Development of a rapid detection paper for cyanide ions in aqueous solutions using an organic reagent

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ABSTRACT

Cyanide is a toxic chemical agent classified as a blood agent, which has been previously used in warfare and acts of terrorism. Cyanide exerts its toxic effects primarily by inhibiting cytochrome oxidase (complex IV) in the mitochondrial electron transport chain, which is essential for aerobic cellular respiration. Numerous methods based on colorimetric reactions have been investigated for the detection of cyanide poisoning; however, these techniques typically suffer from low sensitivity, prolonged detection times, and safety risks due to direct exposure to the chemicals. This paper presents a novel method for detecting cyanide in aqueous solutions. Under the established optimal conditions, cyanide was detected with a low detection limit of about 20 ng/mL. This method has the potential to be developed into a rapid on-site detection kit for cyanide in aquatic solutions.

Keywords: Cyanide; Blood agents; Toxicity; Indicator.

1. INTRODUCTION

Cyanide is the most important compound in the group of blood agents and has been used in warfare and terrorism, etc., especially during World War I [1]. Cyanide poisons the mitochondrial electron transport chain within cells and renders the body unable to derive energy (adenosine triphosphate - ATP) from oxygen. Specifically, it binds to the a3 portion (complex IV) of cytochrome oxidase and prevents cells from using oxygen, causing rapid death [2]. Despite its high toxicity, cyanide is widely used in various fields such as industry, medicine, and agriculture [3]. In industry, cyanide is commonly used in the mining sector, especially in the extraction of gold and silver through the cyanidation process; In medicine, some cyanide derivatives are used as precursors in the synthesis of drugs for treating hypertension or anesthetics in controlled doses and in agriculture, cyanide has been used as a pesticide and fumigant to protect crops and eliminate pests [4-6]. Because cyanide is widely used in various practical applications, the risk of human poisoning remains a constant concern.

Several detection kits for cyanide in water have been developed, such as the M272 Water kit, which can detect cyanide with a sensitivity of 2.0 µg/mL [7]; and the Russian K-54 kit, which is capable of detecting cyanide with an optimal detection limit of approximately 5 µg/mL [8]. However, QCVN 01-1:2018 [9], the regulation on the quality of clean domestic water, requires that the cyanide concentration in water does not exceed 0.05 µg/mL. The cyanide detection kit we previously developed has a detection limit of 50 ng/mL and a detection time of 15 minutes [10].

This kit requires a relatively long detection time and specialized training for users, making it unsuitable for practical application. Recent studies worldwide have also focused on the development of colorimetric sensing systems using pyridinium-based reagents such as 2-HPEAPB for real-time cyanide ion detection. For instance, Chen et al. [11] reported a protonated merocyanine system that exhibited rapid and distinct color changes in ethanol within only a few seconds upon exposure to cyanide, highlighting the suitability of this reagent class for fast and selective sensing. Unlike previous test kits that require long detection times or exhibit limited sensitivity, the present study focuses on the development of a cyanide detection test paper with significantly enhanced sensitivity. By immobilizing the organic reagent 2-HPEAPB onto cellulose filter paper, we achieved a detection limit as low as 20 ng/mL, which is substantially lower than that of existing field detection systems such as the M272 or K-54 kits. This novel test paper allows for visual detection within less than one minute without the need for instrumentation, representing a significant advancement in rapid, on-site monitoring of cyanide contamination.

2. EXPERIMENTAL

2.1. Chemicals and equipment

Chemicals: 4-methylpyridine (98%, Merck), 4-hydroxybenzaldehyde (98%, Merck), acetic acid (99,5%, Acros), allyl bromide (99%, Acros), ethanol (99%, Fisher), acetonitrile (99.9%, Fisher), deionized water (Vietnam), methanol (99.9%, Acros), glycerol (99%, Acros), Tween-20 (AR, Fischer).

Equipments: Electronic analytical balance (Mettler Toledo, Switzerland) with an accuracy of ± 0.1 mg; Ultrasonic cleaner (Elma S100H, Germany); UV-Vis Agilent-8453, wavelength range 190-900 nm (USA), Glass test tubes (12 × 100) mm (China); GF/C filter paper (Whatman), cellulose filter paper (Whatman), cellulose acetate filter (Sigma), Biologix micropipette (20-200 µL, USA) with compatible conical tips and along with various other standard laboratory tools were utilized.

