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## Spectrophotometric analyses of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in water

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### ABSTRACT

A simple and accurate spectrophotometric method for on-site analysis of royal demolition explosive (RDX) in water samples was developed based on the Berthelot reaction. The sensitivity and accuracy of an existing spectrophotometric method was improved by: replacing toxic chemicals with more stable and safer reagents; optimizing the reagent dose and reaction time; improving color stability; and eliminating the interference from inorganic nitrogen compounds in water samples. Cation and anion exchange resin cartridges were developed and used for sample pretreatment to eliminate the effect of ammonia and nitrate on RDX analyses. The detection limit of the method was determined to be 100 µg/L. The method was used successfully for analysis of RDX in untreated industrial wastewater samples. It can be used for on-site monitoring of RDX in wastewater for early detection of chemical spills and failure of wastewater treatment systems.

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### Introduction

Royal demolition explosive (RDX) or hexahydro-1,3,5-trinitro-1,3,5-triazine is also known as “hexogen” and “cyclonite” (Özhan et al., 2003; Uzer et al., 2008). It is an explosive nitroamine that is widely used in military and industrial applications. RDX is used extensively in munitions by the USA military (US EPA, 2012), and commonly used with other explosives since it is sensitive to shock detonation. RDX is produced, loaded, packed and assembled into weapons at US Department of Defense facilities (Freedman and Sutherland, 1998). During the production and handling of RDX, a large amount of wastewater is generated.

According to an announcement from the Agency for Toxic Substances and Disease Registry in 2010, RDX can be released into the environment through: leakage; launching of ammunition;

disposal of munitions; open burning; demilitarization of ammunition, as well as the explosive manufacturing facilities via inadequate treatment, improperly lined impoundments, lagoons and ditches (Ronen et al., 1998). RDX is not retained well by soils and its rate of biodegradation is very slow, so it can migrate readily to groundwater (US EPA, 2012), which results in significant levels of soil and groundwater contamination. If RDX is ingested by humans and animals through the food chain, it can cause seizures, methemoglobinemia, anemia, and severe damage to the liver and kidney (Özhan et al., 2003). The US EPA has classified RDX as a Class C possible human carcinogen (Freedman and Sutherland, 1998) and identified it in more than 31 of the 1699 total hazardous waste sites proposed for inclusion on the National Priorities List.

Several methods have been used for the analysis of RDX in water and solid samples. The US EPA standard analytical

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method SW-846 Methods 8330 (US EPA, 1992) specifies the use of high-performance liquid chromatography with ultraviolet detection (HPLC-UV) (Jenkins et al., 1986). However, HPLC is associated with a long analysis time (Uzer et al., 2008) and can only detect RDX at the mg/L level. Extraction of RDX from water in order to concentrate the chemical, followed by HPLC analysis, can significantly improve the detection to the  $\mu\text{g/L}$  level, but the extraction and concentration procedures take many hours and are labor-intensive. Nitro- and nitroso-compounds can respond specifically to high-performance liquid chromatography–thermal energy analysis (Fine et al., 1984). However, the appearance of unidentified peaks or false-positive values can affect the results, which can be confirmed using gas chromatography–mass spectrometry (Yinon, 1996; Uzer et al., 2008). Mass spectrometers have good potential for the sensitive detection of RDX but are expensive, bulky, and require well-trained chemists and technicians for operation (Uzer et al., 2008). Therefore, simple analytical methods that can be used by operators and engineers for on-site monitoring of RDX in wastewater streams and in treated wastewater are needed for early detection of chemical spills and failure of wastewater treatment systems.

A spectrophotometric method was developed based on the Griess reaction for on-site measurement of RDX (Jenkins and Walsh, 1992). However, the reduction of RDX to nitrite is usually non-quantitative, and nitrite is unstable at low pH. Another spectrophotometric method for an RDX assay was based on the Berthelot reaction (Uzer et al., 2008). In this method, RDX in a water sample was reduced to ammonia and low molecular-weight amines by Zn powder under acidic (HCl) conditions for 2 min. The treated sample was filtered to remove the solids. Then a reagent mixture consisting of NaOH, phenol, and 1.5 mL of bleach (~5% NaClO) was added into the filtrate. The reduced ammonia and amine compounds reacted with phenol and hypochlorite in alkaline medium through the Berthelot reaction to form blue indophenol color compounds that have strong absorbance at 631 nm. In 1859, the Berthelot reaction was first mentioned by a French chemist, who noted the blue coloration that developed after mixing solutions of ammonia, phenol and hypochlorite (Daridon et al., 2001). This reaction is the basis for the analysis of ammonia and amine species using Berthelot's reagent (*i.e.*, an alkaline solution of phenol and hypochlorite). However, phenol is toxic and volatile at room temperature, as well as having a caustic odor.

