

CYANIDE REACTION WITH NINHYDRIN: THE EFFECT OF pH CHANGES AND UV-VIS RADIATION UPON THE ANALYTICAL RESULTS

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Cyanide reaction with ninhydrin has been recommended for instant detection of small traces of cyanide (detection limit 1.5 ng ml^{-1}). However, pH and UV and visible light may induce errors. Therefore, we investigated the effect of pH, UV and visible radiation on cyanide reaction with ninhydrin in order to increase the method robustness. Cyanide can be determined with ninhydrin either at 485 nm or 590 nm, without any color interference.

INTRODUCTION

In pure aqueous solutions of sodium carbonate, ninhydrin was found to be a highly sensitive and selective chemosensor for cyanide, with a detection limit of as little as $0.025 \mu\text{g ml}^{-1} \text{CN}^-$ and a color change that could be observed by naked eye.¹⁻³ Santelli *et al.*,⁴ improved much the assay for detection of small traces of cyanide. Detection limit (1.5 ng mL^{-1}) has become comparable with that of the fluorimetric methods.^{5,6} Therefore, such a cyanide assay has attracted much attention and has been constantly improved so far.^{4,7}

Despite its performance, the commonly used analysis protocols based on ninhydrin-containing reagents are far from exhausting all the opportunities offered by ninhydrin reaction with cyanide. The pH changes as well as the UV and visible radiation may induce errors in cyanide detection and determination. Besides, cyanide reacts with ninhydrin to afford hydrindantin as the main product; afterwards, the resulted hydrindantin may form 2-cyano-1,2,3-trihydroxy-2*H* indene with another cyanide, a very unstable compound especially in the presence of oxygen.^{8,9} The influence of metal ions, pH, oxidizers, and UV irradiation on the formation of hydrindantin or 2-cyano-1,2,3-trihydroxy-2*H* indene on the reaction between cyanide and ninhydrin are less known.

Therefore, this paper aims at presenting only the effect of pH and UV and visible irradiation.

The reaction rate and mechanism are also discussed.

MATERIALS AND METHOD

Reagents

All chemicals were of analytical reagent grade and all solutions were prepared with milliQ grade water with $R = 18.2 \Omega$. A standard solution of cyanide was prepared according to Nagaraja *et al.*¹ A 0.225 % aqueous solution of ninhydrin in 2% sodium carbonate was prepared. Also, a 2.5 N solution of potassium hydroxide was used to increase pH, when necessary. Nitrogen was bubbled into almost all solutions to release the interfering oxygen. KOH of Merck quality was used to bring ninhydrin or cyanide solutions with various pH values.

Instrument

A Libbra S35 PC UV/VIS spectrophotometer with 1-cm matched cells of quartz was used for spectral measurements. The pH values were measured with a HANNA PH 211 microprocessor pH meter. The heating was performed with an ARES heating magnetic stirrer. A Mikro 22 R centrifuge was used as well.

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Procedure

Samples of 1 mL of cyanide solution containing maximum $2 \mu\text{g mL}^{-1}$ were pipetted into the spectrophotometer cuvette, followed by the addition of 0.5 mL of ninhydrin solution. To increase the sensitivity of the reaction or to check the reagent stability, potassium hydroxide solution was added. The resulting solution was bubbled with nitrogen, capped and let to develop color. In the most cases, nitrogen was not used in order measure as quick as possible the absorbance. However, each solution used in the reaction was previously purged with nitrogen. The deep-red or deep-blue color was measured at 485 nm and 590 nm, respectively. A reagent blank with no cyanide was used.

The UV-Vis spectra were recorded in the range from 200 nm to 700 nm.

The influence of UV and solar light was investigated by irradiating ninhydrin reagent for 30 min with a microbiological UV lamp or sun light. After the irradiation, the reagent was reacted with cyanide and the absorbance was measured from 200 nm to 700 nm.

RESULTS AND DISCUSSION

Sensitivity of cyanide determination

Cyanide reaction with the ninhydrin reagent proved to be very sensitive and selective.¹⁻³ Even a very low amount of potassium cyanide, below $2 \mu\text{g mL}^{-1}$, induced a very high absorbance of the resulted adduct between cyanide and ninhydrin. A molar absorptivity of $5 \times 10^4 \text{ mol}^{-1} \text{ cm}^{-1}$ was calculated at λ 485 nm for a solution of 30 μM potassium cyanide. The detection limit was 13 ng mL^{-1} KCN. Therefore, it was easily to observe the apparent color change at concentrations as low as 13 ppb for cyanide anions. The reagent was very selective; only in the presence of cyanide anions, the alkaline solutions of ninhydrin had changes in color. It should be pointed out that all the above experiments were conducted in pure water solutions, no organic solvents were needed any longer, and that many previously reported sensors for cyanide worked in the mixture solution of organic solvents and water. Thus, this point should benefit the potential applications of ninhydrin reagent.

