

## Determination of 2,4,6 Trinitrotoluene in Soil Samples by flow Injection Analysis Followed Chemometrics Methods

Farideh Bataghva\*

Department of Chemistry, Faculty of Science, Malek Ashtar University of Technology, Tehran, Iran

\*Corresponding author: Farideh Bataghva, Department of Chemistry, Faculty of Science, Malek Ashtar University of Technology, Tehran, Iran, E-mail: fbataghva@semnan.ac.ir

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### Abstract

A rapid flow injection spectrophotometric method as an effective method for the selective determination of 2,4,6-trinitrotoluene (TNT) in soil samples is developed. The method is based on a derivatization reaction of TNT with sodium sulfite in a basic acetone medium through janovsky reaction. Due to the high amount of data obtained and the need of evaluate and interpret these data, chemometrics method using PLS calibration was utilized. An acetone-water medium of 80% (v/v) acetone was found to be optimal condition for the determination of TNT. The dynamic linear range and limit of detection were 5-100  $\mu\text{g g}^{-1}$  and 1  $\mu\text{g g}^{-1}$  for 200 mg soil samples. Quantitative results from the determination of TNT in contaminated soil samples shows the good recoveries in the range of 94.6-106.3%.

### Keywords:

2,4,6 Trinitrotoluene, Flow injection analysis, Chemometrics method, PLS calibration, soil sample

### Introduction

One of the most important nitro aromatic compound which is widely used is the energetic compound 2,4,6-trinitrotoluene (TNT). Due to the highly explosive properties, TNT is used for different purposes in military activities or industries like mining or construction. TNT is relatively water soluble compound which can be found in soil and groundwater around places associated with the manufacturing, handling, testing, and disposal of explosive and propellant materials. Due to its risks caused by long- and short-term exposure, there is considerable interest in TNT measurements in military area samples [1].

Up to now, various techniques such as thin-layer chromatography, membrane separation coupled with spectrometric detection, HPLC determination and spectrophotometric (colorimetric) techniques have been utilized for trace determination of TNT in environmental samples [2].

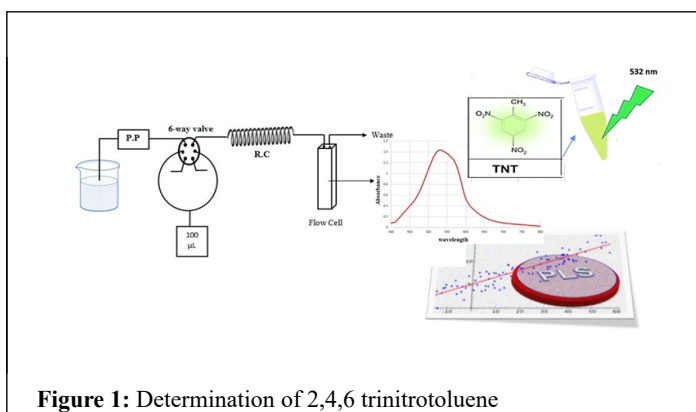
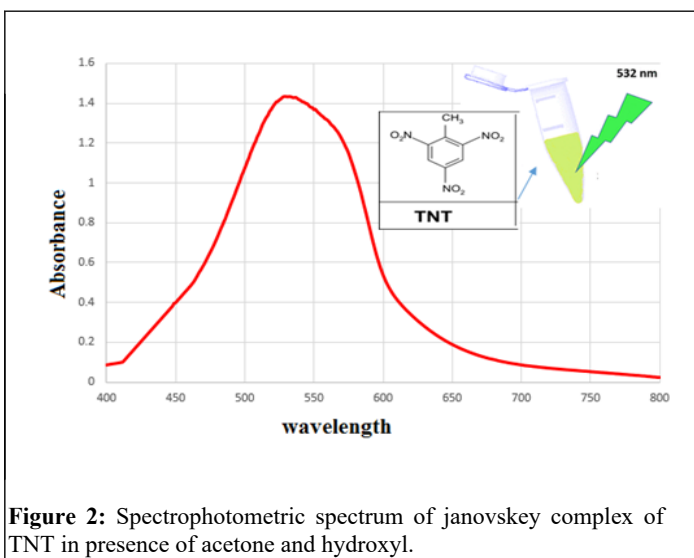