2.2. Research methods

2.2.1. The method for preparing the original standard sample and the intermediate KCN solution

Preparation Method for Stock Solution Referencing TCVN 7723-1:2015 [12]:

KCN Stock Solution, ρ_{CN} = 1000 mg/L: Dissolve 125 mg of potassium cyanide (KCN) in sodium hydroxide solution (c = 0.01 mol/L) in a 50 mL volumetric flask and dilute to the mark with the same sodium hydroxide solution.

Intermediate KCN solution, ρ_{CN} = 100 mg/L: Pipette 10 mL of the above stock solution into a 100 mL volumetric flask and dilute to the mark with sodium hydroxide solution (c = 0.01 mol/L).

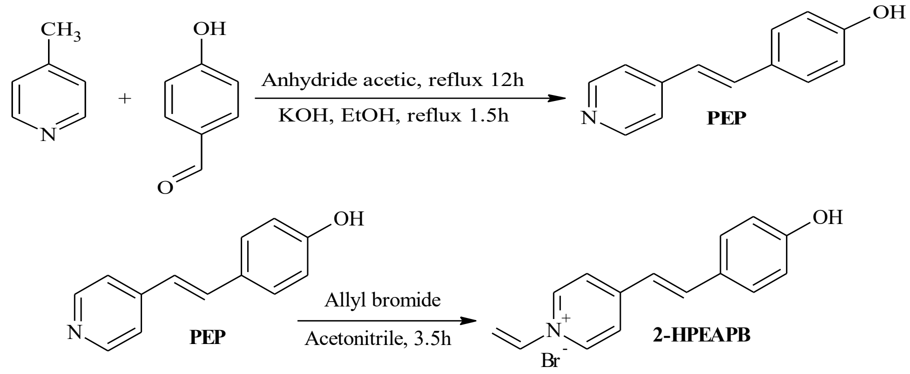
From the intermediate KCN solution (ρ_{CN} = 100 mg/L), a dilution was performed to obtain a concentration of 1 mg/L and subsequently, prepare a series of solutions with cyanide concentrations of 5, 20, 50, 100, 200 and 500 ng/mL.

2.2.2. Synthesis of 2-HPEAPB

Synthesis procedure of the ligand 2-HPEAPB is as follows:

Stage 1: The compound 4-[(E)-2-(4-pyridinyl)ethenyl]phenol (PEP) was synthesized through a condensation reaction between 4-hydroxybenzaldehyde with 4-methylpyridine in the presence of acetic anhydride. Under a nitrogen atmosphere, 4-methylpyridine (2.42 g, 26 mmol) was gradually introduced into a stirred solution of 4-hydroxybenzaldehyde (3.20 g, 26 mmol) dissolved in 30 mL of acetic anhydride. The reaction mixture was then refluxed for 24 hours. After completion, the mixture was allowed to cool to room temperature and subsequently poured into 300 mL of ice-cold water. Stirring was continued for 1.5 hours to ensure complete hydrolysis of the excess acetic anhydride. The resulting solid was collected by filtration, washed thoroughly with cold water, and purified by recrystallization from ethanol. The obtained solid was subsequently refluxed for 90 minutes in 150 mL of ethanol containing 1.4 g of KOH, producing a dark-colored solution. Upon adjusting the pH to 5-6 using acetic acid, a pale yellow precipitate formed. This solid was isolated by filtration and then freeze-dried to obtain PEP.

Stage 2: Conversion of PEP to 2-HPEAPB through an *N*-alkylation reaction using allyl bromide as the alkylating agent, yielding a crude orange-yellow solid product. A mixture of PEP (1.0 g, 5 mmol) and 25 mL of anhydrous acetonitrile was added to a 100 mL round-bottom flask. The solution was stirred and heated to 60 °C before allyl bromide (0.93 g, 7.67 mmol) was slowly introduced dropwise over a period of 10 minutes. The reaction was maintained under reflux for 4 hours, during which the color transitioned from deep red to orange. Upon completion, the reaction mixture was allowed to cool to ambient temperature. The resulting solid was collected by filtration, washed sequentially with acetonitrile and methanol, and dried to obtain 2-HPEAPB as a yellow powder. Final purification was carried out via recrystallization from ethanol.



