

Cyanide detection based on natural dyes reaction from blue butterfly pea flowers (*Clitoria Ternatea*)

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Abstract: A green spectrophotometric method for the determination of cyanide has been proposed using, a green reagent, aqueous extract of blue butterfly pea. The test tube was filled with anthocyanin rich extract (pH 6) and cyanide solution. The reaction was kept constant for 10 minutes at room temperature. The reaction mixture changed color from blue to green as the amount of CN⁻ ions increased. The 620 nm peak intensity increased with CN concentration. Therefore, this wavelength was used for all cyanide analyses. The cyanide calibration curve had a linear range of 0.25-1.00, 1.00-4.00, and 4.00-10.00 mg/L, with a satisfactory correlation coefficient of 0.99 and a LOD of 0.57 mg/L. The recovery ranged from 8.33 to 76.94 percent, indicating that this method is inaccurate at low cyanide concentrations. The intra-day and intermediate precision relative deviations were 0.391-0.871 % and 1.112-1.583 %. An H-bond forms between the C-4 group of the B-carbonyl ring and the HCN molecule according to the B3LYP/TZVP calculation. The method is convenient for cyanide concentrations above the LOQ of 1.09 mg/L, cost-effective, and capable of reducing toxic solvents with acceptable precision. The method could also be used to detect total cyanide in biological, environmental, and industrial waste samples.

Key words: cyanide, blue butterfly pea flower, spectrophotometry, green method

1. Introduction

Due to the critical role of cyanide (CN⁻) ions, their detection has received considerable attention. Cyanide is found throughout the ecosystem and has been linked to toxicity in humans and animals. As a result, there has been considerable interest in developing methods for detecting CN in a variety of samples. While electrochemistry,¹ fluorometry,² chromatography,³ and

flow injection analysis⁴ are all effective method for detecting anions, the simple colorimetric method is the most appealing because it allows for easy observation, cost effectiveness, and rapid detection with the naked eye without the use of large, sophisticated, time-consuming, and expensive analytical instruments. It was critical when a colorimetric technique was developed that altered the color of visible light. This has become increasingly critical in recent years.

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Numerous reports on colorimetric and fluorometric reagent for cyanide detection have been published, including thiazolidinone derivatives,⁵ boronic acid derivatives,⁶ Co(III)-corrinoids,⁷ and aniline derivatives.⁸ Each method's selective and reaction pathways are determined by its structure. However, there are a number of disadvantages to using these sensors, including the fact that they must be performed in organic solvents or mixtures of organic solvents, which are hazardous to humans and the environment.

Anthocyanin is a colored water-soluble dye that is found in flowers and fruits. It has a positive charge at the oxygen atom located in the C-ring of the basic flavonoid structure. An anion may react with anthocyanin structures via nucleophilic addition, complexation, or hydrogen bonding.⁹⁻¹¹

This study describes for the first time the use of anthocyanin extract from butterfly pea petals as a natural dye for cyanide detection. We conducted spectroscopic studies at various pH levels to determine the optimal conditions for anthocyanin-HCN interaction. The method's linearity, accuracy, and precision were evaluated. Additionally, structural and energetic properties of anthocyanin-HCN were determined using B3LYP and TZVP calculations.

2. Experimental

2.1. Chemicals and reagents

The following Sigma Aldrich products were used: potassium cyanide, sodium hydroxide, and hydrochloric acid. The butterfly pea flower (*Clitoria Ternatea*) was collected in Nakhon Ratchasima, Thailand, from a local garden. The chemicals used in this study were all of the Analytical grade.

2.2. Aqueous extraction of natural blue dyes

The petals of blue butterfly pea flowers were dried for 2 hours at 50 °C in an oven. The dried petals were ground to a fine powder using a blender, and stored in a zip-lock bag in a desiccator until needed. 2 g of dried powder was dissolved in 100 mL distilled water at room temperature. The extract mixture was

filtered through a fine cloth, manually pressing it, and finally filtering through filter paper. The natural blue dye solution was kept at 4 °C in a dark-colored bottle until it was used.