Figure 1: Determination of 2,4,6 trinitrotoluene

Although these methods have successfully been applied for explosive determination, they have some limitations such as expensive, complex and unavailable equipment which may not be accessible in every locations. Spectrophotometric methods as very inexpensive and available methods can easily use with good sensitivity for the color materials [3]. In the presence of acetone and a base, TNT can form the red-colored Janovsky complexes to produce 2,4,6-trinitrobenzyl anion (TNT<sup>-</sup>), which can easily monitored in the visible spectrum. Fig. 1 shows the visible spectrum of janovsky complex of TNT. Besides the many advantages of spectrophotometric technique, some analytes may be hard to measure due to overlapping their bands. Chemometrics methods that are a combination of statistics and mathematics, have arisen in order to overcome this problem [4].

In the past decade, Flow Injection (FI) techniques as a powerful method, which can provide various advantage involving automation, miniaturization, versatility, greater accuracy, higher speed, lower sample and reagent consumption (few tenths of microliters), lower risk of contamination or analyte loss and inexpensiveness have attracted great attention. Besides these features, the availability of coupling FI system with different detector could obtain the requirements of chemical analysis, especially in forensic science [5]. The coupling of flow injection system with spectrophotometric detectors can provide comprehensive and also complex analytical data. So in order to analysis the obtained data, using chemometrics methods can be very efficient and important. Partial Least Square Regression (PLSR) is one of the most widely used application of chemometrics [6]. PLSR is the one of multivariate calibration methods that is performed by decomposition of concentration and absorbance matrices in order to identify the unknown variables of the model. PLSR using mathematical calculations can provide a more simple and low-cost procedure compared to complex and expensive sample preparation procedure that can be used for analysis of complex data of FI systems Figure 2 [7].



This paper describes a new procedure for quantitative analysis of TNT in soil samples based on formation of Janovsky complex in an automated flow injection system [8]. The parameters of the flow system like flow rate, reaction coil length and the concentration of the reagents were optimized using design of experiments. According to the numerous and complex data obtained from FIA system and possible existence of interfering materials, PLS calibration as a chemometrics method was used. Finally, the proposed method was utilized for the determination of TNT in the real soil samples [9].

## Experimental

### Instrumentation

The Figure 3 shows the FI manifold used for this work, schematically. The FI system consisted of a multi-channel Heidolph peristaltic pump (Heidolph PD5001), silicon tube with 0.8 mm internal diameter with 2 m length, a six-way Rheodyne injection port with 100  $\mu$ l injection volume, a flow cell with 2.0 mm path length and Agilent-8453 UV/Vis spectrophotometer which recorded the absorbance at 532 nm. Agilent UV-Visible Chem-Station software for data acquisition and instrumentation control was used throughout [10]. The pH values were measured and adjusted by an AMTAST pH-meter equipped with combined Ag/AgCl electrode. MATLAB (Version 7.14) was utilized for Partial Least Square (PLS). All spectra were scaled prior to PLS [11].

