarly, for inorganic analytes, the PCD-Au^{3+} system is found to be much more sensitive for sulphur-bearing analytes in terms of the extent of quenching observed.

Time-resolved Photoluminescence Studies

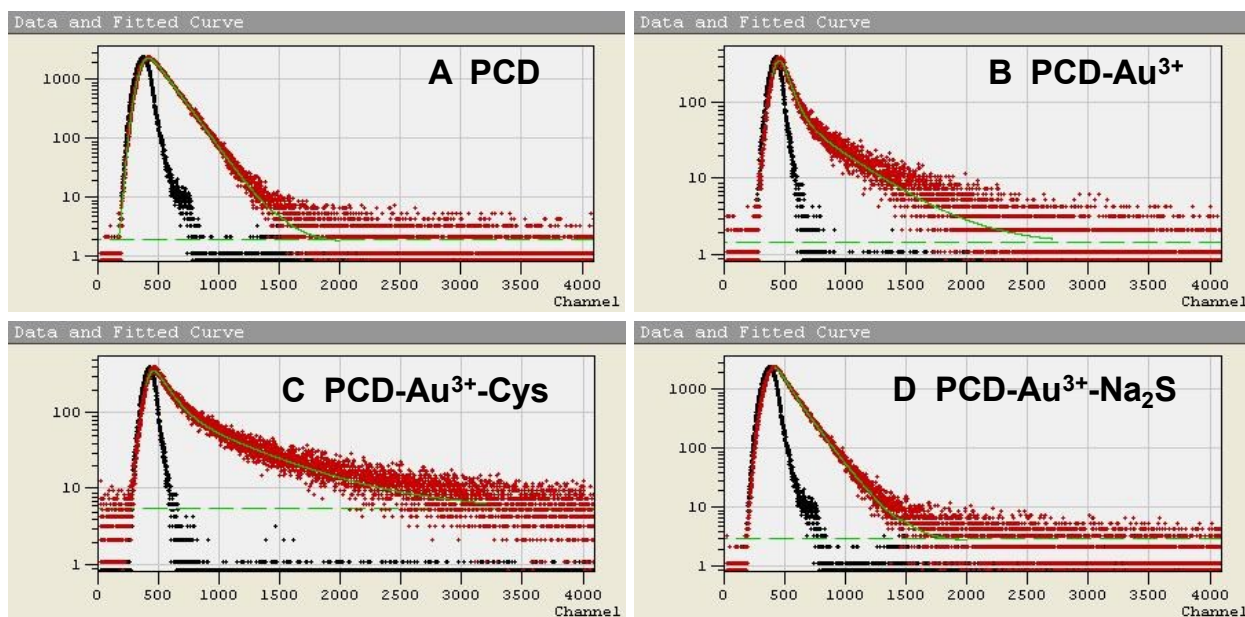


Figure S3: The Time Resolved Photoluminescence Decay curves of (A) PCDs, (B) PCD-Au³⁺, (C) PCD-Au³⁺-Cys and (D) PCD-Au³⁺-Na₂S studied at an excitation source of 336 nm.

Sample	Fraction (%)	Lifetime (ns)
PCD	100	0.747
PCD-Au ³⁺	59.18	0.285
	40.82	1.889
PCD-Au ³⁺ -Cys	43.96	0.543
	56.04	2.951
PCD-Au ³⁺ -Na ₂ S	31.73	0.352
	68.27	0.794

Table S1: The fluorescence lifetime values of different samples obtained from Time-resolved Photoluminescence (TRPL) Analysis.

Mechanistic Evidence from UV-Visible Spectroscopy

UV-visible spectroscopic analysis was carried out in order to draw further evidence in support of the proposed mechanistic pathway. Figure S4 shows the stacked UV-Visible absorption spectra of PCD, PCD-Au³⁺ system and that upon interaction with organic and inorganic sulphur analytes.