2.2.3. Preparation of test paper and evaluation of its capability for cyanide detection in aqueous environments

The preparation process of the test paper was carried out according to the schematic diagram below:

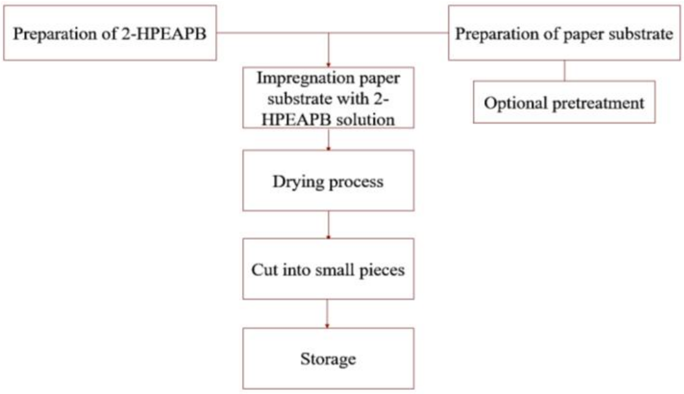


***Figure 1.** The preparation process of the test paper.*

Solution-based colorimetric assay:

For each assay, 90 µL of the 2-HPEAPB stock solution was mixed with 450 µL of the cyanide standard solution of the desired concentration and 150 µL of deionized water in a 10 mL glass test tube. The final volume was adjusted to 3.0 mL with acetonitrile, and the mixture was briefly vortexed. Color changes were monitored visually.

Preparation of test papers:

Filter substrates, including GF/C fiber filters, cellulose filter papers (80–100 g/m²), and cellulose acetate filters, were examined. To improve reagent loading and surface affinity, cellulose filter papers were pretreated with 1–2% sodium carbonate to activate hydroxyl groups, followed by a thin polyethylenimine (PEI) coating. The substrates were then impregnated with a methanolic solution of 2-HPEAPB (3.0×10^{-2} M) containing polyvinylpyrrolidone (0.3%), glycerol (0.5%), Tween-20 (0.02%), and borate buffer (0.02 M, pH 9.0–9.5). Impregnation was performed for 30 min, after which the papers were dried stepwise at 40 °C, 60 °C, and finally 80 °C. The dried sheets were sealed in aluminum pouches with desiccant for storage.

Optimization and evaluation of influential parameters: Substrate type: GF/C fiber filters, cellulose filter papers, and cellulose acetate filter; Reagent concentration: 2-HPEAPB in the range of 10–50 mM; Impregnation time: 10–40 min; Formulation additives: presence or absence of PVP, glycerol, Tween-20, and borate buffer; Drying protocol: stepwise heating vs. single-stage drying.

Paper-based colorimetric assay: Drop the cyanide-containing solution onto the filter paper, observe the color of the paper, and compare it with the control sample using distilled water.

3. RESULTS AND DISCUSSION

3.1. Results of the synthesis of the 2-HPEAPB reagent

The synthesis of 2-HPEAPB is carried out in two stages: the first stage involves the reaction between 4-methylpyridine and 4-hydroxybenzaldehyde in acetic anhydride to produce 4-[(E)-2-(4-pyridinyl)ethenyl]phenol (PEP) (90.4%), and the second stage involves the reaction of PEP with allyl bromide in acetonitrile to obtain the final product, 2-HPEAPB (89.0%). The product is a yellow solid that is well soluble in ethanol. The overall yield of the synthesis reaches 80.5%. In the IR spectrum, characteristic absorption bands are observed, confirming the presence of functional groups in the product. The absorption band appearing in the 3300-2800 cm^{-1} region is characteristic of the stretching vibration of the –OH functional group. The band at 1636 cm^{-1} is typical of the stretching vibration of the C=C bond in alkenes, while the medium-to-weak absorption bands at 1560, 1518, and 1478 cm^{-1} are characteristic of the stretching vibrations of C=C bonds in aromatic rings. The reaction mechanism for the synthesis of 2-HPEAPB is well-established. This is a previously known compound [11], and its identity has been confirmed by comparison of its appearance and IR spectrum with literature data, showing a good match (spectral similarity of 98,5%). Therefore, it is not necessary to perform additional NMR analysis.