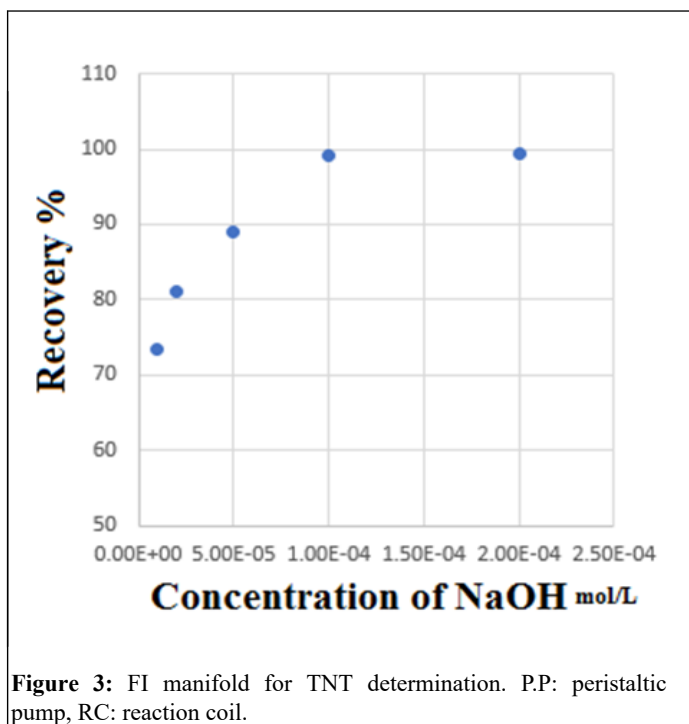
### Materials and Reagents

All chemicals used in the experiments were analytical reagent grade. A 1000 ppm stock solution of TNT was made by dissolving the appropriate amount of solid TNT into solution of 80% (v/v) acetone (ACS grade, Merck, Germany) and 20% (v/v) ultrapure water [12]. The ultra-pure water was prepared by a model Aqua Max-Ultra, Youngling ultra-pure water purification system (Dongang, South Korea). Sodium sulfite and sodium hydroxide were Merck products [13].

### Procedure

According to Figure 3, a solution containing 10<sup>-4</sup> M of sodium sulfite and 10<sup>-4</sup> M of NaOH in 50% V/V acetone/water with flow rate

of 1 mL min<sup>-1</sup> was used as the flow solution. 1.0 g of three contaminant soil samples, were sieved through a 40-mesh sieve to remove larger parts. Then, the finely ground soils were dried in an oven at 110°C for 1 h in order to removing the humidity content, then cooled at room temperature. After that, the samples were accurately weighted and introduced into 30 ml conical tube [14]. Next, certain amounts of TNT in the ranges of 5 to 100  $\mu$ g g<sup>-1</sup> were added to the soil sample and kept for 24 h in the dark, dry and cold place. Then, 2 mL acetone was added to the mixture and the tube was taken place in ultrasonic bath for 15 min. Then the content of tube was centrifuged at 4000 rpm for 5 min and the liquid was collected for further analysis through FI system. An aliquot of 160  $\mu$ L of this liquid was mixed with 40  $\mu$ L of ultra-pure water to reach the percentage of 80:20% of acetone: water [15]. The resulting solution was injected to the 100  $\mu$ L injection loop. Then, the injection valve was turned and the flow solution started to introduce the resulting solution to detector through 2 m reaction coil. The UV/Vis instrument recorded the absorbance of the solution every 10 s in 530 nm. Then the obtained data was collected and transferred to MATLAB for further data analysis [16].



### PLS calibration

As a multivariate calibration method, partial least square as one of the most common techniques highly used due to its suitability and availability. Using FI systems can simplify the procedures but also make data more complex [17]. So, in order to analysis of obtained data from FI system chemometrics methods can be very helpful. Constructing the calibration matrix is the first and most important step in the multivariate calibration for FI systems by PLS technique. Seven different standard solutions containing different amount of TNT were made in 80:20% acetone: water and injected to the FI system. The mentioned procedure was performed and the obtained data were collected and transferred to the MATLAB for further analysis. Table 1 shows the calibration set for the PLS analysis (Table 1) [18].

RUN	Concentration of TNT (mg L <sup>-1</sup> )
1	0.2
2	0.4
3	0.8
4	1
5	2
6	5
7	10

**Table 1:** The calibration set for PLS regression.

## Result and Discussion

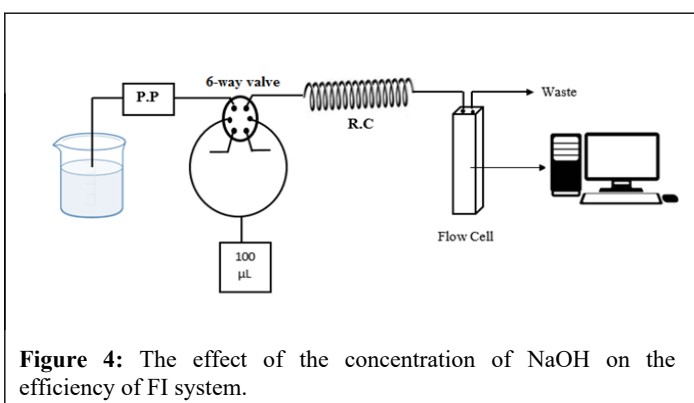
### Optimization of FI parameters

Different parameters such as reaction coil length, injection volume and concentration of the flow solution can affect the efficiency of the FI systems. The injection volume has the most important effect on the FI efficiency according to the fact that with increase the injection volume, the sensitivity of the methods will enhance [19].