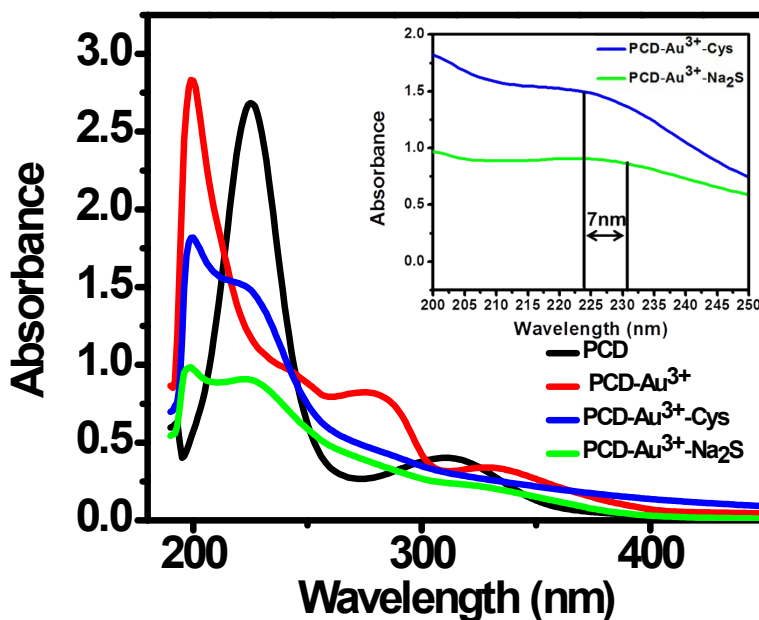


Figure S4: Stacked UV-Visible absorption spectra of PCD, PCD-Au³⁺, PCD-Au³⁺-Cys and PCD-Au³⁺-Na₂S

The absorbance spectrum of PCDs shows two characteristic peaks at 224nm and 313nm. Conjugation of PCDs with Au³⁺ (PCD-Au³⁺) leads to a blue shift in the characteristic absorption peaks of the PCDs giving absorbance at 199nm and 280nm respectively. This blue shift is a clear indication of the successful interaction of PCDs with Au³⁺ ions. Upon addition of sulphur analytes to PCD-Au³⁺ system, further blue shift in the absorption peaks were found to occur demonstrating the successful interaction of Sulphur analyte with the PCD-Au³⁺ system. The absorption pattern of PCD-Au³⁺-Cys and PCD-Au³⁺-Na₂S was found to be same but the spectral peak of Na₂S showed a 7nm red shift as compared to that obtained after addition of Cys. One of the absorbance peaks of PCD-Au³⁺-Cys was found to appear at 224nm while the absorbance peak of PCD-Au³⁺-Na₂S appeared at 231nm.

PL response of bare PCDs towards organic and inorganic sulphur bearing analytes

PL response of bare PCDs towards organic and inorganic sulphur was studied using organic sulphur bearing analyte i.e. DMS and inorganic sulphur bearing analyte i.e. Na₂S. First the PL emission spectrum of PCD was recorded and to this 1mM DMS was added which resulted in intensity drop of PCD to many folds.

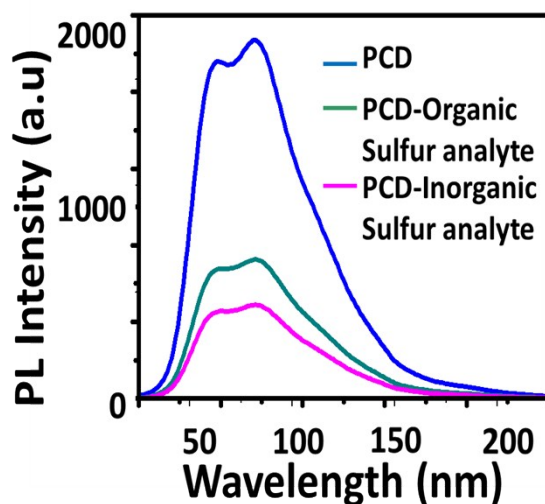


Figure S5: PL emission spectra of PCD, PCD-Organic sulphur analyte and PCD-Inorganic sulphur analyte at a constant wavelength (340nm)

The same experiment was done with Na₂S which also resulted in intensity drop of PCD to a greater extent than organic sulphur containing analytes. Thus, it was observed that bare PCDs could not distinguish between between the organic and inorganic sulphur analytes and showed similar PL response. However, upon conjugation with Au³⁺ the PL behavior of PCDs was different for organic (PL restoration) and inorganic sulphur analytes (PL quenching). This clearly demonstrates the role played by Au³⁺ in the sensing process.