In this work, less toxic and more stable reagents were used and their doses and reaction time were optimized to improve the accuracy and reliability of the spectrophotometric method for analysis of RDX. The noxious and toxic phenol was replaced by sodium salicylate ( $\text{C}_7\text{H}_5\text{NaO}_3$ ), which is a stable reagent without a caustic odor. Disodium pentacyanonitrosylferrate(III), also known as sodium nitroprusside,  $\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]$ , was used as the color stabilizer and as a catalyst to reduce the analysis time (Patton and Crouch, 1977; Tao et al., 2008). Pentacyanoferrate could also be used as a coupling reagent to improve the sensitivity of nitrogen compounds (Kanda, 1995). Cation and anion exchange resin cartridges were developed for pretreatment of water samples to eliminate the interference of ammonium and nitrate with the RDX analysis. The simple and user-friendly analytical method can be used by operators and engineers at wastewater treatment plants and in the field with a portable spectrophotometer for on-site analysis of RDX.

## 1. Materials and methods

### 1.1. Materials

All chemicals were of reagent grade and used without further purification. A standard stock solution of RDX (1000 mg/L) was prepared by dissolving 0.100 g of RDX sample in acetone and diluting to 100 mL with deionized (DI) water. Standard solutions of RDX (50  $\mu\text{g/L}$  to 40 mg/L) were prepared by dilution of the stock solution with DI water. HCl and NaOH solutions were prepared using the chemicals and DI water. Sodium salicylate solution (240 g/L) was prepared by dissolving 24.000 g of solid  $\text{C}_7\text{H}_5\text{NaO}_3$  in 100 mL DI.  $\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]$  solution (18 g/L) was prepared by dissolving 0.090 g solid sodium nitroprusside into 5 mL DI. This solution could be degraded easily under sunlight, so it was prepared every 4 days. Anion exchange resin (SBG I) and cation exchange resin (CGS) were obtained from Resin Tech Inc., West Berlin, NJ, USA. The cation exchange resin was converted to the chloride form using 1 mol/L HCl solution, then washed with DI. Cation and anion exchange resin cartridges were prepared by filling 2.5 mL tips with the exchange resins.

### 1.2. Methods

The wastewater samples collected from an RDX production plant were passed through cation and anion exchange resin cartridges for the removal of ammonium and nitrate. After discarding the first 20 mL of the filtered sample, another 20 mL filtered water sample was collected for analysis.

The reduction of RDX was carried out by adding 0.065 g Zn dust and 0.5 mL HCl (1 mol/L) into 20 mL of water and letting the mixture stand for 10 min at room temperature. The solution was separated from the solid in the mixture by centrifugation at 10,000 r/min for 5 min using a centrifuge (Eppendorf 5804) or filtration using a 0.45  $\mu\text{m}$  syringe filter. Then, 1 mL  $\text{C}_7\text{H}_5\text{NaO}_3$  solution (240 g/L), 0.4 mL  $\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]$  solution (18 g/L; as a color stabilizer and catalyst), 0.8 mL NaOH solution (2 mol/L), and 0.04 mL NaClO solution (5%) were added into 10 mL of the reduced RDX supernatant solution to initiate the Berthelot reaction. After 1 hr of reaction, a stable indophenol blue color was formed. Then, the absorbance of the reacted samples was measured using a portable spectrophotometer (DR 2010, Hach Co., Loveland, CO, USA).

HPLC analyses of wastewater samples were performed to determine the accuracy of the spectrophotometric analyses. The HPLC (LC-10AT, SHIMADZU, Kyoto, Japan) was equipped with a Hypersil C-18 column. A mobile phase (50% MeOH–50%  $\text{H}_2\text{O}$ ) with flow rate of 1 mL/min and a UV wavelength of 254 nm were used in the analysis.

