Detection of Basic Amino Acids under Highly Alkaline Conditions Using a Perylene Amine-Derived Probe

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ABSTRACT: pH plays a crucial part in numerous chemical and physiological processes. In this work, a new perylene diimide derivative that acts as a pH-sensitive dye with Bay Area Carboxylic Acid functionality. The derivative utilizes the outstanding thermal, chemical and photochemical stability found in PDI materials and has remarkable UV-visible absorption and fluorescence emission qualities. Based on these properties, a fluorescent probe (PCA) was synthesised using a perylene tetracarbodiimide (PDI) backbone for the recognition of alkaline pH. In alkaline environments where the pH values are between 10 and 14, the fluorescence intensity significantly decreases, and a blue shift occurs, which is a standard feature of alkaline pH probes. The probe demonstrates exceptional sensing ability within the pH range of 10.00-14.00, with notable stability and reversibility. Encapsulation of the probe in a thin polymer film material enhances the pH sensing capability of the system. New sensors have been developed to detect basic amino acids by utilizing the probes' pH response characteristics. These sensors have also been applied to detect the concentration of arginine.

Introduction:

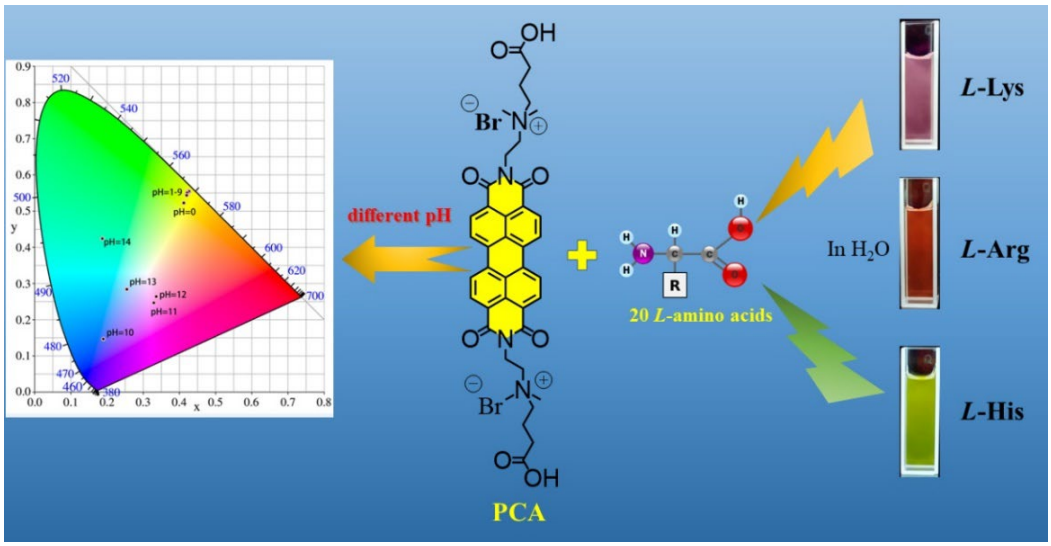
pH is an important chemical parameter that needs to be controlled in various fields including industrial work, as well as environmental and life sciences in order to monitor the availability of inorganic compounds in aquatic organisms and for industrial applications[1]. Moreover, keeping pH stable is essential to maintain the equilibrium of biological environmental systems[2-8]. The effective regulation of pH in turn relies heavily on the precise sensing of pH fluctuations[9-11]. Similarly, amino acids are an essential component of living organisms and are vital for the maintenance of life activities, and indeed, the amino acid content is an

important indicator of health[12]. The basic amino acids (arginine and lysine, histidine) are of unquestionable importance to human health and metabolism; arginine (Arg) plays a central physiological role in cell division, gene regulation, wound healing, hormone release and other transformations; histidine (His) is essential for many enzymatic processes and lysine (Lys) levels are an indicator of inborn metabolic disorders[13-17]. The selective identification of essential basic amino acids is therefore highly desirable for a variety of medical reasons. However, very little has been reported on the 'proportional' detection of basic amino acids, reflecting the limitations when using chemically complex sensor molecules and a range of traditional amino acid detection methods, including high performance liquid chromatography, ion exchange chromatography and gas chromatography[18-24].