Different valve loop with certain volume of 25-200  $\mu\text{L}$  were used and assessed to find the optimum volume. The volume of 100  $\mu\text{L}$  has the best results so it was chosen as the injection volume. The second important parameter is the concentration of the flow solution [20]. Fortunately, the flow solution in this study has no absorbance in the range of visible spectrum so the concentration of 10<sup>-4</sup> (mol L<sup>-1</sup>) was chosen for sodium sulfite.

Another important concentration in this study is the concentration of hydroxy. The pH of the flow solution play a very important role in the formation of janovsky complex so different amount of NaOH were added to the sulfite solution and the most appropriate concentration was chosen as the optimized one [21].

The Figure 4 shows the effect of different concentration of NaOH on the efficiency of the FI system. Finally, the concentration of 10<sup>-4</sup> mol L<sup>-1</sup> was chosen as the optimal value. The recovery% was calculated from following equation [22].



Reaction Coil (RC) is the other parameters on the determination yield. Reaction coil causes good distribution of the analyte into the reagents bulk. If the reaction coil is too short, the analyte cannot have enough time to diffuse and interact with reagents. On the other hand, if RC is too long, the analyte distributes too much to the flow solution and the sensitivity becomes less. So, for this study the RC lengths of 0.5, 1, 1.5, 2, 2.5 and 3 M were assessed and the length of 2 M was chosen as the suitable length. Through optimization of the parameters the flow rate was 1 mL min<sup>-1</sup> for all experiments.

### Analytical figures of merit

The analytical performance of the FI procedure determination of TNT was calculated for the results obtained from spectrophotometric measurements and PLS analysis. Linear calibration curve was obtained over the ranges of 5 to 100  $\mu\text{g g}^{-1}$  with the equation of  $y=0.0183C+0.077$  where C is the amount of TNT in certain weighted soil sample with correlation coefficient of 0.9964 [23].

The limit of detection (LOD) was calculated as  $3sb/m$  where sb is the standard deviation of the blank signal for 5 replications and m is the slope of extraction calibration curve. The LOD for TNT extraction from soil samples was calculated to be 1  $\mu\text{g g}^{-1}$ .

The inter-day and intra-day Relative Standard Deviation (RSD%) for five replicate measurements in three days of TNT at the 10  $\mu\text{g g}^{-1}$  level was 1.2 and 4.4, respectively [24].

### Analysis of real samples

The proposed FI procedure was applied for the extraction and determination of TNT in three soil samples provided from different regions of Tehran (Iran).

A 200 mg of each sample was sieved and the mentioned procedure in the experimental section was applied to each of them.

Moreover, for assessing the validity of the proposed procedure, various amounts of the analyte were spiked to samples and the relative recoveries of 94.6-106.3% were obtained [25]. The obtained results are given in Table 2.

sample	Added ( $\mu\text{g g}^{-1}$ )	Found ( $\mu\text{g g}^{-1}$ )	Recovery %
Soil sample 1	0	BDL	---
	10	9.87( $\pm 0.1$ )	98.7
	20	19.79( $\pm 0.2$ )	98.9
Soil sample 2	0	BDL	---
	10	10.21	102.1
	20	18.92	94.6
Soil sample 3	0	BDL	---
	10	10.63	106.3
	20	20.32	101.6

**Table 2:** Determination of TNT in different soil samples using the proposed FI procedure and PLS regression.

## Conclusion

In the present study, a simple FI method was purposed for the extraction and determination of 2,4,6 trinitrotoluene in soil samples followed by spectrophotometric techniques coupled with partial least square for data analysis.

The method is based on complexation of TNT with hydroxy through janovský reaction in presence of acetone. The situation of the FI system and the important parameters like RC length, concentration of reagents and injection volume were assessed and optimized.

This automated method presented low limit of detections, good accuracies and precisions and good dynamic linear ranges for the analytes. These results proved that the purposed technique is so suitable and convenient for the determination of TNT from the soil samples.

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