One of the advantages of this method proved to be the high reaction rate of cyanide with ninhydrin.

Thus, the reaction was complete in only 15 to 20 seconds, which is very important to detect cyanide in the contaminated and dangerous environment. Contrary to the most of analytical methods for cyanide, ninhydrin-based assay is very simple and effective. To increase the sensitivity of the measurements, we tried to make the reaction at high pH. However, with increasing pH the stability of ninhydrin dramatically decreased.

The effect of pH on the reaction of cyanide with ninhydrin

Ninhydrin is rather sensitive to increasing pH and decomposes over pH 12 (Fig. 1). Thus, ninhydrin is more stable at pH 10, in the presence of sodium carbonate, and began to decompose over pH 11. Therefore, the determination of cyanide with ninhydrin in the presence of sodium carbonate at pH 10 is quite safe. Nagaraja *et al.* proposed to perform the reaction between cyanide and ninhydrin in the presence of carbonate, followed by addition sodium hydroxide to increase pH.¹

It was supposed that the indene derivative formed in the presence of sodium carbonate as a monosodium salt will result in a double sodium salt, which is blue in color. This hypothesis may explain the maxima of absorbance at 380.0 nm which decreased with increasing pH.

However, the part of ninhydrin which was not decomposed on increasing pH reacted with cyanide even at this pH value to afford the colored adduct. Therefore, under high pH conditions ninhydrin is unstable and decomposes quickly. We found that under alkaline conditions, the absorbance at 590 nm of the blue-colored ninhydrin-cyanide adduct decreases with time, depending on the pH value (Fig. 2).

Fig. 3 shows the effect of very high pH values on the absorbance of the indene derivative. There was found a large pH interval where the red-colored form is very stable (from pH 10.00 to pH 11.22).

On increasing pH, one may find an interval from pH 11.22 to pH 11.82 for the two different species (the blue form with an absorbance maximum at 590 nm and the red one with the maximum at 485 nm). The highest absorbance at 590 nm was obtained only at pH 12.8; higher pH values may destroy the indene derivative formed. Therefore, one could determine cyanide either at 485 nm or 590 nm, without any color interference.

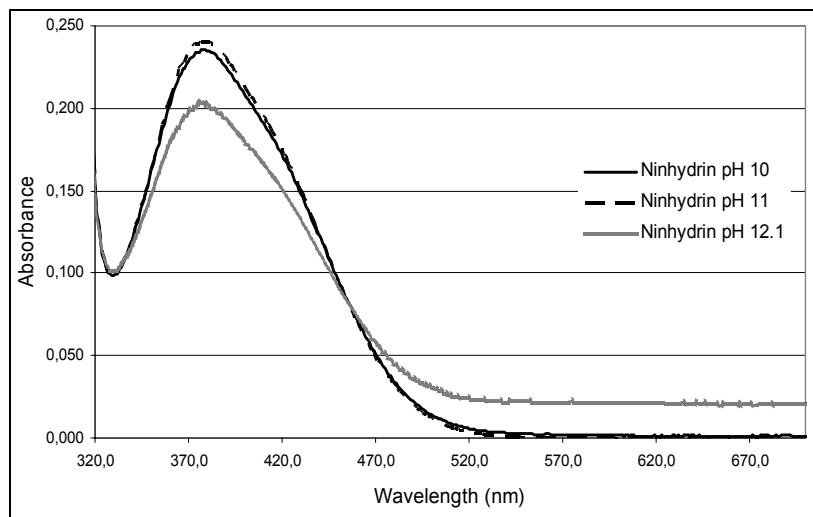


Fig. 1 – Effect of pH on ninhydrin absorbance.