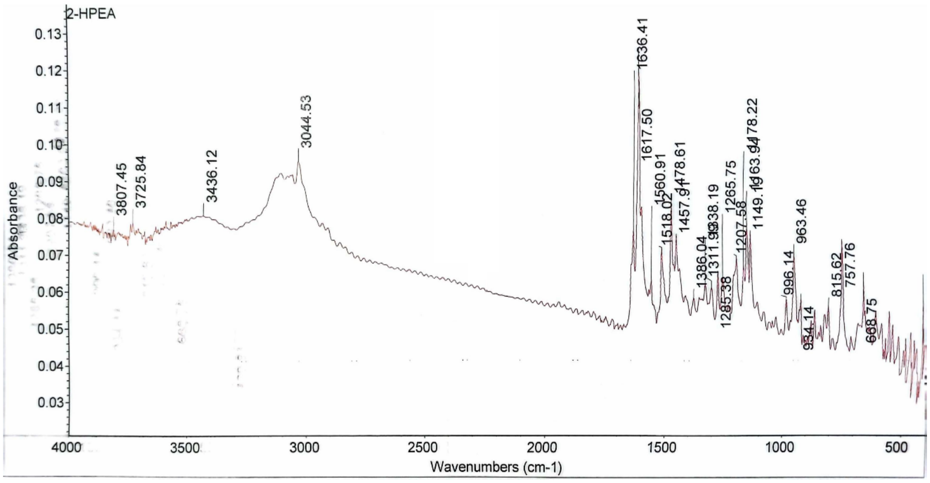


Figure 2. IR spectrum of 2-HPEAPB.

In essence, the detection of cyanide ions in the sample is based on the reaction between the CN^- ion and the reagent 2-HPEAPB. The reaction between 2-HPEAPB and the cyanide ion proceeds via a nucleophilic addition mechanism, which is characteristic of aromatic heterocyclic systems bearing a positive charge.

Pyridinium is an aromatic heterocyclic compound with a delocalized positive charge distributed over the ring. Among the ring positions, C2 exhibits the highest degree of positive character, making it the most susceptible site for nucleophilic attack by species such as the cyanide ion (CN^-).

3.2. Determination of the detection limit of cyanide in solution using the 2-HPEAPB reagent

In this study, the detection capability of cyanide ions (CN^-) in aqueous solution was evaluated using an organic reagent, namely 2-HPEAPB.

The chemosensor exhibited a rapid and selective colorimetric response to CN^- in ethanol, with a clear color change from colorless to light yellow observable by the naked eye within seconds. The blank sample showed no color change, whereas the test samples exhibited a gradual increase in color intensity within the range of 5 ng/mL to 500 ng/mL. The limit of detection (LOD) of CN^- in aqueous solution was determined to be 5 ng/mL based on the visual detection.

Based on the results obtained above, a calibration curve was constructed using a UV-Vis Agilent-8453 spectrophotometer, operating in the wavelength range of 190-900 nm. The samples were prepared at concentrations of 5 ng/mL, 20 ng/mL, 50 ng/L, 100 ng/L, 200 ng/mL, and 500 mg/L. The solutions in test tubes were transferred to 10 mm pathlength quartz cuvettes for UV measurement at a wavelength of 531 nm. The resulting calibration curve is presented in figure 3.

Thus, CN^- ions can be rapidly detected using the 2-HPEAPB reagent. The color change of the solution is easily observable. The detection limit was determined to be 5 ng/mL. The corresponding calibration curve was established as follows: $Y = 0.0006x + 0.0573$ with $R^2 = 0.9998$. The calibration curve obtained using UV-Vis spectroscopy also demonstrates excellent linearity ($R^2 = 0.9998$), which is essential for quantitative analysis.