Perylene derivatives are a class of cyclic aromatic hydrocarbon derivatives (PDIs)[25]. PDIs molecules have good stability, high fluorescence quantum yields, high coloration, photostability, strong π-π conjugation effects, comparable electron affinity to fullerenes, strong electron deficiencies, adjustable solubility as well as excellent optoelectronic properties[26-32]. The modification of the *N*-bound side chain groups enables the detection of metal ions, amino acids etc. [18] under aqueous conditions with large fluorescence changes, high sensitivity and strong visualization. Thus, the strong fluorescence, high photostability and good electron acceptance of PDI derivatives indicate that they can be good materials for incorporation in fluorescent sensors[14, 33-35].

When a π-conjugated system containing free base nitrogen atoms is protonated, its electronic structure changes significantly, contributing to the successful construction of pH-responsive fluorescent probes. Li *et al*,[36] investigated the use of perdiamine-amino acid derivatives for the detection of anions, which exploited the synergistic mode of action of hydrogen bonding and anion-π interactions for the detection of fluoride ions. By contrast, in this work we have extended the development of efficient multi-response fluorescent sensors by synthesising a new compound, PCA, using a π-conjugated perylene system, and have explored the pH properties of perylene diimide carboxylic acid derivatives, and detected alkaline amino acids by exploiting the rapidity of the fluorescence color change. Importantly, the nitrogen atom of PCA can act as a binding site for H^+ or OH^-, enabling the system to monitor alkaline pH in aqueous solutions. PCA has good acid-base stability and is therefore an ideal probe for detecting changes in the alkaline pH of water by removing hydrogen bonds. Luminescence measurements showed that the intensity of the luminescence peak at 550 nm in water varied with the acidity of the solution (pH= 0-14), which is a typical characteristic of pH-responsive probes. Based on the pH characteristics of this probe, a recyclable fluorescent sensor with a wide

alkaline range (pH=10-14) was prepared, and it was applied to polymers to prepare thin films for monitoring strong acidic and alkaline changes in the environment. Furthermore, we successfully used PCA for the selective detection of Arg, Lys and His in water, and the recognition ability depended on the pH of PCA and the alkaline amino acid system response. Unlike other alkaline amino acid probes[37], the PCA probe can detect three alkaline amino acids and can be applied to the concentration detection of arginine with simple preparation, high visualisation and low cost.



Scheme 1. PCA system and components used herein.

RESULTS AND DISCUSSION

1.1. Design and synthesis of probe PCA

A carboxylic acid functionalized water-soluble *p*-diimide derivative (Scheme 1) was synthesized in order to detect pH changes of basic amino acids and strong bases in aqueous solution. Based on the special chemistry of this compound, it is possible not only to detect basic amino acids, but also to exhibit different fluorescence responses over the pH range 10.00 to 14.00. PCA has good water solubility, good stability and strong fluorescence characteristics in water. The reaction of *N,N*-ethylenediamine perylene with the hydroxyl group of 4-bromobutyric acid in refluxing DMF resulted in the formation of PCA. After repeated separation and precipitation with ether, the newly prepared PDI derivative was analyzed; the detailed synthetic process is given in the supporting information. This compound was characterized using a range of spectroscopic methods, including NMR, UV, and fluorescence spectroscopies.

1.2. pH-Sensitive luminescence

Since PCA has good stability in aqueous solution at different pH values, pH-dependent fluorescence can be obtained by adjusting the pH by adding solutions of HCl or NaOH. We speculate that PCA can recognize different pH values due to its special structure and functional groups. Therefore, we explored the performance of PCA under different pH conditions.

The fluorescence spectrum of PCA showed a clear pH dependence when aqueous solutions of PCA (50 μM) configured at different pH conditions using NaOH or HCl were added, as shown in Figure 1a. The fluorescence intensity gradually increases when in solutions with pH = 0-4, while for pH 5-9 the fluorescence intensity gradually decreases. At pH = 10-12, the PCA solution showed a burst of fluorescence intensity, while the fluorescence spectrum showed a blue shift at pH = 13-14; the fluorescence intensity at pH = 14 was higher than that at pH = 13. To further assess the pH dependence of PCA, the UV spectra of PCA at different pH values were analysed as shown in Figure 1b. At pH 1-9, the change in absorbance had the same effect. At pH=10, the UV spectral peak decreased, while at pH=11-14, the UV spectrum of PCA showed a blue shift. Furthermore, as shown in Figure 1c, a fluorescence color change was observed as a distinct change from yellow (pH=0-9), brownish red (pH=10), red (pH=11-12) and then brown (pH=13) and green (pH=14) for the pH 0-14 conditions. The corresponding CIE diagram is shown in Figure S6. These observations show that PCA is a good pH meter under different pH conditions modulated by solutions of HCl-NaOH, and it has been demonstrated that PCA can directly identify strongly basic solutions over a pH range of 10-14 by naked eye.