2.3. Determination of optimal pH of natural blue dyes for cyanide detection

The following procedure was used to determine the optimal pH of blue butterfly pea extract for cyanide binding. 1 mL of the pH 6 extract was added to the vial, and the pH was adjusted to 5-12 using a buffer solution. After combining the extract solutions with varying concentrations of KCN, each mixture solution was diluted with buffer to a volume of 4 mL. It was kept at room temperature throughout the experiment. Each mixing solution's UV-Vis absorption spectrum was recorded from 250 to 700 nm.

2.4. Procedure for cyanide response

The blue butterfly pea aqueous extract (pH6.0) was combined with the KCN solution in a vial, and the volume was adjusted with buffer to obtain the desired cyanide concentration. The absorbance spectrum of each mixing solution was recorded after ten minutes of mixing at various cyanide concentrations (0.25-10 mg/L). This method was validated for linearity, precision, intermediate precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ).

2.5. Structure and energetics

B3LYP/TZVP calculations were used to determine the structural and energetic properties of anthocyanin-HCN. All of the chemical calculations were carried out using the Gaussian03 software package. The interaction between anthocyanin and HCN was quantified using the proton donor-acceptor distance (dDA), as well as the distance between the dC-H covalent bond (dC-H) and the HO hydrogen bond (dHO). The interaction energy (DE) and the asymmetric stretching coordinate (DdDa), which are calculated using the equation $DdDa = |dC-H-dHO|$, respectively.

3. Results and Discussion

3.1. The study of the reaction between butterfly pea petals extracts and cyanide

Butterfly pea petals extract, a natural dye, is composed of flavonol glycoside or anthocyanin. According to anthocyanin's structure it contains a positively charged oxygen atom in the C-ring of the basic flavonoid structure. Typically, they are composed of anthocyanidin or an acyl group-containing aglycone.¹³ Cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin are the most prevalent anthocyanidins.¹³

Anthocyanins, an amphoteric compound, can react with acids or bases. When the solution's pH is increased to alkaline, the wavelength of maximal visible absorption shifts and the red color is also visible in purple and blue. This shift of wavelength and color change is caused by the groups attached to the bond position's basic structure. It is possible to determine whether plant anthocyanin extracts are capable of reacting with cyanide. To identify anthocyanin extract from butterfly pea using colorimetric analysis, cyanide solution at a concentration of 0.25 mg/L is added (pH 6).

Blue butterfly pea aqueous extract (pH 6.0) exhibited a blue hue and an absorption peak in the visible region between 570 and 620 nm (*Fig. 1*). These

wavelengths are consistent with previously defined anthocyanin spectra for *C. ternatea*, which focused on the 500-700 nm region.¹⁴⁻¹⁶

In comparison to the pure anthocyanin extract solution, the spectrum of the anthocyanin rich extract-CN adduct exhibited both a hypsochromic and a hypochromic shift in absorption. This phenomenon could be caused by the presence of cyanide, which interacts with the anthocyanin structure, resulting in the loss of conjugation. Teritins (malonylated delphinidin 3, 3', 5'-triglucosides) have been identified as the major component of *C. ternatea*,¹⁷⁻¹⁹ and they would be the primary reactant for cyanide detection.

3.2. The optimal pH of aqueous extract of blue butterfly pea for detection of cyanide

Anthocyanin's molecular structure is pH-dependent, which contributes significantly to its ability to react with nucleophiles. Although there are numerous reports on anthocyanin reactions with nucleophiles, all of them state that nucleophile reactions occur in an acidic environment.²⁰⁻²² Due to a highly toxic gas, was produced by adding cyanide salts to a low pH solution as hydrocyanic acid (HCN), Prior to the cyanide reaction, the pH of the blue butterfly pea extract was adjusted to between 5 and 12 using a buffer solution, The spectrum of the anthocyanin-