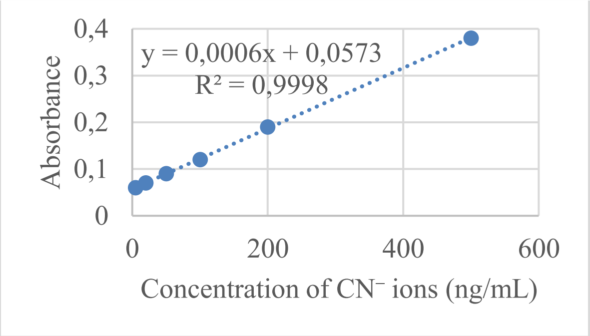


Figure 3. *Calibration curve illustrating the correlation between absorbance and concentration.*

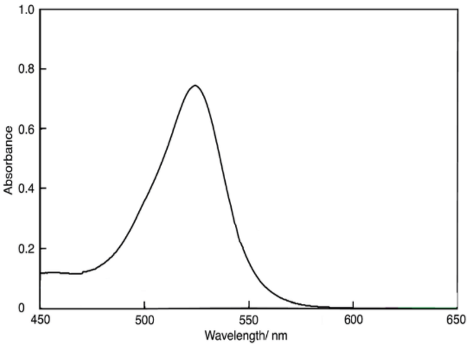


Figure 4. *UV-Vis spectrum of 2-HPEAPB with CN^- ion.*

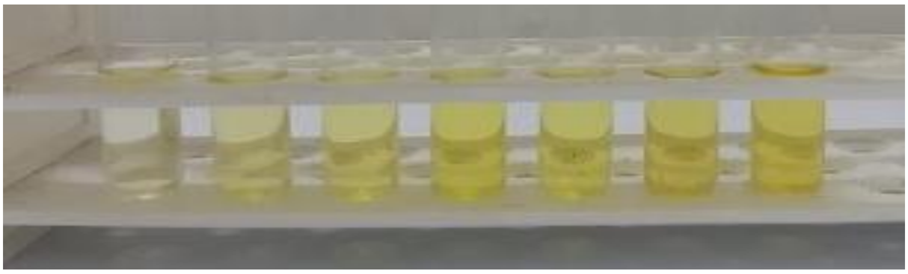


Figure 5. *The color change of the solution according to the concentration of CN^- ions.*

These results confirm that 2-HPEAPB is an effective probe for detecting trace levels of cyanide in liquid media. The reagent offers several notable advantages, including simplicity of use, rapid response time, and the ability to detect low concentrations of CN^- without requiring sophisticated instrumentation. The clear visual color change from colorless to light yellow enables on-site and real-time detection, which is particularly valuable in environmental monitoring or emergency response scenarios involving potential cyanide contamination.

3.3. Detection limit determination of the cyanide test paper

The design model for fabricating the cyanide detection paper was selected based on the reference by Lianfang Chen et al., with several modifications [11]. The test papers evaluated for their ability to immobilize the 2-HPEAPB reagent included GF/C fiber filters, cellulose filter papers, and cellulose acetate filter.

Different filter substrates were evaluated to identify the most suitable carrier for 2-HPEAPB. While GF/C fiber filters provided high porosity, they exhibited brittleness after drying and poor mechanical strength. The cellulose acetate filter showed insufficient impregnation due to low absorbency. In contrast, cellulose filter paper demonstrated superior reagent uptake, homogeneity of color distribution, and mechanical durability. Pretreatment with Na_2CO_3 activated hydroxyl groups on cellulose, while PEI coating introduced cationic functional groups that promoted electrostatic interaction with the cationic pyridinium moiety of 2-HPEAPB. This resulted in improved reagent immobilization and reduced dye leaching.

The concentration of 2-HPEAPB in the impregnation solution was a critical parameter. Low concentrations (< 10 mM) resulted in weak color responses, whereas excessively high concentrations (> 50 mM) caused crystallization and uneven background coloration. An optimized concentration of ~30 mM provided both strong signal intensity and a stable background. The impregnation time also influenced performance: shorter times (< 10 min) led to incomplete loading, while prolonged exposure (> 40 min) caused structural weakening of the substrate. A 30-minute impregnation was found optimal, ensuring sufficient reagent uptake without compromising mechanical strength.