## 2. Results and discussion

### 2.1. Optimization of analytical conditions

$\text{C}_7\text{H}_5\text{NaO}_3$  must be completely ionized in alkaline solution to facilitate the indophenol reaction as a color developer (Tao et al., 2008). Hence, the dose of the reagent should be sufficient to ensure complete color development of the reduced RDX

compounds through the Berthelot reaction. Fig. 1a shows the effect of salicylate dose on the absorbance of a 2 mg/L RDX standard solution at 670 nm. The absorbance increased rapidly with increasing  $C_7H_5NaO_3$  dose (0–0.8 mL). When the dose increased from 0.8 to 1.0 mL, there was no obvious increase in the absorbance. The results suggest that 1 mL of 240 g/L  $C_7H_5NaO_3$  solution should be used for analysis of a 10 mL water sample containing less than 2 mg/L of RDX (Tao et al., 2008).

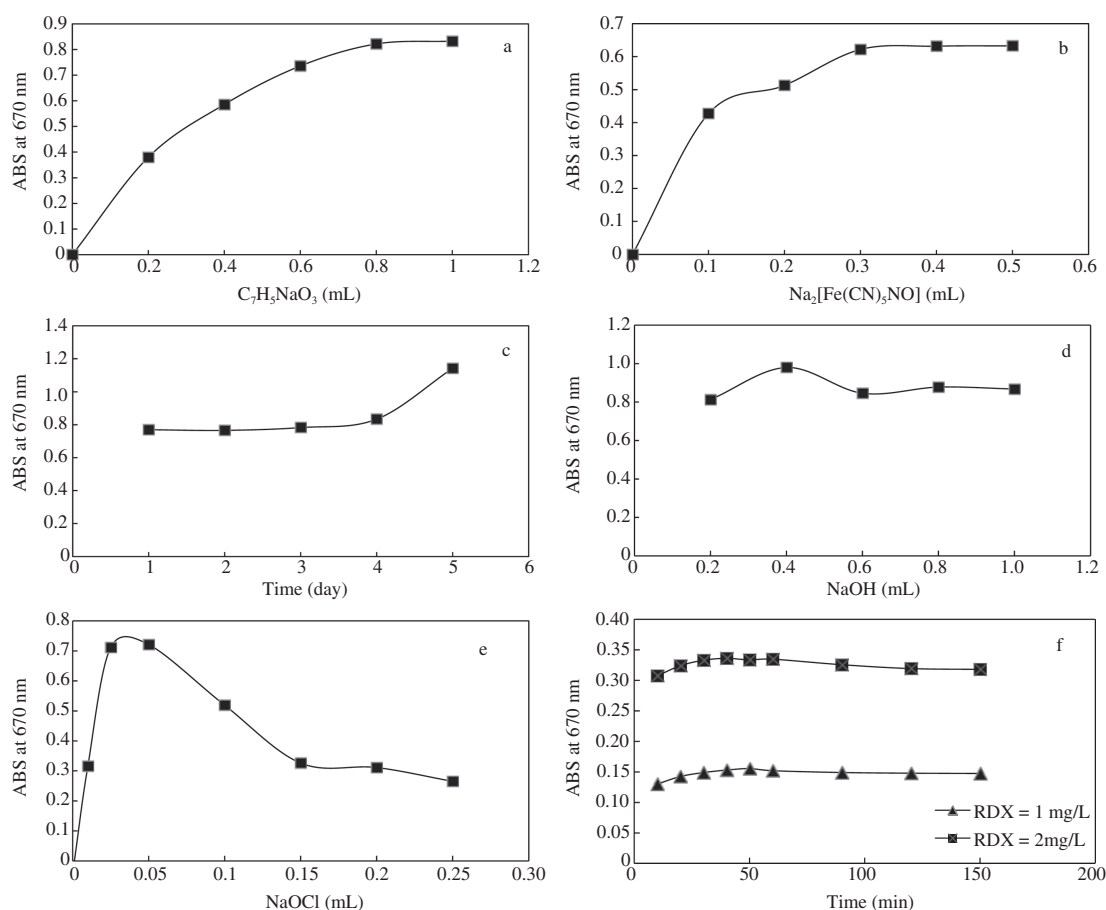
Fig. 1b shows that the absorbance increased rapidly with increasing  $Na_2[Fe(CN)_5NO]$  (18 g/L) dose from 0 to 0.3 mL. As the dose increased from 0.3 to 0.5 mL, the absorbance remained the same, indicating that the optimal dose of  $Na_2[Fe(CN)_5NO]$  was in the range of 0.3–0.5 mL.

The effect of storage time of  $Na_2[Fe(CN)_5NO]$  solutions on the analysis of RDX was also evaluated because  $Na_2[Fe(CN)_5NO]$  is relatively unstable.  $Na_2[Fe(CN)_5NO]$  solutions stored at room temperature for different periods of time were used in the analysis tests. Fig. 1c shows that the absorbance was similar when solutions stored for up to 4 days were used for the analysis. A solution stored for 5 days resulted in high absorbance. The results indicate that solution could be stored for up to 4 days.