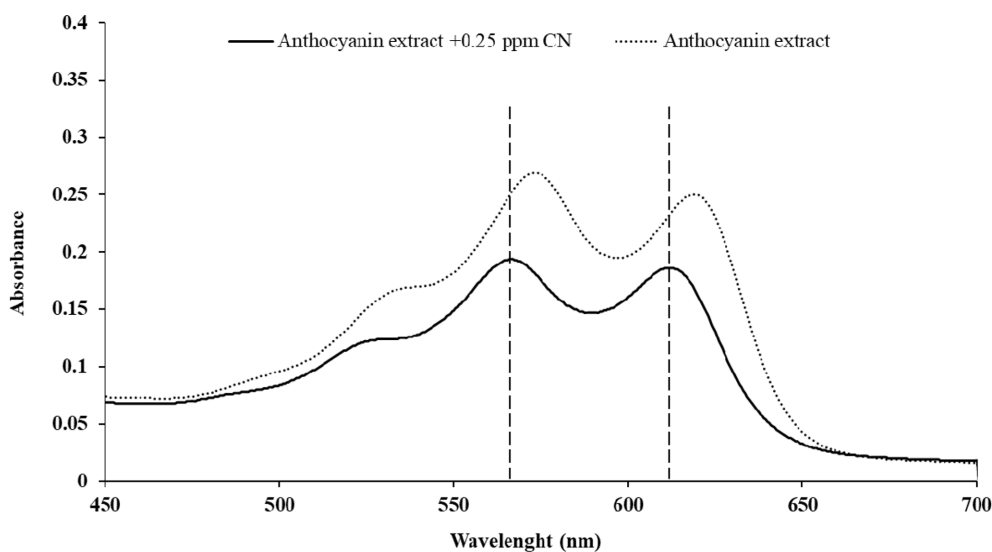


Fig. 1. Visible spectrum change of anthocyanin extract pH 6 from butterfly pea with and without cyanide.

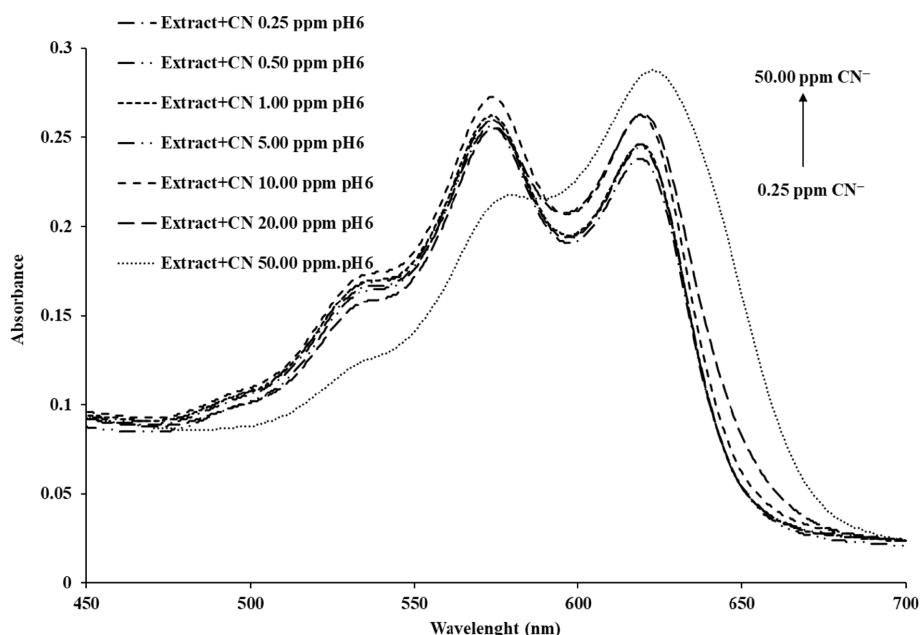


Fig. 2. Changes in visible spectra of a pH6 anthocyanin extract after adding at cyanide various concentrations.

Table 1. Wavelength of maximum absorption and peak appearance of anthocyanin-HCN adduct compared to anthocyanin

The pH of aqueous of blue butterfly pea	Wavelength of maximum absorption	Peak appearance
pH5 + 0.25 ppm CN	570 and 620 nm	Absorbance decrease
pH6 + 0.25 ppm CN	570 and 620 nm	Absorbance decrease and hypsochromic shift
pH7 + 0.25 ppm CN	570 and 620 nm	No obvious change
pH8 + 0.25 ppm CN	620 nm	Absorbance increase
pH9 + 0.25 ppm CN	620 nm	Small hypsochromic shift
pH10 + 0.25 ppm CN	600 nm	Small hypsochromic shift
pH11 + 0.25 ppm CN	600 nm	Small hypsochromic shift
pH12 + 0.25 ppm CN	600 nm	Small hypsochromic shift

HCN adduct at various pH values in comparison to the anthocyanin extract solution is shown in Fig. 2. Between pH values of 5 and 9, cyanide exists primarily as an HCN molecule in aqueous solution. This is because of its pKa value (9.24). Thus, anthocyanins would react with an HCN molecule rather than cyanide ions (CN⁻).