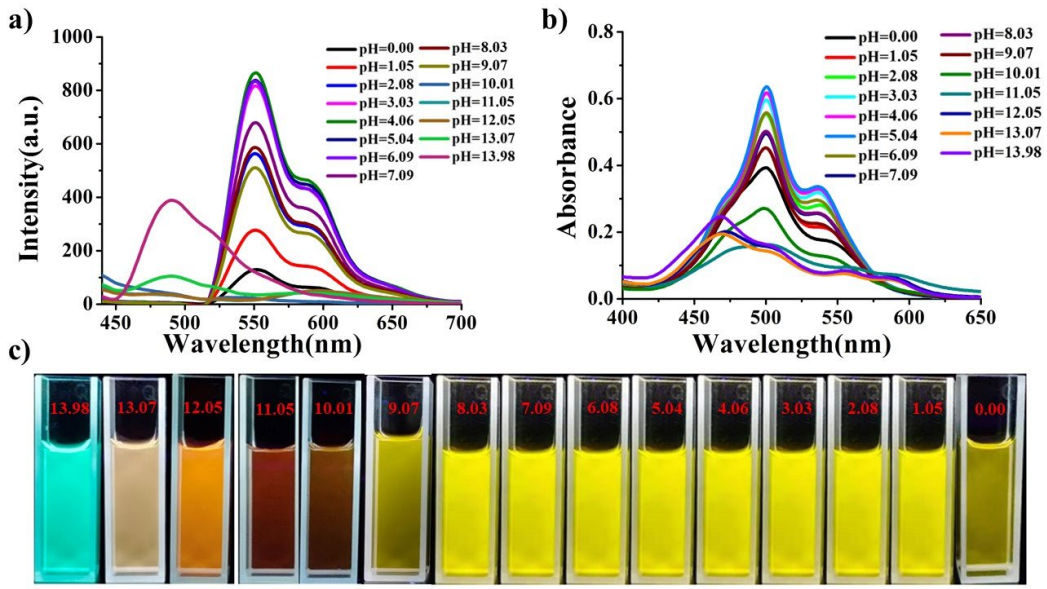


Figure 1: (a) Fluorescence spectra of PCA at different pH values; (b) UV absorption spectra of PCA at

different pH values; (c) Plots of fluorescence color change of PCA at different pH values (observed with a 365 nm UV lamp).

1.3. Reversibility and light stability

To study the reversibility of the PCA after the addition of H^+ or OH^-, the emission intensity (Fig. 2a) and color (Fig. 2b) were recorded at 554 nm at pH = 4.88 and pH = 11.02. As can be seen from the figure, the reversibility of the probe can be assessed by the fluorescence intensity for at least 8 cycles, proving that the PCA can be used as a reversible pH monitor. Considering that the response rate is a key factor affecting the photostability, time-varying curves of the maximum fluorescence emission intensity of the PCA were plotted at pH = 4.88 and 11.02, respectively, at room temperature, while the fluorescence intensity remained almost constant at pH 11.02. The results show that PCA can be reliably used as a pH indicator in practical applications.

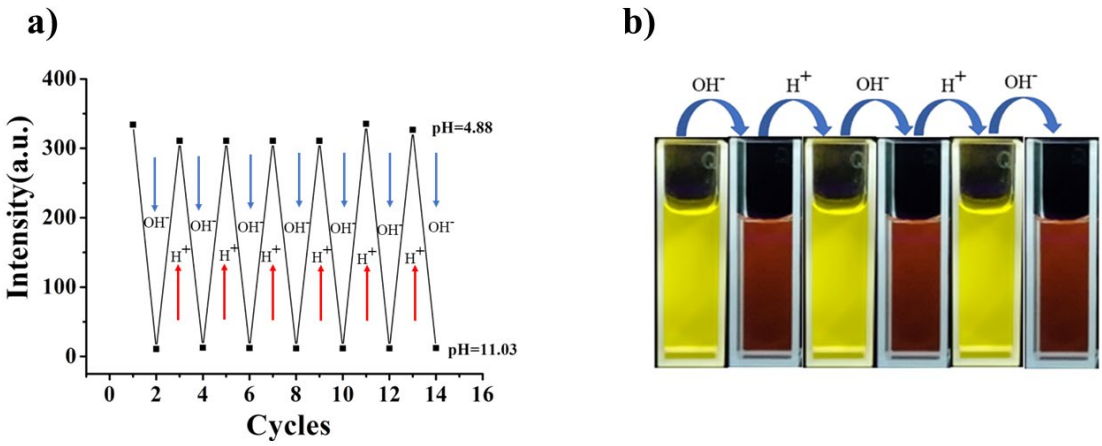


Figure 2. (a) Fluorescence intensity of PCA (10 μM) and (b) color reversibility between pH 4.88 and 11.02 *i.e.*, fluorescence characterization of PCA under different soluble solvent conditions.