NaOH was used for adjustment of the pH of the samples to alkaline conditions to facilitate the Berthelot reaction (Patton and Crouch, 1977). Fig. 1d shows that the absorbance remained similar in a broad NaOH dose range of 0.2–1.0 mL. To ensure alkaline conditions, 0.8 mL of 2 mol/L NaOH solution was selected for RDX analyses.

NaClO was used together with  $C_7H_5NaO_3$  to react with the low molecular-weight amines and ammonium for the development of the blue indophenol color for RDX analyses. Fig. 1e illustrates the absorbance of 2 mg/L RDX standard solution as a function of different volumes of 5% NaClO. The absorbance increased dramatically with increasing NaClO dose from 0 to 0.03 mL. When the dose further increased from 0.05 to 0.25 mL, the absorbance decreased. The results indicate that the optimal dose of NaClO was in the range of 0.03–0.05 mL.

The effect of color development reaction time on absorbance is presented in Fig. 1f. The absorbance increased with increasing reaction time within the first 60 min. The absorbance remained almost unchanged when the reaction time increased from 60 to 150 min. Thus, the optimal reaction time for RDX analysis was 60 min. The reacted samples should be analyzed within a reaction time of 60–150 min to obtain accurate analytical data.



**Fig. 1 – Absorbance of RDX as a function of (a)  $C_7H_5NaO_3$  dose, (b)  $Na_2[Fe(CN)_5NO]$  dose; (c) storage time of  $Na_2[Fe(CN)_5NO]$ ,  $C_7H_5NaO_3 = 1$  mL,  $Na_2[Fe(CN)_5NO] = 0.3$  mL; (d) NaOH volumes,  $C_7H_5NaO_3 = 1$  mL,  $Na_2[Fe(CN)_5NO] = 0.3$  mL; (e) NaClO volumes,  $C_7H_5NaO_3 = 1$  mL,  $Na_2[Fe(CN)_5NO] = 0.3$  mL; and (f) reaction time. RDX = 2 mg/L, sample volume = 10 mL, and reaction time = 1 hr except for panel f.**

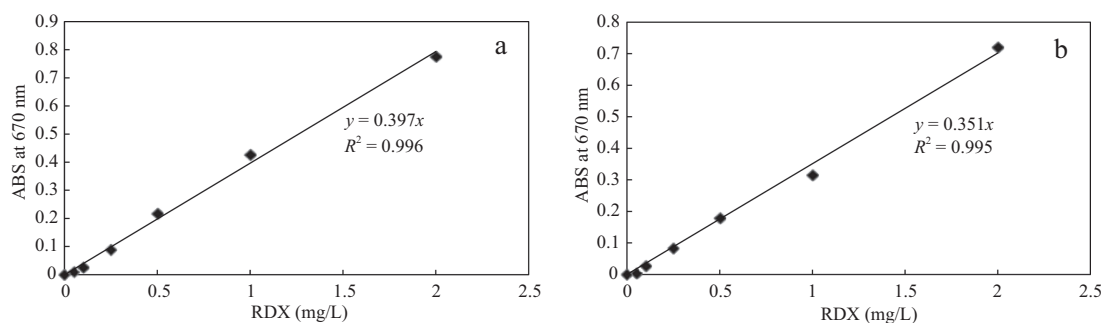


Fig. 2 – RDX standard calibration curves for: (a) DI water standards, and (b) tap water standards.

Based on the optimization results above, a set of analytical conditions was selected for preparation of RDX standard calibration curves and analysis of RDX in water samples in the following tests. The selected conditions for analysis of 10 mL of sample are: 1 mL of 240 g/L  $C_7H_5NaO_3$  solution, 0.4 mL of 18 g/L  $Na_2[Fe(CN)_5NO]$ , 0.8 mL of 2 mol/L NaOH, and 0.04 mL of 5% NaClO. The absorbance of the samples should be measured within a reaction time of 60–150 min. The  $Na_2[Fe(CN)_5NO]$  solution should not be stored at room temperature for more than 4 days.

## 2.2. Standard calibration curves

Fig. 2a and b shows the RDX standard calibration curves for DI water standards and tap water standards, respectively. The linear regression equations and high R-squared values in the figures indicate excellent linear correlation between the absorbance and RDX concentration in a RDX concentration range of 0–2.0 mg/L. The method could be used to detect 100  $\mu$ g/L of RDX in DI and tap water standards, which is more sensitive than the commonly used HPLC method, with a limit of detection more than 500  $\mu$ g/L.