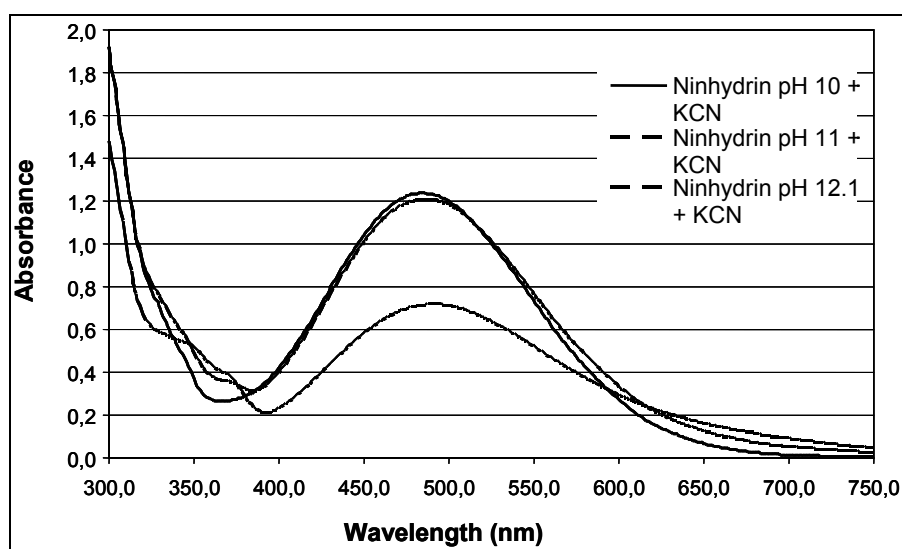


Fig. 2 – Cyanine-ninhydrin complex evolution with the pH.

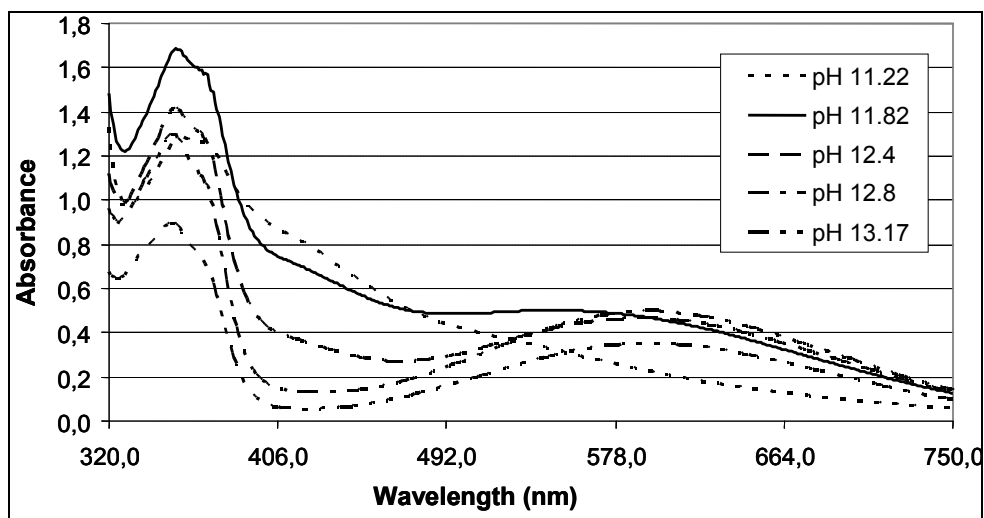


Fig. 3 – Effect of pH on the absorbance of cyanine- ninhydrin complex.

The effect of UV light on ninhydrin

Ninhydrin was damaged by UV or even visible light, as shown by the absorption maximum shifting in UV range from 380 nm to 360 nm. On adding cyanide ions to the irradiated ninhydrin reagent, two complexes with different intensities but the same maxima of absorption were found.

Therefore, we supposed that only the proportion of active ninhydrin was decreased, without affecting the reaction mechanism (Fig. 4). The use of brown bottles to keep the ninhydrin reagent, which was previously purged with nitrogen, avoided the lowering of the absorbance during cyanide determination.