Table 1 showed the wavelength of maximum absorption and peak appearance of anthocyanin-HCN adduct compared to anthocyanin. The anthocyanin-HCN adduct solution at pH 5 decreased the absorbance at the anthocyanins' characteristic absorption wavelengths (570 and 620 nm), which was identical

to the anthocyanin extract solution at the same pH. Under neutral and weakly acidic conditions, this reaction occurs when the anthocyanin chromophore is hydrated at 2-4 positions, resulting in the formation of carbinol pseudobase.²³

The absorption spectrum of the blue butterfly pea extract with HCN at pH 6 revealed a hypsochromic shift in the maximum wavelength range of 5-10 nm (DI = 5-10 nm), indicating that the p-electron in the conjugate structure of quinonoidal anhydrobase was redistributed as a result of HCN molecules' s interaction with the anthocyanin structure.

At pH 7, the anthocyanin extract-HCN adduct also

exhibited the absorption peak at 570 and 620 nm but, there was no obvious change in the spectrum. It is proposed that HCN cannot interact to anthocyanin structure. At pH 8-9, both the anthocyanin extract-HCN adduct exhibited an absorption band peaking at 620 nm which corresponded to anionic quinonoidal base species, as well as UV absorption at 398 nm, which corresponded to anthocyanins in their colorless chalcone form (data not shown).^{16,23,24} The phenomenon of anthocyanin absorption could be due to the anthocyanin structure extending conjugation, implying an interaction between anthocyanin and HCN at pH 8-9.^{20,25,26}

HCN readily dissociates into free cyanide ions (CN^-) at pH greater than pK_a , whereas anthocyanins are primarily found in the chalcone structure (the open pyran ring).²³ The spectrum of the anthocyanin- CN^- adduct at pH 10, 11, 12 exhibits only one peak around 600 nm with hypsochromic and hypochromic shifts at the anthocyanin's maximum absorption wavelength. It has been suggested that the formation of hydrogen bonding in the structure exhibits a hypsochromic shift in the presence of CN^- .^{27,28} It is possible that the charge transfer complex or hydrogen bonding interaction occurs within the anthocyanin skeleton structure as a result of this discovery. These findings indicate that the anthocyanin-rich blue butterfly pea aqueous extract is capable of detecting cyanide.

The visible absorption spectrum of an aqueous extract of blue butterfly pea containing cyanide species was further investigated to determine the optimal pH for cyanide detection. This study examined cyanide concentrations ranging from 0.25 to 50 mg/L. The absorption spectrum of anthocyanin at pH 6 with various concentrations of cyanide added is shown in Fig. 2. Interestingly, as the cyanide concentration increased at a constant pH of 6, the bathochromic shift occurred and the intensity of the 620 nm peak increased significantly. As a result, the optimal pH for cyanide reaction with an anthocyanin-rich extract was determined to be pH 6.

According to the data presented for the addition of various concentrations of cyanide to other pH solutions, the intensity of the absorption spectrum of

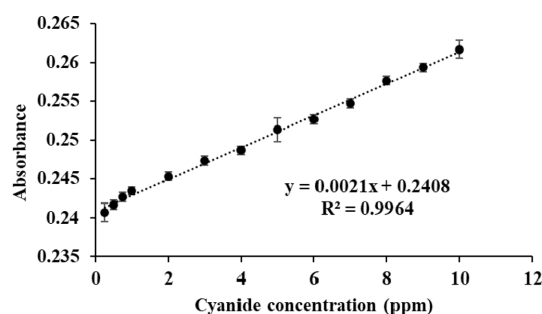


Fig. 3. Linearity relationship between the absorbance (620 nm) and cyanide concentration.

anthocyanin- CN^- did not increase as the amount of cyanide increased (data not shown). As a result, the optimal pH for cyanide reaction with anthocyanin-rich extract was pH 6.