Level of Quantification for different analytes:

Analytes	10 x Standard Deviation of Low Concentration	Level of Quantification
Cys	10X 0.26281	2.6281
DMS	10X 0.0436	0.436
Met	10x 0.0386	0.386
H ₂ SO ₄	10X 0.4016	4.016
Na ₂ S	10X 0.0125	0.125

Table S2: 10 x Standard Deviation of Low Concentration

PCD-AuNPs System

The addition of 100μL of Au nanoparticles to PCD is PCD-Au NPs. The PL properties of PCD are retained upon addition of Au NPs. We have also tried to use this PCD-AuNps system for the detection of organic and inorganic sulfur containing analytes but no change in fluorescent

intensity was observed as shown in figure S6. This confirms that fluorescence sensing of organic and inorganic sulfur containing analytes depends on the oxidation state of gold.

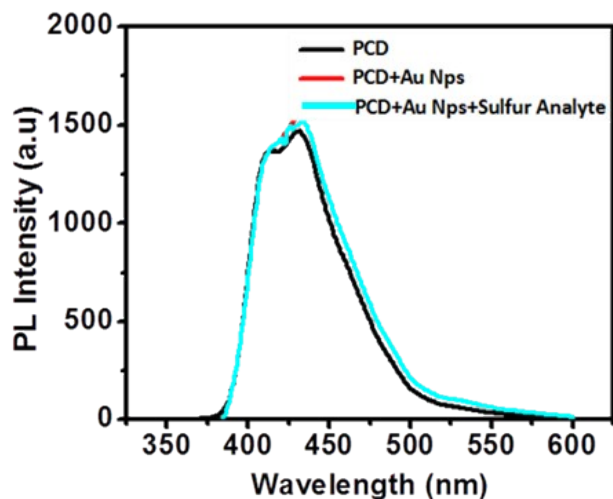


Figure S6: PL spectra of PCD, PCD-AuNps and PCD-AuNps-Sulphur analytes at a constant wavelength (340nm)

pH Influence on Sensor

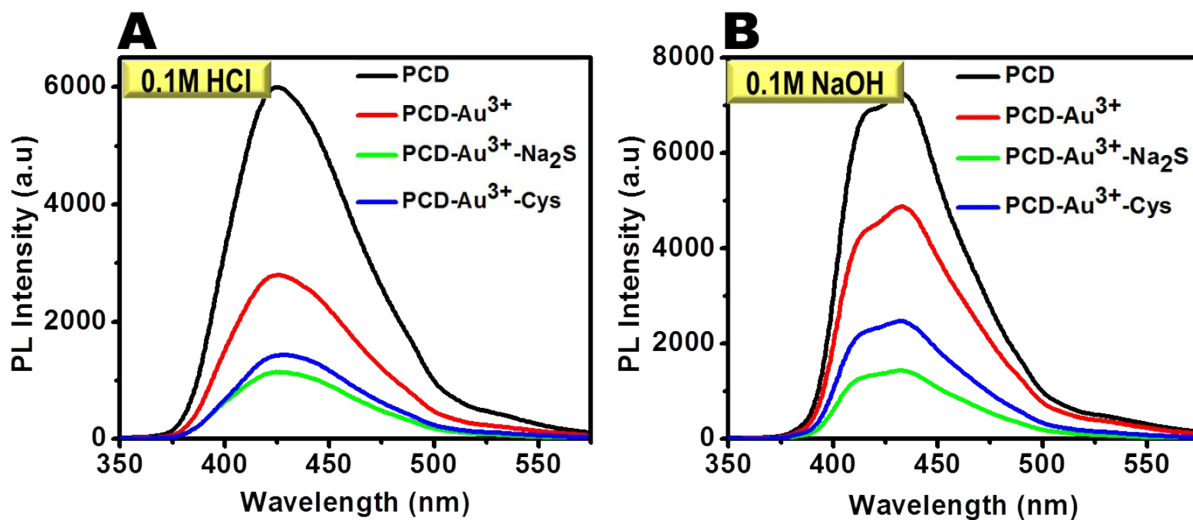


Figure S7: Stacked PL emission spectra of PCDs, PCD-Au³⁺, PCD-Au³⁺-Na₂S and PCD-Au³⁺-Cys at a constant wavelength (340 nm) in (A) 0.1M HCl and (B) 0.1M NaOH

PL response of PCDs, PCD-Au³⁺, organic analytes and inorganic analytes towards different pH medium was studied. 50µl of 0.1M HCl was added to PCD so as to develop an acidic medium and the PL Intensity was recorded. Similarly 50µl 0.1M NaOH was added to PCD to make the system basic. In both cases (acidic and basic conditions) the PL intensity after addition of organic and inorganic analytes was found to decrease and hence no “turn-on” phenomenon was observed. Thus it can be concluded that the system showed the sensing ability only at neutral pH.