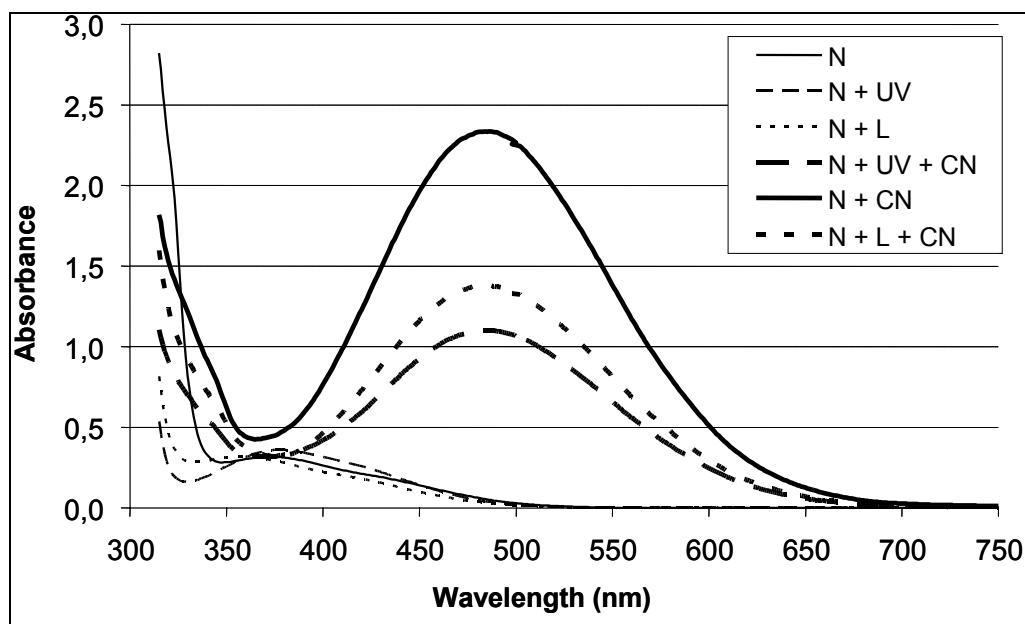


Fig. 4 – Effect of UV-Vis irradiation on the absorbance of ninhydrin and cyanide-ninhydrin adduct in 2% sodium carbonate solution.

Headspace sample of hydrocyanic acid

Head-space sampling of hydrocyanic acid may have many advantages due to complete avoidance of any interference under the conditions of using the ninhydrin reagent, which is very sensitive (Fig. 5). As a consequence, the selectivity of this assay also increased much. Nevertheless, the reaction was delayed and lasted for more than 300 seconds, being inverse proportional with cyanide concentration.

The colorimetric sensors

Within this experiment, we also developed “naked eye” colorimetric sensors by mixing a solution containing cyanide with the reagents in a test tube. The color was compared with standards found in other test tubes. Hence, the low detection limit of the ninhydrin reagent towards cyanide

anions leads to the direct naked eye sensing of this anion. Cyanide can be detected at very low concentration by simple reaction with ninhydrin in the presence of sodium carbonate. If necessary to confirm the presence of cyanide in contaminated waters, the addition of sodium or potassium hydroxide will result in a highly intense colored solution, whereas the change in color might be a sign of cyanide presence.

The determination of cyanide in industrial waste solutions is a challenging task.^{2,3} Usually, cyanide could be found in basic water with the pH range between 8.0 and 9.0.⁷ Because the addition of cyanide to a solution with low a pH value would lead to the formation of hydrocyanic acid, a highly toxic gas, we used only alkaline solutions. Thus, under the experimental conditions of this protocol, it was safe for the operator.

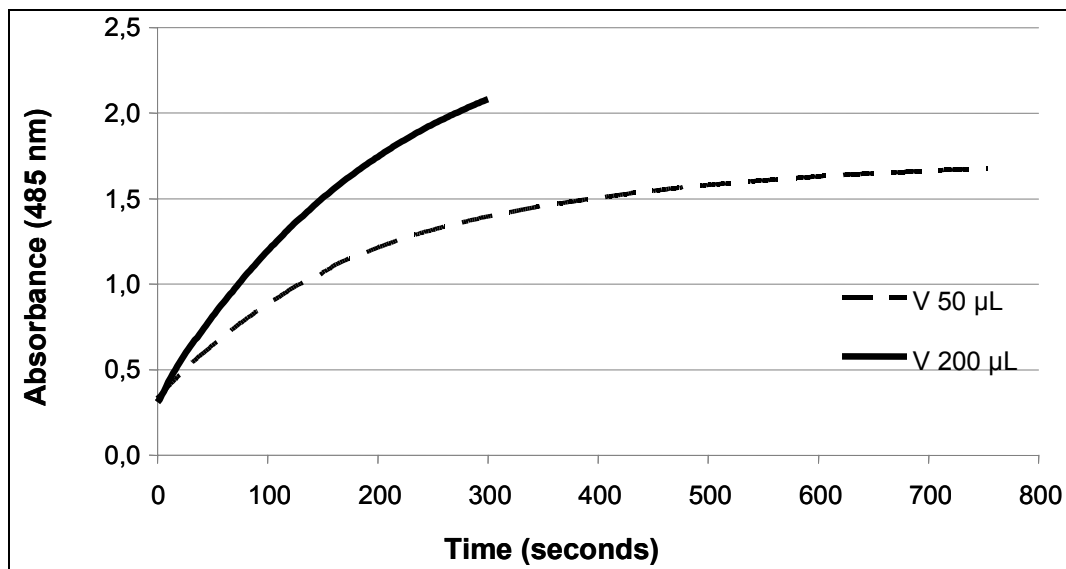


Fig. 5 – Head-space sampling of cyanide and ninhydrin determination.

CONCLUSIONS

The reaction between cyanide and ninhydrin under alkaline conditions can be used as a method to identify and determine cyanide in the contaminated environments (water, air, industrial effluents, etc.). The effect of pH and UV light was investigated. The most robust determination of cyanide can be made in the presence of sodium carbonate, under nitrogen and in the absence of UV-Vis radiation. Reagents are easily available, the reaction is very fast and no special devices are necessary.

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