3.3. Linearity

The absorbance at 620 nm was plotted against the cyanide concentration, with anthocyanin extract serving as the sample blank. The absorbance of the anthocyanin-cyanide adduct was found to be linearly related to the cyanide concentration in the range 0.25-1.00 ppm and 1.00-10.00 mg/L. This corresponded to the findings presented in Fig. 3. The linear range was from 0.25-10.00 mg/L for cyanide with correlation coefficients higher than 0.99.

3.4. Calibration curves and limit of detection

Calibration curves for this study were created using four cyanide ion working solutions and anthocyanin extract as a sample blank. The concentrations

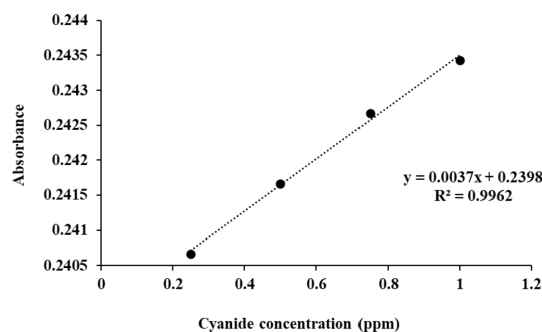


Fig. 4. Calibration curve for cyanide at a range of concentration in butterfly pea extract.

Table 2. Linear range, correlation coefficient, LOD and LOQ of cyanide

Linear range (mg/L)	Regression equation	Correlation coefficient (R)	LOD (mg/L)	LOQ (mg/L)
0.25-1.00	Y=0.0037x+0.2398	0.996		
1.00-4.00	Y=0.0018x+0.2418	0.993	0.57	1.09
4.00-10.00	Y=0.0022x+0.2398	0.998		

of CN⁻ ion working solution used as standards are 0.25, 0.5, 0.75, 1.00 mg/L, as shown in Fig. 4.

We can construct second and third calibration curves using cyanide concentrations of 1.00-4.00 mg/L and 4.00-10.00 mg/L, respectively, based on the linearity of this method. All calibration curves for working solutions were linear and had a correlation coefficient greater than 0.99. The limit of detection (LOD) and limit of quantification (LOQ) were estimated based on the analysis sample blank ten times and found to be 0.57 and 1.09 mg/L, respectively. Table 2 summarizes the regression equation, correlation coefficient (R²), linear range, LOD, and LOQ.

3.5. Accuracy and precision

To evaluate the accuracy of the present method, the recovery was investigated by spiking blank sample with three level of cyanide standard. The recoveries of spiked samples varied from 8.33 to 76.94 %. Recovery is proportional to the amount of cyanide added. The increased cyanide concentration demonstrated that the increased recovery resulted in increased method accuracy. At 0.25 mg/L cyanide added, this value is less than the method's limit of detection (LOD), resulting in low recovery and accuracy.

Because hydrogen bonding is the proposed interaction between anthocyanin and HCN molecule, a lack of 100 % recovery for this method may indicate hydrogen bonding donor interference. Tyramine, hydrogen bonding donor compound found in sample matrix in this study, was used to study the effect of interference.

The results indicated that when tyramine was added to the reaction, the absorbance at 620 nm, which was used to determine cyanide, decreased and the intensity of the absorption spectrum of anthocyanin-HCN adduct remained constant as the amount of cyanide increased (data not shown). It could be concluded that the compounds, which can be hydrogen bonding donor, can interfere with the anthocyanin-HCN interaction.

The precision of the method was determined using repeatability (intra-day) and intermediate precision (inter-day) in three sample concentrations with five replicates. Precision within and between days was satisfactory, with an RSD of less than 2 %²⁹ (Table 3).