1.4. Application to dual-responsive sensing paper

The excellent photochromic properties of the PCA components and the reversibility of the color change prompted us to explore their application in sensing markers. We prepared porous polymeric materials containing PCA. The polymer film was prepared by pouring the PCA dye solution into a polyethylene glycol solid. It was then placed on a printing plate bearing the word "1290". The system was placed in a confined space and the fluorescence changes were observed using a 365 nm UV lamp. Red fluorescence was observed under strong alkaline conditions around pH=11.00. After placing the plate in a concentrated hydrochloric acid solution (*i.e.* fumaric acid) for 30 minutes, the blue-green fluorescence wavelengths of the system

shifted from shorter to longer wavelengths and the plate became fluorescent yellow. When the plate was exposed to triethylamine gas for a further 30 minutes, the system returned to its original red fluorescence (Figure 3). This reversible 'back and forth' switching makes the PCA ideal for high contrast, sensitive optical recording and pH sensing.

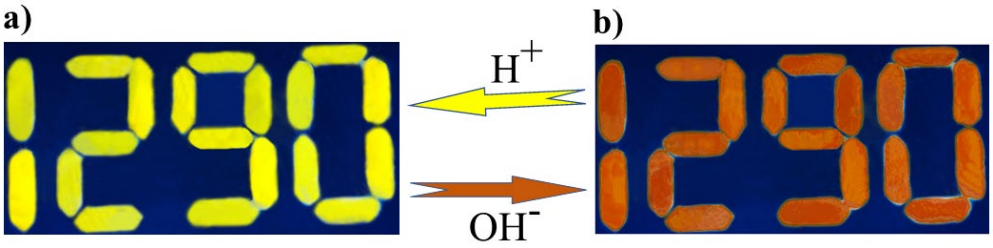


Figure 3: (a) Fluorescence color of PCA (10 μM) and (b) change in fluorescence color between pH acidic and alkaline environments.

2. Probe PCA identifies *L*-type amino acids

Owing to the acid-base fluorescence properties of PCA, we envisaged its potential for detecting basic amino acids in aqueous solutions. To validate this hypothesis, we prepared PCA as a fluorescent probe and conducted fluorescence experiments on each natural amino acid. As a consequence, three basic amino acids were successfully detected in an aqueous environment. As shown in Figure 4, the probe PCA binds to 20 amino acids with an excitation wavelength of 500 nm. Only three basic *L*-amino acids (*L*-Arg, *L*-His or *L*-Lys) lead to a change in fluorescence quenching (*L*-Arg or *L*-Lys is quenched and the fluorescence intensity of *L*-His decreases significantly) when PCA (2×10^{-5} mol/L) is added. By contrast, the His/probe system showed a significant decrease in fluorescence intensity, with all fluorescence emitted at 550 nm. Addition of Lys shifted the original peak to a blue color. The fluorescence of the system was quenched by the addition of Arg. The probes can detect three amino acids, the mechanism may be that when the amino acid solution is added to the PCA solution, the pH of the basic amino acid is higher, causing the PCA probe to deprotonate, thus significantly changing the fluorescence color of the system.