## 2.3. Elimination of interference from co-existing solutes

$NH_4^+$  and  $NO_3^-$  are found commonly in groundwater, surface water, and wastewater, and interfere with the spectrophotometric method for determining RDX. During the analysis,  $NH_4^+$  also forms a blue indophenol color.  $NO_3^-$  can be reduced to ammonium by Zn dust. The ions must be removed from water

samples in order to use the spectrophotometric method for accurate analyses of RDX. Cation and anion exchange resins are commonly used for the removal of  $NH_4^+$  and  $NO_3^-$  from water (McVeigh and Weatherley, 1999; Gendel and Lahav, 2013). The interferences of  $NH_4^+$  and  $NO_3^-$  with RDX analysis were evaluated, and ion exchange resin cartridges were tested for elimination of the interferences.

Fig. 3a illustrates the effects of  $NH_4^+$  on determination of RDX using the spectrophotometric method. The absorbance of the 0.2 mg/L RDX solution was 0.15. After the solution was filtered through the cation exchange cartridge for analysis, the absorbance was 0.14. The slight difference could be due to analytical error and a small amount of RDX removed by the cartridge. When a solution containing 0.2 mg/L RDX and 2 mg/L  $NH_4^+$  was analyzed, the absorbance increased to 3.11. The results demonstrated that  $NH_4^+$  could also react with  $C_7H_5NaO_3$ ,  $Na_2[Fe(CN)_5NO]$  and NaClO under alkaline conditions to produce an indophenol color, significantly affecting the analysis of RDX. When the samples were filtered through the cation exchange resin cartridges and then analyzed, the absorbance was decreased to values similar to that of the 0.2 mg/L RDX solution. Ammonium test kits (TNT 831/832, Hach Co., Loveland, CO, USA) and a spectrometer (DR 2800, Hach Co., Loveland, CO, USA) were used to analyze  $NH_4^+$  concentrations in the samples before and after the cartridge filtration, and determined that the  $NH_4^+$  was completely removed by the resin. The results indicate that the cation exchange resin cartridge could be used to eliminate the  $NH_4^+$  interference.

Fig. 3b shows that  $NO_3^-$  had a similar effect on RDX analysis to that of  $NH_4^+$ . When 20 mg/L  $NO_3^-$  was added to 0.2 mg/L RDX

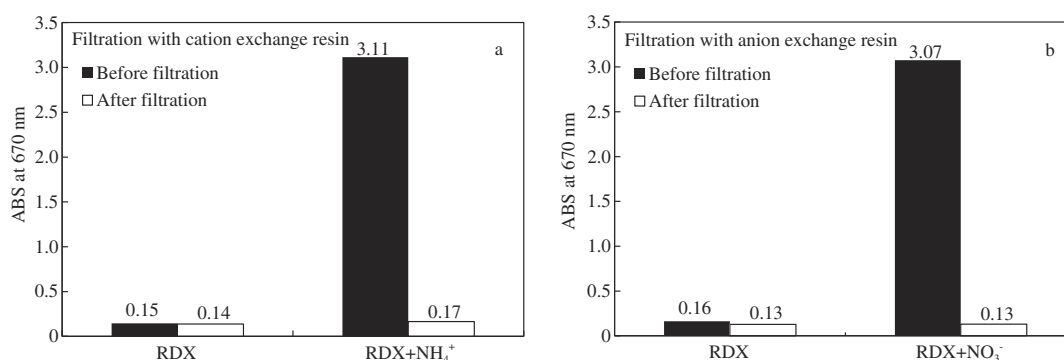


Fig. 3 – Effect of (a)  $NH_4^+$  (2 mg/L), and (b)  $NO_3^-$  (20 mg/L) on RDX analysis, and elimination of the effect by filtration pretreatment of the samples with cation and anion exchange resin cartridges. RDX = 0.2 mg/L, wavelength = 670 nm.