3.6. Structure of Anthocyanin-HCN complex

As previously discussed, it is hypothesized that anthocyanin's neutral quinobinal anhydrobase form interacts with the HCN molecule at pH 6. According to some reports, the nucleophilic adduct of anthocyanin is located in the C-4 position (B-ring) rather than the C-2 position in the C ring. Following the C-4 addition of anthocyanin, the conjugated double bond system was retained, resulting in the light absorption ability and color of solutions. On the other hand, the addition reaction of anthocyanin at the C-2 position was slower than the addition reaction at the C-4 position, and the addition of nucleophilic at this position disrupted the conjugated system of structure, resulting in a decrease in absorption intensity and loss of color.^{16,19,20-22,26,30} The carbonyl oxygen atom at

Table 3. Recoveries and RSD for cyanide (n=5)

Cyanide added (mg/L)	Recovery%	Intra-day (%RSD)	Inter-day (%RSD)
0.25	8.33	0.871	1.239
1.00	52.38	0.839	1.112
10.00	76.94	0.391	1.583

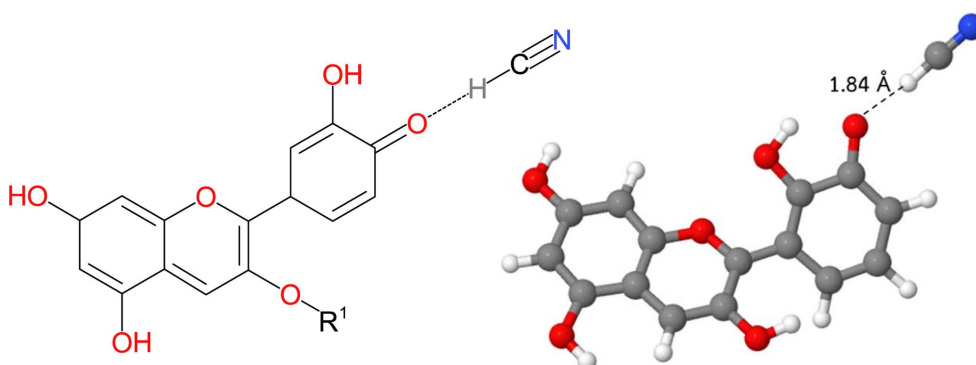


Fig. 5. Optimized structure of Anthocyanin-HCN with B3LYP/TZVP method.

anthocyanin's C-4 position is also expected to form hydrogen bonding interactions with HCN in this case, which is consistent with the absorption spectra and colors obtained for the anthocyanin-cyanide adduct.

The optimized molecular geometrical structure of anthocyanin-HCN is depicted in Fig. 5. This complex is identified by the formation of a hydrogen bond between the anthocyanin's carbonyl oxygen atom and HCN.²⁴⁻²⁶ The B3LYP/TZVP calculation yields a 1:1 anthocyanin-HCN equilibrium structure, with an H-bond forming between the C-4 group of the B-carbonyl ring and the HCN molecule. The intermolecular H-bond distance between O and H is 1.84, which is slightly less than the intermolecular H-bond distances between glycine and HCN (2.04)³⁰ and carbonyl compounds and HCN (1.93-2.71).^{31,32} The length of the bond between the proton donor and acceptor, as well as the asymmetric stretching coordinate, are proportional to the strength of the H-bond and the location of the proton. The smallest dDA value and a DdDA value close to zero revealed the proton's location in the H-bond, indicating a strong H-bond.^{13,33} The moderate dDA and DdDA values of 2.94 and 0.74 for the anthocyanin-HCN complex indicated a strong complex in comparison to other HCN-carbonyl complexes.³¹⁻³³

In this research, we present the novelty of using the natural dyes or anthocyanin-rich extracts from flowers petals for the detection of cyanide. The advantage of this method over other methods is that the reactions with natural dyes are less toxic to

humans and the environment than reactions involving synthetic reagents. Additionally, a comparison of this method's sensitivity and detection range revealed that it has a moderate sensitivity comparable to synthetic reagent used in other method.^{4,34}

4. Conclusions

We demonstrate a colorimetric method for detecting cyanide (CN⁻) ions using a natural dye that is simple, safe, and inexpensive. We observed changes in the visible spectrum of butterfly pea petals extract at 620 nm. The absorbance was proportional to the cyanide concentration with an acceptable correlation coefficient at this wavelength. Intra-day and intermediate inter-day values were less than 2%, resulting in increased precision. However, for cyanide concentrations greater than 10 mg/L, this method provided more accurate results.

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