Additives played a key role in enhancing the performance of test papers. PVP acted as a polymeric binder, reducing cracking during drying and improving uniformity. Glycerol functioned as a plasticizer, maintaining flexibility and reducing brittleness under low humidity. Tween-20 improved wetting and ensured homogeneous spreading of the solution across the substrate. Borate buffer (pH 9.0–9.5) preconditioned the paper environment, providing optimal alkalinity for cyanide detection and stabilizing the chromogenic response. Collectively, these additives contributed to enhanced sensitivity, reproducibility, and long-term stability.

Drying conditions strongly affected color stability. Single-stage drying (e.g., direct heating at 80 °C) often led to uneven reagent deposition and partial degradation of 2-HPEAPB. In contrast, a stepwise drying process (40 °C → 60 °C → 80 °C) resulted in gradual solvent removal, minimizing structural stress and yielding test papers with superior reproducibility, reduced background noise, and extended shelf life.

The observed color changes of the test papers impregnated with 2-HPEAPB at different CN^- ion concentrations are presented in figure 5.

Figure 5. *Colorimetric response of the test paper to different cyanide concentrations.*

From figure 5, the determined limit of detection is 20 ng/mL. The color of the test paper changes immediately and remains stable for 5-10 minutes, making it suitable for on-site analysis. Importantly, the cyanide detection paper was not affected by interfering ions such as NO_3^-, F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, and HSO_4^-, confirming its high selectivity toward cyanide ions.

Unlike conventional test papers prepared by simple impregnation, the present study introduces a systematically optimized fabrication process for 2-HPEAPB-based paper sensors. By combining substrate pretreatment (Na_2CO_3 activation and PEI coating), functional additives (PVP, glycerol, Tween-20, borate buffer), and controlled drying protocols, the resulting test paper exhibits enhanced color uniformity, faster response.

4. CONCLUSIONS

Cyanide in aqueous solution was successfully detected using a paper-based sensor incorporating the organic reagent 2-HPEAPB. Optimization of key fabrication factors – including substrate selection, impregnation concentration and time, additive formulation, and drying protocols – was critical to achieving high performance. The optimized cellulose-based papers, pretreated with Na_2CO_3/PEI and supplemented with PVP, glycerol, Tween-20, and borate buffer, exhibited superior reagent immobilization and color uniformity. Upon exposure to cyanide, the test paper showed an immediate and visually distinct color change, which remained stable for 5–7 minutes under field conditions. With a detection limit as low as 20 ng/mL, the optimized sensor demonstrated markedly higher sensitivity, faster response, and improved robustness compared to conventional field test kits such as M272 and K-54.

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TÓM TẮT

Phát triển giấy thử phát hiện nhanh ion xyanua trong môi trường nước sử dụng thuốc thử hữu cơ

Xyanua là một tác nhân hóa học độc hại được xếp vào nhóm chất độc toàn thân, từng được sử dụng trong chiến tranh và các vụ khủng bố. Cơ chế gây độc chính của xyanua là ức chế enzyme cytochrome oxidase (phức hợp IV) trong chuỗi vận chuyển điện tử ty thể, vốn đóng vai trò thiết yếu trong quá trình hô hấp của tế bào hiếu khí. Nhiều phương pháp dựa trên phản ứng tạo màu đã được nghiên cứu để phát hiện cyanide; tuy nhiên, các kỹ thuật này thường có nhược điểm là độ nhạy thấp, thời gian phát hiện kéo dài và tiềm ẩn nguy cơ mất an toàn do tiếp xúc trực tiếp với hóa chất. Bài báo này giới thiệu một phương pháp mới để phát hiện nhanh xyanua trong môi trường nước. Trong các điều kiện tối ưu đã thiết lập, xyanua được phát hiện với giới hạn phát hiện thấp, khoảng 20 ng/mL. Phương pháp này có tiềm năng được ứng dụng để phát hiện nhanh tại hiện trường đối với xyanua trong môi trường nước.

Từ khoá: Xyanua; Chất độc toàn thân; Độc tính; Chỉ thị.