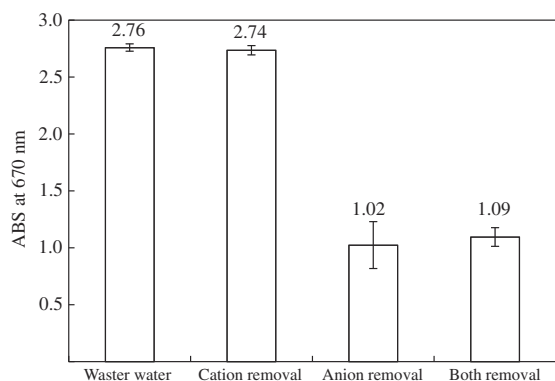
solution for analysis, absorbance of the sample was 3.07. After the RDX + NO<sub>3</sub><sup>-</sup> sample was filtered with the anion exchange resin cartridges, the absorbance decreased to a value similar to that of the 0.2 mg/L RDX solution. The results demonstrated that the anion exchange resin cartridges could be used to eliminate the interference of NO<sub>3</sub><sup>-</sup> from RDX analysis. Analysis of the samples before and after the cartridge filtration using nitrate test kits (TNT 835/836, Hach Co., Loveland, CO, USA) verified that the NO<sub>3</sub><sup>-</sup> was completely removed by the resin.

#### 2.4. Analyses of RDX in wastewater samples

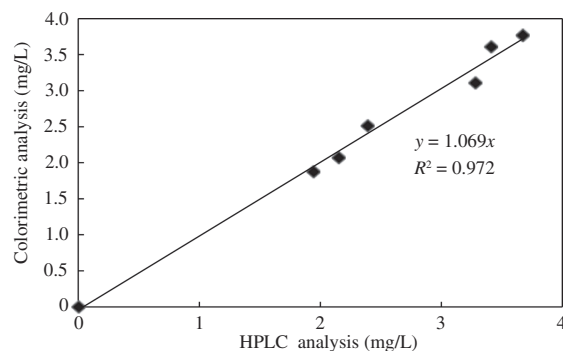
Untreated wastewater samples were collected from an RDX production plant for analysis to assess the accuracy of the spectrophotometric method. Chemical analyses showed that untreated wastewater contained 18 mg/L of NO<sub>3</sub><sup>-</sup> and did not contain NH<sub>4</sub><sup>+</sup>. Fig. 4 shows that the absorbance value of the raw wastewater sample was 2.76. When the sample was filtered through the cation exchange resin cartridge (“cation removal” sample in the figure), the absorbance was reduced slightly to 2.74, which was consistent with the absence of NH<sub>4</sub><sup>+</sup> in the wastewater. After filtration using anion exchange resin cartridges, the absorbance of wastewater samples decreased significantly to 1.02. These results suggested that the effect of NO<sub>3</sub><sup>-</sup> on RDX analysis was eliminated by cartridge filtration. When the wastewater sample was filtered through both cation and anion exchange resin cartridges, the absorbance was almost the same as that of the sample pretreated with the anion exchange resin cartridge. The error bars in the figure indicate that the analytical errors of the method were between 1% and 20%.

A set of standard solutions with a RDX concentration range of 1–4 mg/L was used to prepare a standard calibration curve for the analysis of the untreated wastewater samples. A linear calibration curve with a high R-squared value of 0.997 was obtained (the calibration curve is not presented). The spectrophotometric analyses determined the RDX concentration in the untreated wastewater sample to be 3.28 mg/L. When the sample was spiked with 2.0 mg/L RDX, a RDX concentration of 5.29 mg/L was measured. A recovery of 101% was achieved.

Fig. 5 shows a comparison of RDX concentrations measured with the spectrophotometric method and HPLC for the



**Fig. 4 – Elimination of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> effect using filtration pretreatment of wastewater sample with cation and anion exchange resin cartridges.**



**Fig. 5 – Comparison of RDX concentrations in wastewater samples determined by the spectrophotometric method and HPLC analysis.**

raw wastewater samples. The spectrophotometric results agreed well with the HPLC data, as indicated by the slope of 1.069 and R-squared value of 0.972 of the linear regression line in the figure. The results suggest that the effects of nitrate and ammonium on the spectrophotometric analysis of RDX were eliminated by the exchange resin pretreatment, and there was no detectable effect of other chemicals in the wastewater samples on the analysis.

### 3. Conclusions

On the basis of the Berthelot reaction, a simple spectrophotometric method was developed for analysis of RDX concentrations in water samples. Sodium salicylate and disodium pentacyanonitrosylferrate(III) could be used to replace toxic phenol in the analysis. NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> significantly interfered with the analysis of RDX. These effects could be eliminated with filtration pretreatment of samples using cation and anion exchange resin cartridges. The spectrophotometric method could be used by engineers and operators for on-site analysis of RDX in wastewater.

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