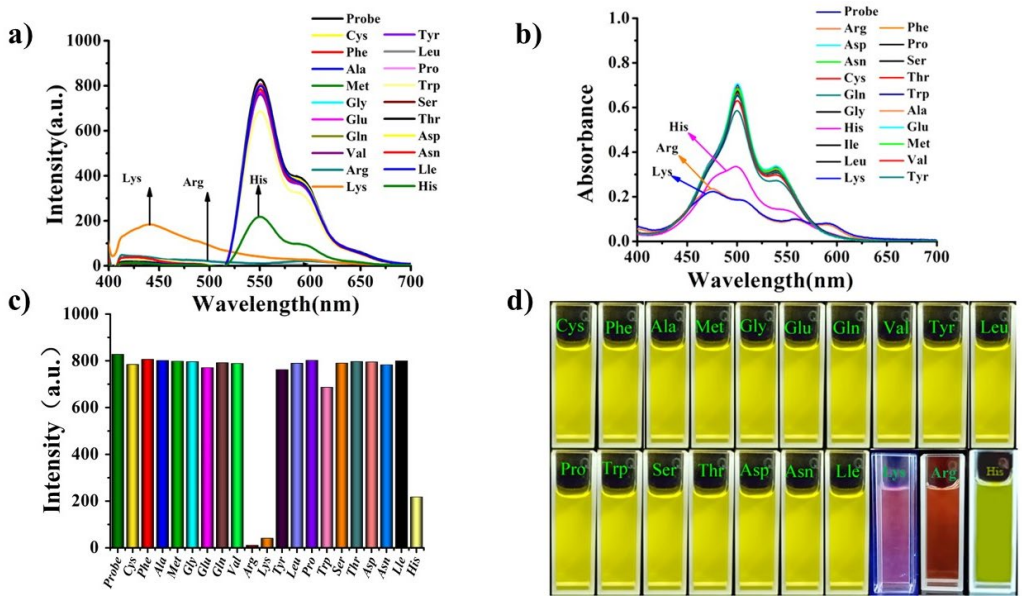


Figure 4. (a) PCA (20 μM) fluorescent probe was added to the fluorescence emission spectra of 20 *L*-type amino acids (600μM); (b) UV-Vis absorption spectra of PCA (20 μM) solution with 20 *L*-type amino acids added separately; (c) Fluorescence change spectrum of PCA added with various amino acids; (d) Fluorescence change of PCA on adding each amino acid at 500 nm.

2.1. Detection limit

Lysine, arginine, and histidine were added dropwise to the probe PCA. λ_{em} = 375 nm was selected as the plot of fluorescence intensity *versus* the corresponding amino acid concentration. The minimum detection limit for these three amino acids was calculated from the detection limit equation (3σ/K). PCA was most sensitive to lysine, with a detection limit of 2.18×10^{-6} mol/L (Figure S3). The detection limits for arginine and histidine were 2.33×10^{-6} (Figure S4) and 2.1×10^{-6} (Figure S5), respectively.

Based on the above fluorescence characteristics of PCA under different pH conditions, and the ability to detect three basic amino acids, the mechanism may be related to the gradual addition of the basic amino acids (*L*-Arg, *L*-His or *L*-Lys) to the aqueous solution of PCA, resulting in a change in the pH of the system. In order to verify this conjecture, we tested the pH of the system in a secondary aqueous solution. PCA was dissolved in the secondary aqueous solution and following the addition of the three basic amino acids, the changes in pH were observed (see Figure S6).

2.2 PCA test strips detect different concentrations of arginine

Given that PDI can identify arginine, we explored arginine in secondary water and an arginine/PCA

solution, respectively, and found that arginine itself is not greatly affected by pH. Thus, for the PCA detection of arginine, the mechanism may be controlled by pH and the arginine chemical structure. Since PCA can detect arginine in conventional aqueous solution, the following experiment was performed: clean test strips were placed into a 10^{-4} mol/L PCA solution. The fluorescence color change was observed with a 365 nm ultraviolet lamp at different concentrations of arginine (10^{-2} mol/L, 10^{-3} mol/L, 10^{-4} mol/L, 10^{-5} mol/L). As shown in Figure 6, the color of the test strip changed from 10^{-2}~10^{-5}mol/L and the fluorescent color from changed from purple-pink-reddish-brown-light yellow with the concentration of arginine, and so the PCA strip can quickly and efficiently detect different concentrations of arginine.

Figure 6. Characterization of fluorescence changes in PCA detection of arginine at different concentrations.

CONCLUSION

In summary, we have synthesized a novel *p*-styryl tetracarboxydiimine derivative, PCA, and used it for the detection of basic amino acids. Three basic amino acids were accurately identified, and PCA test strips were prepared to detect different concentrations of arginine. The system could also be employed as a dual-mode "on-off" fluorescent colorimetric probe for the detection of strong alkaline pH, presenting different fluorescent colors between pH 10.00 and 14.00. This allows for the visual observation of pH under alkaline conditions, showing good reversibility and excellent sensing characteristics. PCA was encapsulated in a polymer matrix, and the pH value of the system was changed, showing good pH sensing performance. This work shows that PCA fluorescent probes have high selectivity, high sensitivity and high reversibility, which

will have great potential for future practical applications.

ASSOCIATED CONTENT

Supporting Information

Preparation of PCA fluorescent probes, details of amino acid detection experiments, UV-Vis spectra, fluorescence spectra, chromatograms.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was financially supported by the Science and Technology Fund of Guizhou Province (ZK[2023]General 040). CR thanks the University of Hull for support.

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